

RESEARCH ARTICLE

GABA quantification in human anterior cingulate cortex

Jan Weis^{1*}, Jonas Persson², Andreas Frick³, Fredrik Åhs⁴, Maarten Versluis⁵, Daniel Alamidi⁶

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

* jan.weis@radiol.uu.se**OPEN ACCESS**

Citation: Weis J, Persson J, Frick A, Åhs F, Versluis M, Alamidi D (2021) GABA quantification in human anterior cingulate cortex. PLoS ONE 16(1): e0240641. <https://doi.org/10.1371/journal.pone.0240641>

Editor: Peter Lundberg, Linköping University, SWEDEN

Received: September 27, 2020

Accepted: January 6, 2021

Published: January 15, 2021

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: <https://doi.org/10.1371/journal.pone.0240641>

Copyright: © 2021 Weis et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the manuscript and its [Supporting Information](#) files.

Funding: J.P. and A. F. were supported by the Swedish Research Council, the Swedish 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, B_0 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 γ -aminobutyric acid (GABA). It should be noted, that both these compounds are not only neurotransmitters. They have other metabolic functions as well. In fact, only a small portion of the Glu

Foundation, and the Swedish Foundation for Humanities and Social Sciences (Riksbankens jubileumsfond). A.F. was supported by The Kjell and Märta Beijer Foundation and J.P. was supported by a postdoctoral grant from the Swedish Brain Foundation. The funders provided support in the form of salaries for authors [J.P., A.F.], but did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The specific roles of these authors are articulated in the 'author contributions' section.

Competing interests: Our co-authors D.A. and M.V. declare no conflict of interests in respect of their commercial affiliation in the Philips Healthcare. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

and GABA are neurotransmitters. Glu is the major excitatory transmitter in the brain [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 ($^4\text{CH}_2$) at 3.01 ppm and C3 protons ($^3\text{CH}_2$) 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). 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. It should be noted that 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 T_1 and T_2 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. The overarching aim is to contribute to the improvement of the GABA quantification methodology.

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 LCMoel'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 K_2HPO_4 , 28 mM KH_2PO_4 , 1 g/L NaN_3 , 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 T_1 -weighted turbo FFE images (TR/TE 8/3.8 ms, isotropic resolution $1 \times 1 \times 1 \text{ mm}^3$) 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 $4 \times 4 \times 2 \text{ cm}^3$, 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



Fig 1. Spectroscopy voxel position. Representative voxel ($4 \times 4 \times 2 \text{ cm}^3$) placement in anterior cingulate cortex and the results of partial volume segmentation of CSF, GM and WM (brown). Transversal images (reconstructed pixel size $0.47 \times 0.47 \text{ mm}^2$, slice thickness 2 mm) were used for segmentation and for reconstruction of coronal and sagittal slices.

<https://doi.org/10.1371/journal.pone.0240641.g001>

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 B_0 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. MEGA-PRESS and PRESS sequence performed water suppression by two selective RF pulses and spoiler gradients. Suppression was accomplished by adjusting the flip angle of the second RF pulse such that the longitudinal magnetization of the water signal was minimal at the time of the first excitation RF pulse.

Reference frequency of the PRESS pulses was centered at tCr/GABA (~3 ppm) in water-suppressed scans. Frequency offset was centered at water in scans without water suppression, ie, there was no chemical shift displacement between the water and GABA PRESS-boxes. Chemical shift displacement between GABA and tNAA (2 ppm) PRESS boxes was approximately 2.2, 3.7, and 1.9 mm in left-right (90° RF pulse, slice thickness 40 mm), ~feet-head (180° RF pulse, slice thickness 40 mm), and ~anterior-posterior (180° RF pulse, slice thickness 20 mm) directions, respectively. Chemical shift displacement (absolute value) between GABA and tCho (~3.2 ppm) PRESS boxes was lower by factor 5 compare to displacement between tCr/GABA and tNAA PRESS boxes. It should be noted that 90° RF-pulse with broader bandwidth (BW) produces lower chemical shift displacement than 180° RF-pulses with narrow BW.

