



## Reduction in V1 activation associated with decreased visibility of a visual target

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The perception of a brief visual target stimulus can be affected by another visual mask stimulus immediately preceding or following the target. The link of this visual masking illusion, with visual cortical activation, offers insights into the neural mechanisms for visual perception. The present study investigated the association of the visibility of a target with cortical activation in humans using psychophysical testing and functional magnetic resonance imaging (fMRI). A visual masking protocol that was suitable for an fMRI study was developed. The event-related fMRI was used to measure activation in primary visual cortex (V1) during visual masking and unmasking stimulation. We found that the visibility of the target stimulus was reduced in the masking condition, due to the presence of mask stimuli, but not in the unmasking condition. We also found that the activation in V1 was modulated by the temporal separation of the mask stimuli from the target and was associated with the visibility of the target that was recorded during psychophysical testing and fMRI. These findings are consistent with what has been observed in the primate visual cortex of monkeys, i.e., the transient on-response and after-discharge of V1 neurons to the target stimulus were suppressed by forward and backward mask stimuli, respectively.

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### Introduction

A brief visual target stimulus that is visible when presented alone can be rendered invisible if it is presented in spatial or temporal proximity to another stimulus (mask). The degree of visibility of the target depends upon both the spatial and temporal separation of the mask from the target. When the mask is placed next to the target with no spatial separation, influence of the mask on the target visibility is maximized. Such influence diminishes with increasing spatial separation between the mask and target. The

temporal separation between the mask and the target can also act to modulate the visibility of the target (Judge et al., 1980; Macknik and Livingstone, 1998). The most common approach to produce a visual masking effect is to use a two-pulse stimulation sequence in which a mask stimulus precedes (forward masking) or follows (backward masking) the display of a target stimulus. However, the masking effect can be enhanced by repetitively alternating the mask and target (standing wave) in which each mask stimulus forward masks the subsequent target and backward masks the previous target (Macknik and Livingstone, 1998).

Origins of the visual masking effect have been investigated by several psychophysical studies. A mask presented to one eye can block the perception of a target presented to the other eye (McFadden and Gummerman, 1973; Olson and Boynton, 1984). Therefore, the retina has been ruled out as the source of the visual masking effect. Neural involvement in the masking effect has been studied electrophysiologically in cats and non-human primates (Macknik and Livingstone, 1998; Schiller, 1968; Wurtz et al., 1980). Macknik and Livingstone correlated visual masking conditions that led to decreased visibility in humans with the electrophysiological responses to these stimuli in monkey primary visual cortex. They found that the mask stimuli that produced forward masking in humans suppressed the transient on-response of neurons to the target in monkey visual cortex. Those that produced backward masking inhibited the transient after-discharge of neurons, the excitatory response that occurred just after the disappearance of the target. Suppression of either transient on-response or after-discharge of primary visual cortical neurons is associated with a reduction in the visibility of a target. Macknik's study demonstrates at the neural level what has been masked or suppressed during visual masking in non-human primates and provides the basis for further investigation of cortical involvement in human visual masking.

If a neural response to a target stimulus is suppressed by a mask stimulus, blood–oxygen level-dependent (BOLD) signals should be decreased. However, the spatial extent of the stimuli that is used in the masking experiment is usually less than 3° of visual angle, and the duration of the stimulus display is between 10 and 30 ms. These demanding experimental conditions challenge reliable

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BOLD signal measurements in the primary visual cortex in response to visual masking and unmasking stimulation. In the present study, we developed stimuli that produced a robust visual masking effect and were suitable for BOLD functional magnetic resonance imaging (fMRI). Then we measured psychophysical and BOLD responses to these stimuli in a group of normal subjects. We found that diminished visibility of a target stimulus was associated with decreased BOLD activation signals in V1 cortex.

## Methods and materials

The study consisted of two parts: the first part aimed to develop and test visual masking stimuli that produce robust visual masking effects and are suitable for an fMRI study; the second part was the primary focus of the study, i.e., by using the spatial dimensions and timing parameters that were determined in the first part of the study, we attempted to measure the visual masking effect during a psychophysical test and fMRI. Twenty and fourteen neurologically normal subjects (18 female, 16 male, aged 19–49 years) participated in the first and second parts of the study, respectively. The study was approved by the Michigan State University Committee on Research Involving Human Subjects. All subjects signed written informed consents prior to the study.

