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Accuracy and stability of measuring GABA, glutamate, and glutamine by proton magnetic resonance spectroscopy: A phantom study at 4 Tesla

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ABSTRACT

Proton magnetic resonance spectroscopy has the potential to provide valuable information about alterations in gamma-aminobutyric acid (GABA), glutamate (Glu), and glutamine (Gln) in psychiatric and neurological disorders. In order to use this technique effectively, it is important to establish the accuracy and reproducibility of the methodology. In this study, phantoms with known metabolite concentrations were used to compare the accuracy of 2D *J*-resolved MRS, single-echo 30 ms PRESS, and GABA-edited MEGA-PRESS for measuring all three aforementioned neurochemicals simultaneously. The phantoms included metabolite concentrations above and below the physiological range and scans were performed at baseline, 1 week, and 1 month time-points. For GABA measurement, MEGA-PRESS proved optimal with a measured-to-target correlation of $R^2 = 0.999$, with *J*-resolved providing $R^2 = 0.973$ for GABA. All three methods proved effective in measuring Glu with $R^2 = 0.987$ (30 ms PRESS), $R^2 = 0.996$ (*J*-resolved) and $R^2 = 0.910$ (MEGA-PRESS). *J*-resolved and MEGA-PRESS yielded good results for Gln measures with respective $R^2 = 0.855$ (*J*-resolved) and $R^2 = 0.815$ (MEGA-PRESS). The 30 ms PRESS method proved ineffective in measuring GABA and Gln. When measurement stability at in vivo concentration was assessed as a function of varying spectral quality, *J*-resolved proved the most stable and immune to signal-to-noise and linewidth fluctuation compared to MEGA-PRESS and 30 ms PRESS.

1. Introduction

Advances in proton magnetic resonance spectroscopy (^{1}H-MRS) methods now permit detection and quantification of key metabolites in the living human brain including the neurotransmitters gamma-aminobutyric acid (GABA) and glutamate (Glu) as well as their metabolic intermediate glutamine (Gln). These neurotransmitters and related metabolites are implicated in many neuropsychiatric and neurological disorders including depression [1–3], schizophrenia [4,5], and epilepsy [6–8]. Despite the increasingly widespread use of these techniques, there is relatively little data about the relative accuracy and reproducibility of the different techniques used to make these measurements.

GABA is the primary inhibitory neurotransmitter in the mammalian brain and is implicated in a broad spectrum of disorders and conditions [6–10]. Measuring GABA using in vivo ^{1}H-MRS is typically achieved using the well-known and robust difference-editing technique [11,12]. However, although this approach is widely accepted and validated for GABA, its utility in simultaneously measuring Glu and Gln is still untested. Double-quantum filtering is another spectral editing method that (unlike difference-editing) provides a single-shot measure of GABA, based on its *J*-coupling constant [11,12]. However, this approach is hindered by severe baseline variation in the GABA-extracted spectrum and low sensitivity, making quantification difficult.

Likewise, several methods have been devised for the optimal, separate and simultaneous measurement of Glu and Gln in vivo. With the advent of improved gradient hardware and high-field magnets, extremely short-echo ^{1}H-MRS sequences have been developed that can achieve echo-times on the order of several milliseconds or less [13–16], thus minimizing the *J*-coupling evolution and maximizing the available signal-to-noise of the Glu and Gln coupled peaks. However, despite short echo-times, Glu and Gln are still highly-overlapped and complex multiplet resonances that are difficult to reliably separate, even under the most optimized conditions. *J*-resolved MRS has been explored as an alternative method for improving the separate measurement of Glu and Gln in vivo. As a variation on this 2D fitting method, a technique developed in our own lab makes use of the widely available proton-dedicated fitting software "LCModel", where the 2D *J*-resolved spectrum is divided into separate 1D spectra and each *J*-resolved spectral extraction is fitted with a theoretically-simulated spectral template. This approach has yielded very favorable results in

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accurately and precisely measuring the coupled resonances of Glu and Gln as described in detail in Jensen et al. [17], but its efficacy in assessing GABA measurement has never been thoroughly tested. To date, the 2D *J*-resolved approach seems the most promising in terms of developing a potentially robust and all-inclusive method for the acquisition and quantification of GABA, Glu and Gln in a single scan, as well as many other important metabolites in vivo.

