Separation and precise estimation of glutamate and glutamine using spectroscopic imaging in human brain at 3.0 T

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TARGET AUDIENCE MR spectroscopists, psychiatrists.

PURPOSE Glutamate (Glu) is a key neurotransmitter of excitatory signaling and is a precursor for production of γ-Aminobutyric acid (GABA, an inhibitory neurotransmitter). Prior studies have indicated alterations in Glu levels in a variety of neurological diseases [1]. Several methods have reported Glu estimates using single voxel spectroscopy with minimal but not negligible contamination from glutamine (Gln) [2-3]. Other studies have reported either composite signal [4] or Gln [5] detection alone. We report for the first time in vivo ^{1}H spectroscopic imaging (SI) of both Glu and Gln signals in normal healthy volunteers at 3T. Absolute estimation and regional distribution mapping of Glu and Gln are reported. Reproducibility results from scan/re-scan are also presented.

METHODS Numerical optimization of PRESS TE was carried out for improving the differentiation of H4 resonances of Glu from Gln between 2.30 to 2.5 ppm. From these simulations we selected an echo time of 97 ms (TE_1 = 32ms and TE_2 = 65 ms), where the Glu signal was well separated from the Gln and GABA signals. At this echo time the largest Glu and Gln (H4 resonances) signals appear as peaks at 2.35 ppm and 2.45 ppm, respectively. PRESS volume localization with TE = 97 ms (TE_1 = 32ms and TE_2 = 65 ms) was used in the SI sequence to acquire *in vivo* data, with a TR of 1.2 sec, a spectral width of 2000 Hz and 1024 complex points per FID. Water signal was suppressed using a four-pulse scheme. The PRESS RF carrier was set to 2.6 ppm. The PRESS 90˚ and 180˚ pulses used in the 90 x 90 x 15 mm^3 volume localization had bandwidths of 4.2 kHz (9.8 ms) and 1.3 kHz (13.2 ms), respectively. The VOI covered most of the central brain region. Typically a 200 x 160 mm^2 field of view (FOV) in the phase encoding directions was used for acquisition with an in-plane resolution of 10 x 10 mm^2, and slice thickness of 15 mm along head-foot direction. Regional saturation bands were used to minimize extraneous signals from subcutaneous regions. Unsuppressed water data was also acquired for eddy current correction and quantification. High-resolution T_1w MPRAGE was acquired at isotropic resolution to perform segmentation and to calculate the gray and white matter fractions in individual voxels of the SI grid. The total scan time was 25 minutes. During post-processing residual water signal in the water suppressed data was removed using the HL-SVD filter of the JMRUI [6]. Frequency-drift and eddy current artifact corrections were performed using in-house Matlab programs. LCModel software [7] with basis-sets created using published chemical shift and coupling constants [8, 9] was used to estimate the metabolite concentrations. The absolute quantification of the metabolites was performed using Cr signal in gray matter dominant region at 8 mM. Six healthy volunteers were scanned twice to test the reliability and reproducibility of estimating major metabolites including Glu and Gln. Written informed consent was obtained from subjects prior to the scans.

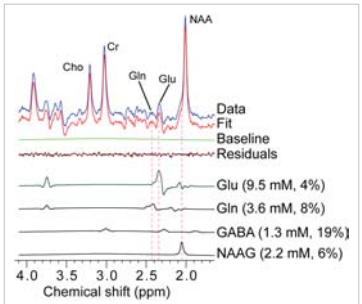

Fig. **1**: *In vivo* spectroscopic data from a healthy volunteer. A representative spectrum (blue) along with LCModel fit (red), residuals (brown) and metabolite signals Glu, Gln, GABA and NAAG from a gray matter dominant voxel.

