ASSESSING ACTIVATION INDUCED CHANGES IN CREATINE KINASE ACTIVITY IN THE HUMAN BRAIN USING 31P SPECTROSCOPY WITH MAGNETISATION TRANSFER

Chen Chen[1], Penny Gowland[1], Susan Francis[1], Mary Stephenson[1], Peter Morris[1], and Andrew Peters[1]
[1]*Sir Peter Mansfield Magnetic Resonance Centre, University of Nottingham, Nottingham, Nottinghamshire, United Kingdom*

INTRODUCTION: The creatine kinase (CK) reaction plays an important role in ATP metabolism, which is crucial for neuronal activity and cell functioning. The CK forward reaction rate constant (k_1) (i.e. unidirectional PCr->ATP rate) can be measured *in vivo* using ^{31}P MRS with magnetisation transfer (MT) techniques[1,2,3]. Unlike fMRI and PET, ^{31}P MRS MT provides a direct measure of neuronal activity at the metabolic level. Previously, a 34 % increase in k_1 has been found during visual stimulation with an 8 Hz flashing photic stimulus[2]. ***Aim*:** to measure the change of CK activity to a visual stimulation paradigm at 3 Tesla using an optimised protocol.

METHODS: ***Optimisation*:** 1) RF pulses for MT saturation were optimised to reduce RF bleed-over effects and keep within SAR limits. 2) TR, number of averages (NSA) and voxel size were optimised to minimise acquisition time (5mins/scan) while providing adequate signal-to-noise ratio (SNR) (250 in lowest PCr peak). 3) Monte Carlo simulation was performed to determine the scan time required to measure a change in k_1 with sufficient power to detect a 10 % change in PCr (this showed that there was not sufficient SNR to estimate the constant rates of the ATPase reaction due to the low SNR of the Pi peak). ***MR measurements*:** 9 healthy subjects (age 22-27 yrs) were recruited and participated in two *in vivo* ^{31}P MT experiments: 1) Progressive saturation transfer experiment (N=6) to saturate γ-ATP to determine T_1 of PCr at baseline; 2) Steady-state saturation experiment (N=9) to quantify the apparent CK forward rate constant (k_1) in the visual cortex at rest and on visual stimulation. Data was collected on a Philips Achieva 3T scanner. A ^{1}H image localizer was acquired using the body coil, ^{31}P spectra were acquired from a localized volume (5x9x7 cm^3) encompassing the occipital lobe using a ^{31}P transmit-receive 14cm loop coil and an ISIS sequence (TR = 12000 ms, samples = 4096, BW = 3000 Hz, NSA = 24, phase cycles = 8). The MT saturation sequence optimized to selectively irradiate only the γ-ATP peak comprised multiple HYPSEC (Philips) RF pulses of constant amplitude and length (2 μT, 114 ms) interleaved with crusher gradients (4 ms, 10 mT/m, bandwidth = 140 Hz). Progressive Saturation of γ-ATP*:* Seven ^{31}P spectra with varying MT saturation time (t_{sat} = 0, 493, 987, 1974, 2961, 4934, 8224 s) were obtained by varying the number of RF pulses in the train. Steady-State Saturation of γ-ATP: Two cycles of 10-min rest period followed by 10-min visual stimulation period were applied. The visual stimulation comprised contrast-defined wedges moving towards or away from a fixation cross, which was presented through goggles (NordicNeuroLab, NNL). This paradigm has been shown to induce large increases in energy consumption[4]. During each period, two steady-state ^{31}P spectra were acquired in the presence (t_{sat} = 9723 s) and absence of γ-ATP saturation. ***Data Analysis*:** ^{31}P spectra were phase corrected and the magnetizations of PCr (M_{pcr}) were quantified with the AMARES algorithm using the jMRUI v4.0 software package[5]. The intrinsic T_1 of PCr (T_1^{int}) for each subject was estimated by fitting the progressive saturation data to Eq. [1]. The forward rate constant during the rest (k_{1,no_vs}) and visual stimulation ($k_{1,vs}$) periods were calculated using Eq. [2], where M^{∞}_{pcr} is the steady-state magnetization of PCr in the presence of γ-ATP saturation and T_1^{int} was set to be the mean of the estimated intrinsic T_1 of PCr at rest. Previous animal studies[6] have shown that $T_1^{int}{}_{PCr}$ is insensitive to physiology and so can be treated as constant. Paired t-tests were used to test for the significant difference in k_1 between visual and rest periods.

