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In vivo single-shot three-dimensionally localized multiple quantum spectroscopy of GABA in the human brain with improved spectral selectivity

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Abstract

A single-shot multiple quantum filtering method is developed that uses two double-band frequency selective pulses for enhanced spectral selectivity in combination with a slice-selective 90°, a slice-selective universal rotator 90°, and a spectral-spatial pulse composed of two slice-selective universal rotator 45° pulses for single-shot three-dimensional localization. The use of this selective multiple quantum filtering method for C_3 and C_4 methylene protons of GABA resulted in improved spectral selectivity for GABA and effective suppression of overlapping signals such as creatine and glutathione in each single scan, providing reliable measurements of the GABA doublet in all subjects. The concentration of GABA was measured to be 0.7 ± 0.2 μmol/g (means ± SD, $n = 15$) in the fronto-parietal region of the human brain in vivo.

Keywords: MRS; Localization; GABA; Human brain in vivo; Homocarnosine

1. Introduction

Successful in vivo application of multiple quantum (MQ) filtering methods to γ-amino butyric acid (GABA) editing faces several technical challenges. For example, to achieve accurate in vivo measurements of GABA, particularly the C_4 methylene protons of GABA at 3.02 ppm, overlapping resonances of creatine (Cr), macromolecules (MM), and glutathione (GSH) around 3 ppm need to be suppressed to minimize contamination from those signals [1–5]. Effective water suppression is also important because residual water can severely distort the baseline as well as generate spurious peaks across the spectrum. When using MQ filtering techniques, uncoupled resonances that cannot form *J*-coupling based MQ coherence such as the methyl group of Cr, can be robustly suppressed [4,6,7]. With the same reason, water suppression is relatively easier with sufficiently strong MQ filtering gradients. However, signals from *J*-coupled molecules such as GSH can be coedited by conventional spectrally non-selective or semi-selective MQ filtering techniques [3,8,9].

The use of double-band frequency selection during MQ preparation has been proposed as a strategy to enhance the spectral selectivity of the GABA signal [2–5]. A double-band spectrally selective 180° pulse set at 3.0 and 1.9 ppm for MQ preparation has been used to suppress the contribution of MM and GSH signals to the GABA signal in each single scan since only the targeted resonances of the C_3 and C_4 methylene protons of GABA are mainly selected for MQ preparation [2–4]. Even with minimized contribution of signals from other metabolites as well as MM, the quantification of GABA is still challenging due to its low concentration in addition to the loss of the center peak of the C_4 methylene

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protons of GABA by editing. GABA quantification can be further compromised by the presence of confounding spurious resonances around the GABA signal. Therefore, better selection of the GABA signal with minimal contribution from other resonances around GABA is very important for accurate quantification of GABA in vivo. When frequency selective pulses are used in a repeated manner, it is known that the spectral selectivity is further enhanced due to the accumulated effect of multiple selections. Therefore, we sought to enhance the spectral selectivity of the MQ filtering method by using an additional double-band frequency selective 180° pulse during the rephasing of the antiphase C_4 methylene protons of GABA. With the two 180° pulses in the MQ filtering sequence used for enhanced spectral selectivity, single-shot three-dimensional spatial localization can be achieved using the following three pulses: (1) a slice-selective 90° pulse for excitation, (2) a slice-selective universal rotator 90° pulse for formation of double quantum (DQ) coherence [3,10,11], and (3) a spectrally semi-selective and slice-selective 90° pulse composed of two slice-selective universal rotator 45° pulses [11,12] for conversion of DQ coherence into antiphase single quantum (SQ) coherence.

Conventional MQ filtering techniques lack internal reference signals for frequency and phase of the MQ filtered spectra since MQ filtering eliminates all metabolite singlets. To overcome this shortcoming, Wilman et al. [13] demonstrated same-scan acquisition technique for the GABA and Cr in brain extracts. For in vivo measurement of GABA, when a spectrally semi-selective $45°_x$–$45°_x$ pulse is used for maximum editing yield of GABA, minimal amount of the Cr methyl group at 3.0 ppm is excited into the transverse plane. Based on the fact that the majority of Cr remains along the longitudinal axis (z-axis) during MQ filtering using the $45°_x$–$45°_x$ pulse for conversion of DQ into observable SQ coherence, we recently proposed a novel three-dimensionally localized simultaneous two echoes of multiple quantum and single quantum (STEMS) method [3], which utilizes a two-echo acquisition scheme for MQ coherence transfer spectroscopy. This method allows simultaneous measurements of GABA and Cr in a single shot [3]. In STEMS, the Cr signal can serve as a reference for the phase of the GABA spectrum and frequency shift, and also as an internal concentration standard. These improvements in MQ filtering techniques can also be applied to measuring many other metabolites with J-coupled spins such as GSH [8,14], glucose [1], β-hydroxybutyrate [15], and lactate [16]. In addition, adaptation of the selective MQ filtering method to two-dimensional (2D) MQ spectroscopy [4,17] and MQ chemical shift imaging (CSI) is also readily conceivable [5,7,9].

