Supplementary Information

Metabolic Model for [1,6-$^{13}C_2$]glucose metabolism in the brain

The differential equations of the mathematical model were adapted from previously published model[1]. Main adaptations are consequent to the unavailability of glucose (Glc) and the addition of a glial-specific dilution flux (V_{dil}). Mass-balance equations and isotope balance equations reflect the two-compartmental scheme for glucose metabolism shown in Fig.1A under metabolic steady-state conditions. It is now well established that cerebral metabolism is highly compartmentalized between neurons and glial cells with two distinct TCA cycle processes[2,3]. The presence of specific enzymes in each respective cell population determines the metabolic specialization of the cells, which are however highly interconnected. Thus, brain functioning relies on a continuous interaction of glial cells and neurons, supporting energetic metabolism and neurotransmission.

Metabolite pool sizes are considered to be stable over time during this type of infusion protocol and determined before glucose infusion, based on ^{1}H Magnetic Resonance Spectroscopy (MRS) quantification in the same voxel as for the ^{13}C MRS. Neuronal glutamate (Glu) is fixed at 90% of the total Glu pool size, while glutamine is considered entirely glial. These assumptions are in accordance with data collected *in vitro*[4,5] and with previous modelling studies performed on *ex vivo* samples[6]. For very small fractions of neural glutamine (0.033-0.5) Gruetter et al.[1] did not observe any variation on the estimated fluxes within the calculated errors. To avoid a possible underestimated measurement of the Gln pool, as pointed out in previous studies[7,8], the Gln pool size in the brain was indirectly determined by the relative fractional enrichments (FEs) at steady-state as previously described[8], leading to an estimated Gln concentration of 5.5 ± 0.7 µmol/g (mean ± SD), which was comparable to values reported previously for the striatum[6,9]. An aspartate pool (1.1 ± 0.4 µmol/g, mean ± SD) is modelled in the neuronal compartment solely, in accordance with immunohistochemical analysis in the rodent brain, which finds co-localization between Asp and Glu in presynaptic vesicles[10]. Glucose passing through the BBB and reaching the tissue compartment is metabolized through glycolysis and supplies mostly (around 97%)[1,11,12] oxidative metabolism, whereas the rest feeds non-oxidative pathways after conversion into lactate. ^{13}C labelling is diluted in the brain lactate pool due to exchange with blood lactate (V_{Ldil}) while a small lactate efflux accounts for net contribution to non-oxidative pathways (V_{out}). The dilution is usually modelled as a symmetric exchange between the blood and the intracellular Lac ($V_{Ldil} \approx V_{out}$) when the brain is at rest[1], while a considerable net efflux, that exceeds glucose oxidation is observed under intense neuronal activation[13]. Under these assumptions, CMR_{glc} is essentially arising from the glucose metabolized through TCA cycles ($CMR_{glc} \approx CMR_{glc(ox)}$), rather than from non-oxidative pathways since:

$$CMR_{glc} = CMR_{glc(ox)} + (V_{out} - V_{Ldil})/2 \quad (1)$$

and

$$CMR_{glc(ox)} = (V_{TCA}{}^{n} + V_{TCA}{}^{g} + V_{PC})/2 \quad (2)$$

More precisely, differences between $CMR_{glc(ox)}$ and total CMR_{glc} are estimated to 5% or less[11] in resting conditions, confirmed by discrepancies between arterial-venous concentrations of oxygen and glucose beyond the stoichiometric relation given by glucose oxidation reaction[12].

This assumption is included in the equations at the lactate pool level:

$$\frac{d^{13}Lac_3 / Pyr_3}{dt} = 2 \cdot CMR_{glc} \cdot AIF - \left(2 \cdot CMR_{glc(ox)} + V_{out}\right)\frac{^{13}Lac_3 / Pyr_3(t)}{Lac} \quad (3)$$

where $CMR_{glc(ox)} = 0.97\ CMR_{glc}$ and AIF arterial input function for Glc FE, simulated as a step function reaching 70%. V_{out} represents the dilution due to glucose efflux to non-oxidative pathways as penthose phosphate pathway and other dilutions at the level of the lactate pool. Lactate labelling flowing through $CMR_{glc(ox)}$ is then distributed between neuronal and glial compartment.

