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# A new strategy for in vivo spectral editing. Application to GABA editing using selective homonuclear polarization transfer spectroscopy

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**Abstract**

A novel single-shot in vivo spectral editing method is proposed in which the signal to be detected, is regenerated anew from the thermal equilibrium magnetization of a source to which it is *J*-coupled. The thermal equilibrium magnetization of the signal to be detected together with those of overlapping signals are suppressed by single-shot gradient dephasing prior to the signal regeneration process. Application of this new strategy to in vivo GABA editing using selective homonuclear polarization transfer allows complete suppression of overlapping creatine and glutathione while detecting the GABA-4 methylene resonance at 3.02 ppm with an editing yield similar to that of conventional editing methods. The NAA methyl group at 2.02 ppm was simultaneously detected and can be used as an internal navigator echo for correcting the zero order phase and frequency shifts and as an internal reference for concentration. This new method has been demonstrated for robust in vivo GABA editing in the rat brain and for study of GABA synthesis after acute vigabatrin administration.


*Keywords:* GABA; Vigabatrin; Spectral editing; Polarization transfer; Quantification

## 1. Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian cortex. GABA has three methylene groups with approximately equal *J* coupling constants. The proton NMR spectrum of GABA consists of a triplet at 2.30 ppm due to GABA-2, a quintet at 1.91 ppm due to GABA-3 and a triplet at 3.02 ppm due to GABA-4. In the proton GABA spectrum at field strength accessible for clinical studies (e.g., 3 T) the GABA-2 signal overlaps with those of glutamate/glutamine (Glx)-4, the GABA-3 signal with those of *N*-acetylaspartate (NAA) and *N*-acetylaspartatylglutamate (NAAG) methyl groups, and the Glx-3 methylene groups, the GABA-4 signal with those of creatine (Cr) methyl group and glutathione (GSH) cysteinyl methylene group. All GABA signals overlap with those of macromolecules (MM) [1]. Even at high field strength available for animal studies GABA editing, if it results in a flat baseline and no overlapping resonances, can still be advantageous for accurate in vivo quantification of GABA.

Due to the importance and difficulty of GABA measurement, many editing methods have been proposed over the past decade. Essentially, most existing GABA editing methods fall into the following categories: (1) subtraction methods for canceling the full or partial intensity of overlapping creatine signal at 3.03 ppm based on the *J* interaction between GABA-3 and

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GABA-4 including the two-step spin-echo $J$ editing methods [2–8] and the longitudinal two-spin order method [9]; (2) multiple quantum filtering methods for selection of GABA-3,4 double quantum (DQ) coherence while suppressing overlapping signals remaining in the single quantum state [10–16]; and (3) two-dimensional (2D) methods for separation of GABA-4 or GABA-3,4 cross-peaks from overlapping signals in the second spectral dimension [16–18]. The subtraction methods are very susceptible to movement, instrument instability, and magnetic field drifts. As a result, alternative methods for separating the GABA-4 signal from that of the overlapping Cr have been developed. Of them, the DQ filtering methods achieving single-shot editing by gradient filtering are much less affected by movement, instrumental instability, and magnetic field drifts. The spectral selectivity of the DQ filtering methods has been significantly improved with the introduction of doubly selective pulses into DQ preparation [12–14]. The 2D spectroscopy methods for GABA detection suffer from lower SNR because of the much shorter $T_2$ and $T_2$* of metabolites in vivo. In addition, similar to the subtraction methods, in vivo 2D methods are susceptible to movement and instrument instability during signal acquisition which generate $t_1$ noise in the 2D spectra. Here we limit our discussion to one-dimensional (1D) methods.

In all 1D GABA methods discussed above, the observed GABA-4 peak originates from the thermal equilibrium magnetization of GABA-4 (and GABA-3 in the case of DQ filtering), which overlaps with the thermal equilibrium magnetization of Cr at 3.03 pm and that of GSH at 2.87–2.96 ppm. As a result, subtraction or DQ gradient filtering has to be used to reveal the GABA-4 signal from those of overlapping Cr and GSH while the MM signal can be separated based on the $T_1$ differences between small metabolites and MM [1]. In this paper, we propose a novel approach for in vivo spectral editing, in which the signal to be edited (target signal) is completely regenerated anew from the corresponding $J$-coupled thermal equilibrium magnetization of a source signal. In the case of GABA editing proposed here, the edited GABA-4 resonance is derived from the thermal equilibrium GABA-3 peak using a novel selective homonuclear polarization transfer approach. For each single scan, the thermal equilibrium signal of the GABA-4 resonance and its overlapping signals (Cr, GSH, and MM) are completely suppressed prior to polarization transfer from GABA-3. Because no DQ filtering gradients are used, the resonance of NAA, an important neuronal marker [19], at 2.02 ppm is detected simultaneously. The intense signal from the NAA methyl group in the selective polarization transfer GABA spectra can serve as an internal phase/frequency and concentration reference [14]. This new strategy for GABA editing has been demonstrated here for in vivo editing of GABA-4 in the rat brain at 11.7 T for accurate quantification of GABA and for the study of GABA synthesis after acute vigabatrin (VGB) administration [2,20]. Application of this method to in vivo editing of GABA and other metabolites at lower field strength is readily conceivable. A preliminary account of this work has been presented as an abstract [21].

