Functional Mapping of CK and ATPase Metabolic Rate Changes Elevated by Visual Stimulation in Cat Brain

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Introduction ATP is the fundamental cellular energy currency, and ATP hydrolysis to Pi and ADP is coupled to all energy requiring process in the cell. Its metabolism in the brain is regulated by two chemically coupled exchange reactions catalyzed by CK enzyme and ATPase enzyme, which can be linked as PCr↔ATP↔Pi. The regulation of ATP metabolic rates plays a crucial role for sustaining brain activity under both resting and activated states. *In vivo* ^{31}P MRS combined with magnetization saturation transfer (MT) method has been recently demonstrated to be capable of noninvasively and simultaneously measuring the Cerebral Metabolic Rate of CK reaction (CMR_{CK}) and ATPase reaction (CMR_{ATP}) in the living brains[1-3]. We have also demonstrated that both CMR_{CK} and CMR_{ATP} are tightly coupled with varied brain activity in the resting brain[4]. In this study, we have applied the *in vivo* ^{31}P MT approach to study whether CMR_{CK} or CMR_{ATP} changes in the cat visual cortex during grafting visual stimulation. We demonstrated for the first time the feasibility of obtaining 3D functional metabolic CK rate mapping in the cat visual cortex, and observing a significant increase of CMR_{ATP} during brain stimulation.

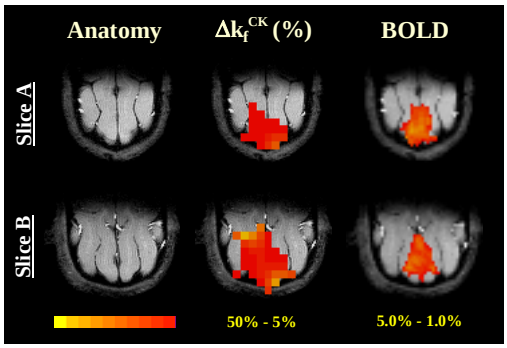

Fig. 1 Anatomic and functional maps of BOLD and relative CK rate constant changes (Δk_1^{CK}) of a representative cat brain in response to visual stimulation.

Methods and Materials Artificial ventilation and gaseous anesthesia (0.9-1.2 % isoflurane in a mixture of 70% nitrous oxide and 30% oxygen) were applied to female adolescent cats for MR studies conducted on a 9.4T horizontal magnet (Magnex Scientific, UK) interfaced with a Varian INOVA console (Varian Inc., Palo Alto, CA). The cat eyes were refracted and focused on the visual stimulus consisting of a binocular high-contrast square-wave moving and rotating gratings (0.3 cyc/deg, 2 cyc/sec and 16°-rotation for every 4sec) to achieve optimal activity in the cat primary visual cortex (V1). The cats were placed in a cradle with head position restrained by the mouth and ear bars. The animal physiological condition was continuously monitored and maintained. A multinuclear RF probe consisted of a ^{31}P surface coil covering the V1 area and a quadrature ^{1}H coil for anatomic and fMRI was used. *In vivo* ^{31}P MT experiments were carried by means of frequency-selective saturation of γ-ATP[1-3] to simultaneously determine the forward unidirectional ATP production rate constant and flux through the oxidative ATPase reaction (k_f^{ATPase} and CMR_{ATP}) and the non-oxidative CK reaction (k_f^{CK} and CMR_{CK}), respectively. The spatial localization of ^{31}P MRS imaging was achieved by using the 3D Fourier series window approach[5]. The acquisition time for each 3D ^{31}P-MRS image was ~7.5 minutes (total scan number=224; FOV=3×3×2.5 cm^3, 7×7×5 phase encodes, voxel size≈0.27ml (nominal voxel size≈70μl)). A 13×13×9 matrix of FIDs were generated from the original 7×7×5 phase encode data for each 3D ^{31}P MT image. Three to four repeated measurements were performed for each cat with and without γ-ATP saturation at rest and during visual stimulation, respectively, for improving SNR. The CK maps were generated based on the changes in the PCr amplitudes. The AMARES method included in the software package MRUI was applied to quantify the Pi intensity and its changes due to MT effect and/or visual stimulation in the central voxels of cat visual cortex. Data from multiple ^{31}P MT imaging slices in three cats were analyzed and experimental results were presented as mean±SD. All animal surgical procedures and experimental protocol were approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

