Modulating Gamma - The Influence of Attention and Disease on Oscillatory Gamma Band Brain Activity

Inaugural-Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Heinrich-Heine-Universität

vorgelegt von

Nina Kahlbrock

aus Hamburg

Düsseldorf, Mai 2011

Aus dem Institut für Klinische Neurowissenschaften
und Medizinische Psychologie
der Heinrich-Heine-Universität

Gedruckt mit der Genehmigung der
Mathematisch-Naturwissenschaftlichen Fakultät der
Heinrich-Heine-Universität

Referent:	Prof. Dr. A. Schnitzler
Korreferent:	Prof. Dr. M. Heil
3. Gutachter:	Prof. Dr. J. Groß
Tag der mündlichen Prüfung:	05.10.2011

Danksagung

In den letzten dreieinhalb Jahren haben mich einige Personen begleitet, die die Zeit meiner Doktorarbeit bereichert haben. Daher möchte ich mich an dieser Stelle bedanken.

Mein besonderer Dank gilt meinem Doktorvater, Professor Dr. Alfons Schnitzler. Vielen Dank, Alfons, dass ich meine Arbeit an deinem Institut durchführen konnte. Vielen Dank für jegliche Unterstützung und wichtige fachliche Anregungen.

Professor Dr. Martin Heil danke ich für die Übernahme des Zweitgutachtens und die fachlichen Diskussionen während dieser Zeit.

Für die Übernahme des Drittgutachtens danke ich Professor Dr. Joachim Groß.

Ganz besonderer Dank gilt Dr. Markus Butz. Markus, vielen Dank für deine unablässige Unterstützung, das Teilen deines Wissens, deine Geduld, deinen Optimismus und vieles mehr.

Weiter möchte ich mich bei meinen Kolleginnen, Elisabeth May und Dr. Jennifer Paszek bedanken. Schön, dass es euch gibt. Vielen Dank für offene Ohren, Kaffeepausen, fachliche Diskussionen, Unterstützung bei Messungen, Doppelkopfabende…

Mein Dank gilt allen Kollegen aus dem Institut für Klinische Neurowissenschaften und Medizinische Psychologie. Ganz besonders bedanken möchte ich mich bei Dr. Joachim Lange, Dr. Hanneke van Dijk, Jan Hirschmann, Dr. Tolga Esat Özkurt, Dr. Holger Krause, Meike Brenner, Matthias Ringleb und Dr. Nienke Hoogenboom.

Diese Arbeit wurde durch unsere Kooperationspartner in der Gastroenterologischen Abteilung der Universität Düsseldorf unterstützt. Mein Dank gilt hier besonders Diethelm Plate und Dr. Gerald Kircheis, die mir in Patientenagelegenheiten stets mit Rat und Tat zur Seite standen.

Natürlich wäre diese Forschung ohne Versuchsteilnehmer nicht möglich gewesen. Mein herzlicher Dank gebührt daher allen Patientinnen, Patienten, Probandinnen und Probanden die an meinen Studien teilgenommen haben.

Für eure immerwährende Unterstützung danke ich euch, Birthe, Till, Ulla und Wolfgang Kahlbrock.

Und dir, Nick, danke ich für alles!

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Glossary

°C	Degree Celsius
ADHD	Attention Deficit Hyperactivity Disorder
BOLD	Blood Oxygen Level Dependent
CFF	Critical Flicker Frequency
EEG	Electroencephalography
fMRI	functional Magnetic Resonance Imaging
GABA	Gamma-Aminobutyric Acid
HE	Hepatic Encephalopathy
HE0	patients with liver cirrhosis but no signs of HE
HE1	overt Hepatic Encephalopathy of grade 1
HE2	overt Hepatic Encephalopathy of grade 2
HE3	overt Hepatic Encephalopathy of grade 3
HE4	overt Hepatic Encephalopathy of grade 4
Hz	Hertz
K	Kelvin
LFP	Local Field Potential
LGN	Lateral Geniculate Nucleus
MEG	Magnetoencephalography
mHE	minimal Hepatic Encephalopathy
MT	Mediotemporal cortex or V5
SQUID	Superconducting Quantum Interference Device
TAP	Tests of Attentional Performance
TMS	Transcranial Magnetic Stimulation
V1	Primary visual area
V2	Secondary visual area
V3	Visual area 3
V4	Visual area 4
V5	Visual area 5

1 Abstract

Attention, the ability to focus on certain aspects of the surrounding world, while ignoring others and oscillatory brain activity are tightly related. This thesis employed Magnetoencephalography (MEG) to extend previous works on attention and its associated oscillatory brain activity. Healthy participants and patients with hepatic encephalopathy (HE), known to have attention deficits, were studied while working on a bimodal task inducing graded levels of visual attention.

Processing advantages of modality specific attended stimuli compared to non-attended stimuli and capacity limitations when dividing attention between different modalities have been shown previously. Thus, graded levels of attention can be achieved by resource allocation between two modalities. Oscillations in the gamma frequency band (30-100 Hz) are related to attention. However, their modulation with attentional modulation has not been studied in a graded manner so far.

In a first study gradual modulation of visual attention was examined in healthy participants using an audiovisual attention design, concurrently recording brain activity using MEG.

Results showed gradual modulation of visual attention, which was reflected by reaction times on the behavioral level. Behavioral effects were closely related to the strength of gamma band oscillations in early visual areas indicating a gradual attentional modulation of oscillatory activity at early stages of visual processing.

Patients with HE show attention deficits which increase with disease severity. Previous works showed that cortico-muscular coupling is pathologically slowed in HE resulting in the commonly observed motor symptoms called (mini-)asterixis. In addition, this slowing correlated with a slowing of the critical flicker frequency (CFF), a parameter shown to be closely related to the severity of HE. Thus, slowed oscillatory activity is assumed to be a key mechanism in the pathophysiology of HE.

In a second study attentional performance of patients with HE was explored in a simplified paradigm, adapted from study 1. Again, brain activity was recorded using MEG. It was determined whether patients with HE show gamma band oscillations and modulation thereof performing the attention task. Relations between severity of HE and patients' performance, their individual gamma band frequencies and their capacity to modulate gamma band oscillation strength were scrutinized.

The present work found that attentional performance and the concurrent modulation of gamma band oscillations are closely related to the severity of HE. The peak frequency of attention-related gamma band oscillations in visual areas was lower, with higher disease severity of HE, as measured by the CFF. Earlier results of slowed oscillatory processes in the motor and visual system of HE patients were thereby extended to the cognitive domain. Moreover, further support was added to the hypothesis that slowed oscillatory activity is a key mechanism in the pathophysiology of HE explaining the broad variety of symptoms. Additionally, the role of the CFF as a sensitive and fine-scaled indicator of HE severity was substantiated.

Taken together, findings of this work underline the relation between visual attention and gamma band oscillations. The close association between neurophysiological effects and actual behavioral measures substantiates the functional importance of gamma band oscillations. This relationship is further corroborated by distinct modulating effects of attention on gamma band oscillations in both, healthy subjects and patients with known attention deficits.

2 Zusammenfassung

Aufmerksamkeit beschreibt die Fähigkeit, auf bestimmte Aspekte der Umwelt zu fokussieren, während andere, unwichtigere Aspekte ignoriert werden und wird mit oszillatorischer Gehirnaktivität in Zusammenhang gebracht. In der vorliegenden Arbeit wurde die Methode der Magnetenzephalographie (MEG) benutzt, um frühere Arbeiten zum Zusammenhang von Aufmerksamkeit und der zugehörigen Gehirnaktivität zu erweitern. Gesunde Probanden und Patienten mit Hepatischer Enzephalopathie (HE), die bekanntermaßen an Aufmerksamkeitsdefiziten leiden, wurden untersucht. Hierzu wurde eine bimodale Aufgabe, die graduelle Stadien visueller Aufmerksamkeit herbeiführte, verwandt.

Bekannt ist, dass beachtete Stimuli in einer Modalität effizienter verarbeitet werden, als nicht beachtete Stimuli in einer anderen Modalität. Außerdem konnten in früheren Studien beim Teilen von Aufmerksamkeit zwischen zwei Modalitäten Einschränkungen der Verarbeitungskapazität gezeigt werden. Daher können graduelle Stadien von Aufmerksamkeit erreicht werden, indem Ressourcen zwischen Modalitäten aufgeteilt werden. Oszillationen in einem Frequenzbereich von 30-100 Hz, die sogenannte Gammaband Oszillationen, werden mit Aufmerksamkeit in Verbindung gebracht. Die Modulation von Gammaband Oszillationen durch Aufmerksamkeit wurde bis jetzt noch nicht in gradueller Weise untersucht.

In der ersten Studie dieser Arbeit wurde in einer Stichprobe gesunder Probanden, Gehirnaktivität mittels MEG aufgezeichnet und das graduelle Aufmerksamkeitsniveau untersucht, während die Probanden eine audiovisuelle Aufmerksamkeitsaufgabe bearbeiteten.

Es zeigte sich, dass die visuelle Aufmerksamkeit der Probanden graduell moduliert wurde. Reaktionszeiten untermauerten auf Verhaltensebene drei Stufen visueller Aufmerksamkeit. Die Verhaltenseffekte spiegelten sich auf neurophysiologischer Ebene in der Stärke der Gammaband Oszillationen in frühen visuellen Arealen wider. Es konnte somit eine durch Aufmerksamkeit initiierte, graduelle Modulation oszillatorischer Aktivität in frühen visuellen Arealen gezeigt werden.

Patienten mit HE zeigen neben motorischen Symptomen Aufmerksamkeitsdefizite, die mit der Schwere der Erkrankung zunehmen. Vorangegangene Arbeiten fanden eine pathologisch verlangsamte kortiko-muskuläre Kopplung bei Patienten mit HE. Diese wurde mit den häufig auftretenden motorischen Defiziten in dieser Patientengruppe in Zusammenhang gebracht und korrelierte außerdem mit der kritischen Flimmer Frequenz (englisch: critical flicker frequency, CFF). Die CFF gibt eine Schätzung der Schwere der HE. Verlangsamte oszillatorische Aktivität wurde daher als Schlüsselmechanismus in der Pathophysiologie der HE vorgeschlagen.

In der zweiten Studie wurden, mit einer vereinfachten Version des Paradigmas aus Studie 1, Aufmerksamkeitsprozesse bei Patienten mit HE untersucht. Gehirnaktivität wurde wieder simultan mittels MEG aufgezeichnet und geprüft, ob Patienten mit HE Aufmerksamkeitsassoziierte Gammaband Oszillationen und deren Modulation zeigen. Des Weiteren wurde der Zusammenhang der Schwere der Erkrankung mit der Aufmerksamkeitsleistung, der individuellen Gammaband Frequenz und der Fähigkeit zur Modulation von Gammaband Oszillationen durch Aufmerksamkeit untersucht.

Es zeigte sich, dass Aufmerksamkeitsleistung und die gleichzeitige Modulation von Gammaband Oszillationen mit der Schwere der HE zusammenhängen. Die Frequenz der aufmerksamkeitsassoziierten Gammaband Oszillationen in visuellen Arealen sank mit zunehmender Schwere der HE, quantifiziert durch die CFF. Somit konnten frühere Studien, die verlangsamte Oszillationen im motorischen und visuellen System zeigten, um die kognitive Ebene erweitert werden. Die Annahme, dass verlangsamte Oszillationen einen Schlüsselmechanismus der HE darstellen, mit dem die unterschiedlichen Symptome der HE erklärt werden können, wurde somit untermauert. Zudem unterstützen diese Ergebnisse die Rolle der CFF als sensitiven Indikator der Schwere der HE.

Zusammenfassend unterstreichen die Ergebnisse dieser Arbeit die wechselseitige Beziehung zwischen visueller Aufmerksamkeit und Gammaband Oszillationen. Der Zusammenhang neurophysiologischer Maße und des gezeigten Verhaltens betont die funktionelle Wichtigkeit der Gammaband Oszillationen. Diese Verbindung wird weiter unterstützt durch die differenzierbare Modulierbarkeit der Gammaband Oszillationen durch Aufmerksamkeit in gesunden Probanden und Patienten mit bekannten Aufmerksamkeitsdefiziten.

3 Introduction

In our complex environment, our brains are confronted with an overwhelming amount of competing input from various sources. In order not to be overwhelmed by all these pieces of information, but to be able to filter out and process relevant pieces while ignoring the rest, attention is needed. Attention affects our actions when we focus on specific aspects of the world and ignore others. Furthermore, attention influences which aspects of the world we perceive. As a cognitive process, the neural bases of attention have been focus of numerous studies. Due to this research dedicated to 'normal' and pathological human behavior and its neuronal underpinnings, we are beginning to understand how the brain works and how attention, behavior, and neural functions are related and interact.

This thesis aimed to extend works on attention, behavior, and related brain processes in the healthy and pathologically changed brain. Oscillatory brain activity, one particular type of brain responses, and their relation to attention were examined. Healthy participants (section 5) and patients with hepatic encephalopathy (section 6) were examined. The focus was placed on gradual modulation of visual attention in a bimodal paradigm in healthy subjects and under the circumstances of a disease, involving attention deficits.

3.1 Attention

> 'Everyone knows what attention is. It is the taking possession by the mind, in clear and vivid form, of one out of what seem several simultaneously possible objects or trains of thought. Focalization, concentration, of consciousness are of its essence. It implies withdrawal from some things in order to deal effectively with others, and is a condition which has a real opposite in the confused, dazed, scatter-brained state which in French is called *distraction*, and *Zerstreutheit* in German.'
>
> - William James (1890)

The study of attention extends back to the beginnings of experimental psychology. William James e.g. stated a very important aspect of attentional selection: *'my experience is what I agree to attend to'* (James, 1890). He thereby emphasized that our perception not only depends on the information entering our senses, but also on which aspects we choose to attend. Later on, attention became to be viewed as a filter or bottleneck (Broadbent, 1958). This filter determined, which one of multiple stimuli would receive further processing and which one would be filtered out. This model was the first to enable studies on limitations of human abilities when dealing with multiple signals at one time.

Although, these are historic concepts, they already show that attention is a concept with multiple aspects and that a clear definition of the type of attention under study is needed when working on a research question.

3.1.1 Types of Attention

Posner and Petersen (1990) argued that attention is not a unitary, but a multifaceted concept. One way to differentiate the different aspects of attention was given by Van Zomeren and Brouwer (1994). They subdivided one aspect of attention according to its selectivity, which is further classified into selective/focused and divided attention. The current work focuses on these two types of attention.

Selective attention plays an important role in managing incoming information. By selectively attending to certain aspects of the surrounding world, the brain is biased towards processing of these relevant pieces of information at the expense of other information (Desimone and Duncan, 1995). For example the visual orienting model interprets attention as a 'spotlight' that can bring certain aspects into its focus (Posner, 1980). Stimuli in the spotlight of attention are processed more efficiently than stimuli outside this area. Other studies on selective visual attention have for example found that subjects respond better to an attended than to a non-attended stimulus. These results have been underlined by enhanced neuronal responses to attended, compared to non-attended stimuli (Munneke et al., 2008).

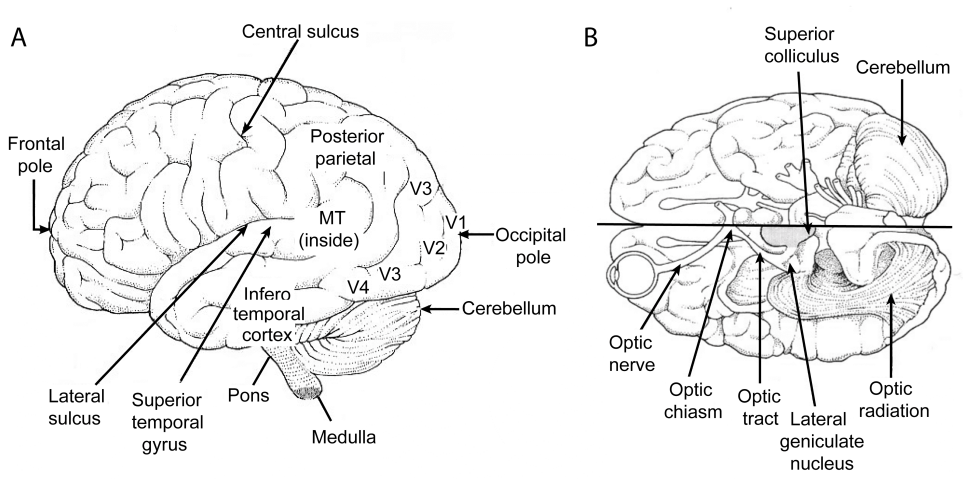
Divided attention describes the fact that attention can also be divided between certain inputs, e.g. when reading a book on a busy airport, while 'out of the corner of an eye' looking for the person you are waiting for. When attention is divided between two simultaneous tasks, performance in each task is limited by the relative amount of attention allocated to it. Performance is expected to improve as a monotonic function of relative amount of attention allocated to the task (Bonnel and Hafter, 1998). Thus, the more you concentrate on reading your book, the more you will understand. However, the likelihood that you thereby miss the person you are waiting for increases. Divided attention has been studied within sensory modalities, mostly in the visual modality (Corbetta et al., 1991; Toffanin et al., 2009). These studies have employed paradigms where subjects had to divide their attention either between certain stimulus features or locations. Other studies have focused on divided attention between modalities. Interactions between the auditory and visual modalities have mostly been studied. Subjects had to, for example, identify changes in concurrently presented visual and auditory stimuli (Bonnel and Hafter, 1998). In case of competing input from two different modalities (e.g. listening for the announcement of flight arrivals, while reading that book on the busy airport), stimuli in the attended modality receive amplified processing compared to stimuli in the non-attended modality (Gherri and Eimer, 2011; Spence and Driver, 1997), again, indicating that attentional resources are limited. Thus, allocating resources to one attended modality gradually subtracts resources from the available supply of all modalities. Considering the monotonic performance improvement with relative amount of attention allocated to a task (Bonnel and Hafter, 1998) and effects of selective and divided attention on stimulus processing, gradual modulation of attention can be studied by resource allocation between two modalities. The level of attention can thereby be measured in one modality and range from high to low with intermediate steps. The present work employed selective attention to a visual and an auditory stimulus and the division between these two. A focus was placed on the visual system (Box 1; for reviews see Kolb and Whishaw, 2003; Levine, 2000).

In selective and divided attention, two different ways of attention allocation have been described. Attention can be allocated voluntarily or involuntarily. The process of voluntary attention allocation is referred to as 'top-down attention'. This type of attention is internally driven by active decision processes, e.g. when you have decided to read the book on the airport and ignore all the loud noise surrounding you.

In 'bottom-up attention', an external stimulus involuntarily draws a subject's attention. This would be the case if the fire alarm went off on the airport. Your attention would be drawn by this noise and would most likely distract your reading (for a review see Theeuwes, 2010). In this work, top-down processes are examined.

Box 1: The Visual System

When a visual signal reaches the eyes, it is conveyed from the eyes via the optic nerves, the optic chiasm and the optic tract to the lateral geniculate nucleus (LGN) of the thalamus. From there, the signal is projected to occipital cortex in the posterior part of the brain. The occipital cortex comprises different visual areas. The area receiving most input from LGN is primary visual area, also named V1, Brodmann Area 17, or striate cortex. In V1 basic information of visual input, like patterns of specific shape and orientation and basic color form and motion information, from both eyes is first processed. The extrastriate cortex comprises other visual areas like the secondary visual area (V2), visual area 3 (V3), visual area 4 (V4) and mediotemporal cortex or visual area 5 (MT/V5). Except for V2 which is functionally similar to V1, the areas of extrastriate cortex code for specific stimulus properties. For instance, the main function of MT/V5 is motion processing. Despite the notion of a hierarchical and parallel flow of information from V1 to V5, it has been shown that V1 also receives information from higher visual areas. These feedback projections are thought to update the current percept (Dakin, 2009). Please note that this brief introduction only covers the parts of the visual system essential for this work. For a complete review of the visual system see e.g. Kolb and Whishaw (2003).



(A) Lateral view of the human brain, showing the major visual and additional areas.

(B) Ventral view of the human brain, depicting the visual pathway. The lower half was dissected in order to also show the structures of the early visual pathway (adapted from Levine, 2000).

3.1.2 Measuring Attention

One way to objectively quantify attention is to measure the time it takes for a subject to react to a certain stimulus or a change in a stimulus, i.e. measure the reaction time. Under the assumption that a short reaction time codes for a high level of attention, reaction times are widely used in neuroscience and psychological research (e.g. Fries et al., 2001; Hoogenboom et al., 2006, 2010; Womelsdorf et al., 2006).

3.1.3 The Neurophysiological Bases of Attention

Using state-of-the-art neurophysiological methods, it becomes feasible to study attentional stimulus processing in the brain. Numerous studies have focused on brain responses in relation to attention. Most of them found enhanced stimulus processing with attention and characteristic neural activity. For instance, functional magnetic resonance imaging (fMRI) works (Gandhi et al., 1999; Munneke et al., 2008) found attention-related increases in the blood oxygen level dependent (BOLD) signal, which measures the hemodynamic response related to neural activity. Single cell recordings in monkeys showed effects of selective attention, by presenting two stimuli in the receptive field of a macaque's V4 neuron (Moran and Desimone, 1985). One of the stimuli was in the preferred color and orientation of that receptive field, the other stimulus was not preferably processed by this receptive field. When attention was directed to the preferred stimulus, the cell responded with a high firing rate. When attention was directed to the non-preferred stimulus, the cell's firing was reduced dramatically. The results of the study by Moran and Desimone constitute the basis of the biased competition model of selective attention (Desimone and Duncan, 1995; Box 2).

Box 2: The Biased Competition Model

The biased competition model is a theoretical framework combining psychological and neurophysiological knowledge about selective visual attention (Desimone and Duncan, 1995). Hereby, enhancing effects of attention on neuronal responses are best understood in the context of competition between all stimuli in one visual field. These competitions can be biased in favor of behaviorally relevant (attended) stimuli. Competition can be resolved by the suppression of the neuronal representations of behaviorally irrelevant (non-attended) stimuli (Desimone, 1998). Recent modeling work extended the biased competition model on a theoretical level to cross-modal attention designs for the visual and somatosensory modalities (Magosso et al., 2010).

The current work focuses on a different aspect of neural communication, i.e. synchronization (section 3.2). Single cell recordings in monkeys and magnetoencephalographic (MEG) studies in humans have confirmed this general mechanism of neuronal communication by showing modulations of synchronized brain activity with attention (Fries et al., 2001; Tallon-Baudry et al., 2005; Vidal et al., 2006).

Besides, selective attention has been shown to affect multiple levels in the sensory processing hierarchy. For example in vision, different areas of extrastriate cortex can be modulated by attention (Fries et al., 2001; Tallon-Baudry et al., 2005). Furthermore, even earliest cortical areas, like V1, assumed to be concerned with most basic stimulus processing have been shown to be modulated by attention (Gandhi et al., 1999; Lakatos et al., 2009; Munneke et al., 2008).

Summary

Attention is a multifaceted concept that can be subdivided into selective and divided attention. Attentional processing is capacity limited, which holds when dividing attention within one modality or between modalities. Gradual modulation of attention can be studied by resource allocation between two modalities.

Attentional processes of the brain can be measured by several means, most important for this work, synchronized brain activity. Modulation of neuronal activity by attention has been found using different methods and on different levels of the sensory processing hierarchy. Important for this work, attentional modulation has been found already in cortical areas representing initial stimulus processing.

3.2 Synchronization and Oscillatory Brain Activity

Attention leads to preferred stimulus processing. Even though it might be assumed, firing rates of single neurons are not always increased with attention (Fries et al., 2001; Moran and Desimone, 1985). The notion that neuronal information can neither solely, nor sufficiently be coded by firing rates expanded the interest to other aspects of neuronal communication: synchronized and rhythmic (oscillatory) activity of multiple neurons (Box 3; Buzsáki, 2006). It was concluded that flexible groups of neurons code sensory information by firing synchronously (Singer, 1999; Varela et al., 2001).

Box 3: Oscillations and Synchronization

Oscillations are periodic variations of certain (time) signals. One well known oscillating object is the pendulum of a clock that seems to never stop moving from right to left. In case of neuronal activity, oscillations can be described as periodic changes in the membrane potential. When an oscillating object entrains another one, acting like a pace maker, it is referred to as an oscillator.

Synchronization describes the time-wise, precise co-occurrence of certain events, thus, if two or more events occur at precisely the same time. An example of synchrony is a chorus singing one song in only one voice.

Even though oscillations and synchronization can be distinguished, they are often found to co-occur: oscillators can synchronize almost effortlessly and usually produce their rhythm in concert with other oscillators, i.e. neuronal populations (for a review see Buzsáki, 2006).

Single cell recordings usually examine isolated responses of single neurons. In contrast, research on oscillations and synchronization focuses on a different level of neural processing, on the population response of multiple neurons.

Simplified, neurons can be classified by their effects on their target neurons. They can have excitatory or inhibitory effects. If, in a local neuronal network, one neuron fires regularly, its output can cause rhythmic fluctuations in the membrane potential of its target cells (i.e. in case of an excitatory neuron, the membrane potential of the target cells gets more positive right after the neuron fired and more negative with time, when the signal is wearing off). If multiple neurons fire synchronously and regularly, their rhythmically fluctuating output signal is combined. Thereby, temporal windows of

increased and decreased excitability are defined in a larger population of target cells. These rhythmic activation and deactivation patterns result in a fluctuating field potential, which can be measured outside the cells (for a review see Schnitzler and Gross, 2005; Figure 1). The basic principle of synchronous firing also applies to larger groups of neurons, coding for example for a complex stimulus (Singer, 1999). Furthermore, synchronization has not only been found in local groups of neurons, but also between them. With synchronized activity, the communication between these groups of neurons has been found to be more effective (Varela et al., 2001). Hence, communication between distant neuronal populations is assumed to be most effective when the local output is rhythmic and synchronized and the neuronal populations synchronize their activity as well (Fries, 2009).

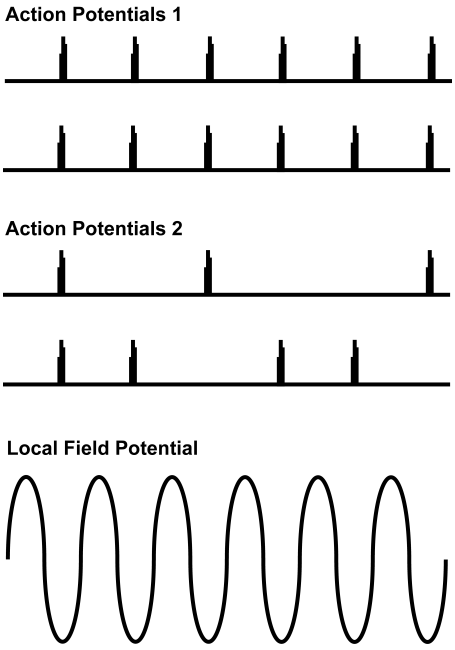


Figure 1: Mechanism of Oscillations

When multiple neurons fire synchronously, either each one consistently at every cycle of the oscillation (Action Potentials 1) or even at no particular frequency (Action Potentials 2), rhythmic variations in neural activity can be the result. These variations can e.g. be measured by the local field potential (LFP), which is recorded extracellularly and represents voltage fluctuations in the membrane potentials of a local neuronal population (adapted from Schnitzler and Gross, 2005).

When decomposing the oscillatory time signal into its frequency components, several frequency bands of interest can be discriminated (delta, 1-3 Hz; theta, 4-7 Hz; alpha, 8-13 Hz; beta, 14-29 Hz; gamma, 30-100 Hz). These frequency bands have been associated with specific functions. For example, oscillations in the beta-, alpha-, theta-, and delta bands are assumed to serve long-range communication between distant areas (Gross et al., 2004; Schnitzler and Gross, 2005). Oscillations in the beta band are known for their role in sensorimotor functions (Pfurtscheller et al., 1996) and most recently in simultaneity perception (Lange et al., forthcoming; Appendix 4). Primarily relevant for this work are oscillations in the gamma band, which have particularly been associated

with attention (Fries et al., 2001; Hoogenboom et al., 2006, 2010; Kaiser et al., 2006; Lachaux et al., 2005; Steinmetz et al., 2000).

3.2.1 Attention and Gamma Band Oscillations

Oscillations in the gamma band (30-100 Hz) have been associated with fundamental brain functions, such as visual feature binding (Gray et al., 1989), visuomotor control (Roelfsema et al., 1997), working memory (Tallon-Baudry et al., 1998), associative learning (Miltner et al., 1999), and attention (Fries et al., 2001).

Modulation of gamma band oscillations by attention has been reported both in monkey (Khayat et al., 2010) and human studies (Gruber et al., 1999; Siegel et al., 2008; Tallon-Baudry et al., 2005; Vidal et al., 2006; Wyart and Tallon-Baudry, 2008). Up to now and in contrast to several fMRI studies (Gandhi et al., 1999; Munneke et al., 2008), attention dependent modulation of gamma band oscillations has mostly been recorded in extrastriate areas (Fries et al., 2001; Gregoriou et al., 2009; Womelsdorf et al., 2006). Results on attention-related changes in gamma band oscillations in primary visual areas are not without ambiguity. A study using single cell recording in monkeys' V1 has shown decreased local field potential gamma band power and decreased gamma band spike field coherence with attention (Chalk et al., 2010). However, previous works in humans using MEG have shown increased induced gamma band oscillations in visual areas V1-V3 during an attention demanding task (Hoogenboom et al., 2006, 2010). Apart from one electroencephalography (EEG) study providing first evidence of gradually increasing gamma band oscillations with task complexity (Simos et al., 2002), all above mentioned studies compared 'extreme' states of attention, i.e. attention versus no attention. Studies on graded attentional modulation of induced gamma band oscillations in early visual areas in humans are lacking up to now.

3.2.2 The Biased Competition Model and Gamma Band Oscillations

It has been shown that attended visual stimuli induce stronger local gamma band oscillations in extrastriate cortex of the monkey than unattended stimuli (Fries et al., 2001). Thus, it was assumed that by enhanced gamma band synchronization, the attended of two competing visual stimuli gets a competitive advantage over the other (Fries, 2005, 2009). Thereby, communication between low-level neurons processing the

attended stimulus and higher-level target neurons is reinforced (Fries, 2009; Fries et al., 2008). This effect has been addressed in the hypothesis of biased competition through enhanced synchronization (Fries, 2005, 2009), which stems from the biased competition model (Desimone and Duncan, 1995).

The conceptual framework of the biased competition model has so far mostly been tested by studies on selective visual attention on single cell level (Moran and Desimone, 1985). This model and in particular the hypothesis by Fries still needs to be realized in cross-modal attention designs. Its application between modalities has recently been addressed on a theoretical level for the visual and somatosensory modalities (Magosso et al., 2010), but graded competition has so far not been addressed.

3.2.3 The Genesis of Gamma Band Oscillations

The interplay between inhibitory interneurons mediated by gamma-aminobutyric acid (GABA; the principal inhibitory neurotransmitter of the mammalian brain) and excitatory cells has been associated with the genesis of gamma band oscillations (Bartos et al., 2007; Wang and Buzsáki, 1996). In particular, the interplay between basket cells - a specific type of inhibitory interneurons - and the excitatory pyramidal cells is assumed to be of importance for this task (Bartos et al., 2007; Vida et al., 2006).

Simplified, the mechanism is assumed to work as follows: excitatory input reaching a group of neurons excites excitatory neurons (e.g. pyramidal cells) and inhibitory interneurons (e.g. basket cells) which fire in response providing excitatory and inhibitory input to their target cells. Due to their abundance, their ability to fire fast, and all their connections, the basket cells inhibit numerous other neurons, including other basket cells. Thereby, large numbers of basket cells are entrained to a common rhythm (Vida et al., 2006). The fast and rhythmically synchronized inhibition of the basket cells influences the local network's excitatory and inhibitory neurons by rhythmically inhibiting them. Thus, the excitatory neurons are left with only a short window of opportunity to exert their excitatory drive, determined by the time of the wearing off of one train of inhibition until the next one arrives (Hasenstaub et al., 2005). These rhythmic and fast fluctuations of excitation and inhibition are thought to constitute a mechanism of gamma band oscillations (for a review see Bartos et al., 2007).

