



## Activity in high-level brain regions reflects visibility of low-level stimuli



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### ABSTRACT

Stimulus visibility is associated with neural signals in multiple brain regions, ranging from visual cortex to prefrontal regions. Here we used functional magnetic resonance imaging (fMRI) to investigate to which extent the perceived visibility of a “low-level” grating stimulus is reflected in the brain activity in high-level brain regions. Oriented grating stimuli were presented under varying visibility conditions created by backward masking. Visibility was manipulated using four different stimulus onset asynchronies (SOAs), which created a continuum from invisible to highly visible target stimuli. Brain activity in early visual areas, high-level visual brain regions (fusiform gyrus), as well as parietal and prefrontal brain regions was significantly correlated with subjects’ psychometric visibility functions. In addition, increased stimulus visibility was reflected in the functional coupling between low and high-level visual areas. Specifically, neuroimaging signals in the middle occipital gyrus were significantly more correlated with signals in the inferior temporal gyrus when subjects successfully perceived the target stimulus than when they did not. These results provide evidence that not only low-level visual but also high-level brain regions reflect visibility of low-level grating stimuli and that changes in functional connectivity reflect perceived stimulus visibility.

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### Introduction

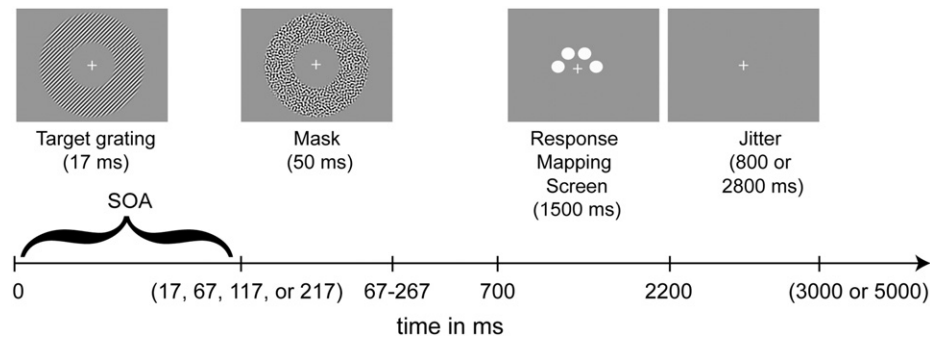
Does activity in high-level brain regions reflect perceived visibility of a low-level visual stimulus? Here we investigated this question by modifying subjects’ visibility of low-level target stimuli (here oriented Gabor gratings) using a visual masking procedure. It has been previously shown that changes in visibility are reflected in high-level brain regions using complex stimuli such as faces (Fahrenfort et al., 2012; Lumer et al., 1998), words (Dehaene et al., 2001), or complex shapes and objects (Grill-Spector et al., 2000; Haynes et al., 2005). Similarly, animal electrophysiology studies (Bridgeman, 1980, 1988; Kovacs et al., 1995; Lamme et al., 2002; Rolls et al., 1999) and recent human ERP and MEG studies (Dehaene et al., 2001; Del Cul et al., 2007; Fahrenfort et al., 2007; Melloni et al., 2007) suggested the involvement of high-level brain regions under visual masking conditions. However, the effect of perception of low-level stimuli on signals in high-level regions has been only rarely addressed (Tse et al., 2005). Hence,

we investigated whether high-level brain regions reflect changes in subjects’ perceived visibility using low-level grating stimuli and visual masking. In addition, we investigated functional coupling between different visual brain regions under different visibility conditions. This has been only rarely studied in the context of visual awareness and perceived visibility (Dehaene et al., 2001; Fahrenfort et al., 2012; Haynes et al., 2005; Imamoglu et al., 2012; Lumer and Rees, 1999).

We used backward visual masking by noise to study changes in subjects’ perceived visibility and how these changes are reflected in neuroimaging brain signals. Visual masking is a widely used procedure to manipulate the perceived visibility of a stimulus (Breitmeyer and Ögmen, 2006; Bridgeman, 1980; Grill-Spector et al., 2000; Macknik and Livingstone, 1998; Rolls et al., 1999). Backward masking by noise is one visual masking type in which the visibility of one briefly presented target stimulus (here a left- or right-tilted grating, Fig. 1 and S1A) is followed by a temporally succeeding briefly presented mask stimulus (here a random noise mask, see also Fig. 1 and S1B). By varying the stimulus onset asynchronies (SOAs), i.e., the delay between the target stimulus and the mask stimulus onset, perception of the target stimulus can be impaired (Breitmeyer and Ögmen, 2006). The psychometric visibility function reflects each subject’s visibility profile. In a typical backward masking experiment this is an ascending function with