The purpose of this study was to develop a single-shot three-dimensionally localized two double-band selective MQ filtering technique with improved spectral selectivity for in vivo GABA measurements in the human brain at 3 T. Two double-band frequency selective refocusing pulses were incorporated into the STEMS method [3]: one in the MQ preparation period and the other in the refocusing period.

2. Materials and methods

All experiments were performed on a 3 T, 80 cm bore horizontal magnet (Magnex Scientific, Abingdon, UK), interfaced to an SMIS console (SMIS, Surrey, UK) using an actively shielded gradient coil (Magnex Scientific, Abingdon, UK) with a 38 cm inner diameter, which was set to 40 mT/m in 400 μs. A circularly polarized ^{1}H RF helmet coil [18] was constructed for 3 T and used for all experiments. Automated localized shimming [19] was used to adjust the currents in all first- and second-order shim coils. The field homogeneity resulted in a ~5–7 Hz full-width at half maximum (FWHM) of the in vivo water signal and a FWHM of ~1 Hz in vitro in a 27–43 ml volume.

The single-shot three-dimensionally localized MQ GABA sequence is composed of three CHESS water suppression interleaved with outer volume suppression (Fig. 1), MQ coherence preparation, conversion of MQ coherence into observable SQ coherence, and DQ gradient filtering [3]. Two identical double-band frequency selective 180° pulses (20 ms, double-band Gaussian pulses) set at 3.0 and 1.9 ppm were used during the MQ preparation period between the two slice-selective 90° (five-lobe optimized sinc, 4 ms) pulses and during the rephasing period between the semi-selective 90° pulse (90° on the C_3 and 0° on the C_4 methylene protons of GABA), and FID acquisition (Fig. 1). The semi-selective 90° pulse was spatially selective and spectrally semi-selective. It consists of two 45° slice-selective universal rotator pulses (three-lobe optimized sinc, 0.8 ms) to enable single-shot three-dimensional localization. As in STEMS [3], a PRESS sequence was appended to the GABA sequence to excite the magnetization of Cr, which remains along the longitudinal axis during the GABA sequence, to obtain the navigator Cr spectra.

2.1. Phantom study

To demonstrate spatial localization using the slice-selective 90° universal rotator pulse and the spectrally semi-selective slice-selective $45°_x$–$45°_x$ pulses, a spherical phantom containing 99.9% of 2-propanol solution was used to acquire MQ filtered axial images of its methyl groups at 1.16 ppm. Phase encoding and frequency encoding gradients were added at the end of the pulse sequence (Fig. 1) in x- and y-axes, respectively. The first slice-selective 90° pulse excites an axial slice (z-axis).

