GABA quantification in human anterior cingulate cortex

Jan Weis, PhD1*, Jonas Persson, PhD2, Andreas Frick, PhD3, Fredrik Åhs, PhD4, Maarten Versluis, PhD5, Daniel Alamidi, PhD6

1Department of Medical Physics, Uppsala University Hospital, Uppsala, Sweden
2Department of Neuroscience, Uppsala University, Uppsala, Sweden
3The Beijer Laboratory, Department of Neuroscience, Uppsala University, Uppsala, Sweden
4Department of Psychology and Social Work, Mid Sweden University, Östersund, Sweden
5Philips, Best, The Netherlands,
6Philips, Stockholm, Sweden

*Correspondence to: Jan Weis
Department of Medical Physics,
Uppsala University Hospital,
SE-751 85 Uppsala, Sweden
E-mail: jan.weis@radiol.uu.se, Tel: +46 – 18 – 611 48 07

Short title: GABA quantification in human brain
Abstract

γ-Aminobutyric acid (GABA) is a primary inhibitory neurotransmitter in the human brain. It has been shown that altered GABA concentration plays an important role in a variety of psychiatric and neurological disorders. The main purpose of this study was to propose a combination of PRESS and MEGA-PRESS acquisitions for absolute GABA quantification and to compare GABA estimations obtained using total choline (tCho), total creatine (tCr), and total N-acetyl aspartate (tNAA) as the internal concentration references with water referenced quantification. The second aim was to demonstrate the fitting approach of MEGA-PRESS spectra with QuasarX algorithm using a basis set of GABA, glutamate, glutamine, and NAA in vitro spectra. Thirteen volunteers were scanned with the MEGA-PRESS sequence at 3T. Interleaved water referencing was used for quantification, B₀ drift correction and to update the carrier frequency of RF pulses in real time. Reference metabolite concentrations were acquired using a PRESS sequence with short TE (30 ms) and long TR (5000 ms). Absolute concentration were corrected for cerebrospinal fluid, gray and white matter water fractions and relaxation effects. Water referenced GABA estimations were significantly higher compared to the values obtained by metabolite references. We conclude that QuasarX algorithm together with the basis set of in vitro spectra improves reliability of GABA+ fitting. The proposed GABA quantification method with PRESS and MEGA-PRESS acquisitions enables the utilization of tCho, tCr, and tNAA as internal concentration references. The use of different concentration references have a good potential to improve the reliability of GABA estimation.
Introduction

Two important neurotransmitters in the mammalian brain are glutamate (Glu) and \( \gamma \)-aminobutyric acid (GABA). Glu is the principal excitatory neurotransmitter in the vertebrate nervous system and is involved in every major excitatory brain function [1]. Glu serves as a metabolic precursor for GABA which is the primary inhibitory neurotransmitter. GABA plays a crucial role in shaping and regulating neuronal activity [2]. Changes in GABA concentrations have been associated with a variety of neuropsychiatric disorders, such as depression, anxiety, epilepsy, schizophrenia, ADHD, chronic pain, etc. [3, 4].

Glu can be quantified with good accuracy by short echo time (TE < 40 ms) magnetic resonance spectroscopy using 3, 4 and 7T scanners [5-7]. However, the quantification of GABA is challenging because GABA spectral lines centered at 1.89, 2.28 and 3.01 ppm are overlapped with the strong signals of total creatine (tCr) (creatine and phosphocreatine), Glu, glutamine (Gln), total N-acetylaspartate (tNAA) (NAA and N-acetylaspartylglutamate (NAAG)), macromolecules (MM) and others. The most widely used approach for GABA detection at 3T is a J-difference Mescher-Garwood (MEGA) spectral editing technique incorporated within a point resolved spectroscopy (PRESS) sequence [8]. MEGA-PRESS exploits the scalar (J) coupling between GABA C4 protons \( (\text{CH}_2) \) at 3.01 ppm and C3 protons \( (\text{CH}_2) \) at 1.89 ppm. J-difference editing involves the acquisition of two spectra measured with TE 68 ms. The first (ON) spectrum is acquired by applying a pair of frequency-selective GABA-editing RF pulses (center frequency 1.89 ppm). These pulses invert the C3 spins and thereby refocus coupled C4 spins (outer two peaks of pseudo-triplet) without affecting the overlapping tCr peak at 3.03 ppm. The second (OFF) spectrum is measured with editing frequency-selective pulses at center frequency 7.46 ppm, which are not expected to have an impact on the spectrum. The unaffected spin evolution results in inverted outer two lines of the C4 pseudo-triplet at TE of 68 ms. Whereas the tCr spectral line is removed by ON and OFF spectra subtraction, the two outer
lines of GABA C4 triplet are summed and the central peak of the GABA triplet is suppressed [4, 8]. Furthermore, MM resonances at ~1.7 ppm are also inverted by editing frequency-selective RF pulses and coupled to MM protons at 3 ppm. These co-edited MM signals overlap with GABA C4 resonances. The resulting GABA peak is therefore referred to as GABA+ to point out the summation of GABA with MM signals. Recent studies showed that approximately 50% of GABA+ intensity originates from MM [3].

