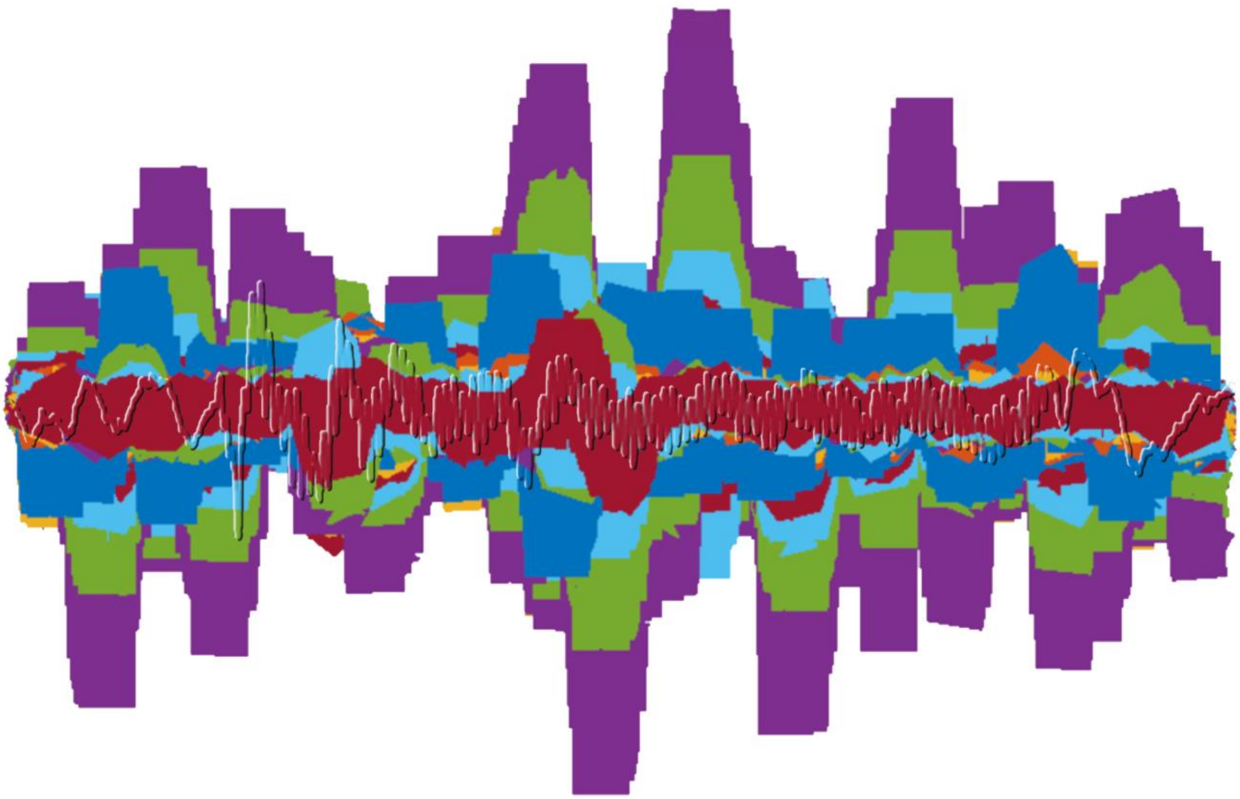
Frequency Tagging at High Frequencies in Downstream Areas Under Influence of Attention



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Abstract

Frequency tagging can be used to study the downstream flow of information from visual cortex to higher order areas. This flow of information is modulated by attention, which might be mediated by cross-frequency coupling of low and high frequencies. To investigate the influence of low frequencies on stimulus processing at high frequencies mediated by attention, the feasibility of using frequency tagging at high frequencies in downstream areas was investigated. Furthermore, the effect of spatial and object-based attention on the magnitude of the elicited response by the frequency tags was examined. Stimuli of faces and houses were presented at high frequencies of 63 Hz and 78 Hz during a catch trial flip detection task. Results show that spatial attentional modulation increased activity in occipital cortex contralateral to the attended stimulus. When examining the effect of object-based attention, no interpretable activity patterns were observed. As a result of interaction between spatial and object-based attention, fusiform gyrus and parahippocampal gyrus in the right hemisphere, but not left, showed enhanced activity in tagged frequencies. With frequency tagging at high frequencies, it will be possible to investigate communication between regions, information processing by phase-to-power cross-frequency coupling under influence of spatial as well as object-based attention1.

Key words: *Frequency tagging; spatial attention; object-based attention; FFA; PPA; magnetoencephalography.*

Introduction

Cognitive neuroscience is shifting from primarily identifying brain regions, to studying the interaction between regions. Communication between brain areas is thought to be reflected by synchronous firing of neurons (Zandvakili and Kohn, 2015). Likely candidates for representing synchronized activation are neuronal oscillations (Fries, 2005). By comparing frequency and relative phase in different brain areas, communicating regions can be identified. Different frequency bands have been associated with various cognitive functions, such as theta band activity (4-8 Hz) in working memory (Hsieh & Ranganath, 2014), alpha band activity (8-13 Hz) in attention (Hanslmayr et al., 2012), and gamma band activity (30-80 Hz) for conscious visual processing (Melloni et al., 2007; Singer & Gray, 1995). It has been suggested that coupling of oscillations with different frequencies plays an important role in the transmission of information (Canolty & Knight, 2010). For example, alpha waves may phasically modulate stimulus processing as reflected by cross-frequency coupling of gamma power to alpha phase. That is, gamma activity is inhibited during the alpha cycle except at the troughs, creating a window for active processing (Osipova, Hermes & Jensen, 2008).

Frequency tagging serves as useful method to study the flow of information in the brain. In frequency tagging, a stimulus is presented in a rapid on-and-off fashion to elicit a response at the same frequency as the stimulus presentation rate. This response is usually observed in occipito-temporal regions (Vialette et al., 2010). Furthermore, areas processing specific features of the stimuli outside of early visual areas can be frequency tagged as well. For example, emotionally valent stimuli presented at 13 Hz evoke higher temporo-frontal alpha activity than neutral images, indicating increased activity in emotional processing areas (Kemp et al., 2002).

