Neural Correlates of Masking

Martijn Schippers[1]
Supervisors: Dr. Jens Schwarzbach[1,2], Prof. Peter Hagoort[1]

[1] *F.C. Donders Centre for cognitive neuroimaging, University of Nijmegen, The Netherlands*
[2] *Laboratory of Functional Neuroimaging, University of Trento, Italy*

This study looks at the phenomenon of visual masking. When two stimuli are presented in rapid succession the perception of one can be blocked by the perception of the other, depending on the timing and features of the stimuli used. There is currently an open discussion as to whether this effect is neurally limited to brain activity in the primary visual areas or whether it also extends to higher parts of the brain. We try to determine the neural correlates of visual masking by showing where in the brain there is a significant difference between consciously (unmasked) and not consciously (masked) perceived stimuli. Two behavioral experiments were performed to determine the optimal stimulus-setup and stimulus-timing for masking effects. The optimal settings found were used in an fMRI experiment. The stimulus visibility was measured while manipulating the time between the start of the stimulus and the start of the mask. This Stimulus Onset Asynchrony (SOA) influences the visibility of the stimulus in a manner that looks like a U-shape (high visibility at short and long SOAs, low visibility in between). We added a control condition where we replaced the mask with a non-masking, but physically almost identical shape. As this control shape does not mask, the SOA should not influence visibility, and hence a U-shape should not occur when plotting visibility versus SOA. These two differently shaped masking functions make it possible to attribute differences in brain activity (as measured by fMRI) to a difference in visibility. As our behavioural data in the scanner did not lead to such opposing masking functions, the found brain activity differences could not be attributed to an effect of masking. We did find activity in the primary visual cortex, the left angular gyrus and extrastriate cortex and the left superior temporal gyrus, but this data can only be taken as an indication where activity might be found in a study where the behavioural data is significantly different. As we present this study as a pretest for such a more elaborately controlled experiment, we present some suggestions as to how this level of control over the masking effect can be achieved.

1. Introduction

Studying visual illusions can provide a useful entrance into the complex mechanics of the human visual system. Through these illusions small functions in the system can be isolated when their mechanics go wrong under very specific circumstances (Eysel, 2003). Knowing these circumstances we can hope to discover more about how that specific function works, and what role it performs in the visual system. These small exploits in the system can therefore be a useful tool to analyze a function of the brain as complex as human vision. While in visual illusions one sees something that is not actually there, closely related to these visual illusions is visual masking, where one doesn't see something that is actually there. In visual masking a stimulus (the *target*) that would by it self be visible can be rendered invisible by presenting another stimulus (the mask) very closely before or after it in time. If and what kind of masking occurs is dependant on a variety of factors. The most important ones are the order and the timing of the stimuli, their location and their shape.

Breitmeyer (1984) defined various distinctions in masking, with one of the main differences lying between forward and backward masking. In forward masking the mask is presented before the stimulus, hence the effect of the mask travels forwards in time, while in backward masking the mask is presented after the stimulus, reversing in time the direction of the masking effect. All kinds of visual stimuli can be used as a mask in visual masking, for instance a light, a pattern or a contour. Within the category of masking by pattern a distinction can be made between para- and metacontrast masking. Paracontrast masking is a form of forward masking, but in this study we will focus on metacontrast masking, which is a kind of backward masking where the contours of the stimulus overlap, but not touch each other (figure 1).

The timing of the stimuli is critical to the kind of masking effect that occurs. Varying the time between target and mask influences the strength of the masking effect, and the durations of the target and mask influences the shape of the masking function. For backward metacontrast masking, there is a range of SOAs (Stimulus Onset Asynchrony, the time between the onset of the target and the onset of the mask, see figure 2) in which masking occurs, at strengths varying depending on the duration of target and mask. Breitmeyer (1984) showed that when the stimulus energy (influenced by contrast, luminescence and most notably duration) of the target is smaller than that of the mask, Type A masking occurs. This type is defined by a linear increase in visibility when the SOA increases (see figure 3). When the stimulus energy of the target is equal or bigger than that of the mask, Type B masking occurs. This type is defined by a high visibility at short and long SOAs, but a dip in visibility at SOAs in between (often, but not exclusively around 33-66ms).

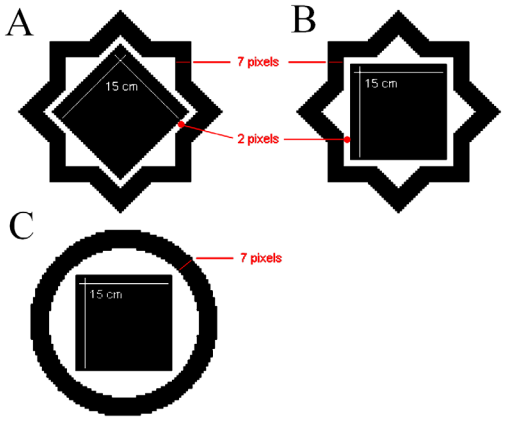


Figure 1. Stimuli. Enlarged view on the stimuli used in both behavioural tests and the scanning session. A is a diamond stimulus with a mask of 2 pixels separation. B is a square, identical to A but rotated with the same mask. Note the overlap in contours between diamond and mask and square and mask. Both targets share an equal amount of contour with the mask. C is a square target with the control as used in behavioural test 2 and the scanning session. The number of black pixels, thickness and separation from the target (measured in the corners) is equal to that of the mask.

Although not directly influencing the kind of masking that occurs, the location of the stimulus and the separation between the edges of the target and the contours of the mask do have an influence on the strength of the masking effect. As subjects in masking experiments are usually instructed to fixate on a fixation cross in the middle (as we indeed did), the farther away from the fixation the stimulus is located, the less visible the target is in the subject's visual field. Less well understood is the effect of target-mask separation on the strength of the masking effect, but from a prior informal pretest this appears to be somewhat of a U-shape in itself. When the contours of target and mask directly touch each other (a separation of 0) the masking is relatively weak, increasing in strength

