

Measurement of CMRO₂ changes by somatosensory stimulation in rat using oxygen-17 at 16.4 TH. M. Wiesner¹, R. Pohmann¹, D. Z. Balla¹, W. Chen², K. Ugurbil², and K. Uludag³¹High-Field Magnetic Resonance Center, Max Planck Institute for Biological Cybernetics, Tübingen, Germany, ²Radiology, Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, Minnesota, United States, ³MBIC, Department of Cognitive Neuroscience, Maastricht University, Maastricht, Netherlands

Introduction The measurement of cerebral metabolic rate of oxygen (CMRO₂) via direct NMR detection of the stable oxygen isotope ¹⁷O in water is a promising tool to study neuroenergetics, brain activity and pathology [1]. Due to the low natural abundance of H₂¹⁷O (0.037%) and the fast relaxation rates of the ¹⁷O nucleus, sequences with short acquisition delays and ultra-high field strengths are mandatory to determine CMRO₂. In this study, we determined CMRO₂ in rat somatosensory cortex during peripheral stimulation using inhalations of enriched ¹⁷O₂ gas at 16.4T.

Methods A 3-D chemical shift imaging sequence (CSI) with an acquisition delay of 538 μs was used for ¹⁷O MRSI. All acquisitions were performed on a 16.4 Tesla magnet (Magnex/Varian Inc.) with 26 cm bore diameter, maximal gradient-strength 1 T/m (Resonance Research Inc.) interfaced to an Avance III - Paravision 5.0 (Bruker BioSpin) console. RF excitation and reception was performed by custom-built hetero-nuclear ¹H/¹⁷O silver wire surface-coils tuned at ω_{1H}=698 MHz / ω_{17O}=94.63 MHz.

Animal preparation: 2 mechanically ventilated male Wistar rats (540g; 300g) anesthetized with i.v. infusion of alpha-chloralose at 50 mg/kg/h were fixated in a stereotaxic frame directly below the ¹⁷O surface coil. Exhaled gases were monitored continuously, arterial blood samples from the tail artery were taken 20 min before in-vivo acquisitions to adjust ventilation, and after the experiment. Body temperature was maintained at rectally measured 37.3±0.1 °C by an electric heat blanket. All procedures and experiments were approved by the local authorities (EUVD 86/609/EEC).

Peripheral stimulation: subcutaneous electric stimulation of single forepaw using 300 μs pulse width, 4 mA at frequencies of 1, 3 and 6 Hz.

Parameters for ¹H EPI: FOV 1.5×1.5×0.7 cm³, matrix 64×64×7 (voxel-volume: 0.1 μl); TR 2s. fMRI task: 40s/60s ON/OFF stimulation paradigms.

Parameters for ¹⁷O MRSI: A weighted 3-D CSI [4] sequence was used with a FOV 2.75×1.25×1.8 cm³; matrix 9×4×4 (voxel-volume: 43.1 μl); 200-μs RF hard pulse with Ernst angle 68°, spectral acquisition 375 points in 3.75 ms [5]; TR 4.92 ms, 6144 FIDs per volume (max. 74 averages in the center of k-space) with an overall duration of 30.2 s per measurement. 109 volumes were acquired while performing 15 min inhalations of enriched ¹⁷O₂ gas (Ventilation mixture: 33% O₂ in N₂O (rat 1) or N₂ (rat 2), enrichment fraction 50-70% ¹⁷O₂, Nukem GmbH) for each determination of CMRO₂ [2,3]. Five consecutive CMRO₂ acquisitions with 3 stimulation and 2 baseline blocks were performed on each animal (Fig. 1c). Stimulation blocks consisted of continuous stimulation from shortly before inhalations until ~20 minutes after end of inhalations.

Post-mortem high-resolution H₂¹⁷O (for coregistration with ¹H-FLASH): Classic CSI (unweighted k-Space) FOV 2.75×1.61×2.5 cm³; matrix 41×24×25 (voxel-volume: 0.45 μl); TR 12 ms with an acquisition duration of 10 ms with 1000 spectral points; total acquired FIDs 3,148,800 with an overall duration of ~10 hours per volume. Data were reconstructed using MATLAB: Spatial zero-filling by a factor of 2 and line-broadening (120 Hz) were used for enhanced SNR and visualization of muscle vs. cortex in the following coregistrations of H₂¹⁷O and proton modality images (Fig. 1b). Coregistration was performed using FSL [6] and Freesurfer [7] by manual alignments based on FOV and contrast. All voxels selected for CMRO₂ difference measurements were masked to be less than 1 cm distant from the coil and to have a significantly higher CMRO₂ than the surrounding muscle tissue. Standard fMRI analysis was conducted on ¹H-BOLD using FSL [6]. ¹H-FLASH images at 59 μm in-plane resolution were used for final coregistration of proton BOLD activations and CMRO₂ differences obtained from fitting the 3D-CSI.

