Comparison of the Repeatability of GABA-Edited Magnetic Resonance Spectroscopy with and without Macromolecule Suppression

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Purpose: The inhibitory neurotransmitter γ-aminobutyric acid (GABA) can be measured in vivo using edited magnetic resonance spectroscopy (MRS), but quantification suffers from contamination by macromolecules (MM). It is possible to suppress this contamination using symmetric editing, but this procedure potentially compromises reliability of the GABA measurement. The aim of this study was to compare the repeatability of GABA-edited MRS with and without MM suppression.

Methods: GABA′ (non-MM contaminated) and GABA′+MM (MM-contaminated) concentration was measured in the occipital lobe (OCC) and anterior cingulate (AC) using symmetric and standard editing ($n = 15$). Each method was performed twice in each region.

Results: Within-participant coefficients of variation for each technique were 4.0% (GABA′+MM) and 8.6% (GABA′) in the OCC and 14.8% (GABA′+MM) and 12.6% (GABA′) in the AC. Intraclass correlation coefficients were better for the suppression method than standard editing in both the OCC (0.72 versus 0.67) and AC (0.41 versus 0.16). These findings were replicated in the OCC of a second cohort ($n = 15$).

Conclusion: Symmetric suppression is shown to be comparable in repeatability to standard GABA-editing. Measuring a purer quantification of GABA becomes increasingly important as more research is conducted on links between GABA concentration, pathology and healthy behavior. **Magn Reson Med 75:946–953, 2016. © 2015 Wiley Periodicals, Inc.**

Key words: GABA; macromolecules; MRS; repeatability; symmetric suppression

INTRODUCTION

As the chief inhibitory neurotransmitter, γ-aminobutyric acid (GABA) plays an integral role in the excitatory–inhibitory balance in the human brain (1,2). It has become relatively straightforward to detect GABA in vivo using proton magnetic resonance spectroscopy (^{1}H MRS). This is in large part due to the simple implementation and increasing standardization of relevant methodology (3). Consequently, there has been a growing interest in clinical investigations of GABA concentration in several mental disorders, including schizophrenia, depression, bipolar disorder, attention deficit hyperactivity disorder, autism and obsessive compulsive disorder (4–9). There has also been a noticeable amount of research conducted on correlational relationships between baseline GABA levels and cognitive and behavioral responses in healthy populations in an attempt to reveal neurochemical predictors of psychological and overt behaviors (10–13). Investigations into possible GABAergic influences on other neuroimaging signals (e.g., BOLD-fMRI, magnetoencephalography) suggest that GABA plays an integral role in the origin and modulation of neurovascular coupling (14–16).

J-difference editing is commonly used to detect GABA in vivo as its coupling and overlap with other metabolites hampers unambiguous detection. Editing takes advantage of the fact that the spins in the GABA molecule are coupled with each other. By running two scans, one where refocusing pulses are applied to the 1.9 ppm GABA resonance ("ON" editing pulses) and another where pulses are placed to be symmetrical about the water resonance ("OFF" editing pulses, 7.5 ppm), the GABA peak at 3.0 ppm is resolved in the spectrum obtained from the difference between ON and OFF scans (3). Nonetheless, a major limitation with this editing technique is that a macromolecular (MM) resonance at 1.7 ppm coupled to another MM resonance also at 3.0 ppm is partially excited by the ON editing pulses, leading to co-editing of the 3.0 ppm MM resonance. Thus, the GABA peak at 3.0 ppm in the difference spectrum contains a degree of MM contamination, which may account for up to 60% of the total signal (17).

One method that can be implemented to account for this contamination involves measurement of the MM baseline by nulling metabolites of interest through an inversion recovery technique (18). This baseline can then be subtracted from a conventional difference-edited spectrum to remove residual MM (19). The disadvantages of this technique, however, include assumptions of T_1 relaxation times of metabolites and MM and increased total acquisition time (both nonnulled and MM-only spectra need to be acquired). Alternatively, the issue with MM can be mitigated by using a symmetric editing-based suppression method (20). By simply placing OFF editing pulses at 1.5 ppm, and keeping ON pulses at 1.9 ppm, the MM signal at 1.7 ppm is equally affected in both scans and thus the coupled MM resonance at 3.0 ppm is removed from the difference spectrum. Because hypotheses relating the measured GABA concentration to behavior or pathology relate specifically to the GABA molecule, rather than loosely defined "macromolecules,"