This study had two main objectives. One was to determine the accuracy of three methods of proton MRS used to measure Glu, Gln, and GABA using phantoms with known concentrations of the metabolites. By testing a series of phantoms with GABA, Glu, and Gln concentrations around the accepted physiological range, it was possible to determine whether accuracy is maintained at lower or higher levels. In particular, we aimed to identify a method that provided excellent results for the three metabolites when measured simultaneously. The second goal was to determine the utility of the different techniques in repeated measures designs.

2. Experimental

2.1. Equipment

All phantom ^{1}H-MRS acquisitions were conducted on a full-body MR scanner (Varian/UnityInova, Varian Inc., Palo Alto, CA) operating at 4 Tesla magnetic field strength using a single-tuned transverse-electromagnetic (TEM)-design volumetric head radio-frequency (RF) coil (Bioengineering Inc., Minneapolis, MN) operating at 170.3 MHz for proton.

2.2. Phantoms

To directly represent the in vivo human brain spectrum, as well as to test the ability of each sequence to accurately and reliably track varying levels of GABA, Glu, and Gln, a series of five in vivo brain analogue phantoms were built in which GABA, Glu, and Gln were varied in concentration. In order to mimic the in vivo scenario, a metal-free water bath to house the metabolite phantoms was constructed to maintain the phantoms at a temperature of 37 °C for a prolonged period of time and yet still adequately load the RF coil like a human head during scanning [17]. The magnet operator was blinded to phantom concentrations during the data acquisition and analysis.

For the metabolite solution, a standard LCModel master-stock solution was first prepared and then proton metabolites added in their respective, physiologically-accepted concentrations, taken from the average of the normal range as listed in Govindaraju et al. [18]. Concentrations of each metabolite are listed in Table 1. In each of the five phantoms, all metabolite levels were identical (derived from the same stock solution) but GABA, Glu, and Gln levels were multiplied by factors of 0.0, 0.5, 1 and 2 to represent a physiologic range, as well as a high-concentration phantom (10x) to act as a calibration source for deriving absolute concentrations from all acquisition methods (Fig. 1).

Pilot images were then acquired in axial, coronal, and sagittal planes to clearly delineate the metabolite ball and allow for the placement of a 2.5 × 2.5 × 2.5 cm voxel centered in the ball. Once the tip-angles were optimized by an automated routine, the voxel was manually shimmed, achieving an unsuppressed water linewidth of 2–3 Hz. The water-suppression (WET) was then automatically optimized to minimize residual water signal [19].

2.3. Data acquisition

The three sequences are all based on a common master-PRESS-localized sequence [20] with identical voxel-localization gradients, excitation/refocus RF-pulses and water-suppression for maximum comparability between methods. The common acquisition parameters were set to TR = 2 s, spectral-width = 2 kHz, complex-points = 1024, voxel = 2.5 × 2.5 × 2.5 cm, scan-time ~18 min. The *J*-resolved method utilized a modified version of the original PRESS sequence which incrementally stepped the echo-times from 30 ms to 650 ms in 20 ms increments (32 echo-steps total), with 16 averages for each echo-time. This sampling scheme provides a 50 Hz window in the indirectly-detected (*J*-resolved) dimension.

The MEGA-PRESS difference editing sequence [21,22] utilized a TE of 68 ms for optimal GABA detection and collected each sub-spectrum in pairs with the exact same phase-cycling for each to ensure optimal subtraction. The sequence utilized a 20 ms duration truncated Gaussian pulse (~100 Hz) to invert the 1.89 ppm GABA region, thus giving rise to an inversion of the coupled sidebands in the GABA groupings at 3.01 ppm and 2.28 ppm. The refocusing pulses were of 1 ms duration (171.4 μs each for ramp-up and ramp-down). For each sub-spectral (or "ON" and "OFF") pair, the only sequence parameter that was altered was the frequency of this Gaussian pulse which alternated between offset frequencies of 0 Hz (1.89 ppm inverted) and 20,000 Hz (1.89 ppm not inverted). Gaussian pulse power was kept constant at optimal saturation power. NEX ("ON"/"OFF") was set to 256/256 (512 averages total).