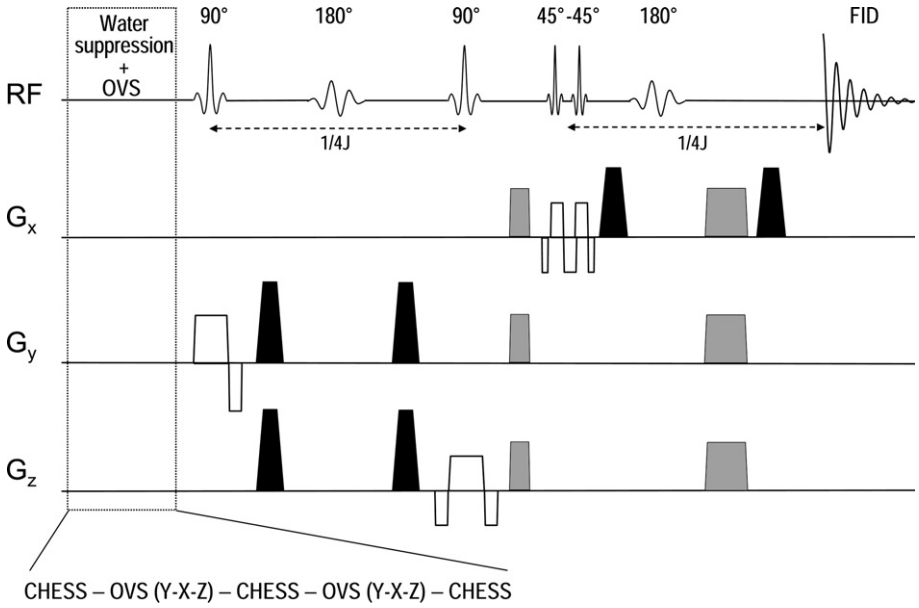

Fig. 1. Single-shot three-dimensionally localized two double-band selective MQ filtering sequence. Three-dimensional localization was achieved by a 90° slice-selective excitation pulse, a slice-selective universal rotator 90° pulse, and a spectrally semi-selective and slice-selective 90° pulse composed of two slice-selective universal rotator 45° pulses. To improve the selectivity of GABA, two double-band frequency selective 180° pulses were used: one during the MQ coherence preparation (the first 1/4J period) and the other during the refocusing period (the second 1/4J period). Localization and corresponding slice selection, prefocusing, and refocusing gradients are shown in white color, the DQ filtering gradients in gray color and the crusher gradients in black color.

The second slice-selective 90° pulse selects a slice along the y-axis. Imaging parameters were FOV = 15 cm, matrix size = 128 × 128, spectral width = 20 kHz, and TE/TR = 156/2000 ms. Images were acquired with either spectrally semi-selective spatially non-selective $45°_x$–$45°_x$ pulses or spectrally semi-selective slice-selective $45°_x$–$45°_x$ pulses for conversion of DQ coherence into observable SQ coherence with slice-selection gradient along the x-axis.

The efficiency and selectivity of detection of the C_4 methylene protons of GABA were tested and optimized using the following phantom samples: (a) 10 mM GABA solution in a 3 L cylindrical bottle, (b) 20 mM GABA, 10 mM Cr, 10 mM NAA, 30 mM glutamine, and 40 mM acetate solution in a 3 L cylindrical bottle, and (c) 10 mM GABA and 100 mM Cr in a 17-cm diameter sphere. The 10 mM GABA solution phantom was used to optimize RF pulses and delays, and trim gradients for a maximum signal yield. The MQ filtering efficiency was also tested using this phantom. The phantoms containing GABA, Cr and other metabolites were used to test the selectivity of MQ filtering methods and SQ coherence suppression.

The suppression of overlapping resonances with coupled spins (MQ coherence suppression) was assessed using the following solution samples in spherical phantoms: (a) 5 mM L-homocarnosine, (b) 10 mM L-lysine, and (c) 10 mM glutathione. Lysine was used to mimic the relevant macromolecule spins resonating at around 3.0 ppm that have coupled spins at 1.7 ppm because the C_5 and C_6 methylene protons of lysine resonate at 1.7 and 3.0 ppm, respectively [20,21].

2.2. In vivo study

Eleven healthy subjects (35 ± 9 years old, means ± SD) were studied with several of them being studied multiple times ($n = 15$), according to the consent approved by the Institutional Review Board of the Nathan Kline Institute. All subjects were positioned supine with the head positioned inside the helmet coil. Transverse T_1-weighted images of the brain were acquired using an inversion prepared three-dimensional gradient echo sequence (MPRAGE: a Magnetization-Prepared Rapid Acquisition Gradient Echo). Then, the volume of interest (VOI, 27–43 ml) was positioned in the fronto-parietal region for localized MR spectroscopy measurements [3]. The gray matter (GM) to white matter (WM) ratio of the VOI from the T_1-weighted images was calculated using the FAST (FMRIB's automated segmentation tool) software (Oxford University, Oxford, UK). After shimming, frequency adjustment and RF pulse power calibration were performed for the MQ GABA sequence. MQ filtered GABA spectra were acquired using either the one-echo acquisition scheme for GABA measurements or the two-echo acquisition scheme for simultaneous measurements of GABA and Cr. The potential MM contribution to the GABA signal was assessed using metabolite-nulled spectra, which were acquired using inversion recovery by placing

a broadband hyperbolic secant inversion pulse (6 ms) before the localization pulses of the MQ GABA sequence. Inversion recovery delay time (TIR) was set based on complete Cr nulling from the non-edited PRESS sequence (TE = 68 ms, TR = 2 s) using the same 6 ms hyperbolic secant inversion pulse. Then the obtained TIR was used for the MQ GABA sequence to acquire metabolite-nulled MQ filtered spectra.