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a more specific measure of GABA is required for proper interpretation. The elegant symmetric suppression method has not been widely adopted as it requires editing pulses to be sufficiently selective so that the pulses placed at 1.5 ppm do not undesirably excite the GABA resonance at 1.9 ppm. This partial excitation of the GABA resonance in OFF sub-spectra would lead to a reduction in GABA signal in the difference spectrum. However, by increasing the echo time (TE) from the widely used 68 ms to 80 ms, more time is available in the acquisition sequence and the duration of editing pulses can be increased to 20 ms (21). (On some platforms, it is possible to use longer editing pulses while maintaining a TE of 68 ms (22)). This alteration provides better frequency selectivity of editing pulses, preventing suppression of the resolved GABA signal, while still correcting for MM.

Several studies have already reported on the reproducibility of edited spectroscopy used to quantify GABA concentration in the human brain (23–25). To date, however, no such research has been conducted on symmetric editing. Therefore, the principal aim of this study was to determine, at 3T, whether the repeatability of the more specific measure of GABA obtained using the symmetric MM suppression technique is comparable to that of the standard GABA-edited MRS technique, which includes a significant MM contribution to the derived GABA concentration.

METHODS

All single-voxel ^{1}H MRS experiments were conducted using a 3 Tesla (T) GE Signa HDx MRI scanner (GE Healthcare, Waukesha, WI) with an eight-channel receive-only head coil and a body coil for transmit.

Phantom Experiments

Two phantom experiments were performed in a 20-mM GABA phantom to select the symmetric suppression acquisition to be used in the study. The phantoms were scanned at room temperature and the effect of temperature on chemical shift was accounted for by adjusting the placement of editing pulses accordingly.

Effect of Partial Excitation

The proximity of the OFF editing pulse to the GABA resonance in the MM-suppressed acquisition will lead to partial excitation of the GABA resonance and signal loss. The impact of this on the GABA signal was measured using two variants of the editing acquisition: (i) a "standard" GABA acquisition with TE = 68 ms allowing an editing pulse duration of 16 ms and (ii) an acquisition similar to Edden et al (21) with TE = 80 ms to allow longer 20-ms editing pulses. Other scan parameters were as follows: 20 × 20 × 20 mm^3 voxel, repetition time (TR) = 1800 ms, 128 averages, 4096 data points, 5 kHz spectral width. One editing pulse position was varied from 1.26 ppm to 2.54 ppm in increments of 0.04 ppm over a series of acquisitions, while the other was fixed at 7.5 ppm. The degree of GABA co-editing (and hence signal loss) in the OFF sub-spectra is reflected in the GABA integral when ON editing pulses are placed at 1.5 ppm.

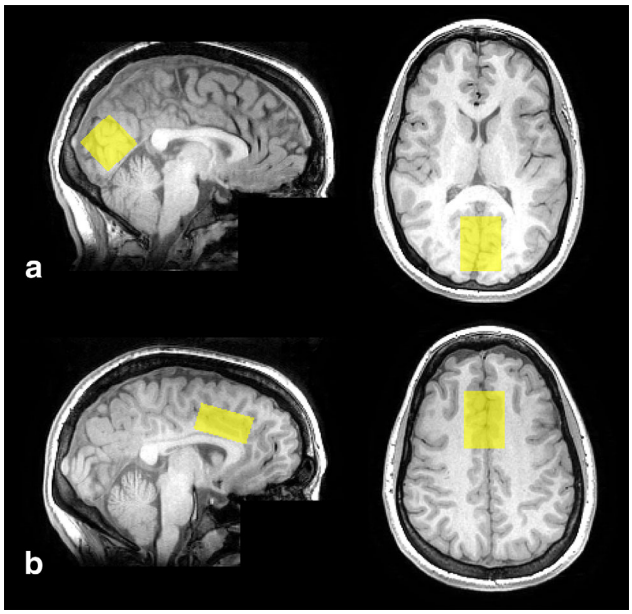

FIG. 1. Representative placement of MRS voxels in the occipital lobe (**a**) and anterior cingulate (**b**) in one participant.

Effect of Increasing TE

To investigate the impact of an increase in TE on the GABA signal, TE was modulated from 60 to 80 ms in 4-ms steps to estimate the overall signal loss between the two methods (other scan parameters were as above). ON and OFF editing pulses (16 ms) were placed at 1.9 ppm and 7.5 ppm, respectively.

In Vivo Experiments

Experiment 1

Fifteen healthy participants (mean age = 26.1 ± 5.1 years; 8 females) were recruited for two 1-h scan sessions. Participants consented to take part in this research, which was approved by the local institutional ethical review board.

