

High-resolution mapping of the neurochemical profile after focal ischemia in mice

M. F. Alf^{1,2}, H. Lei^{1,3}, C. Berthet⁴, L. Hirt⁴, R. Gruetter^{1,3}, and V. Mlynárik¹

¹Laboratory of Functional and Metabolic Imaging, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, ²Institute of Pharmaceutical Sciences, ETH Zürich, Zürich, Switzerland, ³Department of Radiology, University of Lausanne, ⁴Department of Clinical Neuroscience, Centre Hospitalier Universitaire Vaudois

Introduction: In vivo studies of brain metabolism under transient ischemia are essential to understand the mechanisms of related tissue death. The focal model of middle cerebral artery occlusion (MCAO) is very useful for longitudinal examination of ischemic effects both in ischemic core and penumbra and more distant regions. So far, in vivo magnetic resonance spectroscopic (MRS) studies of MCAO in mice have been limited to either single-voxel spectroscopy or MRS imaging (MRSI) with a relatively coarse spatial resolution and restricted spectral information; most MRSI studies up to date only quantify N-acetyl-aspartate (NAA), total creatine (tCr), lactate (Lac), total cholines (tCho) and the sum of glutamate (Glu) and glutamine (Gln). At lower magnetic field strengths, extremely long acquisition times are necessary to achieve sufficient signal-to-noise ratio (SNR) and spectral resolution allows for quantification of only a limited number of metabolites. High-field MRSI of mouse brain is feasible despite other challenges, such as increased field inhomogeneity. A recent LASER-MRSI study¹ on a vertical bore 17.6T magnet quantified a slightly larger set of metabolites. Comparative studies² have demonstrated that standard MRSI (one TR per k-space point) is optimal in terms of sensitivity per time, so it is desirable to stick to this scheme as closely as possible while tailoring the method to fit the needs of setup and investigated model.

Our aim was to adopt an optimized protocol with k-space weighted acquisition and ultra-short echo time (TE) at very high magnetic field strength to measure the spatial distribution of the neurochemical profile after transient ischemia in mice within a reasonable measurement time.

Experimental: Seven male iCD-CR mice underwent a protocol of 30min transient MCAO and were transferred to an MRI system (Varian, Palo Alto, CA, USA) after 3, 8 and 24h of reperfusion. Six mice served as control group. The MRI system was interfaced to a 14.1T magnet with a 26-cm horizontal bore (MagneX Scientific, Oxford, UK). A home-built quadrature surface coil was used as transceiver. T₂-images were acquired with a FSE multislice method³ to position the volume of interest (VOI) for subsequent MRSI and to detect abnormal T₂-hyperintense signals, i.e. ischemic edema. A 1.5mm thick coronally oriented VOI of 4mm×6mm was selected for excitation at 0.4mm anterior to the bregma (Fig. 1). The echoplanar FASTMAP version was used for first- and second-order corrections of the magnetic field in the VOI as in Ref. 4. For MRSI, we combined the SPECIAL technique⁵ (TE=2.8ms) and an accelerated k-space acquisition scheme⁶ with a circular sampling window and a relatively flat 2D Hamming filter weighting the TRs, calculated from the T₁ of metabolites (about 1500ms at 14.1T) and water (T₁≈2000ms) respectively^{7,8}. Maximum TR at the k-space center was 2500ms for metabolites and 1600ms for water. The acquired data were filled into a 32×32 matrix covering a 19mm×19mm FOV in the coronal plane. Corners of k-space were zero-filled and data were multiplied with another Hamming-shaped filter so total filtering corresponded to the standard Hamming filter. The resulting effective full width at half maximum (FWHM) voxel size was 1.4μl. Outer volume saturation and VAPOR water suppression were used for the metabolite acquisition. Acquisition time was 45min for metabolites and 30min for the water reference.