MEGA-PRESS spectra of GABA, Glu, Gln, and NAA aqueous solutions were measured a few hours after preparation. The voxel size was 3x3x3 cm³ 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. All in vivo OFF spectra were averaged and tCho, tCr, and tNAA singlets were fitted using AMARES algorithm (Fig 2). The zero-order phase correction was estimated by AMARES. The in vivo difference (ON-OFF) spectra were fitted by the QuasarX algorithm (QUEST with new constrains and shape peak selection). This time-domain algorithm uses prior knowledge obtained either theoretically by quantum mechanics, or by measuring in vitro aqueous metabolite solutions (Fig 3). Non-linear least-squares algorithm fits a weighted combination of metabolite FIDs to the considered in vivo FID. Our basis set of FID signals was obtained from MEGA-PRESS spectra of GABA (GABA+), Glu, Gln, NAA and NAAG aqueous solutions (Fig 3). 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 3). Gaussian line shapes were used to fit GABA+ spectral lines. The in vivo difference spectra were first averaged and then fitted by the QuasarX algorithm (Fig 4). The zero-order phase correction was estimated by QuasarX.

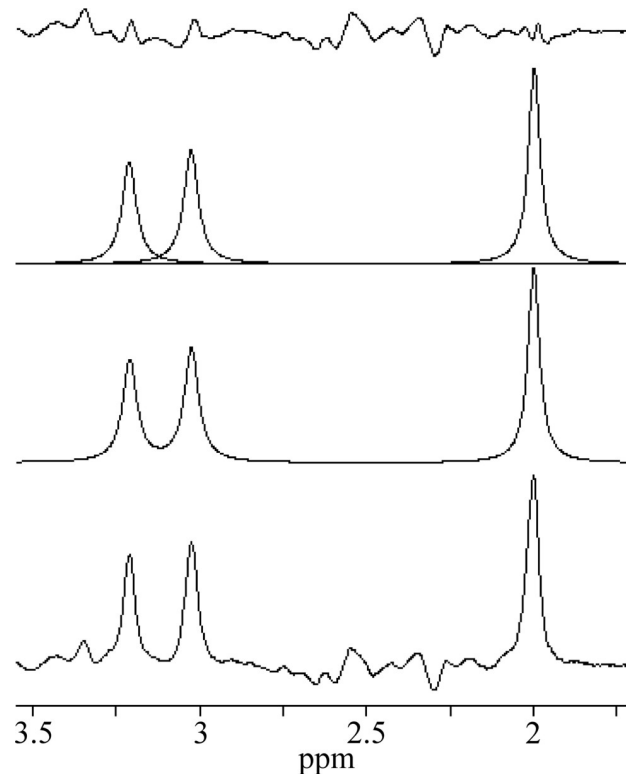


Fig 2. An example of averaged in vivo OFF spectrum. AMARES fits of the tCho, tCr, and tNAA singlets, and residue.

<https://doi.org/10.1371/journal.pone.0240641.g002>

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:

$$C_{\text{GABA}} = \frac{I_{\text{GABA}}}{I_{\text{H}_2\text{O}}} \times \frac{2}{N_{\text{GABA}}} \times \frac{1}{R_{\text{GABA}}} \times W_{\text{conc}} \times \frac{\text{MM}_{\text{cor}}}{\text{eff}_{\text{GABA}}} \quad (1)$$

where I_{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_{GABA} resonance, R_{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_{conc} is the reference water concentration corrected for partial volume and relaxation effects [16,23,24]:

$$W_{\text{conc}} = \frac{W_{\text{H}_2\text{O}}(f_{\text{GM}}R_{\text{H}_2\text{O-GM}} + f_{\text{WM}}R_{\text{H}_2\text{O-WM}} + f_{\text{CSF}}R_{\text{H}_2\text{O-CSF}})}{(1 - f_{\text{CSF}})} \quad (2)$$

and

$$f_x = \frac{c_x \vartheta_x}{0.82\vartheta_{\text{GM}} + 0.7\vartheta_{\text{WM}} + 0.99\vartheta_{\text{CSF}}} \quad (3)$$

where $W_{\text{H}_2\text{O}}$ 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, ϑ_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].