The pyruvate and lactate pools can also be assumed kinetically equivalent with identical FE, since their exchange through lactate dehydrogenase is fast compared to the other metabolic reactions involved[14,15]:

$$\frac{^{13}Lac_3(t)}{Lac} = \frac{^{13}Pyr_3(t)}{Pyr} \quad (4)$$

Neuronal compartment

The neuronal compartment is characterized by the differential equations for its TCA cycle intermediates:

$$\frac{d^{13}OG_4^n}{dt} = V_{TCA}^n \frac{^{13}Pyr_3(t)}{Pyr} - \left(V_{TCA}^n + V_x\right)\frac{^{13}OG_4^n(t)}{OG^n} + V_x \frac{^{13}Glu_4^n(t)}{Glu^n} \quad (5)$$

$$\frac{d^{13}OG_3^n}{dt} = V_{TCA}^n \frac{^{13}OAA_2^n(t)}{OAA^n} - \left(V_{TCA}^n + V_x\right)\frac{^{13}OG_3^n(t)}{OG^n} + V_x \frac{^{13}Glu_3^n(t)}{Glu^n} \quad (6)$$

$$\frac{d^{13}OG_2^n}{dt} = V_{TCA}^n \frac{^{13}OAA_3^n(t)}{OAA^n} - \left(V_{TCA}^n + V_x\right)\frac{^{13}OG_2^n(t)}{OG^n} + V_x \frac{^{13}Glu_2^n(t)}{Glu^n} \quad (7)$$

$$\frac{d^{13}OAA_2^n}{dt} = \frac{V_{TCA}^n}{2}\frac{\left(^{13}OG_3^n(t) + ^{13}OG_4^n(t)\right)}{OG^n} - \left(V_{TCA}^n + V_x\right)\frac{^{13}OAA_2^n(t)}{OAA^n} + V_x \frac{^{13}Asp_2(t)}{Asp} \quad (8)$$

$$\frac{d^{13}OAA_3^n}{dt} = \frac{V_{TCA}^n}{2}\frac{\left(^{13}OG_3^n(t) + ^{13}OG_4^n(t)\right)}{OG^n} - \left(V_{TCA}^n + V_x\right)\frac{^{13}OAA_3^n(t)}{OAA^n} + V_x \frac{^{13}Asp_3(t)}{Asp} \quad (9)$$

Differential equations for glutamate and aspartate are defined as follows:

$$\frac{d^{13}Glu_i^n}{dt} = V_x \frac{^{13}OG_i^n(t)}{OG^n} - \left(V_{NT} + V_x\right)\frac{^{13}Glu_i^n(t)}{Glu^n} + V_{NT} \frac{^{13}Gln_i(t)}{Gln} \qquad i=2,3,4 \quad (10)$$

$$\frac{d^{13}Asp_i}{dt} = V_x\left(\frac{^{13}OAA_i^n(t)}{OAA^n} - \frac{^{13}Asp_i(t)}{Asp}\right) \qquad i=2,3 \quad (11)$$

Glial compartment

The glial compartment differs by a specific dilution flux at the level of the Acetyl-CoA (V_{dil}^{g}), glutamine synthesis (V_{GS}) and pyruvate carboxylase activity (V_{PC}) that is responsible for the replenishment of TCA cycle intermediates. V_{dil}^{g} accounts for the selective entering of glial-specific substrates (acetate, fatty acids and ketone bodies) in the compartment and is expressed as an inflow of unlabeled carbons to the Acetyl-CoA pool:

$$\frac{d^{13}AcCoA_2^g}{dt} = \left(V_g + V_{PC}\right)(1-dilG)\frac{^{13}Pyr_3(t)}{Pyr} - \left(V_g + V_{PC}\right)\frac{^{13}AcCoA_2^g(t)}{AcCoA^g} \tag{12}$$

where *(1-dilG)* expresses the reduced fraction of labelling entering the glial pool due to dilution from glial-specific substrates. We can therefore define the glial dilution flux V_{dil}^{g} as $\frac{\left(V_g + V_{PC}\right)}{\left(1-dilG\right)} \cdot dilG$.

