

Brain GABA Editing Without Macromolecule Contamination

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A new scheme is proposed to edit the 3.0 ppm GABA resonance without macromolecule (MM) contamination. Like previous difference spectroscopy approaches, the new scheme manipulates J-modulation of this signal using a selective editing pulse. The elimination of undesirable MM contribution at 3.0 ppm is obtained by applying this pulse symmetrically about the J-coupled MM resonance, at 1.7 ppm, in the two steps of the editing scheme. The effectiveness of the method is demonstrated in vitro, using lysine to mimic MM, and in vivo. As compared to the most commonly used editing scheme, which necessitates the acquisition and processing of two distinct difference spectroscopy experiments, the new scheme offers a reduction in experimental time (~33%) and an increase in accuracy. Magn Reson Med 45:517–520, 2001. © 2001 Wiley-Liss, Inc.

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γ -Aminobutyric acid (GABA; $\text{NH}_2\text{-C}^4\text{H}_2\text{-C}^3\text{H}_2\text{-C}^2\text{H}_2\text{-C}^1\text{O}_2\text{H}$) is the major inhibitory neurotransmitter in mammalian brain. Editing of GABA by ^1H MRS is a unique tool for in vivo investigation of neurotransmission disorders and responses to pharmacological treatments in humans. Among the different strategies which have been proposed for GABA editing (1–8), the single quantum difference spectroscopy (SQDS) approach, initially proposed by Rothman et al. (1), has been the most fruitful in terms of clinical applications (9–16). All methods focus on the detection of the GABA C^4H_2 ^1H signal at 3.0 ppm and face the problem of co-editing a signal arising from macromolecules (MMs) (17,18). Accurate quantitation of brain GABA concentration requires to correct the peak edited at 3.0 ppm for the contamination from MMs. Until now this correction was performed by measuring, in two separate experiments, the signals G^* and M^* corresponding predominantly to GABA and MMs, respectively (1,5). These two signals were then deconvoluted into pure GABA and MM signals using an in vitro calibration of the editing sequence.

The goal of the present study was to demonstrate that the contamination from MMs can be completely avoided in the editing of GABA C^4H_2 resonance through a single difference spectroscopy experiment, provided a judicious modification of the SQDS editing scheme is introduced. This new scheme, which is intrinsically more accurate and less time consuming than the previous one, was tested in vitro on a phantom designed to mimic MMs, as well as in vivo on the brain of a nonhuman primate.

MATERIALS AND METHODS

All spectra were acquired on a Bruker AVANCE spectrometer interfaced to a 3 T whole-body magnet (Oxford). An in-house-made birdcage probe (15 cm diameter, 8 cm length) was used for in vitro and in vivo experiments. The basic GABA editing module, depicted in Fig. 1a, was similar to that used in Refs. 1 and 5, with a slice-selective 90° excitation, a TE of 70 msec ($= 1/2J$), a Shinnar-Le Roux (19) selective 180° editing pulse (SLR), and a 2-2 semiselective 180° pulse splitting the SLR into two halves and providing a null excitation on water. This module was preceded by a CHESS water suppression, a 3D outer volume suppression, and a 3D ISIS localization. The SLR editing pulse was generated through MATPULSE (20) using the least-squares option with the following parameters: 43 msec duration, 10 Hz bandwidth at maximum excitation, 3% pass band ripple, and 0.5% rejection band ripple. The effect of splitting the SLR pulse in half was analyzed through numerical calculation of the refocused magnetization after a Hahn spin-echo evolution period which included an unsplit SLR refocusing pulse or a 2-2 refocusing pulse or the combination of both, as illustrated in Fig. 1a. The simulations performed for a single resonance of variable frequency are presented in Fig. 1b. The SLR and 2-2 pulses were applied at 0 Hz and 140 Hz, respectively, which corresponds to the frequency offset between GABA C^3H_2 (1.9 ppm) and C^4H_2 (3.0 ppm) resonances. The simulations clearly show that the combination of the SLR and 2-2 pulses provides a perfect refocusing at 0 Hz as well as at 140 Hz.

