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Simultaneous Measurement of Glutamate, Glutamine, GABA, and Glutathione by Spectral Editing Without Subtraction

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Abstract

Purpose—To simultaneously measure glutamate, glutamine, GABA, and glutathione using spectral editing without subtraction at 7 T.

Methods—A novel spectral editing approach was proposed to simultaneously measure glutamate, glutamine, GABA, and glutathione using an echo time of 56 ms at 7 T. By numerical optimization of sequence timing in the presence of an editing pulse, the four metabolites all form relatively intense pseudo singlets with maximized peak amplitudes and minimized peak linewidths in one of the three interleaved spectra. For measuring glutamate, glutamine, and glutathione, the editing pulse targets the H3 protons of these metabolites near 2.12 ppm. Both GABA H2 and H4 resonances are fully utilized in spectral fitting.

Results—Concentration levels ($/[tCr]$) of glutamate, glutamine, GABA, and glutathione from an 8 ml voxel in the pregenual anterior cingulate cortex (pgACC) of five healthy volunteers were found to be 1.26 ± 0.13 , 0.33 ± 0.06 , 0.13 ± 0.03 , and 0.27 ± 0.03 , respectively, with within-subject coefficient of variation (CV) at 3.2%, 8.2%, 7.1%, and 10.2%, respectively. The total scan time was less than 4.5 min.

Conclusions—The proposed new technique does not require data subtraction. The three major metabolites of the glutamatergic and GABAergic systems and the oxidative stress marker glutathione were all measured in one short scan with high precision.

Keywords

Multi-Edit; glutamate; glutamine; GABA; glutathione

INTRODUCTION

Glutamate (Glu) and γ -aminobutyric acid (GABA) are the main excitatory and inhibitory neurotransmitters in the central nervous system. Astroglial glutamine (Gln) is the precursor of glutamate and plays a key role in both glutamate-glutamine and GABA-glutamine cycles. Abnormal functioning of glutamatergic and GABAergic systems has been implicated in many illnesses such as epilepsy, schizophrenia, Alzheimer's disease, major depressive disorder, and anxiety disorders (1,2). Glutathione (GSH) is an antioxidant present in most

cell types including astrocytes. Impaired glutathione metabolism is associated with many illnesses including Parkinson's disease, Alzheimer's disease, multiple sclerosis, schizophrenia, and bipolar disorder (3). Therefore, measuring glutamate, glutamine, GABA, and glutathione reliably will have many applications in the studies of neurological and psychiatric disorders.

It is challenging to simultaneously measure glutamate, glutamine, GABA, and glutathione even at high magnetic fields such as 7 T. It has been shown that the H4 protons of glutamate, glutamine, and the glutamyl moiety of glutathione all form pseudo singlets at ~ 100 ms echo time (TE) without using any editing pulse and can be measured with reasonable precision (4). At this TE, however, detection of GABA is very difficult due to low signal intensity and spectral overlap of GABA with much stronger signals from other metabolites. Although GABA can be successfully detected using difference editing (5–9) at TE = ~ 68 ms, these GABA editing sequences are not optimal for detecting glutamate, glutamine, or glutathione.

It is very common in clinical studies to acquire many different types of imaging and spectroscopy data in a single scan session. There is a constant need to shorten scan time as much as possible while maximizing the information content. Hence, the ability to measure several metabolites of interest in one short magnetic resonance spectroscopy (MRS) scan is highly desirable. An MRS technique that does not require data subtraction is also beneficial for reducing sensitivity to subject motion.

In this work, we propose a new spectral editing approach, named Multi-Edit approach, to simultaneously measure glutamate, glutamine, GABA, and glutathione at 7 T. Different from the conventional subtraction-based two-step editing approach, we use a three-step editing approach where the editing RF pulse is set to OFF, ON at 1.89 ppm, and ON at 2.12 ppm. With the help of the editing pulse, the H2 and H4 protons of GABA and the H4 protons of glutamate, glutamine, and the glutamyl moiety of glutathione all form relatively strong and sharp pseudo singlets at TE = 56 ms. This allows reliable and simultaneous detection of these important markers of glutamatergic and GABAergic systems and oxidative stress in a single MRS scan from a $2 \times 2 \times 2$ cm³ voxel in less than 4.5 min at 7 T.

METHODS

Pulse Sequence

As shown in Fig. 1, a 90° hyperbolic secant pulse was placed at the beginning of the pulse sequence to normalize the longitudinal magnetizations of all metabolites to zero at the beginning of each time of repetition (TR). This null-generating pulse had a duration of 6.8 ms and a bandwidth of 2.5 kHz. The second component of the pulse sequence was the water suppression block which contained eight variable power RF pulses with optimized relaxation delays (VAPOR) (10). Each pulse was a 9 ms sinc-Gauss pulse with a bandwidth of ~ 310 Hz. The main component of the pulse sequence was a point resolved spectroscopy (PRESS) sequence with an editing RF pulse between the two 180° refocusing pulses. The excitation pulse was an amplitude-modulated pulse with duration = 4.5 ms and bandwidth = 3.1 kHz (11). The time delay between the 90° null-generating pulse and the excitation pulse was 3.1 s. The 180° refocusing pulses were also amplitude-modulated with duration = 8.0 ms and

bandwidth = 2.0 kHz. The editing RF pulse was a truncated Gaussian pulse with a duration of 10 ms (Fig. 2). The amplitude profile of the pulse was generated by truncating a Gaussian function to the range of $-\sigma/2$ to $\sigma/2$, where σ is the standard deviation of the Gaussian function. The editing RF pulse had three different settings: OFF, ON at 1.89 ppm, and ON at 2.12 ppm. These three different settings were used in an interleaved order during MRS data acquisition.