The 30 ms PRESS acquisition was identical to the *J*-resolved acquisition described above with the exception of the echo-time being held at 30 ms throughout the duration of the scans rather than stepped. In the 30 ms PRESS acquisition, NEX was set to 512. All other parameters were identical to the *J*-resolved acquisition.

Table 1
Metabolite concentrations in the 1x phantom. GABA, Glu, and Gln were varied by factors of 0.5x, 1x, and 2x the accepted concentration values as listed in Govindaraju et al. All other metabolite levels were held constant.

Metabolites and concentrations in phantoms Metabolite	Concentration (mmol)
N-acetylaspartate (NAA)	10.61
N-acetylaspartylglutamate (NAAG)	1.70
Alanine	0.82
Gamma-aminobutyric acid (GABA)[a]	1.65
Aspartate	1.24
Choline	1.75
Creatine (total)	7.31
Glucose[b]	1.03
Glutamate[a]	3.35
Glutamine[a]	1.44
Glutathione	2.06
Glycerophosphocholine (GPC)	1.03
Glycine	0.72
Myo-inositol	6.13
Scyllo-inositol	0.46
Lactate	0.41
Phosphocholine	0.62
Serine[b]	0.41
Taurine	1.24

[a] Only the 1x physiologic level is listed.
[b] Glucose and serine are not modeled in the GAMMA-simulated LCModel basis sets.

2.4. Data reconstruction/processing/analysis

2.4.1. Generation of MEGA-PRESS, 30 ms PRESS, and J-resolved simulated basis sets

The procedure for generating the 30 ms PRESS and *J*-resolved LCModel metabolite basis sets for each *J*-resolved extraction has been described in detail previously in Jensen et al. [17] based on spectral simulations [23,24]. For the MEGA-PRESS LCModel template, we collected MEGA-PRESS phantom data. Standard LCModel stock-solution phantoms were prepared as described by Provencher [25] for N-acetylaspartate (NAA), GABA, Glu, and Gln.