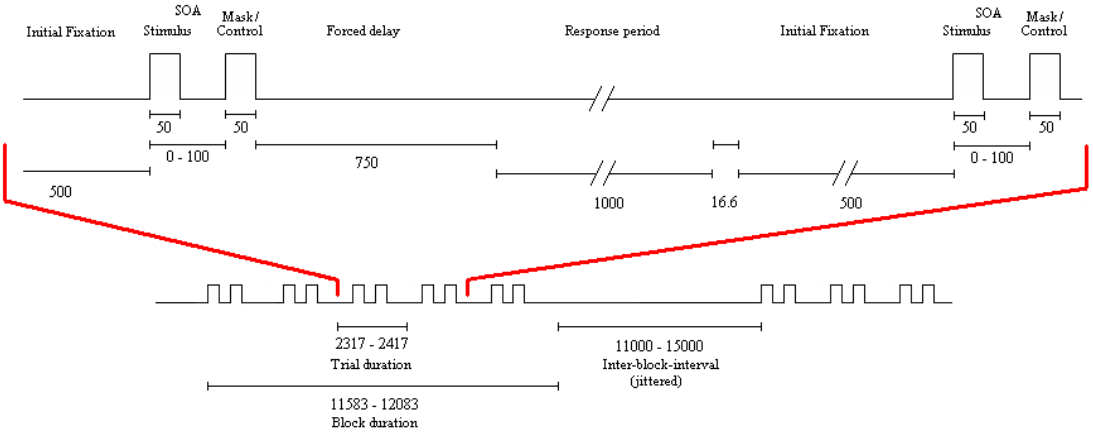


Figure 2. Timing. Overview of the timing of the fMRI experiment. The initial fixation and forced delay display a black fixation cross. The response period displays a red fixation cross indicating the subject can response. The target and mask / control both last 50ms, the SOA (Stimulus Onset Asynchrony) is the time from the onset of the target to the onset of the mask / control. The entire stimulus presentation can last between 50 ms (SOA 0) and 150 ms (SOA 100). Five trials constitute a block, there are 11 different blocks (4 SOAs times mask / control plus 3 different target, mask or control only blocks) in the experiment.

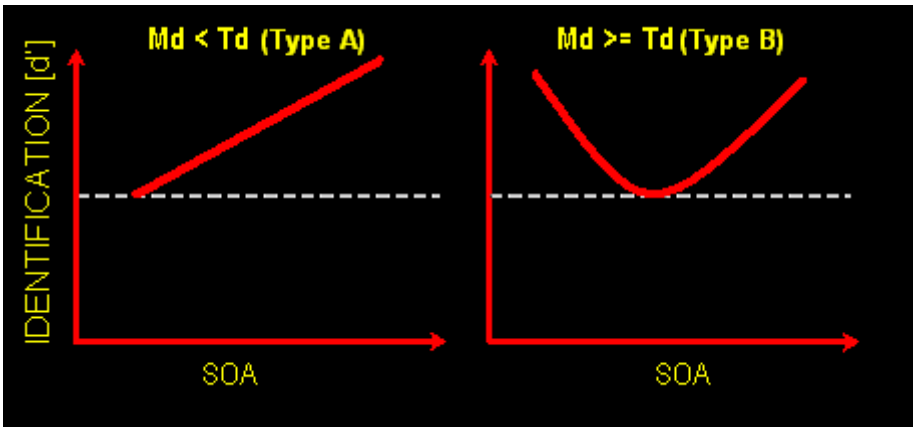


Figure 3. Type A and Type B masking. The identification on the Y-axis is the visibility of the masked target, displayed in d'. The dotted line indicates where visibility is at change level (equal to a 50% score). SOA is the Stimulus Onset Asynchrony. When the duration of the mask is shorter than the duration of the target (Md < Td) Type A masking occurs. When the duration of the mask is equal or bigger than that of the target Type B masking occurs (Breitmeyer, 1984).

when the mask is separated further from the target until a criterion is reached and the strength of the effect weakens again with even further separation.

There are various theories on the underlying mechanisms of metacontrast masking. One of the earliest is Breitmeyer's (1984) dual channel, sustained-transient theory (for a review on its current status, see Breitmeyer and Ogmen, 2000). This postulates that any visual stimulus is processed through two channels. One slow sustained channel that codes for object features like brightness and colour, and one fast transient channel that codes the more coarse patterns of the stimulus such as spatial location and motion. When the timing of the target and mask coincide with the processing speed of both channels a situation can occur where the slow sustained processing of the target is not yet finished when the fast transient processing of the mask already takes place. The former is then disrupted by the latter and processing of the target remains incomplete, preventing it to be consciously perceived.

Another theory on metacontrast masking is Bridgeman's recurrent processing (Bridgeman, 1980, reviewed in Enns & DiLollo, 2000). A visual stimulus causes an initial burst of activity in the striate areas, then spreads to the extrastriate areas through cortico-cortical connections and finally returns to the striate where it finds a match with

the still present initial low-level activity. It is claimed that this recurrent process is necessary to group object features within our attention (Lamme & Roelfsema, 2000, Lamme et al, 2002). When the timing of the stimuli is so that the processing of the target re-enters only after its low-level striate activity has already been replaced by that of the mask, no match is found and the target cannot be fully processed, and hence is not consciously perceived.

A theory that makes a strong claim regarding the locus of masking comes from Macknik (Macknik & Haglund, 1999). They used optical imaging in rhesus monkeys to see whether activity in V1 would reflect the physical stimulus or the masking effect. They found that although target and mask separately both generated correlated activity on the surface of the cortex, the activity of the target disappeared when the target was masked. They make a strong claim that masking only occurs in early visual areas (V1), and not beyond. Based on single cell recording studies in rhesus monkey's V1 their hypothesis is that both the temporal (Macknik & Livingstone, 1998) and the spatial edges (Macknik et al, 2000) of the stimuli generate the strongest neural responses. Regarding the temporal edges of the stimulus the onset and offset of the target are of interest. Backward masking would be caused by a reduction in the after-discharge of the response to the target, highest at an SOA of about 100 ms. Linking this to the effect of the spatial edges the strong neural signal at the spatiotemporal edges is explained by transient activity at the onset and offset of the stimulus in neurons with receptive fields responding to the contours of the stimulus (Macknik et al, 2000).

Lastly, an interesting take on the underlying mechanism of the masking effect comes from Ogawa (Ogawa et al, 2000) who claims any temporal interaction of two visible stimuli can cause masking. They presented subjects with two short (10 ms) visual stimuli and found that at certain inter stimulus intervals (ISI) the brain activity in the visual cortex (as measured by fMRI) of the two stimuli combined was equal to that of only a single stimulus. At an ISI of 200 ms there was no additional effect of the second stimulus whatsoever, while reducing or increasing the ISI gradually reduced this suppression of the neural activity again. As subjects still reported being able to perceive both stimuli at all ISIs, this would technically not be considered masking. However, the neural activity does show a suppression of the first stimulus due to the second, and such a neural refractory process could give an interesting insight in the neural mechanisms of masking.

The aim of the current study is to test the two main claims from the aforementioned theories, using fMRI to measure subjects' brain activity during a masking task. First, if masking really only does occur in V1, as Macknik (Macknik & Haglund, 1999) claims, that should be the only region with a significant difference in brain activity between masked and unmasked stimuli, as all other properties of mask and control are equal and should elicit similar activity. The sustained transient theory also puts the masking effect at low-level visual areas (Breitmeyer & Ogmen, 2000), although less specified. However, it would predict the same outcome in regards to activity changes in regions beyond the primary visual cortex.

Second, if the masking effect really is a refractory process, as Ogawa (Ogawa et al, 2000) claims, the brain activity changes occurring with a masked target should also occur in the similar, but non-masking control condition. This prediction also goes for the recurrent processing theory, as the replacement of the first stimulus' low-level activity by that of the second should, at certain SOAs, occur with all stimuli, instead of only with the mask but not the control.

