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Simultaneous Quantification of Glutamate and Glutamine by J-modulated Spectroscopy at 3 Tesla

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Abstract

Purpose—The echo time (TE) averaged spectrum is the one-dimensional cross-section of the J-resolved spectrum at J=0. In multi-echo TE-averaged spectroscopy, glutamate (Glu) is differentiated from glutamine (Gln) at 3 T. This method, however, almost entirely suppresses Gln resonance lines around 2.35ppm, leaving Gln undetermined. This paper presents a novel method for quantifying both Glu and Gln using multi-echo spectral data.

Methods—One-dimensional cross-section of J-resolved spectroscopy at J=7.5 Hz—referred to as J-modulated spectroscopy—was developed to simultaneously quantify Glu and Gln levels in the human brain. The transverse relaxation times (T_2 s) of metabolites were first determined using conventional TE-averaged spectroscopy with different starting echo time and then incorporated into the spectral model for fitting J-modulated data.

Results—Simulation and in vivo data showed that the resonance signals of Glu and Gln were clearly separated around 2.35 ppm in J-modulated spectroscopy. In the anterior cingulate cortex (ACC), both Glu and Gln levels were found to be significantly higher in gray matter than in white matter in healthy subjects ($p < 10^{-10}$ and $< 10^{-5}$, respectively).

Conclusion—Gln resonances can be clearly separated from Glu and N-acetyl-aspartate (NAA) around 2.35ppm using J-modulated spectroscopy. This method can be used to quantitatively measure Glu and Gln simultaneously at 3T.

Keywords

glutamate; glutamine; quantification; J-resolved spectroscopy; one-dimensional cross-section; TE-averaged spectroscopy; human brain

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Conflict of Interest

The authors have no conflicts of interest to disclose, financial or otherwise.

Introduction

Glutamate (Glu) is the principal excitatory neurotransmitter in the central nervous system (CNS). It is involved in glutamatergic neurotransmission through the Glu-glutamine (Gln) cycle between neurons and surrounding astrocytes (1). Thus, being able to assess changes in both Glu and Gln concentrations in the human brain can provide considerable insight into brain function as well as psychiatric and neurologic diseases.

Proton magnetic resonance spectroscopy (^1H MRS) has been widely used to measure metabolite concentrations in vivo. The detection of Glu and Gln, however, is often hampered by the severe overlap of their resonances. Glu is very similar to Gln in molecular structure, and both are characterized by the coupled spins of C2–C4 hydrogen nuclei. At prevalent field strengths of 1.5 or 3.0 T, it is challenging to separate Glu and Gln by conventional one-dimensional spectroscopic methods. Instead, the resonances are usually assigned to a combination of Glu and Gln, often referred to as Glx.

Many ^1H MRS methods have been proposed to improve Glu/Gln separation (e.g., 2–11). One of the methods that has found numerous applications for Glu MRS is echo time (TE)-averaged spectroscopy (12–14). Because this method cancels out the C4 signal of Gln, it offers unobstructed detection of the Glu signal (12) but leaves few resonance signals to determine Gln concentrations. Instead of additive averaging, different strategies have also been suggested for recovering the resonance signal of the Gln C4 protons from TE-averaged ^1H MRS spectra acquired in vivo (15, 16).

A TE-averaged spectrum is essentially the one-dimensional cross-section of a two-dimensional J-resolved spectrum at $J = 0$. Other cross-sections could potentially uncover metabolites not detected or resolved at $J = 0$. For instance, $J = 7.45$ Hz was previously used to assess in vivo brain gamma-aminobutyric acid (GABA) levels in the human brain (17). The present study explores whether Glu and Gln can be clearly separated and quantified at 3 T using the point-resolved-spectroscopy (PRESS)-based J-resolved spectrum.

Transverse relaxation time T_2 can significantly influence quantified metabolite concentrations for long echo time spectra. Acquisition of a J-resolved spectrum involves a large TE range. Thus, being able to incorporate T_2 s or perform T_2 corrections is critical to quantifying metabolites. Fitting several different TE spectra is commonly used to measure T_2 . One key drawback of this approach, however, is that the short TE spectra and the long TE spectra have significantly different baselines that can result in biased fits for different TEs. In this paper, a new technique is introduced to determine metabolite T_2 s, which takes advantage of J-resolved spectral data as well. The estimated T_2 s are then applied to model spectra to quantify metabolite concentrations including both Glu and Gln.