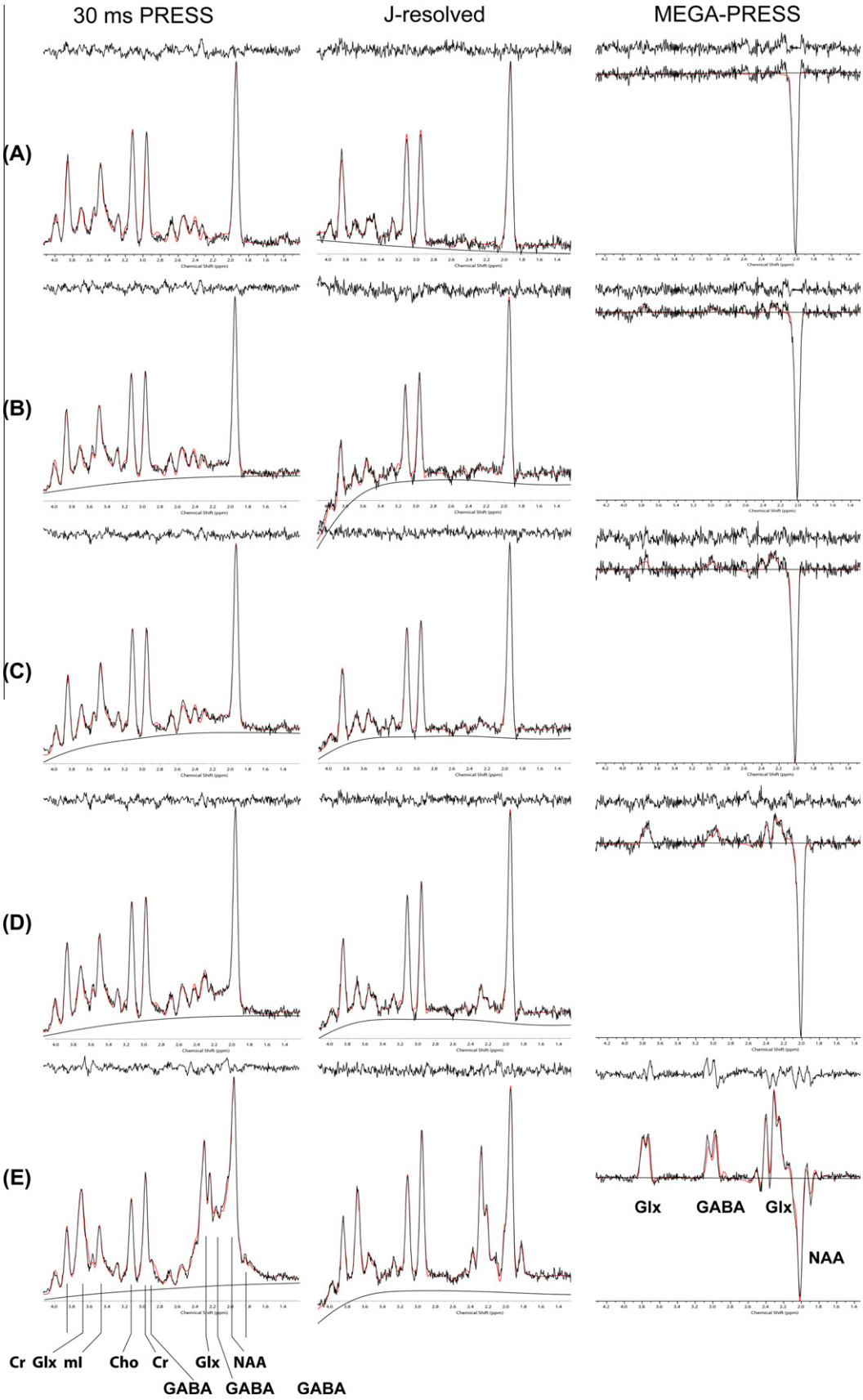

Fig. 1. Representative spectra from all five phantoms containing differing concentrations of GABA, glutamate, and glutamine as acquired using three PRESS-based methods: 30 ms PRESS, *J*-resolved (*J* = 0.0 Hz), and MEGA-PRESS (difference spectrum). All spectra have been Gaussian line-broadened to ~8 Hz (~0.05 ppm) and simulated noise-added in the time-domain to approximate the in vivo case. Spectra are shown with LCModel fits and residuals. The 68 ms sub-spectra for the MEGA-PRESS data are not shown – only the difference-edited spectra are displayed to illustrate the quality of our MEGA-PRESS technique. The respective concentration factors for the phantoms are (A) 0x, (B) 0.5x, (C) 1x, (D) 2x, (E) 10x.

MEGA-PRESS phantom spectra were then acquired at 4 Tesla for each separate metabolite and LCModel basis sets were generated to produce a final LCModel MEGA-PRESS edited template for fitting the edited MEGA-PRESS spectra.

2.4.2. MRS data processing and quantification of absolute metabolite values

For all acquisition methods, the 10x physiologic phantom was used as a calibration source to derive absolute concentration values for GABA, Glu, and Gln. For each acquisition, the baseline measurement for this high-concentration phantom was used to obtain a correction factor for GABA/Cr, Glu/Cr, and Gln/Cr ratios which, when multiplied by the known Cr concentration of 7.313 mM, yielded absolute concentrations for these metabolites. These initial calibration factors served to derive actual concentration values for all subsequent measurements.

All MRS processing was carried out in a fully automated fashion.