Cerebral GABA content is most often expressed as a spectral intensity ratio of GABA+/tCr. A disadvantage of such an approach is the fact that it is difficult to determine whether the alteration is caused by the numerator, denominator, or both. A suitable example is the tCr concentration that is subject to change in patients with schizophrenia, Alzheimer’s and Parkinson diseases [9, 10]. This problem can be minimized by the evaluation of GABA+ spectral intensity ratio to the intensity of other metabolites or to the intensity of the water. The alternative to the unitless spectral intensity ratio is absolute quantification. The most common method for absolute GABA quantification utilizes tissue water as an internal concentration reference [11-13]. However, the quantification is not straightforward because the brain water originates from three tissue compartments: cerebrospinal fluid (CSF), gray (GM) and white matter (WM). Each compartment has different MR-visible water fraction and is weighted by different T₁ and T₂ relaxation times.

Recently, GABA estimation using tCr as an internal concentration reference was suggested as an alternative to water reference [14, 15]. This approach benefits from the fact, that partial volume and relaxation corrections are unnecessary because metabolites originate only from GM and WM compartments and the relaxation times of metabolites are approximately equal in both compartments. Grewal et al [15] assumed tCr to be 7.1 mM. This concentration was estimated for WM using water referenced spectroscopic imaging (PRESS, TR/TE 1500/135 ms) [16]. Similarly, Bhattacharyya et al [14] applied the value 9.22 mM measured by single-voxel PRESS
technique (TR/TE 2700/68 ms). A short TR and long TE caused these approaches to be sensitive to the inaccuracies of tCr and water relaxation times in WM, GM, and CSF.

The goal of this study was threefold: 1) to demonstrate a new fitting approach of MEGA-PRESS spectra using QuasarX algorithm as implemented in jMRUI 6.0 software package [17]. A basis set of GABA, Glu, Gln, and NAA in vitro spectra were measured for this purpose; 2) to quantify GABA using water as the internal concentration reference; and 3) to quantify GABA using total choline (tCho: free choline, phosphocholine, and glycerophosphocholine), tCr, tNAA as the internal concentration references. Contrary to previous studies [14, 15], the reliability of reference tCho, tCr, and tNAA concentrations was improved by using a PRESS sequence with short TE (30 ms), long TR (5000 ms) together with partial volume and relaxation corrections for WM, GM and CSF content in each voxel.

**Material and Methods**

**Study population**

In total, thirteen volunteers (6 females and 7 males) were recruited. The volunteers underwent PRESS and MEGA-PRESS measurements. Mean age of the participants was 37±10 years (range: 24–61). All volunteers were healthy without any history of psychiatric or neurological disorders. Ethical approvals were obtained from local Institutional Review Boards and written informed consent was obtained from each participant.

**Phantoms**

Four phantoms were produced according to the guidelines for the LCModel’s model spectra [18]. The phantoms contained aqueous solutions of GABA (200 mM), Glu (100 mM), Gln (100 mM), and NAA (50 mM). Each phantom contained a single metabolite. Aqueous solutions were prepared using a phosphate buffer consisting of 72 mM K₂HPO₄, 28 mM KH₂PO₄, 1 g/L NaN₃,
and 1 mM sodium trimethylsilyl propanesulfonate (DSS). Solution’s pH was adjusted to 7.2. The chemicals were purchased from Sigma-Aldrich AB (Stockholm, Sweden).

