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Brain GABA Detection *in vivo* with the J-editing ^{1}H MRS Technique: A Comprehensive Methodological Evaluation of Sensitivity Enhancement, Macromolecule Contamination and Test-Retest Reliability

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Abstract

Abnormalities in brain γ-aminobutyric acid (GABA) have been implicated in various neuropsychiatric and neurological disorders. However, *in vivo* GABA detection by proton magnetic resonance spectroscopy (^{1}H MRS) presents significant challenges arising from low brain concentration, overlap by much stronger resonances, and contamination by mobile macromolecule (MM) signals. This study addresses these impediments to reliable brain GABA detection with the J-editing difference technique on a 3T MR system in healthy human subjects by (a) assessing the sensitivity gains attainable with an 8-channel phased-array head coil, (b) determining the magnitude and anatomic variation of the contamination of GABA by MM, and (c) estimating the test-retest reliability of measuring GABA with this method. Sensitivity gains and test-retest reliability were examined in the dorsolateral prefrontal cortex (DLPFC), while MM levels were compared across three cortical regions: the DLPFC, the medial prefrontal cortex (MPFC) and the occipital cortex (OCC). A 3-fold higher GABA detection sensitivity was attained with the 8-channel head coil compared to the standard single-channel head coil in DLPFC. Despite significant anatomic variation in GABA+MM and MM across the three brain regions ($p < 0.05$), the contribution of MM to GABA+MM was relatively stable across the three voxels, ranging from 41% to 49%, a non-significant regional variation ($p = 0.58$). The test-retest reliability of GABA measurement, expressed either as ratios to voxel tissue water (W) or total creatine, was found to be very high for both the single-channel coil and the 8-channel phased-array coil. For the 8-channel coil, for example, Pearson's correlation coefficient of test vs. retest for GABA/W was 0.98 ($R^2 = 0.96$, $p = 0.0007$), the percent coefficient of variation (CV) was 1.25%, and the intraclass correlation coefficient (ICC) was 0.98. Similar reliability was also found for the co-edited resonance of combined glutamate and glutamine (Glx) for both coils.

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Keywords

GABA; glutamate; Glx; ^{1}H MRS; J-editing; mobile macromolecules; phased-array coil; test-retest reliability

INTRODUCTION

Dysregulations of the inhibitory amino acid neurotransmitter system of γ-aminobutyric acid (GABA) have been implicated in the pathophysiology of most neuropsychiatric and of a number of neurological disorders, including schizophrenia [1], mood [2] and anxiety [3, 4] disorders, and epilepsy [5]. Therefore, there is great interest in studying the function of this neurotransmitter *in vivo*. However, detection of brain GABA by ^{1}H MRS presents a number of challenges [6–8]. First, the strongly J-coupled GABA multiplets in the 1.9–3.0 ppm range are overlapped by the much stronger resonances of N-acetyl-L-aspartate (NAA), total creatine (tCr), and combined glutamate+glutamine (Glx), rendering the α, β, and γ resonances of GABA undetectable by conventional MR spectroscopic techniques. Second, the estimated brain GABA concentration of 0.5–1.5 mM is at the lower limit of detection by ^{1}H MRS at commonly available static magnetic field strengths (B_o) of 3T or less. And, third, the GABA C^{4}H resonance at 3.0-ppm that is generally targeted for detection overlaps with a resonance due to mobile macromolecules (MM), which has a coupling partner just 0.2 ppm upfield from the 1.9-ppm GABA C^{3}H resonance, and thus co-edits with GABA in nearly all proposed editing approaches. Collectively, these challenges to reliable brain GABA detection can lead to poor estimates of its concentrations, masking of potential pathologic changes and confounding of interpretation.

In recent years, concerted research activity in several laboratories has led to the development of robust approaches for overcoming these impediments to the reliable detection of brain GABA *in vivo*. These include a variety of spectral editing and post-processing methods for addressing the challenges associated with spectral overlap and MM contamination, and use of phased-array head coils to partially alleviate the low GABA detection sensitivity [9]. Here, we report the results of a study that was designed to (a) evaluate quantitatively the GABA detection sensitivity gains that can be attained with an 8-channel phased-array head coil compared to a standard quadrature single-channel head coil [9] and the tradeoffs that can be made between signal-to-noise ratio (SNR), voxel size and scan time, without sacrificing spectral quality; (b) estimate the magnitude and extent of anatomic variation of the contamination of GABA by the co-edited mobile macromolecule resonance; and (c) evaluate the test-retest reliability of cortical GABA detection for the J-editing spin echo difference technique at 3.0 T. Since the combined glutamate and glutamine (Glx) C^{2}H resonance at 3.71 ppm is co-edited with this method, sensitivity gain and test-retest reliability evaluations of the Glx peak are also reported. While studies evaluating each of these impediments to GABA detection by J-editing have been reported, the differentiating feature of the present study is that it has evaluated all three impediments *simultaneously* in the *same* subjects.

METHODS

Study subjects

For each of the three study aims, we recruited 6 subjects from the same pool of 7 young adult subjects (ages 25.9 ± 2.7 years; 4 females), who gave written informed consent to participate. All subjects were medically and psychiatrically healthy graduate or medical students recruited at Columbia University Medical Center to undergo the brain MRS scans at Weill Cornell Medical College, with approval of the Institutional Review Boards of Columbia University, the New York State Psychiatric Institute at Columbia University, and Weill Cornell Medical College.

MRS Data Acquisition Protocol

All the neuroimaging studies were conducted on a research-dedicated GE 3.0 T MR system using manufacturer-supplied quadrature single-channel and 8-channel phased-array head coils. The basic pulse sequence used to acquire the GABA spectra was the J-edited spin echo difference method first described by Rothman et al. [6], for which several variants have been developed [10, 11]. The sequence used in this study was developed by Sailasuta et al. [11] for 3.0T GE scanners. Briefly, a standard PRESS sequence was converted into a volume-selective J-edited spin echo difference technique for GABA detection by adding a pair of frequency-selective cosine-modulated Shinar-LeRoux inversion radiofrequency (rf) pulses [11], flanked by spoiler gradient pulses of opposite signs, before and after the second 180° rf pulse of the double spin echo sequence. Application of this pair of frequency-selective "editing" pulses at the 1.9-ppm resonance frequency of GABA C^3H on alternate scans, with TE 68ms, alternately inverts the GABA C^4H resonance at 3.0 ppm by allowing or inhibiting its J-modulation. Subtracting the two subspectra thus acquired yields the desired GABA difference spectrum, consisting of the outer lines of the 3.0-ppm C^4H triplet, while the much stronger overlapping tCr resonance – a singlet that is not J-modulated – is eliminated. Due to the high structural, magnetic and chemical similarities between GABA, glutamate (Glu) and glutamine (Gln), this pulse sequence also achieves detection of the combined resonance of Glu and Gln, commonly referred to as Glx, at 3.71 ppm, although with much reduced efficiency. Therefore, in this study we also evaluated the 8-channel coil sensitivity gains and test-retest reliability for Glx, in itself of considerable interest since the excitatory amino acid neurotransmitter system of glutamate has been implicated along with the inhibitory GABA system in a variety of brain disorders. A pulse sequence repetition time, TR, of 1500ms was used throughout. Each application of the sequence also included synchronous acquisition of the unsuppressed voxel tissue water (W) resonance, whose integrated area served as an internal intensity reference.

