

Direct In Vivo Measurement of Human Cerebral GABA Concentration Using MEGA-Editing at 7 Tesla

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Spectral editing of the GABA spin system is hampered by co-editing of macromolecule (MM) coherences. To reduce contamination arising from MMs in spectra edited for GABA, the highest field strength currently available for human experimentation (7 Tesla) and MEGA-based editing were used. Despite judicious choice of experimental parameters, MM contamination was found to arise from field drifts. When the MM contribution was accounted for, [GABA] = $0.75 \pm 0.14 \mu\text{mol/g}$ (mean \pm SD, $N = 16$) relative to $8 \mu\text{mol/g}$ creatine (Cr), whereas without accounting for the MM signal [GABA*] = $0.88 \pm 0.23 \mu\text{mol/g}$ (mean \pm SD, $N = 16$). Incorporating the direct experimental assessment of MM contamination to the edited GABA signal substantially reduced the variance of the measurement, resulting in concentrations that were in excellent agreement with previous ^{13}C labeling experiments. Magn Reson Med 47:1009–1012, 2002. © 2002 Wiley-Liss, Inc.

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GABA (γ -aminobutyric acid ($\text{NH}_2\text{-}\alpha\text{CH}_2\text{-}\beta\text{CH}_2\text{-}\gamma\text{CH}_2\text{-CO}_2\text{H}$)) is the major inhibitory neurotransmitter in the human brain. ^1H NMR spectroscopy has been used to detect human GABA levels in vivo (1,2) and to provide insight regarding the action of antiepileptic medications (3,4). The detection of the GABA resonance is complicated by overlap with resonances from other metabolites, including creatine (Cr) and macromolecules (MMs). To extract the small GABA signal from underneath the strong Cr singlet at 3.0 ppm, investigators have typically used homonuclear editing based on the 7 Hz J coupling between the $\beta\text{-CH}_2$ resonance at 1.9 ppm and the $\gamma\text{-CH}_2$ resonance at 3.0 ppm (3). Editing pulses incorporated into point-resolved spectroscopy (PRESS) (5) have also been utilized to detect the GABA resonance at 3.0 ppm (2) and to study the effects of antiepileptic medications (6). Multiple quantum filtering schemes have also been proposed (7–9).

However, editing of the $\gamma\text{-CH}_2$ GABA resonance near 3.0 ppm is complicated by coediting of MM coherences near 3.0 ppm which couple to a resonance at 1.7 ppm with a similar homonuclear J coupling constant (10). Due to the finite bandwidth of the editing pulse designed to invert the $\beta\text{-CH}_2$ GABA resonance at 1.9 ppm, the MM resonance at 1.7 ppm is inadvertently excited, leading to significant

MM coherence generation (3,10). Single quantum editing has been utilized in conjunction with a numerically optimized editing pulse (1) to improve the selectivity of the editing pulse. In addition, MM contamination is often assessed by mathematical deconvolution (1). Alternatively, Henry et al. (11) were the first to attempt MM contamination-free GABA editing by applying the selective editing pulse symmetrically about the J-coupled MM resonance at 1.7 ppm in the two steps of the editing scheme. Their work highlights the importance of precise and accurate setting of the editing pulse offset, and the vulnerability of all prior methods to offset errors.

The primary goal of this work was to optimize editing selectivity, and thus minimize MM contamination by: 1) implementing symmetric pulsing (11) within the MEGA-PRESS editing technique (2), and 2) performing experiments at 7T, the highest magnetic field currently available for human experimentation. The high magnetic field provided the additional benefits of improved sensitivity and a greater frequency separation between Cr and GABA (12).