A correlation of gamma band oscillations and brain GABA content has furthermore been found in human whole head MEG recordings. The frequency of gamma band oscillations has been shown to positively correlate with resting GABA concentrations in visual (Muthukumaraswamy et al., 2009) and motor cortices (Gaetz et al., 2011), meaning that with more GABA mediated inhibition, gamma band oscillations get faster.

3.2.4 Measuring Oscillatory Brain Activity – Magnetoencephalography

Oscillatory brain activity can be measured from outside the brain. One widely used method for this purpose is MEG (Figure 2). MEG is a noninvasive technique that measures the magnetic field generated by electrical currents of large groups of synchronously active neurons (Ahonen et al., 1993; Hari, 2004). It is assumed to be most sensitive to postsynaptic potentials from pyramidal cells, which are oriented perpendicular to the surface of the cortex.

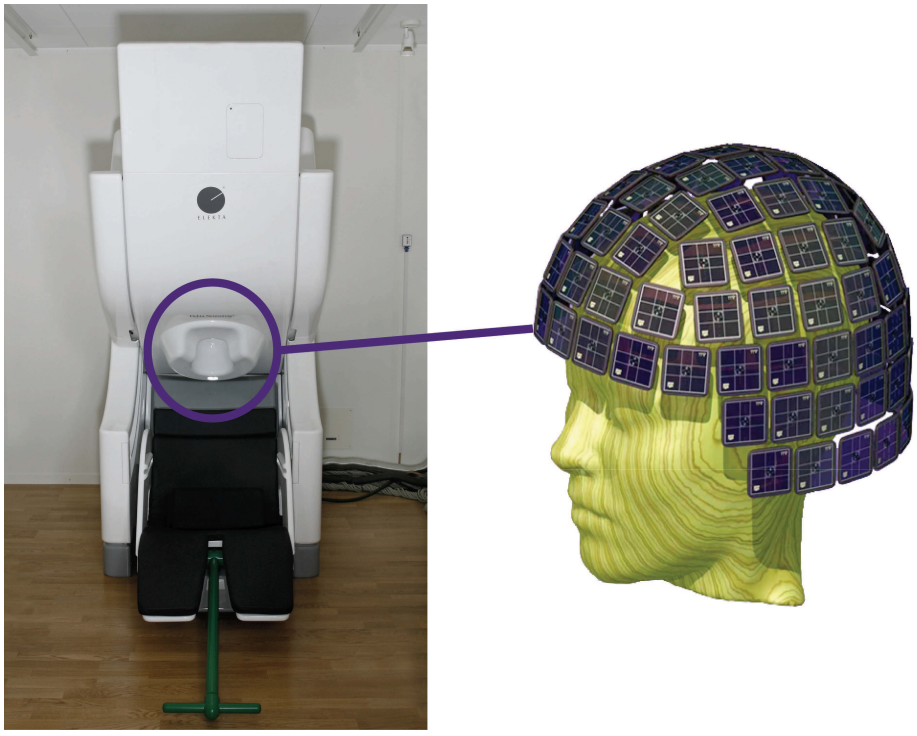


Figure 2: MEG System

Left: Picture of the MEG system at the Institute of Clinical Neuroscience and Medical Psychology of the Heinrich-Heine University Düsseldorf used in study 2 (comprising 306 sensors). Please note, study 1 made use of the previous Neuromag-122 MEG system, comprising 122 sensors. **Right:** The MEG sensors as they are found inside the dewar (image by courtesy of Mika Seppä, Brain Research Unit, Low Temperature Laboratory of Aalto University, Finland).

Depending on the sampling frequency of the recording, MEG has a high temporal resolution in the millisecond range. The brain's magnetic field outside the head is largely unaffected by the skull and surrounding tissue. This is one reason for the good spatial resolution of MEG, which is around 2-3 mm on the cortical surface. Furthermore, recent multi-sensor MEG systems with several hundreds of sensors covering the entire skull provide a high spatial resolution, too. The magnetic field of the brain has a strength of around 10^{-15} Tesla. Comparing this strength with, for example, the earth's magnetic field (10^{-5} Tesla) or the magnetic field produced by electrical devices (10^{-7} Tesla) illustrates how small the brain's magnetic signal actually is. To be able to measure these small field strengths, the MEG system needs to be shielded from external magnetic signals. This is usually achieved in a shielded room (Cohen et al., 1970). Furthermore, the sensors need to be very sensitive. Therefore, superconducting quantum interference devices (SQUIDs; Cohen, 1972) are employed. They perform well with a temperature of approximately 4 K, equaling -269°C, which is realized in an insulated dewar filled with liquid helium (for a review of MEG see Hämäläinen et al., 1993).

Summary

Synchronized oscillatory brain activity of flexible groups of neurons codes for functional information. Oscillations in the gamma frequency band (30-100 Hz) are associated with attention. They are thought to depend on GABA mediated inhibition and the concentration of GABA has been shown to be positively correlated with the frequency of gamma band oscillations.

Oscillatory brain activity can be measured with high temporal and spatial resolution using magnetoencephalography (MEG).

3.3 Hepatic Encephalopathy

Hepatic encephalopathy (HE) is a potentially reversible frequent neuropsychiatric complication in chronic or acute liver disease (Häussinger and Blei, 2007). It encompasses a broad range of neuropsychiatric symptoms of varying severity. These symptoms include alterations in psycho- and fine motor functions, cognitive deficits, altered sleep patterns, and changes in vigilance state ranging from somnolence up to a hepatic coma (for a review see Butterworth, 2000; Prakash and Mullen, 2010). This work concentrates on patients suffering from liver cirrhosis. However, HE can also be caused by acute liver failure or portosystemic shunting (Ferenci et al., 2002; Häussinger and Blei, 2007).

3.3.1 Epidemiology

The epidemiology of HE stays vague, which is most likely due to the given inconsistent definitions of HE in the present literature (Kircheis et al., 2007). Because of this inconsistency, suggested figures for the prevalence of minimal HE, for example ranges between 20 and 80% of patients with confirmed liver cirrhosis (Kircheis et al., 2007).

3.3.2 Symptoms

A wide range of symptoms can be found in patients with HE. In patients with liver cirrhosis, three different types of HE can be distinguished: two overt (refers to stages of HE with overt clinical symptoms) types: episodic and persisting, and minimal HE (Ferenci et al., 2002; Häussinger and Blei, 2007).

The most common presentation of HE is its episodic form. This type of HE comprises patients with known liver disease who develop alterations in their mental state. Episodic HE is mostly caused by promotive factors like gastrointestinal bleeding, infections, kidney failure, constipation, dehydration, or the intake of sedatives like benzodiazepines (Häussinger and Blei, 2007). Typical symptoms comprise a shortened attention span, irritability or depression, sleep abnormalities, loss of orientation to time, overt changes in personality, changes in consciousness (from lethargy over confusion to stupor and coma), and motor symptoms like bradykinesia, dysarthria, rigidity, tremor, and (mini-)asterixis (Häussinger and Blei, 2007).

Patients with persisting HE show multiple, recurrent episodes of HE, as well as progressing changes in their mental status (Ferenci et al., 2002; Häussinger and Blei, 2007). Persisting HE is mostly caused by portosystemic shunts, dietary indiscretion, or constipation. This type of HE is usually rather mild. However, patients with persisting HE can develop a variety of extrapyramidal symptoms, including gait disturbances, chorea, and ataxia. These patients may appear jovial and show spastic paraparesis (Häussinger and Blei, 2007).

Patients with minimal HE have no recognizable clinical symptoms of brain dysfunction. Nevertheless, they show cognitive deficits in detailed psychometric examinations (Butterworth, 2000; Ferenci et al., 2002; Häussinger and Blei, 2007). Subcortical alterations were described as responsible for its development (Häussinger and Blei, 2007). Minimal HE can affect daily life functions, such as fitness to drive (Kircheis et al., 2009; Wein et al., 2004) and quality of life (Groeneweg et al., 1998) and it describes a poorly defined syndrome between normality and overt HE. Nevertheless, minimal HE is of prognostic value, as the probability for the development of an overt HE increases with the presence of minimal HE (Bustamante et al., 1999; Hartmann et al., 2000).

3.3.3 Attention Deficits in HE

Of highest relevance to this work is one key symptom of HE: the gradual increase of attention deficits with increasing disease severity (Amodio et al., 2005; Pantiga et al., 2003; Weissenborn et al., 2001). For example, in a task involving control of selective attention, patients without overt symptoms of HE, showed slowed reaction times and more errors compared to healthy controls (Amodio et al., 2005). Using different psychometric tests and studying different cognitive functions (including attention), Pantiga et al. (2003) found mental impairment in cirrhotic patients in all functions under study. The severity of the impairment and the degree of hepatic dysfunction were related. Attention deficits have been found in all groups of HE patients, even in patients who seem clinically unaffected (Weissenborn et al., 2005). In clinically unaffected patients, attention deficits have even been assumed to be the main symptoms (Weissenborn et al., 2005). The neural underpinnings of attention deficits in HE are thus addressed in this work.

3.3.4 Diagnosis and Clinical Grading of HE

Irrespective of the underlying cause, HE presents itself with varying levels of severity. For overt HE, the diagnosis and determination of the HE grade can be assessed by the clinical status of the patient, using the so-called *West-Haven* Criteria (Conn and Lieberthal, 1979; Ferenci et al., 2002). Thereby, patients with overt HE can be subdivided into four groups depending on the existence and severity of clinical symptoms like attention deficits, psychomotor, cognitive, emotional, motor, and behavioral disturbances. The transition from HE grade 1 to 2 is not easily discernible. Especially in HE grade 1 (HE1), the diagnosis strongly depends on the examiner's and patient's behavior (Häussinger and Schliess, 2008; Kircheis et al., 2007). HE grades 3 and 4 are more easily distinguished as they are characterized by somnolence and hepatic coma, respectively (Häussinger and Blei, 2007). For the classification of lower HE grades (cirrhotic patients with no signs of HE; HE0 and minimal HE; mHE), psychometric computer tests, such as the Vienna test system (Vienna test system, WINWTS, Version 4.50, 1999) are widely accepted (Kircheis et al., 2002). Cirrhotic patients can thus be classified as belonging to one of six HE groups: HE0, mHE, HE1-HE4 (Table 1).

Table 1: Classification of HE

For each of the HE groups potential symptoms are displayed (adapted from Häussinger and Blei, 2007).

	HE Grade	Level of consciousness	Intellectual function	Neurological abnormalities
No HE	0	Normal	Normal	None
Minimal HE	m	Normal	Seemingly normal, Psychometric test abnormalities	None
Overt HE	1	Lack of awareness, Change in personality, Day/night reversal	Short attention span, Easy forgetfulness	Slight tremor, Uncoordination, Asterixis
	2	Lethargy, Unsuitable behavior	Loss of orientation	Asterixis, Abnormal reflexes
	3	Asleep but rousable, Confused when awake	Loss of interpersonal communication	Abnormal reflexes
	4	Unrousable	Absent	Babinsky/clonus, Decerebrate

Patients with HE show changes in EEG recordings, which are, however, rather unspecific (Amodio et al., 2005). Due to their low sensitivity in diagnosing HE, EEG recordings are not routinely used for this purpose (Häussinger et al., 2006; Häussinger and Blei, 2007).

Other neuroimaging methods are employed for differential diagnosis. Computer tomography, for example can rule out other possible causes, like cerebral hemorrhage, in comatose patients (Häussinger, 2004).

A sensitive and objective method to reliably quantify and monitor the severity of HE, is the so-called critical flicker frequency (CFF; Kircheis et al., 2002; Prakash and Mullen, 2010; Romero-Gómez et al., 2007; Sharma et al., 2007). CFF thresholds can be assessed using the Schuhfried Test System (Dr. Schuhfried Inc., Mödling, Austria). With a concave-convex lens system and a luminous diode, the patient is stimulated intrafoveally with rectangle light pulses with a decreasing frequency from 60 Hz downward. The CFF threshold is determined as the frequency at which the subjective impression of a fused light switches to a flickering one (Kircheis et al., 2002). The CFF is independent of training, and education effects and it shows only slight dependence on age (Kircheis et al., 2002). The CFF has been shown to correlate with the grade of HE. A CFF below 39 Hz diagnoses an overt HE with high sensitivity and specificity (Häussinger and Blei, 2007; Kircheis et al., 2002; Sharma et al., 2007).

3.3.5 Pathophysiology

The pathogenesis of HE is not yet finally understood. Research suggests that caused by liver dysfunction elevated levels of cerebral ammonia and an inflammatory response act as causal factors in the development of HE (Butterworth, 2000; Weissenborn et al., 2007). This can thereby lead to astrocyte swelling and the development of a low-grade cerebral edema (Häussinger and Blei, 2007; Prakash and Mullen, 2010). Next to other neurotoxins promoting the development of HE, the accumulation of manganese in globus pallidus is thought to play a central role in its pathogenesis (Häussinger and Blei, 2007). Since the early 1980's, there has been an ongoing discussion about the 'GABA Hypothesis of HE' (for a review see Jones et al., 1984). It has been argued that increased GABA mediated inhibitory neurotransmission contributes to HE (Bassett et al., 1990). However, many studies argued against this hypothesis, for example by showing that brain and cerebrospinal fluid GABA content and synthesis were unaltered in HE patients (Lavoie et al., 1987; Moroni et al., 1987; Zwingmann et al., 2003). An alternative explanation was given by Ahboucha et al. (2004), suggesting a possible role for neurosteroids with GABA-A receptor modulatory properties.

3.3.6 HE and Oscillatory Brain Activity

EEG studies have shown that in HE spontaneous oscillatory activity is progressively slowed, becoming more evident, the more affected the patients were (Davies et al., 1991; Parson-Smith et al., 1957). Kullmann and colleagues reported a significantly slower mean peak frequency for all patients with liver cirrhosis under study even if no signs of HE were present (Kullmann et al., 2001). In line with slowed oscillations in progressing HE, previous works in the motor system demonstrated pathologically slowed thalamo-cortico-muscular coupling with increasing grades of HE. These were associated with the tremor-like motor symptom called (mini-)asterixis (Timmermann et al., 2002, 2003). In addition, this slowing correlated with slowing of the CFF (Timmermann et al., 2008). The physiological mechanism of slowed CFF as clinical measure is still unclear. Human cortical visual areas are able to process flickering stimuli at frequencies higher than the maximum subjectively perceived flicker frequency (Herrmann, 2001). Furthermore, brain responses to flickering stimuli show oscillatory patterns (Fawcett et al., 2004). Thus, it is likely that the observed impairment in perception of an oscillatory visual (flicker-) stimulus, i.e. the decreased CFF, is due to a dysfunction in the cerebral processing of oscillatory visual stimuli. Taking these findings into consideration Timmermann et al. (2005) hypothesized that a slowing of oscillatory activity in various human cerebral sub-systems represents a key mechanism in the pathophysiology of HE.

Summary

HE is a major complication in liver cirrhosis, comprising a variety of symptoms. For instance, attention deficits are present in all stages of the disease. According to diagnostic criteria, cirrhotic patients can be classified into different groups of HE with differing disease severity (HE0, mHE, HE1 - HE4). An alternative method, the CFF, is an objective marker to quantify and monitor disease severity. Motor symptoms in HE have been associated with slowed oscillatory activity, which also correlated with the CFF. Thus, it was hypothesized that slowed oscillations in different sub-systems of the brain, represent a key mechanism in HE pathophysiology.

4 Hypotheses and Aims

The aim of the current work was to examine neural mechanisms of attention in the visual system of the healthy and the pathologically changed brain.

Study 1 aimed to induce graded levels of visual attention, using bimodal stimulation in healthy participants and to show concurrent behavioral effects. It was investigated whether the strength of gamma band oscillations can be gradually varied according to the amount of attention given to a visual stimulus. It was hypothesized that high, medium and low levels of visual attention can be distinguished by the strength of gamma band oscillations. Furthermore, the location of these graded attentional variations was determined and it was scrutinized whether a mirror effect in auditory cortex can be reported.

Study 2 aimed to characterize attention-related behavioral performance and gamma band oscillations in patients with HE and to relate these parameters to disease severity as indexed by HE grade and CFF. It was hypothesized that patients with HE show behavioral deficits in a task involving visual attention. With an adapted version of the paradigm of study 1, patterns of attention-related gamma band oscillations were investigated. The strength and frequency of visual attention-related gamma band oscillations and their relation to HE disease severity were scrutinized in particular.

The overall goal of this thesis was to extend the knowledge about the functional role of gamma band oscillations. Modulations of gamma band oscillations were expected due to experimental paradigm and attention deficits.

5 Study 1: Gamma Band Oscillations and Graded Attention

Study 1 utilized a behavioral paradigm, with which attention was varied by resource allocation between the visual and auditory modalities thereby gradually varying the degree of visual attention (high, medium, and low visual attention). Employing this paradigm and using MEG, gamma band oscillations were measured in the different attention conditions and related to the amount of attention given to the visual stimulus. The location of strongest gamma band oscillations was examined for effects of graded attention modulation. Mirror effects in auditory cortex were examined.

5.1 Introduction

In the complex multisensory environment surrounding us, our brains are confronted with an overwhelming amount of competing input from various sources. As our brain's capacities are limited, one of its most important tasks is to filter out and process relevant information while ignoring the rest. In case of competing input from two different modalities, attention strongly influences how well each stimulus is processed. If attention is focused on one modality, stimuli in the attended modality receive amplified processing compared to stimuli in the non-attended modality (Gherri and Eimer, 2011; Spence and Driver, 1997). Allocating resources to one attended modality gradually subtracts resources from the available supply of the other modality (Bonnel and Hafter, 1998). In this study, gradual modulation of attention was studied by reallocating available resources between competing modality specific stimuli, i.e. auditory and visual stimuli.

Attention and sensory processing have been associated with gamma band oscillations at 30-100 Hz (Fries et al., 2001; Hoogenboom et al., 2006, 2010; Kaiser et al., 2006; Lachaux et al., 2005; Steinmetz et al., 2000). There have been various studies on the modulation of visually induced gamma band oscillations by attention, mostly focusing on extrastriate cortex (Gruber et al., 1999; Siegel et al., 2008; Tallon-Baudry et al., 2005; Wyart and Tallon-Baudry, 2008). However, all these studies compared 'extreme' states of attention, i.e. attention versus no attention. In fact, up to now, studies on graded attentional modulation of induced gamma band oscillations in early visual areas in humans are lacking.

5.2 Methods

The level of visual attention was gradually manipulated. To this end, 16 healthy participants worked on a cued bimodal reaction time paradigm, designed to induce high, medium, and low levels of visual attention. They were simultaneously stimulated with a continuous, moving visual (adapted from Hoogenboom et al., 2006, 2010) and a continuous auditory stimulus and instructed by a cue to either attend to (i) the visual, (ii) the auditory, or (iii) both stimuli. This resulted in three conditions which aimed to induce three levels of visual attention: (i) selective visual condition, high visual attention, (ii) selective auditory condition, low visual attention, and (iii) divided condition, medium visual attention. After a random time interval, one of the stimuli changed its properties and with a time-delay the other stimulus also changed its properties. In the two selective conditions, the subject's task was to react to the change of stimulus properties in the attended modality, i.e. the visual stimulus in the visual and the auditory stimulus in the auditory condition. In the divided condition, subjects had to react to the first changing stimulus, irrespective of its modality (please also see Appendix 2, Fig. 1). As a behavioral measure of attention, reaction times and correctness of responses were recorded. At the same time, the participant's brain activity was measured with a whole-head Neuromag-122 sensors MEG system (ELEKTA Neuromag, Helsinki, Finland). The acquired neurophysiological data were analyzed with respect to the time course, strength and sources of gamma band oscillations and compared between the three different attentional conditions.

5.3 Results

5.3.1 Three Graded Levels of Visual Attention

On the behavioral side, three presumed graded levels of visual attention (high, medium, and low) were reflected by differences in reaction times to visual and auditory stimuli in the different conditions. Reaction times were faster in the selective compared to the respective divided attention conditions with the difference being more pronounced in the auditory than in the visual modality. Overall, reaction times were faster to auditory than to visual stimuli (please also see Appendix 2, Fig. 2).

MEG data showed sustained gamma band oscillations in all three conditions on sensors overlaying visual areas lasting for the whole stimulus period. Confirming the three presumed graded levels of visual attention on the neurophysiological side, the intensity of gamma band oscillations increased with the level of visual attention (from low to high). Differences between conditions lasted up to 1600 ms. Differences were greatest between the selective visual and selective auditory and divided and selective auditory conditions (please also see Appendix 2, Fig. 4).

5.3.2 Gamma Band Modulation in Early Visual, but not Auditory Areas

The location of strongest visual gamma band response was localized in early visual areas (V1 or V2) in 14 of 16 subjects. Also, the modulation of visual attention-related gamma band oscillations was found in these areas (please also see Appendix 2, Fig. 3).

Data reported here were also scanned for mirror effects of auditory stimulation in auditory areas, i.e. high, medium, and low levels of auditory attention-related gamma band responses in auditory areas. However, no systematic sustained stimulus related gamma band responses could be found in auditory cortices.

5.4 Discussion

5.4.1 Gradual Modulation of Visual Attention

Three graded levels of visual attention were distinguished by reaction times. By introducing a third, medium level of visual attention, these data extend earlier behavioral results (Posner et al., 1980; Schroeger et al., 2000; Spence and Driver, 1997) and are in line with behavioral results, showing gradual attention modulation when measuring accuracy (Bonnel and Hafter, 1998).

Tallying earlier findings (Edden et al., 2009; Fries et al., 2001; Hoogenboom et al., 2006; Muthukumaraswamy et al., 2009), subjects showed prominent long lasting visual gamma band oscillations in all conditions when presented with the visual stimulus. Three gradually modulated levels of gamma band oscillations were found, confirming behavioral results on the neurophysiological side and corroborating the role of gamma band oscillations for behavior. By showing that a medium strength of gamma band

oscillations is associated with a medium level of attention, the present study extends previous works that investigated the influence of attention versus no attention on gamma band oscillations (Gruber et al., 1999; Siegel et al., 2008; Tallon-Baudry et al., 2005; Wyart and Tallon-Baudry, 2008). Furthermore, the current data show long lasting gamma band oscillations and modulation thereof. This adds a new dimension to previous studies, which have provided data either averaged over certain time periods, frequency bands or both (Fries et al., 2001; Gruber et al., 1999; Müller et al., 2000; Tallon-Baudry et al., 2005; Vidal et al., 2006).

Gamma band oscillations were different between the selective visual and the divided conditions, but this difference was less prominent and shorter lasting than differences between the other conditions. Thus, from the neurophysiological data, a stronger orientation to the visual stimulus can be assumed in conditions visual and divided. In analogy, behavioral data also showed a stronger orientation to the visual modality, reflected by a greater difference in reaction times between the selective auditory and divided auditory conditions than between the selective visual and divided visual conditions. These data further substantiate the connection between behavioral and neurophysiological data, in line with a study showing a strong positive relation between reaction times and the strength of phase-locked gamma band oscillations (Fründ et al., 2007). The current findings of a stronger orientation to visual than auditory stimuli further tally with a recent study, which showed that the visual system is less vulnerable to competition from auditory stimuli than vice versa (Schmid et al., 2011).

An alternative explanation for the prolonged reaction times in the divided compared to the selective conditions might be increased task complexity in the divided condition. In fact, earlier studies showed that gamma band oscillations can be modulated by overall task complexity (Posada et al., 2003; Simos et al., 2002) and working memory load (Howard et al., 2003). However, if task complexity had been higher in the divided condition, gamma band power would also have been expected to be highest in this condition and similar in the two selective conditions. Instead, a different pattern was found: gamma band oscillations were strongest in the selective visual, medium in the divided and lowest in the selective auditory conditions. This pattern favors the interpretation in terms of a gradual modulation of the attention level rather than an effect of task complexity.

Overall, gamma band oscillations seem to represent a mechanism enabling precise adjustment to the current attentional needs. Their gradual modulation suggests an efficient use of cognitive resources.

5.4.2 Modulation of Gamma Band Oscillations in Early Visual Areas

In this study, the strongest gamma band sources were located in early visual areas (V1 and V2). Modulation of gamma band oscillations was also found in these locations. While previous studies showed pronounced gamma band oscillations in visual areas V1-V3 in humans when attentively monitoring a visual stimulus (Hoogenboom et al., 2006, 2010), attention dependent modulations of gamma band oscillations have primarily been recorded in mid- and high level stages in the visual processing hierarchy (Fries et al., 2001; Gregoriou et al., 2009). In contrast to the present results, one study in the macaque even found decreased local field potential gamma band power and decreased gamma band spike field coherence with attention in V1 (Chalk et al., 2010). In the study by Chalk et al. attention was either directed to a stimulus presented at the center of one receptive field or a stimulus outside of the receptive field. The stimuli in the current study on the other hand were complex gratings, not restricted to the center of one receptive field, but exiting numerous neurons in visual cortex. The data of the current study are thus not directly comparable to those of the study by Chalk et al. (2010). In line with a previous study, from which the here used stimulus was adapted (Hoogenboom et al., 2010), one might speculate that the mechanism of gamma band oscillations can be explained by rhythmically synchronized inhibition through cortical interneurons (Bartos et al., 2007; Vida et al., 2006). Attention would increase inhibitory drive (Lee and Maunsell, 2009), which in turn increases gamma band oscillations.

5.4.3 Biased Competition through Gamma Band Oscillations

By resource allocation between the visual and auditory modalities, the current data found gradual modulation of visual attention, coded by concurrent fast reaction times and modulation of gamma band oscillations. These results could add a new dimension to the biased competition model of selective attention (Desimone and Duncan, 1995). It has been shown that attended visual stimuli induce stronger local gamma band oscillations in extrastriate cortex of the monkey than unattended visual

stimuli (Fries et al., 2001). In addition, it was assumed that by enhanced gamma band synchronization, the attended of two competing stimuli gets a competitive advantage over the other, reinforcing its processing (Fries, 2005, 2009).

From the current results, one could speculate on the application of the biased competition model in an intermodal context, as shown in a modeling study for the visual and tactile modalities (Magosso et al., 2010). With the attention-related fast reaction times and the increase in gamma band synchronization in visual areas, one could assume a connection between the competitive advantage of the visual over the auditory stimulus and the strength of gamma band oscillations. The locally enhanced gamma band synchronization in visual cortex could enhance communication with motor areas, enforcing a reaction. However, the current study cannot directly proof one important aspect of the biased competition model: suppressive effects of one of the two stimuli over the other. Nevertheless, the present results suggest that the biased competition model can be applied to gradual attention modulation in the visual system. Enhanced gamma band synchronization could be seen as an adaptive mechanism enhancing the selective processing of a stimulus in a gradual manner, thereby reflecting the amount of selective attention a stimulus receives.

5.4.4 Gamma Band Oscillations in the Auditory System

In the current data, no systematic sustained stimulus related gamma band responses were found in auditory cortex. A possible explanation might be that MEG sensors are less sensitive to radial sources at the surface of gyri, for example the superior temporal gyrus of the auditory system (Crone et al., 2001). Thus, the signal to noise ratio of an induced auditory gamma band response in this study might have been too low to detect an auditory gamma response in the MEG recording. With the concurrent strong response to the visual stimulus the weak auditory signal might have become even less detectable. Furthermore, general characteristics of the auditory stimulus might have played a role. Intracranial studies have reported short-lasting evoked (Lakatos et al., 2009) and induced auditory gamma band oscillations (Crone et al., 2001). MEG studies have found auditory evoked gamma band activity (Joliot et al., 1994; Pantev et al., 1991; Tiitinen et al., 1993). However, up to now, long-lasting gamma band oscillations in auditory cortex induced by auditory stimuli have not been reported.

A task for future studies could be to find stimuli that reliably induce long-lasting gamma band oscillations in auditory cortex.

5.5 Conclusion

From this study, it can be concluded that visual attention can be modulated gradually. On the behavioral level, gradual attention modulation is reflected by reaction times. These behavioral effects are closely associated with the strength of gamma band oscillations in early visual areas, supporting attentional modulation of oscillatory responses at early stages of visual processing.

6 Study 2: Gamma Band Oscillations in Hepatic Encephalopathy

In study 1, it was shown that gamma band oscillations in early visual areas can be gradually modulated by attention in the healthy brain. In study 2, patients with HE known to experience attention deficits were examined. The first aim of this study was to characterize attention-related behavioral performance and relate it to disease severity. Furthermore, visual attention-related brain activity and its modulation by attention were examined using an adapted version of the paradigm of study 1. The relation between the strength and frequency of gamma band oscillations and HE disease severity was investigated.

6.1 Introduction

A cardinal symptom of HE is a gradual increase of attention deficits with increasing disease severity (Amodio et al., 2005; Kircheis et al., 2007; Pantiga et al., 2003; Weissenborn et al., 2001). Study 1 and previous studies (Fries et al., 2001; Hoogenboom et al., 2006, 2010; Kaiser et al., 2006; Lachaux et al., 2005; Steinmetz et al., 2000) showed that attention is associated with a modulation of gamma band oscillations. For tremor-like motor symptoms, commonly observed in HE, pathologically slowed cortico-muscular coupling has been demonstrated, which correlated with a slowing of processing of visual stimuli, i.e. the CFF (Timmermann et al., 2002, 2003, 2008). The CFF is a measure reliably indicating the severity of HE on a continuous scale and has been introduced as fine-scaled indicator of HE disease severity potentially superior to the classic grouping of HE patients (Häussinger et al., 2006; Kircheis et al., 2007). A CFF value of 39 Hz is described as critical cut-off frequency separating patients with overt HE from healthy controls and HE0 patients (Kircheis et al., 2002). Slowed oscillatory activity is assumed to be one key mechanism in the pathophysiology of HE (Timmermann et al., 2005).

Thus, in this study patients with HE were assumed to show decreasing performance with increasing disease severity in an adapted, i.e. simplified version of the attentional paradigm of study 1. Attention-related gamma band oscillations and their modulation were investigated and it was scrutinized whether a slowed frequency of attention-related gamma band oscillations parallels the severity of HE.

6.2 Methods

26 patients with liver cirrhosis and 8 age-matched healthy controls completed an adapted version of the behavioral task of study 1, requiring shifts of attention between simultaneously presented visual and auditory stimuli. The paradigm was changed from a randomized single trial to a randomized block design to make it accomplishable for all patients. However, the three conditions corresponded to the ones in study 1: (i) selective visual condition, high visual attention, (ii) selective auditory condition, low visual attention, and (iii) divided condition, medium visual attention. Simultaneously, brain activity was recorded using MEG (306-channel, ELEKTA Oy, Helsinki, Finland). As behavioral measures of attention, reaction times and correctness of responses were evaluated. The acquired neurophysiological data were again analyzed with respect to the time course, strength and sources of gamma band oscillations and were compared between the different attentional conditions. Furthermore, the frequency of gamma band oscillations was scrutinized. After examining behavioral effects, only the two selective attention conditions were analyzed and compared, as the strongest differences in visual attention were expected between them.