## 2. Theory

Using the same notation as in [11], we denote the GABA six-spin system using $I_2S_2W_2$ with $I$ for GABA-4 methylene protons at 3.02 ppm, $S$ for GABA-3 at 1.91 ppm and $W$ for GABA-2 at 2.30 ppm, respectively. For convenience, we assume that $J_{23} = J_{34} = 7.3$ Hz. In the following, we also use the weak coupling approximation for the product operator analysis of the selective homonuclear polarization transfer pulse sequence. Similar analysis has been performed for multiple quantum filtering of GABA at 2.1 and 3.0 T [11,14]. For the selective homonuclear polarization transfer pulse sequence shown in Fig. 1, the thermal

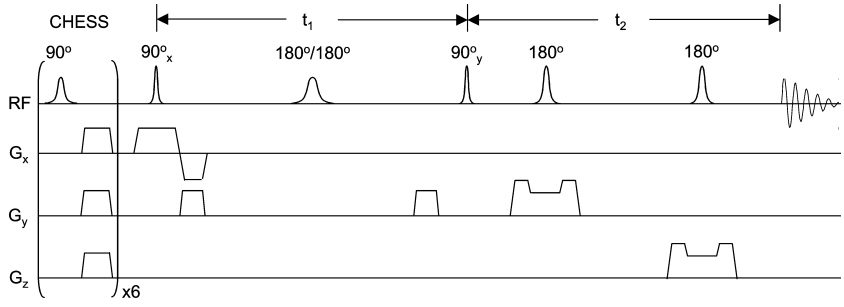


Fig. 1. Selective homonuclear polarization transfer pulse sequence for simultaneous detection of GABA and NAA. The GABA-3 quintet was excited by a slice-selective 90° pulse (five-lobe sinc, 500 μs). During $t_1$ (=1/4$J$) a doubly selective 180° refocusing pulse based on the Hermite pulse (15 ms, $B_{1max}$ = 375 Hz, selecting the GABA-3 resonance at 1.91 ppm and the GABA-4 resonance at 3.02 ppm) allows the GABA-3,4 coupling to evolve and refocuses the spin evolution due to the GABA-2,3 coupling. Another 90° pulse (five-lobe sinc, 500 μs with optional slice selection along $x$-axis) performs homonuclear polarization transfer. The transferred antiphase GABA-4 resonance was rephased during $t_2$. The thermal equilibrium signals from GABA-4, Cr, and GSH at 2.87–3.03 ppm together with that from tissue water were suppressed using CHESS prior to polarization transfer. The OVS pulses were not drawn for simplicity. Note that for clarity, each slice selective 180° pulse represents a pair of adiabatic sech pulses.

equilibrium GABA-4 signal is saturated by CHESS prior to polarization transfer. The first 90° pulse of the polarization transfer scheme excites all remaining thermal equilibrium signals into

$$-(S_y + S'_y + \alpha^*(W_y + W'_y)), \quad (1)$$

where $\alpha \leqslant 1$, representing partial saturation of GABA-2 by the CHESS pulses. The $S_y$ and $S'_y$ spins will be converted to the target GABA-4 signal in the end. During the $t_1$ (=1/4$J$) period, the spectrally doubly selective 180° pulse on GABA-3, and GABA-4 allows $J$ evolution between GABA-3 and GABA-4 but refocuses their chemical shift/$B_0$ evolutions and the $J$ evolution between GABA-2 and GABA-3. At the end of $t_1$, the GABA-3 quintet evolves into

$$-(I_z S_x + I'_z S_x + I_z S'_x + I'_z S'_x). \quad (2)$$

The 90° spectrally non-selective pulse along the $y$-axis produces polarization transfer and generates the GABA-4 antiphase coherence:

$$(I_x S_z + I'_x S_z + I_x S'_z + I'_x S'_z). \quad (3)$$

During the $t_2$ (=1/4$J$) period, the GABA-4 spins rephase into the final GABA-4 inphase doublet represented by

$$(I_y + I'_y + 4I_y S_z S'_z + 4I'_y S_z S'_z)/2 \quad (4)$$

with chemical shift/$B_0$ refocused by the spectrally non-selective 180° pulse(s) during $t_2$. As described previously [11], Eq. (4) represents the full intensity of the outer two lines of the GABA-4 triplet under the weak coupling approximation.