2.4.3. J-resolved

For each dataset, the TE-stepped free induction decays (FIDs) were first zero-filled in f1 out to 64 points, Gaussian filtered, and then Fourier-Transformed in f1 to produce 64 *J*-resolved spectra. Although 32 TE-stepped points were collected, the first 24 were used in the analysis as this provided a theoretically perfect match for our GAMMA-simulated LCModel template. LCModel, utilizing the above-described GAMMA-simulated, *J*-resolved basis sets [17], was then used to fit every *J*-resolved spectral extraction within a bandwidth of 50 Hz (−25 Hz to +25 Hz) with its theoretically matched template. To derive concentration estimates, the data were summed over the 64 raw peak integrals for every metabolite. Ratios of GABA, Glu, and Gln were then calculated to total summed creatine resonance area (Cr+PCr) and multiplied by the appropriate calibration factor for GABA, Glu, and Gln.

2.4.4. MEGA-PRESS

The "ON" and "OFF" spectral subsets were first summed producing single "ON" and "OFF" 68 ms sub-spectra for each phantom dataset. These 68 ms sub-spectra were then subtracted resulting in GABA-edited difference spectra. The respective LCModel templates for the 68 ms "OFF" sub-spectrum and the GABA-edited difference spectra were then applied to derive peak integrals for the edited GABA resonance in the difference spectra as well as Glu, Gln, and Cr from the 68 ms sub-spectra. These GABA, Glu, and Gln areas were then expressed as ratios to 68 ms Cr and multiplied by their respective calibration factors to obtain concentration values.

2.4.5. 30 ms PRESS

The derivation of absolute values for the 30 ms PRESS data was generally similar to that used in the *J*-resolved data. The spectra were fitted with the 30 ms GAMMA-simulated LCModel template and raw GABA, Glu, and Gln resonance areas expressed as a ratio to total creatine. These rations were then multiplied by their respective calibration factors.

2.5. Simulated noise, line-broadening and Monte-Carlo analysis

Since we are interested in approximating the range of spectral quality that would be encountered in typical in vivo brain scans at 4 Tesla, we made use of simulated noise and line-broadening in the processing of our phantom datasets. In the primary analysis, we tested all phantom measurements over all three time-points using Gaussian-weighted signal-to-noise and line-broadening values typically encountered in vivo at 4 Tesla (∼8 Hz, ∼0.05 ppm) to best compare MRS method performance under in vivo-like conditions. In a secondary analysis, we present a Monte-Carlo-based simulation analysis in which a range of different signal-to-noise and line-broadening values (∼8–16 Hz, ∼0.05–0.10 ppm) were tested over repeated measures for our phantom measurements containing the accepted physiological concentrations of GABA, Glu, and Gln (Fig. 2). Combined, both analyses provide a robust and realistic quantitative assessment of the relative performance of each method in simultaneously measuring GABA, Glu, and Gln.

2.6. Analysis I: test–retest over varying concentration

In order to approximate in vivo conditions, we first line-broadened each phantom spectrum to approximately 8 Hz (∼0.05 ppm) with a Gaussian-weighted time-domain filter. This simulated a typical high-quality in vivo voxel spectrum in terms of shim quality. We then we added simulated noise into the line-broadened spectrum such that the final signal-to-noise of each spectrum from each acquisition method approximated that which would be obtained from a 12 cm^3 voxel in vivo for an approximately 15 min scan-time.

2.6.1. Statistical analysis

The relationship between the measured and actual concentration of each metabolite was determined by linear regression using the regression function in Microsoft Excel. The correlation coefficient (R^2) of the regression line was calculated without the 10x phantom with the known target concentration of the metabolite as the independent variable and the measured concentration as the dependent variable. The coefficient of variation for the three measurements for each phantom was calculated and expressed as a percentage. Since it is known that Gln is unstable in solution, we only include the first two measurement time-points for Gln (baseline and 1 week) in the test–retest analysis as it was anticipated that by the 1 month time-point, enough Gln would have decayed that this would add variance to the Gln repeatability measures. Glutamate and GABA, however were measured for all three time-points as they are stable in solution.