METHODS

Fourteen normal volunteers (six males and eight females, average age 30 years), two of whom were studied twice, gave informed consent for this study, which was conducted according to procedures approved by the Institutional Review Board and the U.S. Food and Drug Administration (FDA IDE #G980). All experiments were performed with a 7T, 90-cm bore magnet (Magnex, Oxford, UK) interfaced to a Varian INOVA spectrometer (Varian, Palo Alto, CA) and head gradients (Magnex). Radiofrequency (RF) excitation was accomplished with a surface ^1H quadrature transceiver (13) adapted to tune to 297 MHz. Subjects were positioned supine inside the magnet with the RF transceiver subjacent to their occipital lobe. The protocol for each volunteer began with localizer multislice rapid acquisition with relaxation enhancement (RARE) images (TR = 4.0 s, TE = 23 ms, echo train length = 8, 256×128 matrix, two averages, 2-mm slice thickness, five slices) for selection of a cubic volume of interest ($3 \times 3 \times 3 \text{ cm}^3$) centered on the midsagittal plane in the occipital lobe. Shimming of all first- and second-order coils was achieved using a fast automatic shimming technique with echo-planar signal trains using mapping along projections, FAST(EST)MAP (14), resulting in water linewidths of 10–17 Hz. Following adjustment of the spectrometer frequency, edited spectra were acquired as described below.

Selective double-banded pulses incorporated into MEGA-PRESS (fig. 2B of Ref. 2) accommodated simulta-

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neous localization, water suppression, and editing of the γ -CH₂ resonance of GABA at 3.0 ppm. Instead of applying the editing lobe of the selective RF pulse symmetric about water (at 7.54 ppm) during alternate scans (2), the editing lobe was applied symmetric about the MM resonance at 1.7 ppm in order to reduce MM contamination (11). In summary, the selective double-banded pulse was applied at 4.7 and 1.9 ppm alternating with 4.7 and 1.5 ppm. Additional water suppression using variable power with optimized relaxation delays (VAPOR) and outer volume suppression, OVS, (15) were adapted for the human 7 Tesla system (12) and incorporated prior to MEGA-PRESS. Use of a high magnetic field (7 Tesla) rendered the editing pulse more selective because of the increased frequency separation between GABA and MM at higher field. Each acquisition was stored separately in memory and individually phase-corrected based on the NAA signal. All processing was performed using spectrometer built-in software.

To assess contributions of the MM spin system to the edited signal, a “metabolite-nulled” spectrum (2,10) was collected to complement each edited spectrum. A comparison of metabolite-nulled spectra to spectra acquired without nulling revealed that the MM resonance at 0.9 ppm, which did not overlap with metabolites, was not fully relaxed. Therefore, assuming identical T_1 relaxation, the MM resonance near 3.0 ppm in the metabolite-nulled spectrum was not fully relaxed, thus underestimating MM contamination. The area under the MM resonance at 0.9 ppm was determined both with and without preinversion, providing a correction factor for T_1 relaxation, which was 1.29 ± 0.05 (mean \pm SD, $N = 9$), consistent with an MM T_1 of approximately 0.5 s. Changing this correction factor by 30% affected the resulting GABA concentration by less than 5%. In all cases, the TR was 4.5 s.

The GABA concentration was calculated from the signal intensities according to:

$$[\text{GABA}] = \frac{3(I_{\text{GABA}^*} - I_{\text{MM}})}{2I_{\text{Cr}}\text{Eff}_E} [\text{Cr}] \quad [1]$$

where [GABA] represents the GABA concentration after subtracting the measured MM contamination. GABA concentration calculated without accounting for MM contamination ($I_{\text{MM}} = 0$) is referred to as [GABA*]. I_{Cr} represents

the area under the Cr methyl peak at 3.03 ppm obtained by peak fitting using spectrometer software. I_{GABA^*} represents the area under the edited peak at 3.0 ppm, which was fit as two singlets, each having a fixed line width equal to that determined for Cr, resulting in a total area of I_{GABA^*} . I_{MM} represents the area under the metabolite-nulled (MM) peak near 3.0 ppm multiplied by 1.29, the correction factor for T_1 relaxation. I_{MM} was obtained using a constrained peak fit where the line width was set equal to that of Cr plus 15 Hz. The corresponding experimentally measured value of I_{MM} was subtracted from I_{GABA^*} for each individual scan. The factor 3/2 in Eq. [1] accounted for the different number of protons in the Cr methyl vs. the γ -CH₂ GABA resonances. The Cr concentration, [Cr], was assumed to be 8 $\mu\text{mol/g}$ (3). The editing efficiency, Eff_E , was 0.36, determined by comparing: (a) the unmodulated GABA signal measured relative to glycine in short-echo stimulated-echo acquisition mode (STEAM) spectra with (b) the edited GABA signal measured relative to glycine in spectra of the same voxel in the same phantom. Perfect editing for an AX2 spin system would correspond to $\text{Eff}_E = 0.5$. Signal loss from T_2 decay was assessed to be identical for GABA and glycine in the phantom, and in vivo T_2 of the Cr methyl and of the γ -CH₂ of GABA were assumed to be identical. Given the approximate T_1 of 1.5 s, as determined from the metabolite-nulled experiment, potential differences in T_1 have a negligible effect on the quantification at the TR of 4.5 s.