The Cr methyl proton resonances at 3.03 ppm and the GSH cysteinyl proton resonances at 2.87–2.96 ppm are suppressed together with the water signal by the CHESS scheme. In the case of severe $B_1$ inhomogeneity and/or insufficient CHESS repetitions, residual Cr and GSH signals are left along the $z$-axis by the first 90° pulse together with those generated by $T_1$ relaxation during $t_1$. These signals are then excited by the second 90° pulse along the $y$-axis into the transverse plane and could contaminate the observed GABA-4 signal. If the second 90° pulse is along the $-y$-axis, it inverts the phase of the residual Cr and GSH signals but does not affect that of GABA-4 antiphase coherence denoted by Eq. (3). Therefore, if necessary, a two-step phase cycling scheme cancels the residual Cr and GSH signals originating from the effect of $B_1$ inhomogeneity and $T_1$ relaxation during $t_1$. For the NAA singlet at 2.02 ppm, the 180° pulse during $t_1$ puts it to the $y$-axis right before the $90°_{\pm y}$ pulse for polarization transfer, therefore, it is not affected by the $90°_{\pm y}$ pulse. During the $t_2$ period, the spectrally non-selective 180° pulse(s) refocuses its chemical shift evolution. As a result, the NAA singlet and the edited GABA-4 doublet acquire the same zero order phases.

## 3. Methods

All experiments were performed on a Bruker microimaging spectrometer (Bruker Biospin, Billerica, MA) running Linux-based ParaVision version 3.0.1 software on a Hewlett–Packard computer interfaced to an 11.7 T 89-mm bore vertical magnet (Magnex Scientific, Abingdon, UK), which is equipped with a 57-mm i.d. gradient (maximum gradient strength: 3 G/mm; rise time: 100 μs) for in vivo experiments, and a 2.5-mm broadband inverse (BBI) gradient probe (5 G/mm on $z$-axis) for high-resolution experiments. The in vivo experiments used a homebuilt 15-mm i.d. surface transceiver coil mounted on an integrated homebuilt animal handling system capable of rat head fixation, body support, coil tuning, and RF shielding.

Male Sprague–Dawley rats (150–180 g, $n = 11$) were studied as approved by the NIMH Animal Care and Use Committee. The rats were intubated and ventilated with a mixture of 70% $N_2O$/30% $O_2$ and 1.5% isoflurane. A femoral artery and a femoral vein were cannulated for measuring arterial blood gases ($pO_2$, $pCO_2$), pH, mean blood pressure, and for intravenous infusion of α-chloralose (initial dose: 80 mg/kg supplemented with a constant infusion of 26.7 mg/kg/h throughout the experiment). After surgery, isoflurane was discontinued and pancuronium bromide was administrated (4 mg/kg every 90 min, i.v.) to facilitate immobilization. Rectal temperature was maintained at 37.5 ± 0.5 °C. End-tidal $CO_2$, tidal pressure of ventilation, and heart rate were also monitored. Arterial blood $pO_2$ was maintained at 120–150 mmHg, $pCO_2$ at 25–35 mmHg, mean blood pressure at 180 ± 30 mmHg, and plasma pH at 7.35–7.45. Three-slice (coronal, horizontal, and sagittal) scout RARE images were used to position the rat inside the magnet such that the gradient isocenter was about 0–2 mm rostral to bregma and the center of the voxel was about 0.5–1 mm anterior to the gradient isocenter. Shimming of the rat brain was carried out using FLATNESS [22,23] to correct all first order, second order, and $z^3$, $z^2x$ shims. In group A ($n = 5$), VGB (Sigma–Aldrich, St. Louis, MO, 500 mg/kg, 0.6 mL, i.v.) was administered immediately after the baseline spectra were acquired. In group B (control group, $n = 6$), no VGB was used and only baseline spectra were acquired. Immediately after in vivo data acquisition, the metabolism of the rat brain was arrested using a microwave fixation system (Model TMW-6402C, Muromachi Kikai, Tokyo, Japan), which inactivates enzymatic processes in approximately one second without affecting the level or the distribution of GABA [24]. Perchloric acid (PCA) extracts of the brain tissue corresponding to the spectroscopy voxel were prepared as described previously [25] and then lyophilized repeatedly. The high-resolution spectra of the PCA extracts in $D_2O$ were acquired using added 3-(trimethylsilyl) propionic-

2,2,3,3-$d_4$ acid (TSP-$d_4$) as a chemical shift reference standard.