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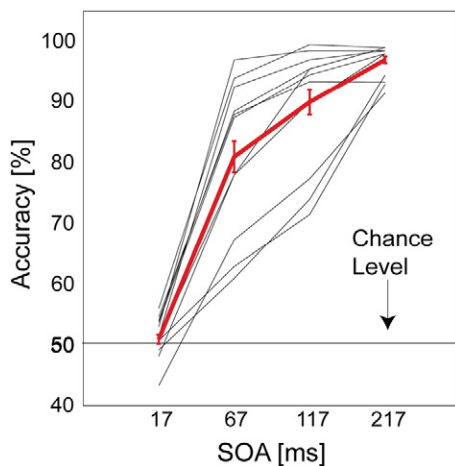
**Fig. 1.** Experimental design. A backward masking by noise experiment with four different visibility levels controlled by the four stimulus onset asynchronies (SOA; 17, 67, 117 or 217 ms) was used. The experiment started with the presentation of the target stimulus (right or left tilted grating) for 17 ms. A blank interval of 0, 50, 100 or 200 ms followed the target presentation that corresponded to the four SOAs respectively (17, 67, 117, or 217 ms). Subsequently, three consecutive different noise frames were presented for a total duration of 50 ms. A response mapping screen (duration 1500 ms) was presented 700 ms after the target onset. A subsequent jitter followed the response mapping screen, completing the total trial duration of 3000 ms or 5000 ms. Subjects' task was to indicate the orientation of the target grating by selecting the corresponding left or right tilted symbol during the response mapping screen using a left or right button press respectively.

increasing visibility levels (Type A masking function, (Kolers, 1962), see also Fig. 2). We used functional magnetic resonance imaging (fMRI) to search for activity in the human brain that followed the Type A psychometric visibility function. Furthermore, using a functional connectivity approach we examined whether the functional coupling in distant brain regions is correlated with changes in individual subject's perceived visibility profile. Our results suggest that not only early visual areas (V1–V4) but also high-level visual areas such as the fusiform gyrus and the superior occipital gyrus as well as a cluster including the superior parietal cortex and superior frontal gyrus are correlated with subjects' visibility profiles. Furthermore, increased stimulus visibility is reflected in increased functional coupling between the middle occipital gyrus and inferior temporal gyrus.

## Materials and methods

### Participants

Fourteen healthy subjects (six female, age range 21 to 36 years) participated in the experiment. All subjects had normal or corrected to



**Fig. 2.** Stimulus visibility. Subjects' psychometric visibility functions for 11 subjects that entered the fMRI analyses are shown. The x-axis represents the four visibility levels (SOA1 = 17 ms, SOA2 = 67 ms, SOA3 = 117 ms, SOA4 = 217 ms). The y-axis depicts the percent correct responses. The red bold line shows the mean curve with the error bars indicating standard error of the mean. A performance range from 50% correct answers (chance level) for the low-visibility condition (SOA1) to 100% correct answers for the high-visibility conditions (SOA4) indicates that the masking worked properly for all subjects.

normal vision and gave written informed consent to participate in the fMRI experiment. The experiment was approved by the Local Ethics Review Board of the Max Planck Institute for Human Cognitive and Brain Science (Leipzig) and conducted according to the Declaration of Helsinki. Three subjects were discarded from the analysis, one due to systematic motion during the experiment and two due to low performance in the behavioral task (Fig. S2).

### Stimuli

The target stimuli were gratings of two orientations: right-tilted (45°) and left-tilted (135°). We used four different phase-shifts (0°, 90°, 180°, 270°) of these targets to minimize retinal adaptation (Fig. S1A). The spatial frequency of the gratings was 2 cpd (cycles per degree of visual angle). The contrast of the target stimuli was 0.3 (standard deviation of the pixelwise luminance divided by the mean luminance). The grating annulus covered the visual field from 4° to 9° eccentricity, sparing the fovea to enhance the stimulus masking effect. The noise mask (Fig. S1B) was created by bandpass filtered noise with the same peak spatial frequency as the spatial frequency of the oriented grating without any orientation preference. We employed a backward masking procedure where the mask was briefly flashed after the grating stimulus (for details on timing see below). The mask had a higher contrast (0.7) than the target stimulus. Three frames with different random versions of the mask were briefly flashed in succession after the grating stimulus for a powerful masking effect. All parameters of the visual stimuli were selected based on behavioral pre-tests.

### Experimental procedure

We used backward pattern masking by noise, where visibility was manipulated by varying the stimulus onset asynchrony (SOA), which is defined as the time interval between the target and mask onsets. Four different SOAs were used to create a range from invisible to highly visible conditions.

