

Reproducibility of prefrontal γ -aminobutyric acid measurements with *J*-edited spectroscopy[†]

Matthew Geramita^a, Jan Willem van der Veen^b, Alan S. Barnett^a, Antonina A. Savostyanova^a, Jun Shen^b, Daniel R. Weinberger^a and Stefano Marengo^{a*}

γ -Aminobutyric acid (GABA) is the chief inhibitory neurotransmitter of the human brain, and GABA-ergic dysfunction has been implicated in a variety of neuropsychiatric disorders. Recent MRS techniques have allowed the quantification of GABA concentrations *in vivo*, and could therefore provide biologically relevant information. Few reports have formally characterized the reproducibility of these techniques, and differences in field strength, acquisition and processing parameters may result in large differences in measured GABA values. Here, we used a *J*-edited, single-voxel spectroscopy method of measurement of GABA + macromolecules (GABA +) in the anterior cingulate cortex (ACC) and right frontal white matter (rFWM) at 3 T. We measured the coefficient of variation within subjects (CVw) and intra-class correlation coefficients on two repeated scans obtained from 10 healthy volunteers with processing procedures developed in-house for the quantification of GABA + and other major metabolites. In addition, by segmenting the spectroscopic voxel into cerebrospinal fluid, gray matter and white matter, and employing a linear regression technique to extrapolate metabolite values to pure gray and white matter, we determined metabolite differences between gray and white matter in ACC and rFWM. CVw values for GABA +/creatinine, GABA +/H₂O, GABA +, creatine, partially co-edited glutamate + glutamine (Glx)/creatinine, partially co-edited Glx and *N*-acetylaspartic acid (NAA)/creatinine were all below 12% in both ACC and rFWM. After extrapolation to pure gray and pure white matter, CVw values for all metabolites were below 16%. We found metabolite ratios between gray and white matter for GABA +/creatinine, GABA +, creatine, partially co-edited Glx and NAA/creatinine to be 0.88 ± 0.21 (standard deviation), 1.52 ± 0.32 , 1.77 ± 0.4 , 2.69 ± 0.74 and 0.70 ± 0.05 , respectively. This study validates a reproducible method for the quantification of brain metabolites, and provides information on gray/white matter differences that may be important in the interpretation of results in clinical populations. Published in 2011 by John Wiley & Sons, Ltd.

Keywords: *N*-acetylaspartic acid; glutamate + glutamine (Glx); creatine; anterior cingulate cortex; white matter; gray matter; MRS; 3 T

INTRODUCTION

γ -Aminobutyric acid (GABA) is the chief inhibitory neurotransmitter of the mammalian brain. Disruption of GABA transmission and metabolism has been linked to many neurological disorders, including anxiety disorders, schizophrenia and epilepsy (1). MRS provides a noninvasive means of measuring GABA in human subjects. However, as a result of GABA's low abundance and overlapping resonances with other metabolites, including creatine, glutamate, *N*-acetylaspartic acid (NAA) and macromolecules (MM), special methods to isolate GABA are needed. At 3 T, various approaches, including multiple quantum filtering (2–4), two-dimensional spectroscopic imaging (5–7) and *J*-editing (8–10), have been employed to isolate the GABA signal.

J-editing methods exploit the *J* coupling between the GABA-4 protons at 3.03 ppm (11) and the GABA-3 protons at 1.93 ppm (11) by employing a frequency band-limited editing pulse during half of the acquisitions to invert the GABA-3 protons and thereby refocus *J*-coupled GABA-4 protons. Subtraction of the 'editing-pulse-off' acquisitions from the 'editing-pulse-on' acquisitions yields a spectrum consisting of the outer two resonances of the GABA-4 triplet and co-edited MMs, with other overlapping resonances suppressed. Although the *J*-editing method does not

* Correspondence to: S. Marengo, Clinical Brain Disorders Branch, Genes, Cognition, and Psychosis Program (GCAP), Intramural Research Program (IRP), National Institute of Mental Health (NIMH), 10 Center Drive, Building 10, Room 3C103, Bethesda, MD 20892, USA.
E-mail: marencos@mail.nih.gov

a M. Geramita, A. S. Barnett, A. A. Savostyanova, D. R. Weinberger, S. Marengo Unit for Multimodal Imaging Genetics, Clinical Brain Disorders Branch, National Institute of Mental Health, Bethesda, MD, USA

b J. W. van der Veen, J. Shen
Magnetic Resonance Spectroscopy Unit, National Institute of Mental Health, Bethesda, MD, USA

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Abbreviations used: ACC, anterior cingulate cortex; CSF, cerebrospinal fluid; CVw, coefficient of variation within subjects; DANTE, delays alternating with nutations for tailored excitation; GABA, γ -aminobutyric acid; Glx, glutamate + glutamine; GM, gray matter; HSVD, Hankel singular value decomposition; ICC, intra-class correlation coefficient; ISIS, image-selected *in vivo* spectroscopy; MEGA-PRESS, Mescher–Garwood point-resolved spectroscopy; NAA, *N*-acetylaspartic acid; RF, radiofrequency; rFWM, right frontal white matter; WM, white matter.

allow the separation of MMs from the GABA peak, and is limited to a single, large voxel with many acquisitions in order to establish a robust signal-to-noise ratio (10), it provides vastly improved separation of GABA + MMs (GABA +) and glutamate + glutamine (Glx) resonances, therefore improving the precision of spectral fitting procedures.

The measurement of GABA *in vivo* in living subjects provides a potentially valuable tool to study the pathology of disease states associated with GABA-ergic dysfunction. However, before attributing variability in metabolite concentration to differences across clinical populations, the reproducibility of the technique must be determined to obtain the amount of variability inherently present in the method of data collection and to validate the acquisition and data processing techniques. A few studies of the reproducibility of GABA measures have been published recently (12–14) at 3 T. All of these studies employed a Mescher–Garwood point-resolved spectroscopy (MEGA-PRESS) sequence (15), with different coils, processing methods, voxel locations and tissue compositions. Here, we used a modified sequence and processing steps, and addressed the gap in the literature regarding the tissue composition of the voxel. To achieve a high signal-to-noise ratio, large voxels must be acquired and will contain varying amounts of white matter (WM), gray matter (GM) and cerebrospinal fluid (CSF). Collecting voxels with the same tissue composition across subjects becomes virtually impossible because of the inherent variability in brain anatomy across individuals, and so differences in metabolite concentrations across WM and GM must also be considered before analyzing differences as a result of pathology. This information might also be relevant for studies in which GABA levels are used to predict functional activation, as the amount of GM in the voxel may be an important confounding factor.