The pulse sequence (Fig. 1) consisted of outer volume suppression (OVS) using 90° sech pulses (2 ms, $\mu = 5$, 1% truncation) along the $x$ (10 mm slab), $-x$ (10 mm slab), $y$ (3 mm slab), $-y$ (5 mm slab), $z$ (10 mm slab), and $-z$ (10 mm slab) directions. Water suppression was accomplished using 90° sech pulses (18 ms, $\mu = 8$, 1% truncation) based on the CHESS method. The saturation bandwidth of the water suppression pulse was 1314 Hz centered at 3.9 ppm to suppress water signal at 4.6 ppm as well as GABA-4, Cr, and GSH signals at 2.87–3.03 ppm. The CHESS and OVS pulses were interleaved and repeated in the following fashion: CHESS-CHESS-CHESS-OVS-CHESS-OVS-CHESS-OVS-CHESS. Immediately after the sixth CHESS pulse, a slice-selective 90° pulse (five-lobe sinc, 500 μs) was applied along the $x$-axis (rotating frame, with slice selection gradient on the $x$-axis). An optimized doubly selective 180° refocusing pulse based on the Hermite pulse (along the $x$-axis in rotating frame, 15 ms, $B_{1\max} = 375$ Hz, centered at GABA-3 at 1.91 ppm and GABA-4 at 3.02 ppm) together with a pair of gradient crushers were used to allow the GABA-3 resonance to evolve under the GABA-3,4 $J$ coupling while refocusing the spin evolution due to the GABA-2,3 $J$ coupling. The optimized double band Hermite-based pulse generated minimal excitation at the macromolecule resonating frequency of 1.72 ppm [12]. The nominal evolution period $t_1 = 1/4J$. Then the antiphase GABA-3, 4 spin state was acted on by a 90° pulse on the $y$-axis (rotating frame, five-lobe sinc, 500 μs) to perform homonuclear polarization transfer. During the rephasing period ($t_2 = 1/4J$), the inphase GABA-4 doublet was formed while spatial selection along the $y$- and $z$-axes was achieved using using slice-selective 180° refocusing pulses (a pair of identical sech pulses per axis [26], 2 ms, $\mu = 5$, 1% truncation). The localized voxel was centered in the brain midline with a dimension of 4.5 mm × 2.5 mm × 4.5 mm. Non-edited spectra were obtained from the same voxel using an adiabatic PRESS sequence (TE = 15 ms) based on slice-selective adiabatic refocusing using two hyperbolic secant pulses per axis [26]. The adiabatic PRESS sequence used the same OVS and water suppression schemes except that the water suppression was achieved using CHESS based on 15 ms Gaussian pulses. The CHESS and OVS pulses were interleaved and repeated the same way as in the selective homonuclear polarization transfer pulse sequence. The voxel definition of the homonuclear polarization pulse sequence along the $x$-axis could be further improved by adding an additional pair of sech pulses in $t_2$ or a 1D ISIS, or by making the second 90° pulse for polarization transfer slice-selective with the corresponding slice selection gradient along the $x$-axis [13,14].

High-resolution experiments for validation of the product operator analysis described in Theory were performed on a GABA solution sample (5 mM in $D_2O$, pH 7.0) using pulse-acquire and a pulse sequence derived from that depicted in Fig. 1. In the high-resolution version of the selective homonuclear polarization transfer pulse sequence, no OVS RF and gradient pulses were applied and all RF and gradient pulses for localization were replaced with hard RF pulses. The rest of the pulse sequence was identical to that in Fig. 1. The effects of the high-resolution version of the homonuclear polarization transfer sequence on the GABA six-spin Hamiltonian were also simulated numerically using Bruker NMR-SIM software written by Pavel Kessler (version 3.1, Bruker BioSpin GmbH, Karlsruhe, Germany), which is based on the Liouville equation and capable of simulating gradient experiments. The NMRSIM software generates a simulated FID from any predefined pulse sequence and spin systems at arbitrary field strength.

Two methods for quantification of GABA concentration were used and cross validated. Using the in vivo GABA-to-NAA ratio method, the GABA-to-NAA intensity ratio was measured in vivo using the selective polarization transfer method (see Fig. 1). The GABA-to-NAA intensity ratio in the NMR spectrum of a phantom sample containing 50 mM NAA and 5 mM GABA was also measured using the same pulse sequence. The in vivo concentration ratio [GABA]/[NAA] was then derived based on the ratio of the GABA-to-NAA intensity ratios measured in vivo and in the phantom sample. It was assumed either: (a) the relaxation difference between NAA and GABA is small; or (b) the difference between the relaxation of NAA in vivo and in the phantom sample is similar to the difference between the relaxation of GABA in vivo and in the phantom sample. With either (a) or (b), the effects of relaxation on the quantification of GABA are cancelled out using the in vivo GABA-to-NAA ratio method.

Using the in vitro GABA-to-NAA ratio method, PCA extracts of rat brain were prepared and analyzed after euthanasia using the microwave fixation system as described above. The GABA-to-NAA ratio from brain extracts was measured using high-resolution NMR spectroscopy under fully relaxed condition on the 2.5-mm BBI gradient probe. Assuming the in vivo relaxation difference between the two metabolites NAA and GABA is small the concentration of GABA can be calculated using rat brain's NAA concentrations reported in the literature.

## 4. Results

### 4.1. Numerical simulation

The GABA editing yield derived in Theory was first verified numerically by a full density matrix simulation

using the NMRSIM software. A comparison of the simulated GABA-4 triplet acquired using a single 90° pulse with the simulated GABA-4 doublet using the high-resolution version of the homonuclear polarization transfer sequence described in Section 3 predicted an editing yield of 91% at 11.7 T (see Table 1). This was verified experimentally using the 2.5-mm high-resolution BBI gradient probe and a 5 mM GABA/$D_2O$ solution. With parameters identical to those used in the numerical simulation, the experimentally determined intensity ratio of the GABA-4 doublet obtained using selective homonuclear polarization transfer to the two outer lines of the GABA-4 triplet obtained using the 90° pulse-acquire sequence was 82%. The central peak in the edited GABA-4 signal was cancelled in the weak coupling limit (Eq. (4)) as described previously [11].