**MRI and MRS acquisition protocols**

All experiments were performed on 3T scanner (Achieva dStream, Philips Healthcare, Best, The Netherlands). The data were acquired with a 32 channel receiver head coil. Whole brain 3D T₁-weighted turbo FFE images (TR/TE 8/3.8 ms, isotropic resolution 1x1x1 mm³) were acquired to guide the positioning of the voxel in the anterior cingulate cortex (ACC) (Fig 1).

The GABA spectra were acquired with a MEGA-PRESS sequence using the following parameters: voxel size 4x4x2 cm³, TR/TE 2000/68 ms, 320 alternating ON-OFF spectra, 14 ms GABA-editing RF pulses at 1.9 (ON) and 7.5 (OFF) ppm, spectral bandwidth 2000 Hz, 1024 time domain data points, and 40 blocks. Each block started with the acquisition of one unsuppressed reference water spectral line followed by four pairs of water suppressed ON-OFF spectra acquired with 4-step phase-cycling. The unsuppressed water signal was used as the internal concentration reference, for eddy current corrections, for B₀ drift correction and for updating the carrier frequency of RF pulses in real time. Reference metabolite concentrations were measured using the standard PRESS sequence (TR/TE 5000/30 ms, spectral bandwidth 2000 Hz, 1024 data points, 32 averages, 16 phase cycle steps) with the same voxel size and position. Two dummy excitations were followed by 16 non-water-suppressed and 32 water-suppressed scans.

**Fig 1. Spectroscopy voxel position.** Representative voxel (4x4x2 cm³) placement in anterior cingulate cortex and the results of partial volume segmentation of CSF, GM and WM (brown). Transversal images (reconstructed pixel size 0.47x0.47 mm², slice thickness 2 mm) were used for segmentation and for reconstruction of coronal and sagittal slices.
MEGA-PRESS spectra of GABA, Glu, Gln, and NAA aqueous solutions were measured a few hours after preparation. The voxel size was 3x3x3 cm\(^3\) and temperature was kept at 22 °C (room temperature) during the acquisition. All other MEGA-PRESS parameters were identical to the in vivo experiments.

**Post processing and quantification**

Reconstructed brain images (matrix 512x512, pixel size 0.47 mm, slice thickness 2 mm) were used for GM, WM, and CSF segmentation (Fig 1). Segmentation was performed by using the automated segmentation tool (FAST) [19]. A binary mask of the water PRESS box was created using the SVMask tool (Philips Healthcare, Michael Schär).

MEGA-PRESS spectra were processed with jMRUI 6.0 software package [17]. Each spectrum was zero filled to 8192 points and the residual water was removed by Hankel-Lanczos Singular Value Decomposition (HLSVD) filter. No apodization of the FIDs was applied in this study. The in vivo spectra were fitted by the QuasarX algorithm (QUEST with new constrains and shape peak selection). This nonlinear least-squares algorithm fits a time-domain model function, made up from a basis set of in vitro spectra, to in vivo data. Our basis set contained MEGA-PRESS spectra of GABA (GABA\(+\)), Glu, Gln, NAA and NAAG aqueous solutions (Fig 2). NAAG spectrum was approximated by shifted NAA spectrum, with the main peak shifted to 2.045 ppm from 2.01 ppm [18]. GABA\(+\) spectrum was made by modifying GABA spectrum. Contribution of MM signals to GABA was empirically simulated by adding the Lorentzian line (linewidth 5 Hz) to the central peak of pseudo-triplet at 3 ppm. The amplitude was adjusted to be about ~10% higher compare to outer two peaks (Fig 2). The zero-order phase correction of in vivo MEGA-PRESS spectrum was estimated by fitting the tCho, tCr, and tNAA singlets in averaged OFF spectrum using AMARES algorithm (Fig 3). The resulting spectrum
was then fitted by the QuasarX algorithm (Fig 4). Gaussian line shapes were used to fit GABA+ spectral lines.

**Fig 2. Basis set of in vitro spectra.** MEGA-PRESS spectra of GABA, Gln, Glu, and NAA aqueous solutions, and simulated GABA+.

**Fig 3. An example of in vivo OFF spectrum.** AMARES fits of the tCho, tCr, and tNAA singlets, and residue.

**Fig 4. Representative in vivo MEGA-PRESS spectrum and fits.** The spectrum was fitted by the QuasarX algorithm using the basis set spectra shown in Fig 2.