### Determination of parameters of stimuli

A three-pulse stimulus sequence, in which one mask preceded and another mask followed the display of a visual target, was developed and tested. The sequence produced a strong visual masking effect, presumably by suppressing both transient on-response and after-discharge of neurons to the visual target. This allowed us to increase spatial dimension of the target stimulus.

The time sequence of stimulus presentation consisted of fixation, forward mask ( $M_f$ ), time delay ( $t$ ), target ( $T$ ), time delay ( $t$ ), backward mask ( $M_b$ ), and fixation (Fig. 1). The fixation comprised a  $0.3^\circ$  cross white mark at the center of the black background field. The target consisted of two vertical bars having the same spatial dimension, being offset left or right from the center of the display with an equal distance from the fixation, and being either in white or light gray on a black background. The width of the bars was  $1.5^\circ$ , and the distance between the centers of the two bars was  $6.5^\circ$ . The forward and backward masks had the same spatial dimension and were comprised of two identical white rectangular boxes as shown in Fig. 1. The inner surface of

the rectangular box was coincident with the outer surface of the vertical bar of the target. The width of the boxes was  $2.5^\circ$ , and the height of the masks was  $1^\circ$  greater than the height of the target. The following parameters were tested: (1) target height  $3^\circ$ ,  $4^\circ$ ,  $5^\circ$ ,  $7^\circ$ , and  $8^\circ$ ; (2) target duration 50 ms, 84 ms, 117 ms, and 150 ms; (3) forward mask duration 50 ms, 84 ms, 100 ms, 117 ms, 134 ms, and 150 ms; (4) backward mask duration 50 ms, 67 ms, 84 ms, 100 ms, 117 ms, and 150 ms; and (5) delay time between the target and each of the masks 0 ms, 34 ms, 100 ms, 500 ms, and 1000 ms. All possible combinations of these parameters were tested to optimize the parameters for producing a robust masking effect, while the spatial dimensions of masks and targets were selected as large as possible and durations of targets and masks were chosen as long as possible for reliable BOLD measurements. During the test, two different types of the targets were used: one with both bars in white and another with one bar in white and another bar in light gray. The subjects were asked to detect whether or not the two bars were at the same gray level. Each condition was administered for 20 trials. Prior to the test, each subject had a pretest to establish the gray level of the light-gray bar for the experiment. During the pretest, only targets were displayed, and the subject was asked to perform the same task. The gray level of the light gray bar of the targets was systematically varied, and these targets were presented randomly. The gray level of the light-gray bar selected for the study was as close as possible to the gray level of the white bar, while the subject was still able to detect the targets with an error rate less than or equal to 10%. During the tests, the subject was seated 2.5 feet away from a personal computer (PC) monitor and was instructed to focus his or her eyes on the fixation mark. The subject entered answers by pressing one of two keys on a PC keyboard at the end of each trial. The result was recorded automatically. Each subject had 10–30 training trials during which the stimuli differed from the tested stimuli in spatial dimension and timing.

The stimulus presentation was programmed with E-Prime (Psychology Software Tools Inc.) and displayed on a LCD. The contrast and luminance of the LCD were calibrated to the same level (<5% in difference) of the LCD used during fMRI.

We found that the target height of  $7^\circ$ , the target duration of 84 ms, and the mask duration of 84 ms or 100 ms produced the most robust visual masking effect. The masking effect decreased if the target extent was greater than  $7^\circ$ . Similarly, if the target duration exceeded 84 ms, and/or if the mask duration was greater than 100 ms or shorter than 84 ms, the masking effect diminished. Thus, we chose a target height of  $7^\circ$ , a target duration of 84 ms, and a mask

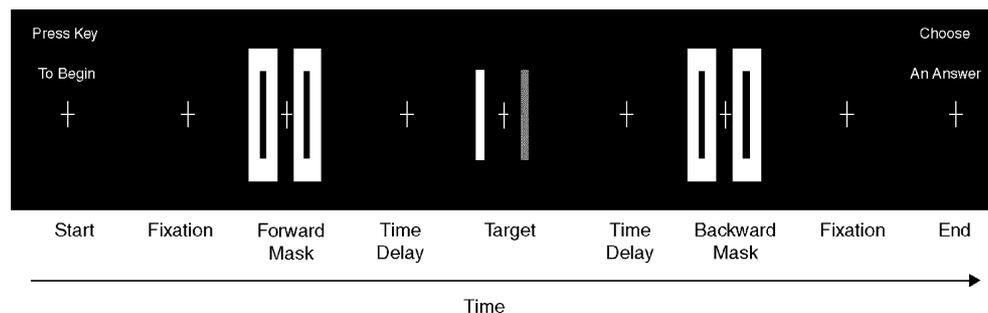


Fig. 1. The stimulus sequence consisted of fixation – forward mask – time delay – target – time delay – backward mask – fixation. Target was displayed for 84 ms, mask 84 or 100 ms, and time delay of 0, 34, 100, or 500 ms. During testing, the subject was instructed to detect whether the two bars were at the same gray level or not.

