

Models of normal brain function

coefficients reflecting perfusion [2,4] were observed at ΔCMRO_2 -activations in one rat.

Discussion/Conclusion: The small CMRO_2 changes evoked by increased neural activity we detected highlight the importance of high NMR sensitivity and baseline stability. Our first directly measured colocalized ^1H -BOLD/ ^{17}O - CMRO_2 changes in rats measured at the ultra-high field strength of 16.4 Tesla motivate further research in the study of neurometabolic-coupling during brain activation.

References:

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Evaluation of glial metabolic fluxes in vivo with [2- ^{13}C] acetate infusion in mouse brain by ^1H -[^{13}C] NMR spectroscopy

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Introduction: ^{13}C NMR spectroscopy combined with administration of ^{13}C labeled glial-specific substrate (i.e. acetate) has been used to investigate glial metabolism and neuronal-glia interaction *in vivo* [1]. The application to transgenic mouse models will be useful to explore glial dysfunctions in models of human disease. Therefore, the aim of the present study was 1) to demonstrate the feasibility of measuring time-resolved ^{13}C labeling in mouse brain *in vivo* with [2- ^{13}C] sodium acetate using a full-sensitivity ^1H -[^{13}C] NMR sequence [3] at 14T; 2) to investigate the glial TCA cycle and neurotransmitter cycle flux in mouse brain.

Subjects and Methods: Five male ICR (CD-1) mice (34-36g) were anesthetized using isoflurane (1.5-2%) and one femoral vein was cannulated for the infusion of [2- ^{13}C] sodium acetate. All spectra were acquired using a previously proposed ^1H -[^{13}C] NMR sequence 'SPECIAL-BISEP'[2] at 14.1T/26cm magnet (Varian/Magnex) with a homebuilt 10mm (^{13}C)/13mm (^1H quad) surface coil. Non-edited and difference spectra were summed up every 94 averages and then quantified with LCModel [3]. Fractional enrichment of metabolites was calculated directly from the relative ratio of the concentration obtained from difference spectra (^{13}C) and that obtained from non-edited ^1H spectra ($^{12}\text{C}+^{13}\text{C}$). A two-compartment metabolic model [4] was used to

fit the measured ^{13}C labeling time courses of metabolites (i.e. GluC4, GlnC4, GluC3 and GlnC3) to calculate the metabolic fluxes.

Results and Discussion: ^{13}C labeling into the C2 position of acetate was detected within 3-7 min and followed by the labeling into GluC4, GlnC4, Glx3 and Glx2, GABAC2 and AspC2 (Fig.1). Fitting *in vivo* measured average time courses (Fig.2), we obtained glial TCA cycle flux $V_{\text{TCA}}^g = 0.13 \pm 0.01 \mu\text{mol/g/min}$, the exchange flux between 2-oxoglutarate and glutamate $V_x^g = 0.16 \pm 0.02 \mu\text{mol/g/min}$, the Glu-Gln cycle flux $V_{\text{NT}} = 0.18 \pm 0.01 \mu\text{mol/g/min}$, while values of the neuronal metabolic fluxes were unreliable and not reported here.

We conclude that it is feasible to directly measure ^{13}C fractional enrichment time courses for GluC4, GlnC4, GluC3 and GlnC3 *in vivo* in mouse brain using ^1H -[^{13}C] MRS with [2- ^{13}C] sodium acetate at 14T and thus to derive glial metabolic fluxes. This study opens the possibility for further study of glial metabolism and neurotransmission disorder in mouse models.

References:

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- [3] Provencher, Magn Reson Med. 30 (1993).
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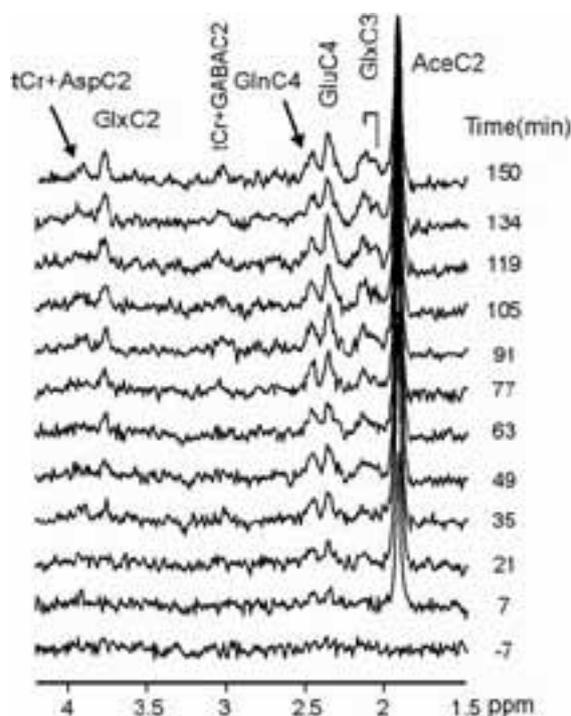