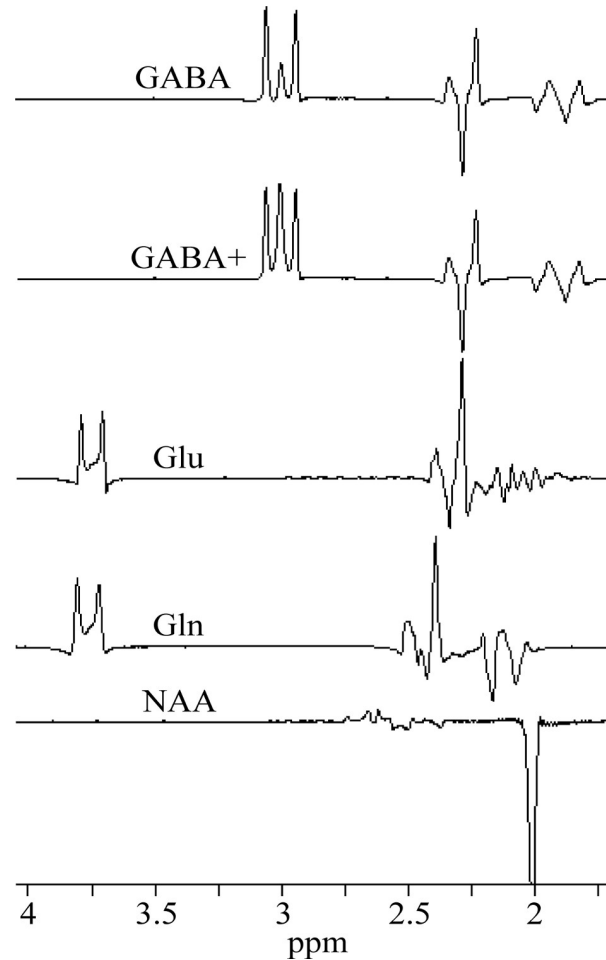


Fig 3. Basis set of in vitro spectra. MEGA-PRESS spectra of GABA, Gln, Glu, and NAA aqueous solutions, and simulated GABA+.

<https://doi.org/10.1371/journal.pone.0240641.g003>

$R_{\text{H}_2\text{O-GM}}$, $R_{\text{H}_2\text{O-WM}}$, and $R_{\text{H}_2\text{O-CSF}}$ are PRESS relaxation attenuation factors $R = \exp(-TE/T_2) \times [1 - \exp(-TR/T_1)]$ of water in GM, WM, and CSF, respectively. The following relaxation times were used for corrections: GABA (T_1 1310 ms, T_2 88 ms) [27,28], water in GM (T_1 1820 ms, T_2 99 ms), WM (T_1 1084 ms, T_2 69 ms), and CSF (T_1 4163 ms, T_2 503 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. $WCONC = W_{\text{conc}}$ [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:

$$C_{\text{GABA}} = \frac{I_{\text{GABA}}}{I_{\text{MET}}} \times \frac{N_{\text{MET}}}{N_{\text{GABA}}} \times \frac{R_{\text{MET}}}{R_{\text{GABA}}} \times C_{\text{MET}} \times \frac{MM_{\text{cor}}}{\text{eff}_{\text{GABA}}} \quad (4)$$

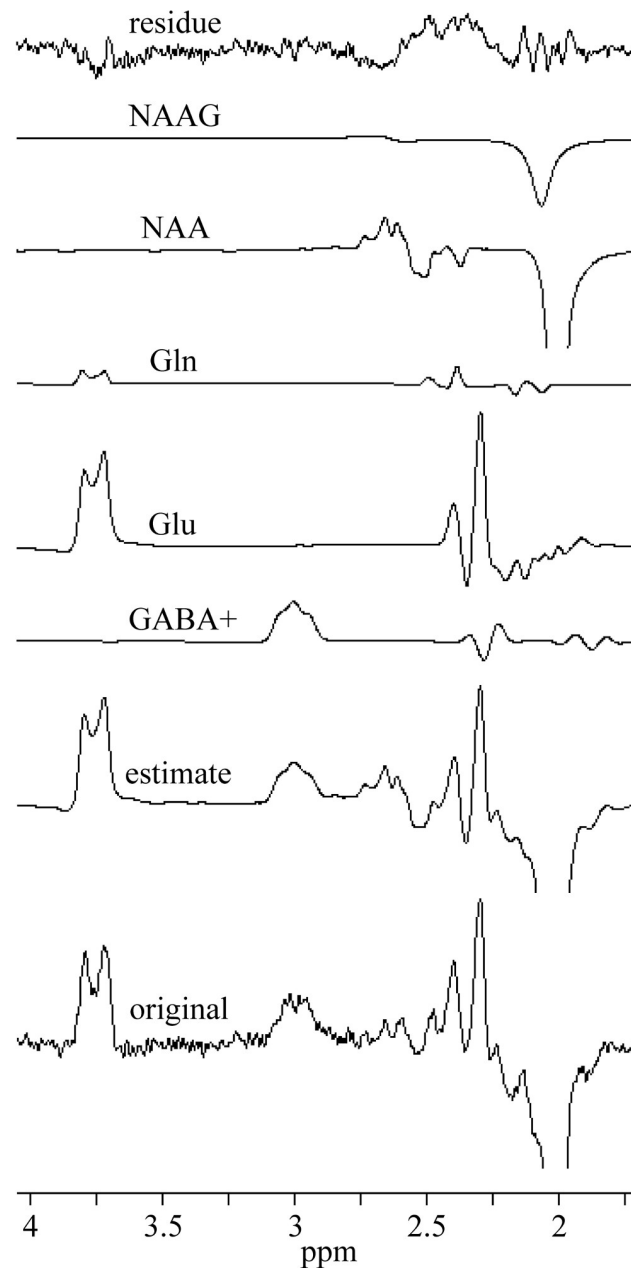


Fig 4. Representative in vivo MEGA-PRESS spectrum and fits. The difference spectra were averaged and fitted by the QuasarX algorithm using the basis set spectra shown in Fig 2.