In this study, we used a single-voxel MRS technique to measure GABA + and other metabolites. Our objectives were to determine the reproducibility of our method of data acquisition and processing for the determination of GABA + concentrations, and to determine whether values of GABA + were significantly different between GM and WM. We placed one voxel in the dorsal anterior cingulate cortex (ACC) where linewidths usually below 7 Hz can be obtained. A second voxel was placed directly adjacent to ACC in the right frontal WM (rFWM). Using the metabolite concentrations and tissue compositions of the two voxels, we extrapolated metabolite values to pure GM and pure WM. This procedure may be helpful in minimizing interpretation difficulties in pathological situations in which voxel composition may be altered significantly, provided that good reproducibility can be achieved.

METHODS

Subjects

Ten healthy volunteers (eight males, two females), ranging in age from 23 to 52 years (mean \pm SD, 39.9 \pm 10.5 years), were recruited as part of the Clinical Brain Disorders Branch 'Sibling Study', a study of neurobiological abnormalities related to genetic risk for schizophrenia (NCT00001486) (16). All volunteers underwent a structured clinical interview for diagnosis based on *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. (DSM IV) criteria and a physical examination in order to rule out any current medical illness, past or current psychiatric or neurological diagnosis. Written, informed consent was obtained in accordance with the National Institute of Mental Health institutional review board. In order to assess between-session reproducibility, participants were scanned twice within a variable time period, ranging from 28 to 226 days (average, 131 \pm 78 days), separating the scans. Although this variable interval of time was mainly based on convenience, it also reflects the typical intervals in time that might be encountered in clinical studies of medication effects in patients with schizophrenia, where long stabilization periods are sometimes required.

MRS

For each session, participants underwent single-voxel MRS in a 3-T GE whole-body scanner using a quadrature transmit-receive head coil (IGC-Medical Advances, Milwaukee, WI, USA). To position the spectroscopic voxels, high-resolution T_1 structural images were acquired using a three-dimensional spoiled gradient pulse sequence (TR = 24 ms; TE = 3.2 ms; flip angle, 17°; in-plane resolution, 0.9 \times 0.9 mm²; 192 \times 256 matrix; field of view, 240 mm; slice thickness, 2 mm; total scan time, 2 min 32 s). MRS spectra were acquired from two voxels, each measuring 2 \times 2 \times 4.5 cm³ (18 cm³). One voxel was placed immediately superior to the ventricles, straddling the midline, in order to contain the largest proportion of GM possible, with the anterior edge of the voxel never exceeding the anterior portion of the genu of the corpus callosum. This resulted in inclusion mainly of the corpus callosum and ACC. Another voxel was placed directly adjacent to the first, in the rFWM, adjusting its position in order to minimize the GM and CSF contribution and always remaining dorsal to the caudate nucleus (Fig. 1).

To measure the level of GABA + and partially co-edited Glx, an interleaved PRESS-based *J*-editing method (9) was used. The method is an extension of that published by Rothman *et al.* (8)

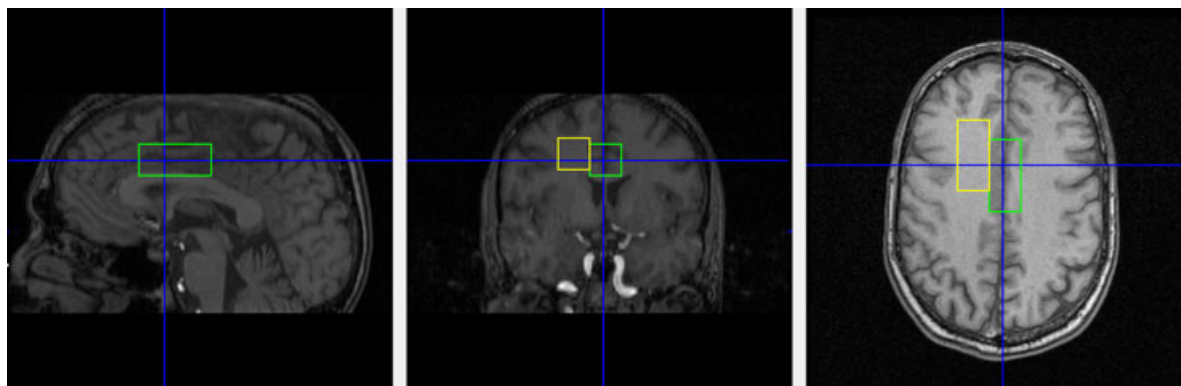


Figure 1. Location of gray and white matter voxels, represented in green and yellow, respectively. Sagittal, coronal and axial views are presented from left to right.

and by Mescher *et al.* (15) (MEGA-PRESS) with a pair of editing pulses with a flat top in the frequency domain. The *J*-based editing method exploits the *J* coupling between the GABA-4 protons at 3.0 ppm and the GABA-3 protons at 1.9 ppm. In addition to water and outer volume suppression pulses, the sequence contains an excitation pulse, two slice-selective inversion pulses and, flanking the second slice-selective inversion pulse, a pair of frequency-selective inversion (editing) pulses. The excitation pulse has a bandwidth of 2370 Hz and a flip angle of 90°. The slice-selective refocusing pulses have a bandwidth of 1385 Hz and, because of radiofrequency (RF) amplitude limitations, a flip angle of 167°. To reduce the error in the voxel location for the metabolites, the frequency of each slice-selective RF pulse was shifted from the value required to excite or refocus tissue water in the desired slice by -2 ppm. The prescribed voxel location therefore corresponds to a chemical shift of 2.65 ppm between the creatine and NAA resonances. The editing pulses last 14.4 ms and have a maximum amplitude (γB_1) of 160 Hz. The editing pulses have a top-hat frequency profile with the top extending from 2.0 to 0.6 ppm, a range that includes both GABA-3 at 1.9 ppm and the M4 MMs at 1.72 ppm. The inversion efficiency in the high-frequency transition band is about 90% at 2.1 ppm and about 10% at 2.4 ppm. For the GABA-3 protons, which are in the flat plateau of the inversion profile, the editing efficiency is therefore insensitive to small variations in frequency, making the method robust for scanning *in vivo*.

We acquired two types of spectra: 'editing-pulse-on' spectra and 'editing-pulse-off' spectra, acquired with the editing pulses centered at a frequency 2000 Hz higher than the water resonance, a region of the spectrum that contains no signals of interest.