To quantify the degree of liver disease, HE, and attentional limitations, participants furthermore underwent a comprehensive clinical assessment including blood tests, fibroscan, psychometric computer tests (Vienna test system, WINWTS, Version 4.50, 1999), assorted subtests of the test battery of Tests of Attentional Performance (TAP; PSYTEST, Herzogenrath, Germany), and CFF measurements (Eberhardt, 1994). Based on these data, patients were firstly subdivided into three groups: (i) patients with liver cirrhosis, but no signs of HE (HE0), (ii) patients with no clinical signs of HE, but pathological psychometric test results (mHE), and (iii) patients with clinical signs of HE of grade 1 (HE1). These three groups are referred to as HE groups in the following. A second subdivision was based on CFF values. All participants showing significant gamma band synchronization in response to the visual stimulus were sorted by their CFF values into one group of participants with low CFF (< 39 Hz, $n = 12$) and one group with high CFF (≥ 39 Hz, $n = 14$). In a second step, the analyses were repeated, including only patients (low CFF: $n = 10$ vs. high CFF: $n = 10$). As the results of the analyses including all participants and only patients are very similar, only results of all participants are reported here (for further details see Appendix 3).

The two groups are referred to as low and high CFF groups in the following. Depending on these classifications, effects of HE severity (according to HE groups, low/high CFF groups, and the CFF by itself) on behavioral and physiological data were examined.

6.3 Results

6.3.1 Behavioral Performance Decreases with CFF

Correctness of responses as a behavioral measure showed that overall, participants were able to perform the tasks and shift their attention between the visual and the auditory modalities. Performance was not different between HE groups. However, both, correctness of responses and reaction times were correlated with the CFF, indicating that behavioral performance is related to disease severity (please also see Appendix 3, Fig. 2). Between the high and low CFF groups, performance differences were observed. Participants with high CFF values performed better than participants with low CFF values, strengthening the notion that behavioral performance depends on disease severity.

6.3.2 HE Patients Show Visual Gamma Band Oscillations

In response to the visual stimulus, all groups of participants showed sustained gamma band oscillations at sensors overlaying visual cortex (please also see Appendix 3, Fig. 3). The strengths of gamma band oscillations were compared between groups. To account for differences in individual gamma band peak frequencies, data were averaged over the time of strongest gamma band response at each participant's peak gamma band frequency. No differences in the strength of gamma band oscillations were observed between groups, neither the HE groups, nor between groups with low and high CFF values.

6.3.3 Attentional Modulation of Gamma Band Oscillations

The expected difference in strength of gamma band oscillations between the selective visual (high visual attention) and selective auditory conditions (low visual attention) was only observed in the high CFF group and in the group of HE0 patients (please also see Appendix 3, Fig. 4). In the low CFF group and in all other groups, no

differences in the strength of gamma band oscillations were observed between these two conditions.

6.3.4 Frequency of Gamma Band Oscillations Decreases with CFF

Individual gamma band peak frequencies correlated with the CFF in the selective visual condition, i.e. the lower the CFF, the lower the gamma band peak frequency (please also see Appendix 3, Fig. 5). This shows that the gamma band peak frequency and HE disease severity are closely related. These results were also confirmed in the CFF based group comparison. The gamma band peak frequencies were higher in the high compared to the low CFF group (please also see Appendix 3, Fig. 6).

6.4 Discussion

6.4.1 Behavioral Performance Relates to HE Disease Severity

Correctness of responses show that overall, both, patients and control subjects were able to shift their attention between the visual and auditory modalities. From study 1, reaction time differences would have been assumed but could not be reproduced. This could be due to the described changes in the paradigm. Another explanation might be the age of the participants (study 1: 25.5 ±4.3 years; study 2: 60.8 ±8.5 years). Research has shown that young adults aim to balance speed and accuracy to achieve the most correct answers, whereas older adults aim to minimize errors, irrespective of the time their responses take (Starns and Ratcliff, 2010). This is in line with the present results.

A worsening of HE was associated with decreased behavioral performance as shown by correlations of behavioral data with the CFF. Additionally, this result was strengthened by differences between the high and low CFF groups. These findings are in line with previous works showing cognitive impairment in patients with HE, mainly in form of attention deficits and deficits in visual perception (Weissenborn et al., 2001, 2003; Pantiga et al., 2003). However, from these previous studies, performance differences would have been expected between HE groups, which were not found in the current work. This could be due to the small group sizes of HE groups, a limitation of study 2.

Then again, this fact and the correlation of behavioral data with the CFF, underline the view that HE groups are not as accurate as the CFF in distinguishing HE disease severity (Häussinger et al., 2006; Kircheis et al., 2007).

6.4.2 HE Patients Show Visual Gamma Band Oscillations

This study is the first to find attention-related visual gamma band synchronization in a cohort of HE patients. The relevance of this finding becomes clear when considering that gamma band oscillations are related to attention (Fries et al., 2001; Hoogenboom et al., 2006, 2010; Kaiser et al., 2006; Lachaux et al., 2005; Steinmetz et al., 2000) and that patients with HE show increased attention deficits with increasing disease severity (Weissenborn et al., 2001, 2003; Pantiga et al., 2003). Moreover, a slowing of brain responses in motor areas has been reported (Timmermann et al., 2002, 2003, 2008). From this evidence, it could be assumed that HE patients - especially more impaired patients - would not show any substantial attention-related gamma band oscillations, at least not in frequencies of the traditional gamma range (30-100 Hz). Nevertheless, the existence of gamma band oscillations in patients with HE simplifies the study of attention-related brain responses in this patient group.

No differences in the strength of gamma band oscillations were found, neither between HE groups nor between groups with low and high CFF values. With the expected and observed differences in attentional performance between high and low CFF groups, these results are in contrast to study 1, showing that the strength of gamma band oscillations is related to attention. However, the previously reported slowed communication of motor areas in patients with HE (Timmermann et al., 2002, 2003, 2008) was of higher strength than the normal communication of these areas. Furthermore, in power spectra of brain responses it has been shown that the spectral power is inversely correlated with its frequency, i.e. the higher the spectral frequency, the lower its power (Buzsáki and Draguhn, 2004). According to this, lower frequencies should have higher power values than higher frequencies. Thus, in agreement with these data and considering that the frequency of gamma band oscillations was related to disease severity, it can be speculated that (i) more impaired patients show weaker gamma band oscillations due to decreased attentional performance, but (ii) that gamma band oscillations should be stronger in lower frequencies. Taking these assumptions

together, the lack of differences in gamma band oscillation strengths between groups might be explained.

6.4.3 Impaired Modulation of Gamma Band Oscillations in HE

The current study demonstrated that the level of visual attention only in the high CFF group modulated the strength of gamma band oscillations. In line with inferior behavioral performance, the low CFF group did not show significant modulation of attention-related visual gamma band oscillations. As previously suggested (Amodio et al., 2005), this might be due to an increased distractibility of more impaired patients by irrelevant inputs, i.e. input from the not attended modality in this study. Furthermore, as discussed in study 1 above, these results give further support to the notion that behavioral measures and gamma band oscillations are tightly related.

6.4.4 Frequency of Gamma Band Oscillations Decreases with HE Disease Severity

The current data found a correlation of the frequency of visually induced gamma band oscillations and the severity of HE as indexed by the CFF, i.e. the lower the CFF, the lower the frequency of gamma band oscillations. As no differences in strength of gamma band oscillations were found this suggests that the frequency, rather than the strength of gamma band oscillations is related to HE disease severity. A slowed frequency of the attention-related gamma band oscillations in more severely impaired patients is in line with previous results showing a reduced mean dominant frequency in HE patients (Kullmann et al., 2001; Van der Rijt et al., 1984). Thus, these data extend earlier findings of slowed oscillatory processes in the motor and visual system (Timmermann et al., 2002, 2003, 2008) to the cognitive domain. Giving first time evidence of a slowed gamma band peak frequency in HE patients performing a cognitive task, further support is added to the hypothesis of slowed oscillatory activity as a key mechanism in the pathophysiology of HE (Timmermann et al., 2005). As the individual gamma band peak frequency has been shown to be stable within subjects for at least four weeks (Muthukumaraswamy et al., 2010), an interesting task for future works would be to determine in a longitudinal study whether disease and thereby induced changes in cognitive performance influence the individual gamma band peak frequency.

In the current study, data were corrected for effects of age. Furthermore, no significant age differences were observed between any of the groups. Thus, the decreased gamma band frequency in patients with HE seems not to be affected by the age of participants as could have been presumed due to previous findings of a negative correlation of age and occipital gamma band frequency in response to visual stimuli (Muthukumaraswamy et al., 2010).

6.4.5 Potential Role of GABA in HE

The frequency of gamma band oscillations has been shown to positively correlate with resting GABA concentrations in visual (Muthukumaraswamy et al., 2009) and motor cortices (Gaetz et al., 2011). The 'GABA Hypothesis of HE' (for a review see Jones et al., 1984) argues that increased inhibitory GABA mediated neurotransmission contributes to HE (Bassett et al., 1990). Following this hypothesis, one would expect higher levels of GABA and hence, a higher frequency of gamma band oscillations in more severely impaired patients. However, the present data show the opposite relation, i.e. a decreased gamma band frequency with increasing disease severity. Recent literature, argues against the 'GABA Hypothesis of HE', by showing that brain and cerebrospinal fluid GABA content and synthesis were unaltered in HE patients (Lavoie et al., 1987; Moroni et al., 1987; Zwingmann et al., 2003). Substances like neurosteroids modulating the GABA-A receptor are now being discussed as contributing factors in HE, rather than GABA content *per se* (Ahboucha et al., 2004). The current data are thus in line with this new hypothesis promoting unchanged or even decreased GABA concentration with increasing grades of HE. Furthermore, one might speculate that in HE patients, the gamma band peak frequency is influenced by a general slowing of oscillations and could, in case of lower GABA concentration, depend on GABA concentration *per se* but less on GABA mediated neurotransmission. However, studies showing direct relations of GABA concentration, GABA-A receptor mediated neurotransmission by other factors and gamma band oscillations in patients with HE, are needed to solve this issue.

6.5 Conclusion

This study showed that performance in a task requiring voluntary top-down shifts of attention is related to the severity of HE. Only patients with normal CFF values are able

to show concurrent modulation of gamma band oscillations. Furthermore, the peak frequency of attention-related gamma band oscillations in visual areas decreases with increasing disease severity of HE as measured by the CFF. Hereby, earlier results of slowed oscillatory processes in the motor and visual system of HE patients are extended to the cognitive domain. Thus, further support is added to the hypothesis that slowed oscillatory activity is a key mechanism in the pathophysiology of HE, explaining the broad variety of symptoms. Finally, the present data emphasize the CFF as being a more sensitive indicator of HE severity than the traditional HE classification.

7 Conclusion

The present work underlines the close relation between visual attention and gamma band oscillations. Neurophysiological effects and actual behavioral performance are tightly associated, which substantiates the functional importance of gamma band oscillations. This close relationship was shown for both, healthy subjects and patients with known attention deficits.

Behavioral results show that visual attention can be modulated gradually. These behavioral effects are found to co-occur with gradual modulations of the strength of gamma band oscillations in early visual areas. This gradual modulation suggests that by being precisely adjustable to current attentional needs, gamma band oscillations represent a mechanism enabling an efficient use of cognitive resources. Pathologically changed attention processes on a behavioral and neurophysiological level in patients with HE further substantiate the relation between visual attention and gamma band oscillations. It is shown that performance in an attention task and the concurrent modulation of the strength of gamma band oscillations are impaired in more severe HE. Furthermore, a relation between the frequency of the attention-related gamma band responses and the severity of HE and thus attentional performance are found. These data emphasize the significant relation between behavioral and neurophysiological data in healthy and attention impaired subjects. The location of these effects in early visual areas is of additional importance, supporting an attentional modulation of oscillatory responses at early stages of visual processing.

By the current results, the biased competition model of selective attention (Desimone and Duncan, 1995) is extended by a gradual dimension. Enhanced gamma band synchronization can be seen as an adaptive mechanism enhancing the selective processing of a stimulus in a gradual manner, thereby reflecting the amount of selective attention a stimulus receives. Assumptions can be made about the application of this model in an intermodal attention design, where the visual stimulus is preferably processed, compared to the auditory one, indicated by enhanced gamma band synchronization.

For the study of HE in particular, the current data extend earlier results of slowed oscillatory processes in the motor and visual system to the cognitive domain. Further support is thereby added to the notion that slowed oscillatory activity is a key mechanism in the pathophysiology of HE.

Based on these data, it can be hypothesized that healthy and pathological attentional performance are encoded by differential mechanisms. Healthy performance seems to be encoded by the strength of gamma band oscillations, as shown in multiple studies (Gruber et al., 1999; Siegel et al., 2008; Tallon-Baudry et al., 2005; Vidal et al., 2006; Wyart and Tallon-Baudry, 2008) and now also in a graded manner (study 1). Pathological attentional performance such as in HE in this work, however, seems to depend on the frequency of gamma band oscillations, as evidenced by the relation of gamma band frequency and HE disease severity.

Furthermore, this work supports the notion that the commonly used grading of HE into groups might be too simple to account for the complex interplay of the various symptoms these patients experience (Häussinger et al., 2006; Kircheis et al., 2007). A continuous measure like the CFF giving one overall measure of the patient's current status seems to be a better choice. Thus, for the clinical setting, it can be concluded that the determination of the CFF poses a valuable measure of HE disease severity, which should be more routinely used to objectively quantify and monitor the progression of HE.

8 Outlook

The current work extended previous studies on the functional role of occipital gamma band oscillations. However, open questions remain which need to be addressed in future studies.

In this work, graded mirror effects of attention-related gamma band oscillations in auditory cortex could not be shown. An interesting task for future studies would be to develop an auditory stimulus that induces long lasting gamma band oscillations in auditory cortices comparable to the gamma band responses shown for visual cortex here. It would thus become feasible to study mirror effects of concurrently antagonistically modulated gamma band oscillations in auditory cortex. Thereby, the biased competition model could be tested in a multisensory setting. Moreover, this would permit conclusions about influences of disease, e.g. HE, on the frequency of gamma band oscillations in the auditory modality. It would certainly be interesting to also investigate attention-related modulation of gamma band oscillations between other modalities.

Furthermore, the observed changes in the attention-related frequency of gamma band oscillations with severity of HE, should be extended to other patient populations with attention deficits. This could be achieved by applying a similar paradigm as the one in of the current work. An interesting approach would be for example to see whether the attention-related frequency of gamma band oscillations is different in children with attention deficit hyperactivity disorder (ADHD). If the gamma band frequency changed in the course of the disorder and correlated with attentional performance de- or increases, further support would be added to the described tight relation between behavioral measures and gamma band oscillations.

Even though the results of this thesis suggest that gamma band oscillations and attention are closely related, a causal relationship cannot be proved. A different and straightforward approach to investigate the functional relevance of gamma band oscillations would be to manipulate gamma band oscillations directly and investigate effects on performance. This could be done by different methodological approaches such as pharmacological or neurophysiological interventions, e.g. transcranial magnetic stimulation (TMS), or neurofeedback. Using neurofeedback training participants learn to

modulate their brain activity, which is concurrently recorded with EEG. Online feedback is given about participant's progress in modulating brain responses. It has been found that gamma band oscillations can be modulated using neurofeedback (Keizer et al., 2010). These preliminary data showed that increases in gamma band oscillations due to neurofeedback training had enhancing effects on intelligence measures and reduced binding costs. The effects of neurofeedback training on gamma band oscillations and concurrent effects on attention have so far not been studied. One way to test this relation would be to let participants perform an attention task and measure their performance and task-related gamma band oscillations in the MEG. After modulating gamma band oscillations during neurofeedback training, effects of these modulations on performance in the attention task and gamma band oscillations could be measured. Interestingly, a previous study showed that children with ADHD improved in daily life behavioral performance after participating in neurofeedback training involving slow cortical potentials and theta and beta band oscillations (Gevensleben et al., 2009). Due to the close relation of gamma band oscillations and attention, this training could be extended to the gamma frequency band. Next to measures of daily functions, changes in performance in attention tests could be considered.

Furthermore, future research could focus on gathering more information on the complex relations of GABA concentration, GABA-A receptor mediated neurotransmission by other factors and gamma band oscillations in patients with HE. As reported previously in healthy adults (Muthukumaraswamy et al., 2009) the combination of magnetic resonance spectroscopy to quantify resting GABA concentration and MEG to measure gamma band oscillations seems to be a promising setup.

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10 Appendix

This work is based on:

Publication 1:

Kahlbrock, N., Butz, M., May, E.S., Schnitzler, A., 2012. Sustained gamma band synchronization in early visual areas reflects the level of selective attention. Neuroimage 59 (1), 673-681. (Appendix 2)[1]

Personal Contribution: Approximately 80%

Publication 2:

Kahlbrock, N., Butz, M., May, E.S., Brenner, M., Kircheis, G., Häussinger, D., Schnitzler, A., under review. Frequency of visual attention-related gamma band activity decreases with severity of hepatic encephalopathy. Neuroimage. (Appendix 3)

Personal Contribution: Approximately 80%

Other aspects are taken from:

Publication 3:

Lange, J., Halacz, J., van Dijk, H., Kahlbrock, N., Schnitzler, A., forthcoming. Fluctuations of pre-stimulus oscillatory power predict subjective perception of tactile simultaneity. Cerebral Cortex. (Appendix 4)[2]

Personal Contribution: Approximately 20%

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[1] Reprinted from Neuroimage, 59/1, Kahlbrock, N., Butz, M., May, E.S., Schnitzler, A., Sustained gamma band synchronization in early visual areas reflects the level of selective attention. pp. 673-681, 2012, with permission from Elsevier.

[2] Lange, J., Halacz, J., van Dijk, H., Kahlbrock, N., Schnitzler, A., Fluctuations of pre-stimulus oscillatory power predict subjective perception of tactile simultaneity. Cerebral Cortex (forthcoming) by permission of Oxford University Press.

Talk and Conference Abstracts:

Kahlbrock, N., 2008. Attention-related cortical activity and hepatic encephalopathy. Talk at: 4th Conference of the SFB 575 "Experimental Hepatology": Regenerative Hepatology, November 6-8, 2008

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Kahlbrock, N., Butz, M., May, E.S., Brenner, M., Kircheis, G., Häussinger, D., Schnitzler, A., June 2011. Frequency of Attention-Related Gamma Band Activity Decreases with Severity of Hepatic Encephalopathy. 17th Annual Meeting of the Organization for Human Brain Mapping, June 26-30, 2011, Québec City, Canada.

May, E.S., Butz, M., Kahlbrock, N., Brenner, M., Schnitzler, A., June 2011. Spatial attention differentially modulates oscillatory activity associated with pain – an MEG study. 17th Annual Meeting of the Organization for Human Brain Mapping, June 26-30, 2011, Québec City, Canada.

Lange, J., Halacz, J., van Dijk, H., Kahlbrock, N., Schnitzler, A., June 2011. Pre-stimulus oscillatory activity predicts subjective perception of tactile simultaneity. 17th Annual Meeting of the Organization for Human Brain Mapping, June 26-30, 2011, Québec City, Canada.

Grants and Prizes

PhD Scholarship

Studienstiftung des deutschen Volkes, 07.2008 – 04.2011

Poster Prize

55. Jahrestagung der Deutschen Gesellschaft für Neurophysiologie und Funktionelle Bildgebung (DGKN), 03.2011

Travel Grant

Boehringer Ingelheim Fonds, 04.2008

Travel Grant

Sonderforschungsbereich 575, Experimentelle Hepatologie, 03.2010

NeuroImage 59 (2012) 673–681

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Sustained gamma band synchronization in early visual areas reflects the level of selective attention

Nina Kahlbrock [a,*,1], Markus Butz [a,b,1], Elisabeth S. May [a], Alfons Schnitzler [a,c]

[a] Heinrich-Heine-University Düsseldorf, Medical Faculty, Institute of Clinical Neuroscience and Medical Psychology, Universitätsstraße 1, D-40225 Düsseldorf, Germany
[b] University College London, Institute of Neurology, 33 Queen Square, WC1N 3BG, London, United Kingdom
[c] Heinrich-Heine-University Düsseldorf, Medical Faculty, Department of Neurology, Universitätsstraße 1, D-40225 Düsseldorf, Germany

ARTICLE INFO

Available online 23 July 2011

Keywords:
Synchronization
Gamma
Attention
Biased competition
MEG
Audiovisual

ABSTRACT

Cortical gamma band synchronization is associated with attention. Accordingly, directing attention to certain visual stimuli modulates gamma band activity in visual cortical areas. However, gradual effects of attention and behavior on gamma band activity in early visual areas have not yet been reported.
In the present study, the degree of selective visual attention was gradually varied in a cued bimodal reaction time paradigm using audio-visual stimuli. Brain activity was recorded with magnetoencephalography (MEG) and analyzed with respect to time, frequency, and location of strongest response.
Reaction times to visual and auditory stimuli reflected three presumed graded levels of visual attention (high, medium, and low). MEG data showed sustained gamma band synchronization in all three conditions in early visual areas (V1 and V2), while the intensity of gamma band synchronization increased with the level of visual attention (from low to high). Differences between conditions were seen for up to 1600 ms.
The current results show that in early visual areas the level of gamma band synchronization is related to the level of attention directed to a visual stimulus. These gradual and long-lasting effects highlight the key role of gamma band synchronization in early visual areas for selective attention.

Introduction

In our complex multisensory environment, it is essential to process relevant information while ignoring the rest. In case of competing input from two different modalities, stimuli in the attended modality receive amplified processing compared to stimuli in the non-attended modality (Gherri and Eimer, 2011; Spence and Driver, 1997). As stimulus processing is believed to be capacity-limited, allocating resources to one attended modality gradually subtracts resources from the available supply of all modalities (Bonnel and Hafter, 1998). Consequently, modulation of attentive processing in one modality can be studied by reallocating available resources between competing modality specific stimuli.

In the brain, attentional modulation of sensory processing has been associated with gamma band (30–100 Hz) synchronization (Fries et al., 2001; Hoogenboom et al., 2006, 2010; Kaiser et al., 2006; Lachaux et al., 2005; Steinmetz et al., 2000). Previous studies reported modulation of visually induced gamma band oscillations by attention in animals (Khayat et al., 2010) and humans (Gruber et al., 1999; Siegel et al., 2008; Tallon-Baudry et al., 2005; Vidal et al., 2006; Wyart and Tallon-Baudry, 2008). In all these studies, states of 'attention' versus 'no attention' were compared. In an EEG study, Simos et al. (2002) provided first evidence that gamma band synchronization gradually increases with task complexity. However, changes remained unspecific and could not be attributed to modality specific regions.

Previous studies on selective visual attention suggest that the attended of two competing visual stimuli gets a competitive advantage over the other by enhancing its gamma band synchronization (Fries et al., 2001, 2008). This effect has been addressed in the hypothesis of biased competition through enhanced synchronization (Fries, 2005), which bases its assumptions on the biased competition hypothesis (Desimone and Duncan, 1995; Reynolds et al., 1999). Nevertheless, the conceptual framework of the biased competition hypothesis has yet to be tested for its application in cross-modal attention designs as for cross-modal designs it has only been addressed on a theoretical level for the visual-tactile domain (Magosso et al., 2010).

While several functional Magnetic Resonance Imaging studies showed that attention modulates processing of sensory information in early visual areas (Gandhi et al., 1999; Munneke et al., 2008), attention dependent modulation of gamma band synchronization has primarily been recorded in mid- and high level stages in the visual processing hierarchy (Fries et al., 2001; Gregoriou et al., 2009;

* Corresponding author at: Heinrich-Heine-University Düsseldorf, Medical Faculty, Institute of Clinical Neuroscience and Medical Psychology, Universitätsstraße 1, D-40225 Düsseldorf, Germany. Fax: +49 211 81 19033.
E-mail address: Nina.Kahlbrock@uni-duesseldorf.de (N. Kahlbrock).
[1] These two authors contributed equally to this work.

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doi:10.1016/j.neuroimage.2011.07.017

Womelsdorf et al., 2006). One study (Chalk et al., 2010) found decreased local field potential gamma band power and decreased gamma band spike field coherence with attention in monkey primary visual cortex. Previous works in humans using magnetoencephalography (MEG) have shown increased induced gamma band synchronization in visual areas V1–V3 during attention demanding tasks (Hoogenboom et al., 2006, 2010). Nevertheless, these neurophysiological results did not show graded attentional modulation of gamma band synchronization in early visual areas. In fact, up to now, studies on graded attentional modulation of induced gamma band synchronization in early visual areas in humans are lacking.

The present study is the first to systematically manipulate the level of visual attention, relate it to behavioral performance and to the intensity of gamma band synchronization in early visual areas. Subjects were simultaneously presented with visual (Hoogenboom et al., 2006) and auditory stimuli in a cued bimodal reaction time paradigm resulting in a gradual modulation of visual attention.

Materials and methods

Subjects

Sixteen healthy right-handed subjects with normal or corrected to normal vision participated in this study (8 female, mean age: 25.5 ± 4.3 years; SD). All subjects gave their written informed consent. The study was approved by the local ethics committee (study no. 2895) and was performed in accordance with the Declaration of Helsinki.

Paradigm

Fig. 1 provides an overview of the paradigm. Each trial started with a cue presented for 1000 ms indicating the specific task of one of three experimental conditions: (i) *selective visual*, (ii) *selective auditory*, or (iii) *divided*, i.e. visual and auditory. Irrespective of the condition, the cue was followed by a 2000 ms fixation period. Then, a visual stimulus (an inwardly contracting grating) and an auditory stimulus (a constant tone) appeared simultaneously. After a randomly assigned period of 500, 1000, 2000, or 3000 ms, either the visual or the auditory stimulus changed its quality (change 1). 750 or 1000 ms later, the other stimulus also changed (change 2). In half of the trials, the visual stimulus changed first followed by a change in the auditory stimulus and vice versa. The order of these changes was randomized. A change of the visual stimulus was implemented as an increase in speed of the stimulus that either continued to move inwards or changed its direction and then moved outwards (inward/outward). A change in the auditory stimulus was implemented as a change in pitch to a higher or lower pitch (high/low). Please see section on stimuli and stimulus delivery for a detailed description of the properties and delivery of the stimuli.

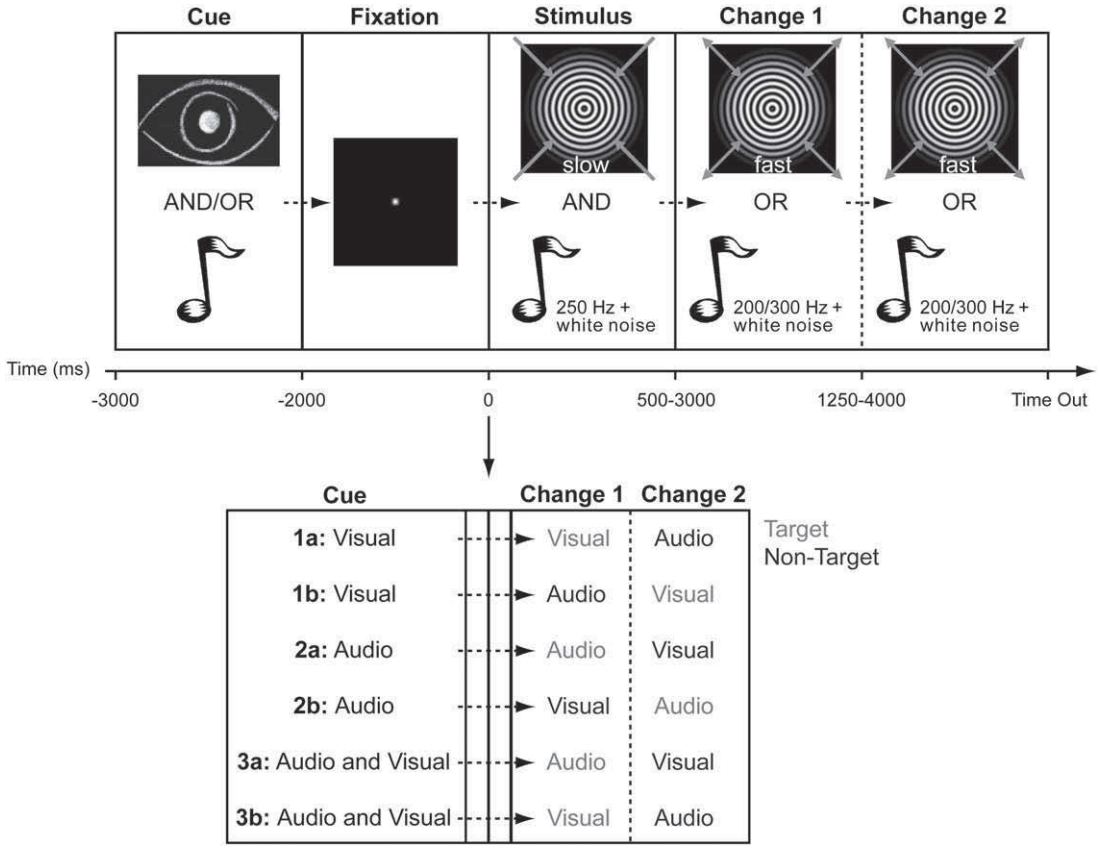

Fig. 1. Paradigm. Upper part: general overview of one trial. Each trial started with a cue indicating the condition (Cue). After presentation of a fixation dot (Fixation), visual and auditory stimuli were presented simultaneously (Stimulus; 0 = start of stimulus). After a randomly assigned period of 500 to 3000 ms, either the visual or the auditory stimulus changed its quality (Change 1). After 750 or 1000 ms also the other stimulus changed (Change 2). Depending on the condition, one of the two stimulus changes served as target. Subjects had to give a speeded response indicating quality of target as soon as it appeared in the cued modality (see section on stimuli and stimulus delivery for exact description of target qualities). A response or a reaction time>2000 ms (Time out) terminated stimulus presentation. Feedback was given after each trial. Periods used for later analysis were Fixation (baseline) and Stimulus. Lower part: detailed description of variable parts of each trial. With a visual cue (1a/1b; condition *selective visual*, n = 108 trials) the change in visual stimulus became target and the change in auditory stimulus non-target. If the cue was auditory (2a/2b; condition *selective auditory*, n = 108 trials) the change in auditory stimulus became target and the change in visual stimulus non-target. Two target positions were possible in the *selective* conditions. When auditory and visual cues were presented together (3a/3b; condition *divided*; 3a: *divided auditory*, n = 108 trials, 3b: *divided visual*, n = 108 trials) the first changing stimulus became target, the second one non-target. Targets are depicted in light grey, non-targets in dark grey. Please note that fixation and stimulus periods consisted of the same stimulation in each trial, only duration of stimulus period varied. Thus, these trial periods are depicted as small empty boxes in the lower part.

Depending on the experimental condition, as indicated by the cue at the beginning of the trial, a change of one of the stimuli became the target. Subjects were required to give a speeded response to the change in the stimulus' quality, i.e. a change in speed of the visual or a change of pitch of the auditory stimulus. In condition *selective visual*, subjects had to exclusively react to the change in the visual stimulus (target), irrespective of its position in the trial (change 1 or 2) and ignore the change in the auditory stimulus (non-target). In condition *selective auditory*, accordingly, subjects had to react to the change in the auditory stimulus (target) only and ignore the change in the visual stimulus (non-target). In the *divided* condition, subjects had to respond to the stimulus that changed first (change 1 = target) and ignore the change in the other stimulus (change 2 = non-target).

The subject's task was thus to react to the target and indicate the quality of this stimulus change by pressing one of four buttons operated with the index and middle fingers of both hands. Thereby, each hand was assigned to one modality (visual and auditory) and each finger to one quality change, i.e. for the visual stimulus an index finger press indicated inward and a middle finger press outward movement, for the auditory stimulus an index finger press indicated a high tone, a middle finger press a low tone. Feedback was given after each trial. If subjects did not respond within 2000 ms after target appearance, the trial was counted as missed. The assignment of the left or right hand to the auditory or visual modality was balanced between subjects, finger assignment was kept fixed.