The amplitude of evoked activity by frequency tagged stimuli is under the influence of spatial as well as object-based attention. When manipulating spatial attention, greater amplitude was observed for attended than unattended stimuli in occipital cortex (Morgan, Hansen & Hillyard, 1996; Müller et al., 2006). Similarly, by presenting spatially overlapping faces and houses in frequencies of 1.5 and 2 Hz, attention on either faces or houses has been found to modulate the amplitude of activity in respectively the fusiform face area (FFA) and the parahippocampal place area (PPA; Baldauf and Desimone, 2014).

These findings correspond to functional magnetic resonance imaging (fMRI) reserach on object-based attention (O'Craven, Downing & Kanwisher, 1999; Serences et al., 2004)

Responses to flickering stimuli have been observed in visual cortex up to 100 Hz (Herrmann, 2001). However, many studies use flickering stimuli in the alpha range, since this frequency range elicits the largest response (Vialette et al., 2010). It is likely that in this case endogenous alpha oscillations are being 'entrained', i.e. locked, to the visual stimulus (Spaak et al., 2014). This is problematic as alpha oscillations have been shown to constitute top-down functional inhibition causing a potential inhibition of stimulus processing (Jensen et al., 2012). For this reason and when studying the role of lower frequency oscillations in stimulus processing with frequency tagging, high frequency stimulus presentation should be used. Studies thus far using frequency tagging at high frequencies make use of computer monitors that have been limiting in stimulus presentation rate (Krolak-Salmon et al., 2002; Lyskov, 1997; Williams et al., 2004).

This study aims to pave the way for studying functional connectivity between brain areas and the role of lower frequency oscillations in information processing. We investigated the feasibility of using frequency tagging at high frequencies as a method to study the downstream flow of information from early visual cortex to higher level areas using a newly developed projector that is capable of presenting stimuli at up to 1440 Hz. To this end we investigated the effects of spatial attention on stimulus processing in early visual cortex. We expected greater activity in the hemisphere contralateral to the attended stimulus. Second, we investigated whether attending to faces or houses would evoke differential activity in the respective face and house processing regions at the tagged frequency. We hypothesized that attention on either faces or houses would increase the activity in the respective processing areas, FFA or PPA.

Materials and Methods

Subjects

Eight right-handed, healthy subjects (mean age, 22.6 ± 1.7 years, 5 female) participated in the study. All participants had normal or corrected-to-normal vision. Participation in the study was compensated by a monetary reward. The type of study was approved by the local ethics committee (CMO region Arnhem/Nijmegen) and was performed according to the declaration of Helsinki.

Procedure

Participants were checked on metal objects on their body and clothing, and were given metal free clothing to change into when necessary. Subsequently, the shape of their head was measured using a 3D digitizer (Polhemus, Colchester, USA). Before the start of the experiment, the eye-tracker was calibrated and participants were familiarized with the task. On a separate occasion, an anatomical T1 MR scan was obtained.

MEG measurement

Magnetic fields induced by ongoing brain activity were measured using a whole-head MEG system with 275 axial gradiometers and a sampling rate of 1200 Hz after a low-pass hardware filter of 300 Hz was applied (CTF MEG systems, Coquitlam, Canada). During the experiment, the head position of the participants was continuously monitored and corrected when necessary (Stolk et al., 2013).

MRI

For the structural magnetic resonance imaging (MRI) scan, a 3T MAGNETOM Skyra (Siemens Healthcare, Erlangen, Germany) was used to acquire an anatomical T_1 weighted image (TR = 2300 ms, TE = 3.03 ms, TI = 1100 ms, 1.0 mm^3 isotropic resolution, 192 sagittal slices).

Apparatus

To present the experimental paradigm, a GeForce GTX960 2GB graphics card with a refresh rate of 120 Hz was used in combination with a PROPixx DLP LED projector (VPixx Technologies Inc., Saint-Brunode-Montarville, Canada). This specific projector was used as it is able to achieve a presentation rate of 1440 Hz. The projector achieves this high rate by dividing the frames received from the graphics card into four quadrants and presenting all quadrants sequentially. Although the resolution of the presented image is hereby reduced, the resulting presentation rate is four times higher (from 120 Hz to 480 Hz). To achieve the maximum presentation rate, each quadrant is transformed into three sequentially presented grayscale images. The grayscale values for each image are contained in the red, green, and blue color values of the original quadrant. This further multiplies the presentation rate by three achieving a maximum rate of 1440 Hz. The projector projected onto a screen with a distance of 75 cm from the participant. A button box was placed at the right hand of the participant to record the responses. To ensure fixation and to monitor eye blinks, an Eyelink 1000 eyetracker (SR Research, Ottowa, Canada) was used.

Stimuli

Twenty grayscale images of neutral faces and houses were adapted with SHINE Toolbox for MATLAB (Willenbockel, 2010) to ensure equal luminance for all images. Background and average luminance of stimuli was specified at half of full black. The presentation rates of the two images were set at 63 Hz and 78 Hz, based on the limitations in frequency of frequency tagging (Hermann, 2001), and such that the two frequencies are close enough to each other to have a comparable magnitude in power and far apart enough to be distinguishable.

Experimental paradigm

During MEG data acquisition, participants performed a vertical flip detection task to ensure attentional engagement to the stimuli (Figure 1). Participants had to fixate on a cross in the middle of the screen during the entire experiment. A cue was presented in order to direct attention to the left or right hemifield and indicating the picture that was most likely to be flipped upside down. Subsequently, a face and a house were presented as circular images with a diameter of 13 cm (10°). The images were presented in the left and right in the lower visual field with the centre of the image at 8.3° of eccentricity. The luminance of both images was sinusoidally modulated at respectively 63 Hz and 78 Hz. Presentation at such high frequencies results in a static percept. The position (left/right) and the frequency (63 Hz/78 Hz) of the faces and houses as well as the cued hemifield (left/right) varied in a semi-random manner such that all combinations of conditions occurred the same amount of times per block. Divided over 4 blocks, there were 640 trials per participant of which 160 trials were catch trials.

