Overcoming experimental challenges in the assessment of metabolic fluxes with [1,6-$^{13}C_2$]glucose in mouse brain

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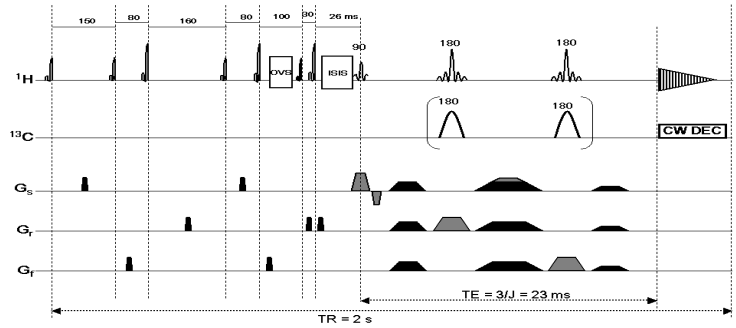
Introduction: The infusion of ^{13}C labeled glucose and acetate allow for *in vivo* monitoring of metabolic conversions by MR spectroscopy (MRS). In brain tissue, both proton observed carbon edited (POCE) *[1]* and distortionless enhanced polarisation techniques (DEPT) *[2]* have been used for time resolved detection of label incorporation into neurometabolites such as glutamate (glu) and glutamine (gln). Although both methods have been frequently performed in humans and rats, the application of these techniques in mice would be very valuable to study neuropathologic diseases in transgenic mouse models. However, the relatively small size and the difficulty to determine an input function from blood samples for each individual mouse during the MR measurements (due to the small blood volume of the mouse) makes this rather challenging. While DEPT can be used to discriminate between the different lables of glutamate (glu C2-4) and glutamine (gln C2-4), additional *in vivo* POCE spectra could allow for the determination of both concentrations of the labeled and non-labelled contents of some of these metabolites and thereby exclude the need for blood sampling. In this study we investigate the potential to perform sequential *in vivo* POCE and DEPT in mouse brain upon ^{13}C labeled glucose infusion using an optimised coil setup, as a step towards quantification of neurometabolic fluxes in brain of various mouse models .

Fig 1: Pulse sequence for localised POCE.
OVS and ISIS localisation were integrated in a VAPOR watersuppresion scheme. ^{13}C editing pulses were inserted in the PRESS localisation pulses [3], TR = 2s, 3x3x4 cm^3, 64 avg. At the ^1H channel a 90^o sinc pulse (1 ms) and two Mao shaped refocussing pulses (2 ms) were uses for voxel selection. A TE of 23.1 ms (=3/J) allowed for sufficient time for the necessary crushers and J-editing . The inversion pulses at the ^{13}C channel were accomplished by 2ms hyperbolic secant pulse shapes, with a more than four fold higher bandwidth than the Mao shaped pulses. During acquisition the [3-^{13}C] lactate signal at 21 ppm was decoupled using 200ms continuous wave decoupling.



Methods: [1,6-^{13}C]glucose was infused i.v. in C57Black6 mice (34-36 gr), using urethane (2.1mg/g) anesthesia following a previous described protocol adjusted for mice *[4]*. All spectra were acquired at 7T (MRRS, Surrey, UK). As the quality of localisation is essental in POCE experiments ISIS, OVS and PRESS have been combined to obtain ^1H MR signals from a 5x5x4 cm^3 VOI in the mouse brain (fig 1). ^{13}C spectra were acquired with DEPT (TR = 2s, 3.4ms interpulse delay, WALTZ16 decoupling, 45^o flipangle), where only ISIS localisation was used to select signals from the identical VOI *[5]*. A double tuned surface coil (∅ = 12mm) was used for optimal signal detection for both ^{13}C and ^1H in combination with a volume coil (∅ = 40mm) for homogeneous ^1H excitation. All ^{13}C excitation pulses have been performed adiabatically using the double tuned surface coil. To demonstrate label incorporation into lactate, a similar experiment was repeated while using isoflurane (1.5%) for anesthetics and ventilation.