Methods

Data Acquisition

Data were acquired using a 3 T GE whole body scanner (General Electric Medical Systems, Milwaukee, Wisconsin, USA) running the 15M4 software platform. A Medical Advances

(Intermetrics General Corporation, Milwaukee, Wisconsin, USA) RF quadrature transmit/receive coil was used with an inner diameter of 25 cm and a length of 20 cm. Ten healthy volunteers (four males, six females; age = 29 ± 7.5) participated in this study. Written informed consent was obtained from all participants. Scan sessions began with a T_1 weighted anatomical scan using the three-dimensional spoiled gradient echo sequence (TR = 7.3 ms, TE = 2.7 ms, flip angle 12° ; in plane resolution $0.9 \text{ mm} \times 0.9 \text{ mm}$; matrix size 192×256 ; field of view $240 \text{ mm} \times 240 \text{ mm}$; slice thickness 2 mm; total scan time 2 min 32 s). Two voxels were prescribed, dominated by gray matter (GM) and white matter (WM) in the anterior cingulate cortex (ACC), respectively. Both voxels had a size of $2.0 \times 2.0 \times 4.5 \text{ cm}^3$.

TE-averaged spectra were acquired using 32 different echo times (the average number (NA) was four). The starting TE was fixed at 35 ms (8.5 ms from the excitation pulse to the first refocusing pulse; 17.5 ms from the first refocusing pulse to the second refocusing pulse), and was step-wise increased by 6.0 ms after each echo. Reference unsuppressed water scans were collected with NA = 2 immediately after the acquisition of metabolite data. Spectra were sampled with a repetition time (TR) of three seconds (a scan time of 9.6 minutes), a bandwidth of 5 kHz, and 4096 complex data points.

In vivo short TE spectra were collected from the same subject group with TE = 35 ms and NA = 64, then processed with LCModel (21) using a LCModel-provided basis set.

Spectral Simulation

Simulation was performed to validate the method theoretically as well as to provide model spectra for spectral fitting. The fitting model consisted of N-acetyl-aspartate (NAA), N-acetyl-aspartyl-glutamate (NAAG), creatine (Cr), choline (Cho), Glu, Gln, glutathione (GSH), and GABA. Because it is impossible to differentiate Cr from phospho-creatine (PCr) at 3 T, the estimated Cr concentration reflects the total Cr concentration including PCr. Similarly, the estimated Cho concentration includes all choline-containing compounds.

All programs were developed in-house using interactive data language (IDL; Research Systems, Inc., Boulder, CO). The time domain signals were generated using simulated pulse sequence that used the real timing values from the scanner. The simulation assumed ideal spin excitation and used two identical Shinnar-Le Roux pulses for refocusing; the two refocusing pulses had a duration of 6 ms (corresponding to a bandwidth of $\sim 1400 \text{ Hz}$). Chemical shifts and coupling constants were obtained from the literature (18). Different TE spectra were stored separately. In the following, $s(t_1, t_2)$ denotes the time domain signal of an individual metabolite component and $s(t_1, t_2)$ the linear sum of all components. After Fourier transform (FT) in the f_2 dimension (data were acquired in this dimension), the TE-averaged spectra and the J-modulated spectra were obtained by

$$S_{avg}(f_2) = \sum_{i=0}^n s(t_1^i, f_2) \quad [1]$$

$$S_{mod}(f_2) = \sum_{i=0}^n e^{-i2\pi \cdot 7.5t_i} s(t_1^i, f_2) \quad [2]$$

where t_1^i stands for i th TE. TE started with 35 ms and was stepwise increased by 6 ms. The number of echoes is represented by n , i.e., 32 for the full echo train. A constant phase of 110 degrees was applied to $S_{mod}(f_2)$.