In this 25% of the trials, one of the two pictures was flipped vertically at the end of the trial. Subsequently, in 80% percent of these catch trials the spatial cue was congruent with the position of the picture that was flipped. Participants were asked to respond by pressing a button with their right index finger when the left image was flipped and pressing a button with their right middle finger when the right image was flipped. In the remaining 20% of the catch trials, the flipped picture was incongruent with cue and had to be ignored. Depending on the participant's performance, the presentation duration of the flipped stimulus was adjusted using a QUEST adaptive staircase procedure (Watson & Pelli, 1983) aimed at achieving 80% correct responses. Starting at 10 ms, presentation duration of the flipped image varied between 2-30 ms. After stimulus presentation, participants had 0.6 s to respond followed by a rest period. In case a cued image was flipped and the corresponding button was pressed, 'CORRECT' was presented during the rest period. When the wrong button was pressed after a cued flip, 'INCORRECT' was displayed and when a cued flip was not followed by a response, 'MISS' was displayed. Furthermore, when an uncued image was flipped and was followed by a button press, 'INCORRECT' was presented. When an uncued flip was ignored, 'CORRECT' was displayed. The trials were ordered semi-randomly, such that all conditions were intermixed, but that one trial in every four trials was a catch trial. The task was presented using MATLAB 2014b (Mathworks Inc, Natrick, USA) and the Psychophysics Toolbox 3.0.11 (Kleiner et al., 2007).

Data analysis

The MEG data were analyzed using Fieldtrip Toolbox version 20160605 (Oostenveld et al., 2011) in combination with MATLAB R2014b (Mathworks Inc, Natrick, USA). Preprocessing was performed by selecting epochs of 1 s before stimulus onset until 1.5 s of stimulus presentation. The signal was down-sampled to 300 Hz and a high-pass filter of 2 Hz was applied to remove slow drifts. SQUID jumps were automatically discarded by rejecting trials exceeding a z-score of 150. Muscle artifacts were discarded by automated artifact recognition based on z-transformed, band-pass filtered data between 110-140 Hz.

Trials exceeding a z-score of 4 were discarded. This resulted in the average rejection of 24% (SEM 3.9%) of the trials for every subject. Artifacts caused by eye blinks were removed with independent component analysis (Bell & Sejnowski, 1995; Jung et al., 2000).

Frequency analysis on the sensor level data was performed on the data from 0.5 to 1.5 s after stimulus onset. The first 0.5 s after stimulus onset were excluded from analysis to avoid possible interaction with the initial evoked response and to let the oscillation reach a steady state (Keil et al., 2003). First, synthetic planar gradients were calculated from this epoch. This increases the comprehensibility of the sensor-level data, as activity is maximal above its source (Bastiaansen & Knösche, 2000). Subsequently, a Fast Fourier transform was performed on the data multiplied with a Hanning taper, resulting in a frequency resolution of 1 Hz.

Source localization was performed using a Dynamic Imaging of Coherent Sources (DICS) beamformer approach (Groß et al., 2001). Individual single-shell models were constructed from structural MRIs combined with the digitized 3D skull surface. Brain volume was divided into a 5 mm^3 grid and normalized to MNI standard space (Montreal Neurological Institute, Montreal, Canada). For every analysis, a common filter based on the all included conditions was created. Separate analyses were performed to localize 63 and 78 Hz activity generated by faces or houses during the epoch of interest. Eventually, the separate frequency conditions were combined by averaging over power for statistical analysis. For both frequencies, a Hanning taper was used.

Spatial attention

Attentional modulation indices of relative power were used to create a contrast between conditions, where activity related to attentional modulation in an experimental condition is represented by either positive or negative values and areas not under influence of attention would have a value of zero. For spatial attention, trials including attention to the right and to the left were averaged separately for every participant and the frequency power (*P*) was calculated for 63 and 78 Hz. To determine where attention on the left visual hemifield would cause greater brain activation than attention on the right hemifield and vice versa, for every sensor (on the sensor level) and for every voxel (on the source level), attention to the right was subtracted from attention to the left. To normalize this difference over subjects, the subtraction was divided by the summation of the two attentional conditions. Hence, the attentional modulation index for spatial attention was calculated as:

$$\frac{P_{attend\ left} - P_{attend\ right}}{P_{attend\ left} + P_{attend\ right}} \tag{1}$$

Object-based attention

For object-based attention, a similar attentional modulation index was created based on attention on object type rather than position. Trials including attention on faces and attention on houses independent of being presented left or right were averaged for each participant. In order to determine in which regions activity would be greater with attention directed to faces or houses and vice versa, activity in trials including attention on houses were subtracted from trials including attention on faces. Subsequently, to normalize, this was divided by the summation of the two conditions. The attentional modulation index for object-based attention was calculated as:

$$\frac{P_{attend\ face} - P_{attend\ house}}{P_{attend\ face} + P_{attend\ house}} \tag{2}$$

Object-spatial attentional interaction effect

To investigate the lateralization of attentional effects, i.e. in which hemisphere is the influence of spatial attention larger on object-based attention, a more complex attentional modulation index was used. First, average activity was calculated separately for the trials containing attention on a face presented left and trials containing attention on a house presented left. The averages were subtracted from each other to determine where activity is greater for a face than a house shown on the left. Subsequently, this was divided by the summation of the two conditions for normalization purposes. The same calculation is applied on the averages of trials containing attention on faces and houses in the right hemifield. In succession, the index for attention on the right was subtracted from the index for attention on the left. The total attentional modulation index that was used to calculate the interaction between spatial and object-based attention was:

$$\frac{P_{attend\ face\ left} - P_{attend\ house\ left}}{P_{attend\ face\ left} + P_{attend\ house\ left}} - \frac{P_{attend\ face\ right} - P_{attend\ house\ right}}{P_{attend\ face\ right} + P_{attend\ house\ right}} \qquad (3)$$

Statistical Analysis

Mean frequency power of 63 and 78 Hz was calculated by averaging over trials and subjects over an epoch of 0.5 – 1.5 s. Statistical analysis on the sensor level and source level was performed with a Monte Carlo permutation dependent samples t-test and corrected for multiple comparisons across sensors or voxels using false discovery rate (Nichols & Holmes, 2001; Genovese, Lazar & Nichols, 2002). A significance level of $p<0.05$ (two tailed) was used for all analyses.