Using high speed full density matrix simulations with high spatial digitization (12,13), we computed the spectra of glutamate, glutamine, GABA, and glutathione for different values of TE, TE₁, and T_d – the time delay between the first 180° refocusing pulse and the editing RF pulse – to find an optimal sequence timing for measuring these four metabolites. The shortest achievable TE was 50 ms for our pulse sequence, but the simulated metabolite peaks are broad and low at this TE. By systematically investigating the signal lineshape and intensity using different values of TE between 50 – 60 ms and different values of TE₁ and T_d for each TE, we found that the H4 peaks of glutamate, glutamine, and glutathione with editing ON at 2.12 ppm and the H2 and H4 peaks of GABA with editing ON at 1.89 ppm change shape simultaneously, i.e., when the glutamate peak with editing ON at 2.12 ppm became sharp and high, the glutamine and glutathione peaks with editing ON at 2.12 ppm and the two GABA peaks with editing ON at 1.89 ppm also became sharp and high. We also found that using the longest achievable TE₁ and T_d for a given TE resulted in the sharpest and highest metabolite peaks of interest. Based on these two observations, the amplitude of the glutamate H4 peak with editing ON at 2.12 ppm was used as the criterion for sequence optimization. Starting from 50 ms, the TE value of the pulse sequence was increased at 1 ms intervals and the longest achievable TE₁ and T_d for the TE were used to compute the glutamate spectrum with editing ON at 2.12 ppm. When TE = 56 ms, the glutamate H4 peak amplitude with editing ON at 2.12 ppm reached the maximum value. Therefore, the sequence timing with TE = 56 ms, TE₁ = 40 ms, and T_d = 15.3 ms was chosen to be the optimal sequence timing for the Multi-Edit pulse sequence. At this sequence timing, the H4 peaks of glutamate, glutamine, and glutathione with editing ON at 2.12 ppm and the H2 and H4 peaks of GABA with editing ON at 1.89 ppm are the sharpest and highest, which is advantageous for quantifying these four metabolites.

To illustrate the effect of TE₁ and T_d on metabolite peak amplitude and linewidth, spectra of NAA, glutamate, glutamine, GABA, and glutathione were numerically simulated at TE = 56 ms using three different pulse sequence timings: (a) sequence timing with the shortest achievable TE₁ and T_d, i.e., TE = 56 ms, TE₁ = 15 ms, and T_d = 11.7 ms; (b) editing pulse placed at the midpoint of TE, i.e., TE = 56 ms, TE₁ = 28 ms, and T_d = 14 ms; (c) optimized sequence timing with the longest achievable TE₁ and T_d, i.e., TE = 56 ms, TE₁ = 40 ms, and T_d = 15.3 ms.

Correction of Bloch-Siegert Phase Shifts

When the editing RF pulse was turned on, the single editing pulse caused frequency dependent Bloch-Siegert phase shifts in the spectra (14). The phase shifts were numerically computed using density matrix simulations. The simulation processes were similar for the two cases in which the frequency of the editing RF pulse was set at 1.89 and 2.12 ppm,

respectively. In each case, the spectra of a single spin at 300 different chemical shift positions, ranging from 1.0 to 4.0 ppm with an increment of 0.01 ppm, were simulated. For each of the 300 chemical shift positions, the phasor of the single peak in the spectrum was determined by fitting a Lorentzian curve to the peak. The final phasor curve as a function of chemical shift was obtained by interpolating the 300 phasor values into the desired spectral resolution. Correction of the phase shifts caused by the editing pulse applied at two different frequencies was performed by multiplying the complex-conjugate of the corresponding phasor functions to both the collected *in vivo* spectra and the simulated basis spectra.

In Vivo Data Acquisition

Five healthy volunteers (three women and two men; age = 34 ± 17 years) were recruited for the study. All volunteers gave informed consent in accordance with procedures approved by our local institutional review board. Experiments were performed on a Siemens 7 T scanner equipped with a 32-channel receiver head coil. T_1 -weighted magnetization prepared rapid gradient echo (MPRAGE) images were acquired with TR = 3 s, TE = 3.9 ms, matrix = $256 \times 256 \times 256$, and resolution = $1 \times 1 \times 1 \text{ mm}^3$ to position the MRS voxel. For each subject, MRS data were collected twice using the proposed pulse sequence from a $2 \times 2 \times 2 \text{ cm}^3$ voxel in the grey matter dominant region of pregenual anterior cingulate cortex (pgACC), which has been implicated in several psychiatric disorders (15). The MRS pulse sequence used TR = 3.5 s, TE = 56 ms, TE₁ = 40 ms, T_d = 15.3 ms, spectral width = 4000 Hz, number of data points = 1024, number of averages = 72, and total scan time = 4 min and 23 s. Before each MRS scan, B₀ field inhomogeneities were minimized by first- and second-order shimming, achieving mean water linewidth of 11.7 Hz.