In order to test the main claims from these theories, we designed a masking experiment that could compare between conditions where a target is followed by a mask, and hence rendered invisible, versus conditions where a target is followed by a control which does not render the target invisible. We adapted the targets from Enns & Di Lollo (1997) into two comparable targets. The first target was an upright diamond, and for the second target we rotated this diamond which produced a square. This way we had two distinguishable targets (a diamond and a square) which are equal in all properties except rotation. Our task in one test was to identify which of these two targets appeared, and in all other tests to judge whether two presented targets were the same or different. We used a mask which contours overlapped both the diamond and the square, and a control ring that has the same properties as the mask, but not the overlapping contours (see figure 1).

Studies that investigate which brain areas are involved in the processing of masked stimuli often make use of neuroimaging techniques. One of these is fMRI, a technique that generates images of the working brain with high spatial resolution

(down to approx. 1mm) but a limited temporal resolution (several seconds). fMRI images the brain by showing the amount of oxygen in the blood. Subjects are placed inside an fMRI scanner that has a high magnetic field, and when radio-frequency pulses are sent through the brain those signals are adjusted differently depending on the amount of oxygen that is present. Although the brain areas involved in a specific task utilize more oxygen while performing the task, it's actually an increase in the blood's oxygenation level that signifies brain activity in a particular area. This is due to the fact that a sudden increase in oxygen demand in a specific brain area leads to various physiological responses, of which an increase in blood flow is the most dominant. While the oxygen consumption in a specific area increases, the effect of the increased blood flow is even stronger so the blood oxygenation level actually rises. The signal from an fMRI scanner is called the BOLD-signal (Blood Oxygen Level Dependant signal, sometimes also called BOLD response). The brain is divided into a three dimensional grid and every so-called voxel (usually about 1x1x1mm to 3x3x3mm in size) has its own BOLD-signal. When the BOLD-signals for every voxel during a certain task are compared with their BOLD-signals while the task is not performed, the voxels that have a significant increase in BOLD-signal during the task are considered to be located on brain areas that are involved in that task.

In our experiment we used fMRI to investigate the neural basis of visual masking. Before we could start with our imaging experiment however, we had to establish the optimal parameters for our stimuli. Masking is a fragile effect, and whether it actually occurs is very dependent on the exact physical properties of the stimuli and the timing of their presentation. Prior to the actual imaging we ran two behavioural tests meant to find the optimal settings of the stimuli's physical properties as well as the level of predictability we needed. We started the first behavioural test with settings from prior unpublished pre-tests, and then proceeded in the second behavioural test with a refinement of the findings from the first test. In this second behavioural test we tested two potential combinations of settings to determine which of the two would produce the best masking result, and would hence be used in the imaging experiment.

2. Material and methods

2.1 Experiments

The first behavioural test was conducted to find the optimal eccentricity (the distance from the fixation cross to the target) and to see which SOAs were most relevant. From these findings we set up two potential combinations of eccentricity and separation. The second behavioural test was used to determine which of these two combination produced the best masking results and was henceforth to be used in the fMRI experiment.

2.2 Subjects

The first behavioural test was performed on 5 subjects (3 male, 2 female) aged 22 to 30. The second behavioural test was performed on 8 subjects (3 male, 5 female) aged 22 to 30. In the fMRI experiment 5 subjects (1 male, 4 female) aged 22 to 26 participated. All subjects had normal or corrected-to-normal vision and gave informed consent according to institutional guidelines of the local ethics committee (CMO region Arnhem – Nijmegen, The Netherlands). Subjects received no financial compensation for their participation.

2.3 Experimental setup

In both behavioural tests subjects were placed behind a standard CRT monitor displaying a 600 by 800 pixels resolution screen at 60Hz. During the experiment subjects sat in an upright position straight in front of the monitor with their eyes approximately levelled with the horizontal midline of the screen. Viewing distance was approximately 60-70cm.

In the fMRI experiment, subjects lay in the scanner in supine position. Head movements were minimized by an adjustable padded head holder. Visual stimuli were projected onto a mirror above the subjects' heads. Responses were recorded via an MR-compatible keypad (MRI Devices, Waukesha, WI). In both setups, stimulus presentation and response collection were controlled by a PC running Presentation Version 9.13 (Neurobehavioural Systems, San Francisco, CA).

2.4 Stimuli

In the first behavioural test (testing eccentricity and SOA) target stimuli consisted of either a black

diamond or a black square on a white background, with a fixation cross (6.7 mm by 6.7 mm, thickness 0.7 mm) in the middle of the screen. The targets were placed at one of three different eccentricities at a 45 degree angle relative to the fixation cross. Total distance from fixation to target was 50, 75 and 100 mm respectively (4.4, 6.6 and 8.8 degrees at a viewing distance of 650 mm) measured from the centre of the stimuli. The diamond and square both measured 15 by 15 mm, and had the same surface size. The masks consisted of an 8 pointed star-shaped figure which contours followed both the target and the mask simultaneously. They were 7 pixels wide, and were placed around the targets with a distance of 2 pixels from the outer border of the target, on a 800 by 600 pixels resolution display (see figure 1).

In the second behavioural test (testing two potential setups) the shape, size and location of the fixation cross and the shape of the target and mask were equal to those in the first behavioural test, but now 2 targets were presented at two different eccentricities. The targets were presented at a 45 (upper right) and 315 (upper left) degrees angle relative to the fixation cross with a distance of 75 or 100 mm (6.6 or 8.8 degrees at a viewing distance of 650 mm). Also, a control condition was introduced. The control consisted of a ring-like shape whose width and surface were equal to those of the mask, and was presented at the same location. Both mask and control were presented at 1 pixel distance from the target when the eccentricity was 75 mm, and at 3 pixels distance when the eccentricity was 100mm.

In the fMRI experiment the location and shape of the fixation cross was kept equal to those in the second behavioural test, but as an additional timing cue it was sometimes presented in red instead of black only. The shape of the target, mask and control were also equal to those in the second behavioural test. Again two targets were presented at a 45 and 315 degree angle relative to the fixation cross with a distance of 100mm only. Mask and control were presented with a three pixels distance from the target. The viewing distance from the subjects eye to the screen was 800 mm, resulting in a distance between target and fixation cross of 7.1 degrees.

2.5 Experimental timing

Due to the fragile nature of the masking effect the timing of the stimuli is of critical importance. As the aim was to recreate a type B masking effect we chose a target-, mask- and control-duration of 50 ms each. The masking functions are distinguished by the difference in masking strength at different intervals (Stimulus Onset Asynchrony) between the onset of the target and the onset of the mask. Masking of any kind usually occurs at SOAs between 0 and 133 ms. For the first behavioural test we used SOAs of 0, 33, 50, 67, 84 and 133 ms. In the second behavioural test and the fMRI experiment SOAs of 0, 33, 67 and 100 ms were used.

