In vivo spectroscopic imaging of N-acetyl-aspartyl-glutamate (NAAG) in human brain at 3.0 T: Reproducibility and regional variation study

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TARGET AUDIENCE: MR spectroscopists, psychiatrists, neurologists.

PURPOSE N-acetylaspartylglutamate (NAAG) is synthesized in the central nervous system, acts as a peptide neurotransmitter and is implicated in various neurological disease models[1,2]. *In vivo* measurement of NAAG singlet signal at 2.05 ppm is challenging due to its close proximity to N-acetyl aspartate (NAA) singlet signal at 2.01 ppm[3]. Previous studies have used increased spectral resolution at high fields[3,4] or spectral editing sequences to estimate NAAG levels with single voxel spectroscopy[5]. However such methods are not translatable in to routine clinical studies. Here we report the ^{1}H spectroscopic imaging (SI) of NAAG levels in human brain with robust separation of NAAG and NAA at 3T. We present *in vivo* measurements from five healthy volunteers along with concentration maps. Each subject was scanned twice to test for repeatability and reproducibility.

METHODS *In vivo* SI data were acquired by PRESS volume localization with TE = 97 ms (TE_1 = 32ms and TE_2 = 65 ms), with a TR of 1.2 sec, a spectral width of 2000 Hz and 1024 complex points per FID. Water suppressed metabolite data were acquired with PRESS RF carrier set to 2.6 ppm and water signal was suppressed using a four-pulse scheme. The PRESS 90˚ and 180˚ pulses had bandwidths of 4.2 kHz (9.8 ms) and 1.3 kHz (13.2 ms), respectively. Axial SI slice was positioned to cover the central brain region above the corpus callosum (volume of interest (VOI) = 80 × 80 mm^2 and field of view (FOV) = 200 x 160 mm^2), 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. Unsuppressed water data were also acquired for eddy current correction. 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 and perform linear regression with white matter fraction. Residual water signal in water suppressed metabolite data were removed using the HL-SVD algorithm of the JMRUI[6]. Residual eddy current artifacts were corrected using unsuppressed water data. Frequency-drifts were corrected using in-house Matlab programs. LCModel software[7] was used for metabolite signal estimation. Basis-sets for LCModel analysis were created using published chemical shift and coupling constants[8]. Absolute quantification of the metabolites was performed using creatine (Cr) in gray matter at 8 mM. Written informed consent was obtained from subjects prior to the scans.

RESULTS AND DISCUSSION Three spectra from left to right, along with residuals and metabolite signals of NAA and NAAG in Fig 1**a** were selected from WM, GM, and WM dominant regions, respectively (shown in Fig 1**c**) in a healthy volunteer. Uniform residuals were observed between 1.8-4.1 ppm. NAAG was estimated to be 2.9 mM and 3.0 mM in WM dominant regions compared GM dominant region level of 0.9 mM. Fig 1**b** represents the spectra (black), NAA signal (blue) and NAAG signal (red) between 1.9 - 2.15 ppm from VOI. The NAAG signal was observed to be higher in WM regions when compared to regions with higher GM values. This is also indicated in the NAAG concentration map (Fig 1**d**), with higher concentration regions corresponding to WM regions (Fig 1**f**). Figure 2 shows average NAAG and NAA concentration maps over the VOI in five healthy volunteers. Figure 3 shows NAAG and NAA estimates as a function of the fractional WM content. NAAG concentration showed statistically significant ($P < 0.001$) positive linear correlation with increasing fractional WM content. This trend was far less in NAA estimates, in agreement with prior studies. Figure 4 shows reproducibility plots comparing the metabolite estimates from the first scan to those from the second scan for all five subjects for NAAG and NAA. The linear regression gave slope close to unity for both NAAG (0.9503±0.0152) and NAA (0.9914±0.0045). The 95% confidence intervals were very narrow and show excellent reproducibility between the two scans. The intraclass correlation coefficient (ICC) for NAAG and NAA were 0.76 and 0.84, respectively, showing high reproducibility. Coefficients of variation (CVs) were 0.17 and 0.06 for NAAG and NAA, respectively. NAA CV value was similar to prior SI studies[9].