Fig.1: Time resolved ^{13}C labeling incorporation into multiple positions of glutamate, glutamine, GABA and C2 position of acetate during intravenous [2- ^{13}C] acetate infusion (14 min temporal resolution, no apodization was applied). Asp, aspartate; Glc, glucose; GABA, γ -aminobutyric acid; Ala, alanine; tCr, total creatine; Ace, acetate; Glx, Glu+Gln.

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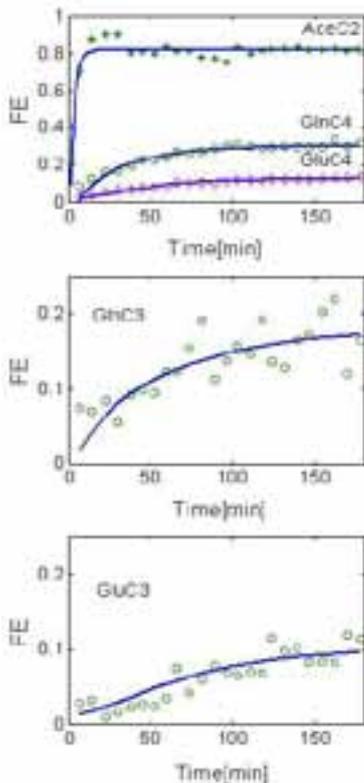


Fig.2: Fits and average fractional enrichment time courses of AceC2, GluC4, GlnC4, GluC3 and GlnC3 ($n=5$).

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Comparison of glucose and lactate metabolism in the barrel field in the awake and stimulated rat.

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Introduction: It's now recognized in the Neuroscience community that glucose is the major energetic substrate for the brain. During the last 20 years, evidence has accumulated to support the concept of a metabolic supply from astrocytes to neurons, lactate more and more thought to be this substitute substrate. However, little information exists about this lactate shuttle in vivo in activated and awake animals. We designed an experiment in which barrel field was unilaterally activated during infusion of both glucose and lactate (alternatively labeled with ¹³C). At the end of the stimulation (1h), S1BF area was removed and analyzed by NMR spectroscopy to compare glucose and lactate metabolism in the activated area versus the non-activated one.

Methods: Wistar rats (200g) were infused with a solution containing [1-¹³C] glucose+lactate or glucose+[3-¹³C]lactate during whisker stimulation (1h, Whisker stimulation was controlled using ¹⁴C-deoxyglucose). At the end of the stimulation, rats were euthanized by microwaves (5KW, 1s). Both activated and non-activated S1BF areas were dissected and analyzed by HR-MAS NMR spectroscopy on a Bruker Avance 500MHz. HR-MAS, high resolution at the magic angle spinning, allows to perform spectra directly on biopsies (50microg), without perchloric acid extraction with a high resolution. Moreover, the use of microwaves to kill animal is a method of choice since no post-mortem metabolism occurs (spectra were compared and lactate was quantified after either microwave or pentobarbital euthanasia).

Results: The whisker stimulation was effective since we observed a 40%-increase in glucose uptake in the activated S1BF area compared to the ipsilateral area.

We also determined that lactate observed on spectra of biopsies of microwave-euthanized animal was not coming from post-mortem metabolism (as evidenced from pentobarbital-euthanized animals).

Specific enrichments (% of ¹³C on a carbon position) of glucose, lactate and some amino acids were measured. Comparison of values in the activated and non-activated areas clearly indicated a difference in glucose and lactate metabolism. Both glucose utilization and lactate synthesis were increased in the activated area.

Discussion/ Conclusion: The use of both HR-MAS spectroscopy and microwaves for euthanasia are two techniques essential to study brain, and more particularly lactate, metabolism on awake and stimulated rats. Results indicated that during activation production of lactate, coming from glucose metabolism, increases thus indicating that lactate can be a supplementary fuel for neurons during brain activation in vivo.