Data Processing and Frequency Drift Correction

The 32-channel free induction decay (FID) data were combined into single-channel FIDs using the generalized least square (GLS) method (16), in which coil sensitivities were computed from the unsuppressed water signals acquired with two averages. Previously acquired unsuppressed water signals from a phantom using the same pulse sequence were used to correct for the phase errors in the combined single-channel FIDs caused by zero-order eddy currents (17). These FIDs were Fourier transformed into the frequency domain to obtain spectra for all 72 data averages. Due to subject motion and system instability during an MRS scan, the measured voxel experienced magnetic field drift in general, resulting in metabolite peaks shifted away from their correct chemical shift positions in the uncorrected spectra. On the other hand, the editing pulse had two fixed frequencies in the scanner electronics and corresponded to two fixed chemical shift positions (1.89 and 2.12 ppm) in the uncorrected spectra. Therefore, Bloch-Siegert phase shifts in the individual spectra were corrected by multiplying each individual spectrum with the complex-conjugate of the corresponding Bloch-Siegert phasor functions which were computed with the editing pulse being applied at 1.89 and 2.12 ppm, respectively.

Next, the frequency drift and zero-order phase change in the individual spectra needed to be corrected to align the individual spectra. We modified a frequency drift correction method recently proposed by van der Veen et al. (18). To find the frequency drifts in the MRS spectra, the total creatine (tCr) and total choline (tCho) peaks in the spectrum of each

individual acquisition were fitted by two Voigt curves. The relative chemical shift between the two Voigt curves was fixed and set to be the chemical shift difference between tCr (3.028 ppm) and tCho (3.210 ppm). Based on the fitting results, the frequency drift of the spectrum was determined and then corrected by shifting the spectrum. Meanwhile, the zero-order phase of each individual spectrum was also determined in the fitting process and removed from the spectrum. Additionally, a spectrum was discarded if the linewidths of the fitted Voigt curves were 50% larger and the amplitudes were 50% smaller than the averaged values. Out of the 10 MRS scans on healthy volunteers, only one scan had 5 individual spectra being discarded by the fitting software. There were no discarded spectra for the other 9 MRS scans. Finally, the frequency and phase corrected individual spectra were separately averaged into three summed spectra, corresponding to the three different settings of the editing pulse: editing OFF, editing ON at 1.89 ppm, and editing ON at 2.12 ppm.

Numerical Computation of the Basis Functions

Basis functions of N-acetyl-aspartate (NAA), N-acetylaspartylglutamate (NAAG), GABA, glutamate, glutamine, glutathione, aspartate (ASP), tCr, tCho, and myo-inositol (mI) were simulated using an in-house developed density matrix simulation program for very fast computation of spatially localized multispin systems (12,13). Other metabolites do not have significant resonances within the target analysis range of 1.8–3.35 ppm and were therefore omitted. Chemical shifts and coupling constants for GABA, glutathione, and the rest of the metabolites were obtained from literature (8), (4), and (18), respectively. Each basis function contained three FIDs corresponding to the three settings of the editing RF pulse. Due to frequency drift, the frequency of the editing RF pulse could be a few Hz off from the intended frequency relative to that of the metabolites. Therefore, we simulated an array of basis functions as a function of frequency offset values of the editing pulse, ranging from –15 Hz to 15 Hz with one Hz increment. These simulated basis FIDs were Fourier transformed to the frequency domain to obtain the basis spectra of the metabolites for the three editing pulse settings and 31 frequency offsets of the editing pulse. Bloch-Siegert phase shifts in these basis spectra were corrected by multiplying the spectra with the complex-conjugate of the corresponding phasor functions computed using density matrix simulations. Next, we computed the basis spectra for fitting the averaged *in vivo* spectra. The frequency offset of the editing pulse in each individual spectrum was equal to the negative value of the frequency drift of that spectrum, which was determined as described above. A histogram of the frequency offsets, which was a distribution of the frequency offsets at 1 Hz intervals, was generated for each of the three editing pulse settings. The basis spectra used in the subsequent fitting process were computed as the weighted average of the array of basis spectra with 31 frequency offset values, where the histogram was used as the weighting function.

Fitting Multi-Edit Spectra Simultaneously

The three sets of averaged spectra, corresponding to the three different settings of the editing pulse, were fitted simultaneously using the basis spectra computed in the previous step. This in-house developed linear combination fitting program used a Levenberg-Marquardt least square minimization algorithm. The unknown variables in the fitting process were amplitude scaling factors, frequency shifts, linewidths, and the Voigt lineshape for all metabolite peaks,

as well as zero-order phases and cubic spline baselines for the three sets of spectra. Spectral data between 1.8 – 3.35 ppm were fitted because this range contains all major metabolites of interest for psychiatric studies.

