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Tissue correction for GABA-edited MRS: considerations of voxel composition, tissue segmentation and tissue relaxations

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Abstract

Purpose—To develop a tissue correction for GABA-edited MRS that appropriately addresses differences in voxel gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) fractions.

Methods—Simulations compared the performance of tissue correction approaches. Corrections were then applied to in vivo data from sixteen healthy volunteers, acquired at 3T. GM, WM and CSF fractions were determined from T1-weighted images. Corrections for CSF content, GM/WM GABA content, and water relaxation of the three compartments are combined into a single, fully corrected measurement.

Results—Simulations show that CSF correction increases the dependence of GABA measurements on GM/WM fraction, by an amount equal to the fraction of CSF. Furthermore, GM correction substantially (and non-linearly) increases the dependence of GABA measurements on GM/WM fraction, for example, by a factor of over four when the voxel GM tissue fraction is 50%. At this tissue fraction, GABA is over estimated by a factor of 1.5.

For the in vivo data, correcting for voxel composition increased measured GABA values ($p<0.001$ for all regions), but did not reduce inter-subject variance ($p>0.5$ for all regions). Corrected GABA values differ significantly based on the segmentation procedure used ($p<0.0001$) and tissue parameter assumptions made ($p<0.0001$).

Conclusion—We introduce a comprehensive tissue correction factor that adjusts GABA measurements to correct for different voxel compositions of GM, WM, and CSF.

Keywords

GABA-edited MRS; Voxel localization; Tissue segmentation; Tissue correction; Voxel Coregistration

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Introduction

Magnetic resonance spectroscopy (MRS) is a non-invasive technique that allows the detection and quantification *in vivo* of endogenous metabolites. It is increasingly being applied to probe the inhibitory neurotransmitter GABA (1). Due to the low concentration of GABA and the overlapping nature of the spectrum, MRS of GABA usually employs an edited experiment with a relatively large volume of excitation (2-4). Such large voxels inevitably contain substantial amounts of gray matter (GM) in which the bulk of GABAergic inhibitory activity occurs, white matter (WM) that contains less GABA, and cerebrospinal fluid (CSF) which contains negligible amounts of GABA. In any group of participants, there will be some degree of variation in the tissue composition of the voxel which, given the differences in GABA concentration of the tissues, will lead to variance in GABA measurements.

One response to this tissue-related variance is simply to accept it – to say that the role of MRS is to estimate the concentration of GABA in the voxel of interest and that changes in tissue composition contribute to that. However, MRS findings driven by bulk tissue changes/differences are generally held to be of less interest, and therefore, correction of measurements for differences in voxel composition has received increasing amounts of attention. There are two alternative approaches - using tissue composition parameters as co-variates in statistical analyses (3), or applying a mathematical correction factor based on voxel composition to give a tissue-corrected concentration estimate (5).

Correcting for voxel tissue composition is potentially beneficial to both the precision and accuracy of GABA quantification (3,6). In terms of precision, a correction for voxel tissue composition is desirable if inter-subject variance in tissue composition is a substantial contributor to variance in GABA measurements *and* if it is possible to experimentally determine appropriate correction factors to remove that variance. Correction for voxel tissue composition can potentially account for several factors including: different tissue concentrations of GABA, different tissue concentrations of MR-visible water, and relaxation of the water signal that differs between tissues. Correction requires some or all of the following to be determined: voxel composition in terms of GM, WM and CSF fractions; water relaxation times for each tissue compartment; MR visible water concentrations for each tissue compartment; GABA relaxation times for each tissue compartment; and contributing GABA concentrations for each tissue compartment. The accuracy with which these are determined, whether through experiment or from literature values, will impact the success of correction. Voxel tissue composition can be determined either by segmentation of T1-weighted brain images using tools such as SPM (7) and FSL (8), or by acquisition of additional data (9). Water and GABA parameters are generally determined from the literature (6,10-15); however, the literature values for these parameters are variable.

While it is generally agreed that the ideal correction factor will account for all these tissue-related factors, correction is often not performed and when it is, there is no standard methodology. The most common approach is to simply remove the impact of the CSF on the concentration estimate by inflating the measured GABA by the fraction of tissue within the voxel (e.g. refs. (16,17)). Another common approach corrects based on the voxel GM

fraction alone (e.g. (18)). Both approaches have their weaknesses – the former effectively ignores difference in GABA concentration between GM and WM, while the latter assumes that WM contains no GABA. One aim of this manuscript is to demonstrate the impact of these assumptions. These corrections are generally applied in manuscripts with the aim that findings based on corrected GABA values will not be impacted by differences in voxel segmentation, and against this benchmark they fail. In order to remove the bias of voxel segmentation from GABA estimates, it is necessary to account for both the voxel fractions of GM and WM and the difference in GABA concentration between the two. In accounting for the differences in GABA concentration in GM and WM, it becomes clear that the segmentation routine and tissue-specific water relaxation and visibility parameters can impact quantitative results.

The purpose of this paper is to investigate the performance of current standard tissue corrections in the context of a metabolite, GABA, with different concentration in gray matter (GM), and white matter (WM), and to develop a tissue correction for GABA-edited MRS that appropriately addresses differences in voxel GM, WM and cerebrospinal fluid (CSF) fractions.

Materials and Methods

Correcting for GABA concentration differences

Consider a voxel containing three compartments corresponding to GM, WM and CSF, with volume fractions f_{GM}, f_{WM}, f_{CSF} respectively, and suppose that the concentration of GABA in each compartment is c_{GM}, c_{WM}, c_{CSF} respectively. The measured concentration of GABA in the whole voxel c_{meas} will be:

$$c_{\mathrm{meas}}=c_{\mathrm{GM}}f_{\mathrm{GM}}+c_{\mathrm{WM}}f_{\mathrm{WM}}+c_{\mathrm{CSF}}f_{\mathrm{CSF}}. \quad [1]$$

Values of c_{meas} can differ either due to differences in the volume fractions or differences in the concentrations. It is helpful that the concentration of GABA in CSF is negligible, so that:

$$c_{\mathrm{meas}} \sim c_{\mathrm{GM}}f_{\mathrm{GM}}+c_{\mathrm{WM}}f_{\mathrm{WM}}.$$

Correction has been based either on the total tissue fraction in the voxel, i.e., $c_{\mathrm{CSFcorr}}=\frac{c_{\mathrm{meas}}}{\mathrm{f_{GM}}+\mathrm{f_{WM}}}$ (also known as CSF-correction in the literature) or the GM fraction, i.e., $c_{\mathrm{GMcorr}}=\frac{c_{\mathrm{meas}}}{\mathrm{f_{GM}}}$. We maintain the slightly conflicting terminology generally used in the literature – CSF correction involves removing the CSF fraction, whereas GM correction only takes the GM fraction into account. Note also that we frame this discussion in terms of concentration and volume fractions, not signal fractions; f_{GM} here is equivalent to f_{GM_vol} in reference (6). These two corrections can be generalized to:

$$c_{\text{tisscorr}} = \frac{c_{\text{meas}}}{f_{\text{GM}} + \propto f_{\text{WM}}}. \quad [2]$$