Subjects were instructed to fixate on the white cross in the middle of the screen during the whole experiment (Fig. 1). At trial onset, a left- or right-tilted grating stimulus (target) was presented for 16.6 ms (corresponding to 1 frame at 60 Hz). After a short delay of 0, 50, 100 or 200 ms (corresponding to SOAs of 17, 67, 117 or 217 ms, i.e., 1, 4, 7 or 13 frames), a mask of three consecutive, different noise frames was flashed for a total duration of 50 ms. The four SOAs created four visibility conditions ranging from invisible (short SOA, 17 ms) to highly visible (long SOA, 217 ms). 700 ms after the trial onset a response mapping screen was presented for 1500 ms. This screen presented two symbols to the

left and right of fixation, indicating which button to press for either left- or right-tilted stimuli. Subjects had to indicate the orientation of the target grating by selecting the corresponding symbol with a left or right response button press respectively. Subjects were encouraged to take their best guess if they could not identify the target or were unsure about its orientation. The position (left vs. right) of the symbols on the response screen was pseudo-randomized in order to decouple orientations from button presses. After the response or timeout, a screen with a fixation cross was presented for 800 or 2800 ms. Each trial was completed either 3000 or 5000 ms after trial onset. The trial order was pseudo-randomized, ensuring 50% left-tilted and 50% right-tilted stimulus presentations. In order to avoid effects of retinal adaptation, both orientations were presented in four phase-shifted versions that were randomly assigned to the trials.

We conducted ten experimental runs in the scanner, each consisting of 80 trials. Every run contained 10 left-tilted and 10 right-tilted stimulus trials (with four different phase-shifts) for each of the four visibility conditions. The mean trial duration was 4000 ms. Prior to the ten experimental runs in the scanner, subjects participated in a practice run outside the scanner. During this practice run, subjects received acoustic feedback on whether their responses were correct. The stimuli were presented using the Cogent stimulus presentation toolbox (<http://www.vislab.ucl.ac.uk/Cogent>) for MATLAB (The MathWorks, Inc.) and were projected from the head-end of the scanner onto a screen placed within the scanner bore (projector resolution: 1024 × 768 pixel, 60 Hz). Subjects viewed the projection via a mirror fixed onto the head coil.

#### FMRI data acquisition and preprocessing

A Siemens Trio 3-Tesla scanner equipped with a 12-channel head coil was used to acquire functional MRI volumes. T2\*-weighted gradient-echo echo-planar images (EPI) containing 33 axial slices (2 mm thick, 1 mm gap, ascending) resulting in a voxel size of 3 × 3 × 3 mm<sup>3</sup> were acquired with the following imaging parameters: TR = 2000 ms, TE = 30 ms, flip angle = 90°, matrix size 64 × 64, field of view (FOV) = 192 × 192 mm<sup>2</sup>. A high resolution T1-weighted structural data set was collected for anatomical localization, with TR = 1900 ms, TE = 2.52 ms, matrix size = 256 × 256, FOV = 256 mm, 192 slices (1 mm thick), and flip angle = 9°. Preprocessing of functional scans was performed using SPM8 (Statistical Parametric Mapping; Wellcome Department of Imaging Neuroscience, Institute of Neurology, London). Specifically, functional images were corrected for motion and slice-acquisition time (using sinc interpolation and by reference to the middle slice = 16) and normalized using the unified segmentation method implemented in SPM8. The data were spatially smoothed with an 8 mm full width at half maximum Gaussian kernel.

#### FMRI data analysis

The first analysis was designed to identify brain regions that responded to the visual stimulation. At the single subject level, we first computed a general linear model (GLM) with four visibility levels (four SOAs) as conditions and six movement parameters as nuisance regressors. The experimental regressors (but not the nuisance regressors) were convolved with a canonical hemodynamic response function. At the group level, we selected brain regions that were activated in any of the four different visibility levels (low to high visibility) by computing an analysis of variance (ANOVA) without a regressor for the mean of each subject. We identified a network of brain regions that were significantly active in any of the four target stimuli presentations ( $p < 0.0001$  (voxel level FWE) cluster extent threshold  $k = 50$  voxels). We used this network (Stimulation mask) to further analyze the task related modulations within this network.

#### Correlations between estimated voxel activity and psychometric visibility function

In this analysis, the similarity between an individual subject's psychometric visibility function and the estimated fMRI responses to the four SOAs was computed at each voxel. Subjects' behavioral responses and brain activity estimates at each voxel were treated as a four dimensional vector, where each entry corresponded to the SOA. We used the Pearson's correlation coefficient to measure the similarity between these two four-dimensional vectors. This resulted in one correlation coefficient  $r(s,x)$  per voxel  $x$  that indicated how much this voxel  $x$  was correlated with subject's  $s$  visibility. The resulting correlation coefficients were then Fisher Z-transformed and entered into a one sample  $t$ -test ( $p = 0.05$ , FWE corrected for multiple comparison across spatial positions). We further applied an inclusive masking procedure (Henson and Friston, 2007; Li et al., 2007) when evaluating our results to select only those correlated voxels that were within the stimulation mask.