In the first scan session, a 30 × 30 × 30 mm^3 voxel was prescribed to the medial occipital lobe (OCC) (Fig. 1a). The ventral face of the voxel was aligned with the cerebellar tentorium and the volume positioned as posteriorly as possible without including the sagittal sinus. Participants then underwent a second scan, on a separate day, where a 20 × 30 × 40 mm^3 voxel was positioned medially in the anterior cingulate (AC) (Fig. 1b). The ventral face of this voxel was aligned parallel to the anterior–dorsal edge of the trunk of the corpus callosum.

For each voxel, two MEGA-PRESS (26) ^{1}H MRS acquisition methods (outlined below) were used to detect GABA. Two scans were performed using each method giving a total of four measurements per session. Scans were interleaved and the order counterbalanced across participants. Participants were not repositioned during the repeated acquisitions in each session. GABA

measures including an MM component are denoted GABA′+MM; GABA concentrations acquired using the symmetric suppression method are denoted GABA′ (to distinguish it from the molecule GABA).

Nonsuppressed acquisition (GABA′+MM). Here, two Gaussian editing pulses (16-ms duration) were placed in an interleaved manner at either 1.9 ppm (ON) or 7.5 ppm (OFF), resulting in a "standard" measurement of GABA plus co-edited MM. Echo time was set to 68 ms.

Symmetric MM suppression (GABA′). In the second acquisition, the OFF editing pulses were placed at 1.5 ppm with the ON pulses kept at 1.9 ppm, thereby suppressing the MM resonance. To ensure editing selectivity, the duration of the editing pulses was increased to 20 ms, with TE increased to 80 ms.

The following parameters were the same for both acquisition methods: TR = 1800 ms, 332 averages, 4096 data points, 5 kHz spectral width, 10-min acquisition time. Eight additional water-unsuppressed scans were acquired as an internal concentration reference.

Experiment 2

Repeatability of the two techniques was also assessed in a second cohort of fifteen healthy participants (mean age = 27.5 ± 4.1 years; 7 females). The scan protocol and acquisition parameters were identical to those in Experiment 1 except spectra were acquired in an OCC voxel only. Additionally, the number of averages used in each acquisition technique was increased to 512 (acquisition time = 15 min) to improve the signal-to-noise ratio (SNR) of GABA′+MM and GABA′ measures.

MRS Analysis

MRS spectra from the phantom and in vivo experiments were processed in Gannet (27), following an analysis pipeline similar to Evans et al (28). Line broadening (0.5 Hz for phantom spectra, 3 Hz for in vivo spectra) was applied to raw time-domain data before Fourier transformation. Frequency-domain data were then automatically corrected for frequency and phase. Using a nonlinear least squares fit, GABA concentration was quantified from the integral of the difference spectrum with a Gaussian function placed over a range 2.79 ppm to 3.55 ppm. Tissue water was used as an internal concentration reference. The ratio of GABA to water (represented in institutional units, iu) was multiplied by a scaling factor to account for the T_1 and T_2 of water and GABA, for MR-visible water concentration and for editing efficiency. Concentration values were not corrected for partial volume effects. Gannet also produces estimates of fit error for both the GABA and water peak model fits calculated as the standard deviation of the fit residuals normalized to peak height. Overall fit error is then defined as the square root of the sum of the squared GABA and water peak fit errors (ε_{fit}).

Statistical Analysis

To quantify the repeatability of the two techniques, coefficients of variation were calculated to represent the measurement error (within-participant coefficient of variation, CV_{wp}) and the population variability (between-participant coefficient of variation, CV_{bp}).

If σ_p is the standard deviation of one participant's measurement values and μ_p is the mean measurement value for each participant, then the CV of each participant is given by:

$$CV_p = 100\frac{\sigma_p}{\mu_p}$$

and CV_{wp} is defined as:

$$CV_{wp} = C\bar{V}_p$$

To estimate the error on this value, a 95% confidence interval (CI) was calculated from a bootstrap of the set of CV_p, sampling with replacement 100,000 times. Similarly, if $\sigma(\mu_p)$ is the standard deviation of participant means, and μ is the mean of all measurements, then:

$$CV_{bp} = 100\frac{\sigma_{(\mu p)}}{\mu}$$

Bayes factors (B) were calculated according to the method described by Dienes (29) to assess whether the difference in CV_{wp} values between each acquisition technique in each voxel is better explained by the null hypothesis (a difference of 0%) or by the alternative hypothesis (a plausible difference in population means). The plausibility of the alternative hypothesis was predicted based on previous studies investigating the reproducibility of GABA-edited spectroscopy at 3T using either occipital (a difference of 9%) or frontal (a difference of 7%) voxels. The plausibility of the predicted population differences was assumed to follow a normal distribution. A B greater than 3 indicates substantial evidence for the alternative hypothesis and a B less than 1/3 indicates substantial evidence for the null hypothesis. If B is between 1/3 and 3 then the evidence is insensitive and no judgement can be passed.

