Reproducibility and regional variation of metabolites in human brain, as measured by ^{1}H MRS at 3T

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TARGET AUDIENCE: Neuro-MR spectroscopists and Neuro-psychiatrists.

PURPOSE Precise and reliable detection of glutamate (Glu), glutamine (Gln) and N-acetyl-aspartyl-glutamate (NAAG) in the human brain is important for research in neuro-psychiatric diseases[1,2]. The proton signals of these metabolites are extensively overlapped and thus the spectral analysis is complicated. The purpose of this study is to demonstrate good separation of the signals and clinically acceptable reproducibility of the metabolite measurements by optimized ^{1}H MRS at 3T.

METHODS *Subject enrollment:* Five male healthy subjects (age 26 ± 2.4) were recruited in this study. In order to evaluate the reproducibility of brain metabolites, each subject underwent two examinations for frontal gray matter (FG), occipital gray matter (OG) and occipital white matter (OW) dominant regions. The time intervals between the two examinations ranged from one to four weeks. *MR experimental:* MR experiments were carried out in a Philips 3T whole-body scanner with an 8 channel receive head coil. A PRESS sequence with TE = 97 ms (TE_1 = 32 ms, TE_2 = 65 ms), which was optimized for the detection of Glu and Gln, was used to evaluate brain metabolite levels. Single-voxel PRESS acquisition parameters included TR = 2 sec, spectral width = 2500 Hz, NEX = 64, and 2048 complex points per FID. The RF carrier frequency was set to 2.5 ppm and a four-RF pulse scheme was used for water suppression. The voxel size was 23x23x23 mm^3 (~12 mL) in all three regions. Unsuppressed water data were acquired, from each voxel, for reference in multi-channel water-suppressed data combination[3], eddy current correction, and metabolite quantification. High-resolution T_1w-MPRAGE was acquired and used for segmentation of gray matter (GM) and white matter (WM) contents within the voxels. Multi-channel combination, frequency drift correction and eddy current correction were performed using in-house written Matlab programs. LCModel[4] was used for spectral fitting. Basis sets were generated from density matrix simulation, incorporating three-dimensional volume localization[5] and published chemical shift and coupling constraints[6]. Metabolite levels were quantified using water signal as reference. Relaxation effects were corrected using published T_1, T_2 values[7,8]. To evaluate reproducibility, Coefficient of Variance (CV) and Intraclass Correlation Coefficient (ICC) were calculated with multi-factorial random effects model from analysis of variance (ANOVA)[9,10].

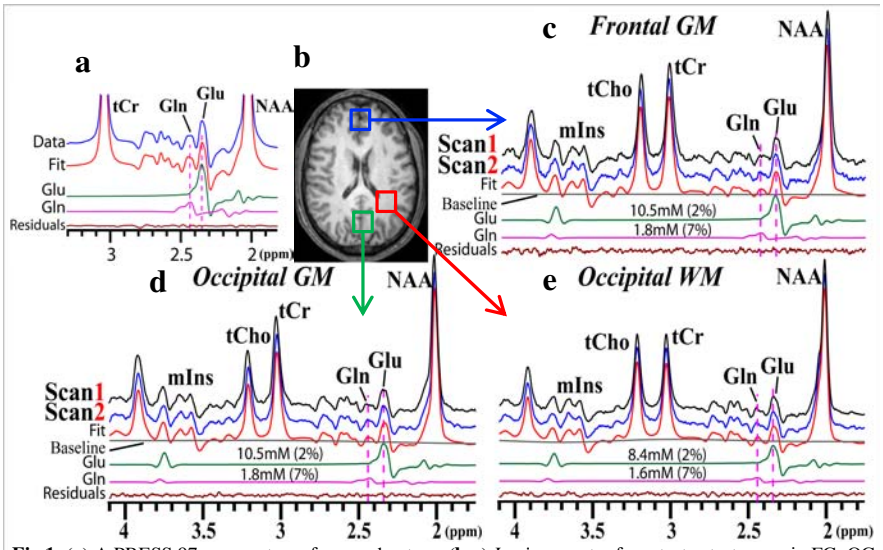

Fig.1: (**a**) A PRESS 97 ms spectrum from a phantom. (**b**-**e**) *In vivo* spectra from test-retest scans in FG, OG and OW dominant regions. LCModel fits, residuals and signals of Glu and Gln are shown for scan 2.