The GABA-4 resonance is a triplet. In the 'editing-pulse-off' spectra, *J*-coupling inverts the outer lines of the triplet. In the 'editing-pulse-on' spectra, the editing pulses, which are separated by half TE, invert the GABA-3 protons, resulting in a spectrum in which the outer lines of the GABA-4 resonance are in phase. The 'editing-pulse-off' spectrum is then subtracted from the 'editing-pulse-on' spectrum to yield an edited or difference spectrum in which creatine and other metabolite signals at 3.0 ppm cancel, but the outer two lines of the GABA-4 signal add. When the inversion pulse is perfect, the method detects the full amplitude of the two side peaks of the GABA-4 protons. There are other *J*-coupled metabolite signals that are also affected by the editing pulses. In particular, the Glx-3 protons at 2.1 ppm are partially inverted by the editing pulse, resulting in a change in the signal of the *J*-coupled Glx-2 protons at 3.8 ppm, and the MM group M4 at 1.72 ppm is completely inverted by the editing pulses, modulating the signal in the *J*-coupled M7 resonance that overlaps the GABA-4 signal at 3.0 ppm. Two groups have measured the contribution of MMs to the 'edited spectrum' signal at 3.0 ppm; Rothman *et al.* (8) at 2.1 T using a sequence with image-selected *in vivo* spectroscopy (ISIS) localization and a delays alternating with nutations for tailored excitation (DANTE) pulse train for editing, and Kegeles *et al.* (17) at 3 T using the same sequence and TR as the present study. Both groups found that slightly more than 40% of the GABA+ signal is due to MMs. The 'edited' spectrum therefore consists of GABA+ (about 60% of which is the edited GABA-4 resonance and 40% of which is the co-edited macromolecular M7 resonance) at 3.0 ppm and partially co-edited Glx-2 at 3.8 ppm.

Each voxel was acquired 776 times. Of these, 384 were water-suppressed 'editing-pulse-on' acquisitions, 384 were

water-suppressed 'editing-pulse-off' acquisitions and eight were nonwater-suppressed acquisitions. In all acquisitions, TE = 68 ms and TR = 1.5 s. The total scan time was about 20 min.

Data processing

A fully automated nonlinear fitting program written in IDL (ITT Visual Information Solutions, White Plains, NY, USA) was used to quantify the GABA+ peak. The editing sequence adds NEX = 2 acquisitions in-scanner and saves the sum individually in a raw data file, resulting in 384 pairs of interleaved 'editing-pulse-on' and 'editing-pulse-off' acquisitions. In the first step of data processing, pairs of acquisitions with variations larger than 10% in the amplitude of the residual water peak, an indicator of subject movement, were eliminated. To correct for phase error caused by subject movement and scanner carrier frequency drift (minimum, 1.1 Hz; maximum, 8 Hz; average across subjects, 3.03 ± 1.65 Hz), the zero- and first-order phases of each acquisition were corrected in the time domain. For the 'editing-pulse-on' acquisitions, the correction applied was the difference between the phase of the residual water signal and the average phase of all the 'editing-pulse-on' acquisitions. An analogous correction was applied to the 'editing-pulse-off' acquisitions (18). The average of the 'editing-pulse-off' acquisitions was then subtracted from the average of the 'editing-pulse-on' acquisitions to create an 'edited' signal. The 'edited' signal was zero-order phased by hand for maximal flatness of the baseline around the GABA resonance. The residual water signal that remained in the averaged 'editing-pulse-off' signal and the 'edited' signal was removed by Hankel singular value decomposition (HSVD) (19). The similar fit to the residual water peak in the 'editing-pulse-off' spectrum before the HSVD correction is shown in Fig. 2A (blue dotted line). Next, using the averaged 'editing-pulse-off' signal, model functions for the choline, creatine and NAA peaks were fitted using two constraints. First, the relative frequency separations of the three peaks were fixed based on literature values (11). The linewidth of the peaks, determined by the local magnetic inhomogeneities, was also constrained by the assumption that all metabolites to be fitted have the same linewidth at a field strength of 3 T. The lineshape consisted of a combination of Gaussian and Lorentzian damping, also called a Voigt lineshape. The fitting algorithm was a Marquardt-Levenberg routine written in IDL. The fit to NAA, choline and creatine is shown in Fig. 2A (red line). The parameters of the NAA, choline and creatine model functions were then used to fit the GABA+ and Glx peaks in the 'edited' spectrum. In this step, the frequency positions of GABA+ and Glx were calculated from the positions of the NAA, choline and creatine peaks using the literature values for the chemical shifts (11). The values for the Gaussian and Lorentzian lineshape parameters were taken from the values determined in the previous step in the fit to the NAA, choline and creatine peaks. By fixing the frequency position and the lineshape, the GABA+ and Glx amplitudes remained the only free parameters to be determined from the fit. Consequently, the processing yielded absolute, unscaled values for GABA+, partially co-edited Glx, as well as ratios of NAA/creatine, partially co-edited Glx/creatine and GABA+/creatine. In addition, the water signal was computed from the nonwater-suppressed acquisitions. The final fit of GABA+ and Glx is shown in Fig. 2B (red line), together with the fitted residual (bottom panel).

To determine the tissue composition of the voxels, spoiled gradient images were segmented into GM, WM and CSF using the