#### Visibility effects within the early visual areas

Whereas the previous analysis was performed for each voxel in the brain, the following analysis was identical except that only early visual regions of interest were studied based on the stimulation mask. This was done to be more sensitive to effects in early visual cortex. We first approximated the location of early visual areas (V1–V4) using the SPM Anatomy Toolbox (version 1.8) (Eickhoff et al., 2005, 2007). This toolbox provides probabilistic cytoarchitectonic maps for the localization of brain functions, including early visual areas (Amunts et al., 2000, 2007; Rottschy et al., 2007; Wohlschläger et al., 2005). These visual areas have been shown to largely overlap with functional retinotopic voxels ((Wilms et al., 2010; Wohlschläger et al., 2005), V1: 85.4%, V2: 74.9%, V3: 67.5%, V4: 78.7%). For each early visual area (V1–V4) and for each subject we selected those voxels that were within the stimulation mask. We then computed the same correlation analysis as described above for these selected voxels (See section “Correlations between estimated voxel activity and psychometric visibility function”) and entered subject's Fisher Z-transformed correlation coefficients into a one-sample  $t$ -test, separately for each visual area.

#### Functional connectivity analysis

In this analysis, we performed a psychophysiological interaction (PPI) analysis (Friston et al., 1997) to identify visibility-dependent changes in functional coupling between low and high-level brain regions. We used a parametric PPI analysis that modeled the psychophysical performance of each subject as a vector with weights [−1.5, −0.5, 0.5, 1.5] corresponding to [SOA1, SOA2, SOA3, SOA4] (Fig. 5, 'PSYCHO'). The mean time course of a seed region located in the occipital cortex was used as the physiological factor (Fig. 5, 'PHYSIO'). We selected this seed region from the effect of interest (i.e., not driven by any specific orientation) activity by drawing a 6 mm spherical cluster around the peak voxel in the right middle occipital gyrus (MNI: [39, −82, 10],  $F_{[3,30]} = 26.76$ ,  $p < 0.05$  FWE corrected for multiple comparison). This region and additional seed regions located in the temporal cortex that were activated by the nonspecific activity were used to test whether functional interactions are affecting perceived visibility (a list of seed regions can be found in Supplementary Table S1). Using these physiological and psychological factors, we examined whether visibility changed the covariation of voxels in the right middle occipital gyrus with other voxels in the brain. An interaction factor was created by multiplication of the psychological and physiological factors. We included the psychological, physiological and interaction term as linear regressors into a voxel-wise GLM. Individual parameter estimates were then used for statistical inference (Haynes et al., 2005; Macaluso et al., 2000).

**Results**

*Behavioral results*

Behavioral results obtained from eleven subjects in the scanner are shown in Fig. 2. Subjects' percent correct responses ranged from about 50% (chance level) at 17 ms SOA (lowest visibility) to 100% correct responses at 217 ms SOA (highest visibility). These results indicate that visibility was successfully manipulated by the backward masking in all subjects. Behavioral results were highly consistent for the two extreme SOAs, whereas the variability observed across subjects was higher in the two intermediate conditions (67 ms and 117 ms SOA). In these intermediate conditions, visual masking was more powerful for some subjects than others (Fig. 2). Over all 8800 trials, subjects failed to give a response in only 66 trials (0.8%). Of the remaining 99.2% responses subjects responded in 79.2% correctly, and in 20% of the trials incorrectly.

*fMRI results*

*Stimulation mask*

Bilateral extrastriate visual cortex (EVC: Left, [−6, −82, −8],  $T_{40} = 19.34$ ; right, [24, −85, 10],  $T_{40} = 20.01$ ), bilateral dorsolateral prefrontal cortex (dlPFC: Left, [−48, 5, 28],  $T_{40} = 20.27$ ; right, [51, 8, 31],  $T_{40} = 16.59$ ), bilateral intraparietal sulcus (IPS: Left, [−36, −52, 49],  $T_{40} = 17.30$ ; right, [33, −55, 55],  $T_{40} = 13.57$ ), and the right putamen ([27, 17, 10],  $T_{40} = 19.13$ ) were significantly activated by the stimuli ( $p < 0.0001$  FWE corrected with a cluster extent threshold  $k = 50$  voxels). We applied this stimulation mask in the following analyses.