### 4.2. In vivo editing

An in vivo adiabatic PRESS proton spectrum acquired from the spectroscopy voxel in the α-chloralose anaesthetized rat brains is shown in Fig. 2 (TR/TE = 2000/15 ms, 4.5 mm × 2.5 mm × 4.5 mm, NS = 256, LB = 1 Hz). The metabolite linewidth $\Delta\upsilon_{1/2} = \sim$10–11 Hz after in vivo FLATNESS shimming. The phosphocreatine methylene peak at 3.93 ppm and creatine methylene peak at 3.92 ppm are clearly resolved without applying any resolution-enhancing window functions. The GABA-2 peak at 2.30 ppm is visible although it partially overlaps with the glutamate-4 (Glu-4) peak at 2.35 ppm. The GABA-2 peak also overlaps with the MM baseline underneath.

Fig. 3 shows the result of GABA-4 editing using the proposed homonuclear polarization transfer scheme without (A, upper trace) and with (B, lower trace) metabolite null (TR/TIR/TE = 2000/635/68 ms, 4.5 mm × 2.5 mm × 4.5 mm, NS = 256, LB = 5 Hz). Both spectra were phased using zero order phase only without any baseline corrections. In Fig. 3A, a clear GABA doublet is observed at 3.02 ppm with a clean baseline indicating

Table 1
Editing yield of the proposed selective polarization transfer method at different field strength simulated using NMRSIM

| Field strength (T) | Pulse[a] duration (ms) | Editing yield[b] (%) | Relative editing yield[c] (%) |
|---|---|---|---|
| 3.0 | 30 | 74 | 94 |
| 7.0 | 20 | 91 | 100 |
| 11.7 | 15 | 91 | 100 |

[a] For 3.0 and 7.0 T, doubly selective 180° Gaussian pulse centered at GABA-4 (3.02 ppm) and GABA-3 (1.91 ppm); for 11.7 T, optimized doubly selective 180° Hermite-based pulse.

[b] Defined as the retention of the outer two lines of the GABA-4 triplet as compared to a 90° pulse-acquire experiment. $T_2$ relaxation was ignored in the simulation.

[c] Relative to that of doubly selective multiple quantum filtering [12–14] using the same double band pulses.

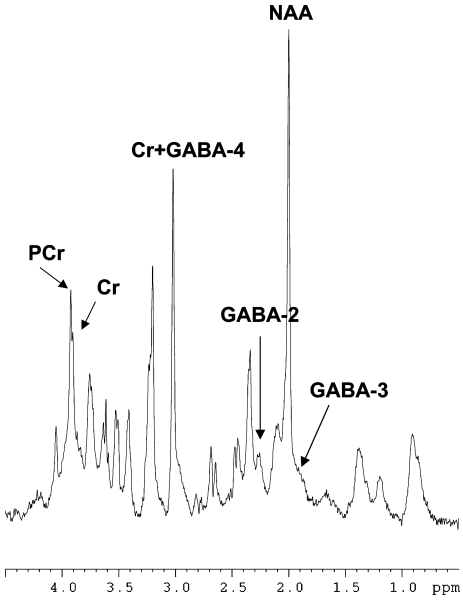


Fig. 2. In vivo non-edited adiabatic PRESS proton spectrum. TR/TE = 2000/15 ms, 4.5 mm × 2.5 mm × 4.5 mm, NS = 256, LB = 1 Hz, AQ = 206 ms (for demonstration of spectral resolution). The phosphocreatine methylene peak at 3.93 ppm and creatine methylene peak at 3.92 ppm are clearly resolved. The spectrum was phased using zero order phase only without any baseline correction.