Results: Water linewidths were 25±3 Hz for the whole excited volume and ≥ 10.2 Hz for individual voxels at SNR values up to 17. Spectra of individual voxels allowed for quantification of 15 metabolites (Fig. 2) plus macromolecules with a CRLB of less than 20%. The resulting values were within error ranges of a previous single-voxel ¹H MRS study⁹. Large concentration changes in the ischemic striatum could be observed for most quantifiable components of the neurochemical profile, e.g. NAA, taurine (Tau) and Glu (Fig.3). The extent of the drop in NAA did not correlate with the increase in Lac (p>0.6). tCr and GABA, NAA and Tau showed a similar pattern of significant temporal decrease. The behavior of GSH was similar to that of Glu, i.e., its decrease compared to the contralateral side was the same at all time points (2.0±0.3 vs. 0.9±0.3 μmol/g in contra- vs. ipsilateral striatum, p<0.0001). GSH and tCho were decreased in the entire MCAO territory whereas changes in Lac, NAA, Glu and GABA were more restricted to the site of T₂-hyperintensity. While in healthy control animals no voxel in the entire MRSI map showed Gln concentrations higher than 4 μmol/g, Gln was significantly increased in striatum and cortex of the ischemic hemisphere (all p<0.05) at 3h and 8h. The concentration peaked at 8h (7.5±0.3 μmol/g in striatum, 8.6±1.4 μmol/g in cortex, see Figure 6) and returned to a normal level in striatum at 24h while remaining significantly increased in cortex (p<0.01).

Discussion and Conclusions: Optimizing the sensitivity per time on a high-end machine yielded metabolite maps of excellent spatial resolution and spectral quantification. To our knowledge this study is the first to provide ¹H-MRSI maps of GABA and GSH and also one of the few mapping Glu and Gln separately at high spatial resolution. Our results are in good agreement with single-voxel MRS and give new insights e.g. concerning the distribution of GSH which we found to be reduced in the entire territory of the occluded MCA which is different from results of a recent microscopy study¹⁰ that suggested increased GSH in the penumbra. Also the protocol is suitable for the further analysis of Gln in different tissues.

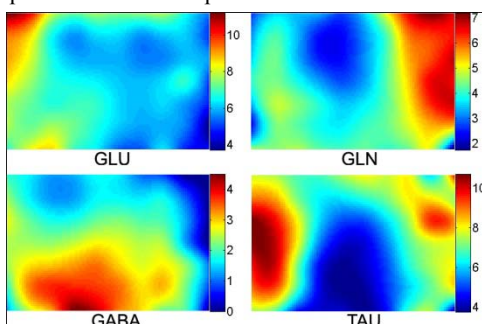


Fig. 3: Maps of example metabolites at 3h, note e.g. high hypothalamic GABA and widely increased Gln in the ischemic area

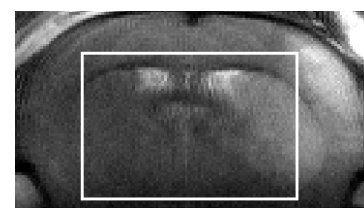


Fig. 1: VOI used for MRSI depicted on T2-weighted image at 24h

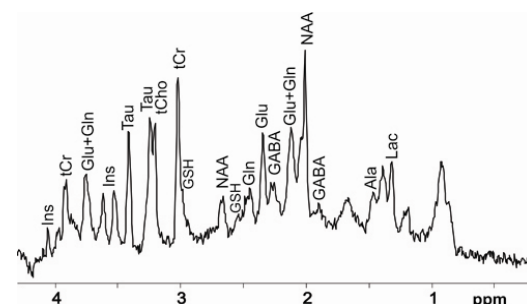


Fig. 2: Spectrum from 1.4μl voxel, healthy striatum

Acknowledgments: This study was supported by EU Grant No. MRTN-CT-2006-035801, by Centre d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV, EPFL and the Leenaards and Jeantet Foundations. MFA was funded by the German Academic Exchange Service (DAAD).

References

1. Weiss K *et al.* MAGMA 22: 53, 2009
2. Pohmann R *et al.* J Magn Reson. 129:145, 1997
3. Hennig J. J Magn Reson 78: 397, 1988
4. Gruetter R & Tkáč I. Magn Reson Med 43: 319, 2001
5. Mlynárik V *et al.* Magn Reson Med. 59:52, 2008
6. Kühn B *et al.* Magn Reson Med. 35:457, 1996
7. de Graaf R *et al.* Magn Reson Med 56: 386, 2006
8. Cudalbu C *et al.* Magn Reson Med 62: 862, 2009
9. Lei H *et al.* J Cereb Blood Flow & Metab 29: 811, 2009
10. Bragin DE *et al.* J Cereb Blood Flow & Metab 30: 734, 2010