In vivo ^{13}C MRS in the mouse brain at 14.1 T and metabolic flux quantification during infusion of [1,6-$^{13}C_2$] Glucose

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Introduction: ^{13}C MRS spectroscopy in the brain in conjunction with the injection of [1,6-$^{13}C_2$] glucose allows a non-invasive assessment of TCA cycle activity and neurotransmission. Moreover mathematical models developed so far allows the differentiation of neuronal and glial compartments (1,2) resulting in a precious and quantitative tool in studying metabolic diseases. Unfortunately, the relatively low sensitivity in ^{13}C detection limited in vivo ^{13}C MRS usually to rat brain studies. To our knowledge only one attempt of direct detection in the mouse brain was performed in a 7 Tesla scanner, although metabolic fluxes were not quantified (3). With the present study, performing our acquisition at 14.1 Tesla we aim to overcome the above-mentioned sensitivity issue. These results could be of particular interest considering the availability of a wider range of disease models in genetically modified mice.

Methods: Data were acquired in three male mice (Swiss Nude, 8 weeks old, 28-30g) anesthetized with isoflurane (1-2%) in a voxel of 112µL (3.6x6.9x4.5mm^3). Mice were fasted for 7 hours and catheterized in the tail vein for glucose infusion. The infusion protocol foresees an exponential decaying bolus of 99%-enriched [1.6-$^{13}C_2$] glucose during 5 minutes and a continuous infusion of 70%-enriched glucose over 5 hours. The rate of the continuous infusion was adjusted in order to keep the targeted glycemia level (around 300mg/dL) based on bench experiments during which the plasma glucose level was monitored over 5 hours. Spectra were acquired on a 14.1T system (Varian/Magnex Scientific) using a home-built 9mm(^{13}C)/15mm(^{1}H quad) surface coil as receiver-transmitter antenna. Direct detection of ^{13}C nuclei was achieved by semi-adiabatic DEPT polarization transfer sequence(4) (TR=2.5s, interpulse delay of 3.8ms (optimized for J_{CH}=130Hz), 45° for the last ^{1}H pulse for the simultaneous detection of CH, CH_2 and CH_3 groups). An ^{1}H spectrum was collected prior to the injection using short-TE localized SPECIAL(5) sequence (TR/TE = 4000/2.8ms) on the same voxel in order to quantify the glutamate and glutamine total concentrations.

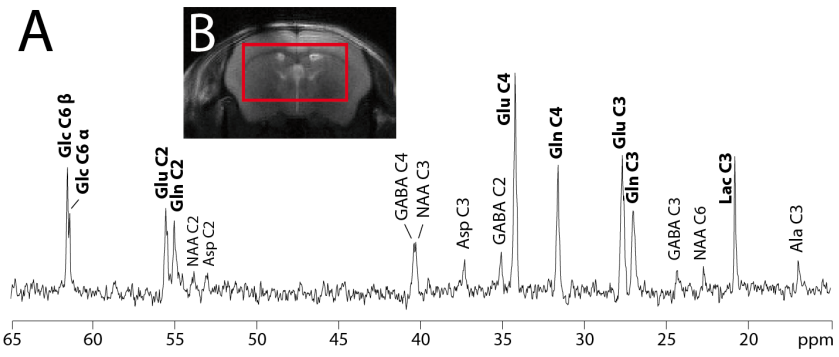

Fig.1: A) In vivo ^{13}C MRS spectrum of the mouse brain at 14.1T after 4.5 hours of [1,6-^{13}C] Glucose infusion (32 min. acquisition); B) T2-weigheted image of the mouse brain with acquisition voxel (112µL).

Results and Discussion: Spectra averaged over 32 minutes acquired after 4.5 hours of [1,6-$^{13}C_2$] glucose infusion showed 17 well-resolved resonances of ^{13}C-labeled metabolites related to glial and neuronal metabolism and neurotransmission (Fig.1). Dynamic acquisition of ^{13}C enrichment of carbon positions C4,C3 and C2 of glutamate and glutamine was achieved with 10 minutes temporal resolution during 5 hours of infusion (SNR of 10-13, data not shown) leading to highly detailed and precise determination of fractional enrichment curves (Fig.2). The quantified metabolic fluxes using two-compartment modeling (1,2) and the respective precision evaluated with Monte Carlo simulation are reported in the table below:

Fluxes	$Vtca^g$	Vpc	Vnt	$Vtca^n$	Vx
[µmol/g/min]	0.11 ± 0.02	0.051 ± 0.005	0.21 ± 0.03	0.33 ± 0.02	0.20 ± 0.03

Conclusion: We conclude that high sensitivity in direct detection of ^{13}C labeled metabolites in vivo was achieved in the mouse brain and enabled a detailed two-compartment analysis of metabolic fluxes involved in energy metabolism for the first time. These results therefore open the way to a wide range of studies on the impact of specific brain diseases on energy metabolism in transgenic mice models. Moreover based on the obtained SNR we can predict that direct detection of Glu and Gln ^{13}C resonances could reach reasonable sensitivity in a voxel size <80µL, which is of particular interest for localized lesions such as brain cancer.

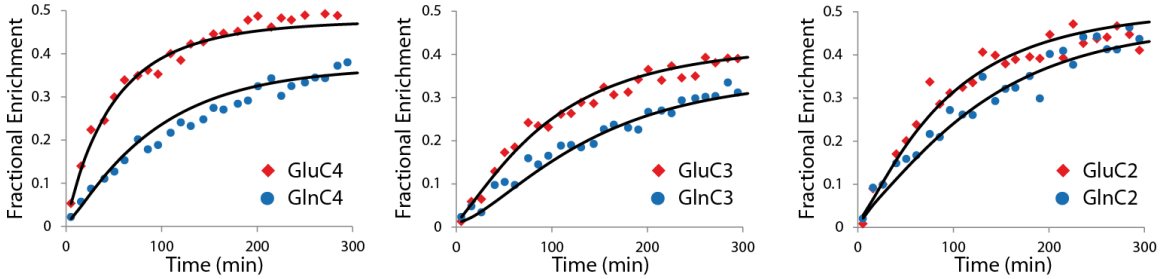

Fig.2: Fractional enrichment curves (n=3) of glutamate and glutamine ^{13}C-enriched carbon positions (C4, C3 and C2) over the 5 hours of [1.6-$^{13}C_2$] glucose infusion. The enrichment curves are fitted with a neuronal-glial compartmental model for the metabolic fluxes quantification.

References: (1) Gruetter R et al., Am J Physiol Endocrinol Metab 2001; (2) Duarte JMN et al., Front Neuroenergetics 2011; (3) Nabuurs C et al., Magn Reson Med 2008; (4)Henry P-G et al., Magn Reson Med 2003; (5)Mlynárik V et al., Magn Reson Med 2006. **Acknowledgements:** Supported by Centre d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV, EPFL, the Leenards and Jeanet Foundations; Grant FP7-PEOPLE-2010-ITN-264780.