CONCLUSION The present study reports reliable separation and estimation of NAAG and NAA at 3T in human brain, with good repeatability and reproducibility.

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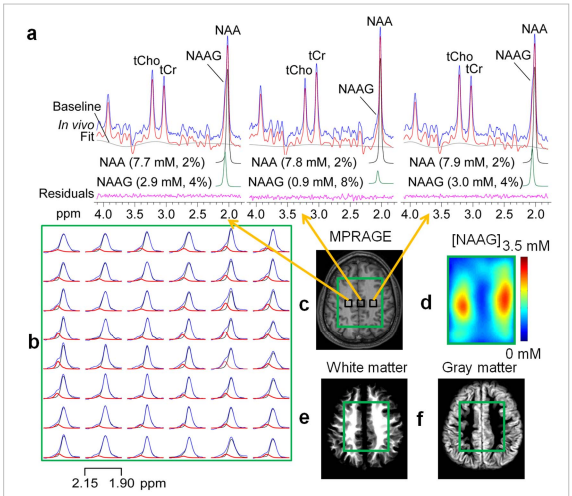

Fig. **1**: *In vivo* spectroscopic data from a healthy volunteer. (**a**) Three spectra (blue) along with LCModel fit (red), residuals (magenta) and metabolite signals NAA (black), and NAAG (green) are selected from the voxels shown in (c, black line). NAA, NAAG estimates and CRLBs for the spectra are also presented. (**b**) Spectra (black) with metabolites signals of NAA (blue) and NAAG signal (red) from the VOI (green line in **c**). (**d**), (**e**) and (**f**) are NAAG concentration, white and gray matter segmentation maps, respectively.

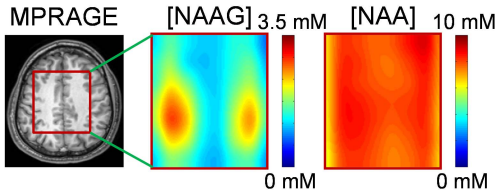

Fig. **2**: Mean metabolite concentration maps from five healthy volunteers (values are shown in mM).

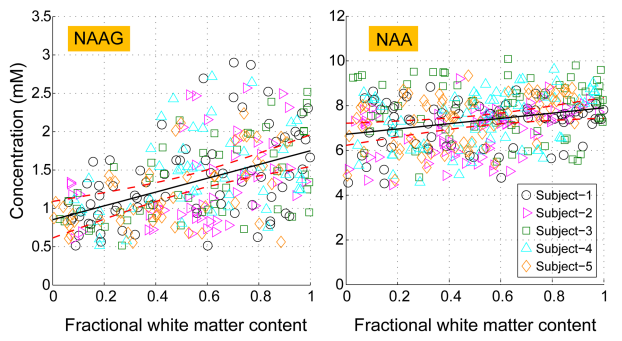

Fig. **3**: Linear regression of NAAG and NAA estimates with respect to fractional white matter content for all the five subjects. The dashed line indicates 95% confidence intervals of the linear fits.

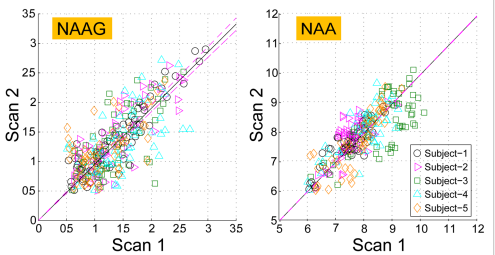

Fig. **4**: Reproducibility plots of NAAG and NAA (axes are in mM). The plots show scan 1 versus scan 2 metabolite estimates, the linear regression line of the data (black line) and 95% confidence intervals of the linear fits (magenta dashed line). The linear fit slopes of NAAG and NAA were 0.9503 and 0.9914, respectively.