The unsuppressed water signal was fitted by Hankel-Lanczos Squares Singular Value Decomposition (HLSSVD) algorithm. The AMARES and QuasarX algorithms provide the Cramér-Rao lower bound (CRLB) standard deviation (CRSD). The fitting error was computed as the percentage ratio of CRSD to the FID’s amplitude. Water scaled GABA concentration in relation to wet weight tissue (mol/kg) was computed according to the equation:

\[ C_{\text{GABA}} = \frac{I_{\text{GABA}}}{I_{\text{H}_2\text{O}}} \times \frac{2}{N_{\text{GABA}}} \times \frac{1}{R_{\text{GABA}}} \times W_{\text{conc}} \times \frac{\text{MM}_{\text{cor}}}{\text{eff}_{\text{GABA}}} \]  

(1)

where \( I_{\text{GABA}} \) is the GABA+ spectral intensity at \(-3 \) ppm, \( I_{\text{H}_2\text{O}} \) is intensity of reference water line, \( N_{\text{GABA}} = 2 \) is the number of protons contributing to \( I_{\text{GABA}} \) resonance, \( R_{\text{GABA}} \) is the GABA attenuation factor, \( \text{MM}_{\text{cor}} = 0.5 \) is a macromolecule correction factor [11, 20, 21], and \( \text{eff}_{\text{GABA}} = 0.5 \) is the editing efficiency [22]. \( W_{\text{conc}} \) is the reference water concentration corrected for partial volume and relaxation effects [16, 23, 24]:

\[ W_{\text{conc}} = \frac{W_{\text{H}_2\text{O}} (f_{\text{GM}} R_{\text{H}_2\text{O}-\text{GM}} + f_{\text{WM}} R_{\text{H}_2\text{O}-\text{WM}} + f_{\text{CSF}} R_{\text{H}_2\text{O}-\text{CSF}})}{(1-f_{\text{CSF}})} \]  

(2)

and
where $W_{H2O}$ is the molal concentration of pure water (55.51 mol/kg), $f_x$ is the mole fraction of water in the voxel’s GM, WM and CSF, $\theta_x$ is the GM, WM and CSF volume fractions and $c_x$ is the relative density of MR visible water in GM (0.82) WM (0.7), and CSF (0.99) [25, 26]. $R_{H2O-GM}$, $R_{H2O-WM}$, and $R_{H2O-CSF}$ are PRESS relaxation attenuation factors $R = \exp(-TE/T_2)x[1-\exp(-TR/T_1)]$ of water in GM, WM, and CSF, respectively. The following relaxation times were used for corrections: GABA (T1 1310 ms, T2 88 ms) [27, 28], water in GM (T1 1820 ms, T2 99 ms), WM (T1 1084 ms, T2 69 ms), and CSF (T1 4163 ms, T2 503 ms) [29-31].

The GABA concentration was also assessed using tCho, tCr, and tNAA as the internal concentration references. The reference metabolite concentrations $C_{MET}$, Glu and other metabolites were measured by PRESS sequence with long TR (5000 ms) and short TE (30 ms) to minimize the influence of the water and metabolites relaxation effects. Concentrations were estimated by LCModel [18]. Partial volume and relaxation corrections were performed by adjusting LCModel control parameter WCONC according to the Eq. 2, i.e. WCONC = $W_{conc}$ [24]. It should be noted that the default LCModel control parameter ATTH2O for water attenuation correction was switched off (ATTH2O = 1) because water relaxation corrections were already performed in Eq. 2. The absolute GABA concentration (mol/kg) was estimated according to the formula:

$$C_{GABA} = \frac{I_{GABA} \times N_{GABA} \times R_{GABA} \times C_{MET} \times MM_{cor}}{I_{MET} \times N_{MET} \times R_{MET} \times C_{MET} \times eff_{GABA}}$$

where $I_{MET}$ is the spectral intensity of reference metabolite in OFF spectrum (Fig 3), $N_{MET}$ is the number of protons contributing to $I_{MET}$ resonance (9 for tCho, 3 for tCr and tNAA), $R_{MET}$ is the metabolite attenuation factor. $C_{MET}$ is the reference metabolite concentration (mol/kg) of considered volunteer. Mean relaxation times of tCho (T1 1140 ms, T2 230 ms), tCr (T1 1110 ms, ...
$T_2$ 163 ms), and tNAA ($T_1$ 1340 ms, $T_2$ 260 ms) were used in relaxation corrections [32]. It should be noted that only small differences in metabolite relaxation times were found between GM and WM [32, 33].