In the previous SQDS editing scheme, the signal G^* corresponding mainly to GABA is obtained from the difference between two spectra (B-A) recorded by running the basic module with two different conditions regarding the selective 180° pulse: In experiment A, the pulse is applied at the frequency of the GABA C^3H_2 resonance (1.9 ppm); in experiment B, it is either turned off (1) or applied at 4.1 ppm (5), i.e., to a position symmetric of 1.9 ppm with respect to 3.0 ppm. Similarly, the signal M^* corresponding mainly to MMs is obtained from the difference B-C: In experiment C, the selective 180° pulse is applied on the MM resonance at 1.7 ppm, which is J-coupled to the MM resonance at 3.0 ppm (17,18). Even at high field, a substantial fraction (noted as F) of the MM peak at 3.0 ppm is co-edited with GABA in B-A. This results from the limited length of the selective editing pulse (due to the fixed TE of the sequence), which imposes a requirement to accept an imperfect selectivity in order to keep the editing efficiency for GABA (noted as E) close to unity. Assuming that the coefficients E and F also apply to M^* , the corrected signals G and M , proportional to the concentrations of GABA and MMs, respectively, are obtained by solving the system of equations:

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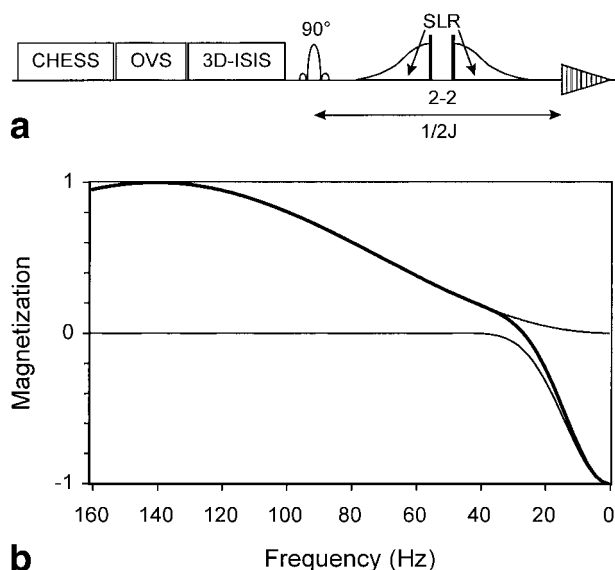


FIG. 1. **a:** Basic module sequence used for GABA editing. The SLR editing pulse was turned off in sequence B, applied at 1.9 ppm in sequence A, and applied at 1.5 ppm in sequence B'. **b:** Numerical calculation of the transverse magnetization refocused along the y axis after a spin-echo evolution, assuming that the transverse magnetization is along the y axis following excitation and that two gradient spoilers are applied on each side of the refocusing pulse. The upper thin line, lower thin line, and thick line correspond to a 2-2 refocusing pulse, to an unsplit SLR refocusing pulse, and to the combination of both, respectively. The simulations were programmed with MatLab (The MathWorks Inc.) using a single resonance of variable frequency. While the refocusing frequency profile is slightly narrower around 0 Hz for the combined 2-2+SLR pulse than for the SLR pulse, the profiles of the 2-2 and combined 2-2+SLR pulses are virtually identical beyond 30 Hz (the upper thin line and the thick line cannot be distinguished) because of the excellent off-resonance performance of the SLR pulse.

$$G^* = E \times G + F \times M \quad [1]$$

$$M^* = E \times M + F \times G. \quad [2]$$

With the new approach, the signal $G^{*'}$ is directly obtained from the difference B'-A, where spectrum B' is recorded with the selective 180° pulse applied at 1.5 ppm, i.e., to a position symmetric of 1.9 ppm with respect to 1.7 ppm. Because the MM peak at 1.7 ppm is affected to the same extent in A and B', the 3.0 ppm MM peak is canceled in B'-A and $G^{*'}$ originates only from GABA. In addition, the frequency selectivity of the SLR 180° pulse is sufficient to yield negligible effect at 1.9 ppm, when applied at 1.5 ppm, so that the efficiency for GABA editing (defined as the ratio of C⁴H₂ resonance intensity in B'-A to the intensity in B', i.e., with J-modulation inhibited) remains close to unity.

The proposed editing scheme was tested on spherical phantoms containing a saline solution of pure GABA or pure lysine, or a 1:1 mixture of both compounds. As reported in Ref. 17, lysine (NH₂-C⁶H₂-C⁵H₂-C⁴H₂-C³H₂-C²HNH₂-C¹O₂H) corresponds most likely to the J-coupled MM signals at 3.0 ppm (C⁶H₂) and 1.7 ppm (C⁵H₂) in the living brain, and this simple amino acid was found to be a

convenient model system with which to mimic MM signals in vitro. To test the new editing scheme in vivo, experiments were performed on a baboon (*Papio papio*) in strict accordance with the European Convention for Animal Care and the guide for care and use of laboratory animals adopted by the National Institute of Health (NIH publication No. 85-23, revised 1985). The baboon was anesthetized with ketamine-xylazine (15–1.5 mg/kg) and its head was positioned into the birdcage probe using a MR-compatible stereotaxic support.