Using Cr as the reference ($[Cr] = 1.0$), the relative metabolite levels in the simulations were: $[tNAA] = 1.3$; $[Glu] = 1.1$; $[Gln] = 0.35$; $[Cho] = 0.3$; $[GSH] = 0.2$; and $[GABA] = 0.15$. The assumed T_2 s were 160 ms, 290 ms, 190 ms, 160 ms, 240 ms, 200 ms, and 200 ms for Cr, NAA, Glu, Gln, Cho, GSH, and GABA, respectively. These T_2 s are generally in line with the available values reported in the literature (30, 34, 36). The linewidths were broadened by 4 Hz to match the in vivo data.

Determination of Metabolite T_2 s

In this study, T_2 was directly incorporated into the spectral model of the multi-echo data. The time domain signal of a specific metabolite component was expressed by:

$$s(t_1, t_2) = e^{-\frac{t_1}{T_2}} e^{-\frac{t_2}{T_2} - \alpha t_2} s^0(t_1 + t_2) \quad [3]$$

where $s^0(t_1 + t_2)$ denotes the computer-generated signal without T_2 decay. The parameter α accounts for the line-broadening induced by magnetic field-inhomogeneity, and is determined by spectral fitting.

T_2 is determined prior to metabolite estimation using the following procedure which minimizes potential bias caused by spectral overlap and baseline distortion. From the entire echo train, three TE-averaged spectra were generated with starting TE of 35 ms, 95 ms, and 131 ms, respectively. Because of the nature of TE-averaging, TE-averaged spectra have simplified spectral structures and fairly flat baselines (12). With assumed T_2 s, these sub-spectra were separately fitted using the corresponding model spectra. The resulting metabolite concentrations were then compared between these three sets to determine whether to increase or decrease the T_2 values. This process was repeated until it reached self-consistency. Because TE-averaging suppresses Gln, the T_2 of Gln was estimated using two J-modulated spectra with starting TE = 35 ms and 77 ms, respectively. The above procedure essentially measured T_2 in the t_1 dimension of the two-dimensional J-resolved data set.

Spectral Fitting and Metabolite Concentrations

In the t_2 dimension, the data points were expanded to 8192 points by zero-filling before Fourier transform. Spectral fitting was performed over the chemical shift region from 1.6 to 3.4 ppm (19).

Lineshape was described by the Voigt model in which a Gaussian decay factor was a common parameter shared by all components in the spectral model. The linewidth of Gln was constrained by that of Glu. The frequency of NAAG was constrained to be 4.8 Hz downfield from the peak of NAA at 1.9 ppm, and its linewidth was constrained according to the linewidth of NAA. The constraints were enforced in a soft manner (20-22). The estimated concentrations of NAA and NAAG were combined to generate tNAA. A spline function was added to the model to account for the residual baseline (23).

Unsuppressed water was used as a reference to derive metabolite concentrations. The reference signals were also recorded using multiple TEs. They were fitted to a bi-exponential decay model, yielding the water intensity at TE = 0, denoted by S_{ref} . In this bi-exponential decay model, the T_2 of the longer decay component was attributed to cerebrospinal fluid (CSF) with $T_2 = 500$ ms (24).

The anatomical images were segmented using SPM5 (25, 26) to determine the fractions of gray matter (GM), white matter (WM), and CSF. An IDL program written in-house was used to extract the coordinates of the spectroscopy data files and their respective tissue fractions from segmentation (26). Finally, metabolite concentration C_m was calculated by

$$C_m = \frac{S_m}{S_{ref}} (f_g W_g + f_w W_w) \quad [4]$$

where S_m is the fitted metabolite signal intensity, f_g and f_w are the volume fractions of GM and WM within the voxels, and W_g and W_w are water densities of pure GM and WM (43300 mM and 35880 mM, respectively) (27). S_{ref} is the water intensity at TE = 0 that has excluded the CSF portion using the bi-exponential fit aforementioned. A comparison showed that the fitted CSF intensities echoed the volumes obtained using SPM5 method.