duration of 84 ms or 100 ms for the psychophysical test and fMRI study.

### Psychophysical test

The stimulus sequence was the same one described in the previous section (Fig. 1). The subjects, who participated in the second part of the study, were only tested with the optimal parameters that produced a robust visual masking effect. The following parameters were used: a height of  $7^\circ$  and a width of  $1.5^\circ$  for the two same size bars of the target with a distance of  $6.5^\circ$  between the centers of the two bars; a height of  $8^\circ$  and a width of  $2.5^\circ$  for the two identical rectangular boxes of the mask; a duration of 84 ms for the target; and durations of either 84 ms or 100 ms for the forward and backward masks. Thus, there were four different duration combinations between the forward mask and backward mask: (A) 100 ms for  $M_f$  and 84 ms for  $M_b$ ; (B) 100 ms for  $M_f$  and 100 ms for  $M_b$ ; (C) 84 ms for  $M_f$  and 84 ms for  $M_b$ ; and (D) 84 ms for  $M_f$  and 100 ms for  $M_b$ , respectively. The time delay between the target and each of the masks was 0 ms, 34 ms, 100 ms, or 500 ms. Again, prior to the test, each participant had a pretest to determine the gray level of the light-gray bar for the experiment subject to the criterion that the participant was able to detect the bars with an error rate equal to or less than 10%.

### Functional MRI protocols

The fMRI scan included three protocols: (1) a retinotopic mapping, (2) a corticotopic mapping, and (3) a visual masking protocol. The retinotopic mapping used phase-encoded polar coordinate stimuli that have been demonstrated to be an objective and reliable methodology to determine the borders of visual areas (DeYoe et al., 1996; Engel et al., 1997; Sereno et al., 1995). During the retinotopic mapping, a half-field black–white checkerboard with a contrast-reversing rate of 4Hz was presented. The checkerboard rotated around a fixation at the center of the visual field and completed a cycle every 36 s. It first rotated counterclockwise for 3.5 cycles, followed by an 18-s fixation, and then rotated clockwise for another 3.5 cycles. Due to the time delay of the BOLD response to the stimulus, the temporal phase of the BOLD signal time course in a voxel was shifted relative to the polar phase of the periodic stimulation corresponding to the spatial location of the checkerboard. This phase uncertainty of the BOLD signals was removed by activation during the counter-clockwise and clockwise rotations of the checkerboard wedge together (Engel et al., 1997; Sereno et al., 1995). The ventral (dorsal) border of V1/V2 was determined by the polar angle phase reversal in the activation of the first band when the center of the stimulus was within  $45^\circ$  of the superior (inferior) vertical meridian.

The corticotopic mapping protocol attempted to determine the cortical regions that were activated by the target independently. During the corticotopic mapping, a target that had the same spatial dimension of the target that was used in the visual masking experiment was displayed for 4 s followed by an 18-s long fixation in each trial, and a total of seven trials were presented (images of the first dummy trial were discarded). In order to increase the activation that was produced by the target, a square-wave grating pattern was created in the vertical direction on each of the two bars with a spatial frequency of 2 degree-per-cycle and a contrast of 35%, and the pattern was contrast-reversing at a rate of 4 Hz.