In addition to minimizing the squared differences between the *in vivo* spectra and fitted spectra, the squared differences between the fitted cubic spline baselines were also minimized in the segments of data that were not significantly affected by the editing pulse. The first spectrum was acquired with the editing pulse turned off, thus the entire baseline was unaffected by the editing pulse. In the second spectrum, spectral signals within 1.89 ± 0.35 ppm were affected by the editing pulse applied at 1.89 ppm. The M7 macromolecule resonances at 3.01 ppm were also indirectly affected by the editing pulse because they are coupled to the M4 macromolecule resonances at 1.72 ppm (19). Resonance signals outside these two regions were not significantly affected by the editing pulse. Therefore, the spline baseline differences between the first and second spectrum in the range of 2.24 – 2.90 ppm and 3.15 – 3.35 were minimized in the fitting process. In the third spectrum, spectral signals within 2.12 ± 0.35 ppm were affected by the editing pulse. Accordingly, the spline baseline differences between the first and third spectrum in the range of 2.47 – 3.35 ppm were minimized in the fitting process.

RESULTS

The real and imaginary parts of the phasor function when the editing pulse was applied at 2.12 ppm are plotted in Fig. 3. The phasor function when the editing pulse was applied at 1.89 ppm was similar, which could be obtained by shifting the curves in Fig. 3 upfield by 0.23 ppm. Based on the phasor functions, we found that the maximum phase change over a 10 Hz range was 13 degrees. In our *in vivo* spectra, the linebroadening of the metabolite peaks due to B_0 inhomogeneity was ~ 10 Hz. Metabolite peak amplitude reduction due to this phase dispersion was ~ 0.2%, which was negligible. Therefore, the phase shifts are the only main effects on the metabolite peaks due to the Bloch-Siegert effect, which can be corrected for.

Numerically simulated spectra of glutamate, glutamine, GABA, and glutathione at TE = 56 ms using three different pulse sequence timings are displayed in Fig. 4. Correspondingly, Table 1 lists the amplitudes and linewidths of five peaks – the glutamate, glutamine, and glutathione H4 peaks in the spectra with editing ON at 2.12 ppm and the GABA H2 and H4 peaks in the spectrum with editing ON at 1.89 ppm – for the three different pulse sequence timings. The peak amplitudes were normalized by the amplitude of the NAA singlet peak in the spectrum with editing OFF and the peak linewidths were full width at half maximum (FWHM) values. Spectra in Fig. 4a were computed using the sequence timing with the shortest achievable TE₁ and T_d: TE = 56 ms, TE₁ = 15 ms, and T_d = 11.7 ms. All five peaks of interest were low and broad. Spectra in Fig. 4b were computed using the sequence timing with the editing pulse placed at the midpoint of TE: TE = 56 ms, TE₁ = 28 ms, and T_d = 14 ms. The five peaks of interest became higher and narrower than in Fig. 4a. Spectra in Fig. 4c were computed using the optimized sequence timing with the longest achievable TE₁ and T_d: TE = 56 ms, TE₁ = 40 ms, and T_d = 15.3 ms. The five peaks of interest became even higher and narrower than in Fig. 4b. The comparison between Fig. 4b and Fig. 4c shows that

the optimized sequence timing yields higher and narrower metabolite peaks than the sequence with the editing pulse placed at the midpoint of the TE.

Numerically simulated spectra of NAA, glutamate, GABA, glutamine, glutathione, and sum of the metabolites using the optimized pulse sequence are displayed in Fig. 5. Metabolite concentration ratios used in the simulations were [NAA] : [Glu] : [GABA] : [Gln] : [GSH] = 10 : 10 : 1 : 2.5 : 2.5. In the spectra with editing ON at 2.12 ppm (right column), the glutamate peak is highly conspicuous in the summed spectrum. Therefore, it is straightforward to determine the line-broadening of the glutamate peak, as well as the glutamate and glutamine levels from the summed spectrum. In the spectra with editing ON at 1.89 ppm (middle column), the GABA peak at 2.28 ppm has relatively high amplitude. Overall, Fig. 5 indicates that the three sets of spectra acquired using the proposed pulse sequence contain sufficient spectral information for determining glutamate, glutamine, GABA, and glutathione levels.

Reconstructed spectra and corresponding fits from two MRS measurements of the same voxel in the pgACC of one healthy volunteer are displayed in Fig. 6. As expected, the *in vivo* spectra resemble the summed spectra in Fig. 5 to a very high degree. The fits match the *in vivo* spectra very well and the fit residuals are small. The glutamine peak at 2.44 ppm is visually distinguishable from the glutamate peak in the *in vivo* spectrum with editing pulse ON at 2.12 ppm (right column). In the *in vivo* spectrum with editing ON at 1.89 ppm (middle column), the GABA peak at 2.28 ppm lies on the right shoulder of the glutamate peak. The amplitude of the GABA peak at 2.28 ppm reaches about 1/3 of the glutamate peak amplitude, which is favorable for reliable quantification of GABA.