Differential equations for TCA cycle intermediates in the glial compartments are defined as follows:

$$\frac{d^{13}OG_4^g}{dt} = \left(V_g + V_{PC}\right)\frac{^{13}AcCoA_2(t)}{AcCoA^g} - \left(V_g + V_{PC} + V_x\right)\frac{^{13}OG_4^g(t)}{OG^g} + V_x\,\frac{^{13}Glu_4^g(t)}{Glu^g} \tag{13}$$

$$\frac{d^{13}OG_3^g}{dt} = \left(V_g + V_{PC}\right)\frac{^{13}OAA_2^g(t)}{OAA^g} - \left(V_g + V_{PC} + V_x\right)\frac{^{13}OG_3^g(t)}{OG^g} + V_x\,\frac{^{13}Glu_3^g(t)}{Glu^g} \tag{14}$$

$$\frac{d^{13}OG_2^g}{dt} = \left(V_g + V_{PC}\right)\frac{^{13}OAA_3^g(t)}{OAA^g} - \left(V_g + V_{PC} + V_x\right)\frac{^{13}OG_2^g(t)}{OG^g} + V_x\,\frac{^{13}Glu_2^g(t)}{Glu^g} \tag{15}$$

$$\frac{d^{13}OAA_2^g}{dt} = \frac{V_g}{2}\frac{\left(^{13}OG_3^g(t) + ^{13}OG_4^g(t)\right)}{OG^g} - \left(V_g + V_{PC} + V_x\right)\frac{^{13}OAA_2^g(t)}{OAA^g} \tag{16}$$

$$\frac{d^{13}OAA_3^g}{dt} = \frac{V_g}{2}\frac{\left(^{13}OG_3^g(t) + ^{13}OG_4^g(t)\right)}{OG^g} - \left(V_g + V_{PC} + V_x\right)\frac{^{13}OAA_3^g(t)}{OAA^g} + V_{PC}\,\frac{^{13}Pyr_3(t)}{Pyr} \tag{17}$$

The dynamics of ^{13}C concentration of glutamate and glutamine in the glial compartment is determined from the equations below, similarly for each carbon position:

$$\frac{d^{13}Glu_i^g}{dt} = \left(V_x + V_{PC}\right)\frac{^{13}OG_i^g(t)}{OG^g} - \left(V_{GS} + V_x\right)\frac{^{13}Glu_i^g(t)}{Glu^g} + V_{NT}\,\frac{^{13}Glu_i^n(t)}{Glu^n} \qquad i=2,3,4 \tag{18}$$

$$\frac{d^{13}Gln_i}{dt} = V_{GS}\,\frac{^{13}Glu_i^g(t)}{Glu^g} - \left(V_{efflux} + V_{NT}\right)\frac{^{13}Gln_i(t)}{Gln} \qquad i=2,3,4 \tag{19}$$

Where $V_{GS} = V_{NT} + V_{PC}$ at metabolic steady state.

Removing TCA cycle intermediates from the model

The set of differential equations was then simplified as already shown in the literature[7,16] and reduced to 12 equations by eliminating the terms referring to the non-measurable TCA cycle intermediates that are enriched in much lower concentration levels compared to metabolites such as glutamate and glutamine.

