

Quantitative comparison of the hemodynamic activation elicited by cardinal and oblique gratings with functional near-infrared spectroscopy

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Evidence has been accumulated for over a century indicating that the visual system of humans and many animals is more sensitive to contour stimulation at vertical or horizontal orientations than oblique orientations. However, the neural basis for this orientation anisotropy is still a subject of debate. In the present study, we recorded brain activity over the parietal-occipital and frontal lobes with functional near-infrared spectroscopy (fNIRS) when human participants were presented with gratings in different orientations. The oblique gratings induced a much larger change in the oxygenated hemoglobin concentration than vertical and horizontal gratings in the left occipital lobe. However, we did not find any significant orientation anisotropy in the frontal lobe. Our study showed that different quantitative changes in the hemoglobin concentrations occurred in response to differently oriented stimuli in the visual cortex and that fNIRS could potentially be a valuable tool in the assessment

Introduction

It is well known that humans and many animals show ‘a small but consistent superiority in performance when visual stimuli are horizontal or vertical as opposed to oblique’ [1]. This orientation anisotropy, known as the oblique effect, appears in a wide variety of perceptual tasks [2–4]. One possible explanation is that more neural machinery is devoted to processing vertical and horizontal (cardinal) contours than oblique ones. For example, many single-unit studies have shown that more neurons respond preferentially to cardinal contours than oblique ones in areas 17 and 21 of cats and the visual cortex of ferrets and primates [5–7]. However, the amount of cardinal overrepresentation in the cortical area is modest or nonexistent in some studies [8,9]. Functional MRI (fMRI) research in humans has also yielded different accounts of how brain activity is modulated by orientation [10–13]. All of these findings suggest that the orientation response in the visual cortex is likely to be more complex than a simple bias in the orientation representation.

The current study aims to further investigate the neural substrates of orientation anisotropy in human adults using functional near-infrared spectroscopy (fNIRS). As a non-invasive imaging modality, fNIRS has been used to assess the cerebral hemodynamic responses within the visual cortex [14–16]. However, studies of visual perception

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are relatively lacking. We investigated changes in the oxygenated hemoglobin (HbO) and HHb concentrations in response to different orientations (0, 45, and 90°) of gratings by recording brain activity in the parietal-occipital and frontal lobes with fNIRS. We expected that visual gratings would activate the fNIRS signals in the occipital lobe. Therefore, we could compare different fNIRS responses associated with differently oriented stimuli.

Methods

Ethics statement

All experimental procedures were approved by the Beijing Normal University Institutional Review Board. Research was carried out according to the principles of the Declaration of Helsinki, and the experiments were conducted with the permission and written consent of each participant.

Participants

A total of 21 college students between 18 and 25 years of age (six men, mean age = 22 years) participated in the present study as paid volunteers. Two students were excluded for a high ratio of noise, and one student was excluded for a lack of activation in the occipital lobe. All of the participants were right-handed, unaware of the purpose of the research, and had normal or corrected-to-normal vision.

Stimuli and apparatus

As shown in Fig. 1, the stimuli consisted of two gratings (diameter 6°, 75% contrast) displayed as two patches (centered 4.5° horizontally from a continuously present fixation cross). One grating was 3 cycle/degree and the other was 2 cycle/degree. The phase of each of the two gratings was randomized by an integral multiple of $\pi/20$. The participants were asked to press one of two buttons with his/her right hand to indicate which grating was lower in spatial frequency (i.e. which one was 2 cycle/degree). No feedback was provided to the participants.

Within each session, the two gratings were presented in the same orientation, which was either 0° (horizontal), 45° (oblique), or 90° (vertical) from horizontal. The stimuli were generated by a Matlab programme (The MathWorks, Natick, Massachusetts, USA) and presented on a 21-inch CRT monitor (1600 × 1200 pixels, 75-Hz frame rate) at an 85-cm viewing distance. The experiment was conducted in a dark room with dim light.

Procedure

The experiment included three separate sessions with different grating orientations of 0, 45, or 90°. The sequence of the three sessions was randomized for each

participant. Each session consisted of five blocks, and each block consisted of 10 trials. In the beginning of each session, the participants fixated on a central cross for 2 min. Then, the block began with two gratings in the same orientation and a randomized phase. The gratings were presented in 20 s (1 trial/2 s, 10 trials), followed by a rest period of 20 s while observing the fixation cross. The trial sequence is shown in Fig. 1. The participants were instructed to maintain fixation on the central cross and blink naturally throughout the experiment. The entire experiment took ~22 min.