Results

To test whether frequency tagging at high frequencies can be used to investigate spatial attention and object-based attention, we manipulated attention to the right and left hemifields, where a face or house was presented with two different frequencies (see Figure 1).

Behavioural data

Behavioural results show that participants responded correctly for 76% (SEM, 2%) of the trials. The false alarm rate was 32% (SEM, 6,9%) for the trials where the image flipped incongruent with the cue and should have been ignored. Figure 1A shows the response rate to targets, i.e. flips congruent with the cue and false alarm rate. It should be considered that the hit rate for congruent targets was targeted at 80% by a QUEST adaptive staircase procedure (Watson & Pelli, 1983). Nevertheless, the percentage button press for uncued targets was significantly lower ($t_7 = 88.5$, $p < 0.001$), indicating that the distractor at the incongruent position was less attended. The reaction times for hits for congruent targets (mean = 0.39 s, SEM = 0.008 s) were about the same as for incongruent distractors (mean = 0.4 s, SEM = 0.013 s; $t_7 = 2.5$, $p = 0.40$).

Frequency tagging

Frequency tagging will elicit activation at the same frequency as the presentation rate of the stimulus. To validate this method, a visualization of the event related field (ERF) is shown for posterior regions, as the stimulus onset is locked to phase. Figure 3 shows the ERFs averaged over sensors at the right back quadrant, participants, and trials including attention on stimuli presented at 63 Hz (Figure 3A) or 78 Hz (Figure 3C) in the left visual field. For visualization purposes, a band stop filter was applied for both ERFs on the frequency that was not of interest, so no crossover of the evoked activation from the other hemisphere would be visible. Hence, for the ERF of 63 Hz, a band stop filter of 75-80 Hz was applied and for the ERF of 78 Hz, a band stop filter of 60-65 Hz was applied. During the trials, 0.5 second after stimulus onset the evoked response reaches a steady state. Spectral analysis was performed on the ERFs to confirm that the observed evoked oscillations had the same frequency as the presentation rate of the stimuli (Figure 3B,D). This was done by contrasting the frequency power of 1 s pre stimulus onset with

0.5-1.5 s post stimulus onset of the ERF, such that (P_{post} – P_{pre})/P_{pre}. Spectral power was significantly greater for 63 Hz in trials attending 63 Hz than 78 Hz in trials attending 78 Hz (t_{14}=4.2 p<0.001).

Spatial attention

The main interest of this study was to look at spatial attention and object-based attention in frequency tagging at high frequencies. To examine whether the tagged frequencies were modulated by attention, relative spectral power between conditions was calculated from the raw trial data with the attentional modulation index for spatial attention (Eq. 1). With this index, regions or sensors where activity is greater for attention to the left visual field compared to right will get a positive value and negative values will be assigned to regions or sensors where activity is greater for attention to the right compared to left.

In figure 4A, clear negative peaks were observed for 63 and 78 Hz in left occipital sensors. Figure 4B shows for right occipital sensors a clear positive peak at 63 Hz, whereas the peak at 78 Hz is absent. These figures indicate that the activity evoked by the frequency tags are modulated by spatial attention in occipital regions.

The same attentional modulation index (Eq. 1) that was used to calculate relative frequency power at 63 an 78 Hz at the sensor level. Subsequently the average relative power of the two frequencies was calculated. A clear modulation of power by attention was observed in left and right occipito-temporal sensors. The t-values of the relative power are depicted in Figure 4C. Sensors with positive values are more engaged for attention to the left hemifield than to the right and sensors with negative values are more engaged for attention on the right hemifield compared to attention to the left. The left hemisphere shows increased activity for attention to the right and the right hemisphere shows increased activity for attention to the left.

On the source level (Figure 4D), the attentional modulation index was averaged over both frequencies for every voxel in a similar manner. A clear modulation by attention was observed in left and right visual cortex as well. Statistical comparison of the modulation index over subjects in the peak voxel of the left and right occipital cortex of the group average shows a trend towards a stronger modulation of spatial attention in the right hemisphere (t_{14}=1.6, p=0.06).

Object-based attention

Next to spatial attention, object-based attention towards frequency-tagged images of faces and houses was manipulated attempting to elicit dissociable activity in face and house related regions such as fusiform cortex and parahippocampal cortex, respectively. The attentional modulation index used for object-based attention (Eq. 2) results in positive values for areas where activity would be greater for faces than for houses. Vice versa, negative values are assigned to areas where activity would be greater in response to houses than for faces. With this attentional modulation contrast, on the sensor level, attention to faces showed right lateralized activation in occipito-temporal sensors and attention to houses resulted in activation over temporal and frontal regions. On the source level, areas where activity was modulated by attention to faces were the right occipital lobe and an area around the parieto-occipital fissure (Figure 5B). Areas where activity was modulated by attention to houses include right mid-temporal and left pre-central regions.

Object-spatial attentional interaction effect

The attentional modulation index to investigate the interaction between spatial and object-based attention (Eq. 3), to find out in which hemisphere the influence of spatial attention is larger on object-based attention, was calculated this way to ensure that every experimental condition would be represented individually and distinguishable in each hemisphere. The expected result of this index would be due to the contralateral hemispheric processing of the visual field. As a result of the attentional modulation index (Eq. 3), stimuli attended in the left visual field are represented in the right hemisphere by positive values for regions that are more engaged in face processing than house processing and by negative values for regions that are more engaged in house processing compared to face processing. Similarly, stimuli that were attended in the right visual field are represented in the left hemisphere by negative values for areas more active to faces than houses and by positive values for areas with enhanced activity for houses compared to faces.