Both the CRT monitor used in the behavioural experiments as the LCD projector used in the fMRI experiment were set to a refresh rate of 60Hz, and the timing of the stimuli was controlled by displaying them for a certain amount of frames. One frame is one screen-refresh, hence it is the minimum amount of time a screen can be displayed. At 60Hz the time of one frame is 16.6 ms. Prior to the actual experiments, the accuracy of this timing method was tested using an oscilloscope with a photodiode sensor. A 60Hz refresh rate dictates the display time of one frame to be 1000 / 60 ms at a specific point on the screen. The oscilloscope test revealed a deviation of no more that 0.2 ms per frame.

In the first two behavioural experiments every trial started with an initial fixation period displaying a fixation cross for a jittered period of between 65 and 100 ms. Then the target was shown, followed by the mask / control at an interval according to the appropriate SOA for that trial. After the stimuli were shown the subject had a 2 second window in which to respond. When the response period ended a new trial started. A pause screen was shown after every 20th trial. The pause lasted until the subject ended it with a button press.

In the fMRI experiment a trial started with a fixation period of 500 ms which showed a black fixation cross, followed by the target and the mask / control with an interval according to the respective SOA. Then a delay period of 750 ms showed a black fixation cross again after which the fixation cross turned red indicating the 1000 ms response period in which the subject could respond. Five of these trials were repeated immediately after each other constituting one block. Between blocks, a jittered period between 11 and 15 seconds (in 1 second steps) showed a blank screen. Slightly varying with the condition the block belongs to (depending on SOA the target and mask might overlap) each block lasted on average 11833 ms, and the total experiment lasted approximately 49

minutes (figure 2).

2.6 Behavioural procedure

In the first behavioural test subjects had to indicate whether the target was a diamond or a square, by pressing one of two buttons on a standard 101-keys keyboard (arrow left for a diamond, arrow right for a square). Subjects were presented with a target, followed by a mask at one of three different eccentricities with an interval of one out of six possible SOA times. These 18 eccentricity-SOA combinations were all repeated 80 times. Added to this was a target-only condition for every eccentricity, where a target without a subsequent mask was shown. These were repeated 40 times each. Hence in total there were 21 conditions in 1560 trials presented in 10 blocks of 156 trials with the voluntary possibility of a short break between every block.

The second behavioural test employed the same stimuli, but now on both sides of the fixation cross and the task changed to a forced choice task where subjects had to indicate whether the two presented stimuli were either the same (two diamonds or two squares) or different (a diamond and a square) using the same buttons (arrow left for the same, arrow right for different stimuli). Two combinations of stimulus eccentricity and target-mask separation were tested (75 mm eccentricity and 1 pixel separation versus 100 mm and 3 pixels) in two separate blocks counterbalanced in order over subjects. Each combination was tested with a mask or a control-ring following the target using four different SOA intervals. Added to this was a target-only condition, adding up to a total of 9 conditions for both combinations who were all repeated 20 times creating two blocks of 180 trials each.

The behavioural part of the fMRI experiment was done with the same double sided stimuli as in the second behavioural test, using an eccentricity of 100 mm and a separation of 3 pixels. Four SOA intervals were used for both the condition with a target followed by a mask and the condition with a target followed by a control-ring. Added to this were three conditions where either a target, a mask or a control were presented by themselves, adding up to a total of 11 different conditions, who were all repeated 50 times to a total of 550 trials. Due to the design of the fMRI experiment all trials were presented in blocks containing 5 trials of the same condition, but with different diamond / square combinations. Every block was repeated 10 times in a fully counterbalanced fashion. This entails that blocks from every condition followed blocks from every other condition an equal number of times.

2.7 Imaging procedures

Images were acquired using a 3T Trio scanner (Siemens, Erlangen, Germany). BOLD sensitive functional images were acquired using a single shot gradient EPI sequence (TR/TE 2s/30 ms, 31 transversal slices, interleaved acquisition, voxel size 3 x 3 x 3 mm). Following the experimental session, structural images were acquired using an MP-RAGE sequence (TR/TE/TI 2250 ms/3.93 ms/850 ms, voxel size 1 x 1 x 1 mm).

Due to its very short duration a single stimulus is unlikely to yield enough stimulus-power to generate a reliable BOLD response. This prevented the use of an event-related design. However in a classical block design the performance of subjects could be influenced by adaptation to the stimulus-timing which would increase the predictability of the targets. Also, long blocks could saturate the processing of visual stimuli. In a similar situation, working with short auditory stimuli, Binder et al (2004) resorted to the use of mini-blocks to counter the limitations of the classical block design while still taking advantage of the amenities of an event-related design. Relatively small blocks of 5 trials increases the stimulus-power while still being short enough to prevent adaptation and remain unpredictable.

2.8 Data analysis

Data from the first behavioural test was initially analysed using Signal Detection Theory (Green & Swets, 1966, 1988). Although SDT is mainly used to detect a signal amidst noise, it can also be used to calculate d' as a measure of visibility of the stimulus. We tried two possible applications, a one- and a two-signal analysis. In the one-signal analysis the Hit, Miss, False Alarm and Correct Rejection rates for both targets (diamond and square) are calculated separately, then averaged and d' is calculated from that. In the two-signal analysis only the Hits and Misses of both targets are calculated, as a False Alarm on one target is equal to a Miss on the other and vice versa.

An extra check was performed by calculating the percentage-correct scores for both targets, and then averaging them to derive a general visibility score. As we found no significant difference between

the two SDT methods and the percentage-correct scores we decided to use the latter for all further analysis of our behavioural data. Henceforth, percentage scores and not SDT were used for the response-bias measure.

The imaging data was analyzed in SPM2 (http://www.fil.ion.ucl.ac.uk/spm/). The first five volumes of each subject were discarded to allow for T1 equilibrium. Prior to analysis the data were spatially realigned and corrected for differences in slice time acquisition using the middle slice in time as reference. Each subject's structural image was coregistered to the first of the functional images. Images were then normalized onto the ICBM template (http://www.loni.ucla.edu/ICBM/) using linear transformations only. Data were spatially smoothed using an isotropic 8 mm FWHM Gaussian kernel.

As a first exploratory analysis we looked for all regions affected by any of the visual stimuli. We performed a fixed effects GLM on all subjects contrasting all the conditions in which a target was followed by either a mask or a control, against the baseline which consisted of all periods in between. The regions that were found to be active in this contrast were used as an ROI later in the analysis. As a specification we performed a fixed-effects analysis with a contrast between all target & mask versus all target & control conditions for all subjects.

Next, we moved to a single subject analysis, creating specific contrast for every subject that reflected their behavioural data from the scanner. Per subject we looked only at the SOAs that showed a significant difference in the behavioural data between target & mask and target & control conditions, and contrasted those. Within the results of this contrast, we then used the previously created ROI (all visual conditions versus baseline) to perform a small volume correction (SVC) to find all regions that were originally activated by any of the stimuli (as seen in the ROI) and that show a significant difference in activity between the well visible target & control conditions and the less visible target & mask conditions.

3. Results

3.1 First behavioural test

Before we started the imaging experiment we first searched for the settings of the optimal visual stimuli, which would induce the biggest masking effect. In our first behavioural test we started out with a single target on the right side of the screen, using a separation of two pixels and three different eccentricities from the fixation cross (50, 75 and 100 mm). We chose 6 SOAs to determine in which time-frame masking would be strongest in this particular setting.