A one-way analysis of variance (ANOVA) was used to calculate the proportional contribution of measurement and population variance to the total variance in the dataset. In addition, as a test of reliability of the two techniques, intraclass correlation coefficients (ICCs) were calculated in SPSS (version 20.0) using a two-way random effects model with measures of consistency. Whereas the CV characterizes measurement variability in one dimension (either within or between participants) and is useful for comparing the variability of measurements with different means, the ICC represents a ratio between between-participant variance and total variance and is a more informative statistic of the test–retest reliability of a measurement.

RESULTS

Phantom Experiments

The GABA signal loss for a symmetric editing scheme (reflected in the signal intensity when editing pulses were placed at 1.5 ppm) was 44% for the TE = 68 ms, 16-ms editing pulse acquisition, but only 20% for the TE = 80 ms, 20-ms editing pulse acquisition. This corresponded to a reduction in the editing pulse bandwidth

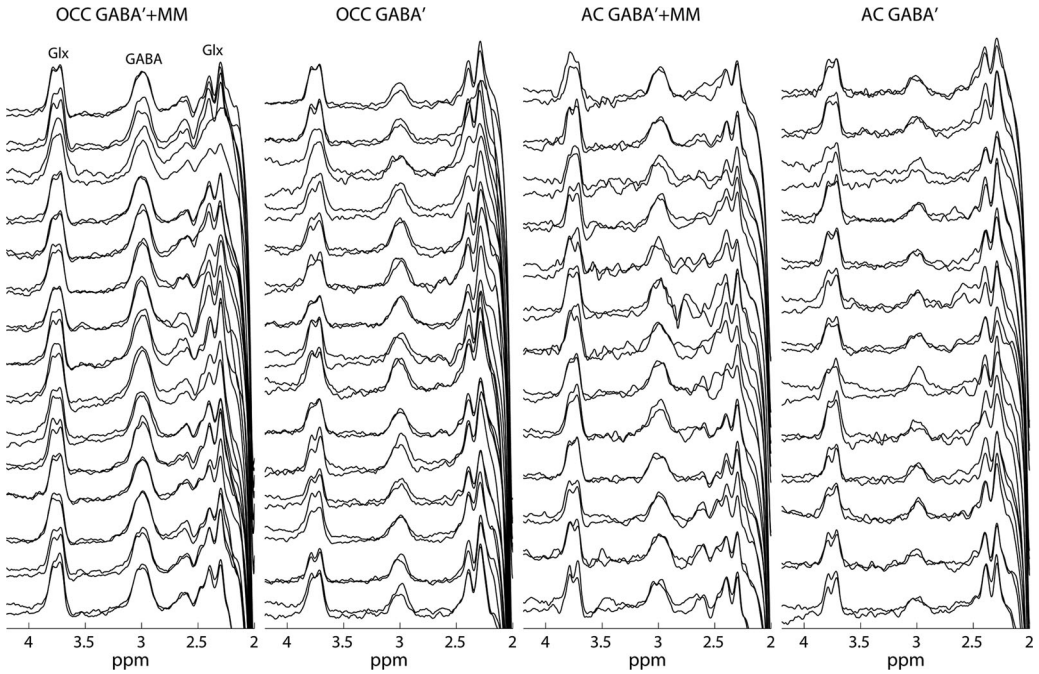

FIG. 2. Individual difference spectra acquired in vivo in all participants from Experiment 1 using standard GABA-editing (GABA′+MM) and symmetric MM suppression (GABA′) in the occipital lobe (OCC; $n = 15$) and anterior cingulate (AC; $n = 13$). Repeated measurements for each technique are overlain. It can be clearly seen that the amplitude of the 3.0 ppm GABA peak is attenuated in GABA′ spectra compared with the same peak in the spectra acquired using standard editing. The composite glutamate + glutamine (Glx) peaks are also shown.

from 82 Hz to 57 Hz. Increasing TE from 68 to 80 ms resulted in a small increase in the GABA integral (approximately 3%), without correcting for T_2 relaxation effects. These results indicate that, overall, the signal loss due to the increase in TE is small in comparison to the signal improvement by improving the frequency selectivity of the editing pulses.