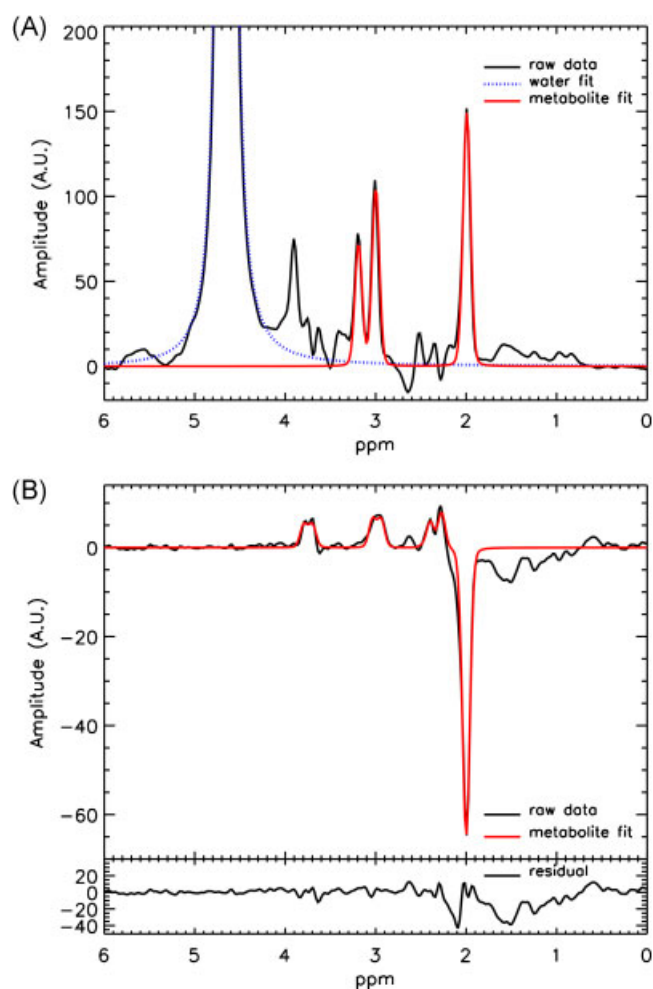


Figure 2. Representative γ -aminobutyric acid + macromolecules (GABA +) spectrum. (A) Fit of the residual water peak (blue dotted line) and of *N*-acetylaspartic acid (NAA), choline (Cho) and creatine (Cre) (red) in the averaged unedited portion of the spectrum. (B) Fit of GABA + and co-edited glutamate + glutamine (Glx) peaks (red) after subtraction of the averaged unedited from the averaged edited spectrum. The bottom panel shows the difference between the fitted and raw values.

segmentation algorithm provided by SPM5 (20). The segmentation produced a separate image for each of the three tissue types (GM, WM, CSF). The three separate images were combined into a single image with every voxel labeled as GM, WM or CSF depending on which tissue type was most probable. Using a program developed in-house, we masked the high-resolution segmented image to consider only tissue inside the spectroscopic voxel and calculated the volume of each tissue class relative to the total voxel volume. With this procedure, we determined the GM, WM and CSF percentages in both of the spectroscopic voxels. Because CSF does not contain metabolites, we divided the absolute creatine, GABA + and partially co-edited Glx values by the percentage of tissue in the voxel (GM + WM) to adjust for the amount of CSF in the spectroscopic voxel. In addition, the absolute creatine, GABA + and partially co-edited Glx values were corrected for transmit gain differences between scans using the same procedure as applied by LCModel for GE scanners (21).

It is common practice to use the brain water signal as an internal reference for metabolite signals; for this purpose, we

used the eight scans of each voxel without water suppression. The use of water as an internal reference is useful if the only reason for the difference in the water signals between voxels is an instrumental effect that would cause a proportional change in the metabolite signals. We can improve the water reference procedure by correcting for the differing compositions of the voxels. Assuming that each voxel contains a mixture of CSF, GM and WM, the water signal in a voxel can be written as:

$$S_k = V(n_{\text{white},k}a_{\text{white}} + n_{\text{gray},k}a_{\text{gray}} + n_{\text{csf},k}a_{\text{csf}})g_k + \varepsilon_k \quad [1]$$

where S_k is the water signal in voxel k , $n_{\text{white},k}$, $n_{\text{gray},k}$ and $n_{\text{csf},k}$ are the fraction of voxel k occupied by WM, GM and CSF, respectively, a_{white} , a_{gray} and a_{csf} are the sensitivity factors that depend on the magnetic properties of WM, GM and CSF, respectively, g_k is a scaling factor for voxel k that is common to all metabolites, V is the volume of voxels and ε_k is the random noise in the measurement. For our spectroscopy sequence, the sensitivity factor for a tissue class α (GM, WM or CSF) is:

$$a_\alpha = \rho_\alpha \exp(-T_E/T_{2,\alpha}) (1 - \exp(-T_R/T_{1,\alpha})) \quad [2]$$

where ρ_α , $T_{1,\alpha}$ and $T_{2,\alpha}$ are the proton density, longitudinal relaxation time and transverse relaxation time, respectively, of tissue class α . Using the published values of ρ , T_1 and T_2 given in Table 1 (22–25), and normalizing the results so that $a_{\text{csf}} = 1$, we find that $a_{\text{gray}} = 0.92$ and $a_{\text{white}} = 0.81$. We can now normalize each voxel by dividing by g_k computed using Equation [1].

We can also use the measured values of S_k , $n_{\text{white},k}$, $n_{\text{gray},k}$ and $n_{\text{csf},k}$ to estimate a_{white} , a_{gray} and a_{csf} . To do this, we rewrite Equation [1] as:

$$S_k = (b_0 + n_{\text{gray},k}b_{\text{gray}} + n_{\text{csf},k}b_{\text{csf}}) + \varepsilon_k + \beta_k S_k \quad [3]$$

where $g_k = \frac{1}{1 - \beta_k}$, $b_0 = Va_{\text{white}}$, $b_{\text{gray}} = V(a_{\text{gray}} - a_{\text{white}})$, $b_{\text{csf}} = V(a_{\text{csf}} - a_{\text{white}})$ and we have used the fact that $n_{\text{white},k} + n_{\text{gray},k} + n_{\text{csf},k} = 1$.

If the gain β_k is thought of as a random variable, the term $\beta_k S_k$ acts as an added noise term, and we can perform a simple linear regression to estimate b_0 , b_{gray} and b_{csf} . The estimates will be biased because of the fact that β_k and S_k are correlated, but, if most of the variance in S_k is a result of the differing voxel composition, the bias should be small.

Fitting our data to Equation [3] yields the values $b_0 = 8.14 \times 10^8$, $b_{\text{csf}} = 1.92 \times 10^8$ and $b_{\text{gray}} = 0.90 \times 10^8$, the resulting estimates for the normalized sensitivities are $a_{\text{csf}} = 1$, $a_{\text{gray}} = 0.90$ and $a_{\text{white}} = 0.81$, in agreement with the estimates computed above using the magnetic properties of the material. Considering the uncertainties in the tissue parameters and

Table 1. Values of proton density (PD), T_1 and T_2 used for water signal correction

	PD (CSF = 1)	T_1 (ms)	T_2 (ms)
White matter	0.77	1084	69
Gray matter	0.86	1820	99
Cerebrospinal fluid (CSF)	1	4163	503

Values for PD from ref. (19), values for T_1 of CSF from ref. (20), values for T_2 of CSF from ref. (21), and values for T_1 and T_2 of gray and white matter from ref. (22).

the crudeness of the model, the excellence of the agreement must be considered fortuitous.