For example, algebraically adding the equation for $\frac{d^{13}Glu_4^n}{dt}$ (Eq.10, *i=4*) to $\frac{V_x}{V_{TCA}^n + V_x}\frac{d^{13}OG_4^n}{dt}$ will originate the expression:

$$\frac{d^{13}Glu_4^n}{dt} + \frac{V_x}{V_{TCA}^n + V_x}\frac{d^{13}OG_4^n}{dt} = \frac{V_x V_{TCA}^n}{V_{TCA}^n + V_x}\frac{^{13}Pyr_3(t)}{Pyr} - \left(\frac{V_x V_{TCA}^n}{V_{TCA}^n + V_x} + V_{NT}\right)\frac{^{13}Glu_4^n(t)}{Glu^n} + V_{NT}\frac{^{13}Gln_4(t)}{Gln} \qquad (20)$$

Since the pool size of TCA cycle intermediates is much smaller than those of glutamate, the variation over time of their respective labeled form follows a similar relationship. Therefore we can assume that $\frac{d^{13}Glu_4^n}{dt} >> \frac{d^{13}OG_4^n}{dt}$ and neglect the latter as follows:

$$\frac{d^{13}Glu_4^n}{dt} = \frac{V_x V_{TCA}^n}{V_{TCA}^n + V_x}\frac{^{13}Pyr_3(t)}{Pyr} - \left(\frac{V_x V_{TCA}^n}{V_{TCA}^n + V_x} + V_{NT}\right)\frac{^{13}Glu_4^n(t)}{Glu^n} + V_{NT}\frac{^{13}Gln_4(t)}{Gln} \qquad (21)$$

By applying a similar procedure to the positions 3 of glutamate in the neuronal compartment (Eq.10, *i=3*) a term of the TCA cycle intermediate OAA_2^n will appear. This can be eliminated by using the differential equation for aspartate at the position C2. A similar approach is applied to the equation for $\frac{d^{13}Glu_2^n}{dt}$ (Eq.10, *i=2*) using the equation for $\frac{d^{13}Asp_3}{dt}$ (Eq.11, *i=3*). The following equations will result from the aforementioned procedure:

$$\frac{d^{13}Glu_3^n}{dt} = \frac{V_{TCA}^n}{V_{TCA}^n + V_x}\left(\frac{d^{13}Asp_2}{dt} + V_x\frac{^{13}Asp_2(t)}{Asp}\right) - \left(\frac{V_x V_{TCA}^n}{V_{TCA}^n + V_x} + V_{NT}\right)\frac{^{13}Glu_3^n(t)}{Glu^n} + V_{NT}\frac{^{13}Gln_3(t)}{Gln} \qquad (22)$$

$$\frac{d^{13}Glu_2^n}{dt} = \frac{V_{TCA}^n}{V_{TCA}^n + V_x}\left(\frac{d^{13}Asp_3}{dt} + V_x\frac{^{13}Asp_3(t)}{Asp}\right) - \left(\frac{V_x V_{TCA}^n}{V_{TCA}^n + V_x} + V_{NT}\right)\frac{^{13}Glu_2^n(t)}{Glu^n} + V_{NT}\frac{^{13}Gln_2(t)}{Gln} \qquad (23)$$

Again, proceeding by substitution, the equation for aspartate labelling at the positions 2 and 3 can be obtained in the following form:

$$\frac{d^{13}Asp_i}{dt} = \frac{V_{TCA}^n}{2\left(V_{TCA}^n + V_x\right)}\left(\frac{d^{13}Glu_4^n}{dt} + \frac{d^{13}Glu_3^n}{dt} + \left(V_{NT} + V_x\right)\frac{^{13}Glu_4^n(t) + ^{13}Glu_3^n(t)}{Glu^n} - V_{NT}\frac{^{13}Gln_4(t) + ^{13}Gln_3(t)}{Gln}\right) \qquad i=2,3 \qquad (24)$$
$$- \frac{V_x V_{TCA}^n}{V_{TCA}^n + V_x}\frac{^{13}Asp_i(t)}{Asp}$$

where the equations for $\frac{d^{13}Glu_4^n}{dt}$ (Eq.10, *i=4*) and $\frac{d^{13}Glu_3^n}{dt}$ (Eq.10, *i=3*) without terms from TCA cycle intermediates can be used to give the explicit form.