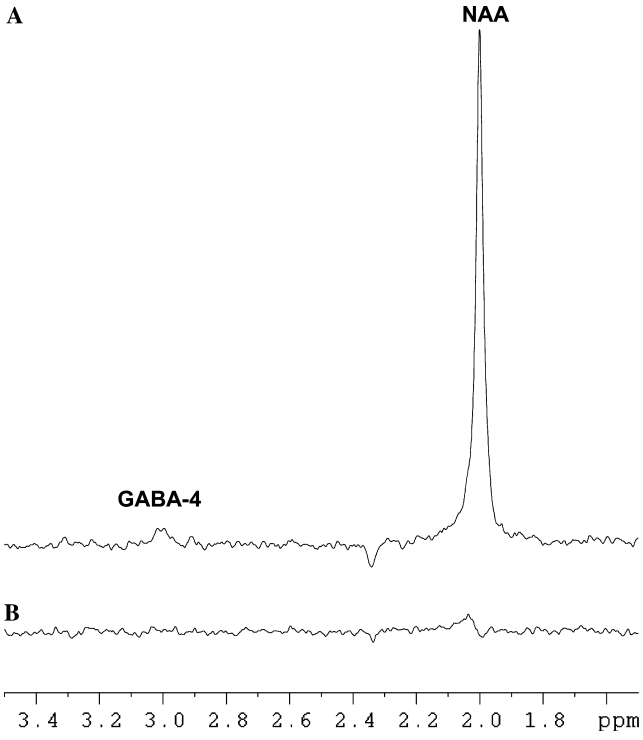


Fig. 3. In vivo edited GABA spectra. (A) GABA-4 editing using the proposed homonuclear polarization transfer scheme shown in Fig. 1 without metabolite null (TR/TE = 2000/68 ms, 4.5 mm × 2.5 mm × 4.5 mm, NS = 256, LB = 5 Hz). The spectrum was phased using zero order phase only without any baseline correction. (B) GABA-4 editing with metabolite null (TR/TIR/TE = 2000/635/68 ms, 4.5 mm × 2.5 mm × 4.5 mm, NS = 256, LB = 5 Hz). The same processing parameters such as phase, baseline and intensity scale were used.

excellent suppression of the overlapping Cr methyl resonance at 3.03 ppm and GSH cysteinyl methylene resonance at 2.87–2.96 ppm as well as outer volume signals. Complete suppression of Cr and GSH signals to the noise level was verified using a phantom sample containing 10 mM GABA, 20 mM GSH, and 50 mM Cr (data not shown). The selective polarization transfer spectrum also clearly demonstrates the phase relationship between NAA and GABA as predicted in Theory. The negative peak at 2.35 ppm was the Glu-4 signal resulted from partial polarization transfer from Glu-3 at 2.11 pm. The TIR was empirically determined to minimize the NAA singlet at 2.02 ppm [1]. No residual MM signal above the noise level was observed in the edited spectra in all rats ($n = 11$). The polarization transfer experiment at metabolite null was also repeated for TR/TIR/TE = 5000/980/68 ms with no residual MM signal detected.

Fig. 4 shows the time course of the in vivo selective homonuclear polarization transfer GABA spectra from the rat cerebral cortex with acute administration of VGB (500 mg/kg, 0.6 mL, i.v.). MR parameters used were: TR/TE = 2000/68 ms, 4.5 mm × 2.5 mm × 4.5 mm, NS = 256, LB = 5 Hz. On acute administration of VGB, the intensity of the GABA doublet at 3.02 ppm obtained from the α-chloralose anaesthetized rat brain significantly increased over time as expected [20]: lower trace (C): 0 h, middle trace (B): 1 h, upper trace (A): 2 h after VGB injection. All spectra were phased using zero order phase only without any baseline correction.

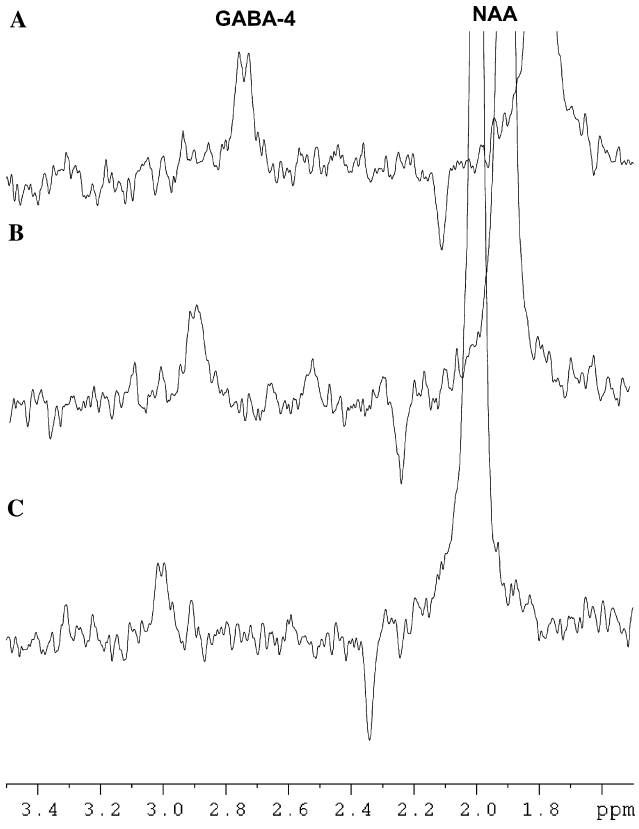


Fig. 4. Time course of homonuclear polarization transfer spectra from the cerebral cortex of the α-chloralose anaesthetized rat brain on acute VGB administration (500 mg/kg, 0.6 mL, i.v.). TR/TE = 2000/68 ms, 4.5 mm × 2.5 mm × 4.5 mm, NS = 256, LB = 5 Hz. Bottom trace (C): 0 h, middle trace (B): and 1 h, top trace (A): 2 h after VGB injection. All spectra were phased using zero order phase only without any baseline correction. The same intensity scales were used. The top and middle traces were shifted horizontally to illustrate the consistency of the phase relationship between the NAA and the GABA-4 peaks.