Structural MRI for Voxel Placement

A three-plane, low-resolution, high-speed scout imaging series was obtained, followed by a series of standardized high-resolution axial, coronal and sagittal T_1- and T_2-weighted scans that were appropriately obliqued to enable optimal prescription of the ^1H MRS voxels of interest. In addition, fast Fluid-Attenuated Inversion-Recovery (FLAIR) scans were performed for detection of potentially exclusionary focal brain lesions.

GABA Detection with Single-channel or Eight-channel Head Coil and J-editing

To evaluate the sensitivity gains achieved for GABA and Glx detection with an 8-channel phased-array head coil compared to a standard quadrature single-channel coil, J-edited spectra were obtained with each coil from a single triply-obliqued 1.0 × 2.0 × 4.8-cm^3 (9.6-cm^3) and a 2.0 × 2.0 × 4.8-cm^3 (19.2-cm^3) voxel placed using anatomic landmarks in the left dorsolateral prefrontal cortex (DLPFC), with voxel faces oriented parallel to the middle frontal gyrus and to the brain surface (Fig. 1A). An upper limit of 30 min was set as the maximum practical total scan time for acquiring each edited spectrum. To compare the relative SNR values, adjusting for differences in voxel size for data acquired with the single- and/or eight-channel coil, SNR was expressed as the ratio of GABA or Glx peak area divided by the root mean square (rms) of the background noise and by voxel size.

Magnitude and Anatomic Variation of MM Contamination of Edited GABA

The short-tau inversion-recovery (STIR) technique with metabolite nulling [12–14] was implemented in this study to measure the levels of mobile macromolecules (MM), which co-edit with the GABA resonance detected by J-editing, in three brain regions: the DLPFC (Fig. 1A), the medial prefrontal cortex (MPFC) (Fig. 1B) including the pregenual anterior cingulate cortex (ACC), and the occipital cortex (OCC) (Fig. 1C). In the present study, we implemented STIR by preceding the J-editing sequence with a single non-selective 180° adiabatic spin inversion pulse, followed by an experimentally determined inversion-recovery delay (TI) of 525ms that allowed the slower-relaxing GABA and other metabolite resonances to reach the zero-crossing point in the rotating frame of reference, before turning on the editing module of the sequence exactly as it would be for GABA detection. A spectrum acquired in this manner would contain only the resonance of the faster-relaxing MM resonance, which would have a net positive magnetization for the value of TI that is optimal for nulling GABA and the other metabolites.

Two sequential MRS acquisitions were performed. First, a J-edited GABA spectrum uncorrected for MM contamination was recorded in 13 min from a 19.2-cm^3 voxel localized in the DLPFC (Fig. 1A), MPFC (Fig. 1B), or OCC (Fig. 1C), using the 8-channel phased-array head coil. Second, without moving the subjects, metabolite nulling by STIR was immediately implemented to record the spectra of the MM resonance at 3.0 ppm. The outcome measures from these scans were: (1) total GABA level ($GABA_T$) uncorrected for MM contribution (i.e., $GABA_T$ = GABA + MM), (2) the magnitude of MM contribution, and (3) the MM/$GABA_T$ ratio, or percent contamination, for each voxel.

Test-Retest Reliability for Brain GABA and Glx Measurement

A triply obliqued 19.2-cm^3 voxel was placed in the left DLPFC (Fig. 1A, left) as described. To estimate the test-retest reliability, edited DLPFC spectra were obtained from each of the 6 healthy volunteers, who were removed from the scanner after the first of the duplicate scans (the test) and returned for the second scan (the retest) 1 hour later, with the voxel replaced using the same anatomic landmarks. DLPFC test-retest data were similarly acquired with the voxel size decreased by half to 9.6 cm^3 (Fig. 1A, right) to assess the effect of SNR on reliability. Test-retest data were acquired with both the single-channel quadrature head coil

and with the 8-channel phased-array head coil to compare the reliability of measuring GABA and Glx with the two different coils.

Eight-Channel GABA ^{1}H MRS Data Processing

The MR signals, $S_n(t)$, recorded with the J-editing technique and the 8-channel phased-array head coil were combined into a single regular time-domain free-induction decay signal, $C(t)$, according to eq. (1) [15]:

$$C(t) = \sum_{n=1}^{n=8} \{W_n * a_n * e^{-(i\delta\phi_n)}\} * S_n(t) \quad (1)$$

where the summation is over all the 8 coil elements, n, of the array; W_n represent the relative coil sensitivities or weighting factors due to physical property differences between coil elements; a_n are the signal amplitudes which may vary as a function of proximity of the coil elements to the voxel of interest, and were all set to 1 in this study as overall coil dimensions were much greater than voxel size; and φ_n are the relative phases for the MR signal in the different coil elements. The relative phased-array coil sensitivities, W_n, and MR signal phases, φ_n, were obtained, respectively, from the magnitude and phase of the first complex time-domain point in the unsuppressed water signal from each receiver coil (Fig. 2A). These were then used as input into a C-language computer program that performed the complex numbers summation in eq. (1) and subtracted the two resulting edit-on and edit-off free-induction decays in the time domain to yield the difference signal (Fig. 2B), which was Fourier-transformed to yield, automatically, fully phase-corrected and combined GABA and Glx difference spectra (Fig. 2C, difference spectrum).

Spectral Data Analysis and Quantification

Details of the MRS data quality assessment criteria and procedures used to retain or reject spectra for further processing and analysis are provided in supplementary material online. In this study, all spectra met our data quality assessment criteria and were processed as illustrated in Figure 2C to obtain the area under the GABA and Glx peaks, which are proportional to the concentration of each neurotransmitter in the voxel of interest. Briefly, the GABA and Glx resonances in the J-edited difference spectra were modeled as a linear combination of pseudo-Voigt lineshape functions and then fitted in the frequency domain using a robust and highly optimized public-domain Levenberg–Marquardt nonlinear least-squares minimization routine, MPFIT [16]. The pseudo-Voigt lineshape function enables more precise analysis of lineshapes that consist of mixtures of Lorentzian and Gaussian functions [17], as is often the case for in vivo spectra.

The GABA and Glx peak areas derived with MPFIT were expressed as ratios relative to the synchronously acquired and similarly fitted unsuppressed intravoxel tissue water signal (W), as well as ratios to the fitted area of the total creatine (tCr) resonance in the spectra acquired with the editing pulses off.

Statistical analysis

The outcome measures assessing the magnitude and anatomic variation of MM contamination of the edited GABA resonance in the DLPFC, MPFC and OCC were evaluated and compared using repeated measures ANOVA. Test-retest reliability of the GABA and Glx measurements was assessed with Pearson's correlation coefficient (R), with the percent coefficient of variation (CV), and with the intraclass correlation coefficient (ICC), the latter being a statistic that compares within- to between-subjects variance such that a maximum ICC of 1.0 indicates identity between test and retest values, and lower values correspond to greater within-subject variability [18].

RESULTS

Eight-channel Sensitivity Gains and Tradeoffs for Scan Time and Voxel Size

Sample edited GABA and Glx spectra obtained with a standard quadrature single-channel head coil and with an 8-channel phased-array head coil are shown and compared in Fig 3. In panels A-C of Fig. 3, single- and 8-channel coil data recorded with a DLPFC voxel size of 19.2 cm^3 or 9.6 cm^3 (Fig. 1A) are compared. In panels D and E of the same Figure, data obtained with the 8-channel coil are compared for different voxel sizes and scan times.