Metabolite concentrations ($/[tCr]$), Cramer-Rao Lower Bounds (CRLB), within-subject coefficient of variation (CV) values, and inter-subject CV values computed from the ten measurements on five healthy volunteers are given in Table 2. The within-subject CVs were computed using the root mean square method (20) based on the two MRS measurements on the same voxel in each subject. The Glu/tCr, Gln/tCr, GABA/tCr, and GSH/tCr ratios were found to be 1.26 ± 0.13 , 0.33 ± 0.06 , 0.13 ± 0.03 , and 0.27 ± 0.03 , respectively; the corresponding within-subject CVs were found to be 3.2%, 8.2%, 7.1%, and 10.2%, respectively; the corresponding inter-subject CVs were found to be 10.9%, 16.1%, 23.1%, and 8.4%, respectively.

DISCUSSION

This work demonstrates that glutamate, GABA, glutamine, and glutathione can be simultaneously measured with short scan time and high precision at 7 T using spectral editing without subtraction. By numerical optimization of sequence timing, glutamate, glutamine, GABA, and glutathione all form relatively intense pseudo singlets with maximized peak amplitudes and minimized peak linewidths in one of the three interleaved spectra. This ensures reliable extraction of their concentrations from the acquired spectra.

The editing pulse is often placed at the midpoint of the TE in many homonuclear spectral editing techniques using a single editing pulse (5,21). For weakly coupled spin systems, the

midpoint of the TE is a convenient choice. However, the glutamate, glutamine, GABA, and glutathione spin systems are not fully weakly coupled systems even at 7 T. For example, the coupling between GABA H2 and H3 is not within the weak coupling limit because the chemical shift difference between GABA H2 and H3 is only 0.39 ppm and GABA H3 is a quintet. Therefore, density matrix simulations were necessary to find the optimal sequence timing to maximize peak amplitudes and minimize peak linewidths. Although the editing pulse was placed off-center, a spin echo was still formed at the beginning of data acquisition by the action of the two slice-selective 180° pulses. This is because the editing pulse flips the z components of the coupled spins to be edited. Any accumulation of phase due to background B₀ inhomogeneity stays on the transverse plane and is cancelled by the spin echo. This situation resembles the heteronuclear spectral editing experiments where the position of the editing pulse in the spin echo is off-center with its position determined by the heteronuclear coupling constant. The bipolar gradients surrounding the editing pulse also dephase any spins excited by the spectrally selective 180° pulse. Outside the bandwidth of the editing pulse, the bipolar gradients cancel because the editing pulse has no effect other than a Bloch-Siegert phase shift.

Without the presence of any editing pulse, glutamate, glutamine, and glutathione can form pseudo singlets at TE = ~ 100 ms while overlapping with the aspartyl moiety of NAA (4,22). At TE = 56 ms, they form weak and broad multiplets without the editing pulse (see Fig. 5). By optimization using full density matrix simulation, we found that glutamate, glutamine, and glutathione form relatively intense pseudo singlets at TE = 56 ms with the editing pulse applied at 2.12 ppm, thus significantly reducing T₂ weighting.

In conventional GABA difference editing techniques, the focus of GABA quantification is GABA H4 at 3.01 ppm and the coedited GABA H2 protons at 2.28 ppm are not fully utilized due to the following reasons. First, the editing pulse has significant remaining spectral interference at GABA H2 when the field strength is significantly lower than 7 T. Secondly, the overlapping glutamate H4 resonances at 2.34 ppm are coedited and direct subtraction of ON and OFF spectra contains the glutamate difference signals along with the desired GABA difference signals. Thirdly, the GABA H2 resonances could be used in the fitting but the overlapping glutamate H4 resonances at 2.34 ppm makes the fit of the GABA H2 peak unreliable because the conventional GABA editing pulse sequences are not designed to maximize the amplitude and minimize the linewidth of the glutamate H4 peak. A small error in the linewidth or amplitude of the glutamate H4 peak would result in a significant error in fitting the GABA H2 peak. The dependence on the GABA H4 signals of the conventional GABA editing techniques makes them vulnerable to creatine subtraction errors, which can be mistaken as part of the GABA difference signals. With the Multi-Edit technique, the linewidth and amplitude of the glutamate H4 peak can be reliably measured in the third spectrum where the editing pulse is applied at 2.12 ppm (see Fig. 6). This makes the fit of the GABA H2 peak much more reliable, resulting in full utilization of the GABA H2 resonances. Full utilization of the GABA H2 signals and reduced dependence on the GABA H4 signals, which overlaps with the dominating creatine signals, can help make this technique less sensitive to creatine subtraction errors. However, the edited GABA signals should still be considered GABA+ with contamination from co-edited macromolecules.

Frequency drift correction is essential to obtain reliable metabolite quantification for spectral editing experiments with long scan time. We developed the frequency drift correction method based on a previous work from our laboratory (23). In the previous work, frequency drifts were corrected using the residual water peak as reference; the frequency drift history was subsequently used to change the chemical shift values of all metabolites when simulating the basis functions. In this work, frequency drifts were corrected using both the creatine and choline peaks as reference. The frequency drift history was used to change the frequency of the editing pulse in the opposite direction relative to all metabolites when simulating the basis functions, which resulted in much higher computational efficiency.