**Statistics**

The reported values are given as the mean ± one SD. $P < 0.05$ of a two-tailed Student’s t-test was considered statistically significant. The relative variances (variance-to-mean ratio) were expressed in %. The two-tailed F-test was performed to compare variances of mean GABA concentrations obtained by different quantification methods.

**Results**

Thirteen volunteers underwent combined PRESS and MEGA-PRESS examinations. All experiments were successful, no spectra had to be discarded. Table 1 summarizes the water-scaled metabolite concentrations and CRLBs acquired by the PRESS (TR/TE 5000/30 ms) sequence. The spectra were processed by LCModel. The mean WM, GM, and CSF volume fractions were $52.0 \pm 3.5\%$, $33.2 \pm 2.5\%$, and $14.8 \pm 3.5\%$, respectively. Spectra of GABA, Glu, Gln, and NAA aqueous solutions and simulated GABA+ spectrum are shown in Fig 2. These spectra were used as prior knowledge for fitting the volunteer’s MEGA-PRESS spectra using the QuasarX algorithm. Figures 3 and 4 show representative in vivo results. The mean QuasarX fitting error of GABA+ intensity was $1.5 \pm 0.2\%$ (range: $1.2 - 1.8\%$). The mean AMARES fitting errors of metabolites were $1.0 \pm 0.1\%$, $0.8% \pm 0.1\%$, and $1.2 \pm 0.2\%$ for tCr, tNAA, and tCho, respectively. The mean spectral intensity ratios GABA+/tCr, GABA+/tNAA, and GABA+/tCho are shown in Table 2. The absolute GABA concentrations were computed according to Eq. 1 and Eq. 4 using tissue water, tCho, tCr, and tNAA as internal concentration references. The concentrations together with their relative variances are summarized in Table
3 and presented in Fig 5. Two-tailed F-tests detected no differences in the variances of GABA concentrations obtained by different methods.

**Fig 5. GABA concentrations.** Concentrations were estimates using H$_2$O, tCho, tCr, and tNAA as the internal concentration references.

**Table 1. Water-scaled metabolite concentrations (mmol/kg) and CRLBs (%).**

| Metabolite | Concentration | CRLB    |
|------------|---------------|---------|
| GABA       | 2.65 ± 0.44   | 18.62 ± 2.96 |
| Glu        | 10.84 ± 0.54  | 5.23 ± 0.44 |
| Glx        | 13.03 ± 1.03  | 6.69 ± 0.48 |
| tNAA       | 12.37 ± 0.68  | 2.23 ± 0.44 |
| tCr        | 9.11 ± 0.57   | 2.0 ± 0.0 |
| tCho       | 2.42 ± 0.20   | 2.85 ± 0.38 |
| mI         | 6.43 ± 0.51   | 3.92 ± 0.28 |

Concentrations were estimated from the PRESS spectra (TR/TE 5000/30 ms).

**Table 2. GABA-to-metabolite spectral intensity ratio (a.u.).**

| Ratio        | GABA+/tCr | GABA+/tNAA | GABA+/tCho |
|--------------|-----------|------------|------------|
|              | 0.070 ± 0.01| 0.052 ± 0.007| 0.088 ± 0.013|

The spectral intensity ratios were evaluated from the MEGA-PRESS spectra.
Table 3. GABA concentrations (mmol/kg).

| Internal concentration reference | H$_2$O | tCho   | tCr    | tNAA  |
|----------------------------------|--------|--------|--------|-------|
| Concentrations                  | 2.57 ± 0.26 (2.7) | 1.63 ± 0.22 (3.1) | 1.46 ± 0.19 (2.6) | 1.61 ± 0.22 (3.1) |

Concentrations were estimated from MEGA-PRESS spectra using H$_2$O, tCho, tCr, and tNAA as the internal concentration references. Relative variances (%) are shown in parentheses.