On the source level, with the spatial-object interaction, only in right fusiform cortex and right parahippocampal cortex expected activation patterns were observed (Figure 6). The right fusiform cortex showed positive values (MNI = [28, -26, -28]) and the parahippocampal cortex showed negative values (MNI = [28, -58, -6]).

Discussion

With this study we investigated the feasibility of frequency tagging at high frequencies as a method to study the downstream flow of information from early visual cortex to higher level processing areas. We examined the modulatory effects of spatial attention in early visual cortex. Furthermore, we examined the role of object-based attention on the propagation of information to downstream areas. Finally, the interaction between spatial attention and object-based attention was examined. Results for spatial attention confirm the hypothesis that attention would cause increased activation in the hemisphere contralateral to the attended stimulus. For object-based attention, no attentional effects of increased activity were observed in the FFA and PPA. Interaction between spatial and object-based attention, however, showed right lateralized increased activation in parahippocampal gyrus for attention on houses and fusiform gyrus for attention on faces. Thus, when attending to a face compared to a house in the left visual field, the right FFA is more engaged than the left FFA when attending to a face compared to a house in the right visual field.

Spatial attention has an amplifying effect on the amplitude of the response to a flickering stimulus of frequencies up to 27.8 Hz (Müller & Hillyard, 2000; Müller et al., 2006; Toffanin et al., 2009). However, not many frequency tagging studies use higher frequencies, since this the spectral power of the evoked response decreases with higher frequencies (Müller, 1997). To our knowledge, this is the first study showing that location specific attentional modulation has an effect on the response amplitude to flickering stimuli presented at high frequencies. This implies that frequency tagging can be used for the analysis of the processing of perceived static images. Furthermore, the role of low frequency oscillations in spatial attention can be investigated without interference or entrainment elicited by the frequency tag in the low frequency bands.

For higher levels of attention, that is feature-based and object-based attention, this is also the first study to present that frequency tagging of objects at high frequencies is possible in downstream areas. Previously, it has been shown that activity in the tagging frequency travels to more temporo-frontal areas for emotionally valent stimuli, indicating emotional processing of the frequency tag in related areas (Keil et al., 2005; Kemp et al., 2002). However, source reconstruction was not included in the analysis. Furthermore, our results show that attentional modulation has an effect on the response amplitude in tagged areas. Preceding studies have made an effort in frequency tagging of FFA and PPA with lower frequencies. An EcoG study attempted to frequency tag FFA by presenting faces at the 70 Hz monitor refresh rate, but only observed a transient evoked response (Krolak-Salmon et al., 2003). Perhaps, the response in the fusiform gyrus was not time-locked and the oscillatory response averaged out. Unfortunately, no frequency analysis was done to examine this. An electroencephalography (EEG) study, however, was successful in frequency tagging the fusiform gyrus by presenting faces at 30-40 Hz with a binocular rivalry paradigm including faces and non-faces. The area active for faces was reconstructed with source modeling of the 30-40 Hz activity and the active area was validated with fMRI results (Wang et al., 2006). More recently, it has been shown that object-based attention can modulate response amplitude for frequency tagged faces and houses in FFA and PPA respectively. Low frequencies of 1.5 and 2 Hz were used and regions of interest (ROI) were obtained by fMRI for faces and houses. Attentional modulation of activity in the FFA and PPA was mediated by the inferior frontal junction (Baldauf & Desimone, 2014). These studies show that frequency tagging is a useful method to investigate where information is processed and to study object-based attention.

The before mentioned studies use fMRI data for ROI analysis and source validation. In the current study, as a result of cross-subject variation, detecting significant activity patterns from all face selective regions averaged over subjects might need a more thorough and specific analysis. First, exact FFA location can differ between subjects (Aguirre, Singh & D'Eposito, 1999), or second, FFA can be absent in one hemisphere (Wojciuilik, Kanwisher & Driver, 1998). Finally, there can be several face selective regions in both right and left fusiform cortex, which can be irregularly shaped (Weiner & Grill-Spector, 2012). To account for this heterogeneity, a per subject between-trials analysis, rather than a between-subject analysis, could be executed as an alternative to the use of fMRI data.

The presented results show that frequency power as the result of frequency tagging at high frequencies is under the influence of spatial and object-based attention. It is suggested that attention is modulated by means of functional inhibition of the unattended localities or objects. This functional inhibition could be represented by activity in the alpha band (Jensen & Mazaheri, 2010). In addition, the coupling of high frequency power to the phase of lower frequencies such as alpha might play a role in visual awareness (Schroeder & Lakatos, 2009, Fiebelkorn et al, 2013), visual processing (Voytek et al., 2010), and working memory (Bonnefond & Jensen, 2015; Lisman & Jensen, 2013) by gating the stream of information represented by high frequency oscillations in the trough of the slow frequency oscillation. Frequency tagging with frequencies in the lower frequency bands might interfere with endogenous ongoing activity by entrainment (Spaak et al., 2014). In order to study effects of cross-frequency coupling between the power of higher frequencies to the phase of lower frequencies with frequency tagging on the propagation of information, high frequencies must be used. It provides a method to investigate how the power of the tagged frequency is influenced by the phase of slower oscillations. However, this method could be limiting by interfering with the oscillations representing stimulus processing by entrainment in the gamma band. In sum, frequency tagging can be used to investigate how mechanisms of attention control the flow of information by cross-frequency coupling to downstream areas.

Lateralization of object selective areas and attentional networks

The results of the interaction between spatial and object based attention only show right lateralized attentional effects in the parahippocampal and fusiform cortex. For the FFA, it has been shown with fMRI that its activity in a response to the presentation to faces is generally lateralized to the right (Dien; 2009; Puce et al., 1995). Furthermore, Baldauf & Desimone (2014) observed right lateralization for attentional modulation in the FFA as well. Likewise, the observed results may be a consequence of the biased activation pattern of bilateral face selective areas.