Single- vs. Eight-channel Coils Varying Scan Time and Voxel Size

Using a single-channel head coil and signal-averaging for 26 min – the longest scan time used in this study – to record GABA and Glx spectra from a 9.6-cm^3 voxel (Fig. 3A, top trace) yielded very poor quality data. By comparison, using the 8-channel head coil with the same voxel size (9.6-cm^3) and signal-averaging time (26 min) as for the single-channel acquisition yielded excellent quality GABA and Glx spectra were be obtained (Fig. 3A, bottom trace). Fig. 3B compares a spectrum from a 9.6-cm^3 voxel obtained with the 8-channel coil (bottom trace) with that obtained from a 19.2-cm^3 voxel – i.e. doubling the voxel size only – with the single-channel coil (top trace) in the same scan time (26 min), and shows the 8-channel coil to yield a slightly higher quality spectrum despite the smaller voxel size. Lastly, comparing spectra obtained from a 19.2-cm^3 voxel in 26 min with the single-channel coil (Fig 3C, top trace) and with the 8-channel coil (Fig. 3C, bottom trace) yielded approximately a factor of 3 higher SNR (see Supplementary Table 1S online) for the 8-channel coil for both GABA (3.04 ± 0.38) and Glx (3.13 ± 0.40).

Eight-Channel Coil with Different Voxel Sizes and Scan Times

In Fig. 3D, 8-channel spectra-only obtained in 13 min from a 19.2-cm^3 voxel (top trace) and in 26 min from a 9.6-cm^3 voxel (bottom trace) – i.e., doubling the scan and halving the voxel size – were compared and found to exhibit nearly identical SNR. Finally, spectra obtained in 13 min (Fig. 3E, top trace) and in 26 min (Fig. 3E, bottom trace) from a 19.2-cm^3 voxel – i.e., constant voxel size, different scan time – show the predicted square-root dependence of SNR on scan time.

Overall, acquiring J-edited GABA and Glx spectra under identical conditions for the superficial DLPFC voxel with the 8-channel phased-array head coil achieved a 3-fold higher

GABA and Glx detection sensitivities compared to the standard quadrature single-channel head coil (Fig. 3C).

Magnitude and Anatomic Variation of GABA Contamination by MM

Reliable quantification of the contamination of the edited GABA signal by MM using metabolite nulling by STIR is highly dependent on the accuracy of determining the inversion-recovery delay, TI, that yields complete nulling of the metabolite resonances. The results of our STIR experiment to determine the optimal TI value for *in* vivo metabolite nulling are shown in Fig. 4A. For the shortest values of TI, all the major brain metabolite resonances were fully inverted, then they progressively recovered as TI was incremented, went through zero-crossing or "nulling" point, before relaxing fully at longer TI values. In the human brain, we found complete nulling of the major brain metabolites to occur for a TI value of 525ms (Fig. 4A). Using this optimal TI value, a pair of J-editing experiments, without (Fig. 4B,a) and with (Fig. 4B,b) metabolite nulling were conducted; the second experiment can be seen to yield a spectrum consisting of only the MM resonance. Subtracting the two spectra thus acquired yielded a difference spectrum in which the GABA C^4H resonance at 3.0 ppm has been corrected for MM contamination (Fig. 4B,a–b). Note that overlaying the MM spectrum (dotted line in Fig. 4B,a) on the MM-uncorrected spectrum shows a left 'shoulder' on the GABA resonance that coincides with the underlying MM resonance, indicating that the clear distortion in the GABA lineshape was due to the underlying MM resonance.

The relative contributions of MM and of "pure" GABA to the total measured GABA ($GABA_T$), all expressed as ratios to the unsuppressed water signal in our three voxels of interest, are shown in Fig. 5. Quantitatively, $GABA_T$ showed significant anatomic variability (OCC: $[3.11 \pm 0.46] \times 10^{-3}$; MPFC: $[2.24 \pm 0.57] \times 10^{-3}$; DLPFC: $[3.29 \pm 0.67] \times 10^{-3}$; $p = 0.03$), as did MM (OCC: $[1.36 \pm 0.21] \times 10^{-3}$; MPFC: $[1.06 \pm 0.21] \times 10^{-3}$; DLPFC: $[1.25 \pm 0.18] \times 10^{-3}$; $p = 0.02$). However, the ratio of the MM to $GABA_T$ (i.e., % contamination) did not differ significantly across brain regions (OCC: 44% ± 10%; MPFC: 49% ± 13%; DLPFC: 41% ± 17%; $p = 0.58$).

Test-Retest Reliability of GABA and Glx Measurement

We found the test-retest reliability of GABA and Glx measurement with the J-editing technique to be very high for both the standard quadrature single-channel coil and the 8-channel phased-array coil (Table 1 and Fig. 6). For instance, for the 8-channel coil, Pearson's correlation coefficient of test vs. retest for GABA/W was 0.98 ($R^2 = 0.96$, $p = 0.0007$, Fig. 6), the percent coefficient of variation (CV) was 1.25%, and the intraclass correlation coefficient (ICC) was 0.98. Similar reliability was found for Glx to voxel water ratios, as well as for both GABA and Glx ratios to tCr (Table 1; Fig. 6).

Discussion

This study has presented the results of a comprehensive assessment (a) of GABA and Glx detection sensitivity gains that can be achieved with an 8-channel phased-array head coil, (b) of the magnitude and anatomic variation of the contamination of GABA by mobile

macromolecules, and (c) of the test-retest reliability of measuring GABA and Glx with the J-editing technique at 3T. It should be noted that in the following discussion of items (a) and (c) above, the GABA levels were not corrected for the degree of mobile macromolecule contamination.

Sensitivity Comparisons: 8-channel vs. Quadrature Single-channel Head Coils

Our determination that implementation of the J-editing pulse sequence with an 8-channel phased-array head coil achieves significant gains in GABA and Glx detection sensitivity has confirmed, in general, the SNR advantage of such coils compared to single-channel coils, and, in particular, the results of van der Veen and Shen [9], who first proposed use of phased-array coils with the J-editing technique to enhance GABA detection sensitivity. The 3-fold higher in SNR achieved in this study for the DLPFC was undoubtedly made possible by the proximity of the voxel in this superficial brain region to the individual elements of the phased-array coil. Simulations by Albrecht et al. [19] assessing the spatial variation of SNR for array coils of different dimensions demonstrated that gains achieved with higher dimension array coils for deeper-lying voxels would be considerably less than those for more superficial voxels. For voxels in the DLPFC, MPFC and OCC that are near the elements of a phased-array coil, sensitivity gains can be achieved and traded for either smaller voxel sizes or shorter scan times, by as much as a factor of 3 for an 8-channel coil, without sacrificing spectral quality. In this study, halving the DLPFC voxel size from 19.2 cm^3 to 9.6 cm^3 (Fig. 1A) while maintaining the scan time at 26 min, allowed sampling of a mostly gray matter DLPFC voxel, which minimized partial volume-averaging with surrounding white matter (Fig. 3B, upper vs. lower traces). In general, the gained detection sensitivity is exchangeable for reductions in voxel sizes or scan times, as illustrated in Fig. 3.

Mobile Macromolecule Contamination of the Edited GABA Resonance

Using metabolite nulling, we have found a significant anatomic variation in the levels of total GABA uncorrected for MM (i.e., $GABA_T$) and those of MM between the DLPFC, MPCF and OCC, with the highest values in both quantities occurring in DLPFC, lowest values in MPFC, and intermediate values in OCC. A prior study examined anatomic variation of MM and reported higher values in cerebellum than in motor cortex, pons, or parietal whiter matter, but did not make a comparison to GABA levels [20]. On the other hand, the ratio of MM to $GABA_T$, i.e. the percent contamination, which ranged between 41% and 49% (mean ± SD: 44% ± 4%) and did not differ across the three brain regions targeted in this study ($p=0.58$), was in excellent agreement with prior determinations (Table 2) of 44 – 57% [6, 20–24]. This relatively stable contribution of MM to the J-edited GABA signal across different brain regions suggests that it may not be a significant confound if not accounted for in studies of normal human brain. However, the possibility remains that the contribution of MM to the J-edited GABA signal might differ significantly between normal and diseased brain – a limitation that could confound interpretation and thus should be acknowledged in studies comparing $GABA_T$ across diagnostic groups.