*Correlations between estimated voxel activity and psychometric visibility function*

The similarity (correlation) between each subject's psychometric visibility function and subject's estimated brain responses for the four

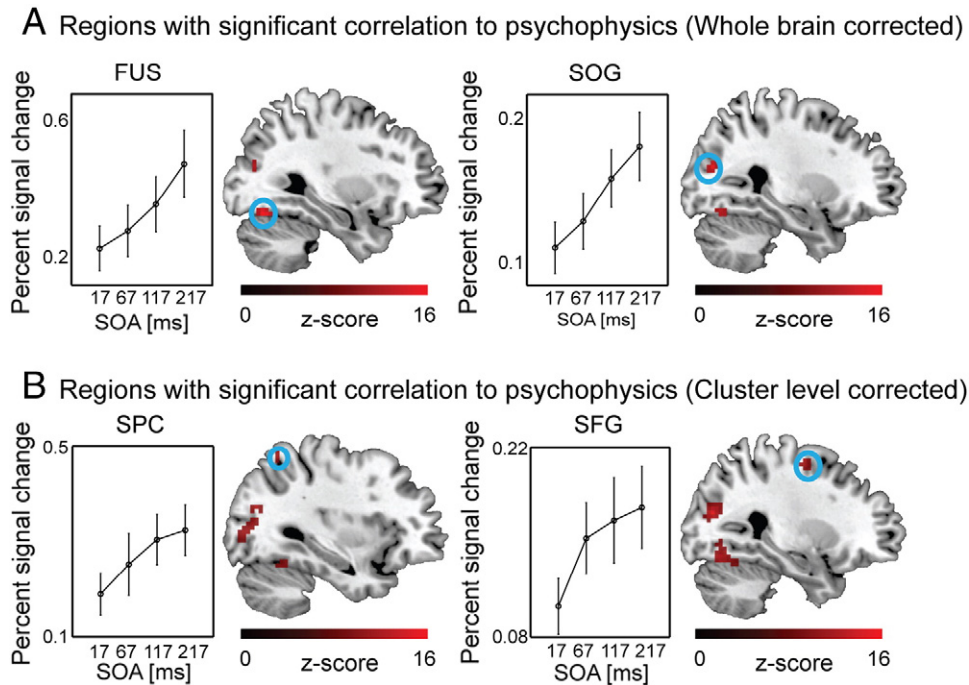
SOAs was significant ( $p < 0.05$  FWE, cluster threshold  $k = 10$  voxels) in the right fusiform gyrus (FUS, [30, −70, −17],  $T_{10} = 16.19$ ) and the superior occipital gyrus (SOG, [27, −76, 19],  $T_{10} = 14.42$ ) (Fig. 3A). When a cluster corrected threshold was used, the superior parietal cortex (SPC, [−33, −58, 58],  $T_{10} = 10.06$ ) and the superior frontal gyrus (SFG, [27, −7, 58],  $T_{10} = 10.18$ ) were significantly correlated with subjects' visibility profiles ( $p < 10^{-5}$  uncorrected with a cluster extent threshold of 20 voxels. We report only those voxels with  $p < 0.05$  FWE cluster-level corrected for multiple comparisons.) (Fig. 3B). The percent fMRI signal changes from these regions, averaged across subjects and computed separately for each SOA, are plotted as a function of SOA in Fig. 3.

*Visibility effects within the early visual areas*

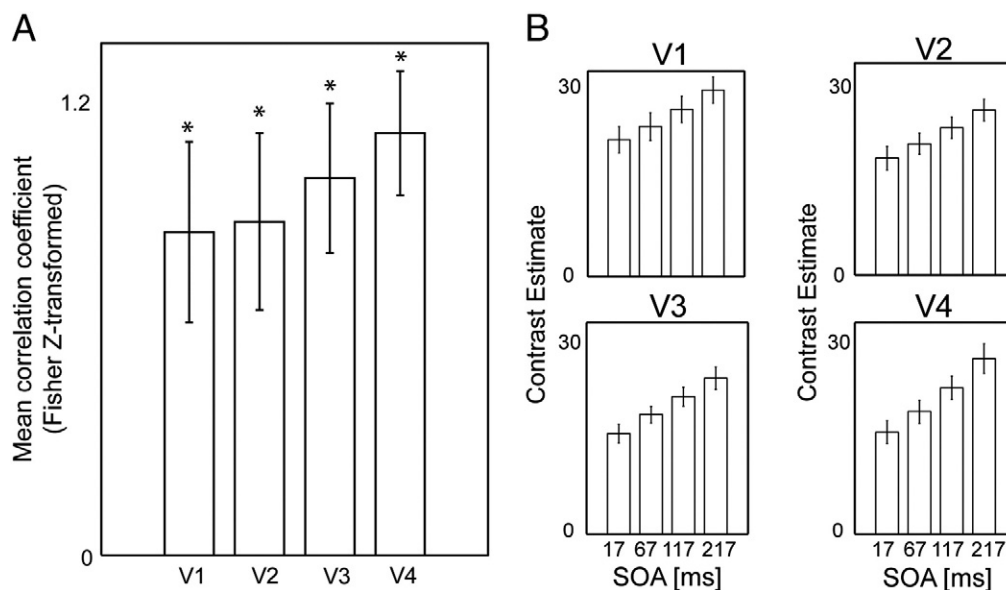
The correlations between perceived visibility and brain activity were significant in each early visual area. (V1:  $T_{10} = 3.52$ ; V2:  $T_{10} = 3.72$ ; V3:  $T_{10} = 4.99$ ; V4:  $T_{10} = 6.71$ ,  $p < 0.05$  (Bonferroni corrected for multiple comparisons)) (Fig. 4A). Fig. 4B illustrates for each early visual area (V1-V4) the estimated brain responses for the four SOAs.