The paradigm consisted of 432 trials: 108 trials in conditions *selective visual* and *selective auditory* each and 216 in condition *divided* (subdivided into 108 trials where the visual stimulus changed first and thereby became target, *divided visual*; and 108 trials where the auditory stimulus changed first, *divided auditory*). Trials from the different conditions were presented in two blocks in a random order. Each block was subdivided into smaller blocks of twelve trials separated by self-paced breaks to avoid fatigue. Prior to data acquisition, subjects were trained on the paradigm until they thoroughly understood the task.

Three levels of visual attention were sought to be obtained by these conditions; high in condition *selective visual*, medium in condition *divided*, and low in condition *selective auditory*. In analogy to Coull et al. (2004), attentional allocation was obtained by varying the likelihood of whether the motor response was based on changes in the visual or the auditory stimulus (*selective visual*: 100% visual, 0% auditory, *selective auditory*: 0% visual, 100% auditory, *divided*: 50% visual, 50% auditory).

Stimuli and stimulus delivery

The fixation point was of a Gaussian (0.56° in diameter), which increased its contrast by 40% after 1000 ms, thereby informing the subject that the stimulation was about to start. The visual stimulus was adapted from Hoogenboom et al. (2006). It consisted of a foveal circular sine wave grating (diameter: 5.6°, spatial frequency: 2 cycles/°, contrast: 100%) continuously contracting towards the center of the screen (velocity: 1.6°/s). The change in visual stimulus (potential target) was characterized by an increase in velocity (3.38°/s). The sine wave grating was then either still contracting towards the center of the screen or changed its direction and expanded outwards.

The auditory stimulus was a binaurally presented 250 Hz sine tone embedded in white noise (white noise reduced by 9 dB compared to sine tone). The change in auditory stimulus (potential target) consisted of a change in pitch of the tone to either 200 Hz or 300 Hz. The auditory stimulus intensity was adjusted to subjectively match the visual stimulus intensity. Thus, auditory stimuli were well audible for all subjects, but at individual volumes.

Stimulus timing was controlled using Presentation® software (version 13.0, www.neurobs.com). Visual stimuli were projected onto a screen with a dlp projector (PLUS Vision Corp. of America) with 60 Hz refresh rate. Participants were seated approximately 76 cm away from the screen. Auditory stimuli were produced using Audacity® (http://audacity.sourceforge.net/). They were sent into the shielded room via a mixing desk and earphone transducers (E-A-RTONE, Aearo Technologies Inc., Indianapolis, USA), which converted the electrical to a sonic signal. The earphone transducers had two equal lengths plastic tubes and earplugs attached which were inserted into participants' ears.

Data acquisition

Neuromagnetic activity was measured in a magnetically shielded room with a whole-head Neuromag-122 MEG system (Elekta Neuromag Oy, Helsinki, Finland). Vertical and horizontal electrooculograms were recorded to later reject epochs contaminated with blink artifacts and eye movements. Individual high-resolution standard T1-weigthed structural magnetic resonance images (MRIs) were obtained from a 3 T Siemens Magnetom MRI scanner (Munich/Erlangen, Germany).

Data analysis: behavioral data

Behavioral data were analyzed by means of error rates and reaction times. Trials were divided into conditions *selective visual*, *selective auditory*, and *divided*. For analyses of the behavioral data, the divided condition was further split up into two subcategories *divided visual* and *divided auditory*. Only correct trials with reaction times faster than 2000 ms were subjected to further analysis. Reaction times were analyzed using repeated measures analysis of variance (ANOVA) with factors modality (auditory versus visual) and condition (selective versus divided).

Data analysis: MEG data general

MEG data were analyzed using FieldTrip, an open source Matlab toolbox (Oostenveld et al., 2011), and Matlab 7.1 (MathWorks, Natick, MA). Continuously recorded MEG data were divided into epochs of interest, starting at the time of first fixation point and ending with appearance of change in either of the stimuli. Please note that for analyses of neurophysiological data, trials of the condition *divided* were not split up, as only periods prior to stimulus change were analyzed. Semi-automatic routines and visual artifact rejection were applied to discard epochs contaminated with eye, muscle, and sensor artifacts. Partial and complete artifact rejection procedures were applied, rejecting either only parts of the trial contaminated by artifacts or the whole trial in case of multiple artifacts. During partial artifact rejection, for each of the different artifact types (eye, muscle, and sensor artifacts), a z-score with specific sensitivity for the respective artifacts was computed. This was done by selecting either only EOG channels or all MEG sensors. Then, data were band-pass filtered in order to only include frequencies in which the artifacts are known to be most dominant. Subsequently, the envelope of the signal was computed using Hilbert transform and normalized by calculating the z-scores for each sensor. Next, one summed z-value was obtained for each moment in time. For this purpose, the z-scores of all selected sensors were added, and this sum was normalized by dividing it by the root of the number of summed sensors. A rejection threshold was then determined separately for each subject and applied automatically to its entire dataset. This adaptation of z-values between subjects was necessary because of differences in noise levels and in the signal-to-noise ratio. After partial artifact rejection, trials were inspected visually and excluded completely in case of remaining artifacts. Power line noise was removed by estimating and subtracting the 50-, 100- and 150-Hz components in the MEG data, using a discrete Fourier transform. The linear trend was removed from each epoch.

Data analysis: individual gamma band peaks

Rhythmic neuronal activity was estimated determining spectral power of the MEG signals. For the time period of 500 to 1000 ms (0 being the start of stimulus presentation) and frequencies of 30 to 100 Hz power spectra were calculated for each participant averaged over all trials of all conditions and over occipital sensors (±1 Hz smoothing, hanning window). Each participant's absolute maximal gamma band frequency was obtained. Please note that to exclude purely stimulus-evoked components, the first 500 ms were excluded from those analyses steps not involving timely evolution of the signal. To include the strongest gamma band peak and the maximally possible amount of trials, periods from 500 to 1000 ms were used to calculate peak gamma band responses and their localization.

Data analysis: individual gamma sources

The source of the strongest gamma band peak (as obtained from the power spectra), averaged over all trials of all conditions, was localized for each individual using Dynamic Imaging of Coherent Sources (Gross et al., 2001), an adaptive spatial filtering technique in the frequency domain. Leadfield matrices were determined for realistically shaped single-shell volume conduction models (Nolte, 2003) derived from the individual structural MRIs. The grid of locations was constructed as a regular 5 mm grid. In order to account for each subject's strongest gamma band response crossspectral density matrices between all MEG sensor pairs at individual gamma band peaks ±5 Hz were determined separately for time frequency windows from −1000 to −500 ms, i.e. before stimulus start (baseline), and from 500 to 1000 ms after stimulus start (stimulus period). Spatial filters were determined based on the crossspectral density matrices averaged over all trials of all conditions of a given subject. Relative changes between pre-stimulus and stimulus periods were calculated and locations of each subject's strongest relative gamma band peak were retrieved. Each subject's source parameters were displayed on their individual brains. Each structural MRI was spatially normalized to a smoothed template MRI based on multiple subjects (Statistical Parametric Mapping; SPM2; http://www.fil.ion.ucl.ac.uk/spm/). Differences between MNI and Talairach coordinates were adjusted (http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach) and individual virtual sensor locations were identified, Brodmann areas were estimated from Talairach and Tournoux (Talairach and Tournoux, 1988) using 'Talairach Client — Version 2.4.2' (Lancaster et al., 2000).

Data analysis: time course of signal at individual gamma sources

To quantify the time course of the signal at each subject's strongest relative gamma source, virtual sensors were generated by linear constrained minimum variance (LCMV) beamformer reconstructions. The time courses of the source wave forms were obtained using covariance matrices for pre-stimulus (−1000 to −500 ms) and stimulus periods (500 to 1000 ms) separately, band-pass filtered for each subject's strongest gamma band peak. Spatial filters were calculated averaged over all trials of a given subject. For each individual, equal numbers of trials for all three conditions and pre-stimulus and stimulus times, were randomly drawn from the available preprocessed trials. Single trial time courses were then projected through those filters, providing single trial estimates of source power. For further analyses, dipole moments' time courses were projected on the direction of maximal power in the individual gamma band frequency. On the resulting source wave forms, time frequency representations of power (TFRs) were calculated for frequencies between 30 and 100 Hz using windows of 400 ms moved in steps of 50 ms. Multitaper spectral estimation was used with ±5 Hz smoothing (3 tapers) in steps of 0.5 Hz. Relative changes of power in the stimulus period (0 to 2000 ms) to the pre-stimulus baseline (−1000 to −500 ms) were calculated. For each subject, average TFRs were calculated for each of the three conditions. Due to the special tuning of the virtual sensors for the subjects' individual gamma band frequencies, lower frequencies were not subjected to further analyses here.

Data analysis: statistical comparison of conditions

To examine differences between the three conditions, average TFRs were subjected to statistical group analysis. The stimulus period relative to the pre-stimulus baseline and the absolute baseline period were analyzed separately. Dependent samples two-sided t-tests for each time- and frequency-point across epochs were performed for all three comparisons (*selective visual*/*selective auditory*, *selective visual*/*divided*, and *divided*/*selective auditory*). Statistical inference was based on a non-parametric randomization test, correcting for multiple comparisons due to a multitude of time- and frequency-points (Maris and Oostenveld, 2007; Nichols and Holmes, 2002). Bonferroni–Holm correction (Holm, 1979) was applied to the alpha level to correct for multiple comparisons between the three conditions.

Data analysis: signal phase-locked to stimulus onset

In the analysis performed earlier (Data analysis: time course of signal at individual gamma sources and Data analysis: statistical comparison of conditions), trials were averaged after conducting time frequency analysis. This approach significantly favors identification of non-phase-locked (induced) activities. Applying time frequency analysis after averaging mainly provides information on phase-locked (evoked) oscillatory bursts (Tallon-Baudry et al., 1996). To determine whether the here observed statistical effects stem from induced or evoked activity, the analysis was repeated for responses phase-locked to stimulus onset and averaged before performing time frequency analysis. Data were aligned to stimulus onset, baseline corrected with a time window of 200 ms before stimulus onset, projected through the common spatial filters, averaged over trials, and subjected to a time frequency analysis. The same non-parametric randomization test as described earlier was applied.

Data analysis: evoked magnetic fields

The analysis was repeated for modulations in evoked magnetic fields. For a direct comparison with the spectral power analysis, the same source locations (virtual sensors) and trial selections were used. Data were filtered with a band-pass filter from 0.03 to 30 Hz. A baseline of 200 ms prior to stimulus onset was subtracted. The statistical group analysis was repeated (dependent samples two-sided t-tests for all three comparisons).

Results

Behavioral data

In all four behavioral conditions (*selective visual*, *selective auditory*, *divided visual*, and *divided auditory*), error rates were below 10%. Mean reaction times were 586.39 ms ± 14.61 for condition *selective visual*, 669.96 ms ± 22.79 for condition *divided visual*, 299.20 ms ± 21.99 for condition *selective auditory*, and 430.74 ms ± 23.50 for condition *divided auditory* (SEM reported here; Fig. 2). For reaction times a repeated measures analysis of variance (ANOVA) resulted in significant main effects for factors modality ($F_{(1,15)} = 426.57$, $p < 0.001$) and condition ($F_{(1,15)} = 90.61$, $p < 0.001$) and in a significant interaction ($F_{(1,15)} = 10.00$, $p = 0.006$) between both factors. Reaction times were faster in the selective compared to the respective divided attention conditions and in the auditory than in the visual conditions. The

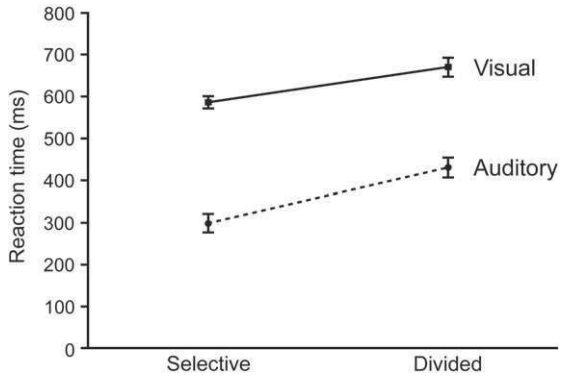

Fig. 2. Reaction times. Reaction times were faster in the selective, compared to the respective divided conditions, in both the visual and the auditory modality ($p<0.001$). Thus, effects of attention between conditions were confirmed. Differences between conditions *selective auditory* and *divided auditory* were more pronounced than differences between conditions *selective visual* and *divided visual* ($p=0.006$). Reaction times were faster in the auditory, than in the visual modality ($p<0.001$; SEM displayed).

difference between selective and divided conditions was more pronounced in the auditory than in the visual modality.

Frequency and location of strongest gamma band source

For MEG data analyses an average of 326 ± 9.48 (SEM reported) trials remained for each subject after rejecting invalid trials and artifacts. Thus, 75% of the previously recorded trials remained. Using only these trials, each subject's strongest gamma band frequency peak was retrieved. Power spectra averaged over each subject's occipital sensors revealed individual peak gamma band frequencies ranging from 54 to 69 Hz (see Table 1 for individual peak frequencies). The maximum gamma band power was localized and a virtual sensor was constructed for each subject. Virtual sensors were mostly localized in early visual cortex. In fourteen of sixteen subjects, the virtual sensor accounting for strongest gamma band activity in response to the visual stimulus was located in Brodmann areas 17 or 18. In one subject, it was located in Brodmann area 19 and in one subject in lingual gyrus, close to the cerebellum (Table 1 and Fig. 3).

Table 1
Characterization of single subject gamma band frequencies and locations. For each subject, individual peak gamma band frequencies and locations of virtual sensors as Brodmann areas and Talairach coordinates are displayed. Please note that for subject 8, the virtual sensor was localized in lingual gyrus, close to the cerebellum, no Brodmann area is specified in this case.

Subject no.	Maximal γ	Brodmann area	Talairach coordinates (x, y, z)		
1	60 Hz	18	−6	−81	20
2	54 Hz	17	14	−82	1
3	66 Hz	18	4	−88	−5
4	65 Hz	18	5	−76	25
5	56 Hz	19	−7	−79	35
6	69 Hz	17	−17	−79	9
7	56 Hz	18	−4	−90	17
8	60 Hz	-	−1	−78	−7
9	58 Hz	18	−17	−95	20
10	54 Hz	18	−6	−98	7
11	58 Hz	17	6	−90	−1
12	66 Hz	17	−8	−84	12
13	64 Hz	18	−21	−99	2
14	54 Hz	17	−11	−84	9
15	60 Hz	17	−2	−84	9
16	56 Hz	18	−19	−95	6

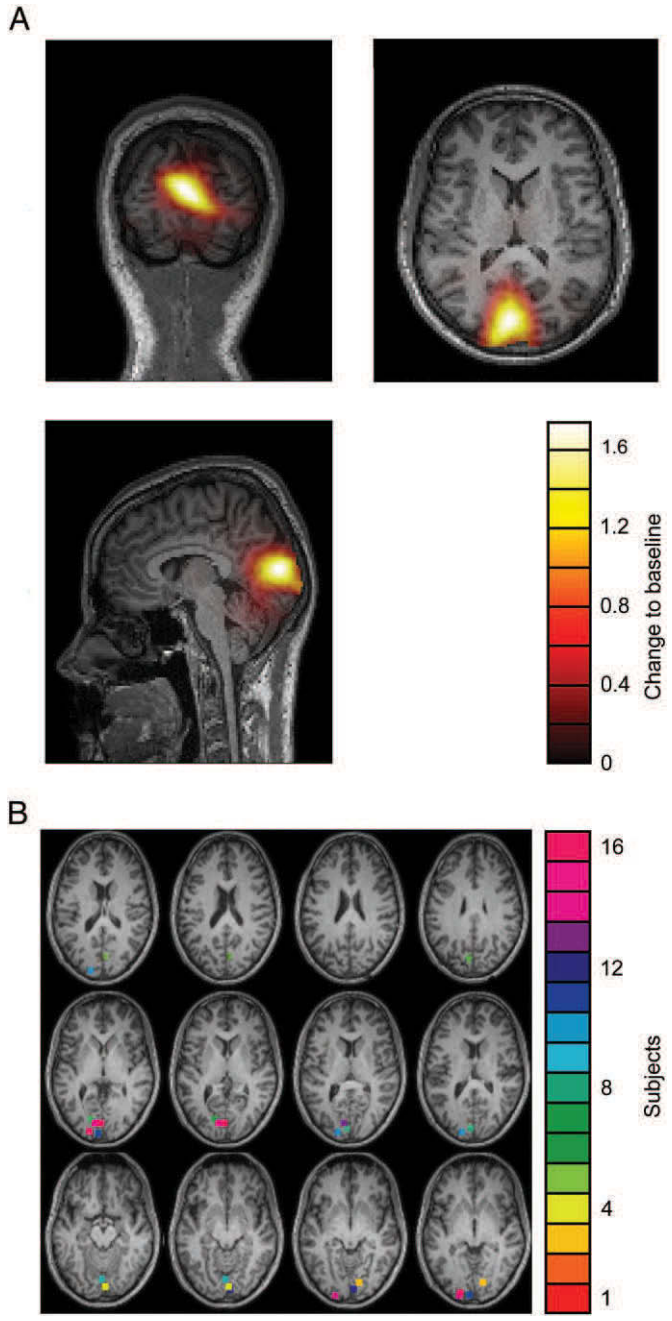

Fig. 3. Localization of gamma band power. A. Gamma band power relative to pre-stimulus baseline activity was localized in early visual areas. Displayed here is the grand average over all correct trials of all conditions for one representative subject (Subject 2). Colors indicate intensity of relative change to the pre-stimulus baseline. Values below 0.5 are masked. B. Virtual sensors were localized in areas V1 and V2 in 14 of 16 subjects. Shown here are virtual sensor locations for all subjects displayed on one individual brain normalized to a smoothed template MRI based on multiple subjects (Statistical Parametric Mapping; SPM2; http://www.fil.ion.ucl.ac.uk/spm/). Each colored point represents the virtual sensor of one subject. Please note that for visualization purposes, the kernels of the virtual sensors were extended to 9 mm. Slice thickness was 3 mm. For that reason, some virtual sensors are present in multiple slices.

Comparing conditions: similar baseline activity between attention conditions

When comparing conditions, the same numbers of trials were retrieved for each of the three conditions (*selective visual*, *selective auditory* and *divided*) and the pre-stimulus baseline and stimulus periods. On average, 79 ± 2.48 (SEM) trials remained per condition.

Between the three conditions no significant differences were found in pre-stimulus baseline activity ($p>0.37$). Thus, further results are based on relative changes in power with respect to a pre-stimulus baseline.

Comparing conditions: differences in gamma band activity between attention conditions

On virtual sensor level, all subjects showed sustained visually induced gamma band synchronization compared to the pre-stimulus baseline in all three conditions (see Fig. 4A for grand averages over all subjects). Pairwise comparisons on group level between all three conditions resulted in significant power differences in the gamma band frequency range. Relative gamma band power was significantly higher in condition *selective visual* than in condition *selective auditory* between 53 and 80 Hz from 400 to 2000 ms ($p<0.001$); it was significantly higher in condition *selective visual* than in condition *divided* between 54 and 74 Hz from 450 to 1550 ms ($p=0.009$); and it was significantly higher in condition *divided* than in condition *selective auditory* between 54 and 75 Hz from 700 to 2000 ms ($p=0.002$; all p-values corrected for multiple comparisons). Thus, relative visual gamma band synchronization was highest in condition *selective visual*, medium in condition *divided*, and lowest in condition *selective auditory* (Fig. 4B). Averaged over all subjects, over time (500 to 2000 ms) and individual gamma peak frequencies ± 5 Hz mean relative power values were 2.21 ± 0.44 (SEM) for *selective visual*, 2.13 ± 0.41 (SEM) for *divided*, and 1.92 ± 0.38 (SEM) for *selective auditory*. Please note that big standard errors are due to a big variance in overall relative gamma power between subjects (range: 0.16 to 6.91).

Signal phase-locked to stimulus onset and evoked magnetic fields

To examine differences in evoked spectral power between conditions, time frequency representations of power phase-locked to stimulus onset were calculated. No statistically significant differences were observed between the three conditions in gamma band power phase-locked to stimulus onset.

Furthermore, it was investigated, whether attentional modulations were preceded by attentional modulations of evoked magnetic fields. Again, no statistically significant differences were observed between the three conditions.

Discussion

The aim of the present study was to gradually modulate visual attention, thereby examining its relation to gamma band synchronization in visual cortical areas. A bimodal paradigm designed to elicit three levels of visual attention (high, medium, and low) was used and three graded levels of visual attention were confirmed on the behavioral side. In response to the visual stimulus, prominent long lasting local gamma band synchronization in visual cortex was found. Gamma band power was gradually modulated in early visual areas according to the amount of attention directed to the visual stimulus. This gradual modulation suggests that by being precisely adjustable

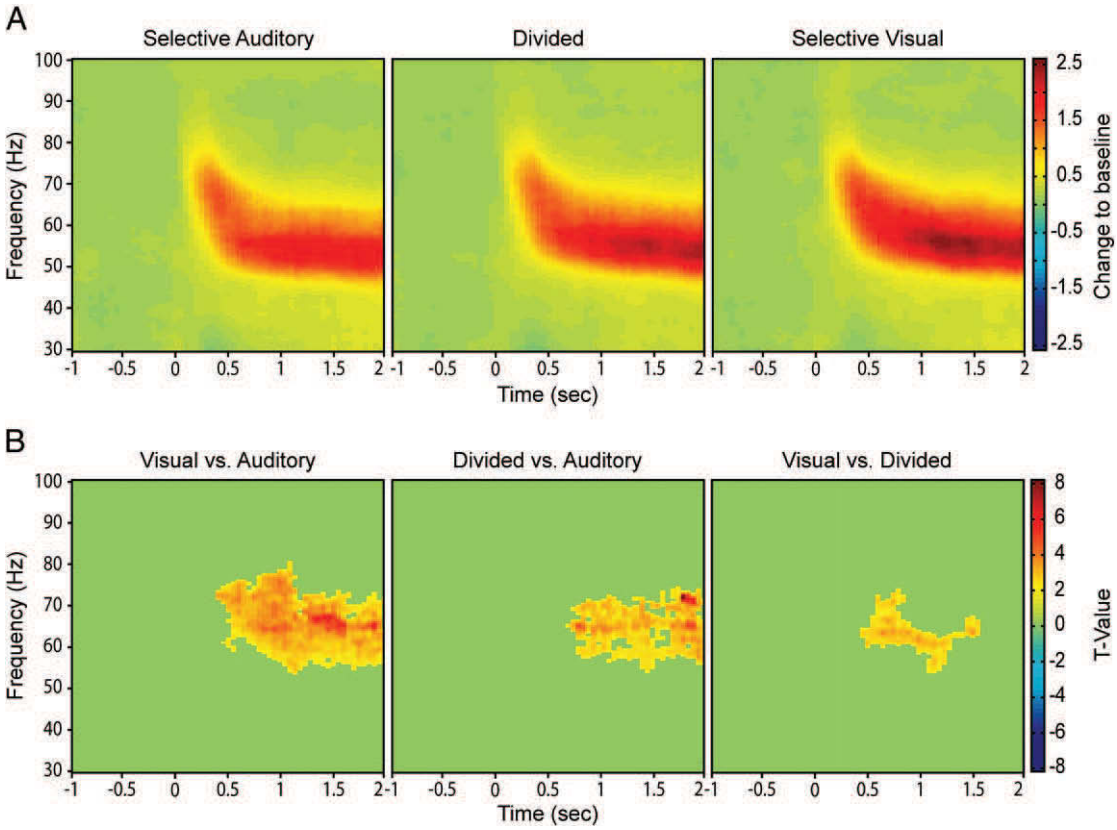

Fig. 4. Virtual sensor time frequency representations and statistics. A. Grand average time frequency representations relative to baseline from each subject's virtual sensor. In all three conditions (*selective auditory*; left, *divided*; middle, and *selective visual*; right) a prominent gamma band response relative to pre-stimulus baseline activity was observed during stimulation (onset of stimulation at t = 0). Color-coding: 0 corresponds to no change and 2.5 to a 250% increase in power relative to baseline. B. Statistical comparisons between all three conditions. Results are shown as t-values; all non-significant frequencies are masked green. Relative gamma band power was stronger in condition *selective visual* compared to condition *selective auditory* ($p<0.001$; left), it was stronger in condition *divided* compared to condition *selective auditory* ($p=0.002$; middle), and stronger in condition *selective visual* compared to condition *divided* ($p=0.009$; right; p-values corrected for multiple comparisons).

to current attentional needs gamma band synchronization represents a mechanism enabling an efficient use of cognitive resources.

Behavioral data and induced gamma band synchronization support gradual modulation of attention

Behavioral data confirmed different reaction times for the three attention levels. Thus, one can assume that graded levels of visual attention were reached by the applied bimodal reaction time paradigm and that attention was shifted between the visual and auditory modalities. Extending earlier behavioral work (Posner et al., 1980; Schroeger et al., 2000; Spence and Driver, 1997), a third medium state of visual attention is introduced, thereby enabling measurement of gradually modulated attention.

In line with earlier research (Edden et al., 2009; Fries et al., 2001; Hoogenboom et al., 2006; Muthukumaraswamy et al., 2009), subjects showed prominent long lasting visual gamma band activity in all conditions when presented with the visual stimulus. Underlining the pivotal behavioral relevance of this neurophysiological effect, gamma band activity in early visual cortex was highest in condition *selective visual*, medium in condition *divided*, and lowest in condition *selective auditory*. Thus, three increasing levels of visual attention are associated with a corresponding modulation on the neurophysiological side. While previous works investigated the influence of 'attention' versus 'no attention' on gamma band synchronization (Fries et al., 2001; Gruber et al., 1999; Mueller et al., 2000; Siegel et al., 2008; Wyart and Tallon-Baudry, 2008), the present study extends these findings by establishing gradual attention modulation. Furthermore, attentional effects on gamma band synchronization have never been shown for such long periods of time (lasting up to 1600 ms). Previous studies have provided data either averaged over certain time periods, frequency bands or both (Fries et al., 2001; Gruber et al., 1999; Mueller et al., 2000; Tallon-Baudry et al., 2005; Vidal et al., 2006). Thus, our data provide new insights into long lasting attention modulation and its relation to gamma band synchronization.

Gamma band power phase-locked to stimulus onset and evoked magnetic field strengths were not significantly different between conditions. Tallying with non-existent predictive properties of evoked magnetic fields for reaction times (Hoogenboom et al., 2010) gamma band modulation in this paradigm is assumed to stem from differences in induced and not in evoked gamma band power. These findings oppose animal data, showing attentional modulation of early evoked gamma band responses in primary visual and auditory areas (Lakatos et al., 2009). However, the described work is based on multiunit recordings from macaque primary areas. This different approach and the restriction to primary areas might be an explanation for these dissimilar results. Furthermore, the present restriction to areas of strongest gamma band synchronization, allows no deeper conclusions about the order of processing in visual areas from these data.

The difference in gamma band synchronization between conditions *selective visual* and *divided* was less prominent and shorter lasting than differences between conditions *selective visual* and *selective auditory* and between conditions *divided* and *selective auditory*. In behavioral analogy, we observed a stronger orientation to the visual modality reflected by a greater difference in reaction times between conditions *selective auditory* and *divided auditory* than between conditions *selective visual* and *divided visual*. While emphasizing the relation between behavioral and neurophysiological data, these findings tally with the ventriloquist effect (Alais and Burr, 2004), stating that vision often dominates audition when attentive processes are involved. A very recent study substantiates this by showing that visual dominance is based on less vulnerability of the visual system to competition from auditory stimuli than vice versa (Schmid et al., 2011).

One might speculate that prolonged reaction times in the *divided* compared to the *selective* conditions reflect an effect of task difficulty. Indeed, earlier studies showed that gamma band oscillations can be modulated by overall task difficulty (Posada et al., 2003) and perceptual load (Howard et al., 2003). However, if task difficulty was higher in the *divided* condition, gamma band power would also have been expected to be highest in this condition and similar in the two *selective* conditions. As this is not the case in the current study, we claim to see modulation of gamma band synchronization that is due to modulation of attention by a limited capacity of attention resources (Bonnel and Hafter, 1998) and not due to task difficulty or perceptual load.

The discrepancy in frequencies, observed between highest relative gamma band power and the statistical difference between these two, might firstly be caused by smearing due to frequency smoothing used in time frequency analysis. Secondly, the strong gamma band power peak in the group average is dominated by power values of some subjects. The statistical effects are most likely due to subjects with higher frequency gamma band power peaks showing consistent modulations between conditions.

Gamma band synchronization in the auditory system

The data reported here were also scanned for effects of auditory stimulation (see Supplementary Figs. 1 and 2 for further information). Auditory evoked responses to auditory stimulation were found (see Supplementary Fig. 2). However, most likely due to stimulus characteristics, we were not able to find any systematic sustained stimulus related gamma band responses in auditory cortex. Certainly, intracranial studies have reported evoked short-lasting (Lakatos et al., 2009) and induced auditory gamma band synchronization (Crone et al., 2001). There have also been MEG studies, showing auditory evoked gamma band activity (Joliot et al., 1994; Pantev et al., 1991; Tiitinen et al., 1993). However, to the best of our knowledge, there are no studies using auditory stimuli inducing long-lasting gamma band synchronization in auditory cortex. One possible explanation might be that, MEG sensors are less sensitive to radial sources at the surface of gyri, e.g. superior temporal gyrus (Crone et al., 2001). Thus one might speculate that the signal to noise ratio of a potentially induced auditory gamma band response in our study might have been too low, especially compared to the visual response, to be seen in the MEG recording. Thus, due to the absence of an adequate auditory stimulus, we confined our analysis to the visual system.

The interpretation of the present findings is limited to some degree by the fact that gamma band activity could not be observed in the auditory system. While a shift of attention between the auditory and visual system can be assumed from behavioral data, explicit corresponding evidence from the neurophysiological data is missing. Hence, the proposed shift of attention between modalities remains speculative. Finding stimuli that permit showing mirror effects of modulated gamma band synchronization in auditory cortex, as shown with steady state stimuli at lower frequencies (Saupe et al., 2009) would be a desirable task for future works. This would permit firm conclusions about the relation between gamma band synchronization and resource allocation between modalities.

Modulation of induced gamma band synchronization in early visual areas

Subjects' strongest induced gamma band sources were located in early visual areas (V1 and V2) in fourteen of sixteen subjects. Modulation of gamma band synchronization was also found in these locations. Relative gamma band power in early visual cortical areas increased with the amount of attention directed to the visual stimulus. While some studies showed pronounced gamma band synchronization in visual areas V1–V3 in humans, when attentively

monitoring a visual stimulus (Hoogenboom et al., 2006, 2010), attention dependent modulations of gamma band synchronization have primarily been recorded in mid- and high level stages in the visual processing hierarchy (Fries et al., 2001; Gregoriou et al., 2009). One study (Chalk et al., 2010) however, found decreased local field potential gamma band power and decreased gamma band spike field coherence with attention in V1 of the macaque monkey. The authors suggest that by a reduction of center surround inhibition, gamma band synchronization decreases with attention, which only holds if an experimental design is used where attention is tightly focused at the center of the classical receptive field. The stimuli used in our study were relatively complex gratings, not restricted to the center of one receptive field, but exiting multiple neurons in visual cortex. The here applicable mechanism behind gamma band synchronization has been described in a recent review, which proposes that gamma band synchronization is driven by rhythmically synchronized inhibition through cortical interneurons (Fries, 2009).

Thus, our results substantiate that graded attentional modulation of gamma band synchronization takes place in early visual areas and support the theory that synchronized inhibition of cortical interneurons can serve as a mechanism for gamma band synchronization.

