# Simultaneous Localized in vivo $^{1}H$ and $^{15}N$ MRS of Glutamine Synthesis in the hyperammonaemic rat brain

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**Introduction:**

An alternative approach to $^{13}C$ MRS for studying glutamate and glutamine metabolism is $^{15}N$ MRS during infusion of $^{15}N$ labeled ammonia. Ammonia is metabolized to glutamine by glutamine synthetase (GS) in astrocytes. Consequently, the activity of cerebral glutamine synthetase in vivo under hyperammonaemia conditions may help understand the mechanism of ammonia toxicity and could provide further insight into the Gln-Glu cycle. Previous NMR studies on cerebral GS under ammonia infusion used either in vivo $^{1}H$ or unlocalized $^{15}N$ spectroscopy (1-3) and all used in vitro brain extracts for absolute quantification. Therefore, the goal of this study was to use in vivo localized $^{15}N$ MRS interleaved with in vivo $^{1}H$ MRS to measure the glutamine synthesis rate under ammonia infusion in the rat brain and to perform a direct absolute quantification of total Gln and 5-$^{15}N$ Gln in the same experiment.

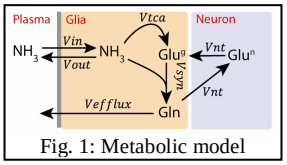


Fig. 1: Metabolic model

**Methods:**

Five SD rats (300-350g) were fasted overnight before the experiment. The femoral artery and vein were catheterized for blood sampling (monitoring blood gases, pH and plasma ammonia levels), as well as $^{15}N$ ammonium chloride (99%-enriched) and α-chloralose infusions. The rats were artificially ventilated. After giving a bolus over 1 min (3), $^{15}N$ ammonium chloride was then infused continuously at a stable rate (4.5mmol/h/kg) for up to 10h. The plasma ammonia concentration increased to 0.95±0.08mmol (Analox GM7 analyzer) and was constant during the experiment. All the $^{1}H$ and $^{15}N$ MRS data were acquired interleaved on a 9.4T system (Varian/Magnex Scientific) using a home-built quadrature $^{1}H$ coil combined with a single 5-loop 10 mm $^{15}N$ coil. The $^{1}H$ spectra were acquired using an ultra-short-TE localized SPECIAL spectroscopy sequence (TE=2.8ms, TR=4s, 160 scans, VOI=5x7x7mm$^3$) (4). Shimming was performed with FASTMAP. The $^{15}N$ spectra were acquired using the SIRENE sequence (5). Unlocalized spectra were acquired in the first hours of infusion (256 scans, TR=5s), followed by a localized spectrum (VOI=7x10x10mm$^3$, 512 averages) used for quantification. $^{1}H$ spectra were quantified using LCModel and the water signal as an internal reference. The $^{15}N$ spectra were quantified using AMARES and an external reference method described previously (6).The metabolic model used to analyze the total Gln and 5-$^{15}N$ labeled Gln time courses is shown on Fig 1. Assuming a negligible Gln efflux (Vefflux) (1), the linear fit of the time-evolution of the total Gln gives the net synthesis flux (Vsyn-Vnt) as well as the initial Gln concentration (Gln(0)). The time-evolution of 5-$^{15}N$ labeled Gln follows the Eq (1):

$$[5\text{-}^{15}\mathrm{N}]\mathrm{Gln}(t) = FE_{plasma}(NH_3)\,[\mathrm{Gln}(0) + (\mathrm{Vsyn} - \mathrm{Vnt})t]\left\{1 - \left(\frac{\mathrm{Gln}(0) + (\mathrm{Vsyn} - \mathrm{Vnt})t}{\mathrm{Gln}(0)}\right)^{\frac{Vin\,Vsyn}{(Vin+Vsyn-(Vsyn-Vnt))(Vsyn-Vnt)}}\right\}$$

where Gln(0) and (Vsyn-Vnt) are determined from the $^{1}H$ data. The last exponent and the plasma $NH_3$ fractional enrichment (FE) were fitted. The value of the fitted exponent cannot give separate information on Vin and Vsyn. Considering a brain uptake index (BUI) of 0.24 for $NH_3$ (7), an average blood flow (BF) of 1 ml/g/min and the measured plasma $NH_3$ concentration of 1mM, Vin = BUI*BF*[$NH_3$] = 0.24 µmol/g/min was used.