### 4.3. Quantification

In a fashion similar to GABA quantification using the GABA-to-Cr ratio as discussed previously [11,14], the in vivo GABA-to-NAA intensity ratio was measured using the proposed selective polarization transfer method. Then the GABA-to-NAA intensity ratio in the NMR spectrum of a phantom containing 50 mM NAA and 5 mM GABA was also measured using the same procedure. The in vivo [GABA]/[NAA] ratio was then derived based on the ratio of the GABA-to-NAA intensity ratios measured in vivo and in vitro. Using this method, the in vivo [GABA]/[NAA] was determined to be $0.14 \pm 0.03$ (mean ± SD, $n = 11$) in rats without VGB administration.

After euthanasia using the microwave fixation system PCA extracts of rat brain were prepared and analyzed as described in Section 3. The [GABA]/[NAA] ratio from brain extracts of the control group was determined to be $0.13 \pm 0.01$ (mean ± SD, $n = 6$) using high-resolution NMR spectroscopy on the 2.5-mm BBI gradient probe. Assuming $[NAA]_{total} = 9.4$ μmol/g wet weight [27], we obtain [GABA] = 1.2 ± 0.1 μmol/g wet weight (mean ± SD). The postmortem GABA concentrations measured from rats euthanized using the microwave procedure closely match those reported in the literature using the same method of euthanasia [28,29]. The postmortem [GABA]/[NAA] measured using high-resolution NMR spectroscopy is in excellent agreement with that measured using the proposed in vivo selective homonuclear polarization technique for GABA editing, providing a direct validation of the in vivo quantification procedure. The kinetics of GABA concentration following acute inhibition of GABA-transaminase was also measured and quantified from the vigabatrin-treated group, which, together with the effects of elevated brain GABA concentration on fMRI signals [30], will be presented elsewhere.

## 5. Discussion

Current in vivo editing methods have focused on revealing the thermal equilibrium target signal from signals overlapping with it (e.g., [2–17,31–36]). The major drawback of that approach lies in technical difficulties of combining the task of preserving the intensity of the

thermal equilibrium signal of the editing target and that of suppressing unwanted overlapping thermal equilibrium signals. The new in vivo spectral editing strategy is fundamentally different from all existing editing methods in that the thermal equilibrium signal of the target together with the overlapping signals are completely suppressed first and then the edited signal in its entirety is regenerated anew from the thermal equilibrium signal of a source resonating at a different frequency. This strategy therefore dramatically simplifies the spectral editing process since the suppression of unwanted signals is completed prior to the start of any coherence transfer steps or spin echoes.

Many existing water suppression methods that selectively suppress the thermal equilibrium water signal can be adapted for single-shot suppression of the target signal and signals overlapping with it. In particular, the CHESS method, which is capable of suppressing water by a factor of $>10^4$ can be used for simultaneous suppression of water and signals at and close to the editing target. The suppression factor rendered by CHESS should be sufficient for most editing experiments, making this strategy potentially very useful when the overlapping signals are intense, for example, in the case of lactate (Lac) editing in breast cancer. For the GABA-4 editing application in brain presented here, the thermal equilibrium GABA-4 signal and the overlapping signals such as those from GSH and Cr in the 2.87–3.03 ppm range were completely suppressed in a single shot. Although we have only applied this method to GABA-4 editing, we recognize the general usefulness of this strategy in editing many other metabolites, for example, β-hydroxybutyrate (bHB) and Lac [32–35].

As shown in Section 2, the theoretical editing yield of the proposed selective homonuclear polarization transfer method is 100% for the two outer lines of the GABA-4 peak when assuming the doubly selective 180° refocusing pulse is a 180° delta pulses on GABA-3 and GABA-4 and a 0° pulse on GABA-2. If the doubly selective 180° refocusing pulse on GABA-3 and GABA 4 partially refocuses GABA-2 during $t_1$, the $J$ evolution between GABA-3 and GABA-2 leads to coherence leakage. The 15 ms doubly selective 180° Hermite-based pulse was simulated numerically. The simulation produced an editing yield of 91% at 11.7 T for the outer two lines of the GABA-4 signal as compared to the 90° pulse-acquire experiment. The double band Hermite-based pulse generated an approximately 12° flip angle on GABA-2 which, together with the $J$ evolution process during the double band pulse, contributed to the signal loss in the regenerated GABA-4 signal. Note that the traditional two-step $J$ editing method also suffers from signal loss due to the $J$-evolution process during the relatively long 180° editing pulse. Therefore, the editing yield of the selective homonuclear polarization transfer method proposed here is similar to that of the traditional subtraction-based two-step editing method and that of a multiple quantum filtering method using the same double band pulse for double quantum preparation [12–14]. The experimentally determined 82% editing yield (at 11.7 T, compared to 90° pulse-acquire) could be due to further signal loss caused by $B_1$ inhomogeneity of the additional RF pulses used in polarization transfer and to $T_2$ relaxation during the spin echo time.