RESULTS

The tests performed on phantoms containing GABA alone, lysine alone, or a 1:1 mixture of both, are summarized in Fig. 2. The effectiveness of the proposed editing scheme relies on the stability of the behavior of the lysine C⁶H₂ signal at 3.0 ppm, when the offset of the selective 180° editing pulse is shifted from 1.9 ppm (experiment A) to 1.5 ppm (experiment B'). This stability, illustrated in Fig. 2 (middle panel), was optimized by applying the 2-2 refocusing pulse at 3.2 ppm, instead of 3.3 ppm as is usually done, in order to achieve null excitation at 1.9 ppm. With this slight modification, null excitation through the 2-2 pulse was obtained at 1.7 ppm (in addition to 4.7 ppm for water suppression), but this did not significantly affect the efficiency of GABA C⁴H₂ editing, which remained close to unity (99%) in the difference B'-A (Fig. 2, left panel). As a consequence, the signal $G^{*'}$ (difference spectrum B'-A) from the GABA:lysine mixture was exactly proportional to the GABA concentration, with negligible contamination from lysine (Fig. 2, right panel).

The effectiveness of the proposed editing scheme in vivo is illustrated in Fig. 3. Both the previous editing scheme and the new editing scheme were run during the same experimental session to detect GABA from a 5-ml voxel placed in the striatum of an anesthetized baboon. As expected, because of the suppression of MM contamination, the new scheme provided a signal $G^{*'}$ at 3.0 ppm significantly less intense than the signal G^* obtained through the previous scheme. However, the GABA concentrations calculated through the two methods (assuming 8 mM of total creatine) were comparable—about 0.5 mM—and the large difference between $G^{*'}$ and G^* was accounted for by an elevated MM concentration—about 2.5 mM.

DISCUSSION

A new editing scheme is proposed to detect GABA without contamination from MMs, which has occurred with previous SQDS editing techniques. The rationale of our approach is to affect the 1.7 ppm MM resonance to the same extent in the two modules of the editing scheme, A and B', so that the 3.0 ppm MM signal is canceled in the difference spectrum B'-A, yielding the GABA signal. Because only two spectra (A and B'), rather than three (A, B, and C) (1,5), are necessary to measure GABA, our method provides a 33% gain in experimental time. The fact that the signal $G^{*'}$ detected in the difference spectrum B'-A is less intense than the signal G^* detected in B-A (see Fig. 3) must not be interpreted as the reflection of a lower accuracy of our method. On the contrary, one can easily demonstrate

