Improved quantification of mitochondrial exchange, TCA cycle rate and neurotransmission flux using $^{1}H\{^{13}C\}$ MRS measurements of C4 and C3 of glutamate and glutamine

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Introduction:

Metabolic modeling of the time courses obtained with ^{13}C MRS following administration of the glia-specific [2-^{13}C]acetate can be used to assess specifically the glial metabolic fluxes and the glutamate/glutamine cycling rate [1]. $^{1}H\{^{13}C\}$ MRS at higher magnetic fields enables highly sensitive detection of both labeled and total glutamate and glutamine, which was recently extended to the separate quantification of glutamate C3 (GluC3) and glutamine C3 (GlnC3). The aim of this study was (a) the analysis on *in vivo* data of the impact of the separated GluC3 and GlnC3 measurement on the precision of the metabolic fluxes and (b) the sensitivity of these fluxes on the assumed distribution of glutamate between glial cells and neurons.

Materials and methods:

Two-compartment modeling of [2-^{13}C] acetate metabolism was applied to extract metabolic fluxes from the dynamic ^{13}C enrichment curves GluC4, GluC3, GlnC4 and GlnC3, measured with $^{1}H\{^{13}C\}$ NMR spectroscopy during 150 minutes, averaged over 5 rats [2], following an infusion which resulted in a constant ^{13}C plasma acetate fractional enrichment (FE) of ~ 90%. [Glu] and [Gln] were measured simultaneously using ^{1}H MRS and used to calculate the respective FE. The model (fig.1) was implemented in Matlab and fitted to the experimental FE data using the standard built-in ODE solver and a modified Levenberg-Marquardt nonlinear regression method, weighted with the inverse of the variance of the experimental noise. The glial and neuronal Krebs cycle fluxes and transmitochondrial fluxes ($Vtca^g$, Vx^g and $Vtca^n$, Vx^n) as well as the apparent neurotransmission flux Vnt were adjusted, without *a priori* constraints.

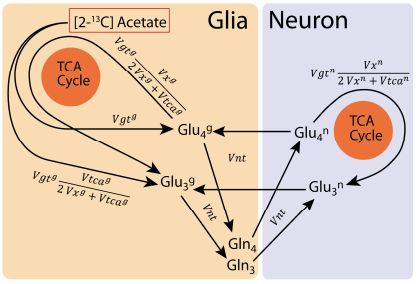

Fig.1: *Metabolic model for [2-^{13}C]acetate brain metabolism*

Uncertainty on the fitted flux values were obtained from Monte-Carlo simulations, based on fits of 500 artificial data [3]. So as to evaluate the changes in the precision on the metabolic fluxes due to the separate measurement of GluC3 and GlnC3, data fitting and Monte-Carlo simulations were applied **(I)** to GluC4 and GlnC4 curves alone, **(II)** to GluC4, GlnC4 and GlxC3 (sum of GluC3 and GlnC3) and finally **(III)** to GluC4, GlnC4, GluC3 and GlnC3. Additionally, the effect of the assumption on the portion of total glutamate located in the glia was analyzed by fitting sequentially the four experimental curves (case I) with increasing glial glutamate concentrations and corresponding decreasing neuronal glutamate concentrations. The variations of the fluxes as well as the evolution of the sum squared error function were observed.

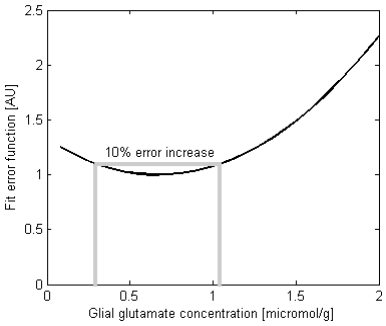

Fig.2: *Fit error function (χ^2) as a function of the assumed glial glutamate concentration*

Results and Discussion:

