# Regional variations of GABA, glutamate, glutamine and NAAG in the human brain, as measured by 1H MRS at 7T in vivo

Changho Choi[1], Sandeep Ganji[1], Zhongxu An[1], and Akshay Madan[1]

[1]*Advanced Imaging Research Center, UT Southwestern Medical Center, Dallas, Texas, United States*

**Target audience:** Neuro-MR spectroscopist

**Purpose:** Precise detection of clinically important metabolites in the human brain remains a major challenge. We aim to accomplish quantitative $^1$H MRS of GABA, glutamate (Glu), glutamine (Gln), glutathione (GSH), and N-acetyl-aspartyl-glutamate (NAAG). Here we report measurements of these technically challenging metabolites in several brain regions, achieved by a GABA-tailored PRESS sequence at 7T.

**Methods:** Nine healthy volunteers were recruited (5 female and 4 male; age 21 - 32). MR experiments were carried out in a Philips 7T whole-body scanner, using a dual-channel transmit and 16-channel receive coil. The PRESS subecho times was optimized for detection of the GABA resonance at 2.29 ppm, with density-matrix simulations that incorporated the slice-selective RF and gradient pulse parameters. Single-voxel MRS data were acquired from gray matter (GM) and white matter (WM) dominant regions in the frontal and occipital brain (4 locations in each subject). Acquisition parameters included TR = 2.5 s, NEX = 128 - 256, voxel size = 10 - 20 mL, 180°-RF pulse bandwidth = 1.4 kHz, and RF carrier frequency = 2.5 ppm. LCModel fitting was conducted using in-house calculated basis spectra of 15 metabolites. GM, WM and CSF contents within the voxels were obtained from $T_1$-weighted image segmentation incorporating the chemical shift displacement effects for each metabolite. Metabolite concentrations were obtained by setting the mean creatine (tCr) estimate from occipital GM-rich regions at 8 mM after normalizing LCModel metabolite-signal estimates to short-TE (14 ms) GM+WM water signal for each voxel. To evaluate the metabolite levels in pure GM and WM, linear regression was conducted for metabolite estimates *vs.* fractional GM contents. Paired two-tailed t-tests were conducted for comparing the data between brain regions. For validation purpose, spectral fitting was also performed without GABA in the basis function.

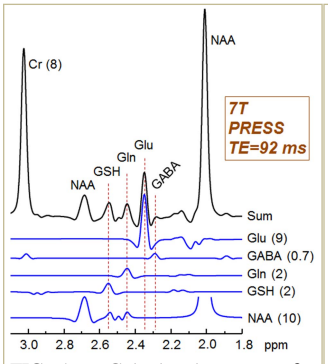


FIG 1. Calculated spectra for concentration ratios shown in brackets. Spectra were broadened to singlet FWHM of 10 Hz.

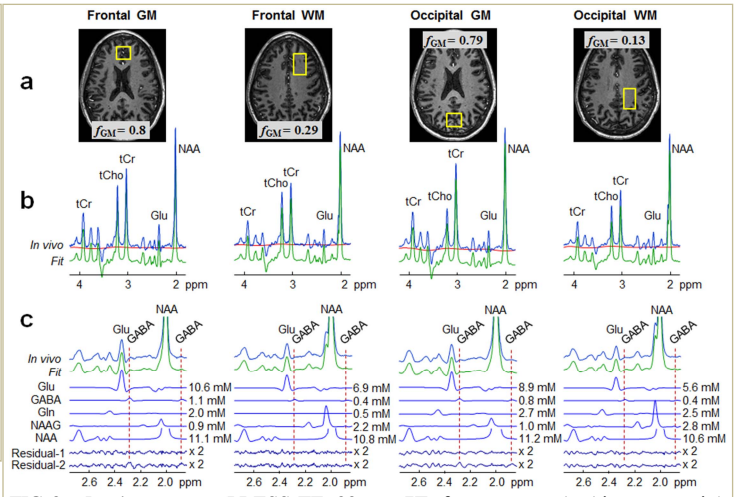


FIG 2. *In vivo* spectra (PRESS TE=92 ms; 7T) from gray and white matter rich regions in the frontal and occipital brain of a healthy volunteer are shown with LCModel analysis results and fractional GM contents ($f_{GM}$) within the voxels. Residual-1 and -2 were obtained from spectral fittings using basis set with and without GABA, respectively. Dotted lines are drawn at 2.29 and 1.89 ppm. Spectra are normalized to GM+WM water. Residuals are 2-fold magnified.

Table 1. Fractional GM contents ($f_{GM}$) and GABA concentrations (mM) and CRLB (%) (Cramer-Rao lower bounds). Data are mean±SD (n=9).

| GABA | GM-rich regions | | WM-rich regions | |
|---|---|---|---|---|
| | Frontal | Occipital | Frontal | Occipital |
| $f_{GM}$ | 0.68±0.07 | 0.70±0.06 (p = 0.16) | 0.23±0.06 | 0.21±0.06 (p = 0.37) |
| mM | 1.07±0.15 | 0.84±0.08 (p = 0.001) | 0.51±0.16 | 0.31±0.06 (p = 0.009) |
| CRLB | 5.1±0.6 | 5.4±0.9 | 9.1±3.0 | 11.9±2.2 |