2.3. Quantification

The concentration of in vivo GABA was quantified using the external reference method. A spherical phantom (17 cm diameter) containing 10 mM GABA and 100 mM Cr solution was placed in the same position to that of the human head in the helmet coil after in vivo GABA measurements. The VOI of the phantom was positioned identical to that in the human brain. The reference spectrum of in vitro GABA was measured with the repetition time (TR) at least five times of the estimated T_1 of GABA. Then in vivo GABA concentrations were calculated by comparing the integration of the in vivo GABA signals with that of the in vitro GABA signals after correction of the coil loading and relaxation time differences [3].

3. Results

In this study, localization was achieved using a slice-selective 90° pulse and two slice-selective universal rotator pulses, providing a single-shot three-dimensional localization of MQ filtered GABA. Since the conventional slice-selective 180° pulse during the refocusing period was replaced by the second double-band spectrally selective but spatially non-selective pulse, the spectrally semi-selective $45°_x$–$45°_x$ pulses were made slice-selective by converting them into 45° slice-selective universal rotator pulses [11]. Localization by the slice-selective universal rotator pulses was evaluated using MQ filtered 2-propanol images (Fig. 2). Fig. 2A shows a MQ filtered 2-propanol image with two-dimensional localization using a conventional slice-selective 90° pulse for slice-selective excitation along the first spatial dimension (z-axis) and a slice-selective universal rotator 90° pulse for slice-selective MQ preparation along the second spatial dimension (y-axis). The MQ filtered 2-propanol image of the three-dimensionally localized volume shows the desired localization accuracy using the spectrally semi-selective and slice-selective universal rotator $45°_x$–$45°_x$ pulses for localization along the third spatial dimension (x-axis) (Fig. 2B).

The efficiency and selectivity of the MQ filtering methods were improved using the two double-band frequency selective pulses as demonstrated in Fig. 3. The MQ filtered GABA spectra were acquired from a phantom containing 20 mM GABA, 10 mM Cr, 10 mM NAA, 30 mM glutamine, and 40 mM acetate using three-dimensionally localized MQ filtering methods. Spatial localization was applied on the two 90° pulses during MQ preparation and the second 180° pulse dur-

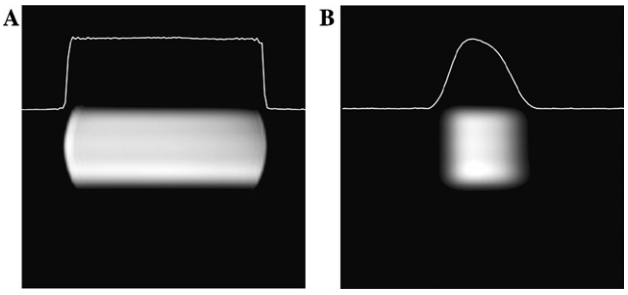

Fig. 2. Validation of localization using slice-selective universal rotator pulses. (A) An MQ filtered image of a spherical phantom containing 99.9% of 2-propanol solution using the pulse sequence shown in Fig. 1 except that the slice selection gradient of the spectrally semi-selective $45°_x$–$45°_x$ pulses was turned off. The sequence parameters were adjusted for optimal detection of the 2-propanol methyl groups. MR parameters were FOV = 15 cm × 15 cm, matrix size = 128 × 128, spectral width = 20 kHz, slice thickness of the first 90° pulse in z-direction = 2.5 cm, and slice thickness of the second 90° pulse in y-direction = 3.5 cm. The bandwidths of both the first and second 90° pulses were 1.56 kHz. (B) DQ filtered image of the 2-propanol solution with identical MR parameters as in (A) except that the slice selection gradient of the spectrally semi-selective $45°_x$–$45°_x$ pulses was turned on along the x-axis (bandwidth = 5.45 kHz). The slice thickness of the $45°_x$–$45°_x$ pulses was 3.5 cm. Intensity profiles at the center of the images are shown above the images.