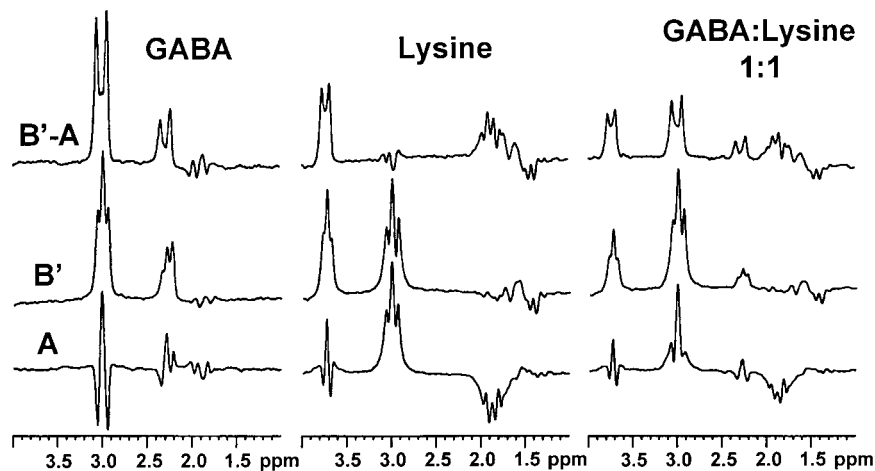


FIG. 2. In vitro tests of the new GABA editing scheme on phantoms containing GABA alone (left panel), lysine alone (middle panel), or a 1:1 mixture of both compounds (right panel). The spectra obtained with sequence A and sequence B' are plotted on the lower row and middle row, respectively, and the difference spectra (B'-A) are plotted on the upper row. For the GABA phantom, the area of the edited pseudo-doublet at 3.0 ppm (B'-A) is 99% of that of the triplet (B'). For the lysine phantom, the area of the residual at 3.0 ppm is 3% of that of the triplet. For the GABA:lysine mixture, the area of the edited pseudo-doublet at 3.0 ppm is 49% of that of the triplet, which is in good agreement with the molar ratio of the two compounds.

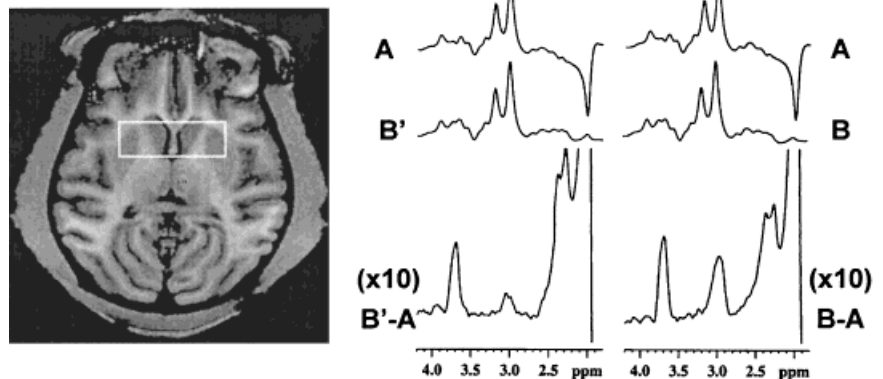
through a simple calculation of error propagation that the new approach potentially offers a gain in accuracy by a factor of 1.3 to 2, in addition to the 33% reduction in total acquisition time. It seems reasonable to assume that when measuring G^* , M^* , or G^{**} on the difference spectra, the absolute uncertainty is the same (noted as U). Hence, with our method the final uncertainty on the GABA signal equals U , because a direct measurement of G^{**} is performed. On the other hand, with the previous method the final uncertainty on the corrected GABA signal G is proportional to $U \times (E+F)/(E^2-F^2)$, because two independent measurements of G^* and M^* are performed, and the system of Eqs. [1] and [2] is solved to obtain the corrected signals G and M . Thus, with E close to 1 and F ranging from 0.25 to 0.5, as reported in Refs. 1 and 5, and as obtained in the present study ($F = 43\%$), the final uncertainty on G ranges from $1.3 \times U$ to $2 \times U$.

A critical constraint for the optimal performance of the new GABA editing scheme in vivo is the symmetry of the excitation of the MM resonance at 1.7 ppm, when the selective 180° pulse is applied either at 1.9 ppm (module

A) or at 1.5 ppm (module B'). This supposes an accurate calibration of the frequency offsets for the semiselective 2-2 and selective SLR pulses. These parameters were therefore carefully optimized in vitro using lysine to mimic MMs. However, an erroneous setting of the frequency offsets in vivo would indeed introduce some contamination of the signal G^{**} due to unequal excitation of the 1.7 ppm MM signal through the two modules, A and B'. It must be noted that this potential source of error is not specific to the new GABA editing scheme. With the previous method, the factors E and F , used to calculate G and M from G^* and M^* , are also determined through in vitro calibrations performed on a phantom containing GABA. Thus, any inaccuracy in the setting of the frequency offsets for the semiselective 2-2 and selective SLR pulses, relative to the in vivo GABA and MM chemical shifts, will also introduce an error in the estimation of G due to the use of inappropriate values for E and F .

In conclusion, the proposed GABA editing scheme offers a significant reduction in experimental time, as well as a potential gain in intrinsic accuracy, without eliminating

FIG. 3. Position of the 5-ml voxel on a T_1 -weighted axial image (left panel) and spectra recorded from the brain of an anesthetized baboon using the GABA editing scheme (right panel) (which has been the most commonly used method until now), and the new editing scheme (middle panel). The same total number of scans (1440) was accumulated for both methods with the same TR (2.5 sec). Spectra were processed with 5 Hz line broadening.



the sensitivity of the SQDS editing method to minor errors in the setting of the RF frequency offsets.

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