In Vivo Experiments

Experiment 1

A total of 120 spectra were analyzed for Experiment 1. Following visual inspection, two AC spectra (from two different participants) were rejected because of excessive head movement in one and excessive fit error in the other; therefore, only 13 participants' data were included in the AC dataset. As shown in Figure 2, there was a clear difference between the GABA′+MM and GABA′ peak amplitudes. Mean concentrations (± SD) averaged across scans and participants were as follows: [GABA′+MM]: 1.13 ± 0.07 iu (OCC), 0.99 ± 0.15 iu (AC); [GABA′]: 0.54 ± 0.08 iu (OCC), 0.43 ± 0.06 iu (AC). The fraction of the total signal retained following MM suppression ([GABA′] / [GABA′+MM]) was 0.48 in the OCC voxel and 0.43 in the AC voxel, in good agreement with previous findings (22,30). Mean ε_{fit} (± SEM) for OCC measures were 4.0 ± 0.1% (GABA′+MM) and 5.2 ± 0.3% (GABA′), and 7.7 ± 0.4% (GABA′+MM) and 10.4 ± 0.6% (GABA′) for AC

Table 1
Measures of Repeatability and Reliability for the Standard GABA-Edited (GABA′+MM) and MM-Suppressed (GABA′) Acquisition Techniques from In Vivo Experiment 1

	OCC ($n = 15$)		AC ($n = 13$)	
	GABA′+MM	GABA′	GABA′+MM	GABA′
Mean ε_{fit} (± SEM)	4.0 ± 0.1%	5.2 ± 0.3%	7.7 ± 0.4%	10.4 ± 0.6%
CV_{wp}[a]	4.0%	8.6%	14.8%	12.6%
95% CI[b]	2.4–5.8%	5.3–13.9%	9.6–21.5%	8.9–16.4%
CV_{bp}	6.1%	15.0%	14.7%	13.6%
σ^2_p	38%	53%	13%	27%
σ^2_e	62%	47%	87%	73%
ICC	0.67	0.72	0.16	0.41

[a]CV_{wp} values were not significantly different between the two acquisition methods for OCC or AC spectra ($P = 0.08$, $P = 0.57$, respectively).
[b]95% CI from the bootstrapping results sampling from the CV_p dataset.
ε_{fit} = fit error; CV_{wp} = within-participant coefficient of variation; CI = confidence interval; CV_{bp} = between-participant coefficient of variation; σ^2_p = between-participant component of variance; σ^2_e = measurement error component of variance; ICC = intraclass correlation coefficient.

Table 2
Measures of Repeatability and Reliability for the Standard GABA-Edited (GABA′+MM) and MM-Suppressed (GABA′) Acquisition Techniques from In Vivo Experiment 2

	OCC (n = 15)	
	GABA′+MM	GABA′
Mean ε_{fit} (± SEM)	3.2 ± 0.1%	4.2 ± 0.2%
CV_{wp}[a]	3.5%	4.6%
95% CI	2.5–4.6%	2.8–7.9%
CV_{bp}	5.8%	14.6%
σ^2_p	64%	86%
σ^2_e	36%	14%
ICC	0.78	0.90

[a]CV_{wp} values were not significantly different between the two acquisition methods (P = 0.69).

measures. Although these were shown to be significantly different for each region ($t(14)$ = –3.74, P = 0.002 and $t(12)$ = –4.11, P = 0.001, respectively), these percentages fall in line with previously reported estimates (28). The average linewidth (± SD) was 8.2 ± 1.4 Hz for the OCC voxel and 7.1 ± 0.5 Hz for the AC voxel.

The repeatability and reliability results (CV, components of variance and ICCs) are reported in Table 1. Paired t-tests showed that CV_{wp} values were not significantly different for OCC ($t(14)$ = –1.92, P = 0.08) or for AC ($t(12)$ = 0.58, P = 0.57) acquisitions. However, the Bayesian analysis revealed that for both voxels the data were insensitive (OCC: B = 1.64; AC: B = 0.62), meaning there was not enough evidence in Experiment 1 to decide whether the within-participant repeatability of symmetric suppression and standard editing is or is not comparable. Taking voxel and acquisition technique as separate factors, a two-way repeated measures ANOVA demonstrated that there was no significant interaction in CV_{wp} between voxel and acquisition technique ($F(1, 12)$ = 3.10, P = 0.10) and no main effect of acquisition technique ($F(1, 12)$ = 0.33, P = 0.58). However, there was a main effect of voxel ($F(1, 12)$ = 9.93, P = 0.008), with the AC voxel showing significantly higher CV_{wp}. CV_{bp} was larger in the suppressed OCC data (15.0%) than in the contaminated data (6.1%) but similar in the AC (13.6% versus 14.7%, respectively).