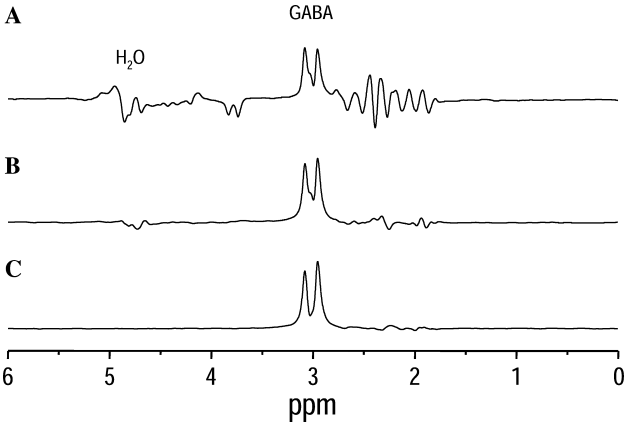

Fig. 3. Comparison of GABA editing using three-dimensionally localized MQ filtering methods of different spectral selectivity. All spectra were acquired from a phantom containing 20 mM GABA, 10 mM Cr, 10 mM NAA, 30 mM glutamine, and 40 mM acetate to demonstrate the improved selectivity of the C_4 methylene protons of GABA using the pulse sequence in Fig. 1. (A) Spectrally non-selective 180° pulses were used for MQ preparation and for rephasing the antiphase C_4 methylene protons of GABA. (B) A double-band frequency selective 180° pulse was used during MQ preparation and a spectrally non-selective 180° pulse was used for rephasing the antiphase C_4 methylene protons of GABA. (C) Two double-band frequency selective 180° pulses were used during the MQ preparation and the rephrasing periods, respectively.

ing the refocusing period for both Figs. 3A and B. For the spatial localization of the proposed method, the two 90° pulses during MQ preparation and the semi-selective universal rotator 90° pulse during the refocusing period were used as described in Section 2. When either the spectrally non-selective 180° pulse or the double-band frequency selective 180° pulse was used during MQ preparation, excellent suppression of Cr was achieved. However, the intensity of GABA signals acquired using a non-selective 180° pulse was ~20% lower (Fig. 3A) than that acquired using the double-band frequency selective 180° pulse (Figs. 3B and C) because the double-band frequency selective 180° pulse refocuses *J* evolution between C_2 and C_3 methylene protons of GABA and, therefore, prevents coherence leakage to the zero quantum coherence formed by the C_2 and C_3 methylene protons of GABA [2,3]. Limited suppression of other signals were also observed using a non-selective 180° pulse during MQ preparation (Fig. 3A) compared to Fig. 3B where the double-band selective pulse was used during MQ preparation. When two double-band selective pulses were used during the MQ preparation and the refocusing periods respectively (see Fig. 1), all residual unwanted signals were further suppressed (Fig. 3C) because only signals at the resonance frequencies of the C_3 and C_4 methlyene protons of GABA can pass the second double-band frequency selective pulse and its crusher gradients with a full yield of the outer two peaks of the GABA triplet.

Fig. 4 shows the assessment of suppression of overlapping resonances with coupled spins. The PRESS spectra (TE = 26 ms) of homocarnosine, lysine, and GSH are shown on the left side of Fig. 4. The corresponding DQ filtered spectra using the pulse sequence shown in Fig. 1 are shown on the right side of Fig. 4. As shown in Fig. 4A, the β methylene protons of homocarnosine at 3.0–3.2 ppm were completely suppressed. The overall homocarnosine suppression ratio was estimated to be 6:1 based on the integrated intensities of the homocarnosine signal in the 2.8–3.2 ppm range obtained using PRESS and the selective MQ filtering method in Fig. 1, respectively. Similarly, the suppression ratio for lysine was estimated to be 8:1 (Fig. 4B). Complete suppression of GSH (Fig. 4C) was also verified with the suppression ratio of over 100:1 as demonstrated previously [3,4]. The suppression ratios reported here are lower limits due to partial *J* evolution in the PRESS spectra (TE = 26 ms), which reduces the integrated intensities of metabolite signals on the left side of Fig. 4.