The visual masking protocol consisted of five different trials: (1) target only (T) displayed for 84 ms; (2) masking condition ( $M_fTM_b$ ) in which a 100-ms forward mask preceded a 84-ms target and a 84-ms backward mask followed the target; (3) mask-only ( $M_fFM_b$ ) in which a 84-ms long fixation period replaced the target in the masking condition; (4) unmasking condition ( $M_fTtM_b$ ) that was similar to  $M_fTM_b$  except a 500-ms time delay (t) was placed between the target and each of the two masks; and (5) second mask-only condition ( $M_fTfM_b$ ) in which a 84-ms long fixation period replaced the target display in the unmasking condition. The first mask-only condition served as a control condition for the masking condition, while the second mask-only condition served as a control condition for the unmasking condition. During each trial, a fixation mark followed the stimulation sequence, resulting in a 20-s long trial. In anticipation that a prolonged fMRI protocol could increase eye stress and fatigue, increase the probability of head movement, and degrade image quality, we limited the number of trials performed. In addition, we needed to have sufficient numbers of trials for each condition to average functional signals and to have statistical power for differentiation of the functional signals for the different conditions. As a trade-off, we only used one of the two targets that were used in the psychophysical test: both two bars in white. All five conditions were pseudo-randomly presented in one scan with three trials for each. Each subject had 6 scans, resulting in a total of 18 trials for each condition. During scanning, the subjects were instructed to focus their eyes on the fixation mark at the center of the visual field at all times and to detect the target. Eight of the subjects were asked to use the motor response pad to indicate whether or not they visualized the target by pressing one of the two response buttons immediately after the display of the stimulation sequence. Their responses were recorded automatically on a PC.

### MRI parameters

Six axial-oblique sections perpendicular to Calcarine Fissure were acquired on a GE 3.0T clinical scanner using a gradient-echo, echo-planar imaging pulse sequence (GE-EPI) with TE/TR = 40 ms/500 ms, flip angle  $70^\circ$ , field-of-view 200 mm, matrix size  $64 \times 64$ , and slice thickness 5 mm. Thus, forty images per anatomic section were acquired for each trial in the visual masking protocol.

### Data analysis

Preprocessing: All functional images were corrected for possible in-plane translation and rotation within each scan and between scans (Cao et al., 1993). Signal intensity time course was corrected for possible slow baseline drifts using 0, 1st and 2nd orders of polynomials, and normalized to allow signal averaging over voxels, over scans, and across subjects (Huang et al., 2001). Then the functional images were sorted according to the five stimulation sequences.

With the Retinotopic Map, the discrete Fourier transformation of the image time series that was acquired during the retinotopic mapping was computed voxel-by-voxel to yield the magnitude and phase spectra. The phase at the stimulus rotation frequency represented the polar location of the stimulus plus a time delay due to the hemodynamic response. This phase uncertainty was removed by averaging two phases that were obtained from the clockwise and counter-clockwise rotations of the stimulus. Finally,

the phase was color-coded and painted onto T1-weighted anatomic images. The border of V1 that was adjacent to V2 was determined by the polar angle phase reversal in the activation of the first band when the center of the stimulus was within 45° of the superior (or inferior) vertical meridian.

**Corticotopic regions of the target:** Corticotopic regions of the target were determined from functional images that were acquired during the corticotopic mapping. The time series of the functional images was cross-correlated (Bandettini et al., 1993) with sine and cosine reference functions having a period of 22 s to obtain a pair of cross-correlation coefficients (ccc) voxel by voxel (Lee et al., 1995). Magnitude and phase of ccc were further calculated (Lee et al., 1995). Activated voxels were at the magnitude threshold of ccc equal to or greater than 0.35 and with phase range of  $[-100, 50]$ . The threshold level of activated voxels was chosen to yield an estimated Type I error rate of  $P < 0.0005$  per voxel. The activated voxels that were coincident with the primary visual cortex (determined by the retinotopic mapping) defined the region-of-interest (ROI) for analysis.

**Visual masking:** Signal intensity time courses of the functional images that were obtained during the visual masking experiment were averaged over the voxels within the ROI first and then over the trials for the same stimulus. The baseline was determined by the average of the last twelve time points, and the data were aligned to trial onset. For each stimulation condition, the area under the time course of signal intensity changes was integrated and then averaged across the subjects. This area was used as a metric for activation.