Table 1. Reproducibility test results

Metabolite	CV	ICC	FG dominant regions		OG dominant regions		OW dominant regions	
			Concentration(mM)	CRLB(%)	Concentration(mM)	CRLB(%)	Concentration(mM)	CRLB(%)
Glu	0.05	0.90	11.31±0.58	2±1	10.94±0.39	2±0	8.30±0.47	3±1
Gln	0.05	0.95	2.21±0.19	7±1	1.84±0.17	7±1	1.52±0.28	9±2
GABA	0.16	0.44	1.46±0.10	8±1	1.41±0.24	7±1	1.07±0.09	10±1
NAAG	0.17	0.91	1.07±0.24	8±2	1.12±0.16	7±1	2.79±0.35	5±1
mIns	0.05	0.90	5.65±0.77	2±1	5.77±0.64	2±0	4.77±0.37	2±1
tCr	0.04	0.83	8.47±0.39	1±0	8.41±0.56	1±0	7.30±0.47	1±0
NAA	0.02	0.87	9.13±0.32	1±1	9.72±0.51	1±0	9.06±0.30	2±1
tCho	0.04	0.96	1.97±0.21	1±1	1.43±0.10	1±0	2.01±0.16	1±1

RESULTS *Phantom and in vivo spectra:* A phantom spectrum and LCModel fitting results are presented in **Figure 1 (a)**. The signals of Glu and Gln at 2.35 ppm and 2.45 ppm were well reproduced in the fit, with ignorable residuals. Representative spectra of test-retest scans in the FG, OG and OW dominant regions in a subject are displayed in **Figure 1 (c-e)**. Spectral pattern was nearly identical between the two scans. Glu and Gln were estimated to be 10.5 and 1.8 mM for FG, 10.5 and 1.8 mM for OG, and 8.4 and 1.6 mM for OW, respectively, with CRLBs between 2% and 7%. *Reproducibility test:* Table 1 presents the CV and ICC values of eight major brain metabolites from FG, OG and OW regions, together with metabolite concentration estimates and CRLB values. The CVs were smaller than 0.05 for most of the metabolites, while being larger for GABA and NAAG (0.16 and 0.17, respectively). The ICCs of Glu, Gln, NAAG, mIns, and tCho were all higher than 0.90 while the Cr and NAA ICC values were slightly lower (0.83 and 0.87). *Linear regression:* **Figure 2** presents the concentration estimates of eight metabolites as a function of fractional GM content. The Glu concentrations in pure GM and WM of occipital brain were estimated to be 12.02 and 7.53 mM, with 95% confidence intervals of 11.23-12.81 and 6.82-8.24, respectively. The Gln concentrations in pure GM and WM were estimated to be 1.97 and 1.44 mM (95% confidence intervals 1.57-2.36 and 1.08-1.79), respectively. The NAAG singlet at 2.045 ppm was well resolved in the data (**Figure 2 (i)**). The NAAG concentrations in pure GM and WM were estimated to be 0.41 and 3.30 mM (95% confidence intervals 0.00-0.82 and 2.91-3.69), respectively. As opposed to Glu, Gln and GABA, which were higher in GM than in WM, the NAAG was significantly higher in WM than in GM ($p < 0.0001$). The coefficient of determination (R^2) in the linear regression was 0.89, 0.31, 0.49 and 0.92 for Glu, Gln, GABA and NAAG, respectively.

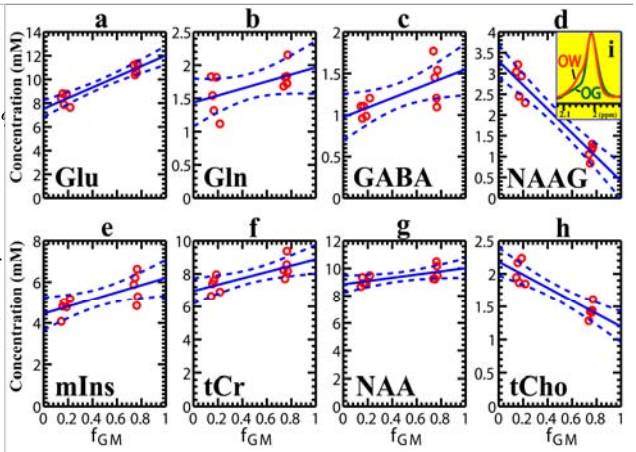

Fig.2: (**a**)-(**h**) Linear regression of metabolite estimates from occipital gray and white matter dominant regions vs. fractional GM contents (f_{GM}). (**i**) Comparison of the NAA and NAAG composite singlet (2.01 & 2.05 ppm) between occipital GM (green) and WM (red) dominant regions.

DISCUSSION AND CONCLUSION The present study reports estimation of Glu, Gln, GABA and NAAG in three brain regions, obtained with a PRESS TE = 97 ms method at 3T. The linear regression revealed that the concentrations of the metabolites are different between gray and white mater regions. The low CVs (0.05) and high ICCs (higher than 0.90) of Glu and Gln, together with low CRLBs (2-3% for Glu, 7-9% for Gln), indicated high reproducibility and precision of the MRS measurements. In conclusion, the present study demonstrates reproducible and precise estimation of Glu, Gln and NAAG, and their regional variation in the human brain by an optimized PRESS 97 ms at 3T.

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ACKNOWLEDGEMENTS This study was supported by NIH CA159128 and CPRIT RP101243.