Precision evolution of the neuroglial metabolic fluxes with the experimental conditions, when using two-compartment modeling applied to [2-^{13}C] acetate dynamic MRS studies

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Introduction:

One or Two-compartment metabolic modeling applied to brain ^{13}C MRS is a choice method to determine non-invasively the local TCA-cycle and neurotransmission fluxes[1,2]. However, the accuracy and precision of the fitted metabolic fluxes obtained with these models, depending on the infused substrate, is still a subject of debate. Previous studies [3] demonstrated for example that the apparent neurotransmission flux Vnt might be unreliable when determined from the two-compartment model applied to [1-^{13}C] or [1,6-^{13}C] glucose infusion studies, depending on the experimental conditions and the resulting data quality. Recently [4,5], acetate infusion studies coupled with two-compartment modeling proved to be a good alternative to measure reliably the glial TCA cycle fluxes and the neurotransmission. The increase in the precision of the fluxes when measuring separately GluC3 and GlnC3 instead of GlxC3 was already analyzed [5]. This study, based on the typical data obtained from ^{1}H{^{13}C} MRS during [2-^{13}C] acetate infusion in rats, uses Monte-Carlo simulation to investigate the evolution of the precision of the fluxes depending :

a. on the duration of the experiment
b. on the time resolution of the data
c. on the noise level of the data.

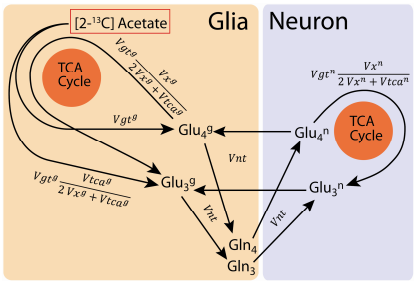

Fig.1: *Metabolic model for [2-^{13}C]acetate brain metabolism*

Materials and methods:

Metabolic fluxes and total glutamate and glutamine concentrations values were assumed as determined previously from ^{1}H{^{13}C} MRS [5] as being Vtca^{g}=0.13, Vx^{g}=0.16, Vnt=0.20, Vtca^{n}=0.73, Vx^{n}=0.38 µmol/g/min, [Gln]=3.5 and [Glu]= 11.5 µmol/g, from which 0.5 µmol/g was assumed to be in the glial compartment. Based on the experimental data, a typical noise level σ=0.05 µmol/g was assumed for the GluC4 and GlnC4 curves and σ=0.1 µmol/g for GluC3 and GlnC3. The typical experiment duration was fixed to 150 min with a time resolution of 5 minutes. The model (**Fig.1**) was implemented in Matlab to generate synthetic ^{13}C turnover curves for GluC4, GluC3, GlnC4 and GlnC3 to which Gaussian noise with corresponding standard deviation was added. Starting from the initial experimental parameters, Monte-Carlo simulations (using in each case 100 artificial curves) were carried out to analyze the evolution of the uncertainty on the metabolic fluxes, varying either the length of the experiment (from 60 to 450min), the time resolution (from 1.5 to 30 minutes) or by varying the noise level (from 0.2 to 5 times the original noise). A modified Levenberg-Marquardt nonlinear regression method, weighted with the inverse of the variance of the experimental noise was used to fit the data.

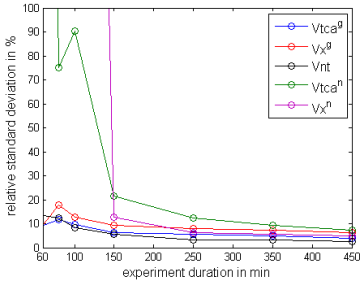

Fig.2A: *Relative standard deviation of the fluxes as a function of the experiment duration*

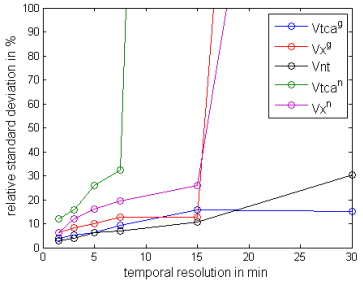

Fig.2B: *Relative standard deviation of the fluxes as a function of the temporal resolution*

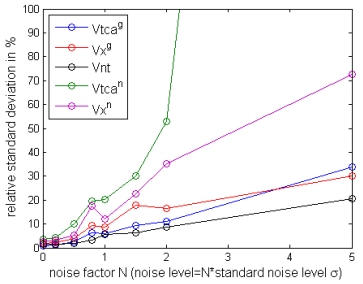

Fig.2C: *Relative standard deviation of the fluxes as a function of the noise factor N. The noise level σ is equal to N times the original noise level (σ=0.1 µmol/g for GluC4 and GlnC4 and σ=0.05 µmol/g for GluC3 and GlnC3)*

Results and Discussion:

a. When varying the experiment duration (**Fig.2A**), a critical point is found around 150 minutes. Below this point, the standard deviations of the neuronal TCA cycle fluxes are diverging, while the determination of the glial and neurotransmission fluxes remains possible, with an uncertainty below 20%. After 150 minutes, the uncertainties are slowly evolving, with a maximal improvement for the neuronal fluxes.
b. The temporal resolution is also affecting predominantly the neuronal fluxes as well as Vx^{g} (**Fig.2B**), which start to diverge with temporal resolution above 7.5 minutes. Vx^{g} is indeed depending essentially on the shape of the beginning of the GluC3 and GlnC3 enrichment curves and cannot be determined if this shape is not well characterized.
c. An increase in the noise level (**Fig.2C**) over a factor 2, corresponding to σ=0.1 µmol/g for GluC4 and GlnC4 and σ=0.2 µmol/g for GluC3 and GlnC3 makes the quantification of the neuronal parameters unreliable, while Vnt and the glial fluxes remain reasonably precisely determined.

We conclude that two-compartment modeling of acetate metabolism applied to ^{1}H{^{13}C} MRS data is a precise method to derive glial and neurotransmission fluxes in less than 200 minutes. Contrary to ^{13}C glucose studies, Vnt is very robustly defined and hardly dependent on experimental conditions. A temporal resolution of about 5 minutes should be used to determine precisely the transmitochondrial fluxes. Since time resolution and SNR are coupled, a suggested way would be to use the best time resolution still enabling a noise level around 0.1 µmol/g. Special care should be taken when reporting the neuronal fluxes, which are strongly dependent on the three experimental parameters analyzed in this study.

References: **1.** R de Graaf, et al. *NMR Biomed*. 16:339 (2003) **2.** P-G Henry et al. *Magn. Reson. Imaging* 24(4):527 (2006) **2.** A Shestov et al. *J. Neurosci. Res*. 85:3294 (2007) **4.** A Patel, et al. *J. Cereb. Blood Flow Metab.* 30(6):1200 (2010) **5.** B Lanz, et al. *Proc. Intl. Soc. Mag. Reson. Med.* 18: 319 (2010)

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