Results and Discussion Figure 1 displays two representative slices of anatomic images, BOLD fMRI and functional CK forward rate constant maps, respectively, from an individual cat brain. It demonstrates the ability of *in vivo* ^{31}P MT approach for 3D mapping functional CK activity and its change during elevated neuronal activity in the cat brain. The activated brain size as mapped by the CK rate measurement was spatially coincided with but larger than that mapped by BOLD fMRI, presumably due to the difference in the spatial resolution between these two types of images where large point-spreading function leads to spatial blurring in the functional CK rate map. Figure 2a shows the anatomic and functional images of BOLD and CK rate constant change from another cat studied. In addition, the paired ^{31}P MT spectra taken from a central voxel of 3D ^{31}P MRS images in resting and stimulated cat visual cortex were also demonstrated in Fig. 2b. It is evident that visual stimulation induces significant increase in [Pi] content and the CMR_{ATP} as well, due to the increase in MT effect on Pi which is exchangeable with γ-ATP resonance. The spectral fitting and data analysis had shown that [Pi] increases 18 ± 6 % while CMR_{ATP} increases 34 ± 13 % (n=7) in the activated cat visual cortex during visual stimulation. In comparison, CMR_{CK} increases 9 ± 6 % (n=9) with an insignificant increase of [PCr] (1 ± 3 %). The changes of CMR_{ATP} in the cat visual cortex during grafting and rotating visual stimulation are in excellent agreement with the changes in $CMRO_2$ (~33%) during the same visual stimulation using the same cat model[6,7]. This striking observation again suggests a tight coupling between the rate increases of oxygen utilization and oxidative phosphorylation in response to elevated brain activity.

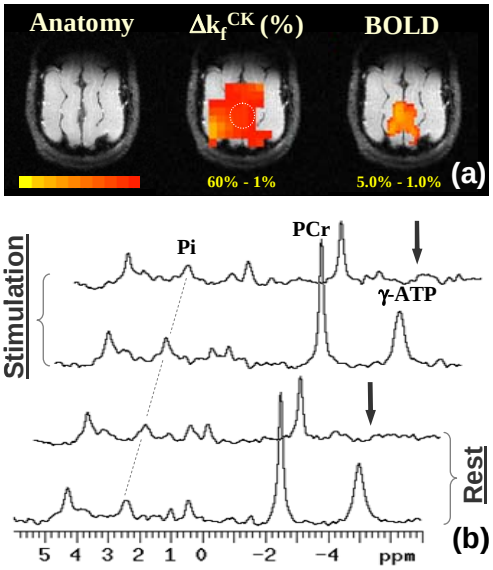

Fig. 2 (a) Anatomic and functional maps of BOLD and CK rate constant changes, and (b) Averaged control and activated ^{31}P spectra with (indicate by arrow) and without γ-ATP saturation in the central voxel from another representative cat brain at rest and during visual stimulation.

Conclusion The findings from this study indicate that (i) the relation between brain activity and oxidative phosphorylation processes (and hence oxidative glucose consumption) can be directly and quantitatively measured *in vivo* in the brain; (ii) the ATP production rates from PCr and from P_i are tightly correlated to brain activity; (iii) the ATP synthesis rate via ATP_{ase} reaction increased more than that via CK reaction with increasing neuronal activity during visual stimulation; (iv) the oxidative phosphorylation has an essential role for supporting increased energy demand during brain activation; and (v) *in vivo* ^{31}P approach for noninvasively determining ATP metabolic rate would provide a useful imaging modality for studying brain function and bioenergetics.

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References [1] Shoubridge et al. (1982) *FEBS Lett* **140,** 289-92; [2] Lei et al. (2003) *PNAS* **100,** 14409-14; [3] Du et al. (2007) *MRM* **57,** 103-114; [4] Du et al. (2006) in *Proc. ISMRM*, Vol. 14, p. 2125; [5] Hendrich et al. (1994) *JMR* **105,** 225-232; [6] Zhu et al. (2006) in *Proc. ISMRM*, Vol. 14, p. 457; [7] Zhu et al. (2007) in *Proc. ISMRM*, Vol. 15, p. 118.