## Results

### Psychophysical test

The visibility of the target was affected by the presence of forward and backward masks, and the effect varied with the time delay between the target and the masks (Fig. 2). Without the masks, all subjects detected the target correctly with a small error rate (mean  $\pm$  SEM =  $4\% \pm 1\%$ ), showing that the target was clearly visible. When a forward mask (100 ms) preceded the target and a backward mask (84 ms) followed it immediately, the two masks dramatically suppressed its visibility, reflected in the significantly increased error rate of  $44\% \pm 3\%$  (50% error rate for random

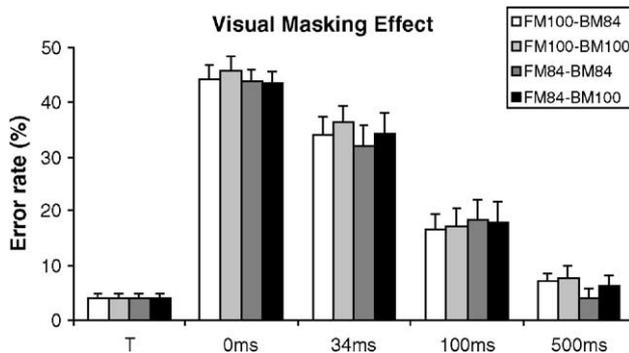


Fig. 2. Results of the psychophysical test. FM100 and FM84 represent the forward mask with time duration of 100 ms and 84 ms, respectively. Similarly, BM100 and BM84 represent the backward mask with time duration of 100 ms and 84 ms, respectively. The labels in the x-axis denote the time delay employed in each stimulation condition, and T is for target only condition.

choice) ( $P < 0.0001$ ,  $t$  test). When two same time delays were placed between the target and the two masks, one time delay prior to the target and the other after it, the masking effect was reduced with increasing the time delay (Fig. 2). The averaged error rates were  $34\% \pm 3\%$ ,  $17\% \pm 3\%$ , and  $7\% \pm 1\%$  for the 34 ms, 100 ms and 500 ms of time delays, respectively. The error rates for the first two conditions were significantly higher than that without the masks ( $P < 0.001$ ), while the error rate for the last one was not ( $P > 0.1$ ). A comparison of the masking condition  $M_fTM_b$  (no time delays between the masks and the target) to the unmasking condition  $M_fTtM_b$  ( $t = 500$  ms, time delays between the masks and the target), revealed that the error rates were significantly different ( $P < 0.0001$ ). The other three combinations of the durations of the two masks had similar effects on the detection of the target (Fig. 2).

The task in our psychophysical test was to discriminate the two bars in the target. After the behavioral tests, the participants were asked whether they saw the target T during the masking condition  $M_fTM_b$ . Some of them reported that they saw the target but could not see the difference between the two bars, indicating the inability to discriminate the bars. However, the others reported that they did not see the target at all, implying that the target was rendered invisible during these trials. In this paper, the term invisibility should be understood as either the invisible of the target or the inability to discriminate the two bars.

### Activation in V1

The averaged signal intensity time courses in V1 for the five conditions are plotted in Fig. 3. The averaged integrated areas of signal intensity changes in V1 for the five conditions are illustrated in Fig. 4. The target only condition produced a measurable BOLD signal change and the area of the BOLD response was  $3.8 \pm 0.4$  ( $\% \times$  second). As expected, the area activated by the target-only was smaller than those produced by the masking and unmasking conditions (Figs. 3 and 4).

The unmasking stimulation  $M_fTtM_b$  produced a significantly greater cortical activation in V1 ( $7.7 \pm 0.6\% \times$  second) than that ( $6.4 \pm 0.5\% \times$  second) observed during its control stimulation  $M_fTtM_b$  in which the fixation F replaced the target T ( $P < 0.005$ , paired  $t$  test). Due to the negligible contribution from the fixation F (a 0.3° cross white mark at the center of the black background field), this observed activation difference was mainly contributed by the neuronal responses of the neurons in the ROI to the target T. Thus, the significantly increased activation for  $M_fTtM_b$  indicates a small or no suppressing effect from the masks, and this is consistent with the unaffected visibility of the target (Fig. 2). The masking stimulation  $M_fTM_b$ , however, produced a slightly less but not significantly different activation in V1 ( $4.4 \pm 0.4\% \times$  second) than that ( $5.2 \pm 0.5\% \times$  second) observed during its control stimulation  $M_fFM_b$  ( $P > 0.1$ , paired  $t$  test). Since  $M_fTM_b$  provided more visual stimulation than  $M_fFM_b$ , this less activation showed that cortical activation was strongly suppressed for  $M_fTM_b$ . During the masking condition, the forward mask  $M_f$  suppressed the on-response of neurons to the target T, the target inhibited the after-discharge of neurons to  $M_f$  and suppressed the on-response of neurons to the backward mask  $M_b$ , and the backward mask inhibited the after-discharge of neurons to T (Macknik and Livingstone, 1998). These suppressing interactions among  $M_f$ , T, and  $M_b$  counteracted the additional stimulation provided by T in  $M_fTM_b$ . As a result,  $M_fTM_b$  provoked a less

