BOLD signal change and its spatial heterogeneity in relation to the reversal frequency of checkerboard stimuli in human primary visual cortex: a high-resolution fMRI study

P. Sun[1], K. Ueno[1], R. Waggoner[1], K. Tanaka[1], K. Cheng[1]

[1]Lab for Cognitive Brain Mapping, RIKEN Brain Science Institute, Wako-shi, Saitama 351-0198, Japan

ABSTRACT In this high-resolution fMRI study, we investigated the effect of stimulus rate on the BOLD signal change and its spatial heterogeneity in human primary visual cortex (V1) using contrast reversing checkerboards. Unlike the generally agreed dependency of the rCBF and BOLD signal change on the stimulus rate that are observed in a number of PET and fMRI studies using flashing LED or checker patterns, we found that the BOLD signal change depends little, if any, on the reversal frequency of checkerboards in Human V1. Using a differential mapping method, however, we revealed, for the first time, that checkerboards of low and high reversal frequency, respectively, activated spatially segregated patches in V1 that measure ~1.5 mm in the narrow dimension.

INTRODUCTION Previous PET and fMRI studies have shown that rCBF or BOLD signal in human V1 increases with the stimulus rate and peaks at ~8 Hz [1-9]. Two major types of visual stimuli were used in these studies, one with a fixed stimulus-on duration and variable inter-stimulus intervals, where the averaged luminance is a function of stimulus rate (e.g., flashing LED or checker patterns), and the other with a varied stimulus rate but a constant averaged luminance (e.g., contrast reversing checkerboards). Although it is generally believed that these two types of visual stimuli have similar effects on rCBF and BOLD signal change [2], a few recent studies, measuring either rCBF [8] or BOLD [10] signal change, have questioned this concept. In particular, a large signal change is observed with contrast reversing checkerboards at very low reversal frequencies. In this high-resolution fMRI study, the BOLD signal change and its spatial heterogeneity in relation to the frequency of contrast reversing checkerboards was explored in anatomically defined, circumscribed portion of V1.

METHODS Eight subjects (6 males, mean age 36.8) participated in the experiments. Circular white/black checkerboards (7.6° in diameter; checker size ~0.8°) with contrast reversal frequency of 0.05, 0.75, 2, 4, 8 and 16 Hz were used. The averaged luminance was kept constant across frequencies. The checkerboards were placed at 225°/315° orientation (7.6° from a central fixation cross) in the left or right lower visual field through a pair of fiber optic glasses. During fMRI scans, the subject detected a brief color change of the fixation cross. The eye positions were monitored. Experiments were conducted on a Varian 4T system with a quadrature surface coil and a segmented EPI pulse sequence (8 segments; volume TR, 4.6 s; TE, 25 ms). Six continuous slices (thickness, 3 mm; in-plane resolution, 0.94 x 0.94 mm), parallel to the calcarine fissure, were prescribed based on V1/V2 borders determined in a separate experiment, and covered the dorsal V1 of the targeted hemisphere. Six scans, each with a reversal frequency (in pseudo-randomized order), were made for each subject. Original data were corrected for rigid head motion and physiological fluctuations. No spatial or temporal smoothing was applied. For each subject, statistical t-test ($p<0.01$) maps showing voxels activated by checkerboards whose contrast was reversed at 0.05, 0.75, 2, 4, 8 and 16 Hz, respectively, were created. Voxels associated with large venous vessels (signal change >5%) were removed. A ROI (across 1-3 slices, depending on the subject) was selected for the frequency that activated most voxels (optimal frequency), and was also used for all other frequencies. The average % BOLD signal change and total activated voxels within the ROI were calculated for each frequency.

