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In Vivo Assessment of Neuronal Metabolic Fluxes in Mouse Brain by ^{1}H-[^{13}C] NMR Spectroscopy

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Introduction

In vivo ^{13}C NMR spectroscopy in conjunction with the administration of ^{13}C labeled substrates has been intensively used to assess cerebral metabolism in human and rat. ^{13}C MRS studies of transgenic mouse models will provide insights into the metabolism in brain pathologies. However, the intrinsically low sensitivity of ^{13}C MRS and the small size of the mouse brain remain limiting factors for measuring ^{13}C labeling into metabolites *in vivo*, hence, most ^{13}C MRS studies in mouse brain were achieved by using extracts [1]. Alternatively, the indirect detection of ^{13}C through ^{1}H combined with [1,6-$^{13}C_2$] or [U-$^{13}C_6$] glucose administration can improve the sensitivity and allow the direct measurement of fractional enrichments (FE). However, the distinct separation of ^{13}C labeling in glutamate (Glu) and glutamine (Gln) using indirect detection requires sufficiently high spectral resolution [2,3]. Furthermore, due to the small blood volume of mouse, the input function from blood sampling during *in vivo* NMR experiments is hardly feasible, and thus the metabolic fluxes derived from *in vivo* measured turnover curves remain to be determined. In the present study, a short-TE full-sensitivity ^{1}H-[^{13}C] NMR sequence[3] combined with high magnet field (14T) was used to enhance the sensitivity and spectral dispersion which allows measuring separated GluC4, GlnC4, GlxC3 time courses in mouse brain with [U-$^{13}C_6$] glucose infusion and the further assessment of metabolic fluxes.

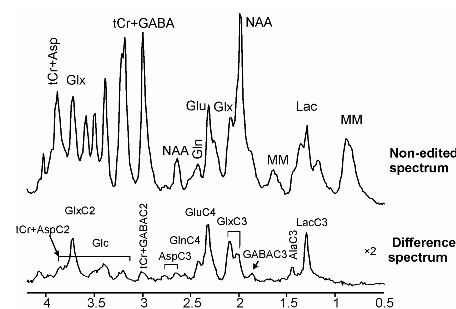

Fig.1: Averaged non-edited (top) and difference (bottom) ^{1}H NMR spectra acquired in the mouse brain during first hour of 67% [U-$^{13}C_6$] glucose infusion. (VOI = 60µl, nt = 960, no apodization was applied). Asp, aspartate; Glc, glucose; GABA, γ-aminobutyric acid; Ala, alanine; tCr, total creatine; Glx, Glu+Gln.

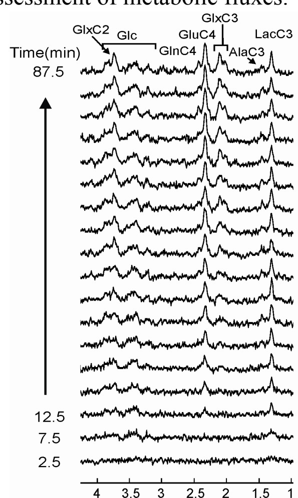

Fig.2: ^{13}C labeling incorporation into Glc, LacC3, GluC4, GlnC4, GlxC3, GlxC2, AlaC3 during the first 90 minutes of [U-$^{13}C_6$] glucose infusion with time resolution of ~ 5min.

Materials and Methods

Animal: Four male ICR (CD-1) mice (34-36g) were fasted over 7 hours and anesthetized using isoflurane (1.5-2%). One femoral vein was cannulated for the infusion of [U-$^{13}C_6$] glucose. After giving a bolus of 99%-enriched [U-$^{13}C_6$] glucose over 5 min, 67%-enriched [U-$^{13}C_6$] glucose was infused continuously for up to four hours.