2.7. Analysis II: Monte-Carlo simulation at physiologic concentration

In order to assess the effects of varying levels of signal-to-noise and spectral linewidth at physiologic concentrations that would be encountered in a typical in vivo study cohort, we tested a broad range of these parameters over repeated measures in a Monte-Carlo based analysis. In this secondary analysis, we based our modeled linewidth and signal-to-noise range on actual in vivo datasets from 4 Tesla for all three MRS methods which range in linewidths from 8 to 16 Hz and NAA signal-to-noise (as determined by LCModel) from 18 to 30 (30 ms PRESS), 15–24 (*J*-resolved 0.0 Hz), 13–22 (68 ms MEGA-PRESS "OFF" spectrum) (Fig. 2). By getting a range of actual in vivo values for LCModel-fitted in vivo brain spectra for each MRS method, we were able to model the in vivo case ranging from full-width, half-maximum (FWHM) of 16 Hz (∼0.09 ppm) and signal-to-noise of ∼18 (regarded as a poorly-shimmed, low-quality spectrum) to FWHM of 8 Hz (∼0.05 ppm) and signal-to-noise of ∼30 (well-shimmed, high-quality spectrum) (Fig. 2). We used the baseline phantom measurements of GABA = 1.65 mM, Gln = 2.88 mM, and Glu = 6.7 mM, which best approximate the respective concentrations of these metabolites in vivo in the brain. For each MRS acquisition method, we performed 200 repeated measures with each measure having a unique and randomly-determined signal-to-noise and line-broadening value applied to the phantom spectra ranging in Gaussian-weighted linewidths from 8 to 16 Hz and relative NAA signal-to-noise values for each MRS method as described above.

2.7.1. Statistical analysis

For each measurement of GABA, Glu, and Gln across the three MRS methods, the 200 simulated repeated measures were binned