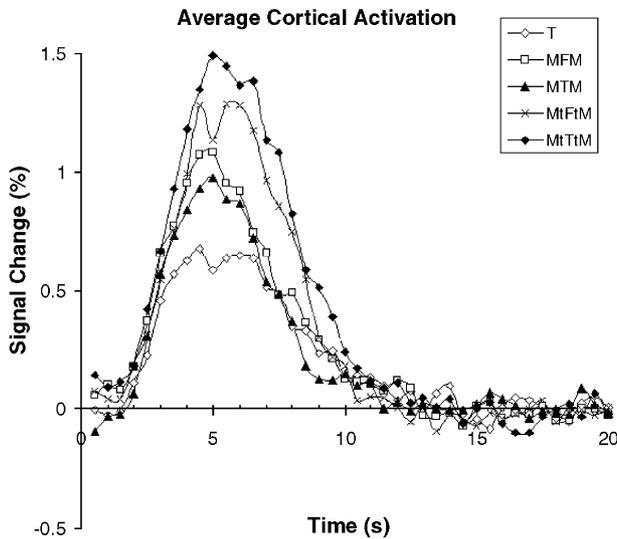


Fig. 3. Averaged signal intensity time courses in V1 over 14 subjects. *T*: target only; *MFM*: the first mask-only condition; *MTM*: masking condition; *MtFtM*: the second mask-only condition; and *MtTtM*: unmasking condition.

activation in V1 than  $M_fFM_b$ . These strong interactions also diminished the visibility of the target in the psychophysical test (Fig. 2). The difference in the activated areas between the unmasking condition subtracted from its own control condition ( $M_{fTtT}M_b - M_{fTtFt}M_b$ ) and the masking condition subtracted from its corresponding control condition ( $M_{fT}TM_b - M_{fF}FM_b$ ) was  $2.1 \pm 0.5$  (%  $\times$  second), statistically significant ( $P < 0.002$ , paired  $t$  test); see Fig. 5.

Eight of the fourteen subjects were asked to report whether they saw the target after each trial by pressing a button during their functional MRI scans. They observed the target for 99% and 100% of the trials during the target-only condition and the unmasking condition, respectively. During the two mask-only control conditions, the subjects did not see the target for 99% of the trials.

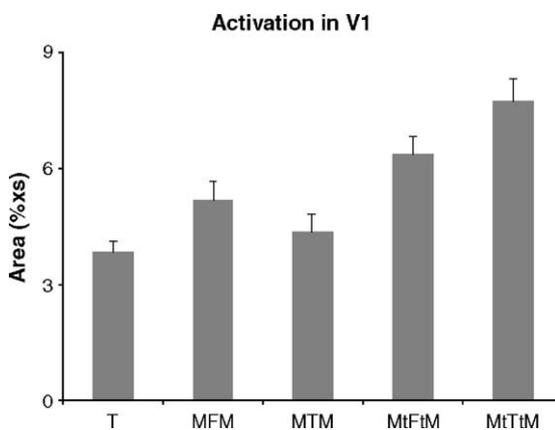


Fig. 4. Averaged integrated areas of signal intensity changes in V1 for the five conditions. Activation in response to the unmasking condition was significantly greater than that observed during its control condition (Paired  $t$  test,  $P < 0.005$ ), while activation provoked by the masking stimulation was slightly less but not significantly different than that produced by its corresponding control condition (paired  $t$  test,  $P > 0.1$ ). *T*: target only; *MFM*: the first mask-only condition for the masking stimulation; *MTM*: masking condition; *MtFtM*: the second mask-only condition for the unmasking stimulation; and *MtTtM*: unmasking condition.

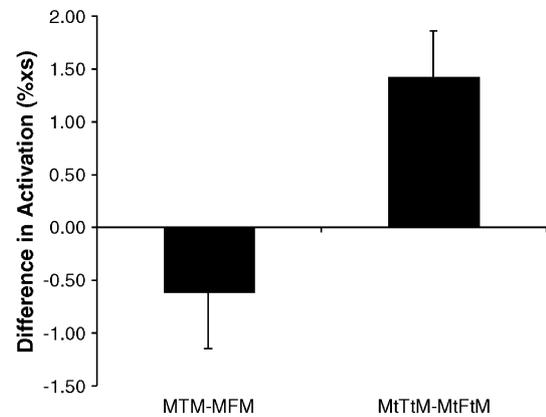


Fig. 5. Averaged differences in the areas under the activation response curves between the masking and its corresponding control conditions ( $MTM - MFM$ ), and between the unmasking and its corresponding control conditions ( $MtTtM - MtFtM$ ). A comparison between the two differences yields a significant difference (paired  $t$  test,  $P < 0.002$ ).