We used the values for b_0 , b_{gray} and b_{csf} calculated above from the actual values of the unsuppressed water signal to derive a water value for the normalization of GABA+ that was independent of the tissue composition of the voxel by scaling it to an average value of GM and WM over all measurements in this sample. This is the value we report for ratios of GABA+ and creatine to H₂O. This method bears similarity to that used in ref. (26), which is based on refs. (27,28).

In order to determine the effect of tissue composition on the metabolite values, we extrapolated absolute values of creatine, GABA+, co-edited Glx, NAA/creatine, GABA+/creatine, creatine/H₂O and GABA+/H₂O from the 68-ms scan to pure GM and pure WM using linear regression of the GM percentage [GM/(GM + WM)] versus metabolite based on the values from the two voxels available.

Statistics

Coefficients of variation within subjects (CVw), defined as the ratio of the square root of the mean sum of squares within subjects over the overall mean $\times 100$ (29), were used for the quantification of reproducibility for each metabolite of interest. This is a more conservative measure than the usually reported average of CVs calculated for each individual. We also calculated intra-class correlation coefficients (ICC), according to ref. (30). Finally, for the ACC region of interest, we computed the average percentage difference between scans and 95% confidence intervals. We also used the standard deviation of the differences between two scans to predict the effect size and number of subjects (N) required to detect a significant difference ($p = 0.05$) between repeated scans of the same individuals (paired t -test) with 0.9 power, assuming a 5% difference in mean values. Effect size and N were calculated using Gpower [version 3.1.2 (31)]. To look for significant differences in metabolite concentrations between pure GM and WM, we calculated the average of the two scans for pure GM and WM and used a paired t -test with Statistica v7.0.

RESULTS

Table 2 reports the concentrations of NAA/creatine, partially co-edited Glx/creatine, GABA+/H₂O, GABA+/creatine, GABA+, partially co-edited Glx, creatine and creatine/H₂O measured by the *J*-editing scan. Values of NAA/creatine, creatine and creatine/H₂O were generated from the averaged 'editing-pulse-off' spectrum, whereas all others were generated from the GABA difference spectrum. CVw and ICCs quantifying the reproducibility of our technique are reported in Tables 3a and 3b. For NAA/creatine and Glx/creatine, the CVw values of the *J*-editing scan were below 6% in ACC. In ACC, CVw was 6.48% for GABA+/creatine and 5.27% for GABA+/H₂O, and, in rFWM, CVw was 8.15% for GABA+/creatine and 8.74% for GABA+/H₂O. Absolute signal intensities of creatine, GABA+ and partially co-edited Glx, corrected for CSF and transmit gain, also generated by our processing procedure, had CVw values of 11% or less. Creatine normalized to water yielded smaller CVw than the absolute, corrected creatine value in both ACC (5.9) and rFWM (6.3) voxels (Table 3a). ICCs (Table 3b) varied from a negative value for GABA/creatine in rFWM to a value of 0.91 for NAA/creatine in ACC

Table 2. Metabolite and ratio values: average \pm standard deviation

	ACC	rFWM
NAA/creatine	1.46 \pm 0.09	1.74 \pm 0.10
Co-edited	0.09 \pm 0.01	0.08 \pm 0.01
Glx/creatine		
GABA/creatine	0.094 \pm 0.008	0.10 \pm 0.007
GABA/H ₂ O	1.74E-4 \pm 1.95E-5	1.45E-4 \pm 1.74E-5
GABA (IU)	1.52E-5 \pm 2.04E-4	1.26E-5 \pm 1.72E-4
Glx (IU)	1.46E-5 \pm 1.79E-4	9.62E-4 \pm 1.42E-4
Creatine (IU)	1.62E-6 \pm 2.36E-5	1.26E-6 \pm 1.64E-5
Creatine/H ₂ O	1.86E-3 \pm 1.85E-4	1.44E-3 \pm 1.54E-4

ACC, anterior cingulate cortex; GABA, γ -aminobutyric acid; Glx, glutamate + glutamine; NAA, *N*-acetylaspartic acid; rFWM, right frontal white matter.

(TE = 68 ms). Table 3c provides mean percentage differences and confidence intervals for the measures related to the ACC voxel and calculations of sample size required to detect a 5% mean difference with 0.9 power.

Tables 4a and 4b contain the averages and CVw values of the voxel compositions for both the ACC and rFWM voxels prior to and after adjusting the GM percentage for the amount of tissue in the voxel. The average GM/tissue percentages in ACC and rFWM voxels were 73.4 \pm 3.2% and 25.4 \pm 5.3%, respectively. In addition, the CVw of GM/tissue percentage was reproducible in both voxels (ACC, 2.76%; rFWM, 3.78%).

Metabolite values, CVw and ICCs for pure GM and WM are reported in Table 5. NAA/creatine, taken from the 'editing-pulse-off' acquisitions, had CVw of less than 5%, whereas CVw values for the absolute values of GABA+, partially co-edited Glx, GABA+/creatine and creatine were slightly higher, but less than 12%, 16%, 14% and 13%, respectively. Extrapolated values of both GABA+/H₂O and creatine/H₂O had comparable CVw to the absolute, corrected values of GABA+ and creatine, but their standard deviations were reduced by about 20%. ICCs were somewhat lower than for the original ACC and rFWM data, but followed the same general pattern. We used a paired t -test to

Table 3a. Coefficients of variation within subjects (CVw)

	ACC	rFWM
NAA/creatine	1.82	2.58
Co-edited Glx/creatine	4.6	6.95
GABA/creatine	6.49	8.15
GABA/H ₂ O	5.27	8.74
GABA	8.59	8.02
Glx	10.23	9.63
Creatine	10.35	8.92
Creatine/H ₂ O	5.9	6.3

ACC, anterior cingulate cortex; GABA, γ -aminobutyric acid; Glx, glutamate + glutamine; NAA, *N*-acetylaspartic acid; rFWM, right frontal white matter.

Table 3b. Intra-class correlation coefficients (ICC)

	ACC	rFWM
NAA/creatine	0.906	0.822
Co-edited	0.841	0.774
Glx/creatine		
GABA/creatine	0.499	-0.351
GABA/H ₂ O	0.788	0.483
GABA	0.605	0.664
Glx	0.317	0.588
Creatine	0.487	0.545
Creatine/H ₂ O	0.68	0.58

ACC, anterior cingulate cortex; GABA, γ -aminobutyric acid; Glx, glutamate + glutamine; NAA, *N*-acetylaspartic acid; rFWM, right frontal white matter.

