

Study of Tricarboxylic Acid Cycle Flux Changes in Human Visual Cortex during Two-hemifield Visual Stimulation with Different Stimulus Frequency using *In Vivo* $^1\text{H}\text{-}\{^{13}\text{C}\}$ MRS and fMRI

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Introduction

The coupling relationships among the stimulus-evoked brain activity, hemodynamic and metabolic responses are critical for understanding the underlying mechanism of fMRI BOLD signal and brain function. They are, nevertheless, highly debated. One particularly interesting question is whether the increase in CMRO₂ during graded brain activation follows BOLD and CBF changes. Previously published PET functional study¹ showed that CMRO₂ changes were larger at 4 Hz reversal frequency (~18%) than at 8 Hz (~4%) using checkerboard visual stimulus despite the opposite trend for CBF changes (~34% at 4 Hz and ~46% at 8 Hz)². The present study aimed to re-examine the CMRO₂-BOLD coupling relationship between 4 and 8 Hz visual stimuli by simultaneously measuring BOLD and the relative changes of oxidative CMR_{glc} in two hemispheric visual cortical regions, which were stimulated at 4 Hz and 8 Hz, respectively (see example in Figs. 1 and 2).

Materials and Methods

Visual stimuli: To increase the experimental efficiency and improve measurement reliability, the visual stimulation consisted of two hemifield reversal checkerboard visual stimuli: the right hemifield with 4 Hz for activating the left hemispheric visual cortex, and the left hemifield with 8 Hz for activating the right hemispheric visual cortex based on the retinotopic relationship (see Figs. 1 and 2A).

Glucose infusion: Uniformly labeled ^{13}C -enriched D-glucose infusion was conducted according to a well-established infusion protocol for measuring the metabolic turnover rate for labeling glutamate (Glu) from the infused glucose^{3,4}. The ^{13}C -labeled Glu signal and its changes were measured by the *in vivo* $^1\text{H}\text{-}\{^{13}\text{C}\}$ editing MRS method for gaining detection sensitivity⁴. The $^1\text{H}\text{-}\{^{13}\text{C}\}$ editing MRS data were acquired before (10 mins), during (around 60 mins) and after (20 mins) the glucose infusion in the presence of visual stimulation. All the procedures were approved by the institutional review board of the University of Minnesota.

NMR experiments: All the NMR measurements were conducted at 4T whole body scanner. The same $^1\text{H}\text{-}\{^{13}\text{C}\}$ dual surface-coil was used for both fMRI and *in vivo* $^1\text{H}\text{-}\{^{13}\text{C}\}$ MRS measurements, consisting of a 10-cm diameter single loop ^1H surface coil and two 15-cm diameter surface coils in quadrature mode for ^{13}C spin inversion and decoupling. A 1-cm diameter sphere containing [^{13}C]-formic acid was placed at the center of the ^1H coil for calibrating the ^{13}C -radiofrequency power. Anatomical images were acquired by the multi-slice T₁-weighted TurboFLASH sequence. The fMRI study using a gradient echo-planar imaging sequence was performed on each subject prior to the ^{13}C measurements. The fMRI maps were used for guiding the voxel position of localized $^1\text{H}\text{-}\{^{13}\text{C}\}$ MRS and for partial volume correction for calculating the relative changes of oxidative CMR_{glc} during the visual stimulation. The measurements of ^{13}C -Glu labeling were based on the $^1\text{H}\text{-}\{^{13}\text{C}\}$ editing techniques using the LASER⁵ localized sequence combined with ^{13}C inversion and decoupling. The B₁ inhomogeneity was corrected using NAA signals and it was assumed that there is no significant difference in [NAA] for the two selected voxels symmetrically along the central fissure of brain. All other procedures were similar to the previous study⁴.

Results and Discussions

Volunteer's performance is crucial for this study. Therefore, we screened some subjects using fMRI and a typical result was illustrated in Fig. 1, showing extensive evoked brain activities in the visual cortex. The subjects with excellent fMRI performance were recruited for the ^{13}C -glucose infusion studies. Fig. 2A demonstrates the experimental setup for performing two-hemifield visual stimulation with different stimulation frequency (4 Hz versus 8 Hz) and the fMRI BOLD map from one subject. Two *in vivo* $^1\text{H}\text{-}\{^{13}\text{C}\}$ spectra shown in Fig. 2B were simultaneously acquired from the two identically-sized voxels illustrated by the two green boxes in Fig. 2A, which were chosen symmetrically along the central fissure and were based on the fMRI maps. The functional MRS (fMRS) results (Fig. 2B) indicate that the total accumulated $^1\text{H}\text{-}\{^{13}\text{C}\}$ -Glu signal within 50 minutes of visual stimulation was significantly larger in the right-hemispheric visual cortex with 8 Hz stimulation than that of the left-hemispheric visual cortex with 4 Hz stimulation. The quantitative fMRS results after correcting B₁ inhomogeneity and partial volume effect based on the fMRI mapping results⁴ are shown in Fig. 2D, and the corresponding BOLD results are summarized in Fig. 2C. These results clearly suggest that both BOLD and oxidative CMR_{glc} (close to CMRO₂) changes are larger at 8 Hz as compared to 4 Hz. Therefore, they indicate a strong coupling between BOLD and CMRO₂ changes in response to the brain stimulation. This conclusion should be true also for the CBF-CMRO₂ coupling because of a strong correlation between BOLD and CBF changes, which has been previously shown in the literature².