Biased competition model applied gradually

Previous studies on selective visual attention suggest that the attended of two competing visual stimuli gets a competitive advantage over the other by enhancing its gamma band synchronization (Fries et al., 2001, 2008). This effect has been addressed in the hypothesis of biased competition through enhanced synchronization (Fries, 2005), which bases its assumptions on the biased competition hypothesis (Desimone and Duncan, 1995; Reynolds et al., 1999). From the present results one might speculate that the conceptual framework of the biased competition model can also be applied to gradual attention modulation in the visual system. In this respect, enhanced gamma band synchronization could be seen as an adaptive mechanism enhancing the selective processing of a stimulus in a gradual manner, thereby reflecting the amount of selective attention a stimulus receives.

From the current results, one could furthermore speculate on the application of the biased competition model in an intermodal context, as shown in a modeling study for the visual and tactile domains (Magosso et al., 2010). With the attention related increase in gamma band synchronization in visual areas, one could assume a connection of the competitive advantage of the visual over the auditory stimulus to the amount of gamma band synchronization. However, one important aspect of the biased competition model is that responses to the non-preferred stimulus are suppressed, when the other stimulus 'wins' the competition for processing. The current study cannot directly proof suppressive effects of one of the two stimuli over the other. To substantiate the application of the biased competition model in an intermodal context, future studies are needed. These could employ bimodal stimuli, inducing long-lasting gamma band synchronization in visual and auditory areas at the same time and could thereby address suppressive and enhancing effects in modality specific cortical areas. Furthermore, interactions with and between other modalities such as the somatosensory system will be of great interest.

Conclusions

The current study is the first to show gradual and long lasting changes of gamma band synchronization in early visual areas related to the level of attention given to a visual stimulus. These attention effects, potentially achieved by resource allocation between the visual and auditory modality, may extend the biased competition model of selective attention and highlight the key role of gamma band synchronization in visual attention.

Role of the funding source

This study was supported by Deutsche Forschungsgemeinschaft (SFB 575, project C4). N.K. was supported by Studienstiftung des deutschen Volkes and a travel allowance of Boehringer Ingelheim Foundation (B.I.F.).

Supplementary materials related to this article can be found online at doi:10.1016/j.neuroimage.2011.07.017.

Acknowledgments

We thank Mrs. E. Rädisch and Mrs. A. Solotuchin for technical support with MRI scans. We are thankful to Prof. Joachim Gross (CCNi, Glasgow), Dr. Hanneke van Dijk, and Dr. Nienke Hoogenboom (both University Düsseldorf) for helpful suggestions and discussion on data analysis. For critically revising the manuscript we thank Dr. Joachim Lange (University Düsseldorf).

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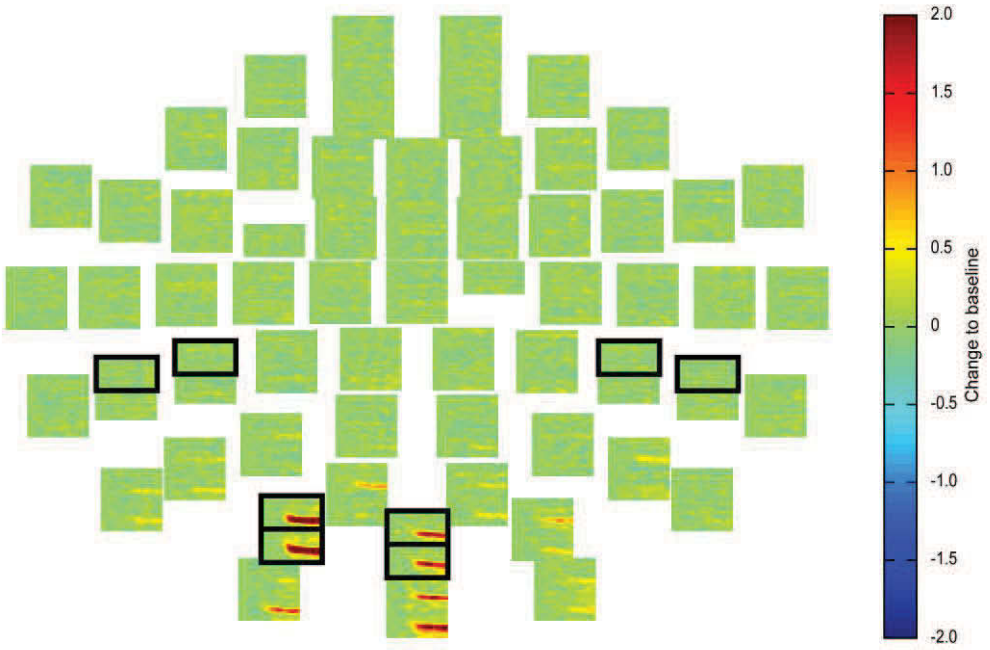
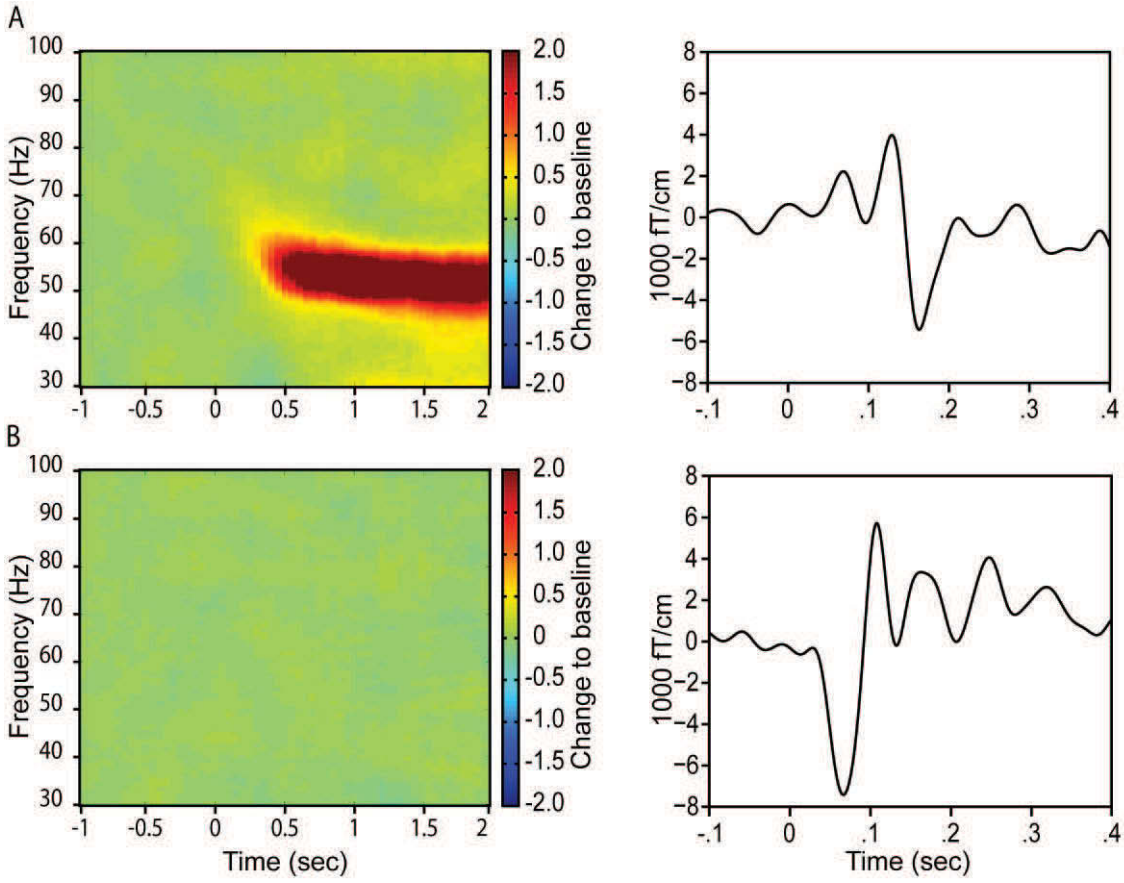
Supplementary fig. 1



Supplementary fig. 1: Sensor Layout of Time Frequency Representations of One Subject

Time frequency representations (30-100 Hz; -1000 to 2000 ms; 0 = start of stimulus; 400 ms time window in 50 ms steps; ±5 Hz frequency resolution; 3 tapers) of all MEG sensors of one representative subject (subject 10) in condition *selective auditory*. Please note that the relative power values (compared to pre-stimulus baseline; -1000 to -500 ms) were limited in this plot (going up to +2, whereas the maximum response over occipital sensors was > +6; color-coding: 0 corresponds to no change and 2.0 to a 200% increase in power relative to baseline), in order to be able to examine responses in auditory areas. A strong gamma band response can be seen over occipital sensors. Over the auditory areas no such response could be observed.

Supplementary fig. 2



Supplementary fig. 2: Occipital and Temporal Areas – High Frequencies and Evoked Responses

A. Left: Power relative to pre-stimulus baseline, averaged over the four occipital sensors (highlighted with a black box in supplementary fig. 1) of one representative subject (subject 10).
   Right: Visual evoked field of the same subject, averaged over the same sensors as in A. To this end, sensor data were filtered with a band-pass filter from 0.03 to 30 Hz. A baseline of 100 ms prior to stimulus onset was subtracted.
B. The same data are displayed as in A, but averaged over the four bilateral temporal sensors as highlighted in supplementary fig. 1. These sensors were selected as they also showed the auditory evoked response in this subject. Please note, that data of all participants were scanned on sensor level in order to identify long-lasting gamma band responses of the auditory cortex in reaction to auditory stimulation. No such response could be found in any subject recorded.

Frequency of visual attention-related gamma band activity decreases with severity of hepatic encephalopathy

***Author's order*:** Nina Kahlbrock[a], Markus Butz[1,a,b], Elisabeth S. May[a], Meike Brenner[a], Gerald Kircheis[c], Dieter Häussinger[c], Alfons Schnitzler[a,d]

***Authors' affiliations*:**

[a] Heinrich-Heine-University Düsseldorf, Medical Faculty, Institute of Clinical Neuroscience and Medical Psychology, Universitätsstrasse 1, D-40225 Düsseldorf, Germany

[b] University College London, Institute of Neurology, 33 Queen Square, London, WC1N 3BG, United Kingdom

[c] Heinrich-Heine-University Düsseldorf, Medical Faculty, Department of Gastroenterology, Hepatology and Infectiology, Universitätsstrasse 1, D-40225 Düsseldorf, Germany

[d] Heinrich-Heine-University Düsseldorf, Medical Faculty, Department of Neurology, Universitätsstrasse 1, D-40225 Düsseldorf, Germany

***Work was performed at*:**

Heinrich-Heine-University Düsseldorf, Medical Faculty, Institute of Clinical Neuroscience and Medical Psychology, Universitätsstrasse 1, D-40225 Düsseldorf, Germany

1 *Corresponding Author*: Markus Butz, University College London, Institute of Neurology, 33 Queen Square, London, WC1N 3BG, United Kingdom, email: m.butz@ion.ucl.ac.uk, phone: +44 203 108 0055

Short Title: Gamma Frequency Decreases with HE Severity

***Keywords*:** MEG, oscillations, slowing, CFF, liver, GABA
***Article Type*:** Research Article

Abstract

Visual attention is associated with pronounced occipital gamma band activity. Hepatic encephalopathy (HE), a frequent neuropsychiatric complication in liver disease, involves substantial attentional deficits. For motor symptoms in HE, previous works demonstrated pathologically slowed cortico-muscular coupling. This slowing correlated with a slowing in processing of visual stimuli, i.e. the critical flicker frequency (CFF), known to reliably quantify HE disease severity.

In this study, 8 healthy controls and 26 patients with liver cirrhosis completed a behavioral task requiring shifts of attention between simultaneously presented visual and auditory stimuli. Brain activity was recorded using magnetoencephalography (MEG). It was tested whether a slowed frequency of attention-related gamma band activity can be observed and how it relates to HE disease severity. Furthermore, attentional modulation of gamma band power was scrutinized.

Results showed a relation between behavioral data and HE severity. Individual visual gamma band frequencies correlated positively with the CFF (r = 0.41). When probing participants with normal and pathological CFF values separately, only participants with normal CFF values showed a modulation of gamma power between attention conditions.

The present results suggest a tight relation between visual attention-related gamma band activity and attentional deficits in HE. Only patients with normal CFF values were able to shift their attention between the visual and auditory domains. The attention-related gamma band peak frequency decreased with disease severity. Thus, further evidence is added to the notion of pathologically slowed oscillatory activity as a key mechanism in the pathophysiology of HE, thereby extending earlier results to the cognitive domain.

1. Introduction

Attention has been associated with cortical gamma band (30-100 Hz) synchronization (Fries et al., 2001; Hoogenboom et al., 2010, 2006; Kaiser et al., 2006; Lachaux et al., 2005; Steinmetz et al., 2000). Previous studies reported modulation of visually induced gamma band oscillations by attention (Gruber et al., 1999; Siegel et al., 2008; Tallon-Baudry et al., 2005; Vidal et al., 2006; Wyart and Tallon-Baudry, 2008).

Work in healthy adults has shown that when stimuli are presented in two different modalities, stimuli in the attended modality receive amplified processing compared to stimuli in the non-attended modality (Gherri and Eimer, 2011; Spence and Driver, 1997). Assuming that stimulus processing is capacity-limited (Marois and Ivanoff, 2005; Posner and Boies, 1971), allocating resources to one attended modality subtracts resources from the available supply of the other modality (Bonnel and Hafter, 1998). Hence, by reallocating available resources between two competing modality specific stimuli, modulation of attentive processing can be studied. Recent work has shown that the gradual modulation of visual attention in a bimodal paradigm is associated with a gradual modulation of visual gamma band activity (Kahlbrock et al., under revision).

Hepatic encephalopathy (HE) is a frequent and potentially reversible neuropsychiatric complication in chronic liver disease. Clinically, it comprises neuropsychiatric symptoms such as motor and cognitive deficits, altered sleep patterns, and changes in vigilance state, ranging from somnolence up to a hepatic coma (for a review see Häussinger and Blei (2007)). Depending on the severity of the symptoms, different grades of HE can be distinguished (HE0, mHE, HE1-HE4; Ferenci et al. (2002); Kircheis et al. (2002)). Interestingly, one key symptom of HE is a gradual increase of attentional deficits with increasing disease severity (Amodio et al., 2005; Kircheis et al., 2009; Pantiga et al., 2003; Weissenborn et al., 2001). For example, Mattarozzi et al. (2005) found performance differences between healthy subjects, patients with minimal (mHE) and overt HE of grade 1 (HE1) in tests of attention and short-term memory. Weissenborn et al. (2005) argue that all three functional attention systems (vigilance and alertness, orienting, and executive functions) of the hierarchy proposed by Posner and Petersen

(1990) are already impaired in mHE. Attention deficits have been observed even in patients who seem clinically unaffected (Weissenborn et al., 2005).

EEG studies have shown that in HE, spontaneous oscillatory activity is progressively slowed, becoming more evident, the more affected the patients were (Davies et al., 1991; Parson-Smith et al., 1957). Kullmann and colleagues reported a significant slower mean peak frequency for all patients with liver cirrhosis under study even if no signs of HE were present (Kullmann et al., 2001).

Motor symptoms are another common aspect of HE. In line with slowed spontaneous oscillatory activity in progressing HE, previous works demonstrated a pathologically slowed thalamo-cortico-muscular coupling with increasing grades of HE. These were associated with the tremor-like motor symptom called mini-asterixis (Timmermann et al., 2003, 2002). Interestingly, this slowing correlated with a slowed processing of visual stimuli, i.e. the so-called critical flicker frequency (CFF; Timmermann et al. (2008)).

The CFF is the frequency threshold at which a flickering light is perceived as flickering and no longer as steady (normally ≥ 39 Hz for healthy people). The CFF has been shown to reliably quantify and monitor the severity of HE, i.e. the lower the CFF the higher the severity of HE (Kircheis et al., 2002; Prakash and Mullen, 2010; Romero-Gómez et al., 2007; Sharma et al., 2007). However, the physiological mechanisms of this clinical measure are still unclear. Human cortical visual areas are able to process flickering stimuli at frequencies higher than the maximum subjectively perceived flicker frequency (Herrmann, 2001). Brain responses to flickering stimuli show oscillatory patterns at twice the frequency of the presented stimulus (Fawcett et al., 2004). Thus, it is likely that the observed impairment in perception of an oscillatory visual (flicker-) stimulus, i.e. decreased CFF in patients with HE, is due to a dysfunction in the cerebral processing of oscillatory visual stimuli.

Taking these findings into consideration, Timmermann et al. (2005) hypothesized that a slowing of oscillatory activity in various human cerebral sub-systems represents a key mechanism in the pathophysiology of HE. For the motor system, this claim has been supported by neurophysiological data. Data for the visual system have so far only been reported as a correlation of slowing motor responses with the CFF. Further results in favor of this hypothesis would strengthen the assumption of slowed oscillatory activity

as a fundamental mechanism explaining the broad variety of clinical symptoms observed in HE.

The present study aimed to test the relation of HE severity and the frequency of attention-related visual gamma band activity and the capacity to modulate attention and concurrently modulate the strength of attention-related occipital gamma band activity. Subjects completed a behavioral task requiring shifts of attention between simultaneously presented visual and auditory stimuli. Attention was modulated by resource allocation between the two modalities, while brain activity was recorded using magnetoencephalography (MEG).

Adhering to the hypothesis of slowed oscillatory brain activity as a key phenomenon in the pathophysiology of HE, a reduction in attention related gamma band peak frequencies was hypothesized. Due to the known attentional deficits in HE, a reduced capacity to modulate gamma band activity with attention was assumed.

2. Methods

2.1 Participants

26 patients with liver cirrhosis, confirmed by sonography or fibroscan (> 13 kPa) and 8 healthy, age-matched controls underwent a comprehensive clinical assessment including blood tests, neuropsychometric computer tests (Vienna test system, WINWTS, Version 4.50, 1999), and CFF measurements (Eberhardt, 1994). As described in Kircheis et al. (2002) and according to *West-Haven* Criteria (Conn and Lieberthal, 1979) and psychometric test results, patients were classified into three groups: (i) HE0, i.e. patients showing no signs of HE, (ii) minimal HE (mHE), i.e., patients showing no clinical signs of HE, but pathological results in two psychometric tests, and (iii) HE1, i.e. manifest HE of grade 1, patients showing clinical signs of HE (see Table 1 for further details about patients' characteristics). The fourth group constituted the healthy control participants, i.e., (iv) controls. 24 patients performed the tests for grading of HE stage within two days, two patients within a week before or after the MEG measurement. All subjects had normal or corrected to normal vision and normal hearing. All of them gave their written informed consent. The study was approved by the local ethics committee (study no. 2895) and was performed in accordance with the Declaration of Helsinki. General exclusion criteria were neurological/psychiatric illness, intake of psychoactive drugs, the existence of an HIV infection, Wilson's disease, Korsakoff's syndrome, and chronic pain syndrome. Patients with liver cirrhosis stemming from alcohol abuse had to be abstinent from alcohol for at least 6 months. To further control for this, blood ethanol levels and carbohydrate deficient transferrine (CDT) were measured.

2.2 Data Acquisition

2.2.1 Determination of the Critical Flicker Frequency (CFF)

CFF thresholds and standard deviations were assessed using the Schuhfried Test System (Dr. Schuhfried Inc., Mödling, Austria). Details of the recording procedure can be found in Kircheis et al. (2002). Only mean CFF values with a standard deviation below 0.5 Hz were considered for further analyses. One control subject did not meet

this criterion and this CFF value was not used in further analyses. In cirrhotic patients, the CFF was measured on both days of recordings. The CFF value of the day of MEG measurement was used for further analyses.

2.2.2 Bimodal Reaction Time Paradigm

A paradigm adapted from Kahlbrock et al. (under revision) was used, as illustrated in Fig. 1. Subjects worked on three experimental conditions presented in separate blocks. A block design was chosen to make the task accomplishable for all HE patients. Each block was subdivided into smaller blocks of twelve trials separated by self-paced breaks to avoid fatigue.

Before each block, subjects were instructed and trained in the specific task of one of three experimental conditions: (i) *visual*, (ii) *auditory*, or (iii) *divided*. Irrespective of the condition, each trial always started with a 2000 ms fixation period. Then, a visual stimulus (an inwardly contracting grating) and an auditory stimulus (a constant tone) appeared simultaneously. After a randomly assigned period of 500, 1000, 1500, or 2000 ms, either the visual or the auditory stimulus changed its quality (change 1). 750 or 1000 ms later, the other stimulus also changed (change 2). In half of the trials, the visual stimulus changed first followed by a change in the auditory stimulus and vice versa. The order of the trials was randomized. A change of the visual stimulus was implemented as an increase in speed of the stimulus that either continued to move inwards or changed its direction and then moved outwards (inward / outward). A change in the auditory stimulus was implemented as a change in pitch to a higher or lower pitch (high / low). Please see section 2.2.3 for a detailed description of the properties and delivery of the stimuli.

Depending on the experimental condition, the change of one of the stimuli became the target. Subjects were required to give a speeded response to the change in the stimulus' quality, i.e. a change in speed of the visual or a change of pitch of the auditory stimulus. In the *visual* condition, subjects had to exclusively react to the change in the visual stimulus (target), irrespective of its position in the trial (change 1 or 2) and ignore the change in the auditory stimulus (non-target). In the *auditory* condition, accordingly, subjects had to react to the change in the auditory stimulus (target) only and ignore the

change in the visual stimulus (non-target). In the *divided* condition, subjects had to respond to the stimulus that changed first (change 1 = target) and ignore the change in the other stimulus (change 2 = non-target).

In detail, the subject's task was to react to the target and indicate the quality of this stimulus change by pressing one of two buttons operated with the index fingers of both hands. Thereby, each hand was assigned to one quality change i.e. in the *visual* condition, the index finger press of one hand indicated inward and the index finger press of the other hand outward movement of the visual stimulus. In the *auditory* condition, the index finger press of one hand indicated a high and the index finger press of the other hand a low tone. In the *divided* condition, the index finger press of one hand indicated a change in the visual and the index finger press of the other hand a change in the auditory stimulus. Feedback was given after each trial. If subjects did not respond within 2000 ms after target appearance, the trial was counted as missed. The assignment of the left or right hand to the target qualities was balanced between subjects.

The paradigm consisted of 540 trials (180 trials of each condition) including 72 catch trials. Catch trials consisted of trials without target appearance and required no response.

As shown in Kahlbrock et al. (under revision), three levels of visual attention were sought to be obtained by these conditions; high in condition *visual*, medium in condition *divided*, and low in condition *auditory*.

2.2.3 Stimuli and Stimulus Delivery

The fixation point consisted of a Gaussian (0.56° in diameter), which increased its contrast by 40% after 1000 ms, thereby informing the subject that the stimulation was about to start. The visual stimulus was adapted from Hoogenboom et al. (2006). It consisted of a foveal circular sine wave grating (diameter: 7.13°, spatial frequency: 2 cycles/deg, contrast: 100%) continuously contracting towards the center of the screen (velocity: 2.16 °/s). The change in visual stimulus was characterized by an increase in velocity (4.32 °/sec). The sine wave grating was then either still contracting towards the

center of the screen (quality change inward) or changed its direction and expanded outwards (quality change outward).
The auditory stimulus was a binaurally presented 250 Hz sine tone. The change in auditory stimulus consisted of a change in pitch of the tone to either 200 Hz (quality change low) or 300 Hz (quality change high). The auditory stimulus intensity was adjusted to subjectively match the visual stimulus intensity. Thus, auditory stimuli were well audible for all subjects, but at individual volumes.
Stimulus timing was controlled using Presentation® software (version 13.0, www.neurobs.com). Visual stimuli were projected onto a screen with a dlp projector (Panasonic, Osaka, Japan) with 60 Hz refresh rate. Participants were seated approximately 96 cm away from the screen. Auditory stimuli were produced using Audacity® (http://audacity.sourceforge.net/). The analogue tonal signal was generated in STIM Audio System (Neuroscan, Abbotsford, Victoria, Australia) and sent into the shielded room. Calibrated earphone transducers (TIP-300 Tubal Insert Phone*,* Nicolet Biomedical*,* Inc., Fitchburg, Wisconsin, USA) then converted the electrical to a sonic signal. The earphone transducers had two equal lengths plastic tubes and earplugs attached which were inserted into participants' ears.

2.2.4 Neurophysiological Data

Brain activity was recorded using magnetoencephalography (MEG; 306-channel, ELEKTA Oy, Helsinki, Finland). Vertical and horizontal electrooculograms were recorded to later indentify epochs with blink artifacts and eye movements. Individual high-resolution standard T1-weigthed structural magnetic resonance images (MRIs) were obtained from a 3T Siemens Magnetom MRI scanner (Munich/Erlangen, Germany).

2.3 Data Analysis

2.3.1 Behavioral Data

Behavioral data were analyzed by means of error rates and reaction times. To confirm behavioral effects as described previously (Kahlbrock et al., under revision) trials were divided according to the different conditions (*visual*, *auditory*, and *divided*). The *divided*

condition was further subdivided into those trials where the visual or the auditory stimulus changed first and therefore became the target (*divided visual* and *divided auditory* respectively). Mean reaction times were calculated for each participant and condition. Only trials with a correct response and reaction times ranging between ±2 standard deviations from the individual mean reaction time of each condition were counted as correct and subjected to further analysis.

Reaction times, percent correct responses, and CFF values were analyzed in two ways. Firstly, the non-parametrical Friedman test was employed to determine differences in distributions of reaction times and correctness of responses between selective and divided conditions. The post-hoc one-sided Wilcoxon signed-rank test was used to test for differences between the single conditions. Secondly, the Kruskal–Wallis one-way analysis of variance was employed to test for differences in distributions of reaction times, correctness of responses and CFF values between groups (Controls, HE0, mHE, HE1). The post-hoc Mann–Whitney U test was used to further test for group differences. To confirm expected behavioral effects (Kahlbrock et al., under revision), reaction times and correctness of responses of all three behavioral conditions were analyzed in these first steps. As the highest contrast of visual attention was expected between the *visual* and the *auditory* conditions, only these two conditions were considered in further analyses.

Partial one-sided correlations, correcting for effects of age, were calculated for reaction times, percent correctness of responses, group membership, and CFF. Bonferroni-Holm correction (Holm, 1979) was applied to all alpha levels to correct for multiple comparisons. All statistical analyses of behavioral data were performed using the statistics software package IBM SPSS Statistics 19 for Windows (IBM Corporation, Somers, USA).

2.3.2 MEG Data General

MEG data were analyzed using FieldTrip, an open source Matlab toolbox (Oostenveld et al., 2011), and Matlab 7.1 (MathWorks, Natick, MA). For artifact rejection, continuously recorded MEG data were divided into epochs of interest, starting at time of first fixation point and ending with time of response or time out in case of a catch trial.

Semi-automatic routines and visual artifact rejection were applied to remove epochs contaminated with eye, muscle, and sensor artifacts. Partial and complete artifact rejection procedures were applied, rejecting either only parts of the trial contaminated by artifacts or the whole trial in case of multiple artifacts. Where applicable, independent component analysis was used to correct for eye artifacts (2 control subjects, 3 HE0 patients, 3 mHE patients, 1 HE1 patient). Power line noise was removed by estimating and subtracting the 50- and 100-Hz components in the MEG data, using a discrete Fourier transform. The linear trend was removed from each epoch. Further analyses were performed on the time window from time of first fixation point until the first stimulus change.

2.3.3 Stimulus Induced Gamma Band Activity

Oscillatory neuronal activity was estimated by determining time frequency representations of power (TFRs) for frequencies between 30 and 100 Hz using windows of 400 ms moved in steps of 50 ms. Multitaper spectral estimation was used with ±5 Hz smoothing (3 tapers) in steps of 0.5 Hz. For each subject, TFRs were calculated for the *visual* and the *auditory* conditions. As previous works showed gamma band activity in response to visual stimuli over occipital areas (Hoogenboom et al., 2006; Kahlbrock et al., under revision), data were averaged over all occipital and the six most caudal parietal sensor pairs (32 sensor pairs in total; Fig. 4A). Group averages were calculated for all four groups under study, i.e., controls, HE0, mHE, and HE1.

2.3.4 Statistical Comparison of Power between Conditions

To determine differences in visual gamma band power between attentional condition, data were first averaged over the above described sensors (Fig. 4A) for each subject. The same number of trials (depending on the condition with the smallest number of trials) was randomly drawn from the available trials for the two conditions. On a single trial basis and using independent samples t-tests, t-values were calculated for the difference in absolute power between these two conditions from -1500 to 1500 ms (0 being the start of stimulus presentation) and 30 to 100 Hz and resulted in time frequency t-maps. Subsequent group level statistics determined time frequency clusters with significant effects at random effects level. To obtain these time frequency clusters,

time frequency t-maps were thresholded at the t-value corresponding to a one-sided t-test with an alpha level of 0.05. The summed t-values per cluster were used as test statistic. Statistical inference was based on a non-parametric randomization test, correcting for multiple comparisons due to a multitude of time- and frequency-points (Maris and Oostenveld, 2007; Nichols and Holmes, 2002). This second step was done for each subject and time frequency points from 500 to 1500 ms (thereby excluding purely stimulus evoked components in the first 500 ms) and 30 to 100 Hz. This analysis was once performed including all participants and repeated separately for each group under study (Controls, HE0, mHE, HE1).

2.3.5 Individual Gamma Band Peaks and Power

For frequencies of 30 to 100 Hz, power spectra were calculated for each participant, condition, and all sensors described in section 2.3.3 (Fig. 4A; ±1 Hz smoothing, hanning window). To exclude purely stimulus evoked components and to include the strongest gamma band peak and the maximally possible amount of trials, periods from 500 to 1000 ms were used. Relative changes compared to the pre-stimulus baseline (-1000 to -500 ms) were calculated. For each sensor, the maximal relative gamma band peak frequency and its power were determined. In order to increase the signal to noise ratio, the ten sensors with highest gamma band power lying in close proximity to each other were identified and averaged for each subject. Their average gamma band peak frequency was determined. The power increase relative to baseline was tested for significance using one-sided independent samples t-tests for each frequency point comparing stimulus with pre-stimulus baseline times. Statistical inference was based on a non-parametric randomization test, correcting for multiple comparisons (Maris and Oostenveld, 2007; Nichols and Holmes, 2002). Only if the previously identified peak gamma band frequency was significantly different from baseline, it was used for further analysis. This procedure was performed separately for the two conditions.

2.3.6 Statistics Gamma Band Peaks

The non-parametrical one-sided Wilcoxon test was employed to determine differences in distributions of gamma band peak frequencies between the *visual* and the *auditory* conditions. This analysis was done including all participants and repeated for each

individual group of participants (Controls, HE0, mHE, HE1). In addition, Kruskal–Wallis one-way analyses of variance were employed to test for differences in distributions of gamma band peak frequencies between groups. Partial one-sided correlation, correcting for age effects, was calculated for gamma band peak frequencies and CFF using IBM SPSS Statistics 19 for Windows (IBM Corporation, Somers, USA). Bonferroni-Holm correction (Holm, 1979) was applied to the alpha level to correct for multiple comparisons.

2.3.7 Sources Gamma Band Peaks

Sources of significant gamma band peaks were localized using *Dynamic Imaging of Coherent Sources* (DICS, Gross et al. (2001)), an adaptive spatial filtering technique in the frequency domain. To this end, each structural MRI was spatially normalized to a smoothed template MRI based on multiple subjects (Statistical Parametric Mapping; SPM2; http://www.fil.ion.ucl.ac.uk/spm/). Leadfield matrices were determined for realistically shaped single-shell volume conduction models (Nolte, 2003) derived from the individual normalized structural MRIs. The grid of locations was constructed as a regular 5 mm grid which was then adjusted to the individuals' head shapes. To obtain the best possible estimate the location of each subject's strongest gamma band response, crossspectral density matrices between all MEG sensor pairs at individual gamma band peaks ±5 Hz were determined for time frequency windows from -1000 to -500 ms (baseline), and 500 to 1000 ms (stimulus period) averaged over all trials of the *visual* and the *auditory* conditions. These common spatial filters were then used to calculate crossspectral density matrices for the two conditions separately. Relative changes between baseline and stimulus periods were calculated.