Table 3c. Mean percentage change and 95% confidence intervals (CI) for anterior cingulate cortex (ACC)

	Mean change (%)	95% CI	Effect size ^a	N ^a
NAA/creatine	-1.73	1.49	2.43	5
Co-edited	-2.38	4.34	0.71	23
Glx/creatine				
GABA/creatine	-0.34	6.95	0.37	77
GABA/H ₂ O	-2.02	5.33	0.66	26
GABA	7.19	6.76	0.51	43
Glx	5.14	9.46	0.41	64
Creatine	7.52	8.78	0.39	71
Creatine/H ₂ O	-2.94	6.12	0.58	34

GABA, γ -aminobutyric acid; Glx, glutamate + glutamine; NAA, *N*-acetylaspartic acid.
^aAssumes a 5% difference in mean values, $\alpha = 0.05$, power = 0.9 and uses the standard deviation of the differences determined from the reproducibility data.

determine significant differences between pure GM and WM as reported in Table 5. NAA/creatine from both scans showed a significantly higher value in pure WM than in pure GM ($p < 0.00001$), with a GM/WM ratio of approximately 0.7. Values

Table 4a. Voxel composition: average \pm standard deviation (%)

	ACC	rFWM
CSF	17.68 \pm 5.01	2.30 \pm 1.16
GM	60.40 \pm 4.3	24.79 \pm 4.97
WM	21.92 \pm 3.14	72.93 \pm 5.76
GM/(GM + WM)	73.40 \pm 3.2	25.40 \pm 5.3
WM/(GM + WM)	26.60 \pm 3.18	74.60 \pm 5.29

ACC, anterior cingulate cortex; CSF, cerebrospinal fluid; GM, gray matter; rFWM, right frontal white matter; WM, white matter.

Table 4b. Voxel composition: coefficients of variation (CVw)

	ACC	rFWM
GM/(GM + WM)	2.76	11.09
WM/(GM + WM)	7.61	3.78

ACC, anterior cingulate cortex; GM, gray matter; rFWM, right frontal white matter; WM, white matter.

of creatine, GABA + and partially co-edited Glx from the *J*-editing scan were significantly higher in pure GM than in pure WM ($p < 0.0001$), showing GM/WM ratios of 1.77, 1.52 and 2.69, respectively. As a result of the steeper concentration gradient in creatine than GABA + between GM and WM, the GABA + / creatine ratio showed a significantly higher ($p = 0.017$) concentration in pure WM, with a GM/WM ratio of 0.88. The dependence of the various measures of GABA + on the percentage GM content is shown in Fig. 3.

DISCUSSION

In this study, we have presented a reproducible method of measurement of major metabolites and GABA + *in vivo* using MRS, and have used it to quantify metabolite differences between GM and WM in healthy volunteers. We have also devised a method to correct the water signal for the relative contributions of voxel tissue composition in individual subjects, which was verified with empirical data. The GABA + / creatine values from the *J*-editing sequence are supported by other studies that have employed the *J*-difference editing technique. GABA + / creatine values are hard to compare across studies because of differences in processing, voxel position and tissue composition. However, three separate studies published by Hasler *et al.* (10,32,33) at 3 T measured GABA + in the medial prefrontal cortex using a similar post-processing procedure. The values of GABA reported in ref. (32) were obtained with the following formula: $GABA = (GABA + / creatine) \times 7.1 \times 2 - 0.635$, where 7.1 mm is the assumed concentration of creatine in a voxel in which GM and WM are equally represented, the factor of two accounts for the fact that only one-half of the GABA spins at 3 ppm are being detected, and 0.635 is the assumed millimolar concentration of MMs in the GABA peak. After controlling for these factors, we found ratios of GABA + / creatine that were about 20% lower than the values reported in all three studies. One possible explanation for this discrepancy is that our voxel was placed in the dorsal ACC, whereas previous studies placed the voxel more anterior in the dorsal anterolateral prefrontal cortex and the ventromedial prefrontal cortex. A more probable explanation, however, is that the calculations in the studies by Hasler *et al.* were performed assuming creatine values from the literature rather than from the single individual, and the studies were performed on different scanners using different software versions, although from the same manufacturer. Using MEGA-PRESS, Waddell *et al.* (34) have recently found GABA + / creatine values of 0.31 in ACC, and Goto *et al.* (35) have found values of 1.29 in the frontal lobe, both substantially higher estimates than those found in our study. The former study used a smaller voxel scanned for a shorter period of time, with a very different processing pipeline that modeled MMs separately. Both studies reported standard deviations much

Table 5. Extrapolation of metabolite values to pure gray and white matter

	Pure gray			Pure white			Gray versus white matter		
	Average \pm SD	CVw (%)	ICC	Average \pm SD	CVw (%)	ICC	GM/WM ratio	t-value	p value
NAA/Cre	1.31 \pm 0.095	2.77	0.86	1.89 \pm 0.12	3.17	0.74	0.70 \pm 0.054	-15.83	<0.000001
GABA + (IU)	1.67E-5 \pm 2.76E-4	11.18	0.56	1.13E-5 \pm 1.99E-4	11.66	0.57	1.52 \pm 0.32	5.74	0.00028
Co-edited Glx (IU)	1.74E-5 \pm 2.61E-4	11.42	0.43	6.94E-4 \pm 1.57E-4	15.59	0.54	2.69 \pm 0.74	11.94	<0.000001
Cre (IU)	1.84E-6 \pm 2.72E-5	12.15	0.54	1.06E-6 \pm 1.33E-5	12.69	0.37	1.77 \pm 0.40	7.88	0.000025
GABA + /Cre	0.090 \pm 0.013	12.71	0.33	0.11 \pm 0.012	13.75	-0.45	0.88 \pm 0.21	-2.94	0.017
GABA + /H ₂ O	1.91E-4 \pm 2.65E-5	7.88	0.69	1.29E-4 \pm 2.11E-5	13.05	0.38	1.52 \pm 0.29	6.38	0.00013
Cre/H ₂ O	2.10E-3 \pm 2.66E-4	8.82	0.53	1.21E-3 \pm 1.87E-4	13.12	0.29	1.78 \pm 0.31	9.96	0.000004

Cre, creatine; CVw, coefficient of variation within subjects; GABA, γ -aminobutyric acid; Glx, glutamate + glutamine; GM, gray matter; ICC, intra-class correlation coefficient; IU, institutional units; NAA, N-acetylaspartic acid; SD, standard deviation; WM, white matter.