The component of the variance associated with differences between participants (σ^2_p) and the component of the variance that is attributed to measurement error (σ^2_e) are expressed as percentages of the total variance across the whole dataset. For the symmetric suppression method, σ^2_p was greater compared to standard editing in both the OCC (53% versus 38%) and AC (27% versus 13%). It also produced comparatively higher ICCs: 0.72 versus 0.67 (OCC); 0.41 versus 0.16 (AC).

Experiment 2

No datasets were rejected as a result of visual inspection of data acquired in Experiment 2, resulting in 60 good quality OCC spectra. Mean concentrations (± SD) were as follows: [GABA′+MM]: 1.15 ± 0.07 iu; [GABA′]: 0.56 ± 0.08 iu. The GABA′ to GABA′+MM signal fraction was 0.49. Mean ε_{fit} (± SEM) was 3.2 ± 0.1% (GABA′+MM) and 4.2 ± 0.2% (GABA′) ($t(14)$ = –3.92, P = 0.002). The average linewidth (± SD) was 8.6 ± 1.2 Hz.

Compared with Experiment 1, there was an overall improvement in both repeatability and reliability for each acquisition technique in the second cohort, particularly for symmetric suppression (Table 2). This is likely a consequence of a reduction in measurement noise following increased acquisition time. As the CV_{wp} data were shown to be nonnormal following a Shapiro–Wilk test (W = 0.79, P = 0.006), a Wilcoxon signed-rank test was performed. Again, the CV_{wp} were not significantly different from each other (z = –0.40, P = 0.69). Moreover, in this experiment B = 0.21, indicating that there was substantial evidence in support of the null hypothesis (a difference of 0%).

Of interesting note is that the MM-suppressed concentrations still revealed increased interindividual variability compared to contaminated concentrations, reflected in the CV_{bp} (14.6% versus 5.8%), σ^2_p (86% versus 64%) and ICC (0.90 versus 0.78).

Pooled Data

Finally, the OCC data from Experiments 1 and 2 were pooled together and examined. The ICC for standard editing was 0.72 and the ICC for symmetric suppression was 0.81. Figure 3 shows the association between GABA′+MM and GABA′ concentration for participants from both cohorts. There was a weak but nonsignificant correlation between the two measures (R = 0.28, P = 0.14, CI = [–0.17, 0.63]). Although it is surprising that the two measurements were not more strongly related, the expected correlation will have an upper bound based on both the intrinsic correlation and the reliabilities of the two techniques. This can be calculated with the following formula: Observed $R_{(\text{variable A, variable B})}$ = True

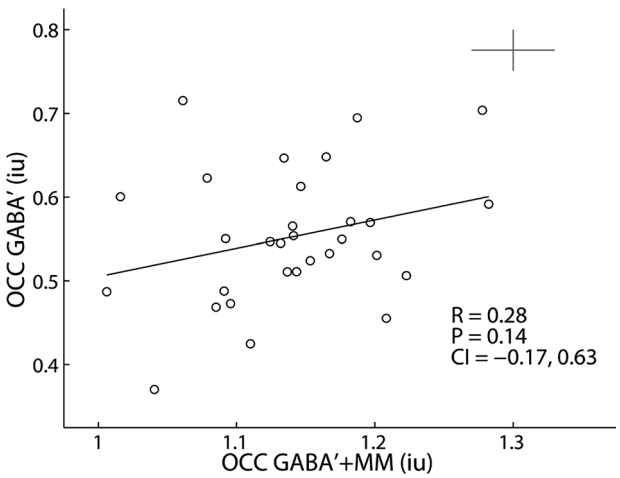

FIG. 3. Scatterplot of OCC GABA concentration for participants pooled from Experiments 1 and 2 (n = 30) acquired with standard editing (OCC GABA′+MM) and symmetric suppression (OCC GABA′). Each point represents the average of the two repeat measurements. Crosshairs indicate the mean scan–rescan difference between the two repeat measurements for each technique. For GABA′+MM this was 0.06 ± 0.05 iu; for GABA′ this was 0.05 ± 0.05 iu. CI: 95% confidence interval of the correlation coefficient calculated by bootstrapping with replacement 10,000 times.