2.3.8 Groups of Participants Sorted by CFF

Due to an ongoing discussion of the validity of the classic definition of HE groups as HE0, mHE, and HE1-HE4 (Häussinger et al., 2006; Kircheis et al., 2007), all analyses were additionally repeated on an alternative group classification scheme. According to Kircheis et al. (2002), the CFF value of 39 Hz is described as being the critical cut-off frequency separating patients with manifest HE from controls and HE0 patients. Therefore, all participants showing significant gamma band activity in response to the

visual stimulus were sorted by CFF into one group of participants with low, i.e. pathological CFF (< 39 Hz, n = 12) and one group of subjects with high, i.e. normal CFF (≥ 39 Hz, n = 14). In a second step, the analyses were repeated, including only patients (low CFF: n = 10 vs. high CFF: n = 10).

Henceforth, the HE groups as classically defined will be referred to as HE groups and groups defined by their CFF values will be referred to low and high CFF groups respectively.

3. Results

3.1 Subjects

No significant age differences were observed between the three HE groups and controls and between the low and high CFF groups.

Mean CFF values and standard errors of the mean for the three HE groups and controls were: 40.6 ±0.8 Hz for control subjects, 40.5 ±0.6 Hz for HE0, 38.4 ±0.5 Hz for mHE, and 36.1 ±0.7 Hz for HE1 patients (also see Table 1 for details and subject dependent data). A Kruskal Wallis test revealed a significant effect of HE group on CFF values ($\chi^2_{(3)} = 18.28$, $p < 0.01$). Post-hoc tests disclosed that CFF values were not different between control subjects and HE0 patients. Trends were observed for a higher CFF in control subjects and HE0 patients than in mHE patients (controls vs. mHE: $U = 12.0$, $p = 0.07$; HE0 vs. mHE: $U = 12.5$, $p = 0.06$). Control subjects, HE0 and mHE patients had a higher CFF than HE1 patients (Controls vs. HE1: $U = 4.0$, $p < 0.01$; HE0 vs. HE1: $U = 1.0$, $p < 0.01$; mHE vs. HE1: $U = 12.5$, $p = 0.02$).

3.2 Behavioral Data Reveal Shifting of Attention and are Similar between HE Groups and Controls

To confirm previously reported effects (Kahlbrock et al., under revision), reaction times and correctness of responses were examined for effects of condition (selective vs. divided) averaged over all subjects and for effects of group (Control, HE0, mHE, HE1). Median reaction times and standard errors of the median were 714 ms ±52 for the *visual*, 598 ms ±52 for the *auditory*, 691 ms ±50 for the *divided visual*, and 602 ms ±52 for the *divided auditory* condition. Differences were observed in the distributions of the four conditions ($\chi^2_{(3)} = 26.10$, $p < 0.01$). However, post-hoc tests showed no differences between the selective and the divided conditions.

Median percent correct and standard errors of the median were 91.0% ±0.7 for the *visual*, 94.2% ±0.9 for the *auditory*, 88.5% ±1.9 for the *divided visual*, and 90.7% ±1.4 for the *divided auditory* conditions. Differences in the distributions of the four conditions were observed ($\chi^2_{(3)} = 31.36$, $p < 0.01$). Post-hoc analyses showed that the median differences between the *visual* and the *divided visual* conditions ($Z = -3.45$, $p < 0.01$)

and the median difference between the *auditory* and the *divided auditory* conditions (Z = -3.35, $p < 0.01$) were different from zero. Thus, participants reacted more correctly in the selective compared to the divided conditions. None of different variables' distributions were different between HE groups and controls.

3.3 CFF Correlates with Behavioral Performance

Behavioral parameters were correlated with the CFF, quantifying the severity of HE. For the *auditory* condition, a negative correlation of CFF and reaction times was detected (r = -0.53, $p < 0.01$; Fig. 2A). This shows that the higher the CFF, i.e., the less impaired the patients, the lower (faster) the reaction times in the *auditory* condition. No correlation of correctness of responses in the *auditory* condition and CFF was found. In the *visual* condition, the CFF correlated positively with the number of correct responses (r = 0.40, p = 0.02; Fig. 2B). This indicates that the higher the CFF, the more correct responses were given in the *visual* condition. No correlation of reaction times in the *visual* condition and CFF was found.

3.4 Low and High CFF Groups Show Differential Behavioral Performance

The comparison of low and high CFF groups showed an overall better performance of the high CFF group (reaction times: *visual*: U = 35, p = 0.02; *auditory*: U = 32, p = 0.01; *divided visual*: U = 41, p = 0.05; divided auditory: n.s.; correctness of responses: *visual*: U = 44, p = 0.04; *auditory*: n.s.; *divided visual*: U = 33, p = 0.03; *divided auditory*: U = 35, p = 0.03; see Supplementary Table for median values and standard deviations). A similar pattern, however not always reaching significance when corrected for multiple comparisons, was observed when comparing only patients with high and low CFF values (reaction times: *visual*: U = 18, p = 0.03; *auditory*: U = 22, p = 0.054; *divided visual*: U = 24, p = 0.09; divided auditory: n.s.; correctness of responses: *visual*: n.s.; *auditory*: n.s.; *divided visual*: n.s.; *divided auditory*: U = 19, p = 0.06; see Supplementary Table for median values and standard deviations).

3.5 Only the High CFF Group is Able to Shift Gamma Band Power between Conditions

In all groups, sustained gamma band activity in response to the visual stimulus was observed at sensors overlaying visual cortex (Fig. 3A). The strongest gamma band peaks were localized in visual areas for all groups under study (Fig. 3B, HE groups and controls). Averaged over these individual gamma band peak frequencies and the time of strongest gamma responses (500 to 1500 ms), no differences in relative gamma band power were observed between any of the compared groups.

Comparing sensor level gamma band power on a time frequency level between the *visual* and the *auditory* conditions for each group separately revealed a cluster from 900 to 1500 ms and 44 to 55 Hz approaching significance ($p = 0.053$) only for HE0 patients. This cluster indicates that gamma band power was stronger in the *visual* than in the *auditory* condition only for this patient group. However, for the high CFF group including all participants, gamma band power was also stronger in the *visual* compared to the *auditory* condition. The cluster spanned a time and frequency area from 680 to 1500 ms and 43 to 57 Hz ($p = 0.037$; Fig. 4B). A similar cluster was also found for the high CFF group of only patients between the attention conditions from 680 to 1500 ms and 43 to 57 Hz ($p = 0.024$). For all other groups, no significant differences were observed.

Tallying previous reports (Kahlbrock et al., under revision), it was not possible to find any systematic sustained stimulus related gamma band responses in auditory cortex. This is most likely due to stimulus characteristics as discussed elsewhere (Kahlbrock et al., under revision). The analyses were thus confined to the visual system.

3.6 Gamma Band Peak Frequency over Occipital Areas Correlates with CFF

Individual gamma band peak frequencies correlated with the CFF in the *visual* condition ($r = 0.41$, $p = 0.04$; Fig. 5). This shows that the higher the CFF, the higher the gamma band peak frequency, denoting that the less impaired the subjects are, the higher their gamma band peak frequency. In the *auditory* condition, no correlation of gamma band peak frequency with CFF was observed ($p = 0.29$).

3.7 Gamma Band Peak Frequency over Occipital Areas Differs between low and high CFF Groups

Individual gamma band peak frequencies were neither different between the *auditory* and the *visual* conditions, nor between three HE groups and controls. However, the separation of all participants into low and high CFF groups revealed significant differences of gamma band peak frequencies for the *visual* ($U = 37.5$, $p = 0.01$; Fig. 6) and the *auditory* conditions *(*$U = 27$, $p = 0.01$). When looking at patients only, a similar trend between low and high CFF groups was observed (*visual*: $U = 26.0$, $p = 0.07$; *auditory*: $U = 18.0$, $p < 0.05$).

4. Discussion

Using a bimodal reaction time paradigm requiring shifts of attention, this study presents first time evidence of visually induced gamma band activity in patients with HE in the visual cortex. The frequency of this visual gamma band activity correlates with the severity of HE as quantified by the CFF. Thus, the current findings suggest that attentional deficits in HE are associated with a slowing in gamma band activity. Furthermore, the current work shows that gamma band power is modulated by the level of visual attention in cirrhotic patients with normal CFF values only, while patients with pathological CFF values are lacking this ability. Behavioral performance was also shown to be related to HE disease severity, as quantified by CFF.

4.1 Behavioral Data

Behavioral data, in particular the high percentages of correct responses, show that participants were able to perform all tasks and thereby shift their attention between the visual and the auditory domain. Expected reaction time differences distinguishing the divided from the selective conditions in the auditory and visual modality, respectively and thereby quantifying the level of attention (Kahlbrock et al., under revision) could not be reproduced. This is most likely due to the slightly different paradigm, which was simplified to a block design for the present study to make it accomplishable for patients with attentional deficits. Furthermore, the age of the current group under study could explain these differences. It has been shown that young adults aim to balance speed and accuracy when working on speeded response task. Older adults, on the contrary, aim to minimize errors, irrespective of the time their responses take (Starns and Ratcliff, 2010). Thus, due to a different strategy in the present study, reaction times might not have been as informative as correctness of responses.

No differences in behavioral performance were observed between the HE groups. However, a worsening of behavioral performance was associated with a worsening of HE as revealed by correlation analyses with the CFF. Additionally, dividing the data into a high and a low CFF group also revealed that less impaired patients show better behavioural performance. This gives further evidence for the notion of a decline of attentional function with increased HE severity.

The fact that a deterioration of patients' disease states was associated with a corresponding impairment in task performance is in line with previous studies showing cognitive impairment, mainly due to attention deficits and deficits in visual perception, in patients with HE (Weissenborn et al., 2001, 2003; Pantiga et al., 2003). Missing differences between HE groups strengthen the view that the commonly used and traditional grades of HE are less informative of disease severity than the CFF (Häussinger et al., 2006; Kircheis et al., 2007). These results further substantiate advantages of the CFF as being finely scaled and objectively recorded compared to the rather vague and subjectively influenced grading into HE groups.

4.2 Gamma Band Brain Activity

This study is the first to show attention related visual gamma band activity in a cohort of HE patients. This finding is by itself of special relevance as one might assume that due to the decreased cognitive abilities (Weissenborn et al., 2001, 2003; Pantiga et al., 2003) and the observed slowing of brain responses in motor areas (Timmermann et al., 2008, 2003, 2002), HE patients, in particular the more impaired ones, would not show substantial cognition related gamma band brain activity at all.

4.3 Impaired Modulation of Attention Related Gamma Band Activity in HE

Gamma band power was modulated by the level of visual attention in participants with normal CFF values. This corresponds with previous works in healthy subjects, reporting attentional modulation of induced visual gamma band responses (Gruber et al., 1999; Siegel et al., 2008; Tallon-Baudry et al., 2005; Vidal et al., 2006; Wyart and Tallon-Baudry, 2008). Interestingly, patients with low CFF values were lacking this ability. As suggested by Amodio et al. (2005), more impaired patients with HE might be more easily distracted by irrelevant inputs, i.e. the non-target modalities' inputs in this study. This might be the reason why patients with low CFF values were not able to efficiently shift their attention between the visual and the auditory domains, as reflected by decreased behavioral performance and less gamma band modulation.

4.4 Slowed Oscillatory Activity as a Key Mechanism of HE Pathology

The present work shows that the frequency of visually induced gamma band activity correlates with the severity of HE. This was revealed by a correlation of CFF values and individual gamma band frequencies in the *visual* condition and by differences in gamma band frequencies between low and high CFF groups. A reduced frequency of the attention related gamma band activity in more severely impaired patients tallies with early results showing a reduced mean dominant frequency of spontaneous oscillatory brain activity in HE patients (Kullmann et al., 2001; Van der Rijt et al., 1984). Moreover, these data extend earlier results of slowed oscillatory processes in the motor and visual system (Timmermann et al., 2008, 2003, 2002) to the cognitive domain. Thus, the present findings add further evidence to the hypothesis that slowed oscillatory activity is a key mechanism in the pathophysiology of HE (Timmermann et al., 2005) explaining the very diverse deficits experienced by these patients.

The correlation of the gamma band peak frequency with the CFF in the *visual* but not in the *auditory* condition is most likely due to the smaller number of participants showing a significant gamma band peak in the *auditory* condition. This is also supported by the fact that gamma band peak frequencies were not different between the two conditions. Based on these data, it can be speculated that healthy and pathological attentional performance are encoded by differential mechanisms. Healthy performance seems to be encoded by the power, i.e. the strength, of gamma band oscillations, as shown in multiple studies (Gruber et al., 1999; Kahlbrock et al., under revision; Siegel et al., 2008; Tallon-Baudry et al., 2005; Vidal et al., 2006; Wyart and Tallon-Baudry, 2008). Pathological attentional performance (in the form of HE in this study), however, seems to depend on the frequency of gamma band oscillations, as evidenced by the relation of gamma band frequency and HE disease severity. A task for future research is to confirm this notion in other patient populations with attention deficits.

4.5 The Potential Role of GABA in HE

The ‘GABA Hypothesis of HE’ (for a review see Jones et al. (1984)) argues that increased inhibitory GABA mediated neurotransmission contributes to HE (Bassett et al., 1990). However, many studies argued against this hypothesis, for example by

showing that brain and cerebrospinal fluid GABA content and synthesis were unaltered in HE patients (Lavoie et al., 1987; Moroni et al., 1987; Zwingmann et al., 2003). An explanation was given by Ahboucha et al. (2004), suggesting a possible role for neurosteroids with GABA-A receptor modulatory properties. From their point of view, increased concentrations of neurosteroids in brains of cirrhotic patients would positively modulate the GABA-A receptor complex as a result promoting GABA-A receptor neurotransmission. Interestingly, the frequency of gamma band activity has been shown to positively correlate with resting GABA concentrations measured with magnetic resonance spectroscopy in visual (Muthukumaraswamy et al., 2009) and motor cortices (Gaetz et al., 2011). The present data show decreased gamma band frequency with increasing disease severity. Thus, they can be seen as indirect support of the hypothesis of unchanged or even decreased GABA concentration with increasing grades of HE. The exact effects of GABA concentration and neurosteroid mediated GABA-A receptor modulations on gamma band frequency in HE, however, need to be studied in more detail.

4.6 Conclusion

In summary, the present work reveals a relation between the attention-related gamma band peak frequency in visual areas and the severity of HE as measured by the CFF. Earlier results of slowed oscillatory processes in the motor and visual system are extended to the cognitive domain. Thereby, the notion that pathologically slowed oscillatory activity is a key mechanism in the pathophysiology of HE is strongly supported. The present data reveal that only patients with high (normal) CFF values are able to show attention related gamma band modulation. More impaired patients with low (pathological) CFF values, however, do not show this modulation, in line with previous findings of pronounced attentional deficits in HE. Finally, the present data emphasize the CFF as being a sensitive indicator of HE severity.

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Acknowledgements

We thank all participants who kindly participated in this study. We thank Mr. Diethelm Plate for his unfailing support in all patient related issues. We thank Mrs. E. Rädisch and Mrs. A. Solotuchin for technical support with MRI scans, and Mrs. A. Solotuchin and Mr. Ulf Zierhut for assistance in data acquisition.

Role of The Funding Source

This study was supported by Deutsche Forschungsgemeinschaft (SFB 575). N.K. was supported by Studienstiftung des deutschen Volkes and a travel allowance of Boehringer Ingelheim Foundation (B.I.F.). M.B. was supported by a Marie Curie Fellowship of the EU (FP7-PEOPLE-2009-IEF-253965).

Disclosures

G.K. and D.H. belong to a group of patent holders for a portable bed-side device for CFF analysis.

Table Legend

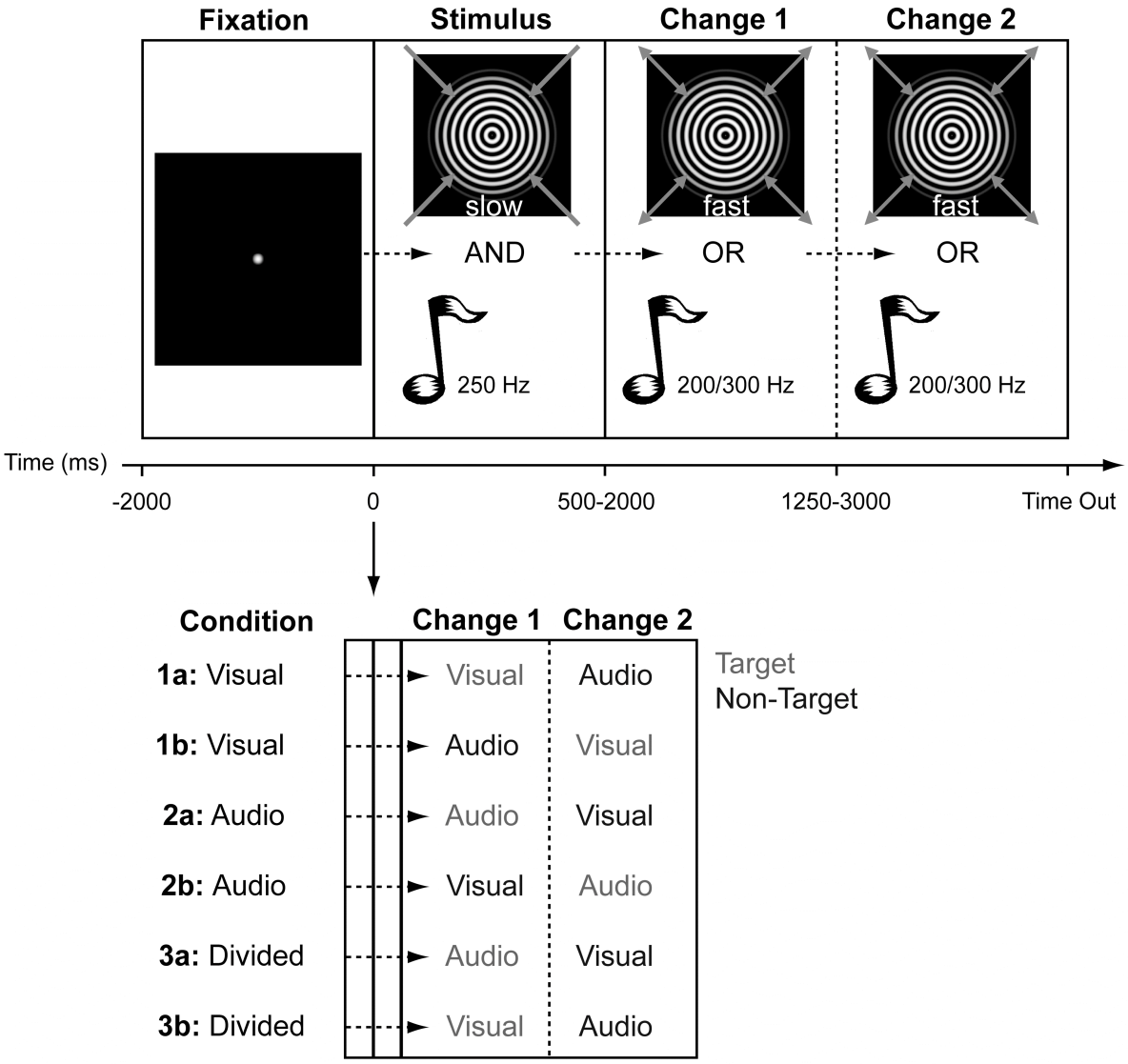
Table 1: Participant Specific Data

group	age	sex	CFF	etiology of cirrhosis	Child-Pugh Score	occipital γ frequency (visual)	occipital γ frequency (auditory)
	51	m	44.3	-	-	-	-
	46	f	41.1	-	-	55.9	51.9
	67	m	39.8	-	-	45.9	45.9
Control	71	m	-	-	-	-	-
	69	f	40.8	-	-	51.9	49.9
	58	f	41.4	-	-	51.9	51.9
	61	f	38.4	-	-	37.9	39.9
	73	m	38.1	-	-	45.9	-
	62.0 ± 3.5		**40.6 ± 0.8**			**48.9 ± 2.6**	**49.9 ± 2.3**
	55	f	39.4	ethanol	A	51.9	51.9
	53	m	43.0	ethanol	B	51.9	55.9
	48	m	41.8	hepatitis C	C	47.9	47.9
HE0	44	f	41.3	PBC	A	61.9	-
	69	f	38.4	hepatitis C	A	45.9	45.9
	60	m	42.3	ethanol	A	41.9	37.9
	70	m	39.0	hepatitis C	A	-	-
	59	m	39.1	hepatitis B	A	47.9	49.9
	52.3 ± 3.3		**40.5 ± 0.6**			**47.9 ± 2.4**	**48.9 ± 2.5**
	60	m	38.6	cryptogenic	B	-	
	59	f	39.1	hepatitis C	A	49.9	49.9
	57	m	38.3	ethanol	A	-	53.9
mHE	65	f	39.0	ethanol	A	51.9	49.9
	56	m	35.7	ethanol	B	35.9	39.9
	57	m	37.2	ethanol	A	53.9	51.9
	62	m	39.9	cryptogenic	A	51.9	51.9
	52	m	39.4	hepatitis C	A	51.9	51.9
	58.5 ± 1.3		**38.4 ± 0.5**			**51.9 ± 2.7**	**51.9 ± 1.8**
	69	m	38.6	ethanol	B	-	-
	67	m	36.2	autoimmune	C	-	-
	70	m	38.3	hepatitis A/B	B	53.9	47.9
	72	m	35.5	hepatitis C	B	45.9	-
HE1	75	f	38.3	cryptogenic	B	43.9	43.9
	65	f	37.7	ethanol	C	43.9	43.9
	45	m	34.8	PSC	C	47.9	47.9
	45	f	32.6	autoimmune	C	47.9	49.9
	63	m	36.4	ethanol	A	37.9	-
	59	m	32.4	ethanol	C	-	-
	63.0 ± 3.3		**36.1 ± 0.7**			**45.9 ± 1.9**	**47.9 ± 1.2**

Participant specific data are displayed for each participant. For age and the CFF, group mean values ±standard errors of the mean are shown. Gender is coded as f = female and m = male. Etiology of liver cirrhosis was assessed by each patient's medical history, PSC = Primary sclerosing cholangitis. Grading of liver cirrhosis was done according to the European Child-Pugh-classification (Pugh et al., 1973). Gamma peak frequencies over occipital sensors are displayed for the *visual* and *auditory* conditions separately. For gamma band peak frequencies, group median values ±standard errors of the median are displayed.

Figure Legends

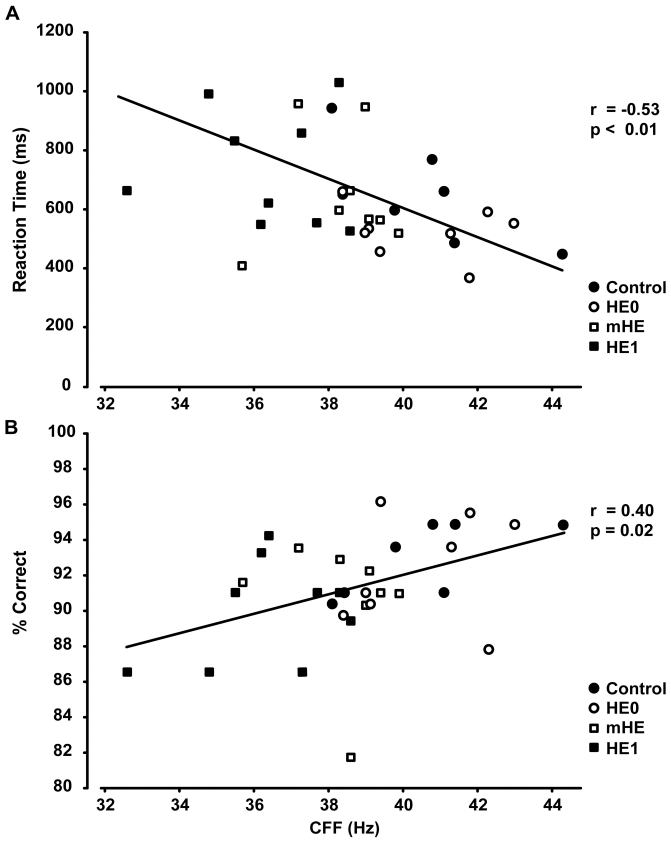
Fig. 1: Paradigm



Upper part: General overview of one trial. Each trial started with the presentation of a fixation dot (Fixation). Thereafter, visual and auditory stimuli were presented simultaneously (Stimulus; t = 0). After a randomly assigned period of 500 to 2000 ms, either the visual or the auditory stimulus changed its quality (Change 1). After another 750 or 1000 ms also the other stimulus changed (Change 2). Depending on the condition, one of the two stimulus changes served as target.

Subjects had to give a speeded response indicating the quality of the target as soon as it appeared in the cued modality (see section 2.2.2 for exact description of target qualities). A response or a reaction time > 2000 ms (Time Out) terminated stimulus presentation. Feedback was given after each trial. Periods used for later analysis were Fixation (baseline) and Stimulus.
Lower part: Detailed description of variable parts of each trial. Depending on the condition, the change of either the visual or the auditory stimulus was target. In the *visual* condition (1a / 1b), the change in visual stimulus was target and the change in auditory stimulus non-target. In the *auditory* condition (2a / 2b), the change in auditory stimulus was target and the change in visual stimulus non-target. Two target positions were possible in these conditions. In the *divided* condition (3a / 3b), the first changing stimulus was target, irrespective if it occurred in the visual or auditory modality. Targets are depicted in light grey, non-targets in dark grey.
Please note that fixation and stimulus periods consisted of the same stimulation in each trial, only duration of stimulus period varied. Thus, these trial periods are depicted as empty boxes in the lower part.

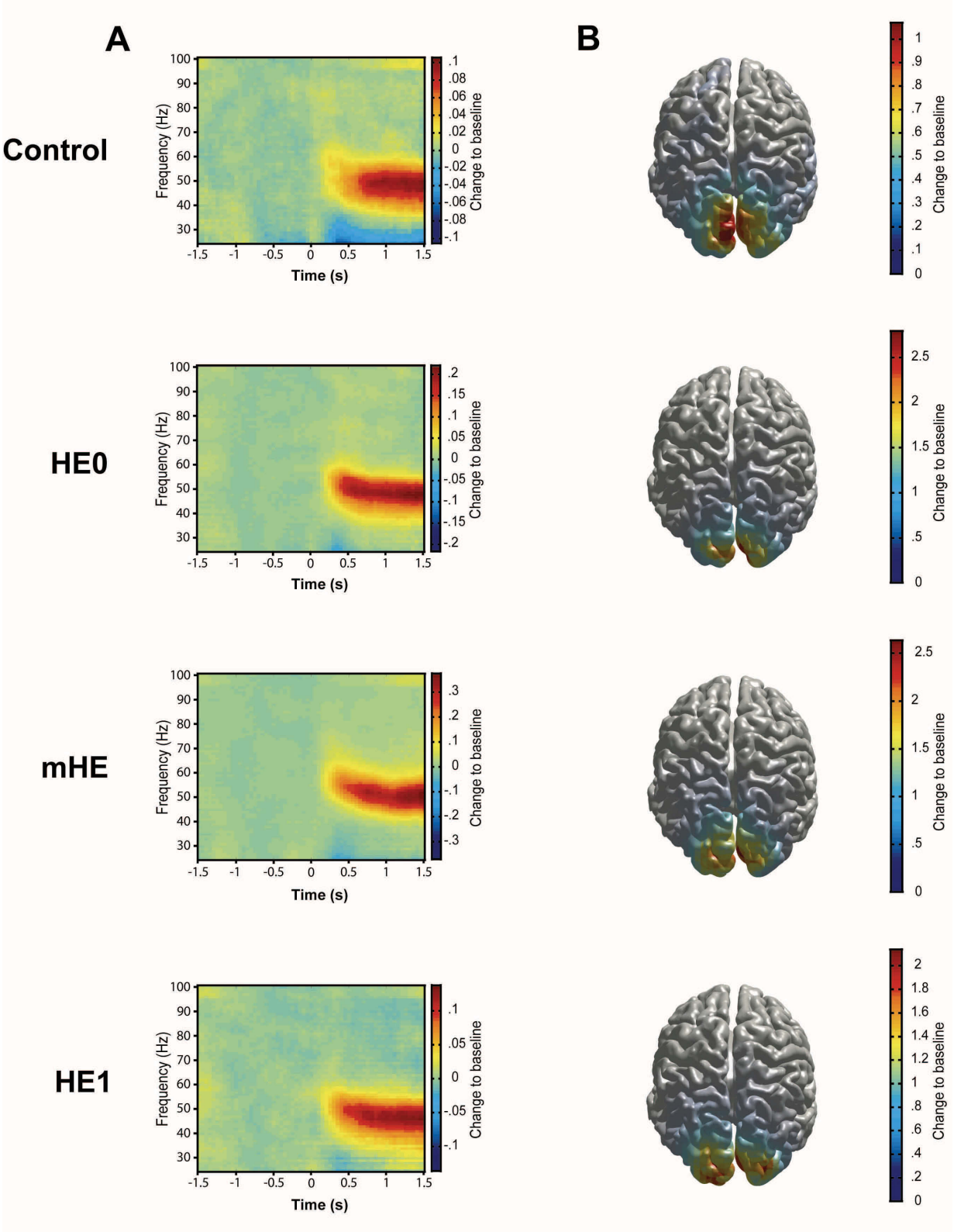
Fig. 2: Disease Severity and Behavioral Performance Correlate



A) In the *auditory* condition, the CFF correlated with reaction times (r = -0.53, p < 0.01, corrected). Thus, the higher the CFF, the faster the response was given. Each subject is represented by its value (Controls = filled circle, HE0 = open circle, mHE = open square, HE1 = filled square).

B) In the *visual* condition, the CFF correlated with the number of correct responses (r = 0.40, p = 0.02, corrected). Thus, the higher the CFF, the more correct responses were given. As in A, each subject is represented by its value.

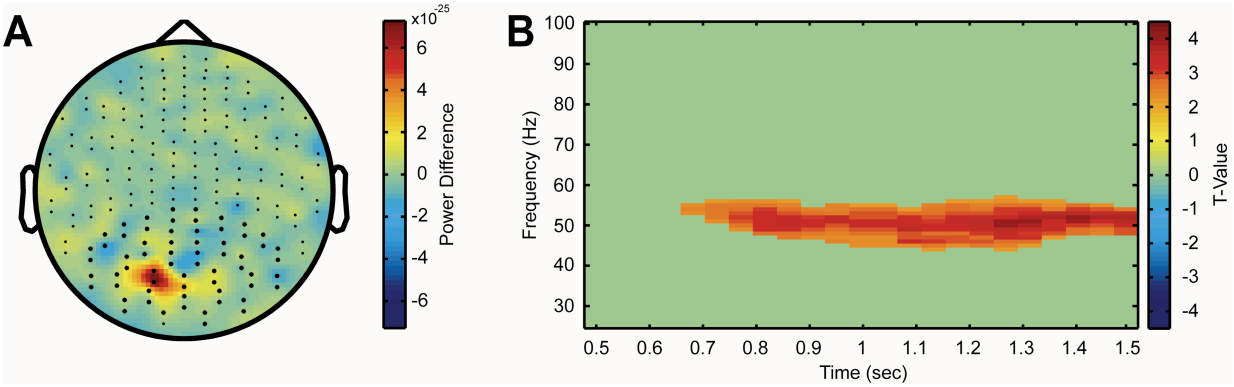
Fig. 3: Patients with HE Show Visually Induced Gamma Band Synchronization



A) For each group of participants the average relative gamma band response in the *visual* condition is depicted (Controls: n = 8; HE0: n = 8; mHE: n = 8; HE1: n = 9). Time-point zero constitutes the time of stimulus onsets. Color-coding: 0 corresponds to no change, 0.3 to a 30% increase, and -0.3 to a 30% decrease in power relative to baseline (-1000 to -500 ms). Please note that due to large differences in relative gamma power, color bar limits are different between the four groups.