RESULTS

The combination of VAPOR water suppression and MEGA-editing resulted in a nearly absent water signal (Fig. 1). The sensitivity improvement at 7 Tesla was observed through detection of the $^1\text{H}_1$ glucose resonance (5.2 ppm) at 69 ms TE in an 8-mL volume (Fig. 1). In the edited spectra (Fig. 2a), the absence of signal at 3.2 ppm, the chemical shift of choline, suggests excellent subtraction accuracy. The flat baseline around 3.0 ppm, the excellent signal-to-noise ratio (SNR), and the narrow lines resulted in excellent fits of the Cr and GABA resonances as judged from the fit residuals (not shown). The chemical shift of the GABA peak (3.01 ppm) was 0.02 ppm upfield from Cr. The chemical shift of the MM peak (2.99 ppm) was 7 Hz upfield from GABA at 7 Tesla. These offsets agree

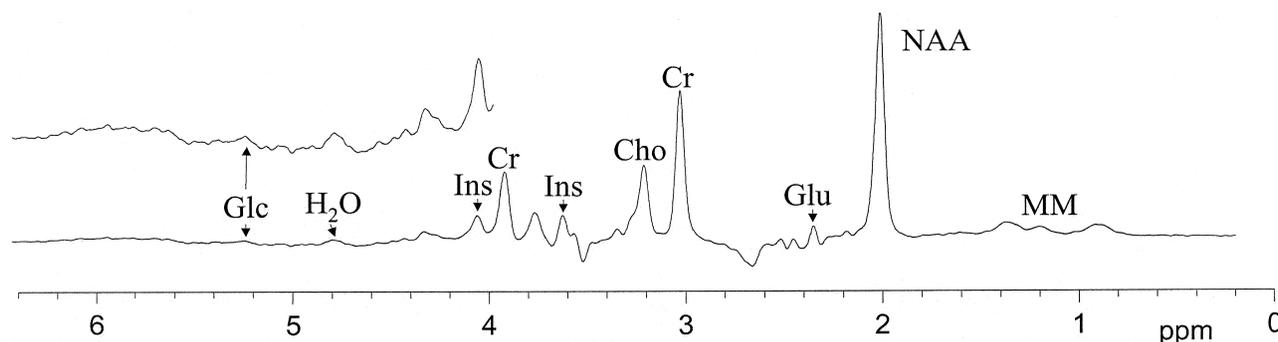


FIG. 1. In vivo ¹H NMR subspectrum in which the selective 180° MEGA pulse was set for water suppression only. TR = 4.0 s, TE = 69 ms, NEX = 128, VOI = 8 mL. The glucose trace is vertically expanded from the subspectrum.