Discussion

To the best of our knowledge, this is the first study whereby a PRESS sequence with short TE and long TR together with a MEGA-PRESS sequence were used to estimate the absolute GABA concentration. Applied PRESS method improved the accuracy of individual reference metabolite concentrations and enabled utilization of tCho, tNAA and tCr as internal concentration references at the expense of a relatively short prolongation of the net measurement time (4 minutes in our case). Spectrum processing approach with QuasarX algorithm and the use of different concentration references have a good potential to improve the reliability of GABA estimation.

The anterior cingulate cortex was chosen because this region acts as a central node in the brain and is important for the regulation of advanced brain functions. The water scaled PRESS spectra were used for the individual reference metabolite quantification. The described approach with short TE and long TR together with the partial volume and relaxation corrections is regarded to be the most accurate. This is because errors due to inaccurate relaxation times were minimized. Low CRLBs of Glu, tCho, tCr, and tNAA LCModel fits (Table 1) indicate very good accuracy of the concentration estimates. The fitting errors of GABA signals were at the boundary of acceptable reliability (CRLB ~ 20%). However, it should be noted that average over a group of LCModel results can significantly reduce the uncertainty [18]. The
concentration estimates of Glu, Glx, tNAA, tCr and tCho are in line with previous studies performed at 3, 4, and 7 Tesla [5, 7].

The relative variances of absolute GABA concentrations estimated using H$_2$O, tCho, tCr, and tNAA concentration references show similar dispersion. The comparison of our GABA concentrations with the literature data is not straightforward due to differences in tissue composition and data processing. Cerebral GABA content from ~1 up to 3.7 mM was previously reported [11-15, 35]. GM/WM ratio is an important issue because GABA content was reported to be from 1.5 to 8.7 times larger in GM relative to WM [14, 20, 34, 36]. Differences in segmentation algorithms, spectrum processing methods, macromolecule correction factor MM$_{cor}$ and accuracy of internal concentration references are also important factors that contribute to the variability of the concentration estimates.

The absolute GABA concentrations estimated using water reference and measured by PRESS (Table 1) and MEGA-PRESS (Table 3) are surprisingly in very good agreement. However, water referenced GABA concentrations were significantly higher than the concentrations estimated with tCho, tCr and tNAA references (Table 3, Fig 5). The main drawback of water referenced quantification using typical MEGA-PRESS (TR/TE 2000/68 ms) acquisition is the fact that partial volume and relaxation corrections (Eq. 2, 3) depend on the precision of WM, GM, and CSF segmentation and on the accuracy of nine experimental constants: water fractions and water relaxation times T$_1$, T$_2$ in GM, WM, and CSF. The advantage of GABA quantification using tCr, tCho, and tNAA as the internal concentration references is the fact that partial volume and relaxation corrections are unnecessary because metabolites originate only from GM and WM compartments and the relaxation times of tCho, tCr, and tNAA are approximately equal in both compartments [32, 33]. It should be noted, that the described metabolite reference method is still subject to all sources of error as in water referenced MEGA-PRESS approach because tCho, tCr, and tNAA were quantified from water.
scaled PRESS spectra (TR/TE 5000/30 ms). However, the main difference is in relaxation
correction accuracy. Standard water referenced MEGA-PRESS approach with a relatively short
TR (2000 ms) and long TE (68 ms) is more susceptible to inaccuracies of relaxation times
compare to the proposed metabolite referenced quantification using PRESS with long TR (5000
ms) and short TE (30 ms). It should be noted, that quantification of tCho, tCr, and tNAA can
be omitted in comparative studies and the most reliable literature values can be applied instead.
Our GABA values can be compared with the concentrations estimated from the water scaled
STEAM and SPECIAL spectra measured at 7 T scanners [5, 7]. The occipital lobe spectra were
measured with long TR and a very short TE (6 ms). High spatial resolution facilitated fitting of
the GABA triplet at 2.28 ppm which is uncontaminated by the macromolecules. GABA levels
in the range of 1.3 - 1.6 mmol/kg were reported. These values were slightly underestimated
because partial volume and relaxation corrections were not taken into consideration.
Nevertheless, we believe that our metabolite referenced results (Table 3) conform to the most
reliable literature values such as the GABA values reported by Mekle et al [5] and Tkac et al
[7]. We hypothesize that our water referenced GABA values are overestimated due to
inaccuracies in partial volume and relaxation corrections.