However, in the interaction of object-based attention with spatial attention, we found right lateralization of the PPA as well. Baldauf & Desimone (2014), however, observed the opposite effect and found stronger activation mediated by attention in the left PPA. In line with these findings, memory studies suggest that face encoding predominantly occurs in the right FFA and encoding and retrieval in the left FFA. For the PPA this activity pattern is reversed. Encoding predominantly occurs in the right PPA and encoding and retrieval in the left PPA (Prince, Dennis & Cabeza, 2009). Since there is only passive encoding in faces and houses in this study, it can be expected that right FFA and left PPA show higher activity. This is not in line with our results. However, the right lateralized FFA and PPA activity was the outcome of object-based attention interacting with spatial attention. As such, the right lateralization of the observed activity pattern might only be partly the result of the functional organization of object selective areas.

The organization of attentional network might underlie the observed activation patterns. The dorsal fronto-parietal network for spatial attention is considered to be right hemisphere dominant (Schulman, 2010). In spatial attention alone, the right hemisphere tends to show a greater response to flickering stimuli (Morgan, Hansen & Hillyard, 1996), which was also observed with a trend in our source data. In addition, in a frequency tagging experiment where spatial location and emotional stimuli were varied, response amplitudes to the flickering stimuli were increased the most in the downstream processing in the right hemisphere. These lateralization effects decreased when the duration of the stimulus presentation time was prolonged (Keil et al., 2005). Other explanations include lack of statistical power as a result of the amount of subjects, trials or presentation time. Summarizing, the right hemisphere might respond greater to the corresponding spatial location after which the processing to downstream areas is increased as well.

Concluding remarks

This study examined the feasibility of frequency tagging of downstream areas with high frequencies under the influence of attention. With combined spatial and object-based attention, we were able to frequency tag right fusiform gyrus and parahippocampal gyrus. This method paves the way for investigateing how the propagation of information is modulated by attentional mechanisms such as cross-frequency coupling.

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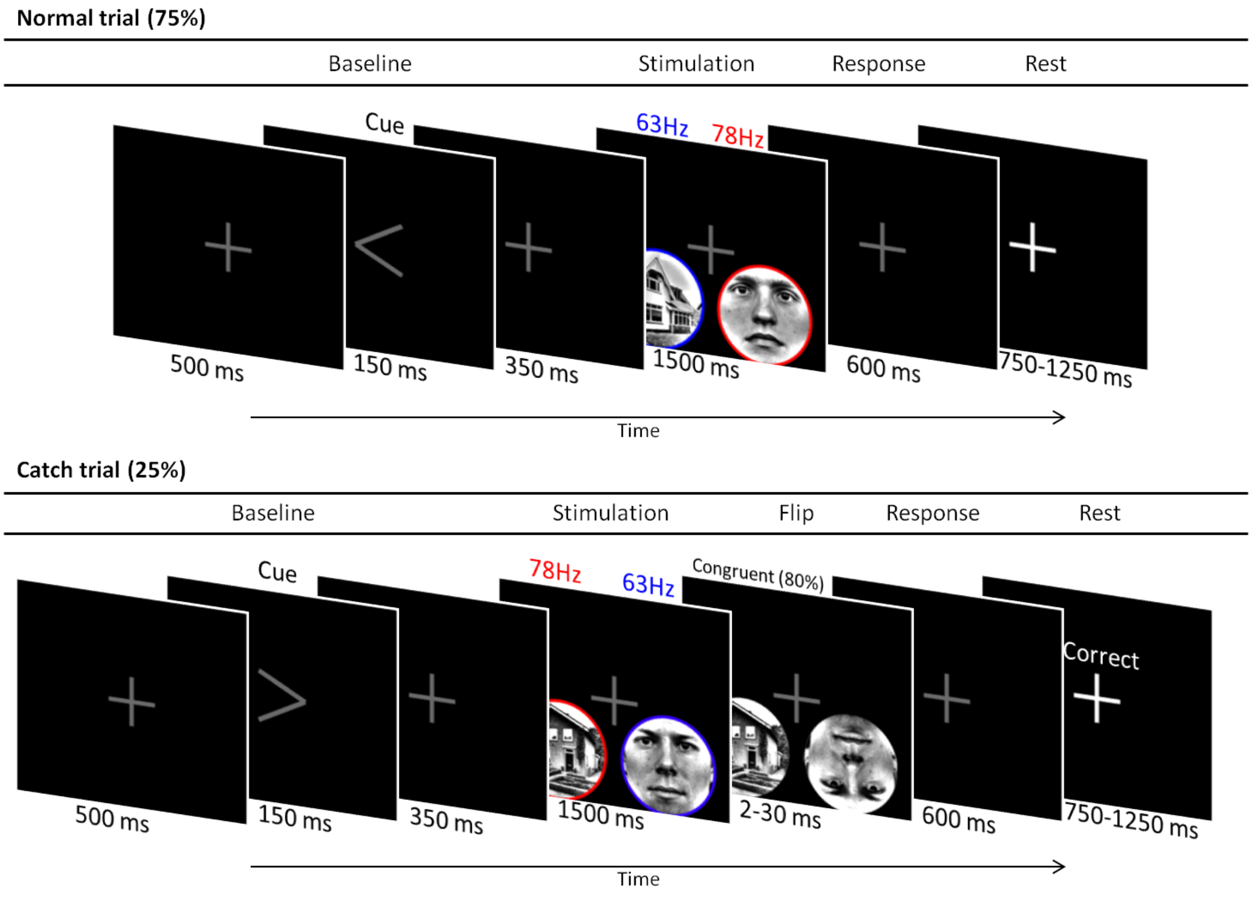

Figure 1 - Schematic layout of the time course of the experimental paradigm. In this catch trial flip detection task, a cue was given, indicating the image most probable to flip. A face and house were presented at 63 and 78 Hz. In 25% of the trials, a one of the images was flipped vertically. In 80% of these catch trials, the flip was congruent to the cue direction and participants had to respond to the position of the flip. In the other 20% of the catch trials, the flip was incongruent to the cue and participants had to ignore this event. The direction of the cue, the position of the stimuli, the position of the frequencies and whether the flip was congruent or incongruent was manipulated in a semi-random manner during the experiment.