$R_{(\text{variable A, variable B})} \times \sqrt{}$ (Reliability$_A$ × Reliability$_B$) (31). Assuming an intrinsic correlation of 0.7, and using the ICCs for the two techniques based on the pooled data, the expected correlation between the two measurements would be no higher 0.53. Furthermore, a range of possible intrinsic correlations (i.e., a 95% CI) was estimated by, first, taking the ICCs and observed correlation coefficient and bootstrapping with replacement each 10,000 times and then randomly sub-sampling from the distribution of bootstrapped values and running the above formula again 10,000 times. The CI of True *R* was shown to be –0.18, 0.90.

DISCUSSION

The main focus of this research was to compare the repeatability of symmetric MM suppression and standard GABA-editing. We used CV_{wp} as an index of measurement repeatability and found that there was no significant difference in CV_{wp} resulting from the symmetric suppression technique and the nonsuppression technique, in either the occipital lobe or anterior cingulate. To further determine whether there was indeed evidence for the hypothesis that the two techniques are comparable in repeatability, Bayes factors were calculated on CV_{wp} values. Whereas the in vivo data in the first cohort was not sensitive enough to make a decision, the second cohort did provide enough evidence in favour of this hypothesis. Therefore, this suggests that symmetric suppression is comparable in repeatability to standard GABA-edited MRS.

Although there was no significant difference between CV_{wp} for the two techniques in the occipital lobe data, mean CV_{wp} in Experiment 1 increased from 4.0% to 8.6% and from 3.5% to 4.6% in Experiment 2 when using symmetric suppression. This is consistent with the overall reduction in the GABA′ integral when using symmetric suppression due to the exclusion of MM. However, there appears to be an increase in the population variance (reflected in higher CV_{bp} and higher σ^2_p), which accounts for the improvement in the ICCs. The increased population variance was also found in the occipital data of the second cohort. Given that CV_{wp} and mean ε_{fit} both decreased relative to the first cohort, it is unlikely that this increased sensitivity was due to noise in the acquisition. This also suggests that the symmetric suppression technique may benefit from longer acquisition time than may be typically used in standard GABA-edited experiments.

Only a few studies have reported ICC values for GABA measurements. Muthukumaraswamy et al (32) reported an ICC of 0.87 for occipital spectra, whereas Geramita et al (24) and Harada et al (33) reported ICCs of approximately 0.70 for anterior cingulate measures. The use of ICCs is useful to an extent but does present difficulties for interpretation. Greater between-participant variance will increase ICC scores if other variance components remain stable (34). As such, the poorer ICC values for spectra acquired in the AC reported here are likely the result of inherently noisier data. This is supported by the fact that the mean ε_{fit} was higher in AC spectra for both acquisition methods and that the AC CV_{bp} percentages were comparable to the CV_{bp} for the MM suppression technique in the occipital session, despite the latter producing a much higher ICC.

CV_{wp} percentages for acquisitions in the occipital lobe are similar to or better than those in other studies (23,28,35). Repeatability results for AC spectra, however, are higher than what has been previously reported for frontal brain regions (24,25,33), but do agree with Evans et al (28). The higher CV_{wp} corresponds to the difficulties in acquiring spectra in frontal regions. Although different protocols and analysis methods make comparisons across research groups challenging, on the basis of the findings here, MM suppression by symmetric editing is comparable in repeatability to standard GABA-editing methods.

In vivo quantification of GABA without MM contamination produced results consistent with previous empirical evidence (17,22,30). Suppression of the 1.7 ppm MM resonance reduced the 3.0 ppm GABA peak by approximately 50% in relation to the peak resolved using the standard editing technique. A comparatively lower mean concentration in the AC is consistent with other studies showing differences in GABA levels in anterior and posterior cortical regions (36,37); but as the voxels were not segmented to control for grey matter, white matter and cerebrospinal fluid composition, this cannot be confirmed.