Fig. 5 shows simultaneous measurements of GABA and Cr in the fronto-parietal region of the human brain in vivo using STEMS [3] with two double-band frequency selective pulses. The first echo for the GABA acquisition uses the pulse sequence shown in Fig. 1. Immediately after acquisition of MQ filtered GABA, a PRESS sequence was used to excite the Cr signal, which remains at the longitudinal axis during the MQ filtering sequence period. The NAA signal intensity at 2.01 ppm was reduced (Fig. 5A) due to the $90°_x$–$90°_{-x}$ refocusing pulses [22] for additional water suppression in the appended PRESS sequence. The $90°_x$–$90°_{-x}$ echo refocus-

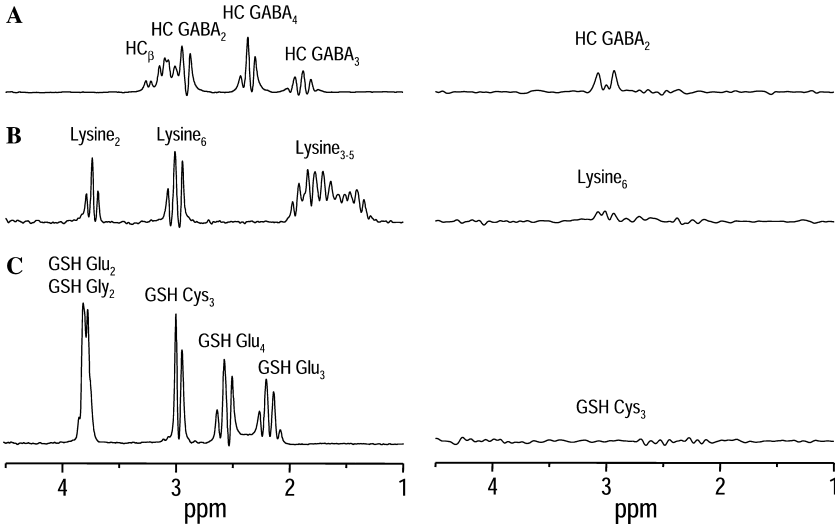

Fig. 4. Suppression of overlapping resonances of GABA: homocarnosine, lysine, and GSH. The left three spectra were acquired using a PRESS sequence (TE = 26 ms, TR = 4 s, VOI =27 ml) from solution phantoms of (A) L-homocarnosine (5 mM), (B) L-lysine (10 mM), and (C) glutathione (10 mM), respectively. The corresponding three spectra on the right are acquired using the proposed three-dimensionally localized two double-band selective MQ filtering method (right). Excellent suppression of GSH (C) and lysine (B) was achieved using the proposed selective MQ method. The resonances of the β methylene protons of homocarnosine at 3.0–3.2 ppm coupled to the α methylene proton resonances at 4.5 ppm showed excellent suppression similar to that of GSH (C). HC_β, the β methylene protons of homocarnosine; HC GABA, the GABA moiety of homocarnosine; GSH Gly, the glycine moiety of GSH; GSH Glu, the glutamate moiety of GSH; and GSH Cys, the cysteine moiety of GSH. All subscripts indicate the position of carbon.

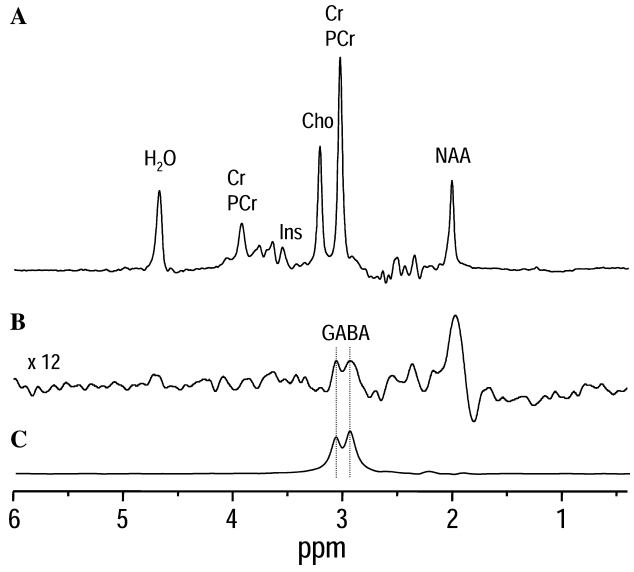

Fig. 5. Simultaneous measurement of GABA and Cr in the human brain in vivo. (A) The Cr spectrum acquired using the single-shot two-echo selective MQ filtering method (TE = 68 ms, TR = 2 s, NT = 572, VOI = 43 ml). (B) The corresponding GABA spectrum after correcting frequency drift using the Cr spectrum as a navigator echo. Each GABA spectrum averaged from four transients was stored separately prior to frequency drift corrections. The vertical expansion of the GABA doublet was scaled 12 times with respect to the Cr signal in (A). (C) The in vitro GABA doublet shows excellent agreement with that of in vivo. The frequency separation of 13.6 ± 0.9 Hz (means $\pm$ SD, $n = 15$) was measured as indicated by two vertical dotted lines. The in vitro GABA spectrum was line-broadened to match the in vivo linewidth. All spectra were processed with zero-order phase correction only and with no baseline correction.