higher than our values of 8.5% (25% and 65% of the mean, respectively). In addition, Yoon *et al.* (36) found similar GABA + / creatine ratios to those reported here in the occipital lobe (mean of 0.105). We report CVw values for GABA + /creatinine of 6.49 in ACC and 8.15 in rFWM. The higher CVw in the rFWM voxel could have been the result of the lower reliability in the placement of the rFWM voxel [as demonstrated by the higher CVw value for percentage GM/tissue in rFWM (3.78%) compared with ACC (2.78%)], a physiologically larger variation in tissue composition

or a lower signal from the GABA + peak and, consequently, greater uncertainty in the measurement. Nevertheless, our CVw values for GABA + /creatinine were lower than those of previous studies that quantified the reproducibility of GABA + measures. Lymer *et al.* (7) reported the lowest CVw at 1.5 T for GABA + at 26%. At 3 T, Bogner *et al.* (12) measured GABA + with multiple processing techniques and internal references, and achieved a minimum CVw of 13.3%. A recent study by Evans *et al.* (13) that acquired GABA + spectra using a MEGA-PRESS acquisition at 3 T

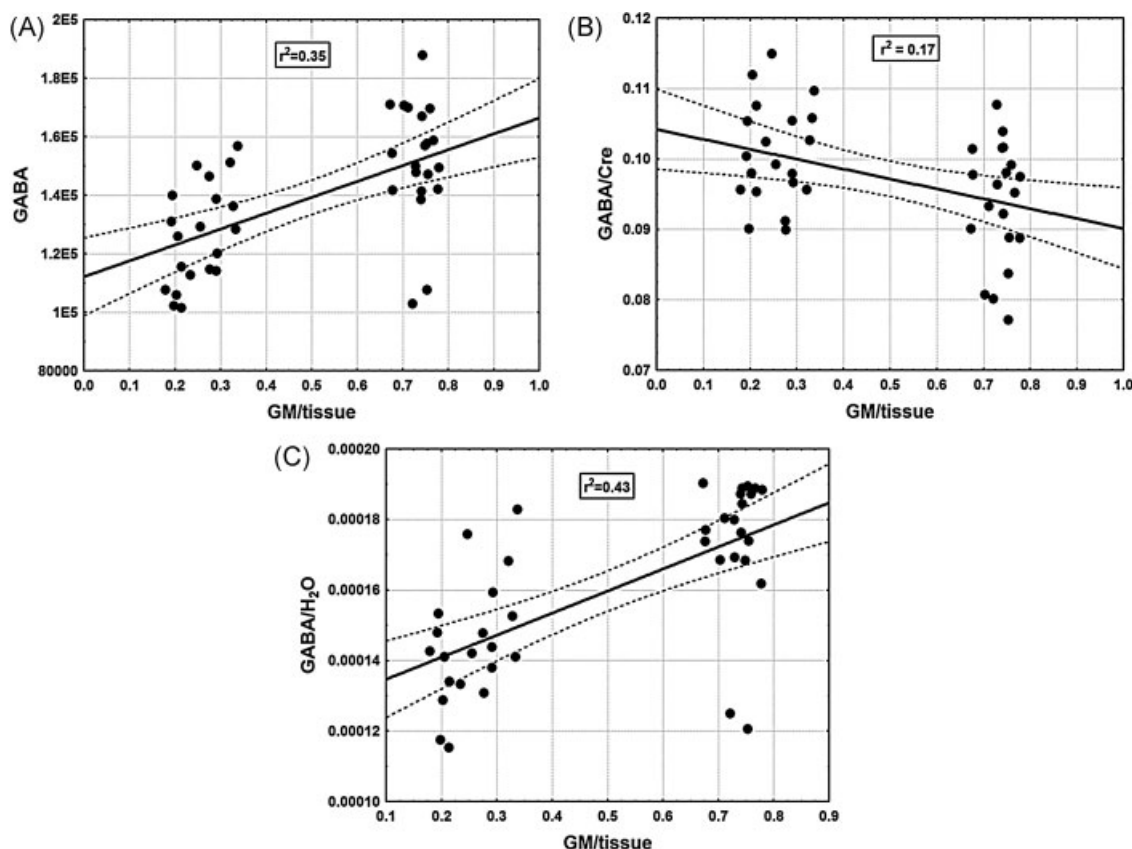


Figure 3. Dependence of γ -aminobutyric acid + macromolecules (GABA +) on gray matter as a percentage of tissue contained in the voxel. (A) GABA + after correction for transmit gain and cerebrospinal fluid (CSF). (B) GABA + /creatinine. (C) GABA + /H₂O.

measured the reproducibility of GABA+ using water as an internal standard in the occipital (6.5%) and sensorimotor (8.8%) cortices. These values compare with those of 5.27% in ACC and 8.74% in rFWM in our study. By acquiring spectra at various points in the day in multiple healthy volunteers, Evans *et al.* (13) found that the MEGA-PRESS sequence was sufficiently sensitive to detect individual changes in the GABA+ signal and that the time of day at which the acquisition took place had little to no effect on the GABA+ signal. In our study, GABA+ values were reproducible over an interval of 131 days on average, although the effects of variable interval were not addressed systematically (a limitation of this study). Our data are also in agreement with an abstract showing a CV of 5.2% for GABA+/water in a lateral frontal cortical voxel acquired with multiple channel surface coils (37). The ICCs reported in Table 3b were unsatisfactory, with most values below 0.7, an arbitrary but generally accepted benchmark. Possible reasons for this are the small number of subjects (therefore limiting the extent of across-subject variance) and, possibly, the uneven interval between scans. GABA+/H₂O had the highest ICC among the GABA-related measures (0.78), similar to that reported by Kegeles *et al.* (37) for a voxel in the prefrontal cortex (0.84). Our ICC for GABA+/creatinine was much inferior (~0.5 versus 0.72) to that reported in eight male subjects in ref. (14). In that study, however, measurements from three different voxel locations were averaged within each subject, providing an estimate of GABA+ for each time point more robust to outliers.

The *J*-editing sequence also provided a measure of the amount of Glx that is partially co-edited with GABA+. However, only a fraction of Glx would be affected by the editing pulse used in our sequence because Glx-3 resonates at 2.1 ppm, on the shoulder of our editing pulse. Our Glx results therefore cannot be compared directly with other studies. The determination of the exact fraction of total Glx was not the purpose of this study. However, CVw values for partially co-edited Glx/creatinine were 4.6% and 6.9% in ACC and rFWM voxels, respectively. These values indicated that the fraction of Glx measured by the *J*-difference sequence remained stable across scans and could be used qualitatively to assess differences in Glx across clinical populations. This was confirmed in GM by an ICC value of 0.84.