The results averaged over all subjects can be seen in figure 4. In a 3 x 7 ANOVA we found a main effect for both eccentricity ($F=9.513$, $p<0.0001$) and SOA ($F=4.591$, $p<0.0001$). A Bonferroni corrected post-hoc test revealed a significant difference between eccentricities 200 and 150 ($p<0.0001$) and between eccentricities 200 and 100 ($p<0.0001$), but not between 100 and 150. In a Bonferroni corrected post-hoc test of SOA we saw a nice U-shape, defined by the lack of significant difference between SOAs 0 and 84, and between SOAs 33, 50 and 67. All other differences were significant (all combinations $p<0.0001$ except between SOA 0 and 67, $p<0.004$). This puts the middle of the U-shape along SOAs 33, 50 and 67 (figure 4). There was no significant difference between SOA 133 and baseline (the target only condition), indicating full visibility was already

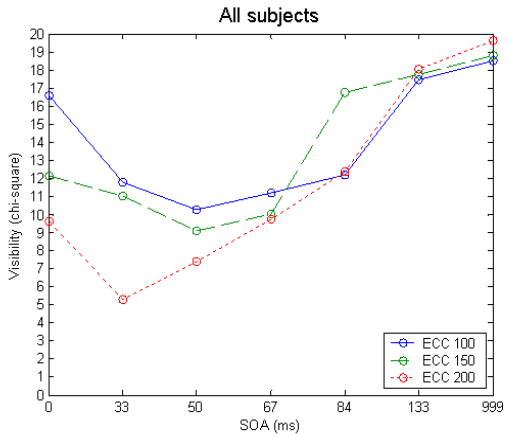


Figure 4. Results of the first behavioural test. Displayed is the visibility of the target per stimulus onset asynchrony (SOA) and eccentricity (ECC). Visibility is measured in Chi-square, where 0 means no visibility (correct responses at change level), and 20 means full visibility. SOA 999 is the target-only condition where there was no mask following the target. As there was nothing to block visibility this was taken as a baseline for full visibility under the experimental conditions. (Note this is not equal to a chi-square of 20, as even with full visibility subjects infrequently made a small number of mistakes.)

restored at that time. Subjects displayed a clear bias towards the right response button (responses averaged over all subjects: 41.84% left response button, 57.84% right response button, 0.29% no response, z= -14.37, p<0.0001 using a binominal test) although the diamond (left button) and the square (right button) both occur in exactly 50% of the trials.

3.2 Second behavioural test

In response to our findings in the first behavioural test, we added a second target on the left side of the screen to counter the response-bias. This also decreased the amount of attention subjects could apply to the stimulus and thereby increased the unpredictability of the stimulus. In order to reduce the number of trials we chose to reduce the number of conditions rather than weaken the statistical strength by reducing the number of repetitions per condition too much. As we found no difference in effect between eccentricities 100 and 150, we removed the smallest eccentricity. We also reduced the number of SOAs to four. These four were chosen to capture the dip of the U-shape as found in the previous test, and allow enough time before and after to be able to still recognize the U-shape itself. To optimize the strength of the masking effect, we decided to try an additional manipulation of separation, thus coming up with two combined conditions, one with an eccentricity of 75 mm and a separation of 1 pixel and the other with a eccentricity of 100 mm and a separation of 3 pixels. This way we had two specific the better results would be used in the fMRI potential stimulus setups, of which the one yielding experiment.

First of all, using two mirrored targets there was no response bias anymore (z = 0.793, p < 0.428 using a binominal test). Figure 5a shows the results for the trials with an eccentricity of 75 and a separation of 1, figure 5b shows them for the trials with an eccentricity of 150 and a separation of 3. In a 2 x 3 x 4 ANOVA we found main effects for SOA (F=20.732 p<0.0001), mask versus control (F=139.781 p<0.0001) and eccentricity/separation (F=9.382 p<0.002). We also found a three-way interaction between SOA, mask/control and eccentricity/separation (F=5.749 p<0.001). There was no significant difference between the two eccentricity/separation combinations at the baseline (p<0.311) but there was for both masked (p<0.014) and control (p<0.050) trials. A test for simple effects revealed an effect of SOA in eccentricity 150 / separation 1 for both masked (F=4.769, p<0.003) and control (F=17.493, p<0.0001) trials, but in eccentricity 200 / separation 3 this effect was only present for masked trials (F=15.852, p<0.0001), and not the controls (F=1.797, p<0.145).

We were aiming for reduced visibility in the masked condition only, but the combination of eccentricity 150 / separation 1 does not provide this. Here, SOA affects both masked and control trials. In the eccentricity 200 / separation 3 combination SOA affects only the masked, but not the control. This difference in shape between the two functions led us to choose this combination for the scanning session.

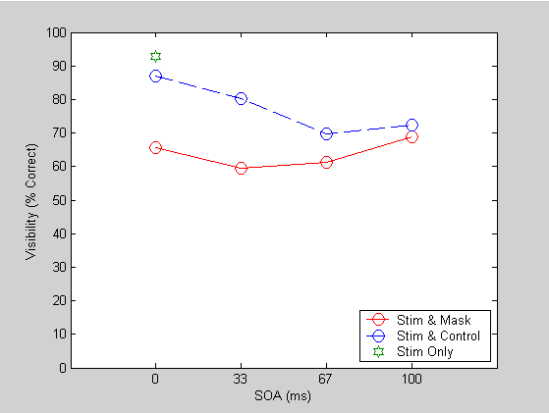


Figure 5A.

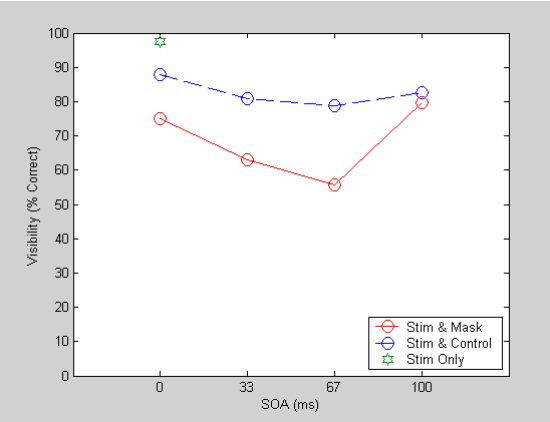


Figure 5B.

Results second behavioural test. A shows the results of trials with an eccentricity of 150 mm and a separation of 1 pixel, B shows the results of trials with an eccentricity of 200 mm and a separation of 3 pixels. The green star indicates the score for trials where the target was not followed by a mask, indicating the visibility baseline for that specific combination of eccentricity and separation. Visibility is measured in the percent of correct responses.

3.3 Behavioural results fMRI

Out of five scanned subjects, one dataset had to be discarded due to recording errors in the behavioural data and one was discarded because the subject's movement in the scanner exceeded our threshold of 4mm. The combined behavioural data of the remaining three subjects is shown in figure 6. Although the only change applied to the behavioural experiment between the second behavioural test and the fMRI experiment was the grouping of trials in blocks, which should not have a dramatic impact on the masking effect, the behavioural data acquired during scanning was quite different from that obtained in the last behavioural test.