Equation for glial glutamate can be derived by summing $\frac{d^{13}Glu_4^g}{dt}$ (Eq.18, *i=4*) to $\frac{V_x+V_{PC}}{V_g+V_x+V_{PC}}\frac{d^{13}OG_4^g}{dt}$ in order to eliminate the terms $\frac{^{13}OG_4^g(t)}{OG_4^g}$:

$$\frac{d^{13}Glu_4^g}{dt}+\frac{V_x+V_{PC}}{V_g+V_x+V_{PC}}\frac{d^{13}OG_4^g}{dt}=-(V_{GS}+V_x)\frac{^{13}Glu_4^g(t)}{Glu^g}+V_{NT}\frac{^{13}Glu_4^n(t)}{Glu^n}+\frac{(V_x+V_{PC})(V_g+V_{PC})}{V_g+V_x+V_{PC}}\frac{^{13}AcCoA_2^g(t)}{AcCoA^g}+\frac{V_x(V_x+V_{PC})}{V_g+V_x+V_{PC}}\frac{^{13}Glu_4^g(t)}{Glu^g} \quad (25)$$

The term $\frac{V_x+V_{PC}}{V_g+V_x+V_{PC}}\frac{d^{13}OG_4^g}{dt}$ is negligible in the small pool approximation, while the rest of the equation can be simplified by adding the equation for the acetyl-CoA in the form $\frac{(V_x+V_{PC})}{(V_g+V_x+V_{PC})}\frac{d^{13}AcCoA_2^g}{dt}$:

$$\frac{d^{13}Glu_4^g}{dt}+\frac{(V_x+V_{PC})}{(V_g+V_x+V_{PC})}\frac{d^{13}AcCoA_2^g}{dt}=-(V_{GS}+V_x)\frac{^{13}Glu_4^g(t)}{Glu^g}+V_{NT}\frac{^{13}Glu_4^n(t)}{Glu^n}+\frac{V_x(V_x+V_{PC})}{V_g+V_x+V_{PC}}\frac{^{13}Glu_4^g(t)}{Glu^g}+\frac{(V_x+V_{PC})(V_g+V_{PC})(1-dilG)}{(V_g+V_x+V_{PC})}\frac{^{13}Pyr_3(t)}{Pyr} \quad (26)$$

After neglecting the term $\frac{(V_x+V_{PC})}{(V_g+V_x+V_{PC})}\frac{d^{13}AcCoA_2^g}{dt}$ the equation can be simplified to the following expression:

$$\frac{d^{13}Glu_4^g}{dt}=-V_{NT}\frac{^{13}Glu_4^g(t)}{Glu^g}+V_{NT}\frac{^{13}Glu_4^n(t)}{Glu^n}-V_{gt}\frac{^{13}Glu_4^g(t)}{Glu^g}+V_{gt}(1-dilG)\frac{^{13}Pyr_3(t)}{Pyr} \quad (27)$$

where

$$V_{gt}=\frac{(V_g+V_{PC})(V_x+V_{PC})}{(V_g+V_x+V_{PC})} \quad (28)$$

is a composite flux, the apparent glutamate turnover flux, that includes contribution from TCA cycle, transmitochondrial flux and pyruvate carboxylase activity[16–18].

A similar approach originates the following equations for the positions 3 and 2 of glial glutamate:

$$\frac{d^{13}Glu_3^g}{dt}=\frac{V_{gt}V_g}{2V_x+2V_{PC}+V_g}(1-dilG)\frac{^{13}Pyr_3(t)}{Pyr}-\frac{(V_x+V_{PC})V_gV_x}{(2V_x+2V_{PC}+V_g)(V_x+V_{PC}+V_g)}\frac{^{13}Glu_4^g(t)}{Glu^g}+\frac{(V_x+V_{PC})\left(V_{PC}+\frac{V_g}{2}\right)}{\left(V_x+V_{PC}+\frac{V_{TCA}}{2}\right)}\frac{^{13}Glu_3^g(t)}{Glu^g}+V_{NT}\frac{^{13}Glu_3^n(t)}{Glu^n}-V_{NT}\frac{^{13}Glu_3^g(t)}{Glu^g} \quad (29)$$