1. The measured average total glutamine was 3.5 µmol/g, the average total glutamate was 11.5 µmol/g from which 0.5 µmol/g was assumed to be in the glia. The Monte-Carlo determination of the uncertainty of the fluxes showed a general improvement of the precision on the fluxes, when using separated GluC3 and GlnC3 curves in addition to the GluC4 and GlnC4 curves:

Flux uncertainty in %	σVtca	σVx^g	σVnt	σVtca	σVx^n	σVgt^g	σVgt^n
GluC4,GlnC4 (I)	-	-	4.1	-	-	2.8	5.4
GluC4,GlnC4,GlxC3 (II)	4.3	11	4.2	12	13	3.0	5.7
GluC4,GlnC4,GluC3,GlnC3 (III)	4.2	6.6	3.8	15	8.5	2.7	5.1

In the case (III), the model resulted in an excellent fit of the four $^{1}H\{^{13}C\}$ MRS time curves (fig.3A), resulting in $Vtca^g = 0.13 \pm 0.06$ µmol/g/min, $Vx^g = 0.17 \pm 0.01$µmol/g/min, Vnt = 0.20 ± 0.01 µmol/g/min, $Vtca^n = 0.76 \pm 0.12$ µmol/g/min, $Vx^n = 0.38 \pm 0.03$ µmol/g/min. The derived composite fluxes [4,5] were respectively $Vgt^g = 0.073 \pm 0.002$ µmol/g/min and $Vgt^n = 0.25 \pm 0.01$ µmol/g/min.

2. The variation of the glial glutamate pool size indicated an optimum of the fitting error function at around 0.7 µmol/g (fig.2), while a value above 1.2 µmol/g gave a significant increase in the fit residual (20 %), visible on the simulated curves by an overestimation of the GluC4 IE and GluC3 IE and underestimation of GlnC4 IE and GlnC3 IE at early time points (fig.3B).

3. The variation of the fluxes was analyzed for glial glutamate concentrations in the range of 10% χ^2 increase (~0.3 µmol/g to 1.1 µmol/g). The maximal variation on the metabolic fluxes was < 19% for Vnt and < 14% for all the other fluxes.

We conclude that the high SNR signal of $^{1}H\{^{13}C\}$ MRS following [2-^{13}C]acetate infusion coupled with two-compartment modeling gives precise information about brain metabolic fluxes. Separation of GluC3 and GlnC3 enables a higher precision on the transmitochondrial fluxes Vx^g and Vx^n. The error function of the fit supports the presence of glial glutamate pool smaller than 1.2 µmol/g. Variations of the assumed glial glutamate concentration around 0.7 µmol/g don't modify drastically the fitted flux values.

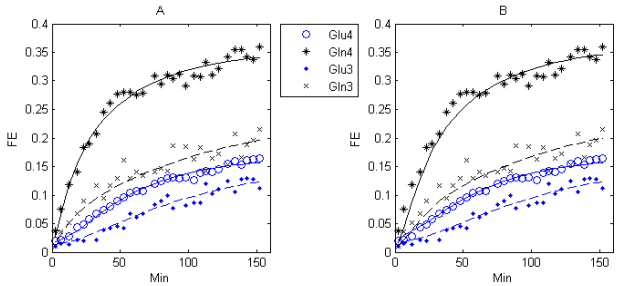

Fig.3: *Fit of GluC4, GlnC4, GluC3 and GlnC3 (A) with [glial glutamate]=0.5 µmol/g and (B) [glial glutamate]=1.2 µmol/g*

References: **1.** V Lebon, et al. J. Neurosci. 22(5):1523 (2002) **2.** L Xin et al. *Proc. Intl. Soc. Mag. Reson. Med.* 17: 130 (2009) **3.** P-G Henry et al. *Magn. Reson. Imaging* 24: 527 (2006) **4.** G Mason, et al. *J. Cereb. Blood Flow Metab.* 12(3):434 (1992) **5.** K Uffmann, et al. J. Neurosci. Res. 85(13):3304 (2007)

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