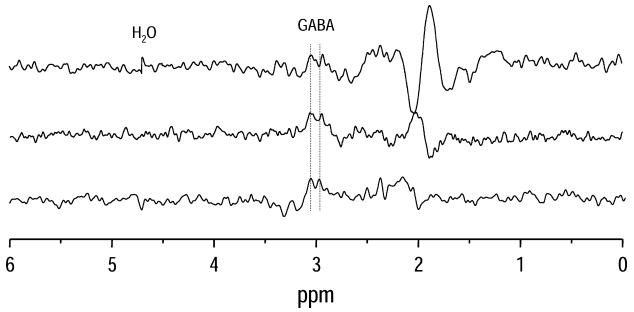

Fig. 6. Consistent measurement of GABA in the human brain in vivo using the proposed two double-band selective MQ spectroscopy. In vivo GABA spectra acquired from three different subjects. Despite the variations in outer volume subcutaneous lipid signal intensity, a clear doublet was observed at 3.02 ppm in all cases. The two vertical dotted lines indicate the frequency separation of GABA, 13.6 Hz, which was consistently observed in all subjects. The acquisition parameters were the same as in Fig. 5. Spectra were processed using zero-order phase correction only and with no baseline correction.

ing pulses were set to refocus Cr at 3.03 ppm and to crush water at 4.65 ppm.

A clear GABA doublet at 3.02 ppm was observed indicating minimal contamination from other resonances such as Cr and GSH (Fig. 5B). Each GABA and Cr spectrum was acquired with an individual trace average of four transients and stored separately. Frequency drift correction of the GABA spectra was performed based on the frequencies of Cr in the interleaved PRESS spectra. The averaged in vivo GABA spectrum (Fig. 5B, NT = 572, VOI = 43 ml) after frequency drift correction using Cr as a navigator echo shows a clear GABA doublet in excellent match with that of phantom solution (Fig. 5C), which was line-broadened to match the in vivo linewidth. The GABA doublet with a frequency separation of 13.6 ± 0.9 Hz (means $\pm$ SD, $n = 15$) was consistently detected in all subjects. The GM/WM ratio of ~1:1 in the VOI of the fronto-parietal region was calculated from the T_1-weighted images. Clean water suppression was also observed as expected. Potential MM contamination to the GABA signal was assessed from metabolite-nulled spectra acquired by adding an inversion recovery pulse with inversion recovery delay times of TIR = 0.66–0.68 s. Using the MQ GABA method proposed here, the residual MM signal was found at the noise level similar to our previous work [3] (data not shown). The GABA concentration was estimated at 0.67 ± 0.18 mM (means $\pm$ SD, $n = 15$) using the external reference method [3]. Fig. 6 demonstrates consistent measurements of GABA in the human brain in vivo using the two double-band selective MQ filtering technique. A GABA doublet at 3.02 ppm was observed in all subjects. Excellent water suppression was also consistently observed. The signal at ~2 ppm shown in Fig. 5B and Fig. 6 was assigned to outer volume subcutaneous lipids.

4. Discussion

Conventional three-dimensional spatial localization uses either STEAM or PRESS techniques. As demonstrated in Fig. 2A, localization along the second spatial dimension was achieved using a slice-selective universal rotator 90° pulse, which selectively prepares the antiphase spins after the action of the first double-band frequency selective 180° pulse and located at the intersection of the first (z-axis) and second (y-axis) slices into MQ coherence. The slice-selective $45°_x$–$45°_x$ universal rotator pulses convert the MQ coherence located at the intersection of the three orthogonal slices into the observable SQ coherence for the C_4 methylene protons of GABA.

For the purpose of spatial localization, the role of DQ filtering gradients is similar to that of the crusher gradients used in STEAM or PRESS for suppression of outer volume signals. With the replacement of the conventional $45°_x$–$45°_x$ pulses by slice-selective universal rotator pulses, the pulse sequence shown in Fig. 1 contains no broadband hard pulses, therefore, avoiding broadband excitation of the entire tissue volume accessible by the RF coil. As a result, the suppression of outer

volume signals (mostly composed of tissue water) should also be made easier than when broadband hard pulses are used as evidenced by the excellent water suppression shown in Figs. 3C, 5B–C, and 6.