Routine use of metabolite nulling to estimate the magnitude of MM contamination in clinical studies is not practical because it doubles the total scan time. In this respect, implementation of J-editing sequence variants (“symmetric editing”) that can achieve MM

correction in a single shot [22, 25] offers a distinct advantage. Symmetric editing has been reported to show test-retest reliability comparable to $GABA_T$ from standard GABA editing [24]. Table 2 includes a summary of studies that have implemented this approach in clinical investigation.

Test-Retest Reliability of Brain GABA and Glx Measurement by J-editing

Our assessment of the test-retest reliability for GABA and Glx measurement using J-editing at 3.0 T has yielded high values for either a standard quadrature single-channel head coil or an 8-channel phased-array coil (Table 1). Although we found the reliability for GABA and Glx measurements expressed as ratios to voxel tissue water to be slightly higher than that for ratios to tCr, the reliability for both referencing methods is excellent and both seem appropriate for use in the normal brain. However, because a number of recent studies have reported changes in tCr levels in various pathological conditions [26–28] the previously assumed stability of this resonance has thus been questioned and caution in its use as an intensity reference is warranted.

Several previous studies [24, 29–34] have evaluated the test-retest reliability of GABA under various conditions. All except Wijtenberg et al. [34] reported levels of GABA plus MM, i.e. $GABA_T$, acquired at 3.0T using the J-editing technique that can contain a contribution from macromolecule signals in the range of 41–57% [6, 21–24] (Table 2). With CV values of less than 4% for the single-channel coil and 2% for the 8-channel coil, our reliability values are to date the highest to be reported. The lowest reliability reported had a CV value of about 15%, with most studies reporting CV values that clustered around 6–8% (Table 3).

Evans et al. [29] found occipital and sensorimotor cortex CV values of 6.5% and 8.8%, while Bogner et al. [30] reported occipital lobe CV values ranging from 13.3%–15%, depending on referencing and fitting methods. O'Gorman et al. [31] studied the left DLPFC and reported CV values ranging from 7%–12%, depending on the fitting method used. Geramita et al. [32] assessed the reliability of measuring GABA in the anterior cingulate cortex and right frontal white matter and reported CV values of 5.3% and 8.7%, respectively. Harada et al. [33] used the J-editing technique and a single-channel head coil to assess the reliability of measuring absolute concentrations of GABA in frontal lobe, ACC and lentiform nuclei voxels, and reported average CV and ICC values of 4.6% and 0.7, respectively. Wijtenberg et al. [30] was the only study to report GABA acquired at 7.0T and free of macromolecule signal. They compared STEAM and J-editing acquisitions in the anterior cingulate and DLPFC with different analysis methods, and found comparable CV values, although on the upper side of those obtained at 3.0T (e.g., CV values by J-editing were 13.6% in anterior cingulate and 13.4% in DLPFC). Recent studies have examined reliability over much longer inter-scan intervals [35]; across multiple brain regions [36]; in elderly subjects [37]; and in comparison of $GABA_T$ to GABA uncontaminated by MM signal [24] (Table 3).

A potentially significant factor for the smaller CV values in the present study could be the very short (one-hour) inter-scan interval, designed for comparison of studies of pre- and post-interventions at a comparable interval. Regional differences, physiological/metabolic variations during inter-scan delays, and/or systematic instrumental and subject repositioning

errors between the scans might account for some of the differences in the test-retest reliability values among the studies. Table 3 provides a summary of these studies with additional methodological details, which in aggregate indicate a relatively high reliability in measuring *in vivo* brain GABA by ^{1}H MRS.

Study Limitations

The presented results were obtained from a relatively small and homogenous sample of healthy young adult subjects. In addition, only a limited number of brain regions was investigated. Therefore, our results might not be generalizable to other specific *in vivo* conditions. A clear example is the potential for estimates of anatomic variation in MM contamination to be different under pathological conditions. Furthermore, our relatively short inter-scan delay of 1 h might have minimized within-subject temporal variations in GABA and Glx that may occur over longer inter-scan delays as mental status and experimental conditions vary. Estimates of test-retest reliability across a short interval such as 1 h are appropriate for studies in which a GABA- and/or Glx-altering intervention is presented and levels are determined prior to and immediately following the intervention (e.g., our recent acute ketamine challenge studies [38, 39]).

Conclusion

This study has established under the same conditions, quantitatively, and simultaneously for the first time (a) the sensitivity gains that can be achieved for GABA and Glx detection with the J-editing technique and an 8-channel phased-array head coil, which can be traded for smaller voxels or shorter scan times; (b) the degree and anatomic variation of the contamination of the J-edited GABA signal by co-edited MM signal; and (c) the test-retest reliability of GABA and Glx measured by J-editing. The J-editing technique is a powerful, sensitive, and reliable tool for advancing our understanding of the major inhibitory and excitatory amino acid neurotransmitters *in vivo* in the normal or diseased brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations used

ACC	pregenual anterior cingulate cortex
ANOVA	analysis of variance
B_0	static magnetic field strength
CV	coefficient of variation

CRLB	Cramer–Rao lower bound
DLPFC	dorsolateral prefrontal cortex
FWHM	full width at half-maximum
GABA	γ-aminobutyric acid
Glx	combined glutamate and glutamine resonance
ICC	intraclass correlation coefficient
MM	mobile macromolecule
MPFC	medial prefrontal cortex
NAA	N-acetylaspartate
ppm	parts per million
OCC	occipital cortex
rf	radiofrequency
ROI	region of interest
SNR	signal-to-noise ratio
STIR	short-tau inversion-recovery
tCr	total creatine
TI	inversion-recovery time
W	unsuppressed intravoxel water signal