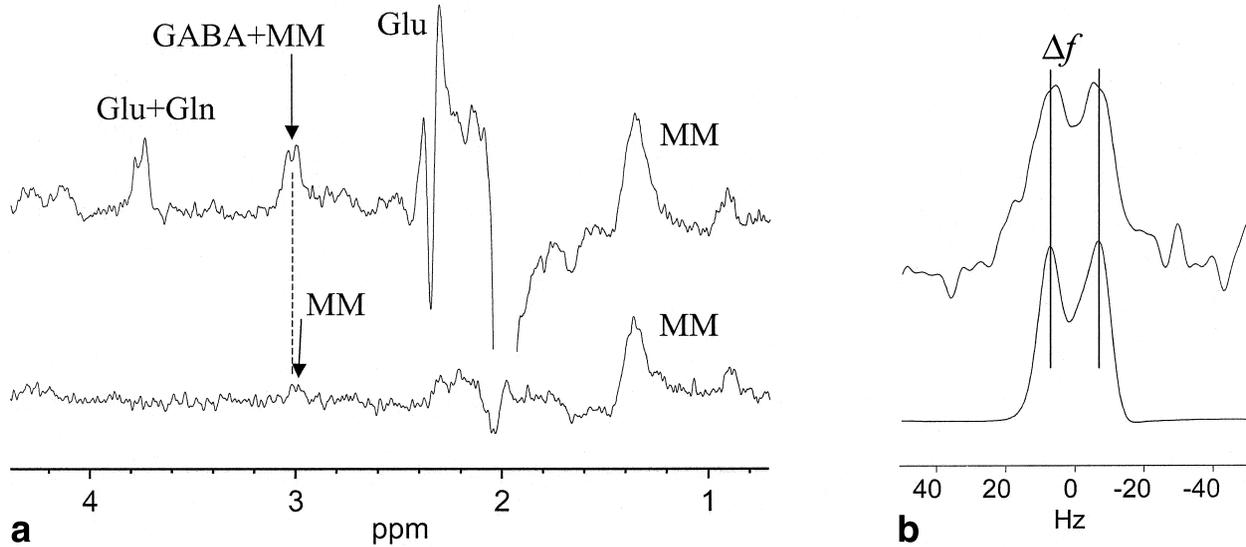


FIG. 2. **a:** Edited in vivo ^1H NMR spectrum (top), and metabolite-nulled spectrum (bottom). TR = 4.5 s, TE = 69 ms, NEX = 256, VOI = 27 mL, and TIR = 0.975 s. **b:** Edited spectra expanded at 3.0 ppm in vivo (top) and accordingly line-broadened GABA phantom (bottom); these two spectra were acquired using identical methods.

with short TE spectra in rats (16), and spectra from dialyzed rat brain cytosol (17). Glutamate, glutamine, and the MM coherences at 0.9 ppm appeared in the edited spectra (Fig. 2a) as a result of coupling partners within the bandwidth of the editing pulses applied at 1.9 ppm. The NAA signal at 2.0 ppm appears with opposite phase because NAA is reduced when the editing pulse is applied at 1.9 ppm.

Complete elimination of the MM signal was not always possible, as assessed in the edited metabolite-nulled spectra (Fig. 2a, bottom trace). The residual contamination was attributed to frequency drifts. While all volunteers were instructed to lie still, the combination of respiration, head motion, and static field drift frequently produced frequency drifts that exceeded 5 Hz and resulted in measurable MM contamination.

Comparison of an in vivo spectrum expanded around 3.0 ppm to an accordingly line-broadened phantom spectrum (Fig. 2b) indicates the presence of a doublet whose separation Δf was consistent with the majority of the signal arising from GABA. In spectra of exceptional quality (i.e., where the subtraction error assessed from the residual choline signal was less than 2%) the fit resulted in a frequency separation, $\Delta f_{in\ vivo}$, of 12.9 ± 1.1 Hz (mean \pm SD, $N = 8$), which is somewhat lower than the Δf measured in the phantom (14 Hz) prior to line broadening. The lower separation, $\Delta f_{in\ vivo}$, was expected because of the effect of broader line widths on the appearance of peak separation in vivo.

The GABA concentration including MM contamination was calculated based on Eq. [1] by setting $I_{MM} = 0$ resulting in $[\text{GABA}^*] = 0.88 \pm 0.23$ $\mu\text{mol/g}$ (mean \pm SD, $N = 16$). Conversely, the GABA concentration without MM contamination was $[\text{GABA}] = 0.75 \pm 0.14$ $\mu\text{mol/g}$ (mean \pm SD, $N = 16$), corresponding to an edited GABA-to-Cr signal ratio of $2.2 \pm 0.4\%$ (mean \pm SD). Figure 3 illustrates the improved confidence when accounting for MM contamination.