RESULTS AND DISCUSSION Figure 1 shows a representative spectrum from gray matter dominant brain region with LCModel fit, residuals and metabolite signals of Glu, Gln, GABA and N-acetylaspartylglutamic acid (NAAG). Glu and Gln were estimated at 9.5 mM with CRLB of 4% and 3.6 mM with CRLB of 8%, respectively. NAAG and GABA signals were also resolved with CRLB's of 6% and 19%, respectively. Spectra 1-3 and 4-6 in figure 2 (**a** and **b**) show data from voxels in gray matter and white matter dominant regions of the brain, respectively, shown in the axial MPRAGE image (Fig. 2 **c**). The residuals of LCModel spectral fitting using the basis-sets without Glu, without Gln, and without Glu or Gln show elevated signals at 2.35 ppm and/or 2.45 ppm when compared to residuals of fitting using the basis-sets which included Glu and Gln (Fig. 2 **d** and **e**). The Gln estimates were higher (with smaller CRLB's) in gray matter dominant regions when compared to white matter dominant regions (with larger CRLB's, Tables **f** and **g** in Fig. 2), suggesting a possible regional variation of Gln in the brain. The gray matter fractions of these pixels are also shown in the table along with the metabolite CRLB's in parentheses. A similar regional dependence was observed in the residuals when fitting without the Gln signal at 2.45 ppm, which was relatively small in white matter dominant regions compared to gray matter dominant regions. Glu showed higher concentrations in gray matter dominant regions compared to white matter dominant regions, consistent with prior reports of Glu in healthy brain [10]. The NAAG concentration was higher in white matter dominant regions compared to gray matter dominant regions. Figure 3 shows the metabolite maps obtained from two scans in a healthy volunteer. The regional variation of metabolite concentrations was consistent between the two scans. The Glu and NAAG regional variations were consistent with prior studies [10-11]. Figure 4 shows plots comparing the metabolite estimates from the first scan to those from the second scan for all six subjects for Glu, Gln, Glu + Gln, choline (Cho), creatine (Cr), N-Acetylaspartic acid (NAA) and NAAG. The linear regression analysis of Glu, Gln and Glu + Gln gave a coefficient of determination (R^2) of 0.64, 0.64 and 0.72, respectively. The major metabolites singlet's Cho, Cr, and NAA showed larger R^2 values of 0.91, 0.80 and 0.87, respectively. NAAG gave an R^2 of 0.51. In conclusion, the present study shows separation and precise estimation of Glu and Gln at 3T in human brain and measures their regional variation.

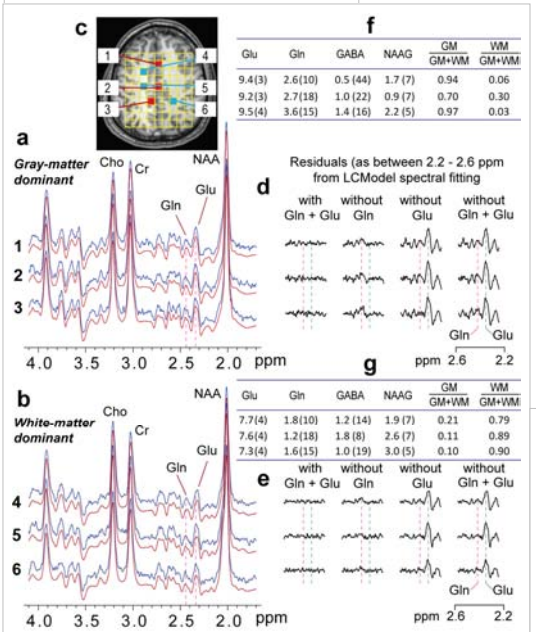

Fig. **2**: (**a**) and (**b**) Spectra (blue), LCModel fits (red) from six selected voxels (shown in the axial MPRAGE images, **c**). The residuals (black) of spectral fitting using the basis-sets with Glu and Gln, without Gln, without Glu and without Glu or Gln are shown in (**d**) and (**e**). The table (**f**) and (**g**) shows the Glu, Gln, GABA and NAAG concentrations (CRLB are shown in parentheses) along with the gray matter and white matter fractions. The gray matter and white matter fractions of each voxels are also

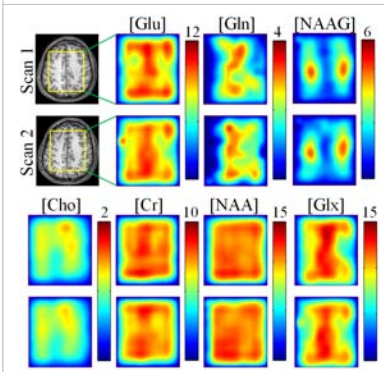

Fig. **3**: Metabolite concentration maps from a healthy volunteer brain in two different scans (values are shown in mM).

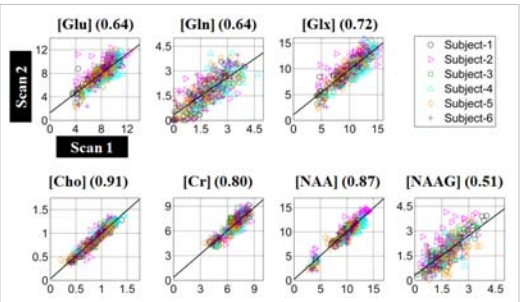

Fig. **4**: Reproducibility plots of various metabolite in six subjects (axes are in mM). The plot shows scan versus rescan metabolite estimates, the linear regression line of the data (black line). Each metabolite plot includes 826 voxel data points from six subjects. The coefficient of determination (R^2) is shown in the top.

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