*Functional connectivity*

The psychophysiological interaction between visibility and responses in the seed region (right middle occipital gyrus) was significant in the right inferior temporal gyrus (ITG, [54, −52, −8],  $T_{10} = 7.89$ ;  $p < 0.001$  uncorrected with a cluster extent threshold of 20 voxels. We report only those voxels with  $p < 0.05$  FWE cluster-level corrected for multiple comparisons.) (Fig. 5). This means, there is a dynamical change in the functional coupling between the low- and high-level visual areas with increasing visibility. In a second step, we also tested additional seed regions located in the parietal cortex, fusiform gyrus, as well as dorsolateral prefrontal cortex. However, these regions did not lead to any significant results. The coordinates of these regions are listed in Supplementary Table S1.



**Fig. 3.** Correlation analysis. Brain regions that were significantly correlated with subjects' visibility function are shown. (A) Whole brain multiple comparison correction with  $p < 0.05$  (FWE) was applied. Left panel: Right fusiform gyrus (FUS, [30, −70, −17],  $T_{10} = 16.19$ ). Right panel: Right superior occipital gyrus (SOG, [27, −76, 19],  $T_{10} = 14.42$ ). (B) Cluster level correction was applied. Only those voxels with  $p < 0.05$  FWE corrected threshold at cluster level ( $p < 10^{-5}$  for individual voxels) are reported. Left panel: Superior parietal cortex (SPC, [−33, −58, 58],  $T_{10} = 10.06$ ) is significantly correlated with subjects' visibility profile. Right panel: Superior frontal gyrus (SFG, [27, −7, 58],  $T_{10} = 10.18$ ) is significantly correlated with subjects' visibility profile. Bar plots within panels show percent fMRI signal change as a function of SOA averaged across subjects (avg ± sem). Please note that the shape of the response curves in these bar plots is expected to increase with increasing SOA because these voxels were selected based on the significant correlation result (Vul and Pashler, 2012; Vul et al., 2009).



**Fig. 4.** Brain responses within early visual areas. Early visual areas were defined using the SPM Anatomy toolbox (see text). (A) Fisher Z-transformed correlation coefficients between voxel activity and subjects' visibility function are plotted for each early visual area (V1–V4) (averaged across subjects and voxels). There was a significant correlation between the brain activity and visibility performance within each early visual area (V1:  $T_{10} = 3.52$ ; V2:  $T_{10} = 3.72$ ; V3:  $T_{10} = 4.99$ ; V4:  $T_{10} = 6.71$ ,  $p < 0.05$  (Bonferroni corrected for multiple comparisons)). (B) Brain activity across four visibility levels computed separately for each early visual area. Error bars correspond to standard error of the mean.