**Conclusion**

QuasarX algorithm together with the basis set of in vitro spectra improves reliability of GABA+
fitting. The proposed GABA quantification method with PRESS and MEGA-PRESS
acquisitions enables the utilization of tCho, tCr, and tNAA as internal concentration references.
Water referenced GABA estimations were significantly higher compared to the values obtained
by metabolite references. The use of different concentration references have a good potential to
improve the reliability of GABA estimation.
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References

1. Meldrum BS. Glutamate as a Neurotransmitter in the Brain: Review of Physiology and Pathology. The Journal of Nutrition. 2000; 130:1007S–1015S.

2. McCormic DA. GABA as an inhibitory neurotransmitter in human cerebral cortex. J Neurophysiol 1989; 62:1018-1027.

3. Mikkelsen M, Barker PB, Bhattacharyya PK, et al. Big GABA: Edited MR spectroscopy at 24 research sites. Neuroimage 2017; 159: 32-45.

4. Harris AD, Saleh MG, Eden RAE. Edited 1H magnetic resonance spectroscopy in vivo: Methods and metabolites. Magn Reson Med 2017; 77:1377-1389.

5. Mekle R, Mlynarik V, Gambarota G, Hergt M, Krueger G, Gruetter R. MR spectroscopy of the human brain with enhanced signal intensity at ultrashort echo times on a clinical platform at 3T and 7T. Magn Reson Med 2009; 61:1279-1285.

6. Oeltzschner G, Zöllner HJ, Jonuscheit M, Lanzman RS, Schnitzler A, Wittsack HJ. J-difference-edited MRS measures of γ-aminobutyric acid before and after acute caffeine administration. Magn Reson Med 2018; 80:2356-2365.

7. Tkac I, Öz G, Adriany G, Ugurbil K, Gruetter R. In vivo 1H NMR spectroscopy of the human brain at high magnetic fields: metabolite quantification at 4T vs. 7T. Magn Reson Med 2009; 62:868-879.
8. Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R. Simultaneous in vivo spectral editing and water suppression. NMR Biomed 1998; 11:266-278.

9. Öngür D, Prescot AP, Jensen JE, Cohen BM, Renshaw PF. Creatine abnormalities in schizophrenia and bipolar disorder. Psychiatry Res 2009; 172:44-48.

10. Pilatus U, Lais C, du Mesnil de Rochmont A, et al. Conversion to dementia in mild cognitive impairment is associated with decline of N-acetylaspartate and creatine as revealed by magnetic resonance spectroscopy. Psychiatry Research: Neuroimaging 2009; 173:1-7.

11. Harris AD, Puts NAJ, Barker PB, Edden RAE. Spectral-editing measurements of GABA in the human brain with and without macromolecule suppression. Magn Reson Med 2015; 74:1523-1529.

12. Harris A, Puts NAJ, Edden RAE. Tissue correction for GABA-edited MRS: consideration of voxel composition, tissue segmentation, and tissue relaxations. J Magn Reson Imag 2015; 42:1431-1440.

13. O’Gorman RL, Michels L, Edden RAE, Murdoch JB, Martin E. In vivo detection of GABA and glutamate with MEGA-PRESS: reproducibility and gender effects. J Magn Reson Imag 2011; 33:1262-1267.

14. Bhattacharyya PK, Phillips MD, Stone LA, Lowe MJ. In vivo magnetic resonance spectroscopy measurement of gray-matter and white-matter gamma-aminobutyric acid concentration in sensorimotor cortex using a motion-controlled MEGA point-resolved spectroscopy sequence. Magn Reson Imag 2011; 29:374-379.

15. Grewal M, Dabas A, Saharan S, et al. GABA quantitation using MEGA-PRESS: Regional and hemispheric differences. J Magn Reson Imag 2016; 44:1619-1623.

16. Gasparovic Ch, Song T, Devier D, et al. Use of tissue water as a concentration reference for proton spectroscopic imaging. Magn Reson Med 2006; 55:1219-1226.
17. Naressi A, Couturier C, Devos JM, et al. Java-based graphical user interface for the MRUI quantitation package. Magn Reson Mater Phys Biol Med 2001; 12:141-152.

18. Provencher SW. Estimation of metabolite concentrations from localized in vivo NMR spectra. Magn Reson Med 1993; 30:672-679.