RESULTS The optimal frequency varied greatly among individual subjects. The linear dependency of either the % BOLD signal change or total activated voxels on reversal frequency was not observed. Across subjects, there was no main frequency effect on %BOLD signal change (one-way ANOVA, $p>0.776$) or on total activated voxels ($p>0.247$). There were some nominal but non-significant differences in that the lowest % BOLD signal change (2.03±0.10%, observed for 0.05 Hz) was ~89% of the highest one (2.28±0.11%, for 16 Hz), and the lowest activated voxel number (normalized to that of the optimal frequency for each subject and averaged across subjects, 77.13±11.90%, for 8 Hz) was ~85% of the highest one (90.72±8.02%, for 0.05 Hz). We also found no significant difference for any of the above-mentioned measures when analyses were performed by including voxels that displayed larger signal change (>5%, associated with large vessels).

In separate sessions with 4 of the 8 subjects, we also conducted control experiments (in-plane resolution was 0.75 x 0.75 mm as a result of reduced FOV; other parameters were the same) comparing a low frequency (0.75 Hz) and a high frequency (15 Hz) directly in a single scan. The size of checkerboards in two scans was the same as in the previous experiment (7.6° in diameter), and in the other two scans the full-screen checkerboards (~19° x 25°, see below) were used. Again, we found no significant difference in % BOLD signal change (repeated measure ANOVA, $p>0.853$) or total activated voxels ($p>0.852$) between low (0.75 Hz) and high (15 Hz) frequencies.

The finding that low (0.75 Hz) and high (15 Hz) frequency checkerboards activated V1 roughly equally well provided us with a unique opportunity to examine if there is any spatial heterogeneity within V1 that process visual information of different temporal frequencies. To explore this possibility, we used full-screen checkerboards and a differential mapping method similar to that used for mapping ocular dominance columns (ODCs, see [11] for details of the method), and revealed highly reproducible patchy pattern in anatomically well defined section of V1, where different patches were preferentially activated by low (0.75 Hz) and high (15 Hz) reversal frequencies, respectively. These patches, which we term *temporal frequency dominant domains* (TFDDs), resembled ODCs in size, measuring ~1.5 mm in the narrow dimension, but displayed distinct spatial arrangements (see Figure).

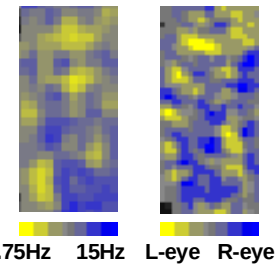

Figure. Left, a TFDD map in anatomically well defined section of V1. Right, an ODC map from the same subject, obtained in approximately the same section in a previous experiment [11]. Frame size = 9 x 19 mm. Top of the figure is to the anterior, and left is to the left.

DISSCUSSION The concept that the rCBF change increases with the stimulus rate and peaks at ~8 Hz for both flashing patterns and contrast reversing checkerboards stemmed from the work of Fox and Raichle [2]. Unfortunately, for 0 Hz with the checkerboard in that study, the measurement was taken from an unstimulated scan (rather than from a scan with a stationary checkerboard), which resulted in a steeper slope of rCBF change for the frequency <1 Hz. It has been recently shown in a few studies that contrast reversing checkerboards of very low frequencies could cause surprisingly large rCBF change [8] or BOLD signal change [10, 12] in human V1. Our results, by measuring activation in circumscribed portion of V1, demonstrate that the BOLD signal change depends little, if any, on the reversal frequency of checkerboards, suggesting that at least in V1, at a coarse spatial resolution, neurons coding varied reversal frequencies are distributed roughly equally.

The TFDDs revealed in the present study suggest that there may exist a distinct functional architecture in V1, where visual information of different temporal frequencies is represented. The existence of such an architecture (map) is not known in both humans and monkeys, although there are some evidence in early monkey physiological studies indicative of separate domains preferentially processing information of low and high temporal frequencies (e.g., see Fig. 2 in [13]). Future works are needed, probably combining with animal studies, to explore the nature of TFDDs and their relationships with other established functional and anatomical architectures, such as ODCs, orientation columns and cytochrome oxidase blobs and interblobs.

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