At TE = 56 ms, the glutamate, glutamine, and glutathione peaks are small when the editing pulse is turned off (Fig. 4c, left column). When the editing pulse is applied at 1.89 ppm (Fig. 4c, middle column), glutamate, glutamine, and glutathione peaks still have low amplitudes. When the editing pulse is applied at 2.12 ppm (Fig. 4c, right column), the glutamate, glutamine, and glutathione H4 peaks become high and sharp, which is favorable for separating the metabolite peaks from each other and from the broad macromolecule baseline. Similar to GABA quantification, one key factor for accurate quantification of the small glutamine H4 at 2.44 ppm is the accurate fitting of the amplitude and linewidth of the relatively large glutamate peak at 2.34 ppm. Therefore, an important goal in the optimization of TE, TE₁, and T_d was to obtain a high and sharp glutamate peak. Fig. 4c shows that this goal was achieved using the proposed pulse sequence timing. From Fig. 5, we can also see that the NAA aspartyl signals around 2.5 ppm had almost no change in the three spectra, whereas the glutamine and glutathione signals changed dramatically in the three spectra due to the three different settings of the editing pulse. This made it easy to separate the glutamine and glutathione signals from the overlapping NAA aspartyl signals. In conventional glutathione difference editing techniques, the editing pulse is applied at 4.56 ppm to edit the glutathione cysteinyl protons at 2.95 ppm, which is sensitive to creatine subtraction errors. The cysteinyl protons form an intense pseudo singlet at TE = ~ 130 ms (24,25). In contrast, here we target the glutamyl H4 protons of glutathione at 2.54 ppm with TE = 56 ms. The glutamyl H4 protons of glutathione are not overlapped by any intense signals. Overall, the Glu/tCr, Gln/tCr, GABA/Cr, and GSH/tCr ratios found in this work are in good agreement with published values (4,22,26–28).

Previously, a single editing pulse was incorporated into PRESS with TE = 68 ms to edit GABA using a two-step subtraction approach (21). The Bloch-Siegert phase shift at 3.0 ppm was made to vanish by carefully choosing the amplitude and duration of the editing pulse. Bloch-Siegert phase shifts at other frequencies were still present. In comparison, the current study computed the Bloch-Siegert phase shifts at frequencies within 1 – 4 ppm using density matrix simulations. The same phase shifts were removed from both the *in vivo* spectra and simulated basis spectra.

The Multi-Edit pulse sequence is not optimal for measuring NAAG because the editing pulse greatly suppresses the NAAG singlet signals. As shown in Table 2, the computed NAAG concentration has large CRLB and within-subject CV. Fortunately, the H4 protons of the glutamyl moiety of NAAG are at 2.185 ppm, which is 0.1 ppm (30 Hz at 7 T) away from the GABA peak at 2.284 ppm. Therefore, errors in NAAG quantification do not have

significant effect on the quantification of GABA, glutamate, glutamine, and glutathione at 7 T.

It should be noted that the spectral information required for reliable determination of glutamate, glutamine, GABA, and glutathione lies predominantly in the two editing ON spectra (see Fig. 5). Unfortunately, the NAA singlet signals are significantly reduced in both cases. Since NAA needs to be measured in most of clinical studies using proton MRS, the editing OFF spectrum was included in our method. If a short-TE experiment is to be included in a clinical protocol, it is possible to achieve a 33% reduction in scan time by omitting the editing OFF spectrum.

CONCLUSIONS

A novel spectral editing approach was proposed to simultaneously measure glutamate, glutamine, GABA, and glutathione. Accordingly, a new post-processing method was developed to simultaneously fit the three sets of spectra acquired by the proposed pulse sequence. By using a single editing pulse, a relatively short TE of 56 ms was achieved. The main targets of the signal detection were the H2 and H4 protons of GABA and the H4 protons of glutamate, glutamine, and the glutamyl moiety of glutathione. No motion-sensitive data subtraction was required. Instead, frequency drifts due to subject motion and system instability were recorded and taken into account in data fitting. Concentrations of glutamate, glutamine, GABA, and glutathione were measured from a $2 \times 2 \times 2 \text{ cm}^3$ voxel *in vivo* in less than 4.5 min with high precision.

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References

1. Puts NA, Edden RA. In vivo magnetic resonance spectroscopy of GABA: a methodological review. *Prog Nucl Magn Reson Spectrosc.* 2012; 60:29–41. [PubMed: 22293397]
2. Ramadan S, Lin A, Stanwell P. Glutamate and glutamine: a review of in vivo MRS in the human brain. *Nmr in Biomedicine.* 2013; 26(12):1630–1646. [PubMed: 24123328]
3. Dringen R, Brandmann M, Hohnholt MC, Blumrich EM. Glutathione-Dependent Detoxification Processes in Astrocytes. *Neurochemical Research.* 2015; 40(12):2570–2582. [PubMed: 25428182]
4. Choi CH, Dimitrov IE, Douglas D, Patel A, Kaiser LG, Amezcua CA, Maher EA. Improvement of resolution for brain coupled metabolites by optimized H-1 MRS at 7 T. *Nmr in Biomedicine.* 2010; 23(9):1044–1052. [PubMed: 20963800]
5. Rothman DL, Petroff OAC, Behar KL, Mattson RH. Localized H-1-Nmr Measurements of Gamma-Aminobutyric-Acid in Human Brain In vivo. *Proceedings of the National Academy of Sciences of the United States of America.* 1993; 90(12):5662–5666. [PubMed: 8516315]
6. Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R. Simultaneous in vivo spectral editing and water suppression. *Nmr in Biomedicine.* 1998; 11(6):266–272. [PubMed: 9802468]
7. Waddell KW, Avison MJ, Joers JM, Gore JC. A practical guide to robust detection of GABA in human brain by J-difference spectroscopy at 3 T using a standard volume coil. *Magnetic Resonance Imaging.* 2007; 25(7):1032–1038. [PubMed: 17707165]
8. Kaiser LG, Young K, Off DJM, Mueller SG, Matson GB. A detailed analysis of localized J-difference GABA editing: theoretical and experimental study at 4T. *Nmr in Biomedicine.* 2008; 21(1):22–32. [PubMed: 17377933]