$$\frac{d^{13}Glu_2^g}{dt} = \frac{V_{gt}V_g}{2\left(V_x+V_{PC}+V_g\right)}\frac{V_{gt}V_g^2}{2\left(V_x+V_{PC}+V_g\right)\left(2V_x+2V_{PC}+V_g\right)}\left(1-dilG\right)\frac{^{13}Pyr_3(t)}{Pyr} + \frac{V_{PC}\left(V_x+V_{PC}\right)}{\left(V_x+V_{PC}+V_g\right)}\frac{^{13}Pyr_3(t)}{Pyr} +$$
$$-\left[\frac{\left(V_x+V_{PC}\right)V_gV_x}{2\left(V_x+V_{PC}+V_g\right)^2}+\frac{\left(V_x+V_{PC}\right)V_g^2V_x}{2\left(V_x+V_{PC}+V_g\right)^2\left(2V_x+2V_{PC}+V_g\right)}\right]\frac{^{13}Glu_4^g(t)}{Glu^g} + \quad (30)$$
$$-\frac{V_xV_g\left(V_x+V_{PC}\right)}{2\left(V_x+V_{PC}+V_g\right)\left(V_x+V_{PC}+\frac{V_g}{2}\right)}\frac{^{13}Glu_3^g(t)}{Glu^g} - V_{gt}\frac{^{13}Glu_2^g(t)}{Glu^g} + V_{NT}\frac{^{13}Glu_2^n(t)}{Glu^n} - V_{NT}\frac{^{13}Glu_2^g(t)}{Glu^g}$$

^{13}C Spectra normalization

For each experiment the FE at the position C3 of Glu (FEGluC3) was determined from the ratio between the total concentration of GluC3 determined at steady-state and the total Glu concentration derived from the quantification of ^{1}H spectrum:

$$FEGluC3 = \frac{GluC3_{ST-ST}}{\left[Glu\right]_{1H}} \quad (31)$$

The ^{13}C concentration of GluC3 can be seen as the product between the raw concentration derived from LCModel in arbitrary units prior to normalization ($GluC3_{raw}$) and the normalization factor (k_{norm}):

$$FEGluC3 = \frac{k_{norm}GluC3_{raw}}{\left[Glu\right]_{1H}} \quad (32)$$

Hence the definition of k_{norm} as:

$$k_{norm} = \frac{FEGluC3\cdot\left[Glu\right]_{1H}}{GluC3_{raw}} \quad (33)$$

FEGluC3 can be derived from the ratio between the doublet of the resonance C4 of Glu (D43) and the sum of the C4 doublet and the corresponding singlet (S4):

$$FEGluC3 = \frac{GluC3}{\left[Glu\right]} = \left[\frac{D43}{D43+S4}\right]_{GluC4} \quad (34)$$

This is possible because metabolism of [1,6-$^{13}C_2$] glucose generates no labelling in GluC5 and only neighbouring carbons generate *in vivo* measurable ^{13}C-^{13}C J-splitting. The GluC4 resonance is therefore only split into a doublet by the presence of ^{13}C in position C3 within the same Glu molecule. This reflects directly the fraction of Glu molecules labelled in position C3, since the labelling at the position C3 will occur with the same probability with and without labelling at the position C4 in the same molecule.

By combining equation 3 and 4 we obtain:

$$k_{norm} = \frac{\left[Glu\right]_{1H}}{GluC3_{raw}}\left[\frac{D43}{D43+S4}\right]_{GluC4} \quad (35)$$

k_{norm} can then be used to scale the measured ^{13}C peak amplitudes into concentrations, after having corrected the amplitude values of each resonance for the corresponding RF efficiency of the ISIS-DEPT sequence, based on phantom measurements (see Materials and Methods).

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