$$M_{pcr}(t_{sat})/M_{pcr}(0) = k_1/R*exp(-R*t_{sat}) + 1/(R*T_1^{int}), \text{ where } R = k_1 + 1/T_1^{int} \quad [1]$$

$$k_1 = (M_{pcr}(0)/M^{\infty}_{pcr} - 1)/T_1^{int} \quad [2]$$

RESULTS AND DISCUSSION: Figure 1 shows the dependence of the normalized PCr magnetization on γ-ATP MT saturation time. The intrinsic and apparent T_1s of PCr at 3T were found to be 4.67±0.91 s and 1.51±0.18 s respectively, consistent with Mlynarik, et al.[7]. The estimated forward rate reaction rate constants of CK for each rest and visual stimulation periods are shown in Figure 2. The average k_1 in the resting (OFF) period was 0.40±0.04 s^{-1}, comparable to 0.42±0.16 s^{-1} measured from the entire human brain[3], and lower than 0.56±0.19 s^{-1} measured from grey matter[2]. The ratio of k_1 measured during visual stimulation to rest ($k_{1,vs}/k_{1,no\_vs}$) is displayed in Figure 3, and has an average value of 1.17±0.12 (p = 2e-6). There was no significant difference between k_1 measured between the two OFF periods (p = 0.36), or the two ON periods (p = 0.09). Visual stimulation induced a 24 ± 14 % (p = 0.0001) and 11 ± 7 % (p = 0.001) increase of k_1 in the visual cortex during the first and the second cycles of visual stimulation. This increase is lower than the 34 % increase reported previously to 8 Hz flashing lights[2]. The forward rate constant (i.e. unidirectional Pi->ATP rate) of ATPase reaction (k_2) was calculated in similar way but using the magnetization of Pi, but no significant increase could be detected in response to visual stimulation (average k_2 in rest and active periods were 0.13±0.08 s^{-1} and 0.15±0.11 s^{-1}; p=0.42), as predicted by Monte Carlo simulations.

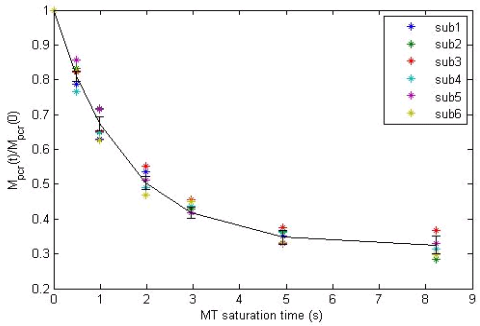

Figure 1: Normalized magnetization of PCr versus MT saturation time, mean and SD of fitted values shown.

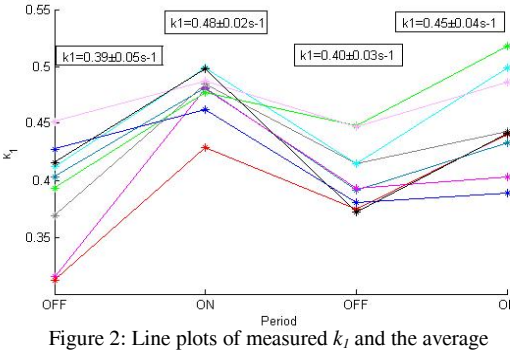

Figure 2: Line plots of measured k_1 and the average values of k_1 measured in each period. Colours represent individual subjects.

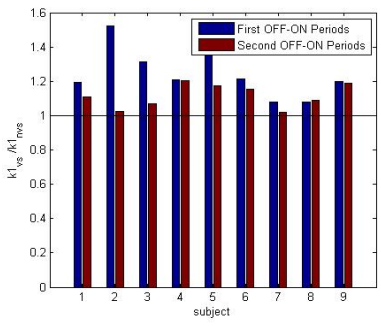

Figure3. Bar plot of the measured $k_{1,vs}/k_{1,no\_vs}$ ratio during the first and second cycle

CONCLUSION: This study showed that the CK forward reaction rate constant (k_1) increased by 17 ± 12 % over the voxel studied, during a relatively long visual stimulation. As predicted, it was not possible to detect a significant change in the forward rate constant of ATPase reaction due to the limited SNR of the Pi peak. In future work, k_1 will be corrected for active grey matter volume based on BOLD fMRI data, and the correlation between the BOLD signal change and % change in k_1 will be assessed for visual stimulation of varying intensity.

REFERENCES: [1] Du F, et al. MRM. 2007;57:103-114. [2] Chen W, et al. MRM. 1997;38(4):551-557. [3] Bottomley PA, et al. MRM.1992;99:443-448. [4] Naressi A, et al. Magma.2001;12:141-152. [5] Yan L, et al. JCBFM.2012;32:1484-1495. [6] Sauter. MR. J. Biol. Chem.1993;268:13166-13171. [7] Mlynarik V, et al. Proc. Intl. Soc. Mag. Reson. Med. 2005;13:2767.