DISCUSSION

The proportion of MM contamination to total edited signal GABA* was only 15%. While symmetric pulsing (11) at 7 Tesla did not completely remove MM contamination, it did remove a substantial portion compared to the contam-

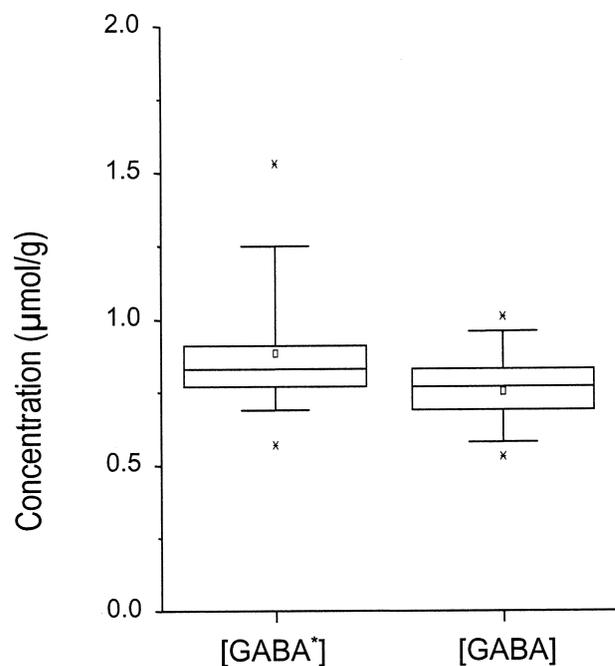


FIG. 3. Box plot of GABA concentrations ($N = 16$) without subtracting the MM contamination ($[\text{GABA}^*]$) and after subtracting the MM signal ($[\text{GABA}]$). The horizontal lines mark the 25th, 50th (median), and 75th percentiles. The square indicates the mean, the error bars the 5th and 95th percentiles, and the stars the outliers. Note the more normally distributed values and smaller coefficient of variation in the measurements after the MM signal is subtracted.

ination of 34–44% assessed using other editing methods (1,3). While the GABA concentration obtained without MM contamination ([GABA]) is within 1 standard deviation (SD) of that including MM contamination ([GABA*]), the SD itself was reduced by a factor of 2 when MM contamination was accounted for. The additional time required for measuring the metabolite-nulled spectrum resulted in increased confidence in the GABA concentration determined for each individual.

An advantage of difference editing methods over multiple quantum methods is that the former retain singlet resonances in the subspectra. In our study this advantage allowed us to determine the overall phase of the spectrum, which was used for individual phase correction, resulting in improved spectral quality. This advantage also allowed us to assess frequency drift, which is a detriment to all methods designed to edit for GABA, including the frequently used mathematical deconvolution of GABA and MM (1,11). Because frequency may vary unpredictably during each study, it appears to be advantageous to assess MM contamination individually in an interleaved fashion.

Since the ratio of the area of the *edited* γ -CH₂ GABA resonance is only 2% of that of Cr, subtraction errors greater than 2% may interfere with the measurement of GABA concentration.

The GABA concentration determined in this study was somewhat less than the previously reported 1.1 $\mu\text{mol/g}$ (3) and 1.3 $\mu\text{mol/g}$ (1) measured in the same brain region relative to 8 $\mu\text{mol/g}$ Cr. However, most previous studies, including our own study at 4 Tesla (2), neither discuss the potential effects of frequency drifts on these calculations nor report the constant detection of a resolved GABA doublet. It is noteworthy that a recent study using the same methodology reported similar low GABA concentrations in nonhuman primates (11). Finally, a neurochemistry textbook (18) states that GABA concentrations range from 0.8 to 2.3 $\mu\text{mol/g}$, which is based on chemical extraction. Since GABA levels can rise in post-mortem tissue, it is not surprising that the *in vivo* method described herein produced GABA levels that were at the lower end of what has been reported in brain extracts.

However, the true GABA concentration may be even lower, since homocarnosine, a dipeptide of GABA and histidine, can coedit with GABA and may contribute as much as 30% of the edited signal at 3.01 ppm (19). Therefore, the GABA concentration may be as low as 0.5 $\mu\text{mol/g}$ in the human occipital lobe. This is in excellent agreement with estimates from ¹³C NMR data, in which the GABA signal was completely resolved (20).

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