Functional near-infrared spectroscopy measurement and data analysis

The continuous-wave system (ETG-4000; Hitachi Medical Co., Tokyo, Japan) has been described in detail elsewhere [15]. The interoptode distance was 30 mm, and the sampling rate was set to 10 Hz. The two 22-channel probe-sets were placed on the scalp above the parietal-occipital and frontal lobes, which were defined by an MRI scan (see details in Fig. 1b).

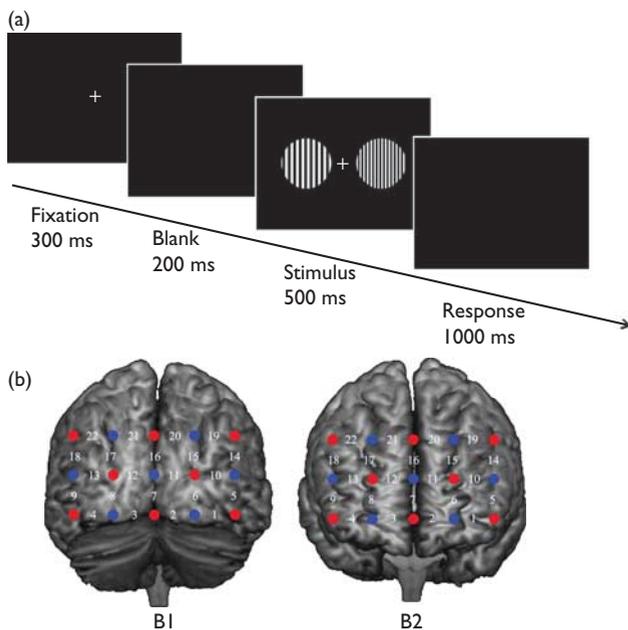
The fNIRS data were processed and analyzed using HomER, a graphical interface program implemented in Matlab [17]. First, the channels with excess noise were discarded (a normalized DC amplitude SD exceeding 20% was discarded). Then, band-pass filtering between 0.01 and 0.2 Hz was applied on the raw data to remove the high-frequency physiological noises and low-frequency baseline drifts. After that, the changes in HbO and HHb concentrations were calculated on the basis of the modified Beer-Lambert law [18]. Finally, the block average was run channel by channel.

The patterns of the activation induced by 0, 90, and 45° were very similar among the three orientations, but with different amplitudes in some regions (Figs 2 and 3). We defined several clusters of channels as the regions of interest (ROIs), where the differences among orientations were evident. To reduce the variability of different participants, the signal change of every single participant was standardized [see Eq. (1)]. After that, the amplitudes of activation from those ROIs were averaged and were then tested by a repeated-measure analysis of variance (ANOVA) comprising the within-participant factor orientation (3: 0, 45, and 90°). The significant interaction effects were analyzed by post-hoc analyses on the individual factor levels. Additional post-hoc two-tailed paired *t*-tests were carried out if the main effect was significant (Bonferroni's corrected $\alpha = 0.05$). For statistical analysis, we defined two indexes for different orientations: the mean amplitude of the task during peak time ± 1 s (mean-peak) and the exact time of the largest amplitude (latency).

$$Y_{ij} = Y_j - (\bar{Y}_i - \bar{Y}_j), \quad (1)$$

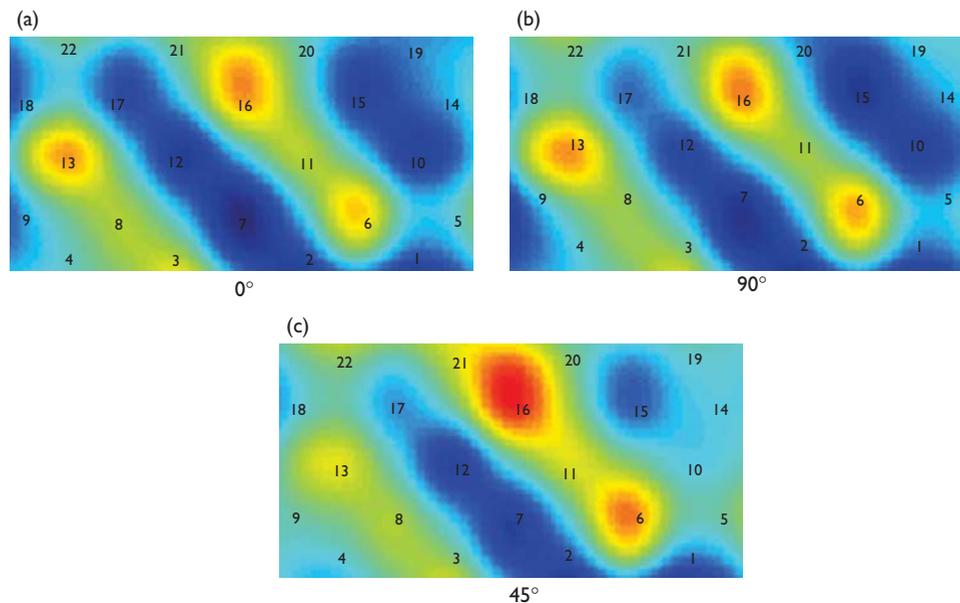
where Y_{ij} is the standardization value of channel j of subject number i ; Y_j is the original value of channel j of