19. Zhang Y, Brady M, Smith S. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. IEEE Trans Med Imaging 2001; 20:45-57.

20. Mikkelsen M, Singh KD, Sumner P, Evans CJ. Comparison of the repeatability of GABA-edited magnetic resonance spectroscopy with and without macromolecules suppression. Magn Reson Med 2016; 7:946-953.

21. Shungu DC, Mao X, Gonzales R, et al. Brain γ-aminobutyric acid (GABA) detection in vivo with the J-editing 1H MRS technique: a comprehensive methodological evaluation of sensitivity enhancement, macromolecule contamination and test-retest reliability. NMR Biomed 2016; 29:e3914.

22. Mullins PG, McConigle DJ, O’Gorman RL, Puts NAJ, Vidyasagar R, Evans CJ. Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. Neuroimage 2014; 86:43-52.

23. Gasparovic Ch, Chen H, Mullins PG. Errors in 1H-MRS estimates of brain concentrations caused by failing to take into account tissue-specific signal relaxation. NMR Biomed 2018; 31:e3914.

24. Quadrelli S, Mountford C, Ramadan S. Hitchhiker’s guide to voxel segmentation for partial volume correction of in vivo magnetic resonance spectroscopy. Magn Reson Insights 2016; 9:1-8.
25. Abbas Z, Gras V, Möllenhoff K, Oros-Peusquens AM, Shah NJ. Quantitative water content mapping at clinically relevant field strengths: A comparative study at 1.5 and 3T. Neuroimage 2015; 106:404-413.

26. Sabati M and Maudsley AA. Fast and high-resolution quantitative mapping of tissue water content with full brain coverage for clinically-driven studies. Magn Reson Imag 2013; 31:1752-1759.

27. Edden RAE, Intrapiromkul J, Zhu H, Cheng Y, Barker PB. Measuring T2 in vivo with J-difference editing: application to GABA at 3 Tesla. J Magn Reson Imag 2012; 35:229-234.

28. Puts NAJ, Barker PB, Edden RAE. Measuring the longitudinal relaxation time of GABA in vivo at 3 Tesla. J Magn Reson Imag 2013; 37:999-1003.

29. Lin C, Bernstein M, Huston J, Fain S. Measurements of T1 relaxation times at 3.0 T: Implication for MRA. In: Proceedings of the 9th Annual Meeting of ISMRM; 2001; Glasgow, 2001:1391.

30. Piechnik SK, Evans J, Bary LH, Wise RG, Jezzard P. Functional changes in CSF volume estimated using measurement of water T2 relaxation. Mag Reson Med 2009; 61,579-586.

31. Stanisz GJ, Odrobina EE, Pun J, et al. T1, T2 relaxation and magnetization transfer in tissue at 3 T. Magn Reson Med 2005; 54:507-512.

32. Träber F, Block W, Lamerichs R, Gieseke J, Schild HH. 1H metabolite relaxation times at 3.0 Tesla: Measurements of T1 and T2 values in normal brain and determination of regional differences in transverse relaxation. J Magn Reson Imag 2004; 19:537-545.
33. Ethofer T, Mader I, Seeger U, et al. Comparison of longitudinal metabolite relaxation times in different regions of the human brain at 1.5 and 3 Tesla. Magn Reson Med 2003; 50:1296-1301.

34. Geramita N, van der Veen JW, Barnett AS, et al. Reproducibility of prefrontal \( \gamma \)-aminobutyric acid measurements with J-edited spectroscopy. NMR Biomed 2001; 24:1098-1098.

35. Mikkelsen M, Rimbault DL, Barker PB, et al. Big GABA II: Water-referenced edited MR spectroscopy at 25 research sites. Neuroimage 2019; 191:537-548.

36. Choi C, Bhardwaj PP, Kalra S, et al. Measurement of GABA and contaminants in gray and white matter in human brain in vivo. Magn Reson Med 2007; 58:27-33.
Figure 2
residue

NAAG

NAA

Gln

Glu

GABA+

estimate

original

Figure 4
Figure 5

The bar graph shows the GABA concentration (mmol/kg) for different internal concentration references. The concentrations are as follows:

- **H₂O**: Significantly higher than the other groups with a p-value of less than 0.001.
- **tCho**, **tCr**, and **tNAA**: The concentrations are not significantly different from each other, with a p-value greater than 0.05.