CSF correction can be applied by setting α to 1; GM correction can be applied by setting α to 0. In fact, α in this equation represents an assumed ratio between the GABA concentrations in WM and GM. The correction in Equation 2 normalizes c_{tisscorr} so that *it represents what the measured concentration would be if the voxel were entirely GM*. This is undesirable, as it does not reflect the tissue that is being studied, and a better correction factor is one that is based on the tissue composition of the voxel of interest:

$$c_{GMWMcorr} = \frac{c_{\text{meas}}}{(f_{\text{GM}} + \propto f_{\text{WM}})} \frac{\mu_{GM} + \alpha\mu_{WM}}{\mu_{GM} + \mu_{WM}}, \quad [3]$$

where μ_{GM} and μ_{WM} are the GM and WM fractions of the group average voxel fractions. In this manuscript, μ_{GM} and μ_{WM} are data-driven and defined as the average GM and WM fractions for each voxel. If the tissue fractions in an individual voxel are equal to the group averages (i.e., if $f_{\text{GM}} = \mu_{\text{GM}}$ and $f_{\text{WM}} = \mu_{\text{WM}}$), this correction simplifies to $1/(\mu_{\text{GM}}+\mu_{\text{WM}})$ and the correction amounts to CSF correction. For other compositions, it further adjusts to account for greater or lower than average f_{GM}.

GABA concentration ratio α simulations

Uncorrected GABA concentrations were simulated for theoretical voxels with 8% CSF and varying amounts of GM and WM, based on $c_{\text{GM}} = 1$, $c_{\text{WM}} = 0.5$ and $c_{\text{CSF}} = 0$ according to Equation 1. Based on Equation 2 above, corrected GABA index concentrations were also calculated based upon α values of 1 (CSF correction), 0 (GM correction), 0.5 (the simulated correct value as defined by on the ratio of GABA concentrations in WM and GM), 0.7 and 0.3 (incorrect estimates of α). For the correction of *in vivo* data, α is not known; we therefore include these incorrect values of α in order to investigate the impact of estimation. In order to demonstrate that findings are not specific to voxels containing 8% CSF, these simulations were repeated for voxels with 30% CSF (chosen to represent atrophy).

These corrections can be compared using two different criteria: the accuracy of the GABA concentration index for a specific tissue fraction (relative to the correct value of 1) and the slope of dependency of the GABA concentration index on tissue fraction.

Experimental Data

In order to compare the correction factors, a dataset was analyzed in which 5 regional GABA measurements have been made in 16 healthy volunteers. This study was approved by the local IRB and all volunteers provided informed, written consent. All scanning was performed at 3T (‘Achieva’, Philips Healthcare, The Netherlands) with a 32-channel head coil. Scanning included a T1-weighted whole brain image, (MPRage, TR/TE = 8 ms/3.7 ms, 1 mm^3 isotropic voxels) and single voxel GABA-edited MRS in 5 voxel locations (visual cortex, OCC, auditory cortex, AUD, sensorimotor cortex, SM, frontal eye fields, FEF and dorsolateral prefrontal cortex, DLPFC). All voxels were 3 × 3 × 3 cm^3, except for the AUD

which was 4 × 3 × 2 cm^3. The GABA-edited MRS was collected using a MEGA-PRESS experiment (19). Editing pulses were applied at 1.9 ppm and 7.46 ppm, interleaving every two transients across a 16-step phase cycle, TR/TE = 2s/68 ms; 320 transients, 2048 data points at a spectra width of 2 kHz; VAPOR water suppression and first-order and second-order shim parameters were derived using pencil-beam projection-based shimming routine. The limited selectivity of the editing pulses (14 ms duration) results in co-editing of macromolecules (MM), thus quantified GABA includes MM contamination, often referred to as GABA+.

Segmentation of T1 images and Coregistration of MRS acquisition volumes

Two segmentations of each T1-weighted image were performed, using both SPM8 (New Segment, (7)) and FSL5.0 (FAST (8)); default parameters were used for both segmentations.

A binary mask of the voxel location was constructed in the same imaging matrix as the T1-weighted image. This was accomplished using the voxel size, orientation and location information from the header of each individual acquisition to determine the coordinates of the voxel corners in the laboratory frame of reference. The convex hull of these points was then used to generate the voxel volume and express it as a binary mask in the T1-weighted image frame of reference. This voxel mask was then applied to the SPM segmentation to determine the voxel GM, WM and CSF fractions. This functionality is now available within the Gannet 2.0 toolbox (20) as Gannet Co Register and Gannet Segment. For the comparison of segmentation tools, a binary mask was similarly reconstructed using SVMask (Michael Schär, Philips Medical Systems) and applied to the segmentation results from FSL.

The tissue fractions for WM, GM and CSF within the MRS voxel were then calculated. The partial volume maps generated from FAST (as opposed to the binary segmentation) were used for the MRS voxel segmentation analysis. The agreement between the SPM- and the FSL-derived tissue fractions for all five voxels was investigated using Bland-Altman methods (21).

Correcting for water signal differences

Water-referenced GABA concentrations (c_G) are usually estimated based on an equation such as:

$$c_G = \frac{c_w \mathrm{MM} \exp\left(-\frac{\mathrm{TE}}{T_{2w}}\right)\left(1-\exp\left(-\frac{\mathrm{TR}}{T_{1w}}\right)\right) I_G}{\kappa \exp\left(-\frac{\mathrm{TE}}{T_{2G}}\right)\left(1-\exp\left(-\frac{\mathrm{TR}}{T_{1G}}\right)\right) I_w}$$

in which I_G and I_w are the signal integrals for GABA (modeled as a 5 parameter Gaussian) and water (modeled as a Gaussian-Lorentzian), c_w is the visible water concentration, the concentration of water is assumed to be 55000 mM, and the MR-visible fraction is assumed to be 0.65, κ is the editing efficiency of GABA, assumed to be 0.5, MM is a correction factor for co-edited macromolecular signal (3), T_{1G} T_{1w} T_{2G} T_{2w} are the T_1 and T_2 relaxation time constants for GABA and water. Literature values for T_{1G}, T_{1W}, T_{2G} and

T_{2W} are assumed (T_{1G} = 800 ms, T_{1W} = 1100 ms, T_{2G} = 88 ms and T_{2W} = 95 ms (20)). Here, relaxation times and the visible water concentration do not differentiate between tissue compartments. All of these constants are standard values from the literature and are that of the standard implementation in Gannet (3,20). TE and TR are the experimental echo time and repetition time (3,20).