B) Localization of strongest relative gamma band sources for each group of participants in the *visual* condition (Controls: n = 7; HE0: n = 7; mHE: n = 6; HE1: n = 7). Only participants with significant gamma band peaks were included. Colors index intensity of relative change to the pre-stimulus baseline. Again, due to large differences in source strengths, color bar limits are different between the four groups.

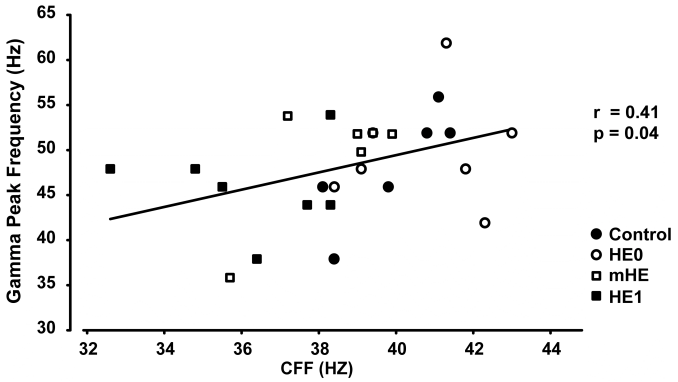
Fig. 4: Patients with High CFF Show Modulation of Gamma Power with Attention



A) Difference plot of gamma band power between the *visual* and the *auditory* conditions for one representative subject (HE0 subject 3, 48 year old male), averaged over the period of 500 to 1000 ms and his individual gamma peak frequency (48 Hz ±5 Hz). Sensors later used for calculation of statistics and peak gamma band frequency are marked by asterisks.

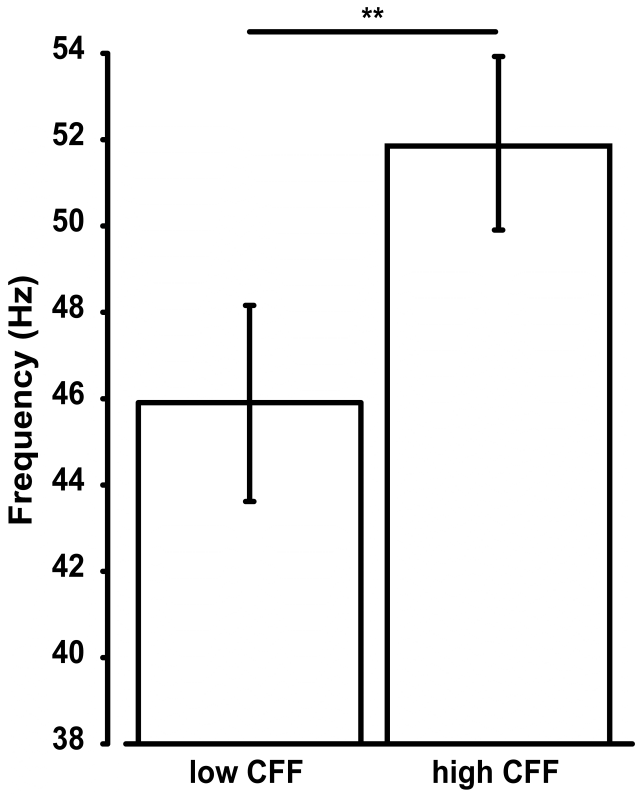
B) High CFF group including all participants: Statistical comparison of gamma band power in the *visual* and the *auditory* conditions. Results are shown as t-values; all non-significant frequencies are masked green. Gamma band power was stronger in the *visual* than in the *auditory* condition ($p = 0.04$, corrected). Please note, that similar results were achieved when looking at the high CFF group including patients only ($p = 0.02$, corrected).

Fig. 5: Gamma Band Peak Frequency over Occipital Areas Correlates with CFF



Individual gamma band peak frequencies of the *visual* condition and CFF values are displayed. Each shape depicts one of the four groups (Controls = filled circle, HE0 = open circle, mHE = open square, HE1 = filled square). Gamma band peak frequencies correlated with the CFF (r = 0.41, p = 0.04, corrected). Thus, the higher the CFF, the higher the gamma band peak frequency.

Fig. 6: Gamma Band Peak Frequency over Occipital Areas Differs between Groups Sorted by CFF



High and low CFF groups including all participants: Median values of gamma band peak frequencies in the *visual* condition. Gamma band peak frequencies were different between the two groups (U = 37.5, p = 0.01, corrected). High CFF group: Median = 51.9 ±2.0, low CFF group: Median = 45.9 ±2.3. Please note, that when comparing high and low CFF groups including only patients, the difference between the groups approached significance (U = 26.0, p = 0.07, corrected). High CFF group: Median = 51.0 ±1.5, low CFF group: Median = 45.9 ±2.1 (standard errors of the median reported).

Supplementary Table: Behavioral Data of Low and High CFF Groups

For the high (≥ 39 Hz) and the low CFF groups (< 39 Hz), including (two upper rows) and excluding healthy controls (two lower rows), numbers of observations (n), median values, and standard deviations (Std) are displayed for reaction times and correctness of responses for each of the conditions.

		Reaction Times				**Correctness of Responses**			
		Visual	**Auditory**	**Divided Visual**	**Divided Auditory**	**Visual**	**Auditory**	**Divided Visual**	**Divided Auditory**
Low CFF Including Controls	N	12	12	11	11	12	12	11	11
	Median	975	753	780	684	91.03	93.91	82.05	84.62
	Std	237	198	223	160	2.58	5.95	10.77	8.50
High CFF Including Controls	N	14	14	14	14	14	14	14	14
	Median	690	562	682	598	92.95	94.55	89.74	91.03
	Std	181	142	133	130	2.46	2.53	5.26	3.29
Low CFF Excluding Controls	N	10	10	9	9	10	10	9	9
	Median	928	753	772	645	91.03	94.54	83.33	88.46
	Std	251	207	120	145	2.84	6.54	9.13	6.06
High CFF Excluding Controls	N	10	10	10	10	10	10	10	10
	Median	690	547	649	587	91.67	94.55	89.74	91.03
	Std	200	151	71	112	2.67	1.62	6.10	2.57

Title Page

Title:

Fluctuations of pre-stimulus oscillatory power predict subjective perception of tactile simultaneity

Authors:

Joachim Lange, Johanna Halacz, Hanneke van Dijk, Nina Kahlbrock, Alfons Schnitzler

Institute of Clinical Neuroscience and Medical Psychology, Medical Faculty, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany

Corresponding author:

Joachim Lange

Institute of Clinical Neuroscience and Medical Psychology, Medical Faculty,

Heinrich-Heine-University

Universitätsstr. 1

40225 Düsseldorf

Tel.: +49 211 8113074

Fax: +49 211 8113015

e-mail: Joachim.lange@med.uni-duesseldorf.de

Running title: Pre-stimulus oscillations predict perception

Abstract

Oscillatory activity is modulated by sensory stimulation, but can also fluctuate in the absence of sensory input. Recent studies have demonstrated that such fluctuations of oscillatory activity can have substantial influence on the perception of subsequent stimuli. In the present study, we employed a simultaneity task in the somatosensory domain to study the role of pre-stimulus oscillatory activity on the temporal perception of two events. Subjects received electrical stimulations of the left and right index finger with varying stimulus onset asynchronies (SOA) and reported their subjective perception of simultaneity while brain activity was recorded with magnetoencephalography (MEG). With intermediate SOAs (30 and 45 ms), subjects frequently misperceived the stimulation as simultaneously. We compared neuronal oscillatory power in these conditions and found that power in the high beta-band (~20-40 Hz) in primary and secondary somatosensory cortex prior to the electrical stimulation predicted subjects' reports of simultaneity. Additionally, pre-stimulus alpha-band power influenced perception in the condition SOA 45 ms. Our results indicate that fluctuations of ongoing oscillatory activity in the beta and alpha bands shape subjective perception of physically identical stimulation.

Keywords

MEG, oscillation, somatosensory, beta, alpha

Introduction

Depending on the surrounding or internal brain states, physically identical sensory stimulation can be perceived quite differently. For example, subjective perception of ambiguous and bistable stimuli fluctuates over time despite identical and constant sensory input to the brain. Moreover, absolute detection thresholds for sensory perception can vary over time or over stimulus presentations. Several studies have shown that fluctuations of oscillatory neuronal activity can predict at least some of the perceptual variability. The state of oscillatory activity just prior to the onset of a stimulus influences whether the subsequent stimulation will be perceived, especially when stimuli are weak and near perceptual threshold. Among all frequency bands, alpha-band (~8-12 Hz) activity has gained much attention in recent years. It has been shown that pre-stimulus alpha-band power and phase in human parieto-occipital areas are correlated with conscious perception of visual stimuli (Thut et al., 2006;Hanslmayr et al., 2007;Van Dijk et al., 2008;Wyart and Tallon-Baudry, 2009;Mathewson et al., 2009;Mazaheri et al., 2009;Romei et al., 2010). Similarly in human somatosensory cortex, it has been shown that attentional or spontaneous fluctuations of pre-stimulus alpha-band activity influences perception of tactile stimuli. Linkenkaer-Hansen et al. (2004) showed that pre-stimulus amplitude of ongoing alpha- and beta-oscillations in human somatosensory cortex correlates with subjects' ability to detect a subsequent weak tactile stimulus, with intermediate levels of amplitudes showing the highest detection rates. Moreover, it has been shown that the phase of alpha-oscillations before stimulus onset influences subsequent perception (Palva et al., 2005). Recent studies have demonstrated that cued attention to somatosensory stimuli modulates pre-stimulus alpha- and beta-band activity in human somatosensory cortex in a spatially (Jones et al., 2010;van Ede et al., 2010;van Ede et al., 2011;Anderson and Ding, 2011) and temporally specific way (van Ede et al., 2011). In

addition, pre-stimulus alpha- and beta-band amplitudes modulate the amplitude of the early, stimulus-evoked M50 component of the event related field (Jones et al., 2009;Anderson and Ding, 2011) and are correlated to behavioral detection rates of subsequent stimuli (Linkenkaer-Hansen et al., 2004;Jones et al., 2010), similar to findings in human visual cortex for alpha-band amplitudes (Van Dijk et al., 2008). In summary, these results are in line with the hypothesis that ongoing fluctuations of oscillatory neuronal synchronization in the pre-stimulus period modulates the gain of neuronal assemblies and thus facilitates subsequent processing of sensory stimulation (Fries, 2005;Van Dijk et al., 2008;Fries, 2009).

Similar to the perception of a single stimulus, simultaneous perception of two tactile stimuli shows a considerable variation. Perception of simultaneity is a powerful cue for determining whether two events define a single or multiple objects. Perception of the relative timing of two events tolerates a moderate degree of temporal delays between sensory stimulations. However, this tolerance of temporal delays introduces a substantial degree of variability. For example, when two tactile stimuli are presented with a stimulus onset asynchrony of ~30-70 ms, subjects' show a considerable variation in their trial-by-trial responses when asked to judge whether the two stimulations were simultaneous or not, i.e. asynchronously, non-simultaneously presented stimuli are frequently misperceived as simultaneous (Geffen et al., 2000;Kopinska and Harris, 2004;Harrar and Harris, 2005;Harrar and Harris, 2008). The neurophysiological basis of this variability is not well understood.

In the present study, we used magnetoencephalography (MEG) to investigate the role of oscillatory neuronal activity for subjective perception of simultaneity and its variability. We employed a simultaneity task to study the role of pre-stimulus oscillatory activity for subjective perception. We focused on the somatosensory domain and compared conditions in which identical stimuli can lead to variable subjective perceptions on a trial-by-trial basis. These conditions offer an intriguing possibility to study the role of oscillatory neuronal

synchronization under constant conditions of sensory stimulation (Rodriguez et al., 1999;Leopold et al., 2002).

Materials and Methods

Subjects

Twenty subjects participated in this study (24.9 ± 3.8 years (mean ± SD), 7 male). None of the subjects had a known history of neurological disorders and subjects gave written informed consent in accordance with the Declaration of Helsinki.

Paradigm and Stimuli

Subjects were seated comfortably with their head placed inside an MEG helmet and fixated a central grey dot on a screen positioned 60 cm in front of them. Each trial started with a decrease of luminance of the fixation dot, which served as the start cue (Fig. 1). After a randomized period of 800-1000 ms, subjects received short (0.3 ms) electrical pulses between the two distal joints of the left and right index finger to stimulate the cutaneous end branches of the digital nerves. The amplitude of the electric pulses was set to 60% of the individually determined subjective (mild) pain threshold level as measured prior to the recordings (mean amplitude 5.5 ± 0.7 mA). Notably, subsequent analyses were performed on within-subject levels, i.e. we always compared conditions of identical stimulation amplitudes (see Data Analysis for details). Stimulation of the fingers was applied with varying stimulus onset asynchronies (SOA) of ± 200, ± 45, ± 30, or 0 ms with negative SOA indicating that stimulation was first on the left finger. The condition of 0 ms was presented twice as often as the other SOA. SOA were chosen based on behavioral pilot-experiments to ensure a balanced distribution of difficulty levels. After another random period of 800-1200 ms, in which only the fixation dot was visible, the fixation dot increased luminance indicating the start of the response window. Subjects were asked to report whether they had perceived the stimulation

as simultaneous or non-simultaneously by button presses. Button configurations were balanced within and between subjects: Half of the subjects responded with the middle fingers of both hands, half of the subjects responded with the index and middle finger of one hand (5 with the right hand, 5 with the left hand). For each subject, the button configuration was switched blockwise, i.e. allocation of response finger and subjective report was balanced within and across subjects. Subjects were instructed to respond within 2000 ms after presentation of response instructions and that response speed was not taken into account. If no response was given after 2000 ms or subjects responded before the presentation of the instructions, a warning was visually presented. The respective trial was discarded from analyses and repeated at the end of the block. Except the warning signal, no feedback was given and subjects were naïve to the different SOA used. Five repetitions of each SOA (i.e. 40 trials) constituted one block with stimuli within one block presented in pseudo-random order. Each block was repeated ten times with self-paced breaks of ~2 min in between. Response instructions for each block were visually presented on the screen before the start of each block. The experimental run was controlled using Presentation software (Neurobehavioral Systems, Albany, USA). Subjects performed a training session of ~5 min before the start of the MEG-experiment.

Data acquisition and analysis

Data recording and preprocessing

Neuromagnetic brain activity was continuously recorded using a 306-channel whole head MEG system (Neuromag Elekta Oy, Helsinki, Finland). Simultaneously, electrooculargram (EOG) were recorded by placing electrodes above and below the left eye and on the outer sides of each eye. The data were recorded at a rate of 1000 Hz. Subjects' head position within the MEG helmet was registered by four coils placed at subjects' forehead and behind both ears. Individual full brain high-resolution standard T1-weigthed structural magnetic resonance

images (MRI) were obtained from a 3T MRI scanner (Siemens, Erlangen, Germany) and offline aligned with the MEG coordinate system using the coils and anatomical landmarks (nasion, left and right pre-auricular points).

MEG data were offline analyzed using FieldTrip (http://www.ru.nl/donders/fieldtrip), an open source matlab toolbox for neurophysiological data analysis (Oostenveld et al., 2011). Power line noise was removed from the continuous data using a discrete Fourier transformation of 10-s long signal periods to estimate the amplitudes and the phases of the 50, 100, and 150 Hz components. These components were subtracted from the continuous data as described earlier (Hoogenboom et al., 2006;van Ede et al., 2010;Lange et al., 2011;van Ede et al., 2011). This was done separately for all 10-s periods around all periods of interest. Continuous data were segmented into trials, starting with first appearance of the fixation dot and ending with appearance of instruction text. Artifacts caused by eye-movements or muscle activity were removed using a semi-automatical algorithm and the linear trend was removed from each trial.

Time-frequency analysis

Time-frequency representations (TFR) were computed applying a Fourier transformation on adaptive sliding time windows containing five full cycles of the respective frequency f ($\Delta t = 5/f$), moved in steps of 25 ms (similar to (Mazaheri et al., 2009;Van Dijk et al., 2010;Haegens et al., 2011). Data segments were tapered with a single Hanning taper, resulting in a spectral smoothing of $1/\Delta t$.

Next, we determined regions of interest in sensor space. We chose four sensors in the left and four sensors in the right hemisphere covering bilateral primary somatosensory cortex (SI) and four sensors in the left and four sensors in the right hemisphere covering secondary somatosensory cortex (SII) (Fig. 3).The choice of sensors was based on previous studies (Bauer et al., 2006;Haegens et al., 2010;van Ede et al., 2010;Hagiwara et al., 2010;van Ede et

al., 2011). This set of sensors defined the somatosensory region of interest for subsequent analyses for all subjects. The set of sensors in the left and in the right hemisphere were symmetrically distributed with respect to the midline of the sensor array (Fig. 3B,E,H,K). For each subject separately, we sorted trials with respect to the SOA. Within each SOA-bin, we compared trials with reports of subjective simultaneity to trials in which the stimulation was perceived as non-simultaneous. Thus, we compared two conditions with identical physical stimulation that only differed with respect to the subjective perception. To this end, we averaged spectral power over the sensors of interest (see above) for each perceptual condition and compared both conditions by independent samples t-tests. This comparison was done independently for each time-frequency sample and thus resulted in a time-frequency t-map for each subject. Note that this comparison is not an actual statistical test but serves as a normalization of inter-individual differences. This comparison was done separately for sensors in the left and right SI and SII. Only conditions with SOA of 30 and 45 ms were included in the analysis as only these conditions revealed a reasonably high number of trials for both perceptual conditions (simultaneous and non-simultaneous). Behavioral and neuromagnetic data revealed highly symmetrical patterns for positive and negative SOA (e.g. Fig. 2 for behavioral data), i.e. no statistically significant differences were found when restricting the analyses to contra- or ipsilateral sites with respect to the site of the first stimulation. To increase statistical power, we pooled data regarding the site of the stimulation, i.e. we report data in terms of contra- and ipsilateral to the site of the first stimulation. All t-values of the time-frequency t-map were transformed to z-values using SPM2 resulting in time-frequency z-maps (e.g. Van Dijk et al., 2008;Mazaheri et al., 2009). For group level statistics, we used the z-maps obtained for single subjects as inputs and determined their consistency across subjects. We used a non-parametric permutation approach that identifies clusters in time-frequency with significant changes. This effectively corrects for multiple comparisons (Maris and Oostenveld, 2007;for details see Lange et al., 2011). For statistical

testing, the entire time window (-500 to 800 ms) was used. To generate topographical representations of statistically significant effects, we repeated the abovementioned statistical comparison, but this time for each sensor independently, resulting in time-frequency z-maps for each sensor separately (instead of averaging over sensors). For each sensor, we averaged the z-values over all individual time-frequency samples that correspond to the statistically significant time-frequency clusters in the abovementioned analysis (as can be seen in e.g. Fig. 3A,B). Finally, we plotted the averaged z-values in a topographical representation (Fig. 3B,E,H,K).

Correlation of pre-stimulus power and detection rates

Next, we aimed to further investigate the correlation of pre-stimulus power to perception of simultaneity. First, we averaged spectral power over time, frequency, and sensors. Sensors of interest were defined as mentioned above (left and right SI and SII). Time-frequency bands of interest were determined by the significant time-frequency clusters in the abovementioned cluster-based statistical analysis on group level (Fig. 3A,D,G,J), resulting in four different time-frequency bands in the beta-band. Since the significant clusters slightly differed in time and frequency for the different sensors of interest, time-frequency bands used to compute pre-stimulus power for the correlation analysis differed for each set of sensors of interest. The exact time-frequency bands for each analysis can be found in Fig. 4.

Due to the relevance of pre-stimulus alpha-band power in somatosensory perception (Linkenkaer-Hansen et al., 2004;Jones et al., 2009;Jones et al., 2010;van Ede et al., 2011;Anderson and Ding, 2011), we also included the alpha-band into the analyses. The exact time-frequency-bands used for each correlation analysis can be found in Fig. 4. The averaging was done for each subject separately (with a common and fixed time-frequency-sensor triplet for all subjects, based on the group-level statistics). Subsequently, we sorted the single trials of each subject according to averaged power and divided all trials into six bins with equal

number of trials. For each bin, we calculated the mean number of simultaneity reports and normalized the result for each subject. Finally, we computed the mean and standard error of the mean (SEM) over subjects and fitted linear and quadratic functions to the data to determine the best fit (Linkenkaer-Hansen et al., 2004;Van Dijk et al., 2008;Jones et al., 2010).

Correlation of pre-stimulus power and ERFs

To study a potential relation between pre-stimulus alpha- and beta-band power and post-stimulus event related fields (ERFs) (Jones et al., 2009;Jones et al., 2010;Anderson and Ding, 2011), we correlated pre-stimulus power and ERFs in line with the abovementioned analysis of pre-stimulus power and detection rates. To this end, we averaged power over time, frequency, and sensors. Sensors were chosen as defined above. Time-frequeny bands were based on the significant clusters found in Fig. 3A,D,G,J. Since the significant clusters slightly differed in time and frequency for the different sensors of interest, time-frequency bands used to compute pre-stimulus power differed for each set of sensors of interest. The exact time-frequency bands for each analysis can be found in Fig. 5. Time-frequency bands were defined on group level and the same time-frequency band was used for all subjects. Subsequently, we divided trials in two bins (low and high pre-stimulus alpha/beta-power) and then computed the ERFs in the post-stimulus period over the same sensors used for the power analyses (Jones et al., 2009;Jones et al., 2010). ERFs were computed by first applying a low pass filter of 30 Hz, rectifying the signals by taking the root-mean-sqaure of the signal in the time-domain (e.g. Bauer et al., 2006;Van Dijk et al., 2008;Mazaheri et al., 2009), and then averaging ERFs over trials and subjects. Statistical analysis was performed by applying dependent-sample t-test between low- and high power conditions for each time point.

Source reconstruction

To determine the cortical sources of the significant effects on sensor level, we applied an adaptive spatial filtering technique in the frequency domain (Gross et al., 2001).
The leadfield matrix was computed for grid points in a realistically shaped single-shell volume conduction model, derived from the individual subject's structural MRI (Nolte, 2003). To this end, a regular three-dimensional 1-cm grid in the Montreal Neurological Institute (MNI) template brain was created and each subject's structural MRI was linearly warped onto this template. The inverse of this warp was applied to the template grid, resulting in individual grids based on individual subject's volume conduction model. The individual source parameters estimated in this way were combined across subjects per grid position. We aimed to determine the sources for the statistically significant effects revealed on sensor level (Fig 3). To this end, we computed cross-spectral density (CSD) matrices between all MEG sensor pairs from the Fourier transforms of the tapered data epochs at the frequency of interest for each subject separately. The data epoch and the frequency of interest were based on the significant time-frequency clusters of the abovementioned group-analysis on sensor level (Fig.3A,D,G,J). Since the significant clusters differed in time and frequency for the different sensors of interest, time-frequency bands used for source reconstruction differed for each condition. The exact time-frequency bands for each analysis can be found in Fig. 6. Common spatial filters for each subject were computed using the CSD between all MEG sensor pairs, averaged over all trials of a given condition for the respective subject (pooled over subjective perceptions). For each subject, the CSD matrices of single trials were then projected through those individual filters, providing single trial estimates of source power (Hoogenboom et al., 2010). Statistical testing on source level was performed in line with testing on sensor level (see above). Results were displayed on the template brain and cortical sources were identified using the AFNI atlas (http://afni.nimh.nih.gov/afni), integrated into Fieldtrip.

Results

Behavioral results

Subjects were asked to report their subjective percept of simultaneity for electrical stimuli delivered to their left and right index finger with different stimulus onset asynchronies (SOA). They made negligible errors for SOA of 0 and 200 ms (Fig.2). However, intermediate SOA were perceived as simultaneous in some trials and as not simultaneous in other trials (SOA of -30 ms: 51.8 ± 5.5% (mean ± SEM) simultaneity reports; SOA of +30 ms: 54.9 ± 5.2% ; SOA of -45 ms: 30.2 ± 4.8%; SOA of +45 ms: 33.6 ± 4.8%).

Condition contrasts

Next, we studied the role of oscillatory activity for the perception of simultaneity. Within each SOA we sorted trials with respect to subjects' perceptual reports. We compared spectral power between reports of simultaneity and reports of non-simultaneity in sensors over sensorimotor areas.

For SOA of 30 ms, we found spectral power in sensors over ipsilateral primary somatosensory cortex (SI) to be statistically significantly enhanced in the frequency band 27.5-40 Hz if subjects perceived the stimulation erroneously as simultaneous. Notably, this effect occurred between -225 and -125 ms, i.e. the effect appeared already before any electrical stimulation was delivered, and the effect was only present in ipsilateral sensors (Fig. 3A). In line with these findings, the topographical representation of this effect revealed a focus on sensors over ipsilateral SI (Fig. 3B). In sensors over secondary somatosensory cortex (SII), power was statistically significantly enhanced in the frequency band 17.5-40 Hz between -475 and -275 ms (Fig. 3D). The topographical representation revealed a focus over ipsilateral SII (Fig.3E). No significant differences were observed in contralateral sensors (Fig.3C,F).

For SOA of 45 ms, a similar finding was observed. In sensors over ipsilateral SI, oscillatory activity between 15-35 Hz and -150 to 50 ms was enhanced if subjects perceived the following stimulation erroneously as simultaneous (Fig. 3G,H). In sensors over ipsilateral SII, oscillatory activity between 25-40 Hz and -350 to -200 ms was significantly enhanced (Fig.3J,K). Contralateral sensors did not show any significant differences (Fig.3I,L).

Correlation of pre-stimulus power and detection rates

We found spectral power in alpha- and beta frequency bands to be enhanced before and around the onset of stimulation, when subjects incorrectly perceived the two subsequent stimuli as simultaneous. To study the relation between subjective perception of stimuli and pre-stimulus oscillatory activity, we performed a correlation analysis. To this end, in each trial spectral power was averaged over sensors, time, and frequencies. Next, trials were sorted for spectral power and divided into six bins (Linkenkaer-Hansen et al., 2004;Van Dijk et al., 2008;Jones et al., 2010). The perceptual reports were normalized per subject and then averaged over subjects. For the four cluster in the beta-range in ipsilateral SI and SII (see Fig. 3A,D,G,J), we found a significant linear relationship between pre-stimulus power and subjects' perceptual reports in ipsilateral sensors for all conditions (SOA of 30 ms: SI: $r^2 = .90$, $p < .01$; SII: $r^2 = .87$, $p < .01$; SOA of 45 ms: SI: $r^2 = .86$, $p < .01$; SII: $r^2 = .91$, $p < .01$; Fig. 4A-D), i.e. high pre-stimulus power was correlated with a high number of erroneous simultaneity reports. In contrast, we did not find a significant correlation in contralateral sensors (Fig. S1). Additionally, we performed the same analysis for the alpha-band in the condition SOA of 45 ms. We observed a quadratic relationship between subjective perception and pre-stimulus oscillatory activity in SI, with intermediate power bins showing the lowest probability of simultaneity reports ($r^2 = .89$, $p < .05$; Fig. 4E). In other words,

intermediate alpha amplitudes were associated with a more veridical perception of non-simultaneity. No significant correlation was found in contralateral sensors (Fig. S1).

Correlation of pre-stimulus power and ERFs

We additionally analyzed the correlation between pre-stimulus alpha-/beta-band activity and post-stimulus event related fields (ERFs) (Jones et al., 2009;Jones et al., 2010;Anderson and Ding, 2011).

First we sorted trials in the condition SOA of 30 ms for power in ipsilateral sensors over SI in the time-frequency-band between 27.5-40 Hz and -225 to -125 ms (i.e. the significant cluster in Fig 3A). Trials with low pre-stimulus beta-band power revealed a significant increase of the ERFs between 150-168 ms and 216-232 ms (Fig. 5A). Trials with high pre-stimulus beta-band power in sensors over SII revealed increased ERFs between 93-148 ms (Fig. 5B).

For the condition SOA of 45 ms, we found no significant effects of pre-stimulus beta-band power on ERFs for sensors over SI (Fig. 5C). Sensors over SII revealed increased ERFs for trials with high beta-band power between 107-162 ms (Fig. 5D). Additionally, we sorted trials for pre-stimulus power in the alpha-band. Sensors over SI revealed increased ERFs for trials with high pre-stimulus power between 64-75 ms. Furthermore, trials with low pre-stimulus power revealed increased ERFs between 250-278 ms (Fig. 5E).

Source localization

To identify the cortical sources of the significant effects found in TFRs on sensor level (Fig 3), we applied a beamforming approach. For both conditions (SOA of 30 and 45 ms), the comparatively late (~-200-0ms) significant components (Fig. 3A,G) revealed a significant source in ipsilateral sensorimotor areas with the peak located in ipsilateral SI (SOA of 30 ms: $p<.05$; SOA of 45 ms: $p<.05$, Fig. 6A,C). For both conditions, the earlier component (~-450

to -250 ms) was located in ipsilateral SII (SOA of 30 ms: $p<.05$; SOA of 45 ms: $p<.05$, Fig. 6B,D).

Discussion

We studied the contribution of oscillatory neuronal activity to subjective perception of brief electrical stimuli applied bilaterally to the index fingers with varying stimulus onset asynchronies (SOA). We were interested whether fluctuations of spectral power predict subjective perception. Crucially, the paradigm enabled us to study the role of neuronal oscillatory under conditions of constant physical stimulation while only the subjective perception was changed intrinsically.

When SOA was 30 or 45 ms, subjects frequently misperceived the stimulation as simultaneous. Erroneous perception of simultaneity was associated in both conditions (SOA 30 and 45 ms) with an increase of power in the beta-band (~20-30 Hz) in sensors over primary (SI) and secondary (SII) somatosensory cortex. The increase was evident in the cortical hemisphere ipsilateral to the site of the first stimulation, but not in contralateral sites. Notably, this increase was visible before the onset of stimulation and the significant differences appeared earlier in sensors over SII than in sensors over SI. Source reconstruction confirmed a priori sensor selection by revealing significant cortical sources of the earlier effects (found in sensors presumably over SII at ~-450 to -250 ms) in ipsilateral SII. The relatively later effects (~-200-0 ms, observed in sensors presumably over SI) were located in ipsilateral sensorimotor areas with the peak located in SI. It should be noted that the source reconstruction was performed on pre-stimulus data, i.e. in the absence of any stimulation. Without stimulation, absolute power levels have a smaller signal-to-noise ratio than power values in response to stimulation. Low signal-to-noise ratios naturally limit beamforming results by making also the sources noisier and thus spatially smeared. Furthermore, the

observed significant effects are relatively short-lived which also limits beamforming techniques. Despite these limitations and although the significant sources appear spatially smeared, their origins can be clearly assigned to SI and SII and are in good agreement in terms of location and quality with other findings of pre-stimulus power changes (Haegens et al., 2010;van Ede et al., 2011;Haegens et al., 2011). In addition, the topographical representations imply weak activations in other cortical areas, presumably frontal and parietal areas (Fig. 3). However, none of these areas was found to be significantly activated in the source analysis. Reasons for the lack of significance might be that the effects in these areas were less strong than in the somatosensory areas or less consistent over subjects. Further studies need to unravel the contribution of non-sensory areas to the perception of simultaneity.

Notably, all reported effects were observed prior to onset of stimulation. We found pre-stimulus power in the beta (~20-30 Hz) band in both ipsilateral SI and SII to be linearly correlated to perceptions of non-simultaneity, i.e. veridical perception was highest for low pre-stimulus amplitudes. In addition, alpha-band power in ipsilateral SI revealed a quadratic relation to perception of simultaneity for condition SOA of 45 ms, i.e. veridical perception was highest for intermediate states of pre-stimulus alpha-power.