NMR spectroscopy: All MRS experiments were perfromed on a 14.1T/26cm magnet (Varian/Magnex) using a homebuilt ^{13}C /^{1}H surface coil. ^{1}H-[^{13}C] spectra were acquired using a previously proposed full signal intensity ^{1}H-[^{13}C] NMR sequence 'SPECIAL-BISEP'[3], which was a hybrid sequence composed of a preceding ^{13}C editing block based on the inversion BISEP and a spin-echo coherence-based localization. ^{13}C editing was achieved by turning on and off the AFP in the ^{13}C channel on alternate scans and the difference spectrum contained ^{13}C coupled ^{1}H resonances.OVS and VAPOR [4] water suppression were applied prior to SPECIAL-BISEP to suppress water signal and lipid contamination in ^{1}H NMR spectra. FASTMAP was used to optimize B_0 inhomogeneity and resulting in water linewidth of 21-24 Hz for a volume of 60µl containing cerebral cortex and striatum. Adiabatic ^{13}C decoupling was applied during 145 ms acqusition time. ^{13}C-edited and non-edited spectra were acquired with 8 scans in an interleaved mode and TR was 4 s.

Data analysis: Spectra were quantified with LCModel [5] and fractional enrichment of metabolites was calculated directly from the relative ratio of the concentration obtained from difference spectra and that obtained from non-edited ^{1}H spectra (AFP off). Under the isoflurane anesthesia, lactate (Lac) can be detected in ^{1}H-[^{13}C] NMR spectra. Given that rapid activity of lactate dehydrogenase allowing fast exchange between pyruvate and lactate, turnover of LacC3 is mimicking that of pyruvate and thus used as the input function for the metabolic model. One compartment metabolic model (glutamatergic neuron) [6] was used to fit the measured ^{13}C labeling time courses of metabolites (i.e.GluC4, GlnC4, GlxC3) to calculate the metabolic fluxes and the reliability of the fitting was evaluated by Monte-Carlo simulation.

Results and Discussion

Non-edited and difference proton spectra acquired from the VOI of 60µl using SPECIAL-BISEP at 14T demonstrate a high signal-to-noise ratio and spectral resolution (Fig.1), e.g. the separation of GluC4 and GlnC4. ^{13}C labeling in Glu, Gln, GABA, Asp, Glc, Ala and Lac was detected as shown in Fig.1. Note that ^{13}C labeling into Glc and Lac can already be observed during the second 5 min acquisition after the start of [U-$^{13}C_6$] glucose infusion. Subsequently, carbon positions of Glu, Gln and GABA were labeled, respectively (Fig1, Fig2). The average fractional enrichment curves of GluC4, GlnC4, GlxC3 from 4 mice were fitted using LacC3 as the input function (Fig.3) and resulted in values of neuronal TCA cycle flux $V_{TCA(N)}$ = 0.93 ± 0.03 µmol/g/min (mean ± sd), which is similar to the results obtained from mouse brain extracts [7]. Additionally, the exchange flux between 2-oxoglutarate and glutamate V_x = 0.78 ± 0.04 µmol/g/min and the Glu-Gln cycle flux V_{NT} = 0.21 ± 0.01 µmol/g/min were obtained.

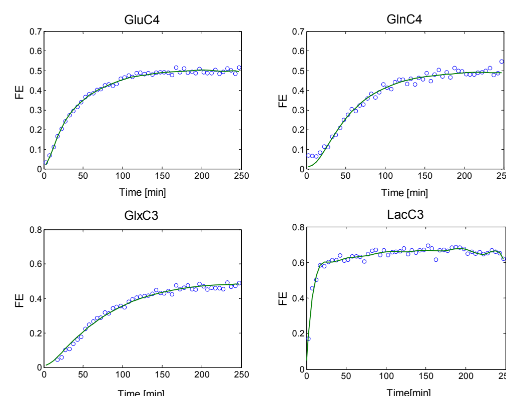

Fig.3: Fits and average time-resolved ^{13}C fractional enrichment curves of GluC4, GlnC4, GlxC3 and LacC3.

We conclude that high quality ^{1}H-[^{13}C] NMR spectra can be acquired from a volume as small as 60µl in mouse brain *in vivo* at 14T. This allows us to obtain ^{13}C labeling turnover curves of GluC4, GlnC4, GlxC3 with time resolution of ~ 5 min as well as LacC3 which offers precursor information to determine metabolic fluxes without additional blood sampling. The metabolic fluxes were obtained for the first time using *in vivo* measured turnover curves in mouse brain. This study can be extended to transgenic mouse models for further investigation of brain pathologies.

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