During the masking condition, however, the observation of the target was reduced to 72% of the trials, yielding a big error rate of 28%.

## Discussion and conclusions

The aim of the study was to measure the neural response to visual masking using BOLD fMRI. We explored a large range of spatial dimensions for the target and a large range of temporal durations for the masks and developed a three-pulse stimulation sequence that produced a robust masking effect (Fig. 2). This stimulation sequence has a spatial dimension and temporal duration that are feasible for BOLD fMRI investigations of visual cortical response to the masked or unmasked target. In addition, a time delay between the target and the masks can robustly modulate the effect of visual masking.

When these stimulation sequences were employed, an 84-ms target stimulus, which was clearly visible for the subjects, was perceptually diminished when the masks preceded and followed the target immediately. Under this masking condition, the subjects were only able to detect the target correctly for 56% of the total trials. Since the number of trials in which the two bars in the target were at the same gray level was count-balanced with the number of trials in which the two bars were not at the same gray level, and the detection of the target was a binary task (e.g., yes or no), randomly selected answers would yield a 50% chance for correctly detecting the target. Thus, the observed 56% correct rate for detecting the target implies that the visibility of the target was almost diminished under the masking condition. With an increased time delay between the target and the masks, the rate of correctly detecting the target increased, showing an increased visibility of the target. When the time delay was 500 ms, the influence of the masks on the target vanished, and the target became clearly observable again.

When the same stimulation sequences were used in the same subjects, BOLD responses were measured in the primary visual cortex. During the fMRI, the five different conditions were present in a pseudo-random fashion, and the task for the subjects was changed to whether they saw the target instead of detecting the contrast of its two bars, as in the psychophysical test. The task change served two purposes: (1) monitoring the subjects' performance and attention and (2) providing some useful infor-

mation for the visibility of the target during the different conditions. This change was mainly due to the time limitation for the fMRI and the requirement of a relatively large number of trials for statistically significant differences among the conditions. The task difference between the psychophysical test and the fMRI was reflected in the different error rates for the psychophysical test (44%) and the fMRI (28%) during the visual masking condition. Since a partially suppressed target could remain the target still visible but significantly obscure the correct detection of the contrast of its two bars, these different error rates imply that the target was partially suppressed under the visual masking condition.

For visual cortical activation, the masking condition  $M_fTM_b$  was controlled by the first mask-only (control) condition  $M_fFM_b$ . Similarly, the unmasking condition  $M_ftTM_b$  was controlled by the second mask-only condition  $M_ftFM_b$ . The masking condition and its control condition had the same stimulation duration, and the only difference is that  $T$  in the former was replaced by  $F$  in the latter. It is also the same for the unmasking and its control condition. Cortical activation for  $M_fTM_b$  was mainly from three contributions: the forward mask stimulus  $M_f$ , the backward mask stimulus  $M_b$ , and the interaction between them. This interaction includes the suppressed transient on-response of neurons to the second stimulus  $M_b$  due to the first stimulus  $M_f$  and the inhibited transient after-discharge of neurons to the first stimulus  $M_f$  due to the second stimulus  $M_b$  (Macknik and Livingstone, 1998). In comparison with  $M_fFM_b$ , cortical activation for  $M_fTM_b$  had three additional contributions: the target stimulus  $T$  and two additional interactions between  $T$  and the two masks ( $M_f$  and  $M_b$ ), respectively. So the difference in activation between  $M_fTM_b$  and  $M_fFM_b$  accounted for the three additional contributions. Similarly, the difference in activation between  $M_ftTM_b$  and  $M_ftFM_b$  also accounted for three contributions: the target  $T$  and two interactions between  $T$  and the two masks, respectively. Thus, the difference between these two differences provided a measure for examining the effect of the time delay  $t$  to the interactions between the target and the masks, since  $t$  was the only variable. The effect was significant as seen in Fig. 5, showing that the interactions significantly varied with the time delay. When  $t = 0$  (the masking condition), the interactions were strong, reflected in the reduced activation and in the observed strong masking effect (Figs. 2 and 4). For the long time delay  $t = 500$  ms (the unmasking condition), however, the interactions were weak, reflected in the diminished masking effect (Fig. 2).