One potential concern in the interpretation of the results might be that the effects are caused by motor preparation. It is well known that alpha- and beta-band power in sensorimotor cortex can be modulated by motor preparation and execution (e.g. Hari and Salmelin, 1997). For several reasons, however, it is unlikely that our reported effects are related to motor preparation:

To minimize the influence of pre-movement power changes, we had included a jittered interval of 800-1200 ms after stimulus presentation before occurrence of the response cue. Subjects responded on average 539 ± 36 ms after the response cue. Thus, while subjects responded on average ~1500 ms *after* stimulus presentation, significant differences in

oscillatory activity were found ~0-400 ms *before* stimulus presentation. In contrast, no significant differences were found in the post-stimulus period prior to button presses. Consequently, we did not find any correlation between pre-stimulus power and reaction times (data not shown). Furthermore, response configurations were balanced across and within subjects so that the response hand and the site of first stimulation were unrelated. Taken together, due to the setup and the timing of the significant effects it is highly unlikely that the observed effects are due to motor preparation.

Recent studies investigated the influence of attention on pre-stimulus alpha- and beta-band power and their impact on tactile detection (Linkenkaer-Hansen, 2004; Jones et al., 2009, 2010; van Ede et al., 2010, 2011; Anderson and Ding 2011). These studies reported pre-stimulus effects to be lateralized contralaterally to the side of the stimulation. While in these studies stimulation was applied unilaterally and the side of stimulation was cued, we applied stimulation bilaterally and the site of the first stimulation was unknown (i.e. randomized from trial to trial). Therefore, we did not expect attention to be lateralized. In line with this, we found pre-stimulus power modulations bilaterally rather than lateralized. In addition, fluctuations of pre-stimulus power modulations do significantly affect perception of subsequent stimuli and that these effects are lateralized with respect to the site of the first stimulation. While there are also post-stimulus modulations of oscillatory activity in both hemispheres in response to bilateral stimulation, Fig. 3 reveals that these modulations do not differ for the two subjective reports. In other words, post-stimulus modulations of spectral power do not correlate with subjective perception of simultaneity.

In line with our findings, Jones et al. (Jones et al., 2010) reported a linear relationship for veridical perception of tactile stimuli and pre-stimulus alpha-/beta-band power. While Jones et al. explicitly studied the effects in SI, we observed the effects in both, SI and SII. While we and Jones et al. found a linear relationship, Linkenkaer-Hansen et al. (Linkenkaer-Hansen et al., 2004) reported a quadratic relationship between pre-stimulus beta-band activity in

sensorimotor areas and subjects' performance in a tactile detection task. A possible reason for these different findings of Linkenkaer-Hansen et al. might be that they used much broader time- and frequency bands for their analyses.

In addition, studies reported that intermediate amplitudes of pre-stimulus alpha-band oscillations in sensorimotor areas were optimal for perception of weak tactile stimuli (Linkenkaer-Hansen et al., 2004;Zhang and Ding, 2010). In line with these studies, we found a quadratic relationship between pre-stimulus alpha-band activity and simultaneity reports in sensors over SI, i.e. veridical perception of simultaneity was highest for intermediate states of pre-stimulus alpha-band activity. In contrast, a linear relationship between pre-stimulus alpha-band activity and detection probabilities of tactile stimuli has been reported by Jones et al. (Jones et al., 2010). Differences might be attributable to different tasks: While Jones et al. employed a cued attention task, subjects in our study were asked to report subjective simultaneity.

Several studies reported a correlation of pre-stimulus power and post-stimulus event related fields (ERFs) (Jones et al., 2009;Jones et al., 2010;Zhang and Ding, 2010;Anderson and Ding, 2011). In line with previous studies (Jones et al., 2009;Jones et al., 2010;Anderson and Ding, 2011), we found that trials with a high pre-stimulus power in the alpha-band revealed increased ERFs between 64-75 ms in ipsilateral SI, which is likely to represent the early evoked M20/M50 component to electrical or mechanical stimulation. Note that the time scale is always presented relative to the presentation of the *first* stimulus, while the reported effects are always in the hemisphere contralateral to the site of the *second* stimulation. Due to this shift in stimulation parameters, we expect ERFs to be shifted by 30 or 45 ms, respectively. In their computational study, Jones et al. (2009) suggested that an increased M50 component might be caused by greater levels of recruited inhibition, subsequently decreasing the effect of excitatory cells. Notably, we found an increased early ERF component only in ipsilateral SI and only for the condition SOA of 45 ms, suggesting that the proposed inhibition processes

induced by pre-stimulus alpha-band power influence only the (interhemispheric) processing of stimuli spaced 45 ms, but not stimuli spaced 30 ms. We suggest that with higher pre-stimulus power, i.e. with early inhibiting post-stimulus processes, the second stimulus might be processed less efficiently, leading to a lower temporal precision and thus more incorrect reports in the perception of simultaneity.

In addition, we found that trials with low pre-stimulus beta-band power revealed a lower M100 peak (at ~130 ms for SOA of 30 ms and at ~145 ms for SOA of 45 ms, see above for discussion of the temporal shift of the M100 component). Studies in human and non-human primates have demonstrated subsequent attenuation of ipsilateral somatosensory responses after contralateral tactile stimulation (Simões and Hari, 1999;Simões et al., 2001;Hlushchuk and Hari, 2006;Tommerdahl et al., 2006;Wühle et al., 2011;Reed et al., 2011) with the maximum attenuation for peaks at ~100 ms (Simões et al., 2001;Wühle et al., 2011). Our results suggest that the attenuation is meditated by pre-stimulus states of the beta-band. The correlation of beta-band power and ERFs was only found in sensors over ipsilateral SII, but not in SI. Since SII receives input from both body sides and bilateral SI, it is a likely candidate for integration of bilateral sensory input. One potential explanation might be that the stronger attenuation of the M100 component reflects stronger interhemispheric interaction which in turn is modulated by pre-stimulus states in the beta-band.

The abovementioned studies (Linkenkaer-Hansen et al., 2004;Jones et al., 2010) have argued that pre-stimulus alpha- and beta band activity influences the perception and detection of tactile stimuli. In line with this hypothesis, we suggest that subjective perception of simultaneity strongly depends on the veridical perception of the second stimulus. If pre-stimulus alpha- and beta-band activity is at optimal states, the likelihood to detect the second stimulus is high. This in turn promotes veridical perception of the two stimuli as temporally separate. We report beta-band effects in SI and SII, while most previous studies reported pre-stimulus effects mainly in SI (Linkenkaer-Hansen et al., 2004;Jones et al., 2009;Jones et al.,

2010;van Ede et al., 2010;Zhang and Ding, 2010;van Ede et al., 2011;Anderson and Ding, 2011). One crucial difference is that we used bilateral stimulation while the above-mentioned studies always used unilateral stimulation. Pre-stimulus activity in SII might therefore be relevant for bilateral integration of tactile stimuli or gating of information, but less crucial for unilateral perception. However it should be mentioned that pre-stimulus effects in the beta-band have been reported also in SII before (Linkenkaer-Hansen et al., 2004). Another crucial difference is that previous studies explicitly or implicitly incorporated a spatial attention task where subjects had to direct attention to one body side. It might be possible that spatial attention is more strongly confined to SI while bilateral interaction is more strongly relying on SI and SII.

Our main finding was that for both conditions (SOA of 30 and 45 ms) pre-stimulus beta-band activity was increased in SI and SII when stimulation was erroneously perceived as simultaneously. Several studies have reported involvement of beta-band oscillations in top-down modulations of attention or the perception of bistable stimuli (von Stein et al., 2000;Engel et al., 2001;Gross et al., 2004;Buschman and Miller, 2007;Kranczioch et al., 2007;Pesaran et al., 2008;van Elswijk et al., 2010). In their computational study, Jones et al (2010) suggested that pre-stimulus alpha-band activity modulates feed-forward, bottom-up processing while beta-band activity reflects both feed-forward and feedback modulations of cortical processes. Similarly, Engel and Fries (Engel and Fries, 2010) suggested that beta-band activity plays a role in endogenous top-down modulation of cognitive processes. According to this hypothesis, low amplitudes of beta-band oscillations should promote bottom-up, stimulus-driven processing while high amplitudes should increase the threshold for the responses to novel unexpected stimuli. In line with this hypothesis we suggest that fluctuations of pre-stimulus beta-oscillations determine the threshold for detecting stimuli. An increase of beta activity impairs bottom-up processing, therefore renders distinct temporal detection of first and second stimulus more unlikely und thus biases (incorrect) simultaneous

reports. Several studies also found inter-areal coherence mainly in the beta-band (Gross et al., 2004;Kranczioch et al., 2007;Hipp et al., 2011). A recent study found increased pre-stimulus beta-band activity in superior temporal gyrus associated with the (incorrect) perception of the bistable McGurk-illusion (Keil et al., 2011). We suggest that the perception of bistable stimuli (such as McGurk effect, attentional blink or our paradigm of simultaneity perception) is strongly influenced by ongoing network fluctuations in the beta band.

Similar to the attentional blink paradigm, in our paradigm the second of two subsequent stimuli is frequently misperceived. Both paradigms require thus a high temporal resolution of sensory perception. We propose that low states of beta-oscillations prior to the sensory stimulation promote a processing of stimuli while states of high beta amplitudes increase the threshold for sensory processing and make perception less accurate, especially for weak, near-threshold stimuli (Engel and Fries, 2010). In our case, less accurate (temporal) perception might bias simultaneity reports.

Prolonged SOA will lead to more veridical reports, i.e. prolonged SOA will decrease the degree of ambiguity or bistability (Fig. 2). Subjective perception for prolonged SOA thus might be less influenced by small fluctuations of ongoing fluctuations of oscillatory activity. Additional components might thus be necessary to further increase perceptual threshold. One component might be inhibited bottom-up processing of sensory input in SI by alpha-band activity (Jones et al., 2009). In line with this hypothesis, we additionally found increased pre-stimulus alpha-power for subjective perception of simultaneity in condition with SOA of 45 ms.

In summary, we found that pre-stimulus activity in the alpha- and high beta-band predicts the subjective perception of electrical simultaneity. We propose that states of pre-stimulus alpha- and beta-band activity determine perceptual detection thresholds for tactile and electrical stimuli (Engel and Fries, 2010). Modulations in the beta-band were found in SI and SII, while alpha-band modulations were found in SI. We suggest that these regions communicate in the

respective frequency bands and thus control bottom-up and top-down information flow. The results mount on recent evidence and extend findings emphasizing the role of pre-stimulus oscillatory activity for perception.

Captions

Fig. 1

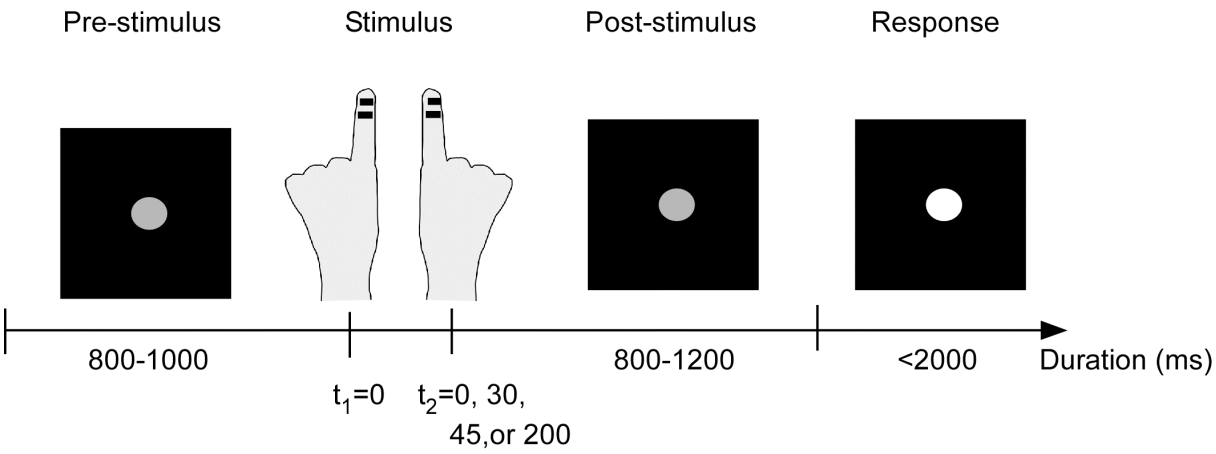


Fig. 1: Schematic illustration of the paradigm. Subjects fixated a central gray dot throughout the entire trial. After 800-1000 ms tactile stimulation was given to one index finger (right or left), followed by stimulation of the other finger after a randomized SOA (0, 30, 45, or 200 ms). After a jittered period (800-1200 ms), the luminance of the fixation dot increased and subjects reported their subjective perception of simultaneity by pressing a button, upon which the next trial began (indicated by a luminance decrease of the fixation dot).

Fig. 2

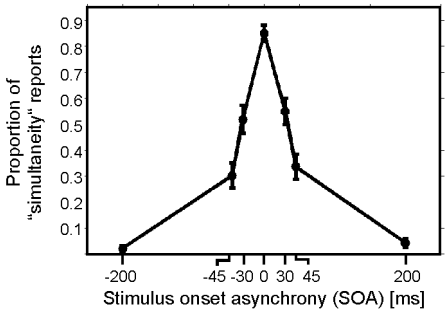


Fig. 2: Behavioral results presented as proportion of simultaneity reports depending on SOA of left and right index finger stimulations. Negative SOA indicate that stimulation was applied first to the left index finger. Data are presented as mean ± 1 SEM.

Fig. 3

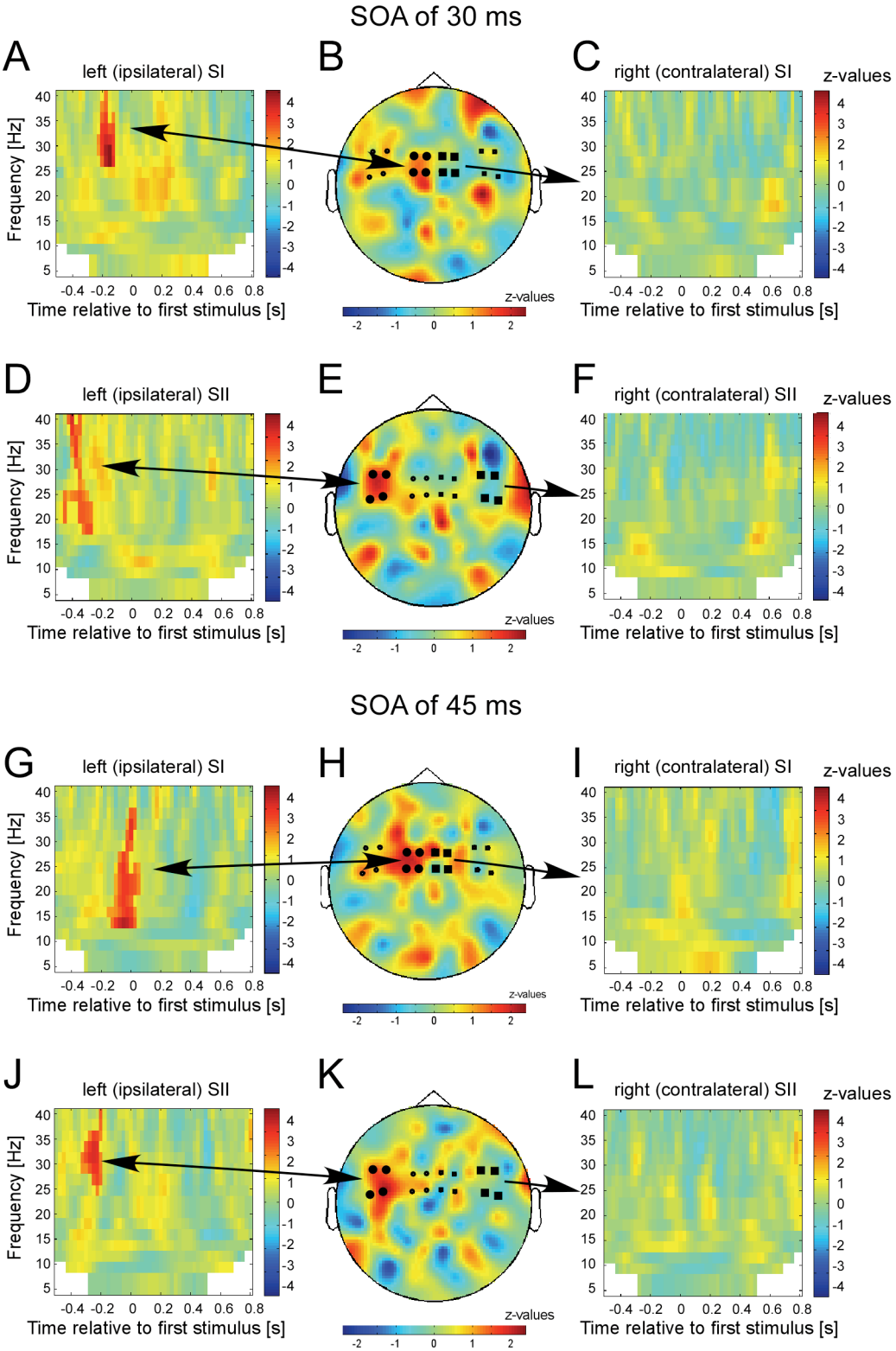

SOA of 30 ms
A
left (ipsilateral) SI
B
C
right (contralateral) SI
z-values
D
left (ipsilateral) SII
E
F
right (contralateral) SII
SOA of 45 ms
G
left (ipsilateral) SI
H
I
right (contralateral) SI
J
left (ipsilateral) SII
K
L
right (contralateral) SII
Frequency [Hz]
Time relative to first stimulus [s]

Fig. 3: Results of the statistical comparison of trials with subjective simultaneity vs. non-simultaneity for conditions SOA 30 ms (A-F) and SOA 45 ms (G-L) for different sensor groups:

A) TFR for the four sensors over the left (ipsilateral) primary somatosensory cortex (SI) as indicated by the larger black circles in B). Z-values in non-significant pixels are lowered by 60% in order to highlight significant clusters. Colorbars represent z-values. Positive z-values indicate higher power if subsequent stimulation was misperceived as simultaneously. B) Topographical representation of the significant cluster as highlighted in A). Only time-frequency samples that correspond to the statistically significant time-frequency clusters in A) were averaged to generate the topographical representation (see Methods for details). C) TFR for the four sensors over the right (contralateral) SI (as indicated by larger black squares). No significant clusters were found. D) Same representation as in A) but for four sensors over left (ipsilateral) secondary somatosensory cortex (SII). E) Topographical representation for the significant cluster as highlighted in D). F) TFR for the four sensors over the right (contralateral) SII (as indicated by larger black squares).

G-L) Same representation as in A-F) but now for condition SOA of 45 ms.

Fig. 4

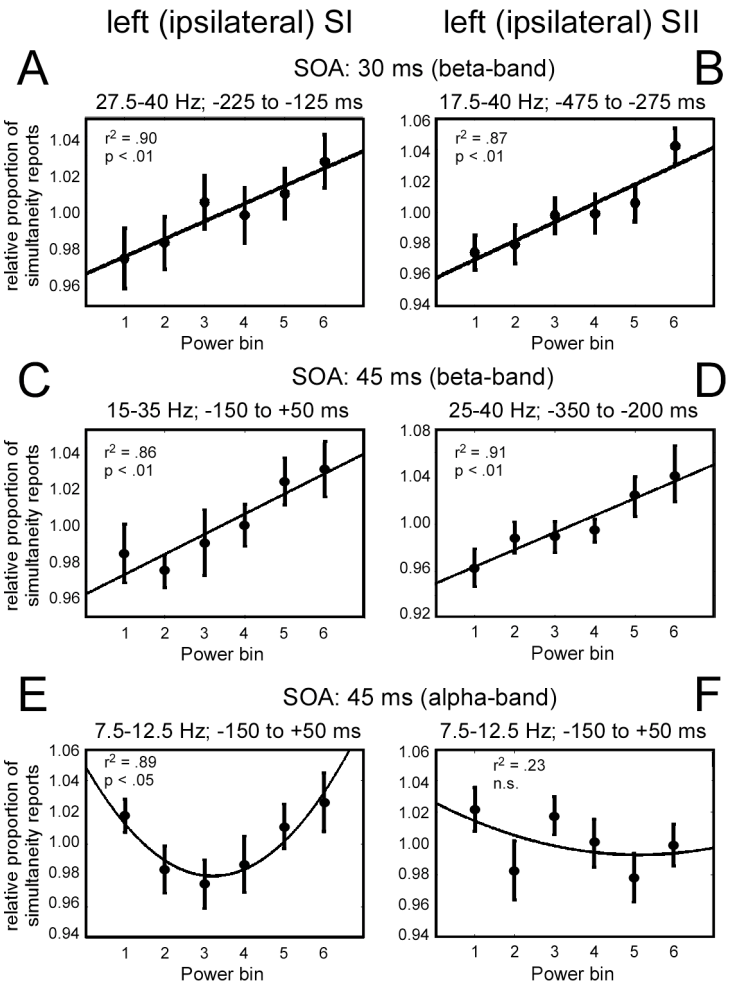


Fig. 4: Regression analyses of the dependence of subjective perception on pre-stimulus oscillatory activity for the four significant clusters in the beta-band (as shown in Fig. 3) and for the alpha-band. The exact time-frequency bands to determine averaged pre-stimulus power bins are based on significant clusters in Fig. 3 and are presented at the top of each figure. A) Results for the significant cluster in the beta-band for condition SOA 30 ms in sensors over ipsilateral SI (as highlighted in Fig. 3A). B) Same analysis as in A, but for the significant cluster in sensors over SII (as highlighted in Fig. 3D). C-D) Same analysis as in A-B), but for the significant clusters in the beta-band for condition SOA 45 ms (as highlighted in Fig. 3G and 3J). For all regression analyses a significant linear relationship was found ($p<.01$). E) Same analysis for the alpha-band for condition SOA 45 ms in sensors over

ipsilateral SI. A significant quadratic relationship was found. F) Same analyses as in E) but for sensors over SII. No significant relationship was found.

Fig. 5

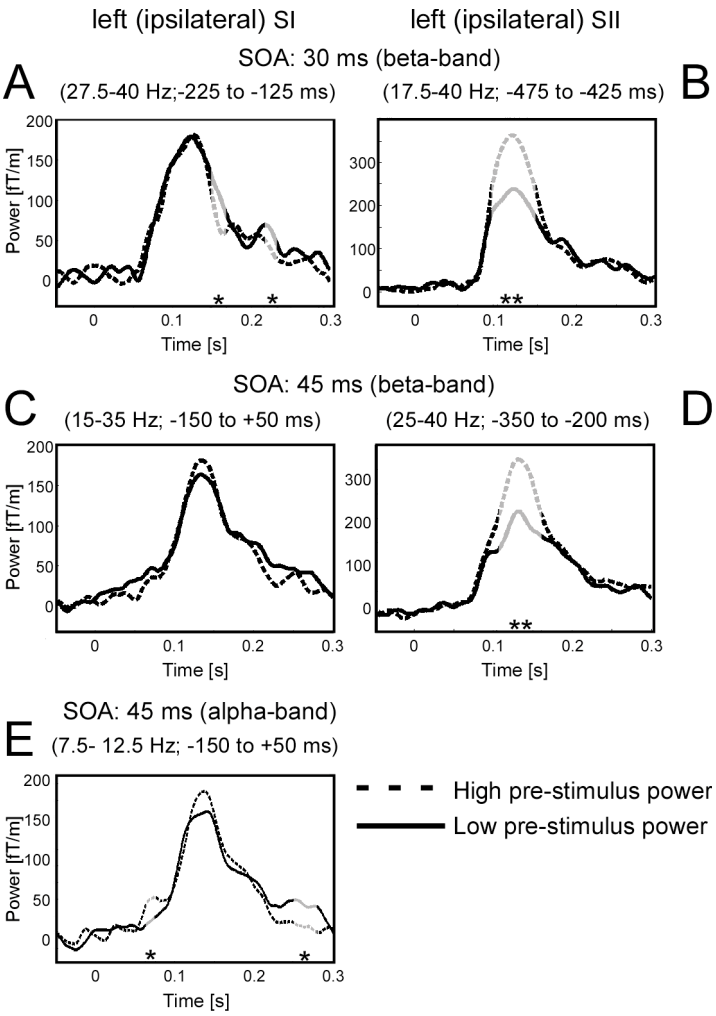


Fig. 5) Dependence of post-stimulus ERF amplitudes on pre-stimulus power for the four significant clusters in the beta-band (as shown in Fig. 3) and for the alpha-band. The exact time-frequency bands to determine averaged pre-stimulus power bins are based on significant clusters in Fig. 3 and are presented at the top of each figure. A) Results for the significant cluster in the beta-band for condition SOA 30 ms in sensors over ipsilateral SI (as highlighted in Fig. 3A). B) Same analysis as in A, but for the significant cluster in sensors over SII (as highlighted in Fig. 3D). C-D) Same analysis as in A-B), but for the significant clusters in the beta-band for condition SOA 45 ms (as highlighted in Fig. 3G and 3J). E) Same analysis for the alpha-band for condition SOA 45 ms in sensors over ipsilateral SI. Significant differences (* $p<.05$; ** $p<.01$) are indicated by grey shaded areas.

Fig. 6

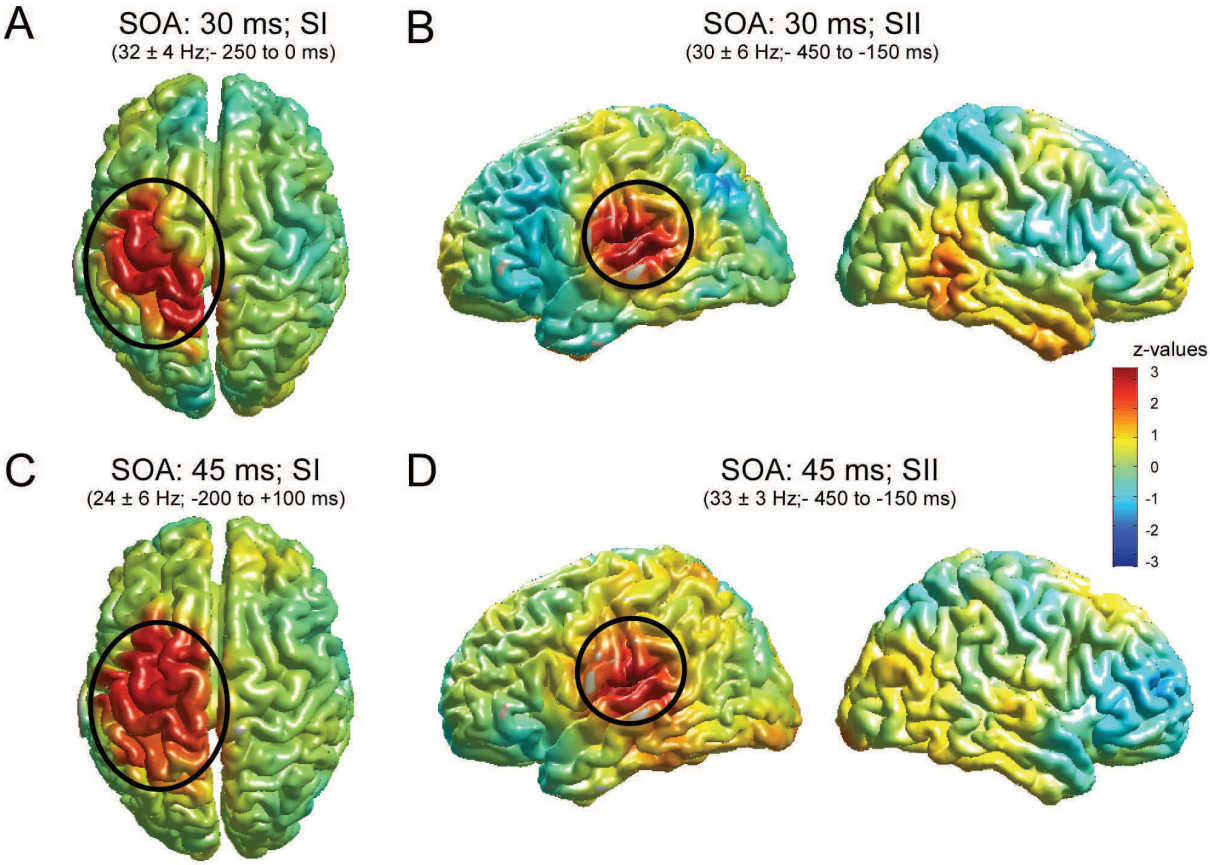


Fig. 6: Source analysis of significant clusters as found in Fig. 3. The exact time-frequency bands used for source reconstruction are based on significant clusters in Fig. 3 and are presented at the top of each figure. A) Results for the significant cluster in the beta-band for condition SOA 30 ms (as highlighted in Fig. 3A). Z-values in non-significant regions are lowered by 60% in order to highlight significant clusters. Additionally, significant clusters are highlighted by ovals. B) Same as in A), but for beta-band effect as highlighted in Fig. 3D. Left column: view of the left hemisphere, right column: view of the right hemisphere. C-E) Same as in A-C) but for beta-band effect in SOA 45 ms (as highlighted in Fig. 3G and 3J). The colorbar applies to all figures.

Fig. S1

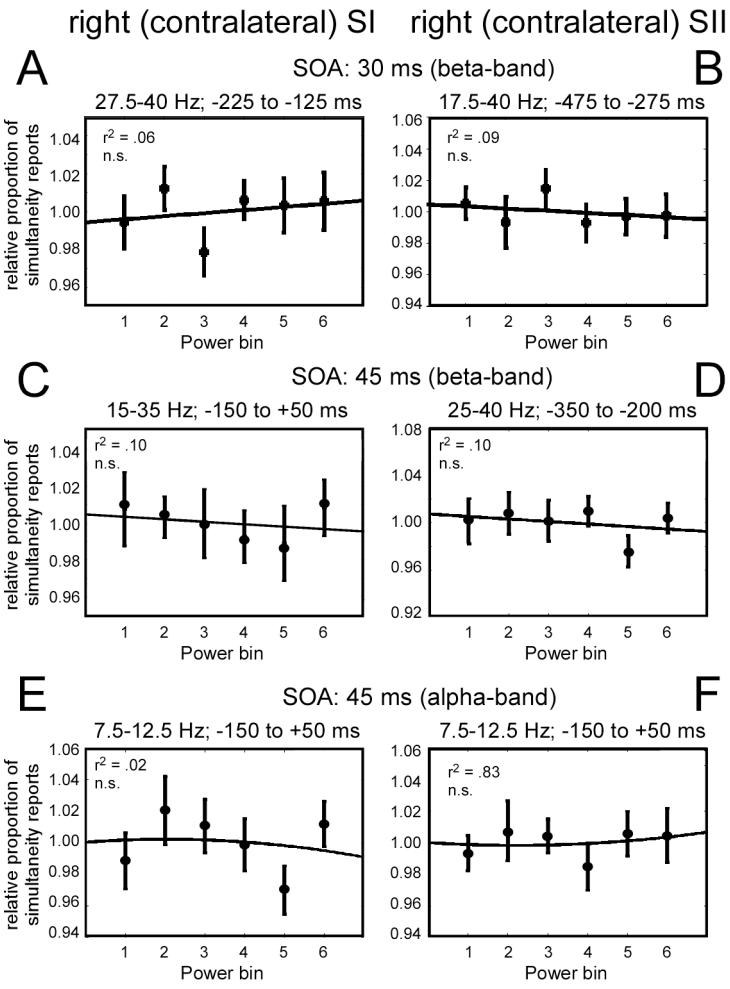


Fig. S1: Same analyses as in Fig. 4 but for contralateral sensors. No significant correlations were found.

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