In vivo ^{13}C NMR spectroscopy at 14.1 T

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Introduction: The combination of dynamic ^{13}C NMR spectroscopy with the infusion of ^{13}C-enriched substrates is a powerful method to probe metabolic fluxes *in vivo*. The increase in magnetic field opens the opportunity for gain in sensitivity and spectral resolution, which was now explored to study brain metabolism.

Methods: Male Sprague-Dawley rats (n=4, 282±12 g) were prepared and maintained during the NMR experiment as previously described [1]. Under α-chloralose anaesthesia, [1,6-^{13}C]glucose was infused to induce a step function in plasma fractional enrichment (FE) [2], while ^{13}C spectra were measured. Localized ^{1}H and ^{13}C NMR spectroscopy was performed on a 14.1 T, 26 cm VNMRS spectrometer (Varian, Magnex) with a coil consisting of a ^{1}H quadrature surface coil and a ^{13}C linearly polarized surface coil [2]. Field homogeneity was adjusted by FASTMAP [3]. The VOI size was 5x8x8 mm^3. Total metabolite concentrations were determined by ^{1}H NMR spectroscopy using SPECIAL [4]. ^{13}C NMR spectra were acquired using semi-adiabatic distortionless enhancement by polarization transfer (DEPT) combined with ^{1}H localization [2]. Both ^{1}H and ^{13}C spectra were quantified with LCModel [5].

Results: Excellent sensitivity was evident from the measured ^{13}C spectra. High spectral resolution allowed complete separation of C2 and C3 resonances of glutamate and glutamine as well as good observation of their multiplets resulting from homonuclear coupling in multiply labelled metabolites (Fig.1A). FE in aliphatic carbons of glutamate and glutamine were determined in 4 animals with high reproducibility (Fig.1B) which may increase accuracy in determination of cerebral metabolic fluxes in appropriate mathematical models. Multiplets were clearly observed and isotopomer analysis can be performed *in vivo* for different metabolites (Fig.1C), including glutamate, glutamine, aspartate, *N*-acetylaspartate (NAA) and GABA. We also observed that FE of glutamate and glutamine carbons could be accurately determined with 3 minutes of time resolution. However, there was increased uncertainty in the quantification of isotopomers in these conditions. Labelling incorporation into carbons of lower concentrated metabolites, like aspartate, glutathione (GSH) and GABA, could be reliably determined by reducing temporal resolution (Fig.1D). For example, here we show for the first time the direct detection of ^{13}C labelling incorporation into carbons of GABA with a time resolution of 25 minutes, only determined before by ^{1}H-[^{13}C] NMR spectroscopy or in brain extracts.

Conclusion: Direct detection of ^{13}C enrichment of cerebral metabolites was improved at 14.1 T with gain in sensitivity and especially in spectral resolution. Upon infusion of [1,6-^{13}C]glucose, numerous ^{13}C-enriched metabolites could be quantified over the entire time course. In addition, LCModel allowed maximizing the amount of information that can be extracted from ^{13}C spectra [5]. All this together will increment the reliability of complex mathematical models describing cerebral energy metabolism.

References: [1] Duarte *et al.* (2009) J Neurochem 111:368. [2] Henry *et al.* (2003) MRM 50:684. [3] Gruetter (1993) MRM 29:804. [4] Mlynárik *et al.* (2008) J Mag Reson 194:163. [5] Henry *et al.* (2003) NMR Biomed 16:400 [6] van Eijsden *et al.* (2010) J Neurochem 112:24.

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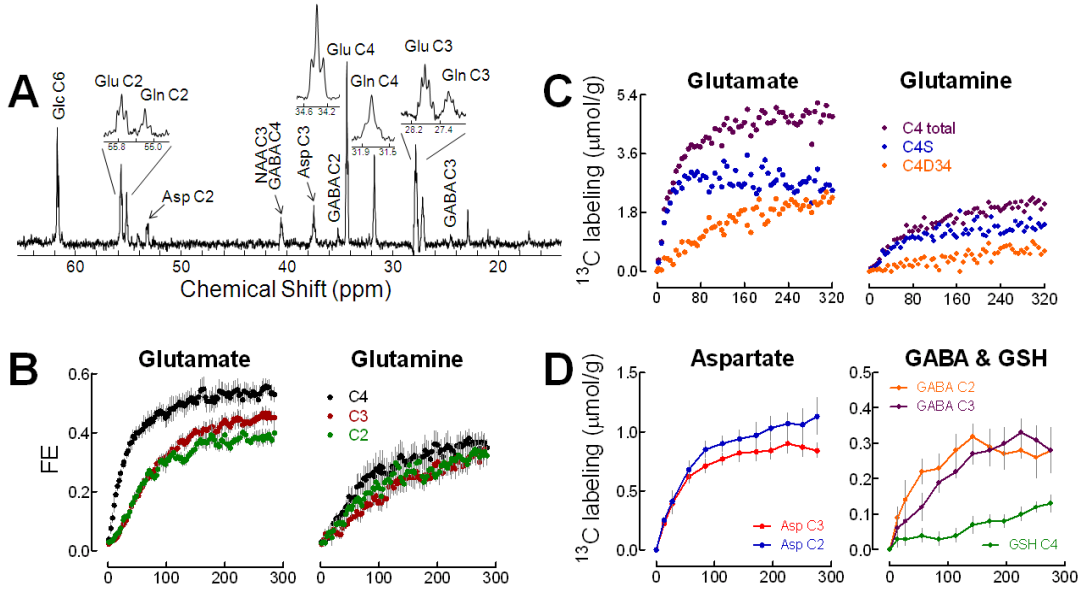

Figure 1. A) Typical *in vivo* ^{13}C NMR spectrum at 14.1 T acquired for 40 minutes after 4 hours of [1,6-^{13}C]glucose infusion (2 Hz Lorentzian line broadening). **B)** Fractional enrichment (FE) of glutamate and glutamine carbons (n=4). **C)** Time courses of individual isotopomers of glutamate and glutamine in a single experiment. **D)** Concentration of ^{13}C label in carbons of aspartate, GSH and GABA. Abscissas are time in minutes and data are mean±SEM.