Results

Estimated brain CMRO₂ from H₂¹⁷O time courses using a three-phase model [2] was calculated as 1.42 and 1.8 μmol/g brain tissue/min. Baseline fluctuations in brain CMRO₂ from systemic physiological fluctuations were below expected stimulation dependent ΔCMRO₂ effects (Fig. 1a; rat 1: ±4%; rat 2: ±2% SD of baseline CMRO₂). Peripheral somatosensory stimulation effects were detected in both ¹H-BOLD and H₂¹⁷O- CMRO₂ modalities. (CMRO₂ change in the stimulated S1: +5.4% in rat 1, +12.1% in rat 2). In one rat also the secondary somatosensory cortex showed colocalized fMRI/CMRO₂ activation (Fig. 1b). In dependence of the frequency of stimulation a highest ΔCMRO₂ was observed in primary somatosensory cortex (Fig. 1c) at a frequency of 1 Hz (+11.5% above baseline CMRO₂) and decaying with higher frequencies (3 Hz: +8.8%; 6Hz: +5.1% ΔCMRO₂). Established dependence on stimulation frequency [8] was found in ¹H-fMRI of S1 (+1 Hz: 10%; 3Hz: +8%; 6 Hz: +4% BOLD).

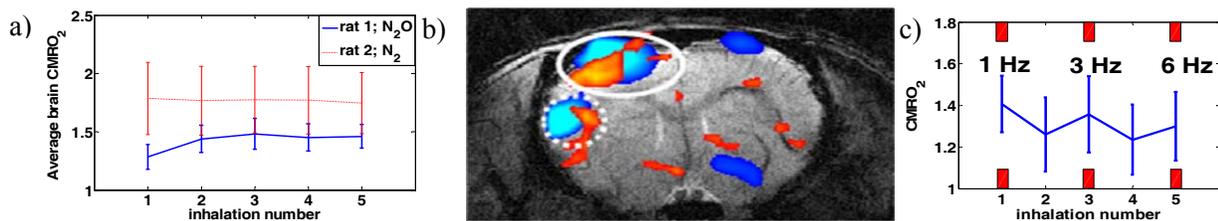


Figure 1(a) Average absolute brain CMRO₂ (b) Colocalized brain activations of fMRI ¹H-BOLD (red) and ¹⁷O-CMRO₂ (cyan) of primary (line) and secondary (dotted) somatosensory cortex. (c) CMRO₂ in S1 during stimulation frequencies 1, 3, 6 Hz vs. baseline (stimulation blocks in red). Absolute measurements of CMRO₂ are given in μmol/g brain tissue/min.

Conclusions: We have observed the first directly measured CMRO₂ changes in rats by peripheral somatosensory stimulation at an ultrahigh field strength of 16.4 Tesla. The results show that apart from the high sensitivity required also the stability of baseline metabolism is crucial in detecting small CMRO₂ changes evoked by increased neural activity. Lower global baseline metabolism was found when ventilating with a N₂O vs. N₂ mixture of equal O₂ fraction due to the increased anesthetic effect of N₂O in agreement with previous work [9]. The direct detection of changes in CMRO₂ can also help to investigate the metabolic changes in negative BOLD activations on the ipsilateral hemisphere. Our results are also in accordance with previous results using ¹⁷O for CMRO₂ measurements from other species at 9.4T [10].

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References: [1] Zhu et. al. PNAS (2002), [2] Atkinson & Thulborn NI (2010), [3] Zhang et. al. MRM (2004), [4] Brown et. al. PNAS (1982), [5] Pohmann et.al. JMR (1997). [6] Smith et. al. NI (2004) [7] Greve & Fischl NI (2009) [8] Huttunen NI (2008), [9] Crosby et. al. Anesthesiology (1983), [10] Zhu et. al. JCBFM (2008).