Incorporating different c_w T_{1w} *and* T_{2w} for each tissue compartment *i* leads to the following water-corrected GABA estimate:

$$= \frac{\mathrm{MM}I_{\mathrm{G}}}{\kappa I_{\mathrm{w}}} \left(\frac{\sum_i^{GM,WM,CSF} c_{\mathrm{w,i}} \exp\left(-\frac{\mathrm{TE}}{T_{2\mathrm{W,i}}}\right)\left(1-\exp\left(-\frac{\mathrm{TR}}{T_{1\mathrm{w,i}}}\right)\right) f_i}{\exp\left(-\frac{\mathrm{TE}}{T_{2\mathrm{G}}}\right)\left(1-\exp\left(-\frac{\mathrm{TR}}{T_{1\mathrm{G}}}\right)\right)} \right), \quad [4]$$

where the sum is over the three compartments (GM, WM and CSF) each with individually specified relaxation parameters and visible water concentration. Tissue compartment values for GABA are not available in the literature, therefore the assumption of a single value for T_{1G} and T_{2G} for all tissue (GM, WM and CSF) is maintained; T_{1G} = 800 ms (3) and T_{2G} = 88 ms (11).

The following literature-derived relaxation parameters of water were used: white matter $T_{1w,WM}$ = 832 ms and $T_{2w,WM}$ = 79.2ms; gray matter $T_{1w,GM}$ = 1331 ms and $T_{2w,GM}$ = 110 ms (13); CSF $T_{1w,CSF}$ = 3817 ms (15) and $T_{2w,CSF}$ = 503 ms (12). MR-visible water concentrations ($c_{w,i}$) of 36.1 mol/dm^3, 43.3 mol/dm^3 and 53.8 mol/dm^3 for WM, GM and CSF, respectively were derived from published MR-visible water fractions (6).

Correcting for GABA concentration differences between compartments (as addressed above) leads to the compartment corrected GABA estimate:

$$= \frac{\mathrm{MM}I_{\mathrm{G}}}{\kappa I_{\mathrm{w}}} \left(\frac{\sum_i^{GM,WM,CSF} c_{\mathrm{w,i}} \exp\left(-\frac{\mathrm{TE}}{T_{2\mathrm{w,i}}}\right)\left(1-\exp\left(-\frac{\mathrm{TR}}{T_{1\mathrm{w},i}}\right)\right) f_i}{\exp\left(-\frac{\mathrm{TE}}{T_{2\mathrm{G}}}\right)\left(1-\exp\left(-\frac{\mathrm{TR}}{T_{1\mathrm{G}}}\right)\right)} \right) \left(\frac{1}{f_{\mathrm{GM}} + \propto f_{\mathrm{WM}}} \right). \quad [5]$$

Then normalizing to a group-average voxel, as developed in Equation 3 results in the fully corrected GABA estimates:

$$c_{\mathrm{fullcorr}} = \frac{\mathrm{MM}I_{\mathrm{G}}}{\kappa I_{\mathrm{w}}} \left(\frac{\sum_i^{GM,WM,CSF} c_{\mathrm{w,i}} \exp\left(-\frac{\mathrm{TE}}{T_{2\mathrm{w,i}}}\right)\left(1-\exp\left(-\frac{\mathrm{TR}}{T_{1\mathrm{w,i}}}\right)\right) f_i}{\exp\left(-\frac{\mathrm{TE}}{T_{2\mathrm{G}}}\right)\left(1-\exp\left(-\frac{\mathrm{TR}}{T_{1\mathrm{G}}}\right)\right)} \right) \left(\frac{\mu_{\mathrm{GM}} + \propto \mu_{\mathrm{WM}}}{(\mathrm{f}_{\mathrm{GM}} + \propto \mathrm{f}_{\mathrm{WM}})\,(\mu_{\mathrm{GM}} + \mu_{\mathrm{WM}})} \right). \quad [6]$$

Quantification of GABA

Experimental data were processed with Gannet 2.0 (20) - here we refer to the water-referenced GABA concentration values (output from Gannet as MRS_struct.out.GABAconciu) as the uncorrected GABA concentrations c_{uncorr}. Tissue-specific water relaxation and concentration values were also used to generate the

compartment corrected GABA estimate, Equation 4, and $c_{fullcorr}$ according to Equation 6, with $\alpha = 0.5$ and the region-average values for μ_{GM} and μ_{WM}.

These tissue corrections have also been added to the Gannet analysis toolkit. Consistent with the processing pipeline in GannetLoad and GannetFit, these additional modules perform batch analysis. Using the T1-weighted images corresponding to the MRS data files and routines from SPM, GannetCoRegister and GannetSegment perform the MRS voxel registration, T1-weighted image segmentation and calculate f_{GM}, f_{WM} and f_{CSF} based on the voxel mask and segmentation results. GannetQuantify then uses the signal integrals for GABA and water as determined in GannetFit and applies the tissue specific relaxation and water visibility constants for each tissue fraction to quantify GABA using Equation 4. This is saved to the output structure (MRS_struct.Quantify.QuantGABA_iu). The compartment correct GABA level is also determined as per Equation 5 using $\alpha = 0.5$ and saved to the output structure (MRS_struct.Quantify.QuantCorrGABA_iu). Lastly, the normalization to the group-average voxel is determined using Equation 6. By default, μ_{GM} = average of f_{GM} values for all files supplied in the batch analysis and μ_{WM} = average of f_{WM} values for all files supplied in the batch analysis. This is saved to the output structure (MRS_struct.Quantify.QuantNormTissCorrGABA_iu).

Using the compartment corrected GABA estimate, the regression of measured GABA and GM fraction was used to determine α from the experimental, in vivo dataset. Segmentation results from SPM was used for this section.

In order to investigate the impact of parameter choices, fully corrected GABA values were also calculated for using a second set of literature-derived water relaxation parameters: white matter $T_{1w,WM}$ = 1084ms and $T_{2w,WM}$ = 69 ms; gray matter $T_{1w,GM}$ = 1820 ms and $T_{2w,GM}$ = 99 ms (14); CSF $T_{1,CSF}$ = 4300 ms (15) and $T_{2,CSF}$ = 503 ms (12).

Statistical Methods

In order to test for mean differences between uncorrected and fully corrected GABA indices, paired t-tests were performed, both for each region individually and for all data pooled across regions. F-tests were used to test for differences in variance for the same comparisons after normalizing to the group mean. Paired t-tests were used to test for mean differences in GABA indices arising from segmentation with FSL and SPM, and Bland-Altman plots were used to examine consistency of segmentation methods (21). F-tests were used to test for differences in variance between GABA values corrected based upon FSL and SPM segmentation. Paired t-tests were used to test for mean differences in GABA values arising from using different literature relaxation values.