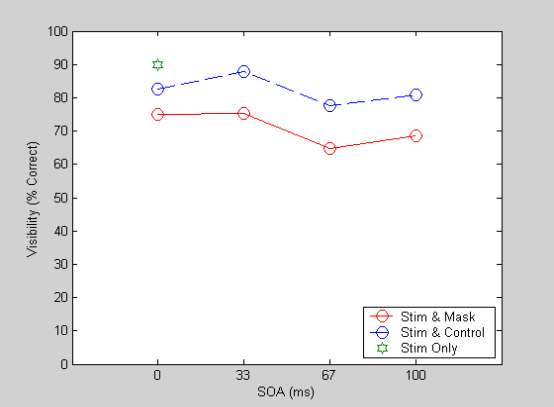


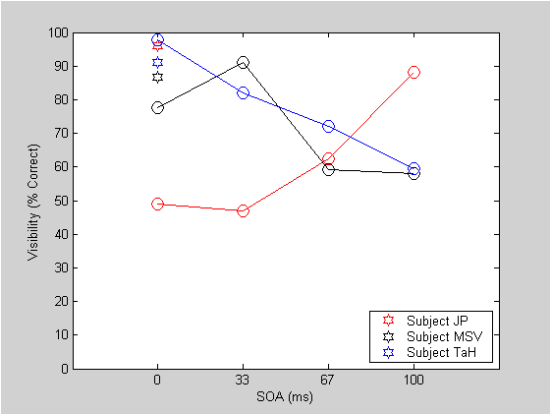
Figure 6. Behavioural results fMRI experiment, all subjects.Shown is the visibility of the targets in the scanner. The green star indicates the score for trials where the target was not followed by a mask, indicating the visibility baseline for that specific combination of eccentricity and separation. Visibility is measured in the percent of correct responses.

Using a 3 x 4 ANOVA we found main effects for SOA ($F=4.384$, $p<0.004$) and mask/control ($F=21.503$, $p<0.0001$), but no interaction between them ($F=0.296$, $p<0.828$). Hence, although the masked targets were less visible than the controls, both masked and control trials were affected by SOA in a similar manner. Bonferroni corrected post hoc comparisons revealed that the masked condition differed from both the control and the baseline (mask/baseline $p<0.0001$, mask/control $p<0.0001$), but that the difference between control and baseline was marginal ($p<0.071$).

There was no U-shape in the masking function, and its shape did not differ from that of the control condition. Because of this a potential effect in the BOLD response between these two conditions can not be attributed to a difference in the masking function. There was a large inter-subject variability, as seen in figure 7. This will make it more difficult to look for BOLD correlates averaged over all subjects, as the bigger differences in visibility do not occur in the same conditions for all subjects.

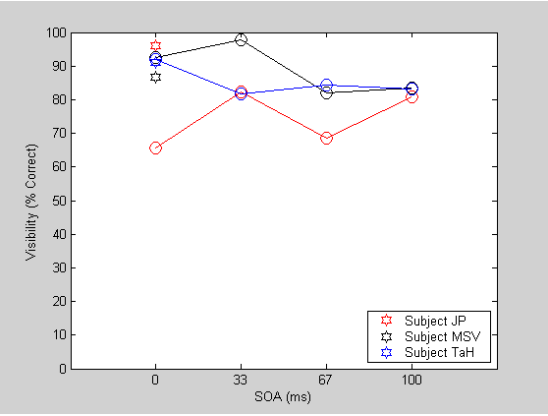
3.4 Imaging results fMRI

The initial fixed effects GLM of all visual conditions versus baseline on all subjects revealed a multitude of activated areas, including the visual cortex (stimulus related) and the motor cortex



Single subject target and mask.

Figure 7A.



Single subject target and control.

Figure 7B.

Behavioural results fMRI experiment, single subject. A. shows the percent correct scores per subject for all three subjects of trials with a target followed by a mask. B. shows the percent correct scores per subject for all three subjects of trials with a target followed by a control. Note the large inter-subject variability between the scores per condition.

(response related) (figure 8). This contrast should find all areas activated by any of our stimuli, and we created our ROI from this contrast.

The contrast of all target & mask conditions versus all target & control conditions in a fixed effects analysis did not reveal anything significant, nor did any correlation between the conditions appear. The behavioural data shows that the visibility of the target is reduced more when it is followed by a mask than when it is followed by a control. However, this effect does not occur in all SOAs. Therefore we should not create a contrast that includes all masked and all control conditions, but one that has only those conditions with SOAs that induce a significant difference in the behavioural data between target & mask and target & control. However, SOAs that show a large difference between target & mask and target & control in one subject showed no significant difference in another subject, and vice versa. This means it is not possible to create such a contrast for a fixed effects analysis of all subjects. For this reason we conducted single subject analyses (figure 9). From the behavioural data recorded in the scanning session we found for each subject which SOAs had a significant difference between target & mask and target & control. We created subject specific contrasts of target & mask conditions versus target & control conditions for those specific SOAs (see figure 10 for an example of one subject). On these subject specific contrasts we then applied a SVC (small volume correction), using the subject specific contrast of all visual conditions versus baseline as an ROI (figure 11).

Looking at subject JP as an example, at a threshold of 0.01 and a clustersize > 100 we found

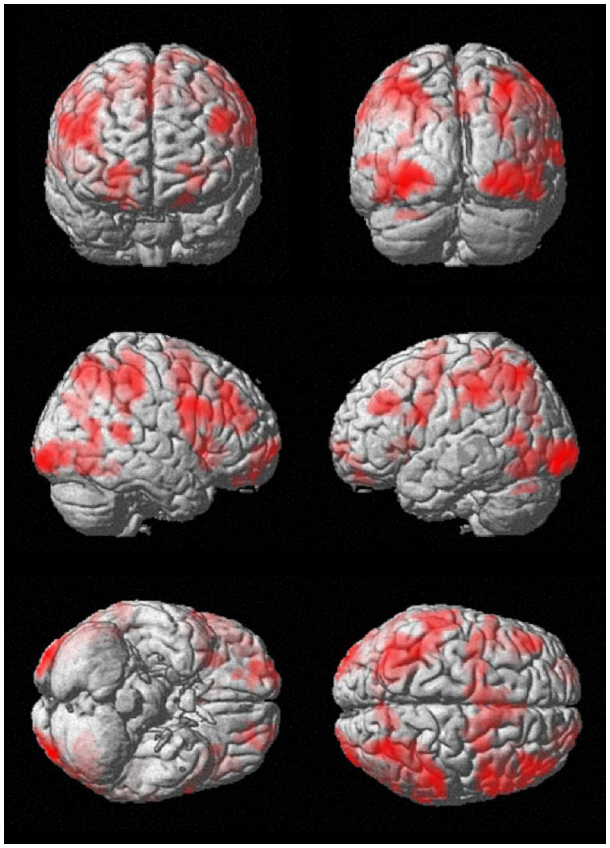


Figure 8. All conditions versus baseline, all subjects. This figure shows the averaged activity of three subjects in a fixed effects analyses contrasting all visual conditions versus baseline, projected on the brain of subject JP. This shows all areas that are somehow activated by any of the presented visual stimuli, or the associated task of pressing a button. The central occipital gyrus (area V1) is not clearly active, but the surrounding visual areas are. Also visible is the activity generated in the motor-cortex, due to the pressing of the response buttons. This contrast is used as the ROI in further analysis.