To measure the Gln concentration, TE-averaged spectroscopy was first used to determine the Glu concentration. Compared to NAA, Cr, and Cho, Glu is weakly represented and is more vulnerable to estimation errors. This study assumed that metabolites experience similar field-inhomogeneity-induced line broadening, and thus tied the line broadening of Glu to that of Cr in a soft constraint. The TE-averaged spectroscopy has a much stronger Cr peak than the J-modulated spectroscopy and should result in more reliable estimation of Cr and Glu amplitude and linewidth than the latter (see Results). For this reason, the Glu level was estimated by TE-averaged spectroscopy, and Gln/Glu ratio was determined by J-modulated spectroscopy, thus yielding the Gln concentration.

Monte Carlo Analysis

Simulated short TE and J-modulated spectra were used to assess Glu and Gln measurement reliability using J-modulated spectroscopy. Random noise was added to the simulated data. The spectral lineshapes were Lorentzian, and all metabolite peaks were broadened by 4 Hz. Since there were 32 echoes in the TE-averaged and J-modulated spectra, the short TE spectrum was simulated using 32 excitations with 32 different noise realizations. The same noise level was added into each single scan.

Results

The J-modulated spectrum at $J = 7.5$ Hz yielded the best separation of Gln from other metabolites. Figure 1a shows the simulated J-modulated spectrum ($J = 7.5$ Hz) decomposed into individual contributions from each metabolite component in the spectral model. It can be seen from the decomposed spectra that Gln only possibly overlaps with Glu and NAA aspartate in the region of 2.35 ppm. GABA contributed small peaks (not shown in Fig. 1) and had negligible influences on the quantification of Glu and Gln.

In Figure 1b, a series of spectra consisting of Glu (purple), Gln (red), and NAA aspartate (green) were simulated with J values from 0 (i.e., the TE-averaged spectrum) to 10.5 Hz. Note that a phase of 110 degree was applied to all spectra. $J = 7.5$ Hz was the optimal frequency that separates Gln, Glu, and NAA aspartate around 2.35 ppm with minimal interference for quantification. Notably, the Glu peak intensity in the J-modulated spectrum ($J = 7.5$ Hz) was comparable to that in the TE-averaged spectrum ($J = 0$).

Figure 2 compares J-modulated spectroscopy and short TE spectroscopy ($TE = 35$ ms). Simulated short-TE data were included here to assess the effect of overlapping metabolites in the absence of baseline distortion. Note that in vivo, uncertainty in fitting short-TE data was substantially higher due to severe baseline variations.

Metabolite levels were estimated for the short TE, TE-averaged, and J-modulated spectra using Monte Carlo simulations ($n = 50$). The short TE spectrum used 32 excitations, matching the echo number of the J-modulated spectrum. As shown in Table 1, in the absence of an uneven baseline the J-modulated spectrum yielded the smallest deviations in Gln levels, but had higher deviations for NAA, Cr, and Cho due to the substantially reduced signal intensities at $J = 7.5$ Hz. The Glu deviations were comparable in all three situations.

In vivo data were acquired from two voxels in the region of ACC dominated by GM and WM, respectively, as shown by Fig. 3. Averaged over 10 subjects, GM voxels were composed of 62% (± 8.4) GM and 27% (± 16) WM; WM voxels were composed of 26% (± 12) GM and 71% (± 7.4) WM.

Figure 4 shows the TE-averaged spectra with three different starting TEs: 35 ms, 95 ms, and 131 ms. T_2 s were estimated using these three starting TEs for tNAA, Cr, Cho, and Glu. The T_2 s determined in the t_1 dimension are listed in Table 2 ($n = 10$, age = 29 ± 7.5). In the spectra of the WM voxel, the acetyl proton peak of NAAG was visible as shown by the left shoulder of the peak at ~ 1.9 ppm. For Gln, T_2 was estimated using the two J-modulated spectra with starting TE = 35 ms and 77 ms, as shown in Figure 5.

Figure 6 shows the differences in Glu and Gln between GM and WM. The data were acquired from two ACC voxels of a healthy subject (female, age 30), dominated by GM and WM, respectively. Higher Glu levels were found in the GM-dominated voxel by both the TE-averaged (Fig. 6a and 6b) and the J-modulated methods (Fig. 6c and 6d). Higher Gln levels in GM were also revealed in J-modulated spectra. In addition, the acetyl peak of NAAG was clearly higher in WM than in GM, consistent with earlier reports (28). The estimated metabolite concentrations across all subjects were summarized in Table 2.