Results

GABA concentration ratio α

Figure 1A plots corrected (solid lines) and uncorrected (dashed lines) GABA index against the tissue WM/GM fractions, for simulated voxels. The $\alpha = 0.5$ line has value 1 and zero slope. CSF correction (that corrects for the total tissue in the voxel without differentiating between GM and WM), $\alpha = 1$, underestimates the GABA index by an amount that depends

on f_{WM}, and increases the slope of the line (by $1/(1-f_{CSF})$). Conversely, with increasing f_{WM}, GM correction ($\alpha = 0$) overestimates GABA and shows a tendency to blow up. The slope of the GM correction line at a tissue fraction of 50% is more than four times steeper than that of the uncorrected line and the GABA level is over estimated by a factor of 1.5. Intermediate values of α outperform the extremes, in terms of both GABA index and slope; that is, $\alpha = 0.7$ has reduced slope and error than $\alpha = 1$ and $\alpha = 0.3$ has reduced slope and error than $\alpha = 0$. $\alpha = 0.7$ has less slope and error than $\alpha = 0.3$. Figure 1B shows the same simulation results for a CSF fraction of 30%. The corrected lines overlay exactly between Figures 1A and 1B.

In-vivo data

One spectrum from the OCC voxel, two spectra from the AUD and two spectra from SM were excluded due poor data fitting, often resulting from frequency drifts or step-changes of greater than 20 Hz (22). Example spectra are shown in Figure 2. The average water linewidths were 10.2 ± 1.5 Hz. Quantified GABA, using tissue specific relaxation parameters (Equation 4) were plotted against the GM fraction (shown in Figure 3) for all regions and α was calculated to be 0.43 based on the in vivo current dataset.

Quantification of GABA

Figure 4A compares the uncorrected GABA estimates with the fully-corrected GABA estimate for each brain region and pooling all GABA results. The corrected GABA measurements are significantly increased compared to the uncorrected measurements ($p < 0.001$ for all five voxels and all pooled). Figure 4B compares the coefficients of variation across subjects. Statistically, there is no significant difference in variance in the fully corrected data compared to the uncorrected data ($p = 0.90, 0.99, 0.71, 0.98$, and 0.92 for OCC, AUD, SM, FEF and DLPFC voxels respectively, and 0. 51 for all data pooled).

Figure 5 examines the correlation of the fully corrected, measured GABA compared to the uncorrected measurements. In Figure 5A, a strong correlation between the fully corrected data and the uncorrected data is shown for all voxels. In Figure 5B, the magnitude of the full correction factor is plotted against the uncorrected data to illustrate the variance of the correction factor. The coefficient of variation for the full correction factor is 1.7%, 1.3%, 3.3%, 2.1%, 2.1% and 5.4% for the OCC, AUD, SM, FEF, DLPFC and all data pooled, respectively.

Segmentation of T1 images by FSL and SPM

Figure 6 shows Bland-Altman plots for WM, GM and CSF segmentation results. The results of SPM and FSL are correlated; however, there are systematic differences in the GM and CSF segmentation results. FSL calculates larger f_{CSF} values than SPM. Across all voxels, FSL-corrected GABA values are significantly higher than the SPM-corrected GABA values ($p < 0.001$ for all comparisons). The choice of segmentation method does not significantly change the variance in corrected GABA values ($p = 0.89, 0.86, 0.58, 0.59$, and 0.70 for OCC, AUD, SM, FEF and DLPFC voxels respectively).

Impact of Water Relaxation Times

Figure 7 compares the corrected GABA concentrations when applying the different sets of relaxation times for each set tissue type. The GABA measurements using the Stanisz values are reduced compared to the GABA measurements using the Wansupura values ($p<0.0001$).

Discussion

The correction of MRS-derived concentration estimates for voxel tissue composition is a long-standing recommendation (5); however, the implementation of these procedures is not consistent and the implication of this inconsistency is not widely recognized. Correcting for different amounts of CSF is mathematically trivial due to its negligible GABA concentration. Correcting for tissue composition for a compound such as GABA, which is known to have different concentration in GM and WM, has received less attention. In this manuscript, we propose a correction approach that accounts for differences in GABA and MR-visible water concentrations and water relaxation behavior between the three tissue compartments. This approach is more successful than either CSF correction (which under-corrects) or GM correction (which over-corrects, and also increases the dependency of GABA measurements on voxel composition). With this correction, the dependency of the corrected GABA index on tissue fractions is removed and the estimate of GABA is accurate when using the correct α. When an assumed α close to the true value is used the dependency of GABA on the tissue fraction is reduced and a more accurate estimate is reported compared to other correction strategies. It is interesting that the corrections do not substantially reduce inter-subject variance in this cohort of young healthy adults. Corrections for differences in voxel composition are likely to have greater impact in cohorts that include disease- or age-related atrophy. This correction is now incorporated in an automated fashion within the Gannet toolkit for GABA-edited MRS.

The quantification of GABA is impacted by the composition and tissue characteristics of the voxel. This includes not only the voxel fractions of WM, GM and CSF, which can be determined by segmentation of an anatomical image, but also the relative concentrations of GABA in each tissue. In order to correct for different concentrations of GABA in GM and WM, it is necessary to estimate the concentration ratio between these compartments – a quantity we refer to as α. Clearly, the efficacy of this correction depends on the accuracy of the chosen value of α In this paper, an α-value of 0.5 has been selected. This assumption corresponds to WM having a GABA concentration that is half that of GM, a common assumption (3). It is important to recognize that α should reflect not just the ratio of GABA concentration between tissue compartments, but the ratio of GABA-edited MRS signal between compartments. This is relevant as GABA values are often contaminated by approximately 50% co-edited MM signal (3,23,24). Values for α can be determined based upon literature concentration ratios from two sources: assays of tissue extracts (both post-mortem and biopsy samples), and MRS studies that extrapolate from GM-rich and WM-rich voxels to 100% GM and WM. A crude average of tissue extract (25-27) and MRS studies measuring MM-suppressed GABA (28) gives $c_{WM}/c_{GM} = 0.35 \pm 0.20$, and of those measuring GABA+ (28-32) give $c_{WM}/c_{GM} = 0.41 \pm 0.23$. Consistent with the literature, calculating α from the current dataset results in $\alpha = 0.42$. Based on this value, the literature

and our simulations, which show that it is better to over-estimate rather than under-estimate α,we round this value to 0.5.

Correcting GABA measurements for the voxel tissue content results in an increase in estimated GABA, primarily as a result of addressing the CSF fraction that contributes to the water signal but not the GABA signal. Correction for tissue-specific water relaxation and visibility parameters also increases the GABA measurement, without reducing variance.

Consistent levels of variation between the uncorrected and fully corrected data may occur either because the correction factors are small and thus have little impact, or because variance removed by the correction is counterbalanced by additional variance introduced by it. Fully corrected GABA measurements and uncorrected measurements are highly correlated, however the correction does have some impact on the measurements. The correction factors have a relatively small inter-subject variance and show no hint of correlation with uncorrected GABA concentration, illustrating that variance in uncorrected GABA concentration is not substantially explained by the correction factors. Therefore, within this cohort of healthy volunteers, measured variance in voxel composition does not substantially contribute to variance in GABA measurements, and correcting for it does not remove inter-subject variance.