Fig. 1



(a) The trial sequence of the spatial frequency discrimination task. (b) The sketch map of the two 22-channel functional near-infrared spectroscopy probe sets placed on the participants' scalp. In the parietal-occipital probe (B1) and the frontal probe (B2), the red dots represent the light sources and the blue dots represent the detectors. The numbers show the positions of 22 channels separately. Accordingly, channel 7 in the parietal-occipital probe (Fig. B1) was placed above the ERP marker position Oz (10–20 system) and channel 7 in the frontal probe was placed 5 cm above the nasion (Fig. B2).

Fig. 2



The mean-peak value of the three orientations across the parietal-occipital lobe. The signal change in the oxygenated hemoglobin mean-peak value (mean-peak \pm 1 s) is shown separately for 0° (a), 90° (b), and 45° (c).

subject number i ; \bar{Y}_i is the mean value of all channels of subject number i ; and \bar{Y}_{ij} is the mean value of all channels of all participants.

Results

Behavioral results

On average, the performance on the spatial frequency discrimination task was stable and at a high level ($\sim 98.6\%$ correct response ratio) in all three orientations (0°: $98.78 \pm 1.22\%$; 45°: $99.11 \pm 1.97\%$; 90°: $97.89 \pm 1.71\%$). For the reaction time, a repeated-measures ANOVA did not show any significant differences among the three orientations [$F(2,51) = 0.099$, $P = 0.960$; 0°: 488.48 ± 52.11 ms; 45°: 483.96 ± 53.49 ms; 90°: 487 ± 57.64 ms].

Functional near-infrared spectroscopy results

Figure 3 shows the HbO changes induced by gratings with three orientations in the parietal-occipital lobe. We selected three areas (where the differences among orientations were evident) as ROIs: area 1, including channels 16, 21, and 22; area 2, including channels 4, 8, and 13; and area 3, including channels 2, 6, and 11. The within-session t -values of HbO data between the task and rest period are shown in Table 1. It can be seen that the gratings of all three orientations led to significant activation.

The time courses of HbO changes induced by different orientations in the occipital lobe are shown in Fig. 3. The HbO changes in the three orientations were all characterized by a peak at 7–9 s. The repeated-measure ANOVA

showed that the significant difference was in area 1 with $F(2,15) = 4.023$, $P < 0.05$, whereas other channels showed insignificant effects of orientation. Post-hoc multiple comparisons showed that the HbO activation of the oblique orientation was significantly larger than the cardinal ones [45 vs. 0°: $t(17) = -2.46$, $P < 0.05$; 45 vs. 90°: $t(17) = -2.653$, $P < 0.05$]. However, the HbO activation did not differ between the vertical and the horizontal orientations [0 vs. 90°: $t(17) = -0.052$, $P = 0.96$]. As for the latency of the peak, the three orientations led to no significant difference [$F(2,15) = 1.191$, $P = 0.316$].

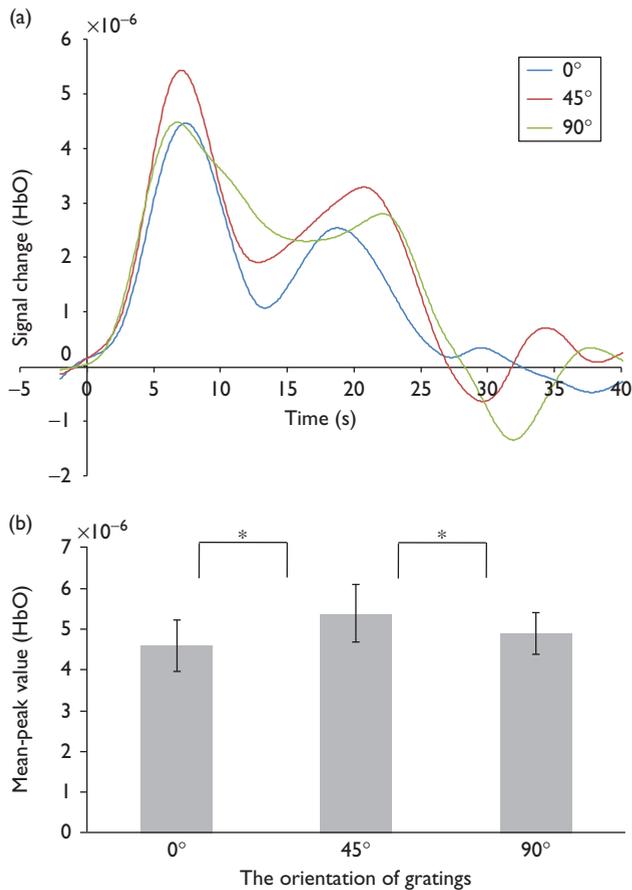
The gratings did not lead to significant activation in most channels of the frontal lobe. The only significantly activated channel by all orientations was 22 [0°: $t(17) = -2.89$, $P < 0.01$; 45°: $t(17) = -2.19$, $P < 0.01$; for 90°: $t(17) = -2.36$, $P < 0.01$]. However, when we used the repeated-measures ANOVA for the factor of orientation, there was no significant difference in the HbO signal among the three orientations [$F(2,51) = 1.217$, $P = 0.360$].