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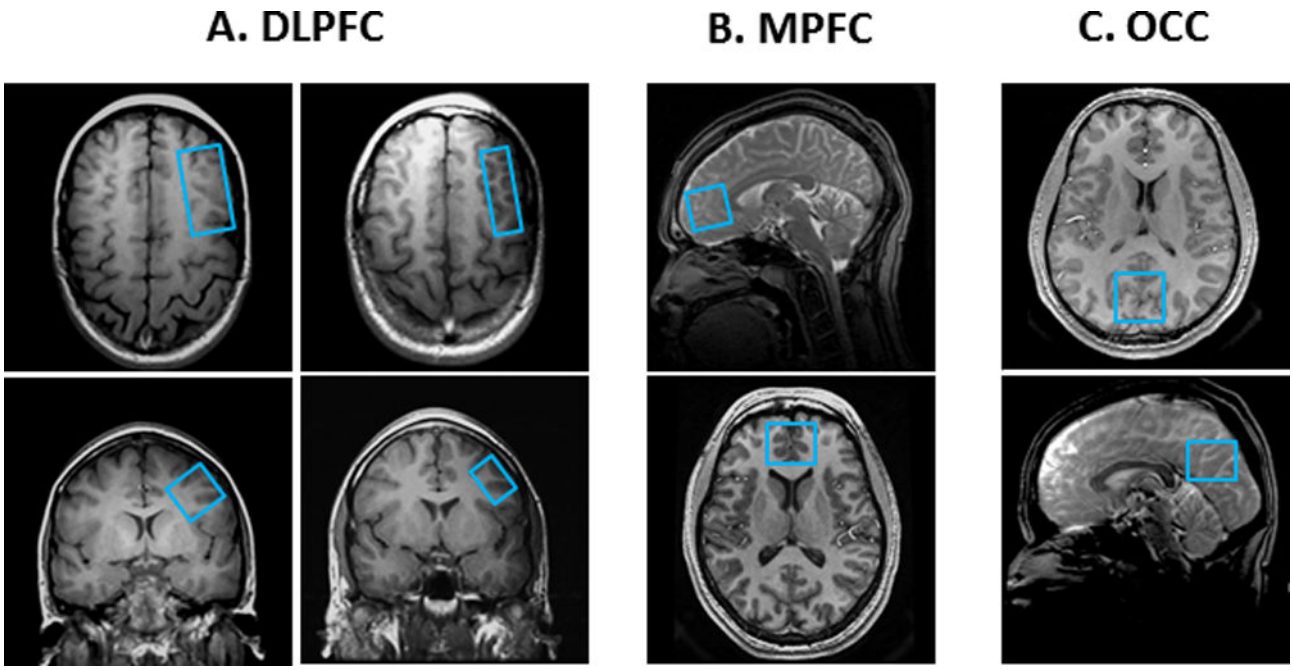

Figure 1.

Depiction on high-resolution MR images of the MRS voxels targeted in this study. (A) Dorsolateral prefrontal cortex (DLPFC) voxels: Left, 19.2-cm^3 voxel; Right, 9.6-cm^3 voxel; (B) 19.2-cm^3 medial prefrontal cortex (MPFC) voxel; (C) 19.2-cm^3 occipital cortex (OCC) voxel.

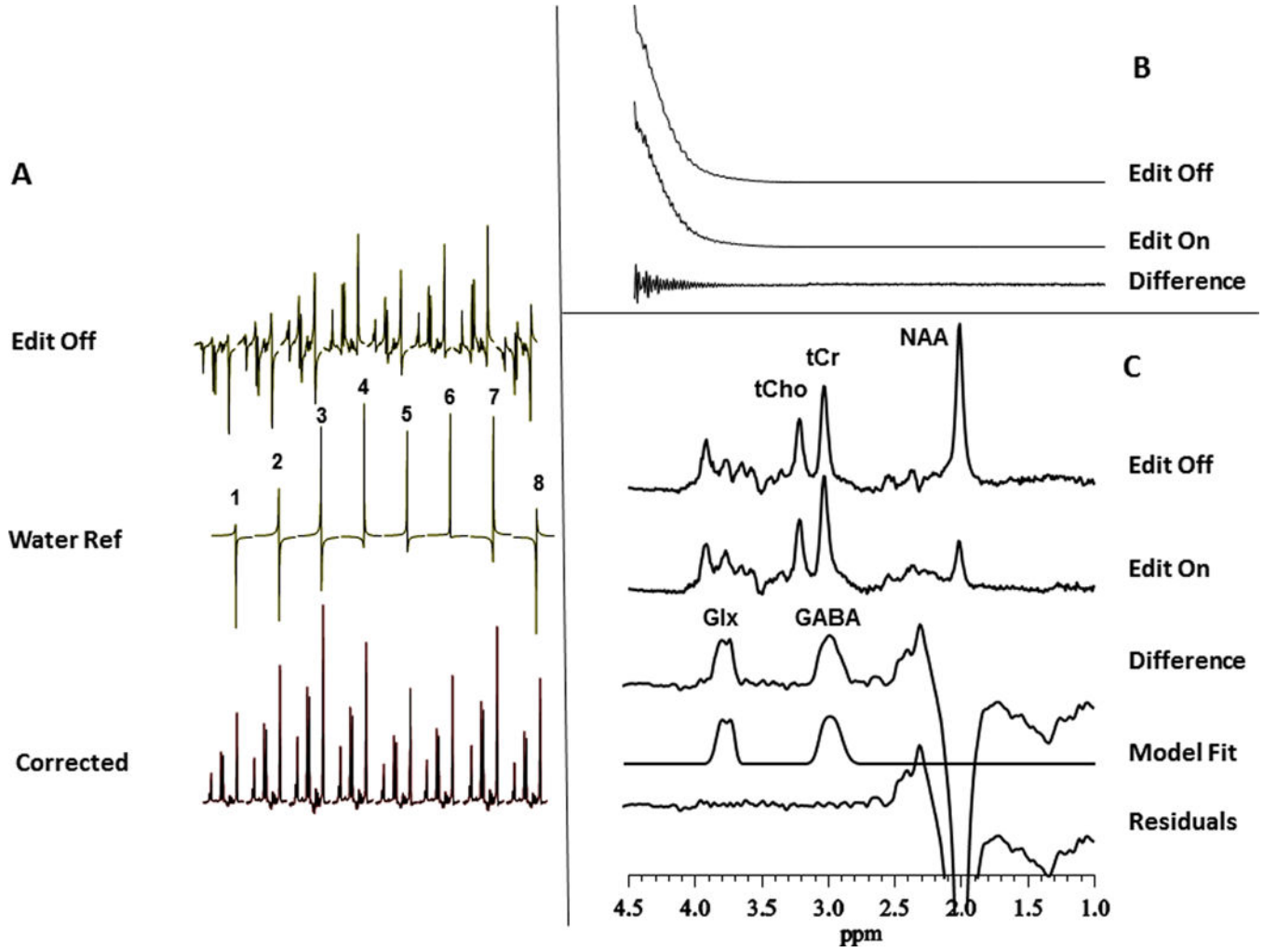

Figure 2.

Reconstruction of the recorded J-editing data:

(A) Individual spectra from each of the 8 coil elements of the phased-array used in this study. The top traces illustrate the variations in the amplitudes and phases in the water-suppressed metabolite spectrum with the coils in the array. The middle traces present the amplitude and phase variations as in the top traces but for the unsuppressed water voxel resonance. Note that the variations of the metabolite signal amplitudes and phases (top traces) are identical to those of the unsuppressed water resonance (middle traces), justifying use of the amplitudes and phases of the robust unsuppressed water signal from each receiver coil to combine the data into a single regular 1D signal. The bottom traces show the spectra for each of the 8 coil elements after application of the correction factors from the water signal. Each spectrum shows a pure absorption lineshape, indicating the appropriateness of the phase angles derived from the unsuppressed water resonance for phase correction of the metabolite or edited spectra. The 8 spectra thus reconstructed can simply be summed, after correction for potential frequency shifts, to produce the final single spectrum from the 8-channel coil.

(B) Alternatively, the reconstruction of the phased-array data can be performed entirely in the time-domain. The free-induction decay (FID) signals for the data acquired in each receiver coil with the editing pulse alternately off or on are combined automatically in the time domain also using weighting factors from the corresponding and synchronously acquired unsuppressed voxel water signals. Subtraction of the two FIDs thus derived yields a difference FID that can be processed in the usual manner. Also, note the near complete elimination of the strong water signal after subtraction, which leaves a free-induction decay with a clear interferogram reflecting the chemically shifted GABA and Glx signals; a large residual water signal after subtraction would generally indicate subject head movement during an acquisition.