Results and Discussion: Figure 2 shows spectra obtained from the same VOI using POCE and DEPT subsequentially. The subtraction spectra obtained from non-edited minus edited spectra (fig 2, bottom) show signals of Glu H4, Glx H3 and Glx H2/Glc H6, which demonstrates the feasibility of using J_{CH} editing techniques in mouse brain. As ^{13}C CW decoupling was off-resonance, these signals showed up as doublets. The DEPT spectrum shows ^{13}C MR signals of the same labeled compounds, demonstrating the ability to distinguish between glu and gln due to the higher spectral resolution of ^{13}C acquisition. Labeled signals of lactate could not be obtained from this mouse with either POCE or DEPT, indicating proper physiology. However, detection of lactate incorporation was feasible, when ventilating the mouse and using isoflurane for anesthetics (fig 3). The combination of a volume Tx coil for ^1H and the adiabatic ^{13}C pulse shapes enabled optimal RF transmission in the region of interest. No lipid contamination was seen in either POCE (at 1.3 ppm) and DEPT (at 30 ppm) spectra (fig 2), confirming proper localisation for both methods. Further improvements on POCE are required on shimming, as the detection of isotopic enrichment of the glc ^1H signal at 5.23 ppm was hampered by the linewidth of water in the VOI (shimming the first and second order manually resulted in linewidths of 18-20Hz).

Fig 2:
A) POCE spectra of mouse brain.
Summed (top) and difference (bottom) spectra of acquisitions with (eight) and without (eight) J_{CH} editing. VOI = 100uL. tCr = total creatine, Cho =Choline, NAA = N-aspartyl aspartate, Tau = taurine, Gln = glutamate, Glu = glutamine, Glc = glucose, Glx = glutamate and glutamine
B) DEPT spectrum of mouse brain. *Same VOI =100uL, nsa = 256, J_{CH}=145*

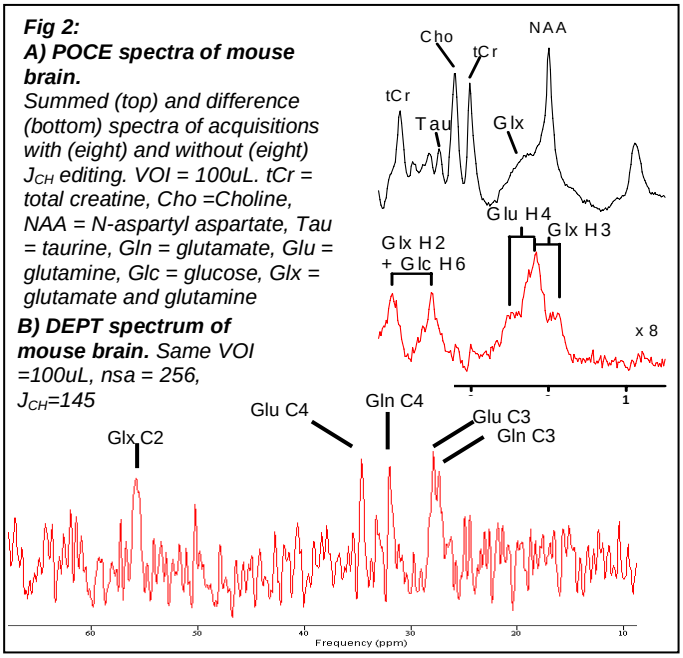
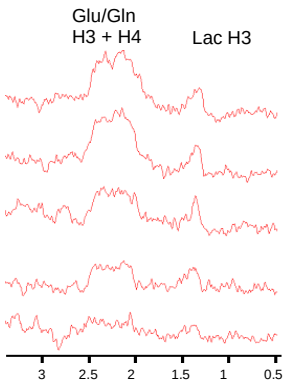


Fig 3: Time resolved detection of ^{13}C labeled lactate from brain of a mouse who was ventilated.
Spectra was acquired with POCE, acquisition time was 21min per represented difference spectrum, VOI =100uL. Ventilation was performed with 150 strokes/min and 8 mL/kg. Note the ^{13}C label incorporation in lactate (Lac) and the glutamine glutamate resonances. The latter show up undecoupled as the ^{13}C CW decoupling was focused at 21 ppm for the lactate.



Conclusion: These preliminary results demonstrate the first *in vivo* POCE MR spectra obtained from mouse brain. The interleaved acquisition of DEPT enabled distinction of labeled Glu and Gln resonances in the ^{13}C spectra obtained from the same VOI. The incorporation of label into lactate was shown upon ventilation of the mouse. Although further optimalisation on shimming is required to enable the detection of an input function from the POCE MR spectra, both the signals from labeled and non-labeled fractions of the metabolites could be obtained.

References: *[1] Rothman et al. PNAS 1985; [2] Doddrel et al. JMR, 1982; [3] Chen et al, MRM 1998; [4] Fitzpatrick, JCBF&M 1990 [5] Nabuurs et al. MRM, in press.*