In the current manuscript, we defined μ_{GM} and μ_{WM} (in Equation 3) as the group-average f_{GM} and f_{WM} for each voxel, thus μ_{GM} and μ_{WM} were data-driven. In group comparison studies in which there are no anatomical differences between groups, it may be appropriate to use mean values across both groups. In studies that compare patients with atrophy with healthy controls, it may be most appropriate to calculate μWM and μGM from just the control group. In a longitudinal study of patients with varying degrees of atrophy due to differences in disease duration, pre-defined values for μ_{GM} and μ_{WM} may be most appropriate.

FSL and SPM are two widely used software packages for imaging analyses and both include segmentation tools, applying different segmentation strategies (7,8). In this study the segmentation results from SPM and FSL are generally significantly consistent, but there are differences in the segmentation results, in particular in GM and CSF. Differences in segmentation impact corrected GABA values largely through differing CSF fractions, which should be considered when comparing literature data. Discrepancies between the segmentation results from FSL and SPM have previously been documented (33,34), but their impact metabolite quantification is often overlooked. SPM8 segmentation analysis is integrated within Gannet.

Literature-derived relaxation constants (12-15) impact GABA quantification most strongly through the T_{1W} of GM and the T_{2W} of WM (at TE of 68 ms and TR of 2s). Acquiring water reference data with short TE and long TR reduces the dependence of GABA values on water relaxation parameters. Selection of relaxation constants will not impact the detection of group differences within a study, but this emphasizes that literature values derived from different relaxation constants cannot be compared.

A major limitation of this approach is the need to determine α. The linear fitting approach to calculate α, which has been used previously (29), assumes that GABA levels within GM or WM do not vary across the brain. This assumption has been applied previously (e.g., Refs (29,30,32) and is likely an oversimplification. However, the assumption is necessary to compute α from the data, and the value determined from this process agrees with previous MRS studies (28-32) and tissue extract studies (25-27). Simulations show that it is better to estimate α than to settle for CSF correction or GM correction, and that over-estimation of α is preferable to underestimation. Correction based upon an α-value of 0.5 will out-preform CSF correction for the full range of literature c_{WM}/c_{GM} ratios (0.09 to 0.67). In future, more accurate, and possibly region-specific, estimates of α may become available. A second limitation is the fact that compartment-specific relaxation parameters for GABA are not currently not available. In common with all in vivo MRS studies, there is the further limitation that no gold standard exists with which to compare the in vivo results.

In conclusion, we have demonstrated a GABA quantification correction that addresses differences in tissue relaxation characteristics and GABA concentration of the different tissue compartments that make up the MRS voxel. We showed the impact of differences in GABA concentration between WM and GM and recommend tissue correction should be performed by setting α = 0.5 to minimize the dependence of GABA measurements on tissue fraction. CSF correction alone does not address known differences in GABA concentration between WM and GM, whilst GM correction tends to over-correct. This correction makes it less likely that inter-subject differences in voxel GM, WM and CSF fraction drive differences in measured GABA concentration. We highlight an often-overlooked issue that tissue correction generally corrects to a hypothetical all-GM voxel, which does not reflect the tissue studied and propose a normalization method with μGM and μWM that provide GABA measurements more reflective of the tissue of characterization. The application of this normalization in future work will be study dependent. Among healthy young adults, this proposed correction does not substantially impact inter-subject variance in GABA measurements, largely because differences in voxel composition are relatively small. Both the segmentation method (i.e., FSL versus SPM) and literature-derived constants (ie., Wansapura versus Stanisz) impact corrected GABA values; therefore caution must be applied when comparing results between studies in the literature. The approach presented in considering tissue compartments for metabolite quantification will generalize to other metabolites with substantially different WM and GM concentrations.

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References

1. Puts NA, Edden RA. In vivo magnetic resonance spectroscopy of GABA: a methodological review. Prog Nucl Magn Reson Spectrosc. 2012; 60:29–41. [PubMed: 22293397]
2. Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R. Simultaneous in vivo spectral editing and water suppression. NMR Biomed. 1998; 11(6):266–272. [PubMed: 9802468]
3. Mullins PG, McGonigle DJ, O'Gorman RL, et al. Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. NeuroImage. 2014; 86:43–52. [PubMed: 23246994]

4. Rothman DL, Petroff OA, Behar KL, Mattson RH. Localized 1H NMR measurements of gamma-aminobutyric acid in human brain in vivo. Proc Natl Acad Sci U S A. 1993; 90(12):5662–5666. [PubMed: 8516315]
5. Kreis R, Ernst T, Ross BD. Development of the human brain: in vivo quantification of metabolite and water content with proton magnetic resonance spectroscopy. Magn Reson Med. 1993; 30(4): 424–437. [PubMed: 8255190]
6. Gasparovic C, Song T, Devier D, et al. Use of tissue water as a concentration reference for proton spectroscopic imaging. Magn Reson Med. 2006; 55(6):1219–1226. [PubMed: 16688703]
7. Ashburner J, Friston KJ. Unified segmentation. NeuroImage. 2005; 26(3):839–851. [PubMed: 15955494]
8. 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(1): 45–57. [PubMed: 11293691]
9. Ernst T, Kreis R, Ross BD. Absolute quantitation of water and metabolites in the human brain. I. Compartments and water. J Magn Reson B. 1993; 102
10. Puts NA, Barker PB, Edden RA. Measuring the longitudinal relaxation time of GABA in vivo at 3 tesla. J Magn Reson Imaging. 2013; 37(4):999–1003. [PubMed: 23001644]
11. Edden RA, 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 Imaging. 2012; 35(1):229–234. [PubMed: 22045601]
12. 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(3):579–586. [PubMed: 19132756]
13. Wansapura JP, Holland SK, Dunn RS, Ball WS Jr. NMR relaxation times in the human brain at 3.0 tesla. J Magn Reson Imaging. 1999; 9(4):531–538. [PubMed: 10232510]
14. Stanisz GJ, Odrobina EE, Pun J, et al. T1, T2 relaxation and magnetization transfer in tissue at 3T. Magn Reson Med. 2005; 54(3):507–512. [PubMed: 16086319]
15. Lu H, Nagae-Poetscher LM, Golay X, Lin D, Pomper M, van Zijl PC. Routine clinical brain MRI sequences for use at 3.0 Tesla. J Magn Reson Imaging. 2005; 22(1):13–22. [PubMed: 15971174]
16. Petrou M, Pop-Busui R, Foerster BR, et al. Altered excitation-inhibition balance in the brain of patients with diabetic neuropathy. Acad Radiol. 2012; 19(5):607–612. [PubMed: 22463961]
17. Foerster BR, Pomper MG, Callaghan BC, et al. An imbalance between excitatory and inhibitory neurotransmitters in amyotrophic lateral sclerosis revealed by use of 3-T proton magnetic resonance spectroscopy. JAMA neurology. 2013; 70(8):1009–1016. [PubMed: 23797905]
18. Stagg CJ, Best JG, Stephenson MC, et al. Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. J Neurosci. 2009; 29(16):5202–5206. [PubMed: 19386916]
19. Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R. Simultaneous *in vivo* spectral editing and water suppression. NMR in Biomedicine. 1998; 11:266–272. [PubMed: 9802468]
20. Edden RA, Puts N, Harris AD, Barker PB, Evans CJ. Gannet: a batch-processing tool for the quantitative analysis of GABA-edited MRS spectra. J Magn Reson Imaging. 2014 in press. 10.1002/jmri.24478
21. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. The Lancet. 1986; 327(8476):307–310.
22. Harris AD, Glaubitz B, Near J, et al. Impact of frequency drift on gamma-aminobutyric acid-edited MR spectroscopy. Magn Reson Med. 2013 In Press. 10.1002/mrm.25009
23. Near J, Simpson R, Cowen P, Jezzard P. Efficient gamma-aminobutyric acid editing at 3T without macromolecule contamination: MEGA-SPECIAL. NMR Biomed. 2011; 24(10):1277–1285. [PubMed: 21387450]
24. Harris AD, Puts NA, Barker PB, Edden RA. Spectral-editing measurements of GABA in the human brain with and without macromolecule suppression. Magn Reson Med. 2014 In Press. 10.1002/mrm.25549