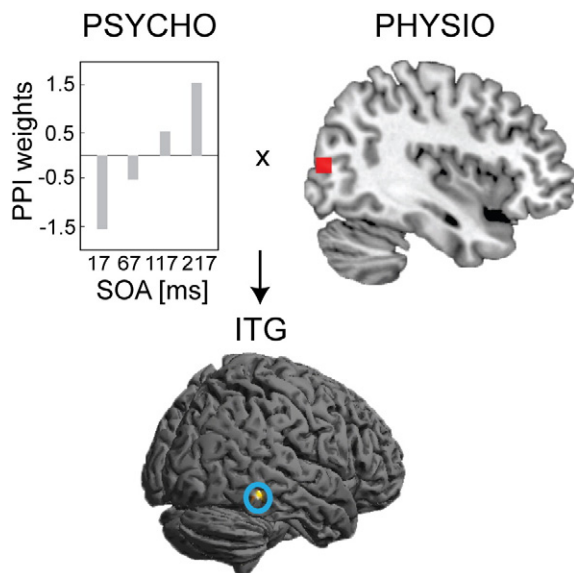
## Discussion

We studied the neural correlates of visibility of low-level visual stimuli and examined how subjects' brain activity changes with gradual changes in stimulus discriminability. We manipulated the delay between target and mask to create a continuum from invisible to visible conditions. In our first analysis (*Correlations between estimated voxel activity and psychometric visibility function*), we correlated subjects' psychometric visibility functions to their brain activity acquired at the different visibility levels. Consistent with previous studies, we found parametric visibility-related changes in brain activity during visual backward masking in several regions (e.g. Dehaene et al., 2001; Grill-Spector et al., 2000; Haynes et al., 2005; Kovacs et al., 1995; Macknik and

Livingstone, 1998; Macknik and Martinez-Conde, 2007; Tse et al., 2005). The individual subjects' visibility functions were reflected in brain activity at multiple hierarchical stages spanning from early visual areas, via high-level visual cortex (fusiform gyrus), to parietal and prefrontal regions. Our results suggest a linear relationship between subjects' visibility and their brain activity, which has been suggested earlier for low-level (Windley et al., 2013) and high-level tasks (Bar et al., 2001; Grill-Spector et al., 2000).

Changes in perceived visibility under masking conditions were reflected in signals as early as V1, for which there have previously been conflicting findings (see e.g. (Haynes et al., 2005; Lamme, 2006; Lamme et al., 2002; Macknik and Haglund, 1999; Macknik and Livingstone, 1998; Ress and Heeger, 2003; Super et al., 2001; Tse et al., 2005; von der Heydt et al., 1997)). Our results are in line with studies that show visibility-related modulations in primary visual cortex. For example, trial-by-trial visibility fluctuations of near-threshold stimuli are reflected in fMRI signals in V1 (Ress and Heeger, 2003), and it has been shown that V1 activity correlates with perception under binocular rivalry (Polonsky et al., 2000; Tong and Engel, 2001), visual masking (Pins and Ffytche, 2003) and the attentional blink (Williams et al., 2008). Furthermore, pattern information in fMRI signals from V1 correlates with changes in orientation perception under masking (Haynes and Rees, 2005a) and with changes in conscious perception under binocular rivalry (Haynes and Rees, 2005b). We also found masking-related reduction in signal amplitude in visual regions beyond V1, especially a correlation between visibility and brain activity in high-level visual areas (fusiform gyrus, Fig. 3A), which is consistent with previous human fMRI and EEG studies (Bar et al., 2001; Beck et al., 2001; Grill-Spector et al., 2000; Leopold and Logothetis, 1999; Pins and Ffytche, 2003; Tong et al., 1998).

Visibility was also reflected in brain signals in fronto-parietal regions (Fig. 3B), thus consistent with studies suggesting an important role of distributed fronto-parietal activity during conscious perception (Beck et al., 2001; Dehaene et al., 2001; Del Cul et al., 2007; Haynes et al., 2005; Imamoglu et al., 2012; Lau and Passingham, 2006; Lumer et al., 1998; Sergent et al., 2005; Tononi et al., 1998). There has been some debate as to whether prefrontal cortex activity is strictly necessary for consciousness, in the sense of a neural correlate of consciousness (NCC) or rather reflect top-down influences (Aru et al., 2012). The NCC is typically defined as the minimal set of neural processes that are jointly



**Fig. 5.** Psychophysiological interaction analysis. Functional connectivity between the right middle occipital gyrus (MOC, [39, -82, 10]) and the right inferior temporal gyrus (ITG, [54, -52, -8]) was significantly enhanced with perceived visibility. ( $T_{10} = 7.89$ ;  $p < 0.001$  uncorrected with a cluster extent threshold of 20 voxels. We report only those voxels with  $p < 0.05$  FWE cluster-level corrected for multiple comparisons).

sufficient for a specific conscious percept (Baars, 1988; Crick and Koch, 1990). For example, Eriksson et al. (2008) demonstrated that prefrontal cortex activity was decreased after training in a difficult object identification task (using constant stimuli), suggesting that prefrontal cortex activity is influenced by top-down regulatory processes.

Other studies using bistable stimuli have been similarly questioning the necessity of prefrontal cortex activity during perceptual alternations (de Graaf et al., 2011; Fraessle et al., 2014; Knapen et al., 2011). Bistable stimuli (as e.g. in binocular rivalry), in which a constant stimulus gives rise to alternating perceptual experiences have been widely used to study the NCC (Leopold and Logothetis, 1999; Sterzer et al., 2009). For example, a recent binocular rivalry study demonstrated that in the absence of subjective reports (subjective reports were measured by correlated pupil size) fMRI brain activity during perceptual alternations of rivalrous stimulus was observed in occipital and parietal regions but was either absent (middle frontal gyrus) or reduced in prefrontal regions (Fraessle et al., 2014).