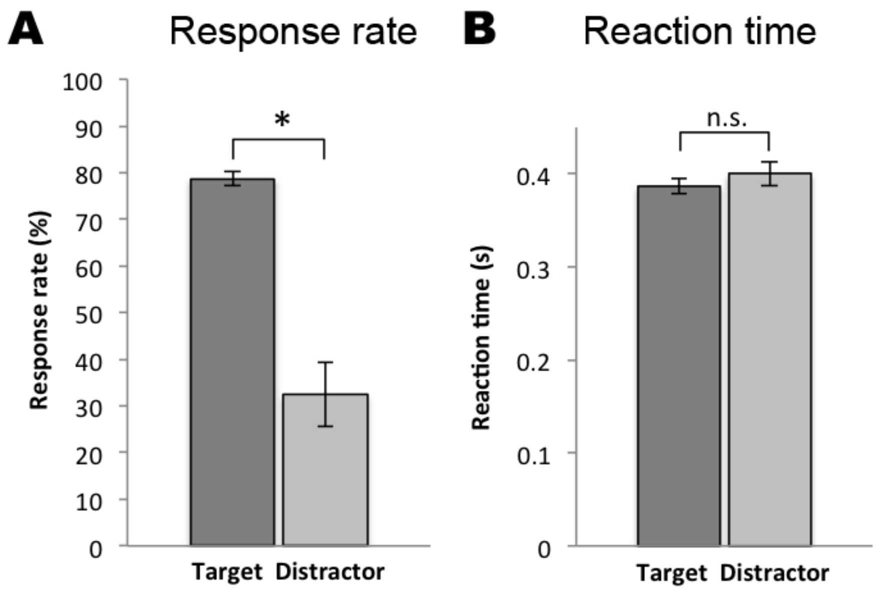

Figure 2 – Behavioural results of the catch trial flip detection task. **A** Response rate (+SEM) of target flips congruent to the cue and distractor incongruent flips, which had to be ignored. Difference in response rate to targets and distractors was significant ($t_7 = 88.5$, $p < 0.001$). **B** Mean reaction time (+SEM) of the button press after the stimulus flip of targets and distractors. Reaction times did not differ for button presses as a reaction on targets and distractors ($t_7 = 2.5$, $p = 0.40$).

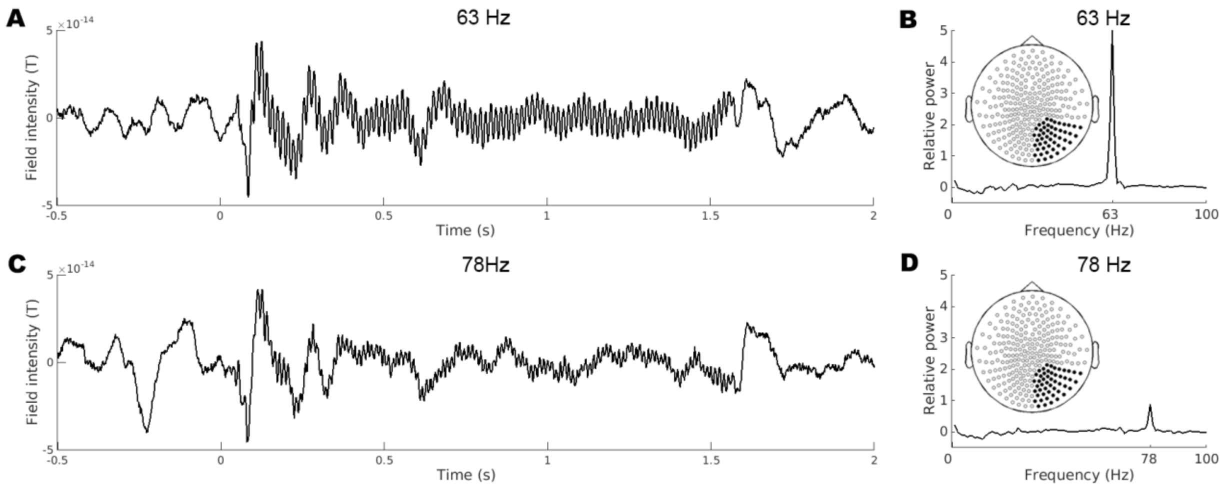

Figure 3 - ERFs and power spectrum averaged across all 8 participants and trials where attention was directed towards the left visual field and a stimulus was presented at 63 Hz (**A, B**) or 78 Hz (**C,D**). Recorded in occipital, temporal and parietal sensors located in the right, back quadrant**. A** A clear evoked 63 Hz oscillation was observed as result of stimulus presentation at 63 Hz. **B** Validation of the oscillation frequency by spectral analysis and a depiction of the used sensors. A clear spectral peak was observed at 63 Hz. **C** A 78 Hz oscillation was observed with smaller amplitude than the 63 Hz evoked oscillation as a result of stimulus presentation at 78 Hz. **D** The frequency spectrum for the 78 Hz oscillation correspondingly shows less spectral power at the tagged frequency (t_{14}=4.2 $p<0.001$).