<https://doi.org/10.1371/journal.pone.0240641.g004>

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 (T_1 1140 ms, T_2 230 ms), tCr (T_1 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 \pm 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. The OFF and difference (ON-OFF) spectra of all volunteers are shown in S1 Fig (Supporting information). 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 3. These spectra were used as prior knowledge for fitting the volunteer's MEGA-PRESS spectra using the QuasarX algorithm. Figs 2 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 were 0.070 ± 0.01 , 0.052 ± 0.007 , and 0.088 ± 0.013 , respectively. The absolute GABA concentrations (mmol/kg) were computed according to Eqs 1 and 4 using tissue water (2.57 ± 0.26 [2.7]), tCho (1.63 ± 0.22 [3.1]), tCr (1.46 ± 0.19 [2.6]), and tNAA (1.61 ± 0.22 [3.1]) as internal concentration references. The square brackets depict relative variances. Concentrations are visualized in Fig 5. Two-tailed *F*-tests detected no differences in the variances of GABA concentrations obtained by different methods.

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 precision 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.

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

	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).

<https://doi.org/10.1371/journal.pone.0240641.t001>

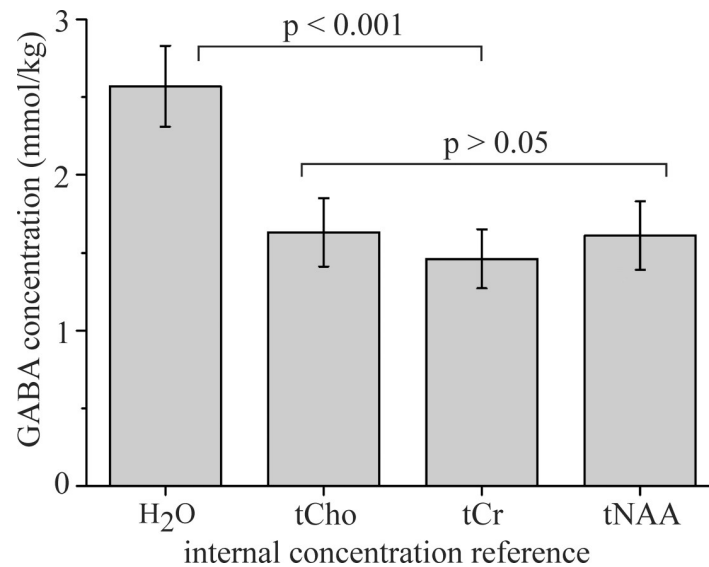


Fig 5. GABA concentrations. Concentrations (mmol/kg) were estimated using H₂O (2.57±0.26), tCho (1.63±0.22), tCr (1.46 ± 0.19), and tNAA (1.6±0.22) as internal concentration references.

<https://doi.org/10.1371/journal.pone.0240641.g005>

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 LCMoel fits (Table 1) indicate very good fitting results of the LCMoel algorithm. 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 LCMoel 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 H₂O, 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 [11–15,34,35]. 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 and MEGA-PRESS are surprisingly in very good agreement. However, water referenced GABA concentrations were significantly higher than the concentrations estimated with tCho, tCr and tNAA references (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 (Eqs 2 and 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 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.

Supporting information

S1 Fig. MEGA-PRESS spectra. OFF spectra (left) and corresponding difference spectra (right) of all volunteers. (TIF)

Author Contributions

Conceptualization: Jan Weis, Jonas Persson, Andreas Frick, Fredrik Åhs, Maarten Versluis, Daniel Alamidi.

Data curation: Jan Weis, Jonas Persson, Andreas Frick, Daniel Alamidi.

Formal analysis: Jan Weis, Jonas Persson, Andreas Frick, Fredrik Åhs, Maarten Versluis, Daniel Alamidi.

Funding acquisition: Jonas Persson, Andreas Frick, Fredrik Åhs.

Methodology: Jan Weis, Jonas Persson, Andreas Frick, Fredrik Åhs, Maarten Versluis, Daniel Alamidi.

Project administration: Andreas Frick, Daniel Alamidi.

Resources: Maarten Versluis, Daniel Alamidi.