The first double-band pulse during MQ preparation selectively prepares the C_3 and C_4 methylene protons of GABA into antiphase coherence. Thus, only the C_3 and C_4 methylene protons of GABA are converted into the DQ spin state. The second double-band pulse selectively refocuses the resonances at 3.0 and 1.9 ppm. As a result, only the antiphase C_4 methylene protons of GABA are rephased into an inphase doublet prior to data acquisition. Similar to the WATERGATE (WATER suppression by GrAdient-Tailored Excitation) methods [23,24], signals at other resonant frequencies are further suppressed by the crusher gradients of the second double-band frequency selective 180° pulse. This was verified by the result shown in Fig. 3C. It should also be noted that, if any residual GSH signal passes the MQ filter, the second double-band frequency selective 180° pulse keeps it in the antiphase spin state because the cysteinyl methylene protons of GSH at the 4.56 ppm was not acted on by the second double-band frequency selective pulse, leading to further suppression of GSH as demonstrated in Fig. 4C.

The use of two double-band frequency selective pulses in MQ filtering provided complete suppression of GSH and the β methylene protons of homocarnosine, and significant suppression of lysine, which mimics MM. The suppression of the β methylene proton resonances of homocarnosine at 3.0–3.2 ppm can be explained by their coupling to the α methylene protons at 4.5 ppm, which lies outside of the bandwidth of the two double-band frequency selective 180° refocusing pulses used in Fig. 1. The situation of the cysteine moiety of GSH is very similar to that of homocarnosine since the methylene protons of GSH also resonate at around 4.5 ppm. As a result, the suppression ratio for the cysteinyl methylene protons of GSH was measured to be over 100:1, comparing favorably to the previously reported suppression ratio of 51:1 using a single double-band frequency selective pulse [2]. The lower limit of the suppression ratio for lysine was estimated to be 8:1. Considering relatively short T_2 relaxation time of MM, the dispersion of chemical shifts and J coupling constants, it is expected that the suppression of MM in vivo would be much greater than that of lysine in phantoms as evident by the fact that the residual MM signals in the metabolite-nulled MQ filtered spectrum was at the noise level (data not shown). In summary, the results shown in Fig. 4 clearly demonstrated the advantage of using two double-band frequency selective 180° refocusing pulses for DQ filtering for enhanced spectral selectivity.

GABA doublet was observed consistently at 3.02 ppm in vivo using the two double-band selective MQ filtering method (TR = 2 s, TE = 68 ms, VOI = 27–43 ml). The reliable measurement and quantification of GABA in vivo using the proposed method was demonstrated by an excellent match between the in vivo GABA doublet and that from the GABA solution phantom. In addition, concentration measurements of GABA were consistent with our previous results from the same brain region [3,25]. All GABA spectra show excellent water suppression and relatively clean baseline. Spurious peaks have been found at ~2 ppm, which was absent in all phantom spectra. To trace the origin of the spurious signals, phase encoding gradients were added to the pulse sequence shown in Fig. 1 to obtain chemical shift images of the selected single voxel (data not shown). The signals at ~2 ppm were found to be spatially originated from subcutaneous lipid, which could pass the MQ filter since lipids can also form DQ coherence between adjacent methylene protons in the aliphatic side chains. The outer volume lipid can, therefore, be explained by the imperfect B_1 profile of the helmet coil, which results in imperfect slice selection and outer volume suppression. Improved outer volume suppression using a homogeneous volume transmission coil and/or additional outer volume suppression pulses should minimize the residual outer volume lipid signals at ~2 ppm.

5. Conclusions

We have demonstrated that it is feasible to use a combination of a slice-selective 90° pulse, a slice-selective universal rotator 90° pulse and a spectrally semi-selective and slice-selective 90° pulse composed of slice-selective universal rotator 45° pulses for single-shot spatial localization in MQ filtering. The two double-band frequency selective 180° pulses can enhance GABA selectivity with excellent suppression of tissue water and other metabolite signals. Further optimization of the slice-selective RF pulses and outer volume suppression should reduce the signals observed at ~2 ppm although they do not interfere with GABA measurements at 3.0 ppm. In addition, this technique can be further developed into chemical shift imaging techniques for mapping the distribution of GABA in the human brain in vivo.

Acknowledgments

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