9. Harris AD, Saleh MG, Edden RAE. Edited H-1 Magnetic Resonance Spectroscopy In Vivo: Methods and Metabolites. *Magnetic Resonance in Medicine*. 2017; 77(4):1377–1389. [PubMed: 28150876]
10. Tkac I, Andersen P, Adriany G, Merkle H, Ugurbil K, Gruetter R. In vivo H-1 NMR spectroscopy of the human brain at 7 T. *Magnetic Resonance in Medicine*. 2001; 46(3):451–456. [PubMed: 11550235]
11. Murdoch JB, Lent AH, Kritzer MR. Computer-Optimized Narrow-Band Pulses for Multislice Imaging. *Journal of Magnetic Resonance*. 1987; 74(2):226–263.
12. An L, Li SZ, Wood ET, Reich DS, Shen J. N-Acetyl-Aspartyl-Glutamate Detection in the Human Brain at 7 Tesla by Echo Time Optimization and Improved Wiener Filtering. *Magnetic Resonance in Medicine*. 2014; 72(4):903–912. [PubMed: 24243344]
13. Zhang Y, An L, Shen J. Fast computation of full density matrix of multispin systems for spatially localized in vivo magnetic resonance spectroscopy. *Medical Physics*. 2017; 44(8):4169–4178. [PubMed: 28548302]
14. Emsley L, Bodenhausen G. Phase-Shifts Induced by Transient Bloch-Siegert Effects in Nmr. *Chem Phys Lett*. 1990; 168(3–4):297–303.
15. Devinsky O, Morrell MJ, Vogt BA. Contributions of Anterior Cingulate Cortex to Behaviour. *Brain*. 1995; 118:279–306. [PubMed: 7895011]
16. An L, van der Veen JW, Li SZ, Thomasson DM, Shen J. Combination of multichannel single-voxel MRS signals using generalized least squares. *Journal of Magnetic Resonance Imaging*. 2013; 37(6):1445–1450. [PubMed: 23172656]
17. Klose U. In vivo Proton Spectroscopy in Presence of Eddy Currents. *Magnetic Resonance in Medicine*. 1990; 14(1):26–30. [PubMed: 2161984]
18. Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain metabolites. *Nmr in Biomedicine*. 2000; 13(3):129–153. [PubMed: 10861994]
19. Behar KL, Rothman DL, Spencer DD, Petroff OAC. Analysis of Macromolecule Resonances in H-1-Nmr Spectra of Human Brain. *Magnetic Resonance in Medicine*. 1994; 32(3):294–302. [PubMed: 7984061]
20. Bland JM, Altman DG. Statistics notes: Measurement error proportional to the mean (vol 313, pg 106, 1996). *Brit Med J*. 1996; 313(7059):744–744. [PubMed: 8819450]
21. Keltner JR, Wald LL, Christensen JD, Maas LC, Moore CM, Cohen BM, Renshaw PF. A technique for detecting GABA in the human brain with PRESS localization and optimized refocusing spectral editing radiofrequency pulses. *Magnetic Resonance in Medicine*. 1996; 36(3):458–461. [PubMed: 8875418]
22. An L, Li S, Murdoch JB, Araneta MF, Johnson C, Shen J. Detection of glutamate, glutamine, and glutathione by radiofrequency suppression and echo time optimization at 7 tesla. *Magnetic Resonance in Medicine*. 2015; 73:451–458. [PubMed: 24585452]
23. van der Veen JW, Marengo S, Berman KF, Shen J. Retrospective correction of frequency drift in spectral editing: The GABA editing example. *Nmr in Biomedicine*. 2017; 30(8)
24. Srinivasan R, Ratiney H, Hammond-Rosenbluth KE, Pelletier D, Nelson SJ. MR spectroscopic imaging of glutathione in the white and gray matter at 7 T with an application to multiple sclerosis. *Magn Reson Imaging*. 2010; 28(2):163–170. [PubMed: 19695821]
25. An L, Zhang Y, Thomasson DM, Latour LL, Baker EH, Shen J, Warach S. Measurement of Glutathione in Normal Volunteers and Stroke Patients at 3T Using J-Difference Spectroscopy with Minimized Subtraction Errors. *Journal of Magnetic Resonance Imaging*. 2009; 30(2):263–270. [PubMed: 19629994]
26. Andreychenko A, Klomp DW, de Graaf RA, Luijten PR, Boer VO. In vivo GABA T2 determination with J-refocused echo time extension at 7 T. *NMR Biomed*. 2013; 26(11):1596–1601. [PubMed: 23893556]
27. Choi IY, Lee P. Doubly selective multiple quantum chemical shift imaging and T-1 relaxation time measurement of glutathione (GSH) in the human brain in vivo. *Nmr in Biomedicine*. 2013; 26(1): 28–34. [PubMed: 22730142]