(C) Frequency-domain illustration of the J-editing method for GABA and Glx detection by ^{1}H MRS *in vivo*: (top to bottom) single-voxel subspectra acquired in 13 min with the editing pulse on or off and 256 (512 total) interleaved averages; difference between the two subspectra showing the edited brain GABA and Glx resonances; a model fit of the difference spectrum to obtain the GABA and Glx peak areas; and residual of the difference between the experimental and fitted model spectra. GABA, γ -aminobutyric acid; Glx, glutamate + glutamine; NAA, N-acetyl-L-aspartate; tCho, total choline; tCr, total creatine.

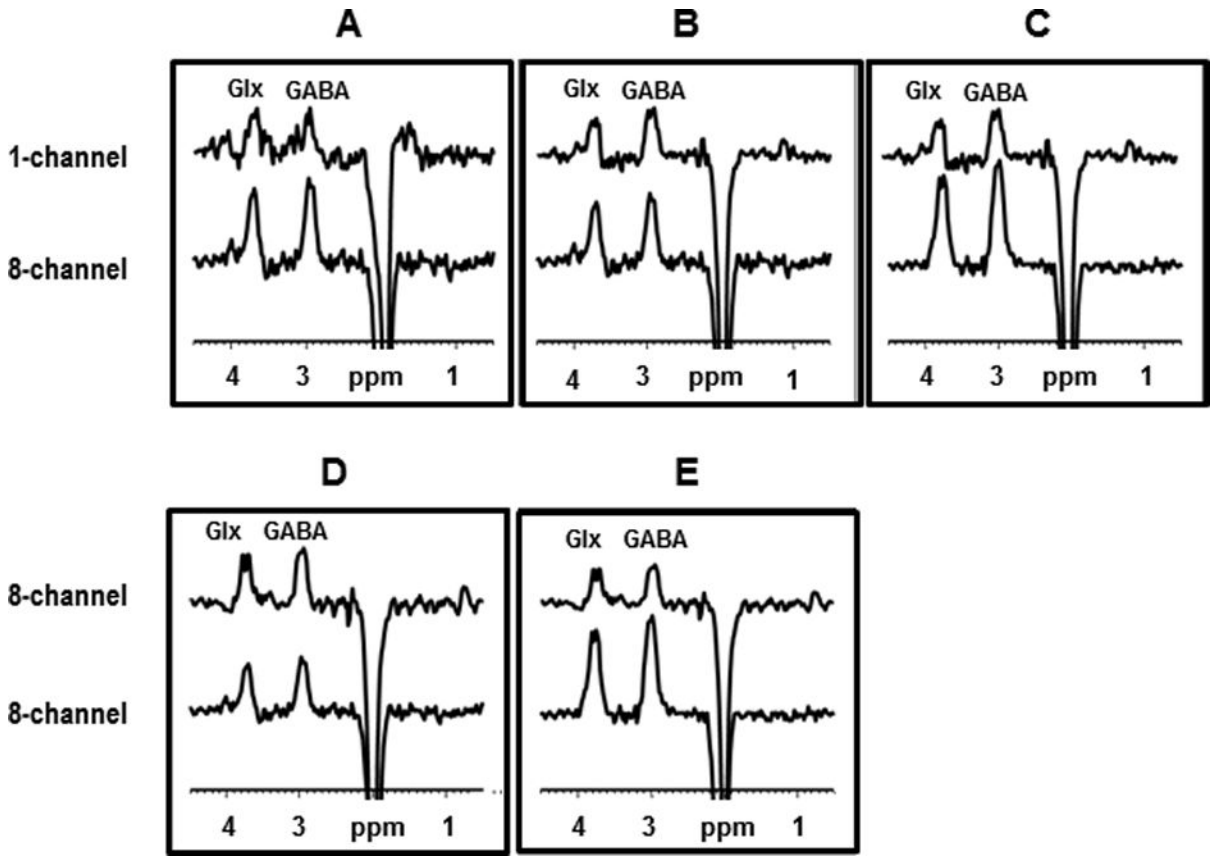

Figure 3.

DLPFC spectra contrasting the effects of scan duration, voxel size, and coil type used. The panels show spectra acquired with single-channel (upper panel) and 8-channel (lower panel) coils from (A) a 9.6-cm^3 voxel in 26 min; (B) a 19.2-cm^3 voxel (upper panel) and a 9.6-cm^3 voxel (lower panel), each in 26 min; (C) a 19.2-cm^3 voxel in 26 min (both upper and lower panels); and (D) with an 8-channel coil from a 19.2-cm^3 voxel in 13 min (upper panel) and from a 9.6-cm^3 voxel in 26 min (lower panel); and (E) with 8-channel coil from a 19.2-cm^3 voxel in 13 min (upper panel) and in 26 min (lower panel).

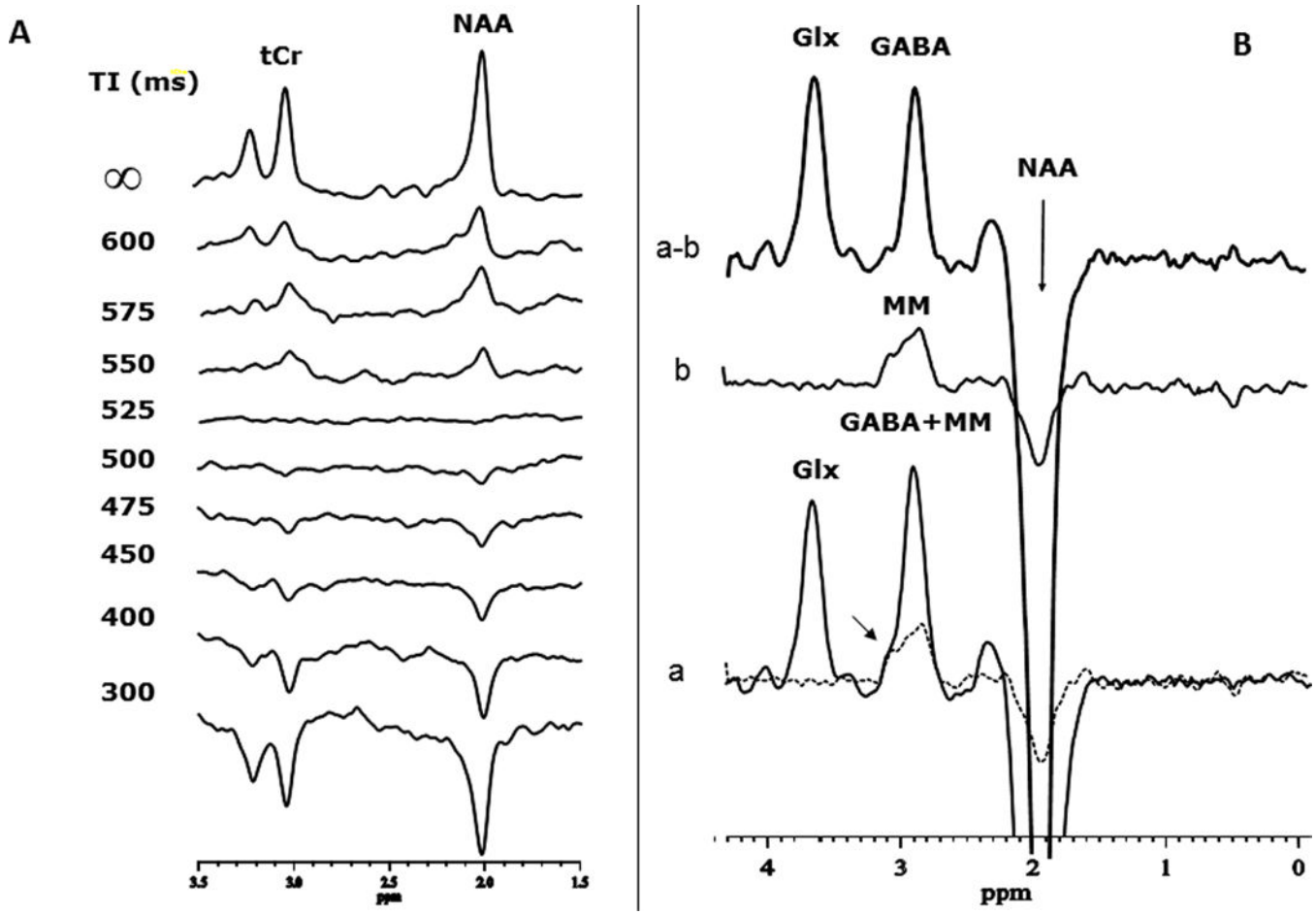

Figure 4.