Table 3c provides 95% confidence intervals for the differences between repeated scans and the number of subjects (*N*) required to detect a 5% mean difference in the assessed variables based on the standard deviation of the differences obtained in this study. This shows that, when the metabolites are referenced to creatine or water (except for GABA+/creatinine), the *N* value required to detect a small difference in repeated scans is within acceptable ranges. When metabolites are corrected for transmit gain and tissue composition only, however, *N* rises above 60. Among the GABA measures, GABA+/H₂O is best in terms of providing an efficient measure.

The *J*-editing methodology yielded values for NAA/creatinine and partially co-edited Glx/creatinine that were comparable with those reported in studies using similar scanning parameters and processing procedures. In ACC, the NAA/creatinine ratio of 1.29 ± 0.086 and partially co-edited Glx/creatinine ratio of 2.13 ± 0.202 were similar to the values reported by Ham *et al.* (38). At 3 T with TE = 35 ms and processed with LCModel, this study reported metabolite values in ACC of 1.19 ± 0.19 for NAA/creatinine and 2.24 ± 0.49 for Glx/creatinine. In the rFWM voxel, the values of 1.49 ± 0.12 for NAA/creatinine and 1.83 ± 0.264 for partially co-edited Glx/creatinine were corroborated by those reported by Garcia *et al.* (39). In the parietal WM, the authors used

a PRESS sequence with TE = 30 ms, processed the spectra with LCModel and found similar values of NAA/creatinine (1.51 ± 0.31) and Glx/creatinine (1.55 ± 0.49). Both our values and the values in the literature cited showed similar trends for higher Glx/creatinine in predominantly GM voxels and higher NAA/creatinine in predominantly WM voxels.

We also employed a linear regression technique to quantify metabolite variation between GM and WM. The method of extrapolating metabolite concentrations to pure GM and pure WM based on the GM/tissue percentage of the voxel has been validated previously using MRSI (40–42). Although the use of this method with single voxels was more prone to error because of the limited number of data points, it still afforded a reasonable solution to the problem of GM/WM partial voluming, as demonstrated by the acceptably low CVw, especially for 'pure' GM (~6% for creatine, less than 3% for NAA/creatinine, ~11% for GABA+ and <9% for Glx/water). The large difference in the GM/tissue percentage between the two voxels and our ability to reproducibly maintain the tissue composition added support for our extrapolation to pure GM and WM. ICCs were higher for pure GM than for pure WM. The reduction in the standard deviation of the extrapolated values for GABA+/H₂O and creatine/H₂O, when compared with absolute GABA+ and creatine values, indicates that the water signal correction has the intended effect of reducing the variance of the measurements across individuals.

We extrapolated absolute signal intensities and values referenced to water in addition to metabolites referenced to creatine in order to determine whether tissue variation was caused by the reference chemical or the desired metabolite. We found that GABA+ was 1.47 times more concentrated in pure GM than pure WM. Two other studies have reported GM and WM differences in GABA+. Using a two-dimensional, *J*-resolved spectroscopic imaging method, Jensen *et al.* (41) reported that GABA+ was 2.18 times more concentrated in GM, whereas Choi *et al.* (42), using a quantum filtering-based chemical shift imaging method, reported that GABA+ was 8.2 times more concentrated in GM than WM. Together, these studies indicate that a significant proportion of GABA+ is concentrated near synapses; however, the large variability in the GM/WM ratio of GABA+ indicates that the exact difference in GABA+ concentration between WM and GM may depend strongly on the brain area in question. This study was the first to report tissue differences based on measurements taken in ACC. Jensen *et al.* (41) and Choi *et al.* (42) measured tissue differences in the temporal and occipital lobes including subcortical structures, and in the prefrontal and parietal lobes, respectively. Petroff *et al.* (43) measured tissue differences in GABA by taking biopsy samples during neurosurgery, and found a GM/WM ratio of 1.68 in the temporal lobe. Although actual tissue samples offer a more direct measure than *in vivo* MRS, GABA synthesis and catabolism are strongly affected by post-mortem processes, most probably in a tissue-specific manner. Despite the spread of the reported values, the significant increase in GABA in GM relative to WM highlights the importance of quantifying and determining the effect of tissue composition on single-voxel MRS studies.

The information relating to the GM/WM gradient of GABA has implications for the functional imaging literature that attempts to predict blood oxygenation level-dependent [for example, ref. (44)] or other types of signal obtained with functional MRI (45) on the basis of resting GABA concentrations. Although partial voluming of GM and WM can be a confounding variable in these studies, it has rarely been addressed in detail in

this context. However, two studies reported a lack of correlation between GABA levels (using water as a reference) and the volume of GM in the studied voxel, expressed either in absolute terms (46) or as a percentage of overall tissue content (26). It is possible that the range of GM/WM mixture was too limited to generate a significant correlation with the limited number of subjects available in these studies, but partial voluming of GM and WM might be more relevant if correlative studies of GABA and functional MRI were performed in pathological conditions in which a wider variation across individuals occurs.

We have reported that creatine is between 1.77 and 2.00 times more concentrated in pure GM than pure WM. Other studies have reported lower GM/WM ratios of creatine that range from 1.20 to 1.41 (40,47–49); however, these studies used spectroscopic imaging techniques to extrapolate metabolite values to pure GM and WM. In addition, regional differences in the concentration of creatine, as well as differences in T_2 decay between acquisition methods, may also explain the differences in creatine between GM and WM. Moreover, spectroscopic imaging methods measure metabolite concentrations in a larger number of voxels with varying amounts of GM, and this allows a more precise linear extrapolation. The GABA + /creatinine ratio was less sensitive to partial volume effects than ratios to water or absolute values of GABA +. This could actually constitute a relative advantage when using ratios, provided that the relative proportion of GM/WM is constant across the studied populations. For example, we would agree with Donahue *et al.* (45), who studied ratios of GABA + to creatine in relation to various functional indices, that partial voluming of GM and WM was an unlikely contributor to the associations uncovered.

CONCLUSIONS

GABA measurements using the *J*-editing technique described here yielded reproducible estimates of both ratios to creatine and absolute values expressed in institutional units, allowing for improved interpretation of results deriving from voxels containing mixtures of GM and WM. This technique and processing steps should therefore be applicable in large clinical populations.

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