**Results:** The PRESS subecho times were optimized as ($TE_1$, $TE_2$) = (31, 61) ms for resolving the J-coupled resonances between 2.1 - 2.6 ppm. At this TE of 92 ms, the sidebands of the GABA, Glu, Gln and GSH multiplets were substantially attenuated and as a result, the overall linewidth of the multiplets was similar to that of a singlet and the multiplets were completely separated (**Fig. 1**). Specifically, the 2.29 ppm signal of GABA was clearly resolvable from the adjacent large Glu signal at 2.35 ppm. For *in vivo*, metabolite signals were well discernible in all spectra, with drastically attenuated macromolecule baseline signals (**Fig. 2**). As predicted by the simulation, the GABA and Glu multiplets between 2.2 and 2.4 ppm were clearly resolved (**Fig. 2c**). The signals of GABA and Glu were larger in spectra from GM rich regions compared to spectra from WM regions. With GABA in the basis set, the *in vivo* spectra were well reproduced by the fits, leading to minimal residuals (**Fig. 2c**, Residual-1). However, when GABA was excluded from the basis set, unfit signals were clearly noticeable at 2.29 and 1.89 ppm in the residuals (**Fig. 2c**, Residual-2), indicating that the signals at 2.29 and 1.89 ppm were mainly attributed to GABA. The GABA residual difference between the fitting methods was larger in spectra from GM regions than in spectra from WM regions. Gln was not reliably measurable in frontal WM regions while it was well detected in other brain regions. The PRESS method also afforded reliable detection of NAAG signals at 2.045 (singlet) and 2.18 ppm (multiplet), which were notably large in spectra from WM regions. GABA signals were detected with CRLB < 15% in all the 4×9 data sets (see **Table 1**). For each of the GM- and WM-rich regions, the GABA levels in frontal and occipital brain were significantly different ($p < 0.01$), while the fractional GM contents ($f_{GM}$) were about the same ($p > 0.1$). Linear regression of the estimates *vs.* $f_{GM}$ showed that GABA increases with $f_{GM}$, with slightly stronger correlation in frontal than in occipital brain (**Fig. 3**). The GABA level in pure GM (*i.e.*, at $f_{GM} = 1$) was significantly different between frontal and occipital brain ($p = 0.009$). Glu was also higher with increasing $f_{GM}$, giving significantly different pure-GM Glu level between frontal and occipital brain (13.6 *vs.* 10.6 mM; $p = 6\times10^{-4}$). Of note, the Gln signal was not detected in frontal WM regions in 8 subjects (other than the subject in Fig. 2), indicating that Gln may be very low in this brain region. In contrast, Gln in occipital brain was measured to be ~2.6 mM irrespective of $f_{GM}$. NAAG showed inverse correlation with $f_{GM}$, the concentration and its $f_{GM}$ dependence being about the same between frontal and occipital brain. GSH at $f_{GM} = 1$ was different between frontal and occipital brain (1.27 *vs.* 0.84 mM; $p = 0.02$). Creatine and choline were different between GM and WM as well as between frontal and occipital brain, while NAA was fairly uniform between the brain regions.

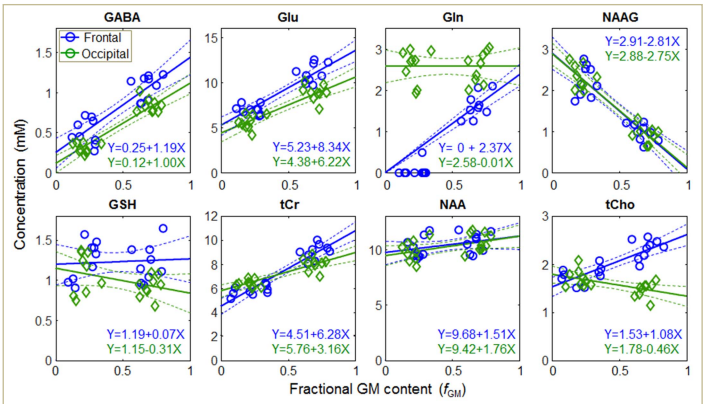


FIG 3. Linear regression of metabolite estimates *vs.* fractional GM contents was performed, separately for frontal brain data (18 circles in blue) and occipital brain data (18 diamonds in green). The functions of the fits are shown for each metabolite. The fitting for frontal-brain Gln estimates was undertaken with Y = *a*X, excluding 8 null estimates from WM-rich regions. Dashed lines indicate 95% confidence intervals of the linear fits.

**Discussion & Conclusion**: It was our experience that, although short-TE MRS at 7T may greatly benefit from minimal $T_2$ signal loss, spectral fitting results were often not highly consistent, which was largely due to the effects of spectral overlaps and the interferences of macromolecule signals. We have shown that an optimized intermediate-TE PRESS at 7T can provide complete signal separation between 2.1 - 2.5 ppm and thus reliable detection of GABA as well as Glu, Gln, GSH and NAAG in several brain regions. Of note, the GABA measurement by this method does not have contamination from homocarnosine and macromolecules, as opposed to MEGA editing of GABA. The observation of GABA and Glu level differences between frontal and occipital brain may be of high interest. The finding of markedly different Gln levels between frontal and occipital WM regions may be novel. Further research is required to find how these metabolic differences are related to brain functions.

*This study was supported by NIH MH093959 and CPRIT RP101243.*