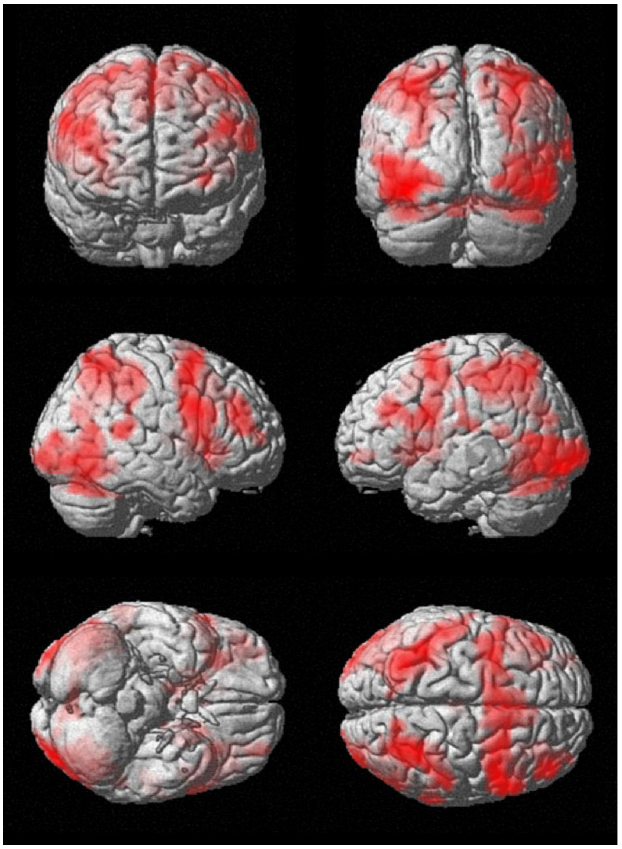


Figure 9. Masked versus control for subject JP. Activity of subject JP in a contrast of all masked conditions versus all control conditions ,using the subject specific behavioural data as the basis for the contrast.

significant activity in the left and right primary visual cortex, activity on the border of Brodmann areas 39 (angular gyrus) and 19 (extrastriate cortex) on the left hemisphere and activity on the border of Brodmann areas 47 (orbito-frontal) and 38 (superior temporal gyrus) also on the left hemisphere, where the locus seems to lie in area 38. Activity in the contra-lateral sides of BA 47/38 and BA 39/19 was also significant (BA 47/38 $p<0.08$ and BA 39/19 $p<0.012$) but these regions fell just outside of the ROI.

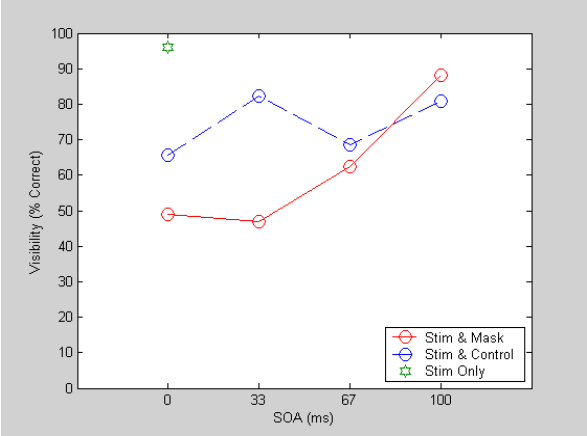


Figure 10. Visibility of the targets in the fMRI experiment, subject JP. The green star indicates the score for trials where the target was not followed by a mask, indicating the visibility baseline for that specific combination of eccentricity and separation. Visibility is measured in the percent of correct responses. The subject specific contrast used in figure 9 was devised from this data, emphasizing SOAs 0 and 33 as they have the largest difference between target & mask and target & control conditions.

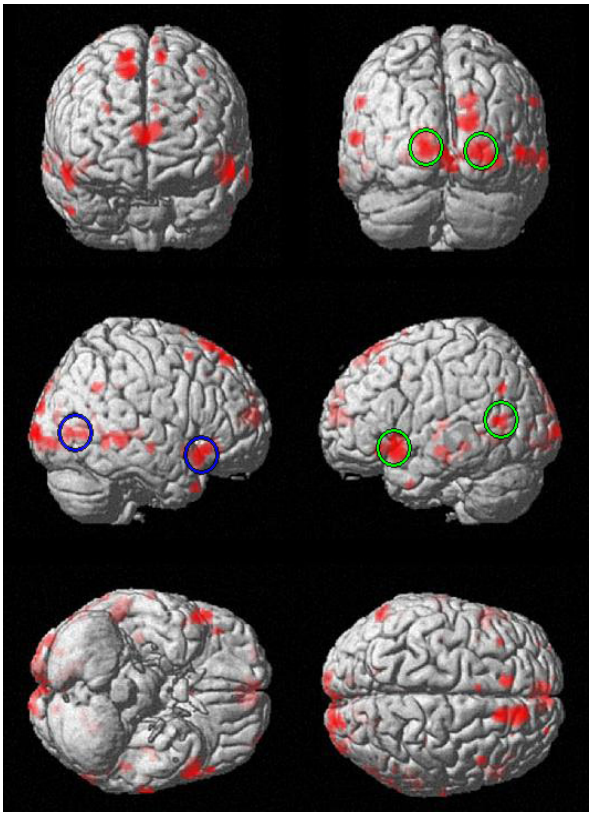


Figure 11. Masked versus control conditions after SVC, subject JP. This shows the activity that remained after applying a small volume correction (SVC) on the subject specific contrast of masked versus control conditions (figure 9). The ROI used is shown in figure 8. The areas that are significant and fall within the ROI are marked by green circles. The two areas on the right that are controlateral to the activated areas on the left are also significantly active. They fall outside the ROI however. These two areas are marked by blue circles.

4. Discussion

The initial two behavioural tests enabled us to create a set of target and mask that induced a good backward masking effect. Unfortunately in the scanner we were unable to control environmental factors as stringent as we did in the behavioural tests. The difference between the behavioural results from the second test and the behavioural results from the scanner, both using the same settings for target and mask, can be explained by this lack of control. The contrast and luminescence of the stimuli could not be tested to be equal to the behavioural setting, the lighting in the room was dimmer and the position and angle of view of the subject was restricted due to the limitations of stimulus presentation in the scanner. Due to this the results of the scanning session can only be taken as an indication for further research, which would also carry the need for an increase in subjects to be scanned.