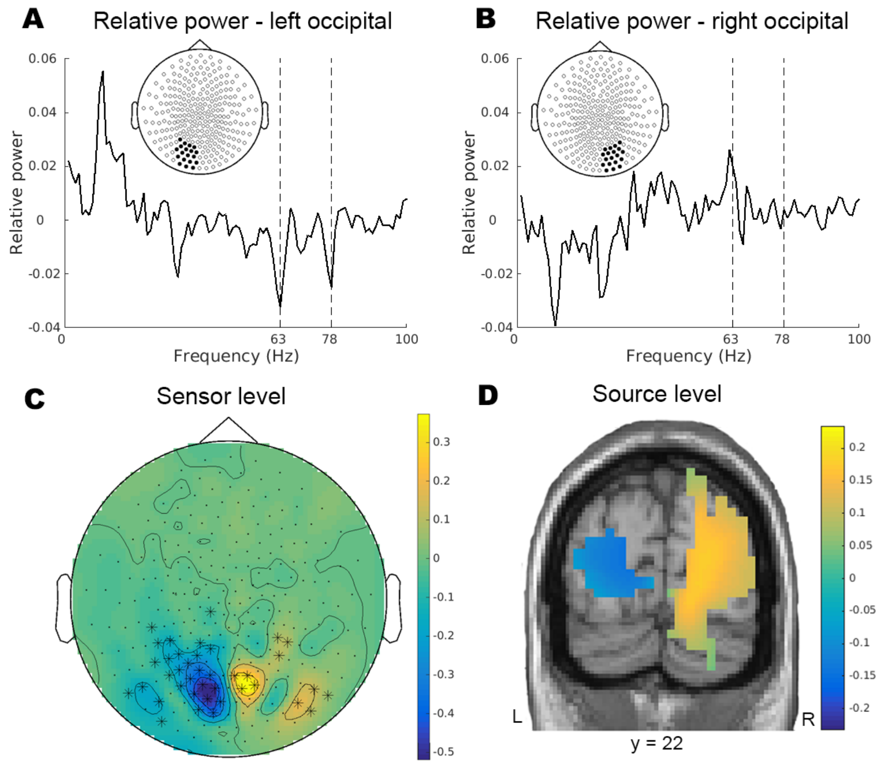

Figure 4 – Relative power of spatial attention modulation. Modulation of spatial attention increases the spectral power of the evoked response by the flickering stimuli in the attended visual hemifield. The attention modulation index $(P_{attend\ left} - P_{attend\ right})/(P_{attend\ left} + P_{attend\ right})$ at an epoch of 0.5-1.5 s after stimulus onset was used. **A** Relative spectral power recorded at the left occipital electrodes. A decrease in relative power at the tagged frequencies was observed for attention to the left versus attention to the right. **B** Relative spectral power recorded at the right occipital electrodes, showing an increase in spectral power at 63 Hz for attention to the left versus attention on the right. Attentional modulation for 78 Hz is less pronounced. **C** Topographical representation of relative power and significant sensors (*; $p<0.05$) for spatial attention on 63 and 78 Hz on the sensor level. **D** Source level analysis of the relative power as a result of modulation of spatial attention at 63 and 78 Hz shows a clear activity increase in right occipital regions as a result of attention to the left and activity decrease as a result of attention to the right in left occipital regions ($p<0.05$).

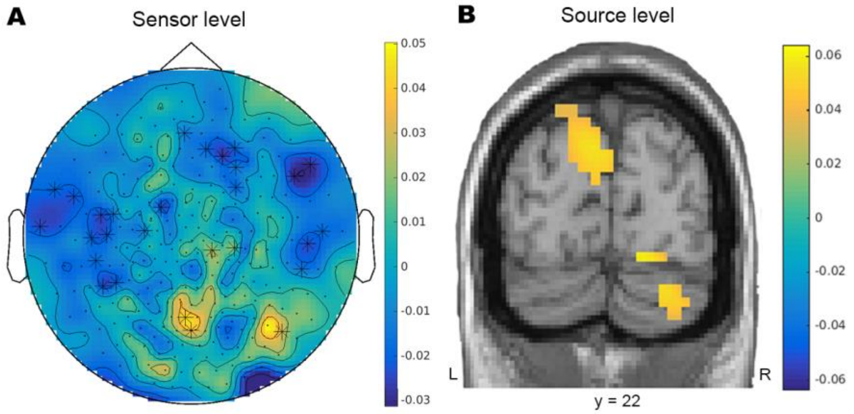

Figure 5 – Relative power of object-based attention modulation. The attention modulation index *($P_{attend\ face}$ – $P_{attend\ house}$)/($P_{attend\ face}$ + $P_{attend\ house}$)* at an epoch of 0.5-1.5 s after stimulus onset was used. **A** Topographical representation of relative power and significant sensors (*; p<0.05) for object-based attention at 63 and 78 Hz. Attention to faces gives right lateralized activation in occipito-temporal sensors. Attention to houses results in activation over temporal and frontal regions. **B** Source level activity object-based attention shows occipital activation for faces and right mid temporal and left pre-central activity for houses.

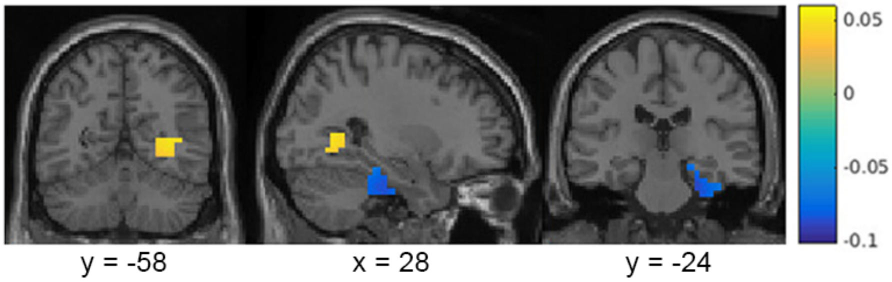

Figure 6 – Interaction of spatial attention with object-based attention results in activation for faces in the fusiform cortex (MNI = [28, -26, -28]; $p<0.05$) and activation in the parahippocampal cortex for houses (MNI = [28, -58, -6]; $p<0.05$). The attendional modulation index $(P_{attend\ face\ left} - P_{attend\ house\ left})/(P_{attend\ face\ left} + P_{attend\ house\ left}) - (P_{attend\ face\ right} - P_{attend\ house\ right})/(P_{attend\ face\ right} + P_{attend\ house\ right})$ was used at an epoch of 0.5-1.5 s after stimulus onset.