Excellent suppression of the Cr and GSH signals was achieved in a single shot using the surface transceiver coil. Because broadband pulses and multiple repetitions of CHESS were used, the suppression of the Cr and GSH signals was not sensitive to $B_0$ or $B_1$ inhomogeneity, subject movement, instrumental instability or magnetic field drifts. Therefore, the selective homonuclear polarization transfer method should be an attractive choice for chemical shifting imaging of GABA. As described in Section 2, contamination from the Cr and GSH signals could occur due to severe $B_1$ inhomogeneity and $T_1$ relaxation during $t_1$. If necessary, optional phase cycling could be employed to further enhance the suppression of the residual Cr and GSH signals. Residual Cr and GSH signals introduced to the transverse plane due to $B_1$ inhomogeneity of the slice selective refocusing pulses will not survive the crusher gradients flanking the slice-selective 180° pulses. The signals from the GABA-4, Cr, and GSH at 2.87–3.03 ppm were suppressed together with water using the CHESS method composed of single-band 90° pulses. Several other methods could also be used to achieve the same goal. For example, the signals at and around 3.0 ppm can be suppressed together with water using two single band 90° pulses-based CHESS or using saturation of thermal equilibrium signals via simple selective RF irradiation (if magnetization transfer is not of concern).

One of the drawbacks of the DQ filtering experiments for editing is that there are no dominant singlets in the DQ filtered spectra for zero order phase reference. This could be problematic when the sensitivity is low (e.g., due to reduced GABA peak in diseased brain or in animal models of human diseases). In chemical shift imaging an internal phase reference would also be very convenient. For GABA editing, this potential problem has been overcome by using a two-echo method for simultaneous detection of DQ filtered GABA in the first echo and Cr in the second echo [14]. Other two-echo schemes have also been reported [35,36]. Unlike in the DQ methods where the second 90° pulse returns all singlets to the $z$-axis, in the selective polarization transfer method the second 90° pulse acts as a spin-locking pulse for the NAA resonance, rendering it with the same phase as that of the regenerated GABA-4 peak. Therefore, the detection of GABA and NAA in a single spectrum allows determination of the zero order phase of the spectrum while the first order phase error can be elimi-

nated by balancing the echo times. The NAA signal could also be used as a concentration reference. In particular, the NAA singlet at 2.02 ppm is completely refocused by the numerically optimized Hermite-based doubly selective 180° pulse. Since NAA is an important neuronal marker [19], simultaneous measurement of GABA and NAA in a single spectrum is also of value when the scanning time is very limited, e.g., in chemical shift imaging.

Even at 11.7 T, macromolecule baseline and partial spectral overlapping around the GABA-2 resonance make reliable quantification of GABA in vivo rather difficult (see Fig. 2). We have found that spectral editing was necessary to obtain a flat baseline and a clean GABA peak for accurate in vivo quantification of cerebral GABA and GABA synthesis [30]. Spectral separation of the GABA signal from overlapping resonances using editing is particularly important when shimming is suboptimal. Although this new GABA-4 editing method has only been demonstrated for in vivo measurement of GABA in the rat brain at 11.7 T, it could also be very useful for human studies at lower field strength because DQ-based GABA-4 editing using similar doubly selective refocusing pulses has already been demonstrated at 2.1 and at 3 T [12–14]. The GABA-4 editing yield using the proposed selective homonuclear polarization transfer method was also simulated using the NMRSIM software for the field strength of 3 and 7 T (see Table 1), which should be a useful guide for extension of this method to editing GABA in the human brain. For low strength applications, it may be difficult to achieve 100% refocusing of both NAA and GABA-3 using the Gaussian pulse-based doubly selective pulses suggested in Table 1. The intensity of NAA could vary if the frequency of the Gaussian pulse-based doubly selective refocusing pulse is not placed accurately. This may degrade the usefulness of the NAA signal as an internal concentration standard unless careful pulse design and frequency calibration are performed for a specific low field strength. Nevertheless, the usefulness of the NAA signal as a phase reference or an internal navigator is not affected if the doubly selective refocusing pulse is imperfect.

## 6. Conclusion

We have shown that in vivo spectral editing can be achieved by regenerating the target signal from the thermal equilibrium magnetization of remote spins which are *J*-coupled to the target spins. The thermal equilibrium target signal and its overlapping signals are suppressed in a separate process. For GABA-4 editing using selective homonuclear polarization transfer based on this principle, complete suppression of the overlapping Cr and GSH resonances was achieved in each single scan while detecting GABA-4 methylene group at 3.02 ppm with an editing yield similar to that of the two-step *J* editing based subtraction method and to that of the single-shot doubly selective DQ filtering method. The simultaneous detected NAA resonance at 2.02 ppm can be used as an internal navigator echo for zero order phase and frequency shift corrections and as an internal standard for concentration reference. The method proposed here should also be readily adapted for obtaining chemical shift images of GABA and for editing other metabolites in vivo at lower field strength.

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