Determination of the contamination of the edited GABA signal by mobile macromolecule (MM) signals using metabolite nulling by STIR: (A) Sample *in vivo* localized inversion-recovery study to determine the optimal inversion-recovery delay (TI) for nulling the metabolite signals in the STIR experiment, which was about 525ms in this study. (B) J-editing experiment conducted (a) without STIR and (b) with STIR. Note that when metabolites are nulled with STIR, a clear and substantial MM resonance is detected at the GABA chemical shift, although this was likely made possible by the good shim and SNR of these particular spectra; (a–b) shows the edited spectrum with the GABA amplitude corrected for MM contamination

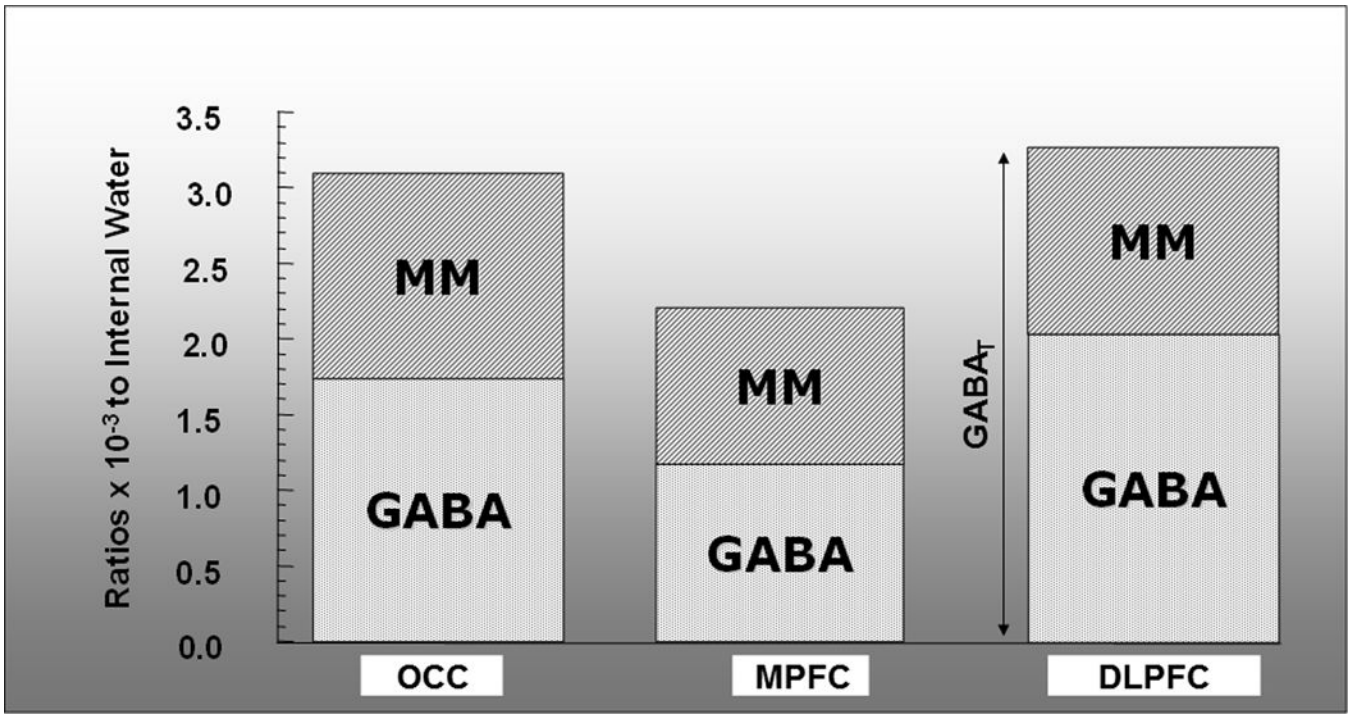

Figure 5.

$GABA_T$ measurements in each of 3 voxels (OCC, MPFC, DLPFC) showing the contributions in each voxel of GABA and MM. Percent contribution of MM to $GABA_T$ ranged from 41% to 49% across these regions.