Significantly higher concentrations of Glu and Gln were observed in GM with very high statistical significance ($p < 10^{-10}$ and $< 10^{-5}$, respectively).

Finally, in Table 3, the estimated concentration ratios of Glu to Gln were compared with those obtained by using short TE LCModel analysis. The [Gln]-to-[Glu] ratios estimated using J-modulated spectroscopy were smaller than those estimated using short TE LCModel analysis (21), and the standard deviations were considerably reduced.

Discussion

In this paper, we presented a novel method for quantifying Glu and Gln simultaneously at 3 Tesla. Using a one-dimensional cross-section of J-resolved spectroscopy at $J = 7.5$ Hz, we found that the resonances of Glu and Gln were clearly separated around 2.35 ppm. This cross-section complements the conventional TE-averaged spectroscopy method, which essentially averages out Gln resonances around 2.35 ppm. Our results demonstrate that in vivo simultaneous quantification of Glu and Gln is feasible with multi-echo spectral data at 3 T.

Whereas the estimated metabolite concentrations reported here were in broad agreement with literature values (e.g., 2,21,29), The [Gln]-to-[Glu] ratios of both GM and WM were about 20% smaller than estimated by the short TE LCModel analysis (Table 3). In addition to differences inherent in the method, this disparity may also be attributable to differences in assumed basis spectra including T_2 values. Note that J-modulated spectroscopy essentially has a long TE and hence the estimated concentrations heavily rely on T_2 s. Table 3 shows the appreciable reduced standard deviations for J-modulated spectroscopy. These were attributable to not only the increased scan number (the J-modulated spectra used twice as many scans as the short TE spectra did) but also the better separated Glu and Gln peaks.

As showed by the Monte Carlo simulation (Table 1), the measurement reliability of Gln was significantly improved using J-modulated spectroscopy. However, higher deviations for other metabolites were found which could be attributed to reduced signal intensities. It also should be pointed out that the favorable short-TE results are somewhat misleading; actual short-TE measurements in vivo are hampered by large uncertainties associated with the baseline, including macromolecule signals unaccounted for by a pre-determined number of metabolites. In the current study, all metabolite concentrations were measured using TE-averaged spectroscopy except for Gln, since the TE-averaged results for those metabolites exhibited smaller estimated deviations based on the Monte Carlo simulations. J-modulated spectroscopy was only used to determine the concentration of Gln.

Both Glu and Gln levels were found to be significantly higher in GM than in WM (5,30-33), and Cr also showed a similar trend, echoing previous reports (33). Although no T_1 corrections were performed in this study, the differential T_1 saturation effect is expected to be minor considering the long TR used in this study and the high uniformity of metabolite T_1 s at 3 Tesla (34,35).

The three TE-averaged spectra, with starting TE = 35 ms (the first echo), 95 ms (the tenth echo), and 131 ms (the sixteenth echo), respectively, were constructed to measure the T_2 s of

NAA, Cr, Cho, and Glc. These TE values were selected empirically using these two criteria: i) the TE-averaged spectrum for each starting TE value is properly simplified with sufficient signal intensity; ii) they generate significantly different T_2 weighting among the TE-averaged spectra. Due to the low Glc signal intensity and the overlap between Glc and Glu, only two T_2 points were selected for Glc T_2 measurement, with TE starting at 35 ms and 77 ms, respectively.

The T_2 measurement method described here benefits from the fact that all TE-averaged spectra have fairly flat baselines and reduced spectral overlap and, as such, the estimation biases caused by the interference of spectral overlapping and the baseline are greatly reduced. A similar approach was recently reported that uses several sub-arrays of J-resolved spectral data and the effective TEs to estimate metabolite T_2 s (36). Considering the effects of regional differences, our metabolite T_2 values generally agree with the available literature values (30,34,36).