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Reference: 1. Manouchehr S V, JCBFM, 1999. 2. Zhu X H, MRM, 1998. 3. Gruetter R. Dev Neurosci, 1998. 4. Chen W, MRM, 2001. 5. Marjanska M, ISMRM 2004.

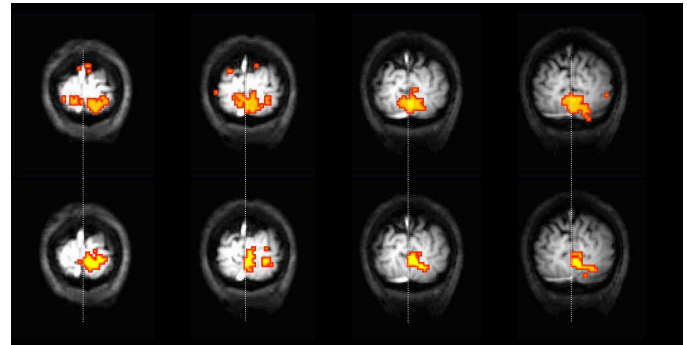


Fig 1. Visual cortex activities indicated by BOLD signals in response to the two-hemifield (upper panel: 8Hz left hemifield and 4Hz right hemifield) and one-hemifield (lower panel: 8Hz left hemifield) visual stimulus, respectively, from one subject.

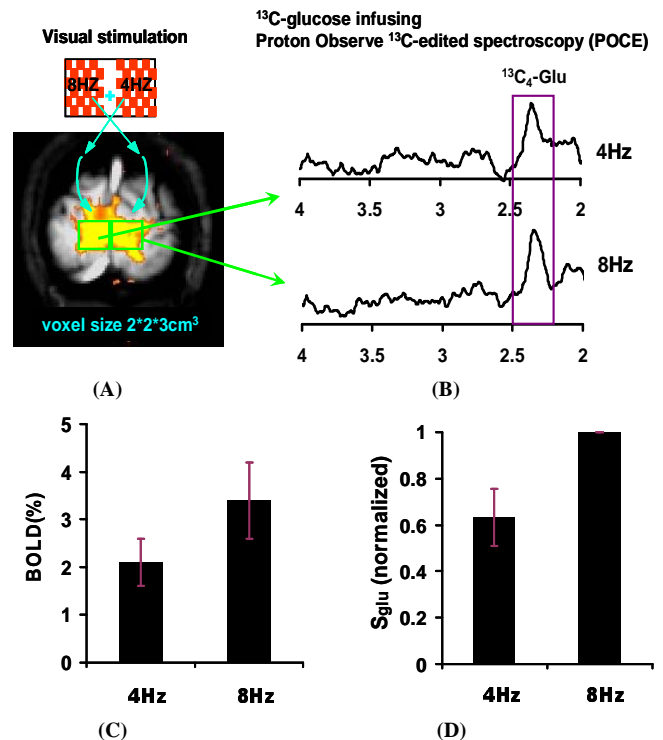


Fig 2. Functional brain study with two-hemifield visual stimulation and its hemodynamic and metabolic response detected by BOLD and fMRS, respectively. (A) One representative fMRI map and two selected voxels for acquiring two $^1\text{H}\text{-}\{^{13}\text{C}\}$ spectra. (B) *In vivo* $^1\text{H}\text{-}\{^{13}\text{C}\}$ spectra showing the ^{13}C -labeled Glu integrals from two hemispheric visual cortical regions which were stimulated by 8 Hz and 4 Hz visual stimulation, respectively. These spectra were simultaneously acquired during 50 minutes of ^{13}C -glucose infusion. (C) BOLD responses (n=8) and (D) relative changes of [^{13}C] Glu signal (n=2) with significant difference between 8 Hz and 4 Hz stimulations (p<0.01).