Elevated ATP Synthase and Creatine Kinase Activities in Human Visual Cortex during Visual Stimulation: A 31P NMR Magnetization Transfer Study at 7T

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Introduction: The cerebral adenosine triphosphate (ATP) metabolism is mainly controlled by ATP synthase (ATP_{ase}), and creatine kinase (CK) reaction is coupled with ATP production/utilization and plays a critical role in transferring ATP energy between mitochondria and cytosol. These reactions constitute a chemical exchange system involving ATP, phosphocreatine (PCr) and inorganic phosphate (Pi)[1,2]. With advantage of the increased sensitivity and the large chemical shift dispersion provided by ultra-high field strength, *in vivo* ^{31}P MRS with magnetization transfer (MT) technique has significantly advanced our understanding of neuroenergetics associated with the ATP_{ase} metabolic rate in the resting human brain[2] and its correlation with varied brain activity from light anesthesia to iso-electric state in the animal brain[3]. In this study, we further investigated the central roles of ATP metabolism in response to functional stimulus in the human visual cortex at 7T, and its temporal change during and after the stimulation.

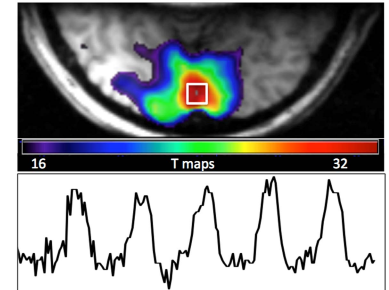

Figure 1. A functional activation map (top) and corresponding BOLD signal (bottom) generated based on block-design fMRI data from a representative subject

Methods: Six healthy volunteers (age 27–60 yrs, 3M/3F) participated in this study and provided written informed consent. All MRI and *in vivo* ^{31}P MRS-MT measurements were conducted at 7.0 Tesla/90 cm bore human scanner (Siemens). The RF probe consists of a butterfly-shape ^{1}H surface coil for anatomic imaging and B_0 shimming with FASTMAP algorithm[4], and a 5-cm-diameter single-loop ^{31}P coil for collecting ^{31}P MRS dat a. For the functional study, a full-screen black-white checkerboard visual stimulus flashing at 8 Hz with eye fixation point in the screen center was used to activate visual cortex with a large view angle for both fMRI and ^{31}P-MT experiments.

FMRI study and Data Analysis: Gradient-echo echo-planar images (GE-EPI) (TR/TE = 2000/23ms, matrix size = 64x64 matrix size, slice thickness = 3mm, 15 slices, total acquisition time = 6 min) were acquired using a functional paradigm (baseline block (60s), followed by five repeated blocks between stimulate (20s) and control condition (40s)). The fMRI data analysis was done using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/ spm8) and statistical significance was calculated after correcting multiple-comparison (FWE, corrected $p < 0.05$, T threshold = 13).

In vivo ^{31}P-MT measurement: To minimize the neuronal adaptation effects and to explore the tempora l changes of the ATP_{ase} and CK fluxes, we designed a visual stimulation paradigm consisting of a long baseline block (300s, 150 FIDs), followed by 25 repeated blocks switching from stimulation (20s, 10 FIDs) to 1st post-stimulation (20s, 10 FIDs) and 2nd post-stimulation (20s, 10 FIDs). All ^{31}P MR spectra were obtained using single-pulse-acquire sequence (2s repetition time, 5kHz spectral bandwidth, 300 µs hard excitation pulse with optimized Ernst flip angle). The B_1 insensitive selective train to obliterate signal (BISTRO) scheme[6] was applied on the γ-ATP peak (or on the symmetric side of Pi for control) for measuring the MT effects on the PCr and Pi and for determining chemical exchange fluxes. The acquired ^{31}P-MT data were analyzed using JMRUI software package (Version 4.0) and the AMARES fitting algorithm[7]. The concentrations of brain ATP, PCr and Pi metabolites were determined from their control magnetization values after correcting for the saturation factor due to the short TR. Absolute concentration of PCr and Pi were then calibrated using a cerebral ATP concentration of 2.8mM as an internal reference for each subject[8]. Finally, the forward reaction rate constants (k_f) were calibrated according to: $M_c/M_s \approx 1 + k_f \cdot T1^{nom}$ where M_c and M_s are control and γ-ATP saturated magnetization and T_1^{nom} is the nominal T_1 that is field dependent[5]. The fluxes can be calculated by $F_{f,ATPase}=k_{f,ATPase}$x[Pi] and $F_{f,CK}=k_{f,CK}$x[PCr]. Paired t-test was applied for statistical comparison between baseline and stimulation conditions, and a p value of < 0.05 was considered statistically significant.