Software: Maarten Versluis, Daniel Alamidi.

Supervision: Daniel Alamidi.

Validation: Jonas Persson, Fredrik Åhs, Daniel Alamidi.

Visualization: Jan Weis.

Writing – original draft: Jan Weis.

Writing – review & editing: Jan Weis, Jonas Persson, Andreas Frick, Fredrik Åhs, Maarten Versluis, Daniel Alamidi.

References

1. Zhou Y, Danbolt NC. Glutamate as a neurotransmitter in the healthy brain. *J Neural Transm* 2014; 121:799–817. <https://doi.org/10.1007/s00702-014-1180-8> PMID: 24578174
2. McCormic DA. GABA as an inhibitory neurotransmitter in human cerebral cortex. *J Neurophysiol* 1989; 62:1018–1027. <https://doi.org/10.1152/jn.1989.62.5.1018> PMID: 2573696
3. Mikkelsen M, Barker PB, Bhattacharyya PK, et al. Big GABA: Edited MR spectroscopy at 24 research sites. *Neuroimage* 2017; 159: 32–45. <https://doi.org/10.1016/j.neuroimage.2017.07.021> PMID: 28716717
4. Harris AD, Saleh MG, Eden RAE. Edited 1H magnetic resonance spectroscopy in vivo: Methods and metabolites. *Magn Reson Med* 2017; 77:1377–1389. <https://doi.org/10.1002/mrm.26619> PMID: 28150876
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. <https://doi.org/10.1002/mrm.21961> PMID: 19319893
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. <https://doi.org/10.1002/mrm.27233> PMID: 29752742
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. <https://doi.org/10.1002/mrm.22086> PMID: 19591201
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. [https://doi.org/10.1002/\(sici\)1099-1492\(199810\)11:6<266::aid-nbm530>3.0.co;2-j](https://doi.org/10.1002/(sici)1099-1492(199810)11:6<266::aid-nbm530>3.0.co;2-j) PMID: 9802468
9. Öngür D, Prescott AP, Jensen JE, Cohen BM, Renshaw PF. Creatine abnormalities in schizophrenia and bipolar disorder. *Psychiatry Res* 2009; 172:44–48. <https://doi.org/10.1016/j.psychresns.2008.06.002> PMID: 19239984
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. <https://doi.org/10.1016/j.psychresns.2008.07.015> PMID: 19427767
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. <https://doi.org/10.1002/mrm.25549> PMID: 25521836
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. <https://doi.org/10.1002/jmri.22520> PMID: 21509888
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. <https://doi.org/10.1016/j.mri.2010.10.009> PMID: 21232891
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. <https://doi.org/10.1002/jmri.25324> PMID: 27264205

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. <https://doi.org/10.1002/mrm.20901> PMID: 16688703
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. <https://doi.org/10.1007/BF02668096> PMID: 11390270
18. Provencher SW. Estimation of metabolite concentrations from localized in vivo NMR spectra. *Magn Reson Med* 1993; 30:672–679. <https://doi.org/10.1002/mrm.1910300604> PMID: 8139448
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. <https://doi.org/10.1109/42.906424> PMID: 11293691
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; 29:932–942. <https://doi.org/10.1002/nbm.3539> PMID: 27173449
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. <https://doi.org/10.1016/j.neuroimage.2012.12.004> PMID: 23246994
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. <https://doi.org/10.1002/nbm.3914> PMID: 29727496
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. <https://doi.org/10.4137/MRI.S32903> PMID: 27147822
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. <https://doi.org/10.1016/j.neuroimage.2014.11.017> PMID: 25463455
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. <https://doi.org/10.1016/j.mri.2013.08.001> PMID: 24050900
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. <https://doi.org/10.1002/jmri.22865> PMID: 22045601
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. <https://doi.org/10.1002/jmri.23817> PMID: 23001644
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. *Magn Reson Med* 2009; 61:579–586. <https://doi.org/10.1002/mrm.21897> PMID: 19132756
31. Stanisz GJ, Odobina EE, Pun J, et al. T1, T2 relaxation and magnetization transfer in tissue at 3 T. *Magn Reson Med* 2005; 54:507–512. <https://doi.org/10.1002/mrm.20605> PMID: 16086319
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. <https://doi.org/10.1002/jmri.20053> PMID: 15112302
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. <https://doi.org/10.1002/mrm.10640> PMID: 14648578
34. Geramita N, van der Veen JW, Barnett AS, et al. Reproducibility of prefrontal γ -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. <https://doi.org/10.1016/j.neuroimage.2019.02.059> PMID: 30840905
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. <https://doi.org/10.1002/mrm.21275> PMID: 17659613