The control condition  $M_ftFM_b$  evoked a smaller BOLD response than  $M_ftTM_b$ , showing the non-linearity of the BOLD response under these conditions (Fig. 4). The difference between these two conditions is reflected in their different stimulation frequency –  $M_ftFM_b$  has a temporal frequency of approximately 5.4 Hz and  $M_ftTM_b$  has a temporal frequency of approximately 0.84 Hz. Cortical activation for  $M_ftFM_b$  and  $M_ftTM_b$  was attributed to the forward mask stimulus  $M_f$ , the backward mask stimulus  $M_b$ , and the interaction between them. Since the only difference between  $M_ftFM_b$  and  $M_ftTM_b$  is the time delay between  $M_f$  and  $M_b$ , e.g., the former has a time delay of 84 ms and the latter has a time delay of 1084 ms, it is plausible to assume that the interaction is the underlying physiological mechanism responsible for the stimulation frequency effect and the non-linearity of the BOLD response. This interaction represents the suppressed transient on-response of neurons to  $M_b$  due to  $M_f$  and the inhibited transient after-discharge of neurons to  $M_f$  due to  $M_b$  and varies with the time delay, yielding the stimulation frequency effect and the non-linearity of the BOLD

response. As the interaction always reduces cortical activation and decreases with increasing the time delay, it predicts that cortical activation for  $M_ftTM_b$  increases with decreasing its stimulation frequency. This prediction is consistent with our observation (Fig. 4). When the time delay is large enough, the interaction vanishes, yielding the linearity of BOLD response which has been observed in many fMRI studies.

The diminished visibility of target in visual masking is associated with decreased activation in V1. Although the unmasking condition produced a significantly more activation than the masking condition, it might be problematic for direct comparison of BOLD measurements between  $M_ftTM_b$  and  $M_fTM_b$  due to the possible non-linearity of BOLD response. In consideration of the difference between  $M_ftTM_b$  and  $M_ftFM_b$ , if the BOLD response was linear, the difference of their BOLD measurements should equal to the BOLD measurement for  $T$ , since there were almost no interactions among the target and the masks for the long time delay ( $t = 500$  ms). The measured difference, however, is much smaller than the BOLD measurement for  $T$ , showing that the BOLD response was not linear (Fig. 4). In order to overcome this problem, several comparisons were made. First, we compared  $M_ftTM_b$  with  $M_ftFM_b$ . Both stimuli had the same stimulation duration, and  $M_ftTM_b$  provoked a significant more BOLD response than  $M_ftFM_b$ . Second, we compared  $M_fTM_b$  with  $M_fFM_b$ . In spite of much more visual stimulation provided by  $M_fTM_b$  than  $M_fFM_b$ , the former produced a lesser BOLD response than the latter did. Thus, the additional stimulation must be counteracted by the suppressing effect of the strong interactions among the target and the masks. In consideration that the visibility of the target was intact under the unmasking condition but significantly reduced under the masking condition, these comparisons suggest that the significantly more BOLD response provoked by  $M_ftTM_b$  is associated with the visibility of  $T$  under the unmasking condition, and the reduced BOLD response in  $M_fTM_b$  is associated with the diminished visibility of  $T$  under the masking condition, respectively. We also compared the difference between  $M_ftTM_b$  and  $M_ftFM_b$  with the difference between  $M_fTM_b$  and  $M_fFM_b$ ; they are significantly different (Fig. 5). In addition, the target itself produced measurable BOLD signals. All of these observations combined indicate that the decreased visibility of the target is associated with the decreased BOLD signals in the primary visual cortex, suggesting the neural involvement in the visual masking effect. These findings are consistent with what have been observed in V1 of monkeys using multi-unit recording, i.e., forward masking suppressed the transient on-response of neurons to the target stimulus while backward masking inhibited the transient after-discharge of neurons in response to the disappearance of the target (Macknik and Livingstone, 1998). An interesting application of this paradigm is to study visual cortical inhibitory functions in disease conditions using fMRI. A psychophysical study of visual masking in migraine suggests that hypovisual cortical inhibitory functions might be, in part, responsible for migraine with visual aura (Palmer and Chronicle, 1999).

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