The amplitudes of the HHb response were much smaller than those of the HbO (the mean-peak amplitude for 0, 45, and 90° was 0.11, 0.11, and 0.09, respectively), and we did not find any significant difference in orientation in the HHb data.

Discussion

In the present study, we did not find any orientation anisotropy in the behavioral results. This result is different from previous studies in which the orientation

Fig. 3



The time course and mean-peak value of the oxygenated hemoglobin (HbO) signals for different orientations. (a) The zero point represents the beginning of the task. The horizontal axis starts from the baseline (-2 s) to the end of the entire block (40 s). (b) The mean-peak value and SE of HbO for the first peak. * $P < 0.05$.

Table 1 The activation of the oxygenated hemoglobin data (mean value of the task period vs. the rest period) indicated by the *t*-value in the regions of interest of the parietal-occipital lobe

Area	0°	45°	90°
Area 1 (channel 16, 21, and 22)	6.27**	6.70**	10.30**
Area 2 (channel 4, 8, and 13)	7.69**	6.75**	8.37**
Area 3 (channel 2, 6, and 11)	7.86**	6.56**	7.24**

** $P < 0.001$.

judgments were faster and more accurate at cardinal than other orientations [2,4]. In fact, other studies further suggested that the orientation anisotropy effect depends on the type of task that the participant has to perform [19,20]. The absence of orientation anisotropy in the current study might be caused by the easy spatial discrimination task.

For the fNIRS results, the present study showed that there were significant changes in the HbO signal in the parietal-occipital lobe but not in the frontal lobe. This is

in line with many previous single-unit recording studies in animals and fMRI studies in human beings. For example, orientation anisotropy was mainly found in areas 17 and 21 of cats, the visual cortex of ferrets [5,9] and V1, V2, V3, and even V4 in humans [10,11]. In terms of a more superior area, the orientation anisotropy effect is absent because the information has already been integrated (e.g. [21]).

The main result of the present study showed that the HbO activity elicited larger HbO responses to oblique orientations than cardinal ones, which was different from previous studies [10]. However, recent fMRI research in humans has also yielded different accounts of orientation anisotropy [11,21,22]. For example, Mannion *et al.* [21] showed that the field-independent orientation anisotropic effects were decreased to horizontal orientations and increased to oblique ones. Another MEG study found that the sustained gamma response was larger for oblique stimuli than cardinal stimuli [22]. Our result was consistent with these recent findings, showing that HbO activity had the greatest responses to oblique orientations in the left occipital cortex, which was somewhere between Brodmann Area 7 (Somatosensory Association Cortex) and Brodmann Area 19 (V3), according to our fMRI structural image of one participant. All of these results suggest that the processing of orientation is more complex than we expected and occurs at both early and late stages of visual information processing.

We did not find significant differences between 0 and 90° in both the behavioral data and HbO magnitude. This finding was consistent with most previous studies (e.g. [4,21]), indicating that the two cardinal orientations might have very similar neural substrates. In addition, we did not find any orientation anisotropy in the HHb data, which might result from the very small HHb responses, and suggested that HbO was the most sensitive indicator of changes in regional cerebral blood flow in the fNIRS measurements [23].

Conclusion

Our study showed that there were quantitative changes in the hemoglobin concentration in response to differently oriented visual stimuli. We established this by showing lower HbO responses to the horizontal and vertical orientations and higher HbO responses to the oblique orientations in the visual cortex.

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Conflicts of interest

There are no conflicts of interest.

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