In the search for a causal role of fronto-parietal network in conscious perception, transcranial magnetic stimulation (TMS) applied to dorso-lateral prefrontal cortex (DLPFC) has been shown to reduce subjective reports of visibility in correctly classified trials in a visual discrimination task (Rounis et al., 2010). Similarly, TMS applied to DLPFC disrupted perceptual alternations during voluntary control but had no effect on involuntarily occurring perceptual alternations (de Graaf et al., 2011). Other TMS studies have implicated a causal role of parietal regions by demonstrating that perceptual alternations in binocular rivalry can be disrupted by TMS applied over parietal cortex (Carmel et al., 2010; Kanai et al., 2010; Zaretskaya et al., 2010).

Although, the implications for the underlying mechanisms of conscious perception using perceptual alternations in bistable stimuli is unclear (Andrews, 2001; Sterzer et al., 2009), our signal changes in prefrontal cortex might not be part of the NCC proper but might rather represent processes that follow conscious perception such as motor preparation of reports (Aru et al., 2012; de Graaf et al., 2012) or task difficulty (Eriksson et al., 2008).

We also observed that visibility was reflected in the functional connectivity between middle occipital gyrus and inferior temporal gyrus, which is in line with other previous works (Dehaene et al., 2001; Fahrenfort et al., 2012; Haynes et al., 2005; Imamoglu et al., 2012). Others have already pointed out that awareness might depend on the integration of information between multiple brain regions, especially recurrent processing theories (Lamme, 2006). For example, it has previously been suggested that a late onset of response modulations by visual masking in early visual areas indicates a breakdown in recurrent processing between early and late visual brain regions (Enns and Di Lollo, 2000; Lamme et al., 2002). Similarly, the finding of TMS-unmasking, where a masked target is rendered visible due to a TMS-pulse affecting the mask, indicates an involvement of feedback processing in masking (Ro et al., 2003; see also Amassian et al., 1993). While compatible with reentrance, with the limited temporal resolution of fMRI our interpretation may not be complete. Overall, our findings indicate that perceived visibility requires neuronal integration between the early visual and high-level visual regions.

Our study focused on stimulus discriminability as an “objective” criterion of awareness, and did not employ additional subjective measurements (Merikle and Daneman, 2000). This was done in order to not further complicate the task requirements in each trial. Additional subjective measurements such as perceptual awareness scale and confidence judgements might further help to distinguish between graded and dichotomous consciousness (Overgaard et al., 2006). However, please note that introducing multiple psychometric judgments on each trial will lead to a superposition of brain responses to each required task, the separation of which is problematic when relying on fMRI with its sluggish response. Furthermore, the absence of discriminability is commonly assumed as a hard criterion for an absence of awareness (Dehaene and Naccache, 2001; Merikle and Daneman, 2000).

An interesting question is whether our findings in sensory regions might be related to top-down influences from attention or expectations (Aru et al., 2012; de Graaf et al., 2012; Melloni et al., 2011). Expectations (e.g. Melloni et al., 2011) are an unlikely explanation for our findings in this experiment because on each trial subjects did not know which of the two oriented gratings and which visibility level they would be presented with, all being equally probable. Thus, expectation would have to be unspecific in our case. However, it has been previously shown that visual attention can modulate visibility under metacontrast masking conditions (Ramachandran and Cobb, 1995). Object substitution theory has been proposed to account for the relationship between masking and attention (Enns and Di Lollo, 2000). According to this account, perception of a mask directly interferes with access to briefly present and unattended targets. This means, no masking effect occurs when attention is shifted onto the target location, but strong masking effect occurs on unattended targets (Weidner et al., 2006). Furthermore, attention can be reflected in enhanced brain activity as early as V1 and thus could affect brain activity measured during conscious perception (Somers et al., 1999). While we cannot fully exclude the effects of attention in our study, it is important to point out that attention effects on masking have typically been studied in the presence of competition between multiple stimuli in the visual field. In contrast, in our study only one position of the visual field was used for stimulus presentation, thus presumably processing occurred under full-blown attention.

In summary, the results presented in this paper broaden our understanding of visual awareness in two important ways. First, visibility profiles of masked low-level features are correlated with activity in visual, high-level visual as well as parietal and frontal areas. Second, functional connectivity changes between the occipital and temporal cortex are observed using low-level visual stimuli suggesting the role of functional integration in conscious perception.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.neuroimage.2014.08.045>.

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