**Results and Discussion:** The increase in the total Gln pool at different time points during infusion was apparent in the $^{1}H$ spectra (Fig. 2). The total Gln (0) concentration was 2.5±0.3 mmol/kg$_{ww}$, increasing to 15±3.3mmol/kg$_{ww}$ at the end of the infusion, which was in the range of previous studies (1-3). The total Glu concentrations remained unchanged during the experiment. Fig. 3 shows a series of in vivo unlocalized $^{15}N$ spectra acquired in the rat brain at different time points during infusion. The 5-$^{15}N$ Gln peak (-271ppm) was visible in the first and all subsequent scans, whereas the 2-$^{15}N$ Gln/Glu peak (-342ppm) was observed after about 1.5h. The time courses of total Gln and 5-$^{15}N$ Gln were highly reproducible in all five rats. The application of the model to the in vivo data shows an excellent fit (Fig. 4). Based on the model presented in Fig. 1 we obtained a net synthesis flux (Vsyn-Vnt) of 0.021±0.006µmol/min/g. By fitting the in vivo 5-$^{15}N$ Gln time course to Eq (1), the apparent glutamine synthesis rate, Vsyn, was 0.29±0.1µmol/min/g, and the plasma $NH_3$ FE was 71±6%. Finally, the apparent neurotransmission rate, Vnt, was 0.26±0.1µmol/min/g. While the apparent glutamine synthesis and neurotransmission rates were higher that previous unlocalized $^{15}N$ NMR studies, they are within the range of $^{13}C$ NMR measurements (8). We conclude that it is feasible to combine localized in vivo $^{15}N$ with $^{1}H$ MRS to measure the glutamine synthesis rate under ammonia infusion in the in vivo rat brain. This technique allows a robust absolute quantification of total Gln and 5-$^{15}N$ Gln in the same experiment. Moreover, in contrast to previous studies, the net synthesis flux (Vsyn-Vnt) was directly measured.

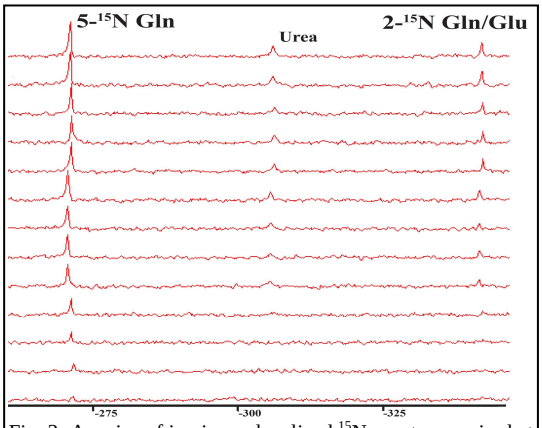


Fig. 3: A series of in vivo unlocalized $^{15}N$ spectra acquired at 9.4T in the rat brain at different time points (from bottom to top: 23, 47, 69, 115, 173, 193, 218, 321, 376, 398, 420, 463, 529min). The $^{15}N$ chemical shifts were referenced to nitromethane.

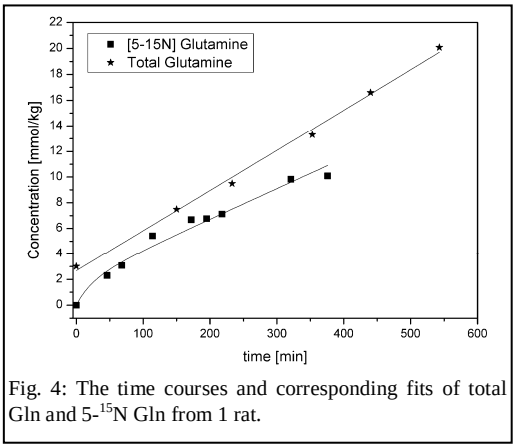


Fig. 4: The time courses and corresponding fits of total Gln and 5-$^{15}N$ Gln from 1 rat.

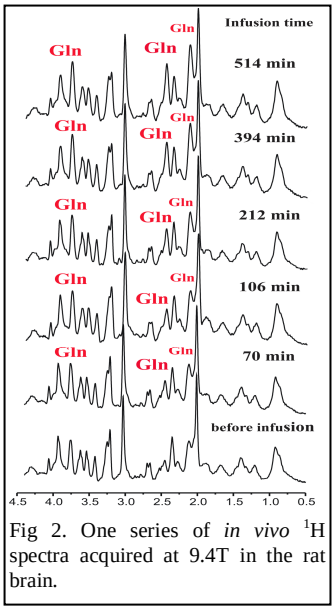


Fig 2. One series of *in vivo* $^{1}H$ spectra acquired at 9.4T in the rat brain.

**References:**

[1] Kanamori K et al., Biochem J 1993 ;293 :461. [2]Shen J et al., Dev Neurosci1998 ;20 :434. [3] Kanamori K et al., NMR Biomed 1993;6:21. [4] Mlynarik V et al., J Magn Reson 2008;194:163. [5] Choi IY et al., Magn Reson Med 2000 ;44 :387.[6] Gruetter R, et al., Magn Reson Med 1991;20:327. [7] Cooper AJL et al., J Biol Chem 1979;12:4982. [8] Sibson NR et al, Proc Natl Acad Sci 1997;94:2699.

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