25. Banay-Schwartz M, Palkovits M, Lajtha A. Heterogeneous distribution of functionally important amino acids in brain areas of adult and aging humans. Neurochem Res. 1993; 18(4):417–423. [PubMed: 8474566]
26. Petroff OA, Ogino T, Alger JR. High-resolution proton magnetic resonance spectroscopy of rabbit brain: regional metabolite levels and postmortem changes. J Neurochem. 1988; 51(1):163–171. [PubMed: 3379399]
27. Petroff OA, Pleban LA, Spencer DD. Symbiosis between in vivo and in vitro NMR spectroscopy: the creatine, N-acetylaspartate, glutamate, and GABA content of the epileptic human brain. Magn Reson Imaging. 1995; 13(8):1197–1211. [PubMed: 8750337]
28. 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(1):27–33. [PubMed: 17659613]
29. Zhu H, Edden RA, Ouwerkerk R, Barker PB. High resolution spectroscopic imaging of GABA at 3 Tesla. Magn Reson Med. 2011; 65(3):603–609. [PubMed: 21337399]
30. Choi IY, Lee SP, Merkle H, Shen J. In vivo detection of gray and white matter differences in GABA concentration in the human brain. NeuroImage. 2006; 33(1):85–93. [PubMed: 16884929]
31. 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 Imaging. 2011; 29(3):374–379. [PubMed: 21232891]
32. Jensen JE, Frederick Bde B, Renshaw PF. Grey and white matter GABA level differences in the human brain using two-dimensional, J-resolved spectroscopic imaging. NMR Biomed. 2005; 18(8):570–576. [PubMed: 16273508]
33. Eggert LD, S S, Jansen A, Kircher T, Konrad C. Accuracy and Reliability of Automated Gray Matter Segmentation Pathways on Real and Simulated Structural Magnetic Resonance Images of the Human Brain. Plos One. 2012; 7(9):e45081. [PubMed: 23028771]
34. Klauschen F, Goldman A, Barra V, Meyer-Lindenberg A, Lundervold A. Evaluation of automated brain MR image segmentation and volumetry methods. Human brain mapping. 2009; 30(4):1310–1327. [PubMed: 18537111]

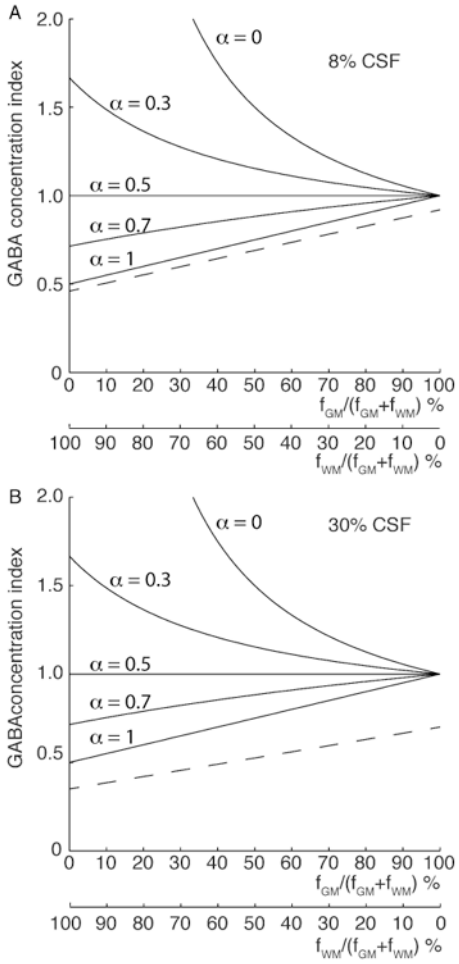

Figure 1.

Correcting for differing GABA concentration in GM and WM. The simulated voxel has (A) 8% and (B) 30% CSF. The GABA index is simulated across all normalized GM (and WM) tissue fractions, assuming no GABA in CSF, GABA concentration in GM = 1 and in WM = 0.5. The simulated, uncorrected GABA index (shown as a dashed line) is then corrected for differential tissue content using Equation 1, using values of α of 1 (corresponding to CSF correction), 0 (corresponding to GM correction), 0.5 (the true correction value for this simulation), 0.7 and 0.3 (corresponding to over- and under-estimates). The correction aims to normalize the GABA index to an all GM voxel. The efficacy of correction for a given value of α can be evaluated by the ability of the accuracy of correction at a specific GM tissue fraction (i.e., difference between a GABA index and 1) and the dependency of the GABA index on the voxel tissue fractions, denoted by the slope of each correction factor.