Additional to these limitations, subjects consistently reported fatigue after the scanning session, both in general and in regards to their sight. A duration of 45 minutes without breaks (which were used in the behavioural test but not during scanning) seems too long to get reliable results throughout the session. We therefore like to present this study as a pretest for a future masking experiment that carries more control over environmental factors in the scanner. Yet before we proceed to our recommendations, we will look at what our preliminary results could still say about our intended aim.

The first part of our aim was to see whether

the masking effect would occur only in V1 (the primary visual cortex), or also in areas beyond V1. Both Macknik's theory (Macnik & Haglund, 1999) and the sustained-transient theory (Breitmeyer & Ogmen, 2000) claim there is no effect of masking on visual processing beyond area V1. However, we found two regions outside the visual cortex (the angular gyrus and the orbitofrontal cortex) more strongly activated by unmasked than by masked stimuli. This casts some doubt on whether the masking effect is actually confined to area V1, as predicted by a re-entry theory (Macknik & Livingstone, 1998, lamme et al, 2002) and, in lesser extent, the sustained-transient theory. In line with these findings, a recent study by Noguchi and Kakigi (2005) using MEG also showed masking effects in areas beyond the visual cortex. Studies on the phenomenon of masked priming also indicate there is information processing beyond the primary visual areas even when that information is masked. Masked priming occurs when information that is masked, and thus not consciously perceived, still has some effect on later cognitive processes. Usually this means facilitating a related choice or task performed directly after the masked information is presented, which is called priming. Vorberg et al (2003) for example were able to dissociate a masking effect from a priming effect, showing that masked information indeed exerted its influence on behaviour even when it was not consciously perceived. Areas in the brain that process masked information have for example been found in the parietal lobe. Dehaene et al (1998) presented masked numbers to subjects, and although they reported not seeing them they still had an influence on reaction time in a subsequent number classification task. Their imaging results show larger task-related motor activity when the prime was congruent with the required response, indicating the masked information reached the motor cortex to exert its influence.

The second part of our aim was to see if masking is a refractory process. Comparing masked and unmasked (control) conditions we found several regions both in and beyond the primary visual cortex that were more active for control conditions than for masked conditions. As the presented visual stimuli were physically equal except their rotation but cause different neural activity, this is an indication that not all high frequency successive visual stimuli cause a suppression of the processing of one of the two stimuli as claimed by Ogawa (Ogawa et al, 2000). Various differences apply to the study by Ogawa and a regular masking study. Firstly, Ogawa uses a stimulus duration that is considerably smaller (10ms) than is usually used in masking (Breitmeyer, 1984). Their stimuli never reach full invisibility in their subjects. Furthermore, they describe an effect of the first visual stimulus to the second, which is opposite to backward masking where it's the first stimulus that is suppressed. The signal suppression reported by Ogawa occurs around 200ms after stimulus presentation, which is much later than the dip of an average U-shape masking function which occurs between 33-100 ms depending on the subject. Even the standing wave of invisibility to which Macknik designs his experiments has a masking peak at around 100ms (Macknik & Livingstone, 1998). At these times (<100ms) Ogawa actually finds a BOLD response which, for two rapid successive stimuli, is twice that of a single stimulus. The use of different stimuli and variations in the experimental setup could explain variants in masking peak, although not as drastic as these.

In the comparison between masked and unmasked targets, two areas were active beyond the primary visual cortex, the angular gyrus and the orbitofrontal cortex. The angular gyrus, located closely to the extrastriate cortex (part of the visual system) is thought to serve as a connection between visual information and language (Ramachandran & Hubbard, 2001, Hubbard & Ramachandran, 2003, Ramachandran, 2005). Within the context of this experiment some interpretation of the target should occur in order to classify it as a diamond or a square. Such a conscious classification, necessary for the subject's experimental task, can only take place when the target is consciously available. Hence an increase in activation in conditions where the target is followed by a control would be in accordance with the idea that there is a masking effect in higher cognitive areas, but goes against the notion that, at least in this case, there is an influence of masked information on subsequent information processing.

The orbitofrontal cortex is often linked to processes of decision making and reward evaluation (Bechara et al, 1994, Rolls et al, 1994). It is though to facilitate an early 'bad' emotional feeling someone can have in regards to a stimuli or situation before there is conscious information to confirm this. In this sense, unconscious processing of information should be the domain of the masked targets, while in our experiment we found an increase in this area associated with the unmasked (thus visible) targets.

The only difference in regards to reward evaluation between masked and unmasked trials is that subjects usually knew that they gave the correct response in the control, while they didn't in the masked condition as they did not consciously perceive the stimuli. While we did not add any reward to the experiment, there was some pressure on the subject to perform the task well, and the absence of any feedback in the masked condition compared to a possible self-evaluation in the control condition might explain this orbitofrontal activation.

As this is a pilot study, we would like to suggest some additions for any future follow-up experiments using this set-up. Using only a single masking function (i.e. Type A or Type B masking) does not yet conclusively show that the diminished visibility at certain SOAs is actually due to masking, instead of just a repetition effect at a certain frequency of stimulus presentation. Hence, incorporating both masking functions is a valuable addition to such an experiment. As both masking functions have different temporal characteristics (plotting visibility versus SOA, Type A shows a straight increasing line while Type B displays a U-shape, see figure 3), this can help to show that an activity increase or decrease in certain brain areas is actually due to masking instead of a frequency effect. When brain regions are identified whose activity show a dependency on stimulus visibility the BOLD response in those areas should roughly follow the same pattern as the masking function itself. This way, while using physically similar stimuli like in the experiment described here, this should be a good way to show masking is actually a phenomenon in itself instead of just a refractory process of successive high frequency visual stimuli.

Of course, this strategy will only work if one gets clear masking functions in the behavioural data from the scanning session. Controlling environmental factors in the scanner is therefore critical. We suggest performing the prior behavioural test (to find the optimal stimuli and timing parameters) inside the scanner itself, or a dummy scanner that is equal in subject positioning and environmental light to the actual scanner used to perform the fMRI experiment. One of our main limitations was the lack of control over the equipment used to present the stimuli to subjects inside the scanner. The behavioural tests were performed on a CRT-monitor, on which the stimulus timing was tested using an oscilloscope. In the scanner however we were forced to use an LCD beamer on which stimulus timing could not be checked using the oscilloscope. Wiens and Öhman (2005) cast some doubts on the appliance of such a projector for masking experiments, especially when very short stimuli are used. Also the luminescence of the stimuli and the screen should be controlled for using a photometer (which measures light).

Regarding the data analysis, as the masking effect displays a strong inter-subject variability (see figure 7) we would like to make an argument for single subject analyses in masking experiments. Ress & Heeger (2003) present an example of the appliance of a single subject analyses in a study involving the primary visual areas (V1 to hV4). Especially when the visibility varies over SOA per subject it is important to be able to create individual contrasts. That way even when small neuroarchitectonial differences make subjects susceptible for masking at slightly different SOAs one can still extract the main effect and later combine them in a group analysis.

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