Metabolite quantification is affected by many factors, including signal intensity, spectral resolution, lineshape, fidelity of the spectral model, a priori knowledge, baseline, and sampling artifacts. Of these, spectral resolution is critical to reliable and accurate spectral quantification of Glc and Glu. In most cases, in vivo spectra at 3 Tesla do not have sufficient spectral resolution to resolve Glc and Glu. Additional complications due to lineshape imperfections and macromolecule-induced baselines make the quantification of Glc and Glu even more difficult. Using two different cross-sections of the same multiple-echo data set, the proposed method spectrally separates Glc and Glu, leading to significant improvement in quantification of Glu.

Constraining spectral parameters can greatly reduce estimation uncertainty. The constraints on the line-broadening parameter α in Eq. 3 were critical to determining Glc and Glu, especially Glu. The rationale for constraining the line-broadening parameter α is that field inhomogeneity causes similar line-broadening for all metabolite resonance lines. While many constraints were enforced in a soft manner as described in the Methods, allowing small individual variations, line broadening of Glu was strongly tied to that of Glc because of the low Glu signal intensity. The CH resonances of Glc and Glu at 3.7 ppm are essentially inseparable at 3 T and, further, they strongly overlap with the GSH CH₂ resonances and require incorporating myo-Inositol and the T_2 s of Glc and Glu CH resonances into the spectral model. Inclusion of this region increases fitting uncertainty, and thus, this region was not included in the fitting model.

The J-modulated spectrum is one of the many cross-sections of a 2D J-resolved spectrum. Fitting to the two-dimensional spectrum enables using the whole dataset, as in the ProFit method (37, 38). A 2D fit, however, requires a much larger parameter set. For example, all metabolite T_2 s are needed to fit the 2D J-resolved spectrum because different cross-sections are related by T_2 s. In addition, artifacts such as eddy currents or frequency drifts can distort the spectrum in both dimensions and may require additional parameters to explicitly account for them. A large parameter set tends to be more problematic for a non-linear fitting process. Whereas the 2D fit is the ultimate goal in using 2D J-resolved spectral data, using one-dimensional sections such as the TE-averaged spectrum and/or the J-modulated spectrum

proposed in this study has the important benefit of simplified spectral structure and greatly suppressed baseline. As shown by the current work a large amount of information including Glu and Gln levels can be extracted using the 1D methods.

In conclusion, we have developed a novel J-modulated spectroscopy method, and found that Gln resonances were clearly separated from Glu and NAA resonances around 2.35ppm at $J = 7.5$ Hz. This method is useful for simultaneously and quantitatively measuring Glu and Gln at 3 T. Metabolite T_2 s were incorporated into the fitting model. In adult healthy subjects, Glu and Gln levels were both found to be higher in GM than in WM.

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Fig.1.

(a) Simulated J-modulated spectrum. Individual contributions from glutamate (Glu), glutamine (Gln), N-acetyl-aspartate (NAA), and glutathione (GSH) are displayed separately under the top trace. An additional phase of 110 degrees was applied to the spectrum. Around 2.35 ppm, the Gln resonance is clearly separated from resonances of Glu, NAA, and GSH. All individual component intensities were scaled by using total creatine as the reference ($[Cr] = 1.0$), $[Glu] = 1.1$, $[Gln] = 0.35$, $[NAA] = 1.3$, $[choline (Cho)] = 0.3$, $[GSH] = 0.2$, and $[\text{gamma-aminobutyric acid (GABA)}] = 0.15$. GABA contributes small peaks (not displayed), but these were found to be negligible for the purpose of quantifying Glu and Gln. **(b)** From 0 to 10.5 Hz, 7.5Hz was found to be the optimal J that led to minimal overlaps between Glu (purple), Gln (red), and NAA aspartate (green).

Fig.2.

Simulated J-modulated spectrum and short echo time (TE) spectrum (TE = 35 ms). The short TE spectrum was generated using 32 excitations. Using total creatine as a reference ([Cr] = 1.0), [NAA] = 1.3, [Glu] = 1.1, [Gln] = 0.35. Spectral overlap among NAA, Glu and Gln seen in the short-TE spectrum was substantially reduced in the J-modulated spectrum.

Fig 3.

T_1 weighted images show the locations of the gray matter (GM) voxel and the white matter (WM) voxel in anterior cingulate cortex (ACC).

