Study of Tricarboxylic Acid Cycle Flux Changes in Human Visual Cortex during Two-hemifield Visual Stimulation with Different Stimulus Frequency using In Vivo $^1$H-$^1$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 particular issue in the fMRI question is whether the increase in CMRO$_2$ during graded brain activation follows BOLD and CBF changes. Previously published PET functional study$^1$ showed that CMRO$_2$ 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 CMRO$_2$-BOLD coupling relationship between 4 and 8 Hz visual stimuli by simultaneously measuring BOLD and the relative changes of oxidative CMR$_{ox}$ 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 $^1$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$^3$. The $^1$C-labeled Glu signal and its changes were measured by the in vivo $^1$H-$^1$C editing MRS method for gaining detection sensitivity$^4$. The $^1$H-$^1$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 $^1$H-$^1$C dual surface-coil was used for both fMRI and in vivo $^1$H-$^1$C MRS measurements, consisting of a 10-cm diameter single loop $^1$H surface coil and two 15-cm diameter surface coils in quadrature mode for $^1$C spin inversion and decoupling. A 1-cm diameter sphere containing $^1$C-formic acid was placed at the center of the $^1$H coil for calibrating the $^1$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 $^1$C measurements. The fMRI maps were used for guiding the voxel position of localized $^1$H-$^1$C MRS and for partial volume correction for calculating the relative changes of oxidative CMR$_{ox}$ during the visual stimulation. The measurements of $^1$C-Glu labeling were based on the $^1$H-$^1$C editing techniques using the LASER$^1$ localized sequence combined with $^1$C inversion and decoupling. The $^1$C homogeneity 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 study$^4$.

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 $^1$C-glucose infusion studies. Fig. 2A demonstrates the experimental setup for performing two-hemifield visual stimulation with different stimulus frequency (4 Hz versus 8 Hz) and the fMRI BOLD map from one subject. Two in vivo $^1$H-$^1$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 $^1$H-[4-13C]-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 $^1$B inhomoogeneity and partial volume effect based on the fMRI mapping results$^5$ 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$_{ox}$ (close to CMRO$_2$) changes are larger at 8Hz as compared to 4 Hz. Therefore, they indicate a strong coupling between BOLD and CMRO$_2$ changes in response to the brain stimulation. This conclusion should be true also for the CBF-CMRO$_2$ coupling because of a strong correlation between BOLD and CBF changes, which has been previously shown in the literature$^2$.

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