The phantom experiments that were conducted yielded similar findings to Edden et al (21). The authors observed a negligible decrease in modelled signal (~1%, with T_2-weighting) when modulating TE from 68 to 80 ms in vitro, whereas we saw an increase of approximately 3%. Extrapolating from the estimated signal loss in vivo (~7%) by Edden et al, we would expect around 4% signal loss in vivo. However, it appears that the frequency selectivity of editing pulses has a much larger effect. By increasing editing pulse duration from 16 to 20 ms, the editing pulse bandwidth was decreased, leading to improved efficiency of the GABA-editing experiment. Although both TE and editing pulse duration were manipulated in this experiment, the effect of TE is shown to be minimal and does not significantly contribute to the signal change.

As the focus on the relationships between endogenous GABA and cognitive–behavioral responses continues to grow, it is important to reiterate that the GABA concentration quantified with standard GABA-edited MRS contains an MM contribution. While it is argued that MM are not likely to have any functional importance to such responses (12), the degree to which the MM contribution to the 3.0 ppm GABA signal differs within particular regions and across individuals is not fully known. This would be a significant issue only if the interindividual variability of this contribution was large enough to drive correlations, however, and it is still unclear whether this is the case. Nonetheless, quantification of GABA concentration in the AC in one symmetric suppression study showed that suppressing MM resulted in higher between-participant variability (~15%) compared to not suppressing MM (~10%) (22). Although we saw a similar degree of interindividual variability in both our MM-suppressed and MM-contaminated AC concentrations, the findings from this previous study are reflected in our OCC data. However, any conclusions drawn about this

variability are only speculative because of the difference in MM T_2 relaxation effects between the suppression and standard editing techniques caused by the increased TE used in the former method. That voxels were not segmented into different tissue components also prevents further conclusions on this. It should be additionally noted that the age range of participants in Aufhaus et al (22) was larger compared to the current study, which may have contributed to the larger variability in the suppressed measurements.

When OCC GABA′ and GABA′+MM concentrations were compared, only a weak association between the two was apparent. This finding was unexpected as the measurements would be thought to show a certain degree of correlation. Given the good reliability of both standard editing and symmetric suppression as demonstrated here, there are two possible explanations. Either one measurement is more sensitive to systematic effects (e.g., the effect of frequency drift on editing efficiency), or the interindividual variability in the MM contribution to the GABA peak is large enough to affect the correlation. At this stage, this is only speculation and limited to the OCC data, necessitating further investigation.

A limitation of this study is that concentration measures were not corrected for tissue composition in each voxel. While the scan-to-scan repeatability would not be affected by variation in tissue composition because participants were not repositioned during each scan session, it is still possible that between-participant variability could be influenced by tissue differences. GABA concentration has previously been reported to be higher in grey matter compared to white matter (38,39), which would affect the SNR of the GABA resonance and thus the reliability of the acquisition across participants. It is also a question whether GABA′+MM or GABA′ levels correlate more strongly with the fraction of grey matter in the voxel. Such an investigation was beyond the scope of the present study, however.

Ultimately, what is desired is to be able to optimally detect pure GABA in vivo. Future spectroscopic studies, particularly those involving correlational designs, would benefit from using symmetric editing to suppress MM. To illustrate, whereas Gao et al (40) recently reported an age-related decline in GABA′+MM concentration in a large healthy cohort, Aufhaus et al (22) showed that when MM is suppressed using symmetric editing the relationship between age and GABA concentration no longer holds. Suppression would also prove beneficial in clinical populations as differences in the MM baseline have been shown in at least neurological pathologies such as multiple sclerosis, stroke and tumors (41–43). What differences may occur in neuropsychiatric disorders, to which disruptions in GABAergic mechanisms have been tied, remains unknown. A further issue is the effect of motion-induced frequency drift, which may be especially problematic in patient populations. Negative drift, for example, will lead to more MM contamination in the GABA signal in standard editing (44), potentially impacting on the reproducibility of the technique. This is of particular concern in symmetric editing given the closer proximity of the OFF editing pulses to the MM resonance. All in all, these points highlight the need to account for the 1.7 ppm MM resonance in edited spectroscopy.

CONCLUSIONS

To conclude, symmetric editing-based MM suppression is shown to have comparable repeatability to that of standard GABA-editing. By slightly modifying the MEGA-PRESS sequence, this technique successfully attenuates a major limitation of GABA-edited MRS. A growing interest in measuring GABA concentration in vivo in both healthy and clinical populations is apparent. The need for both accurate and reliable quantification is therefore essential when attempting to draw conclusions between GABA measures, pathology and healthy behavior. Here, we have shown that symmetric suppression produced higher ICCs in two regions and in two separate cohorts, suggesting that it is perhaps more sensitive to interindividual differences in MRS-measured GABA.

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