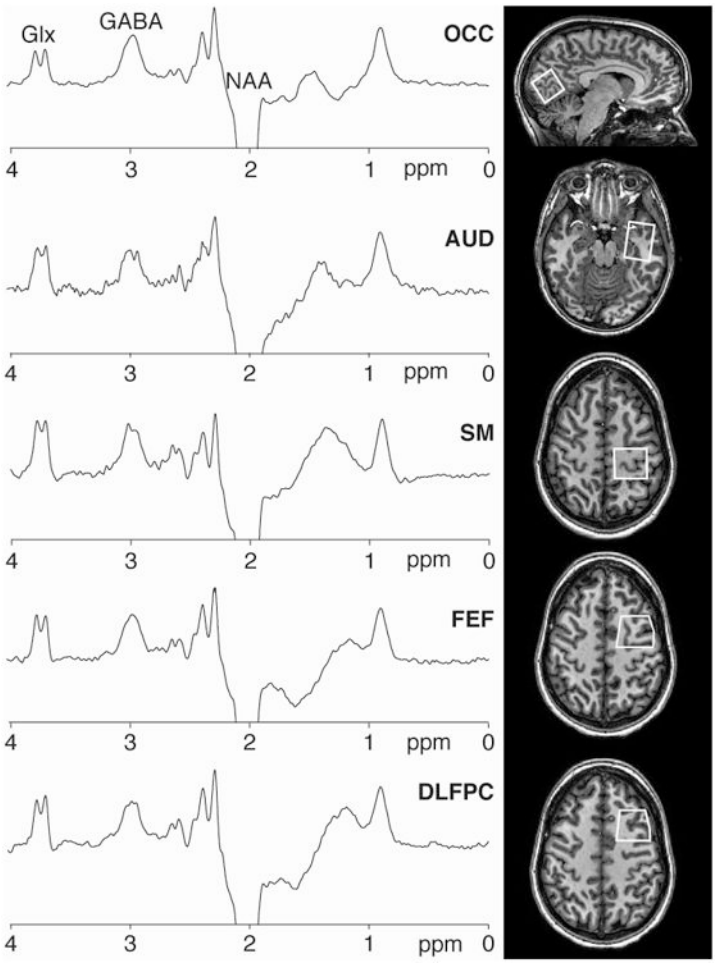

Figure 2.

Example spectra from one subject, after spectral registration for frequency correction for each of the 5 voxels included in the in-vivo dataset. The voxel location for each spectrum overlaid on the T1-weighted image is shown.

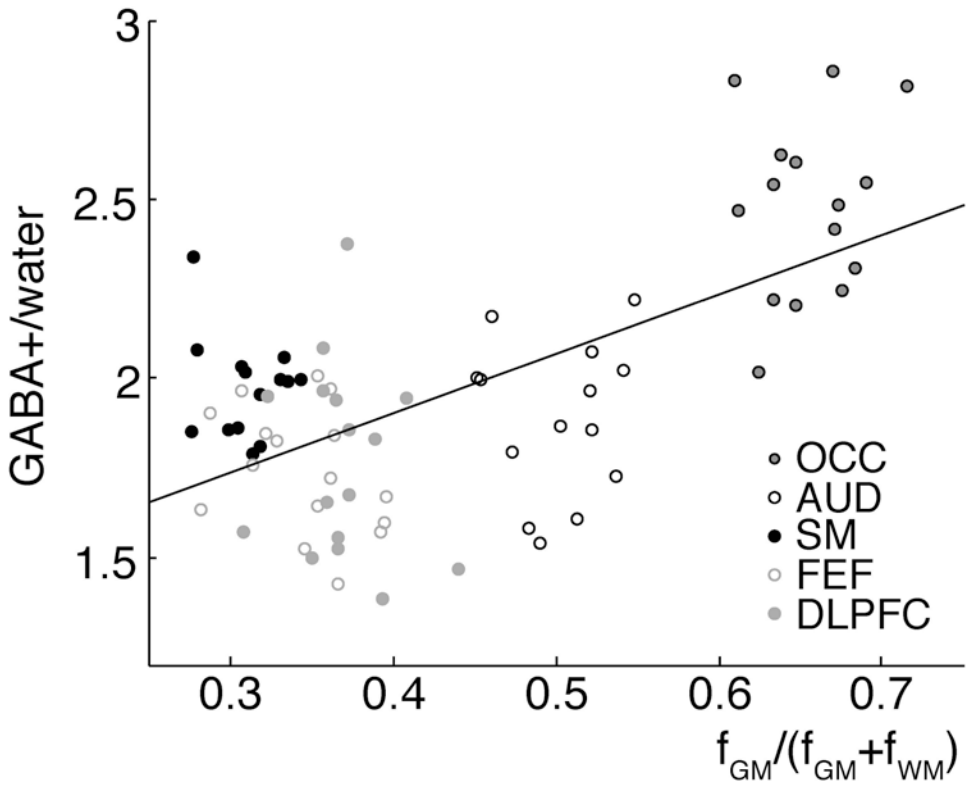

Figure 3.

GABA versus gray matter fraction. GABA was quantified using tissue-specific relaxation parameters (Equation 4). All data was pooled for the linear fit, the equation of the fit is GABA = $1.66f_{GM} + 1.24$, giving a calculated $\alpha = 0.43$.

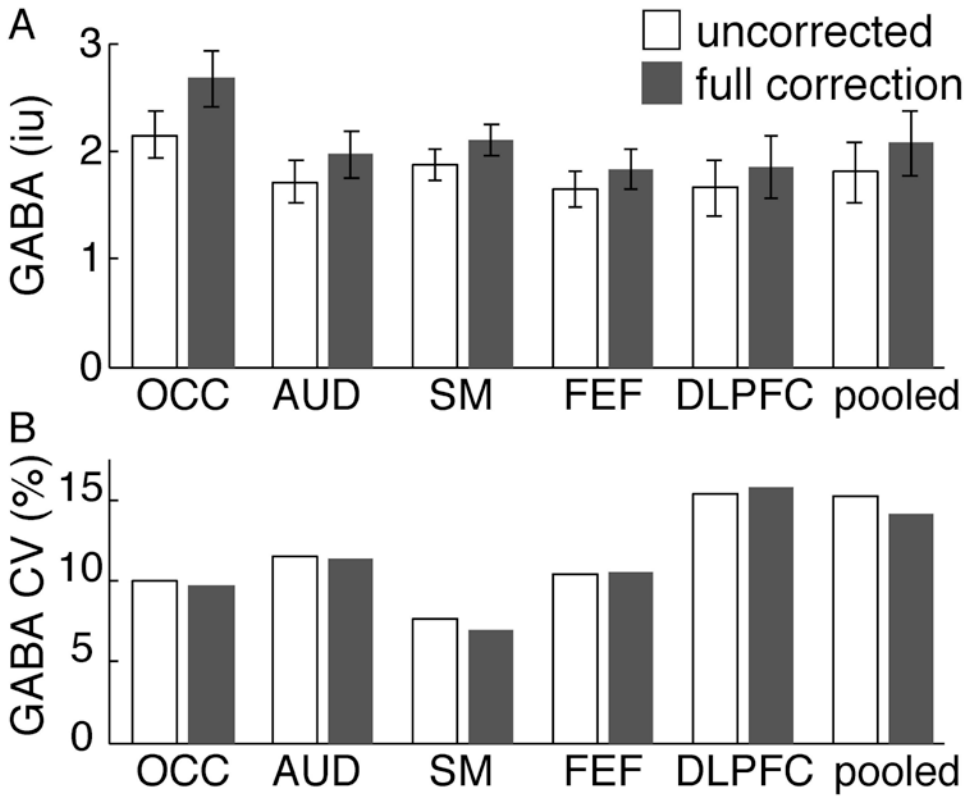

Figure 4.