28. Napolitano A, Kockenberger W, Auer DP. Reliable gamma aminobutyric acid measurement using optimized PRESS at 3 T. *Magnetic Resonance in Medicine*. 2013; 69(6):1528–1533. [PubMed: 22807127]

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Fig. 1. Schematics of the pulse sequence. $TR = 3.5\text{ s}$, $TE = 56\text{ ms}$, $TE_1 = 40\text{ ms}$, $T_d = 15.3\text{ ms}$, editing RF pulse setting = OFF, ON at 1.89 ppm, and ON at 2.12 ppm.

Fig. 2.

Pulse shape and refocusing profile of the 180° editing RF pulse. The duration of the pulse was 10 ms. The amplitude profile of the pulse was generated by truncating a Gaussian function to the range of $-\sigma/2$ to $\sigma/2$, where σ is the standard deviation of the Gaussian function.

Fig. 3.
Plots of the real and imaginary parts of the Bloch-Siegert phasor function when the editing pulse was applied at 2.12 ppm.

Fig. 4.

Numerically simulated spectra of glutamate, glutamine, GABA, and glutathione at $TE = 56$ ms using three different pulse sequence timings. The same concentration was used for all four metabolites. All spectra were broadened to a singlet half-height width of 9 Hz. **a.** Sequence timing with the shortest achievable TE_1 and T_d : $TE = 56$ ms, $TE_1 = 15$ ms, and $T_d = 11.7$ ms. **b.** Editing pulse placed at the midpoint of TE : $TE = 56$ ms, $TE_1 = 28$ ms, and $T_d = 14$ ms. **c.** Optimized sequence timing with the longest achievable TE_1 and T_d : $TE = 56$ ms, $TE_1 = 40$ ms, and $T_d = 15.3$ ms.

Fig. 5.

Numerically simulated spectra of NAA, glutamate, GABA, glutamine, glutathione, and sum of the metabolites using the optimized pulse sequence timing: $TE = 56$ ms, $TE_1 = 40$ ms, and $T_d = 15.3$ ms. Concentration ratios were $[NAA] : [Glu] : [GABA] : [Gln] : [GSH] = 10 : 10 : 1 : 2.5 : 2.5$. All spectra were broadened to a singlet half-height width of 9 Hz.

Fig. 6.

Reconstructed spectra and corresponding fits from two MRS measurements of the same voxel in the pregenual anterior cingulate cortex (pgACC) of one healthy volunteer. TR = 3.5 s, TE = 56 ms, TE₁ = 40 ms, T_d = 15.3 ms, spectral width = 4000 Hz, number of data points = 1024, number of averages = 72, and total scan time = 4 min and 23 s. Bloch-Siegert phase shifts caused by the editing pulse have been removed from both the *in vivo* and fitted spectra.

Peak amplitudes and linewidths of numerically simulated spectra of glutamate, glutamine, GABA, and glutathione at TE = 56 ms using three different pulse sequence timings.

Table 1

	TE ₁ /T _d = 15 ms/11.7 ms		TE ₁ /T _d = 28 ms/14 ms		TE ₁ /T _d = 40 ms/15.3 ms	
	Amplitude	Width (Hz)	Amplitude	Width (Hz)	Amplitude	Width (Hz)
Glu H4	0.121	23.2	0.162	15.9	0.182	15.1
Gln H4	0.151	22.9	0.189	20.5	0.198	16.4
GSH H4	0.165	22.5	0.208	19.3	0.220	18.1
GABA H2	0.253	21.0	0.322	17.8	0.360	15.9
GABA H4	0.248	23.4	0.319	20.3	0.349	16.8

Table 2

Metabolite concentrations (/[tCr]) in the pregenual anterior cingulate cortex (pgACC) of five healthy volunteers. The same voxel in each subject was scanned twice.

	Concentration (/[tCr])	CRLB (%)	Within-subjectCV (%)	Inter-subjectCV (%)
NAA	1.26 ± 0.10	0.6 ± 0.1	4.8	7.5
NAAG	0.08 ± 0.03	6.3 ± 1.2	21.4	19.3
Glu	1.26 ± 0.13	1.5 ± 0.2	3.2	10.9
Gln	0.33 ± 0.06	5.6 ± 0.9	8.2	16.1
GABA	0.13 ± 0.03	7.4 ± 1.3	7.1	23.1
GSH	0.27 ± 0.03	5.1 ± 0.7	10.2	8.4
tCho	0.30 ± 0.02	0.5 ± 0.1	3.4	8.1