Fig. 4.

Examples showing T_2 measurement using TE-averaged spectra in (a) gray matter (GM) voxel and (b) white matter (WM) voxel. The three TE-averaged spectra had starting TEs of 35 ms, 95 ms, and 131 ms, respectively.

Fig.5.

J-modulated spectra with starting echo times (TEs) of (a) 35 ms and (b) 77 ms. Unlike the spectrum with TE = 35 ms, which was multiplied by a 110 degree phase factor, an overall phase of 150 degrees was applied to the spectrum with a starting TE of 77 ms, in order to turn up the phases of glutamate (Glu) and glutamine (Gln) peaks. These two spectra were used to estimate the Gln T_2 . The simulated sub-spectra in (b) show partial overlap between Glu and Gln around 2.35 ppm.

Fig.6.

Examples of (a, b) TE-averaged spectra and (c, d) J-modulated spectra acquired from a gray matter (GM) voxel (a, c) and a white matter (WM) voxel (b, d) in anterior cingulate cortex (ACC). The GM voxel was composed of 58% GM and 30% WM; The WM voxel contained 69% WM and 27% GM. The TE-averaged spectra and the J-modulated spectra both show higher glutamate (Glu) levels in the GM voxel. Higher glutamine (Gln) levels in the GM voxel were revealed by the two J-modulated spectra (c and d). The J-modulated spectra (c and d) were scaled up by $\times 2$. The fitted spectra and the baselines are displayed in red, along with the fit residuals.

Table 1

Monte Carlo analysis of estimated metabolite levels and deviations in short TE, TE-averaged and J-modulated spectra* ($n = 50$).

Method	Glu	Gln	NAA	Cr	Cho
Short TE	1.09(±0.05)	0.32(±0.11)	1.30 (±0.01)	1.00(±0.01)	0.30(±0.01)
TE-averaged	1.13(±0.08)	0.43(±0.16)	1.30 (±0.01)	1.00(±0.02)	0.30(±0.01)
J-modulated	1.06(±0.11)	0.32(±0.07)	1.29(±0.05)	0.99(±0.08)	0.30(±0.02)

* Included metabolites are [Glu] = 1.1, [Gln] = 0.35, [NAA] = 1.3, [Cr] = 1.0, and [Cho] = 0.3. The noise level was 0.02. The assumed T_2 s were 190 ms, 160 ms, 290 ms, 160 ms, and 240 ms, respectively. All Lorentz-type linewidths were broadened by 4 Hz. Fifty different noise realizations were used.

Abbreviations: TE: echo time; Glu: glutamate; Gln: glutamine; NAA: N-acetyl-aspartate; Cr: creatine; Cho: choline.

Table 2

Estimated metabolite T_2 s and concentrations* ($n=10$, age = 29 ± 7.5).

	Glu	Gln	tNAA	Cr	Cho
GM T_2 (ms)	185(± 12)	145(± 26)	318(± 8)	175(± 7)	282(± 12)
WM T_2 (ms)	146(± 18)	134(± 33)	287(± 6)	153(± 9)	226(± 9)
GM Conc.(mM)	11.1(± 0.89)	3.02(± 0.46)	12.5(± 0.52)	10.2(± 0.45)	2.35(± 0.16)
WM Conc.(mM)	8.21(± 0.95)	1.90(± 0.54)	12.8(± 0.61)	8.39(± 0.48)	2.22(± 0.18)

* Standard deviations are given in parentheses. For Glu and Gln, T_2 s were measured by the resonances of the C4 protons; tNAA T_2 was the combined T_2 of acetyl protons of NAA and NAAG; the CH₃ resonances were used to estimate Cr and Cho T_2 .

Abbreviations: Glu: glutamate; Gln: glutamine; tNAA: total N-acetyl-aspartate; Cr: creatine; Cho: choline; GM: gray matter; WM: white matter.

Table 3

Concentration ratios of glutamine to glutamate estimated with J-modulated method and LCModel analysis.

	J-modulated	LCModel
Gray matter	0.27(± 0.040)	0.33(± 0.061)
White matter	0.23(± 0.067)	0.28(± 0.093)