Comparison of corrections. A) Uncorrected GABA concentrations (white) and fully-corrected concentrations are shown for all five brain regions and all data pooled. Correcting for voxel content and correcting for compartment-specific water relaxation result in significantly increased GABA measurements (t-tests $p < 0.001$). B) Coefficients of variation (CV) across subjects are unaffected by the corrections (F-tests $p > 0.05$).

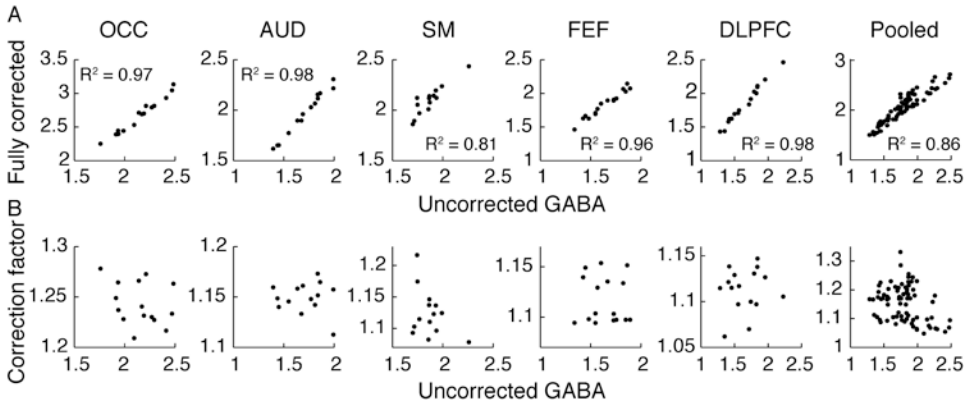

Figure 5.

Investigation of the impact of correction factors. A) Correlations of uncorrected and fully corrected concentrations show strong relationships ($0.81<R^2<0.98$). B) Plot of the magnitude of the full correction factor against uncorrected GABA concentrations c_{uncorr} showing no bias in correction factors

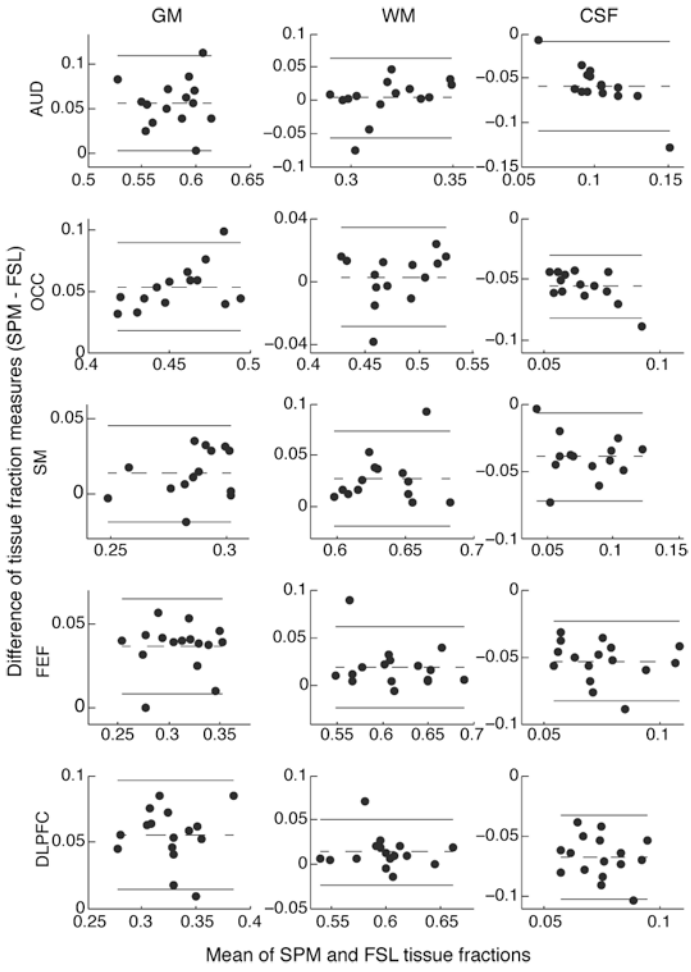

Figure 6.

Bland-Altman plots assessing the agreement between FSL and SPM voxel segmentation results. While there is agreement between these two segmentation results, FSL biases towards CSF and SPM biases towards GM. WM fractions are relatively consistent between the two methods.

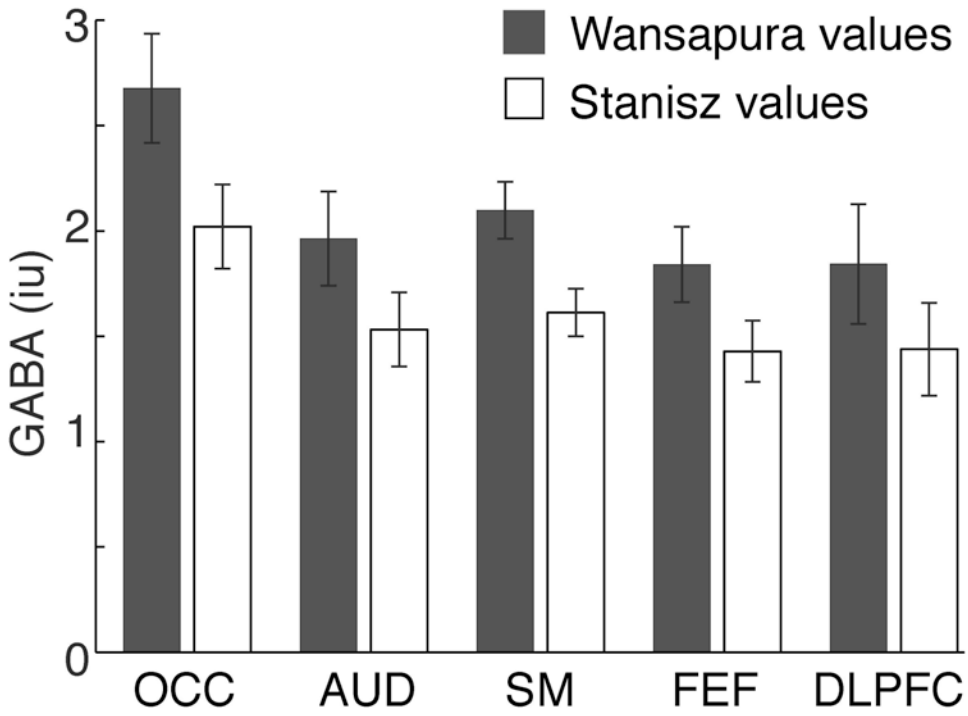

Figure 7.

Comparison of resulting GABA estimates when using two different sets of relaxation parameters. In all regions, there is a significant difference in GABA quantification between the two parameter sets. The Wansapura values (applied in Figures 3, 4 and 5) tend to increase the GABA concentrations more than the more recent Stanisz values.