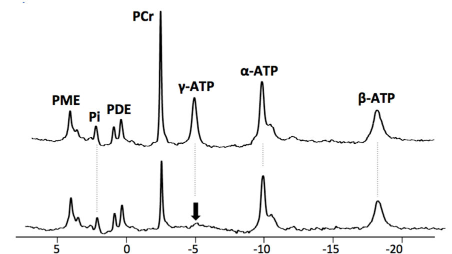

Figure 2. Localized *in vivo* ^{31}P MR spectra of human visual cortex in the absence (top panel) and presence (low panel) of saturating at-ATP resonance (pointed by the black arrow) acquired from a representative subject.

Results and Discussion: Figure 1 shows a typical fMRI map and elevated neuronal activity covering most of the primary visual cortex in response to visual stimulation. The majority of *in vivo* ^{31}P MRS signal measured by the surface coil with 5 cm diameter coincided with the activated visual cortex region. Figure 2 displays typical ^{31}P spectra of human visual cortex acquired in the absence and presence of γ-ATP saturation, showing excellent spectral quality (line width of PCr ~ 8 to 10 Hz) and high detection sensitivity which ensures reliable detection and quantification of the PCr and Pi signals and their changes *in vivo*. Simulated nominal T_1 values of PCr (3.18s) and Pi (2.44s) were used to quantify the chemical exchange rate constants under partially relaxed acquisition condition. For instance, the forward rate constant of $f_{k,CK}$ measured in the absence of the stimulation was 0.31 s^{-1} (Fig. 3), which is in excellent agreement with previously reported value[9]. During the visual stimulation, the reaction rate constant of $k_{f,CK}$ (PCr→ATP) was increased 8.33±0.01% (p=0.012) and $k_{f,ATPase}$ (Pi→ATP) was elevated substantially 28.1±0.05% (p = 0.003) compared to baseline. The $F_{f,ATPase}$ flux increased 21% from 7.9±1.6 µmol/g/min at rest to 9.5±2.0 µmol/g/min with activation, and the $F_{f,CK}$ flux increased 7% from 63.3±3.8 µmol/g/min to 67.6±5.5 µmol/g/min. The enhanced ATP_{ase} activity as observed in this human study reflects the increasing ATP energy demand, which is consistent with the results of a similar cat brain study showing an increase of >20% [10]. Interestingly, in contrast to the rapid recovery of $k_{f,ATPase}$ during the post-stimulation periods, $k_{f,CK}$ showed a persistent elevation during the same periods as demonstrated in Fig. 3. This finding indicates distinct roles of CK and ATP_{ase} activities in supporting neuronal activation. **Conclusion:** The changes of CK and ATP_{ase} forward reaction rate constants and their fluxes in the human visual cortex were directly studied under resting and activated states using *in vivo* ^{31}P-MT technique at 7T. Significant increase of $F_{f,ATPase}$ reflects a high demand of oxidative ATP synthesis/utilization in the activated brain region for supporting the elevated neuronal activity and brain function.

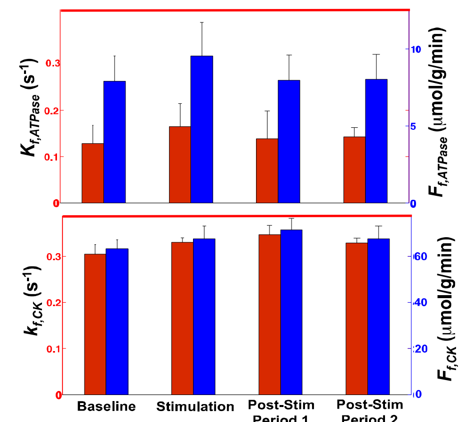

Figure 3. The forward reaction rate constants and fluxes of ATP_{ase} reaction (top panel) and CK reaction (low panel) measured at baseline, stimulation and two post-stimulation periods, respectively.

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References: [1] Slater *et al. Biochem J*; **55**:530-544 (1953); [2] Lei *et al.*, *PNAS*; **100**:14409-14414 (2003); [3] Du et al., *PNAS*; **105**: 6409-6414 (2008) [4] Gruetter *etal.*, *MRM*; **29**:804-811 (1993); [5] Xiong *et al.*, *Circ. Res*; **108**: 653-663 (2011); [6]. de Graaf *et al.*, *NMR Biomed*; **9**:185-194 (1996); [7] Vanhamme *et al*, *JMR*; **129**:35-43 (1997); [8] Zhu *et al. Proc. ISMRM;* **17**:4287 (2009). [9] Du *et al.*, *MRM*; **57**: 103-114 (2007). [10] Zhu *et al., Proc. ISMRM* **16**: p.408 (2008).