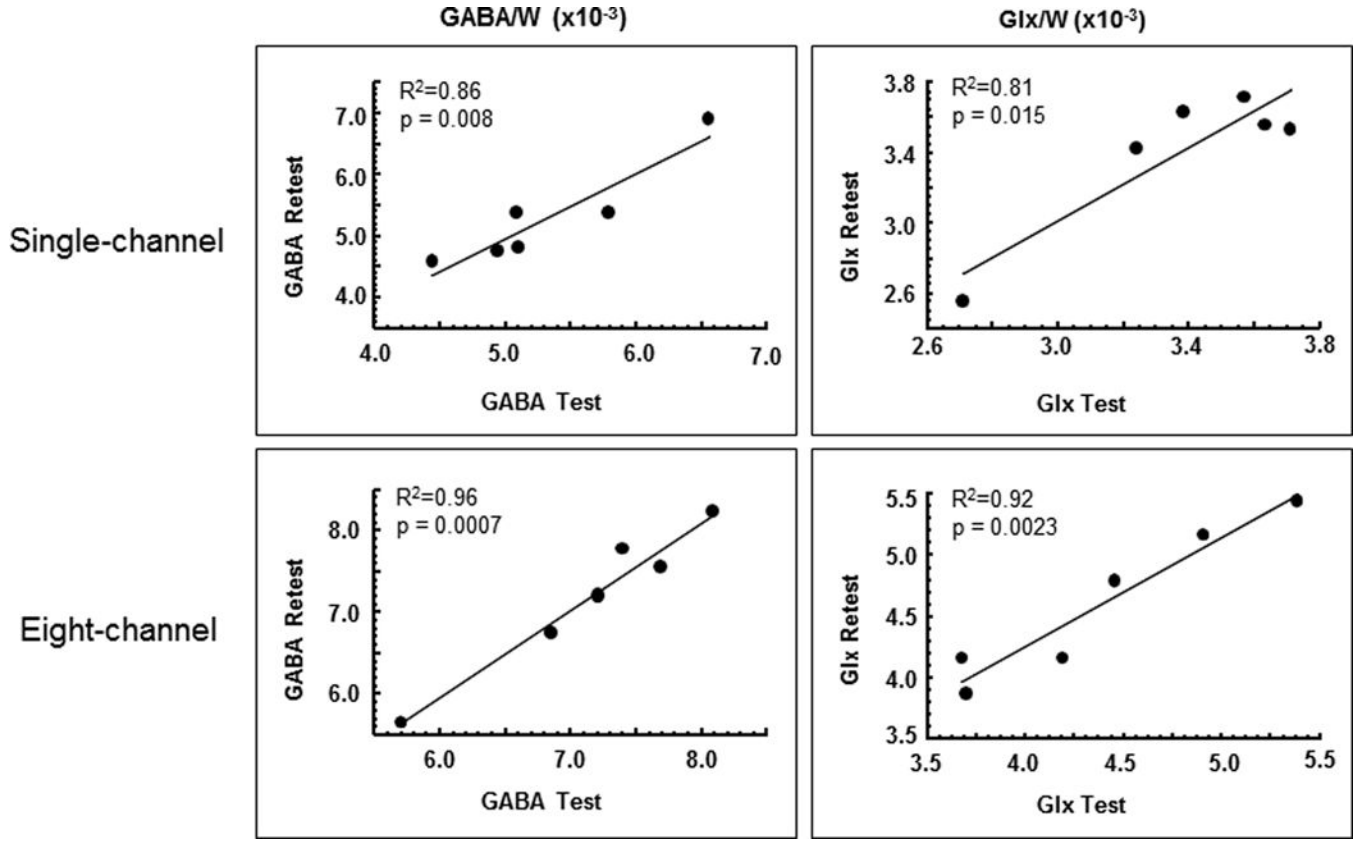

Figure 6.

Regression plots of GABA and Glx levels as ratios to the unsuppressed voxel tissue water (W) in the test vs. retest scans for the single-channel and 8-channel coils. All cases showed very high test-retest reliability for the 1-hour duration between test and retest examined in this study.

TABLE 1

Test-Retest Results

a. Single-Channel Coil				
	GABA/H_2O	**GABA/tCr**	**Glx/H_2O**	**Glx/tCr**
R	**0.93**	0.90	**0.90**	0.94
R^2	**0.86**	0.81	**0.81**	0.88
p (R^2)	**0.008**	0.014	**0.015**	0.006
ICC	**0.93**	0.89	**0.90**	0.86
%CV	**3.64**	6.05	**3.49**	4.65

b. Eight-Channel Coil				
	GABA/H_2O	**GABA/tCr**	**GIX/H_2O**	**Glx/tCr**
R	**0.98**	0.98	**0.96**	0.95
R^2	**0.96**	0.96	**0.92**	0.90
P(R^2)	**0.0007**	0.0006	**0.0023**	0.0041
ICC	**0.98**	0.97	**0.95**	0.96
%CV	**1.25**	1.72	**2.84**	2.32

Table 2

Mobile Macromolecule Contamination

Reference	Field strength	Subjects	Method	Brain region(s)/voxel	Results
Rothman el al. [6]	2.1T	2 HCs 2 epilepsy patients	Symmetric editing	OCC	44% MM contamination
Henry et al. [25]	3.0T	(in vitro)	Symmetric editing	(in vitro)	elimination of MM contamination
Shen et al. [21]	2.1 T	7 HCs	Multiple-quantum editing	OCC	Reduced MM contamination
Near et al. [22]	3.0T	5 HCs	Symmetric editing, “MEGA-SPECIAL”	OCC	46% MM signal eliminated
Edden et al. [40]	3.0T	10 HCs	Increased TE (80 ms) and symmetric editing	Medial parietal lobe, $(3.5\ cm)^3$	suppression of coedited MM
Aufhaus et al. [23]	3.0T	45 HCs	Symmetric editing	dACC	50% contamination eliminated
Rowland et al. [41]	3.0T	60 SCH, 77 HCs	Symmetric editing	dACC	uncontaminated GABA lower in older SCH
Mikkelsen et al. [24]	3.0T	30 HCs	Symmetric editing	dACC, OCC	52–57% MM contamination
This study	3.0T	6 HCs	Metabolite nulling	DLPFC, MPFC, OCC	41–49% MM contamination

Table 3

GABA Test-Retest Reliability

Reference	Field strength	Subjects	Acquisition sequence	Interval	Analysis	Brain regions/voxels	Results, CV for GABA
Evans et al. [29]	3.0T	8 HCs	MEGA-PRESS, 8-channel	2.5 hours, 5 scans per subject	Frequency-domain spectral fitting Unsuppressed water normalization	OCC, Sensorimotor/27 cm^3	Occipital, 6.5%; Sensorimotor, 8.8%
Bogner et al. [30]	3.0T	11 HCs	MEGA-PRESS	Variable; at least 1 day; 2–4 scans per subject	AMARES, jMRUI [42] Referencing to both tCr and unsuppressed water	OCC/22.5 cm^3	GABA/tCR,13.3%; GABA/water, 15.0%
O'Gorman et al. [31]	3.0T	14 HCs	MEGA-PRESS	4 scans within-session	LCModel [43], MATLAB, jMRUI; water referencing	DLPFC/30 cm^3	GABA, 7–12% (Glx,6–18%)
Geramita et al. [32]	3.0T	10 HCs	MEGA-PRESS, single-channel	28–226 days (131 ± 78 days)	Frequency-domain spectral fitting Referencing to both tCr and unsuppressed water	dACC, rFWM /18 cm^3	dACC GABA/W, 5.3%; rFWM GABA/W, 8.7%. dACC GABA/tCr, 6.5%; rFWM GABA/tCr, 8.2%
Harada et al. [33]	3.0T	15 HCs	MEGA-PRESS, single-channel	1 week	LCModel	Lentiform nuclei, frontal lobe, ACC/ 27 cm^3	4.6%
Wijtenburg et al. [34]	7.0T	4 HCs	STEAM, MEGA-PRESS, 32-channel	8 ± 2 days	STEAM: LCModel MEGA-PRESS: Frequency-domain spectral fitting	AC, DLPFC/STEAM:27 cm^3 MEGA-PRESS: 34.2 cm^3	AC STEAM, 3.5% DLPFCSTEAM, 16.2% AC MEGA-PRESS, 13.6% DLPFCMEGA-PRESS, 13.4%
Near et al. [35]	3.0T	17 HCs, male	MEGA-PRESS	229 ± 42 days	jMRUI; ReferencingtotCr	OCC /27 cm^3	0.2–12.3%; mean 4.3%
Gaetz et al. [36]	3.0T	5HCs	MEGA-PRESS	~5 days, 5 scans per subject	jMRUI; referencing to tCr, NAA, and Glx	Motor cortex/27 cm^3 Auditory cortex/24 cm^3 Visual cortex:/27 cm^3	6–14%; average ~10% over regions and referencing methods
Mikkelsen et al. [24]	3.0T	15 HCs × 2 cohorts	MEGA-PRESS with and without symmetric editing	15 minutes	Gannet[44]	dACC/ 24 cm^3 OCC/27 cm^3	$GABA_T$: 4.0% OCC, 14.8% dACC; GABA symmetric editing: 8.6% OCC, 12.6% dACC
Long et al. [37]	3.0T	5HCs, elderly	MEGA-PRESS	2–28 days	LCModel comparing 2 basis sets	Cerebellum, left and right/ 15.6 cm^3	4–13%
This study	3.0T	6 HCs	MEGA-PRESS, single-channel and 8-channel	1 hour	Frequency-domain spectral fitting Unsuppressed water normalization	DLPFC/9.5 cm^3	GABA, 3.6% single-channel; 1.3%, 8-channel (Glx, 2.8–3.5%)

HC=healthy control; SCH=schizophrenia patient; DLPFC=dorsolateral prefrontal cortex; dACC=dorsal anterior cingulate cortex; OCC=occipital cortex; rFWM=right frontal white matter; tCr=total creatine/phosphocreatine; W=unsuppressed water; AC=anterior cingulate.