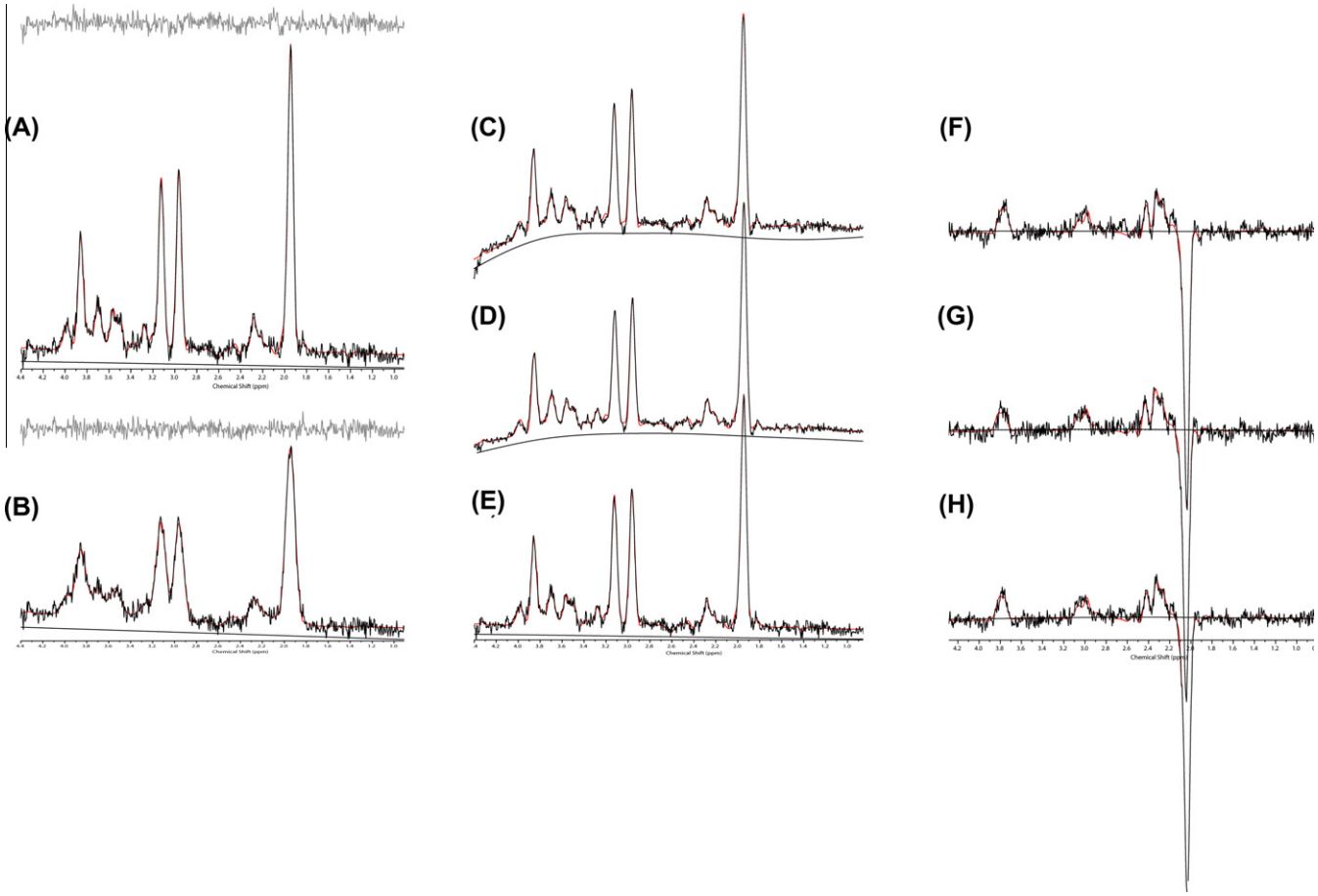

Fig. 2. Representative *J*-resolved (*J* = 0.0 Hz) phantom spectra from the 2x phantom with (A) ~8 Hz (~0.05 ppm) and (B) ~16 Hz (~0.10 ppm) Gaussian line-broadening. Noise has been added to simulate the in vivo conditions. *J*-resolved (*J* = 0.0 Hz) phantom spectra from the same 2x phantom over the three time-points: (C) baseline (D) 1 week and (E) 1 month as well as the corresponding MEGA-PRESS difference spectra (F, G, H) from the same time-points.

into ten discrete linewidth segments, with each segment representing 20 repeated measures across a linewidth range of 2.4 Hz (~0.014 ppm) and a NAA signal-to-noise range of 18–30 (30 ms PRESS), 15–24 (*J*-resolved 0.0 Hz), 13–22 (68 ms MEGA-PRESS "OFF" spectrum) (Fig. 3). Within each segment, the average and standard deviation for each derived metabolite concentration (n = 20 repeated measures/segment) were calculated and plotted against the known target concentration value for each metabolite measurement series for the three MRS methods (Fig. 3). The standard deviation in the mean (SDm) was calculated for each metabolite for each method across the range of linewidths using all 200 points providing a quantitative measure of measurement stability versus spectral quality.

3. Results

3.1. Analysis I: test–retest over varying concentration

Since we are interested primarily in the accuracy of these methods at or around the physiological range of GABA, Glu, and Gln, the analyses were performed without the 10x phantom. Both the *J*-resolved method and MEGA-PRESS gave excellent results for GABA and Glu for correlations between measured and targeted concentration values (Table 2 and Fig. 3). However, 30 ms PRESS yielded very poor results for GABA, only returning one measured value of GABA for the 2x phantom (all other measures were zero). In addition, 30 ms PRESS actually yielded a negative correlation between measured and target values between phantoms indicating that 30 ms PRESS is not at all adequate for making GABA or Gln measures at typical in vivo concentrations and acquisition parameters. The 30 ms PRESS approach, however, did result in a very strong correlation between measured and target values for Glu (R^2 = 0.987), suggesting that it is suitable for reliable Glu acquisition only (Table 2 and Fig. 3). Overall and not surprisingly, MEGA-PRESS yielded the best correlation between measured and target GABA concentrations (R^2 = 0.999), followed by *J*-resolved (R^2 = 0.973), due largely to the clear isolation of the edited GABA doublet from overlying Cr at 3.00 ppm. All three methods performed well for Glu with R^2 values above 0.9, owing mostly to the relatively high concentration of Glu in vivo. For Gln detection, *J*-resolved showed the strongest correlation with R^2 = 0.855, followed closely by MEGA-PRESS (R^2 = 0.815). To summarize, our results strongly suggest that for pure GABA measurements, MEGA-PRESS is the best choice, but for simultaneous measures of GABA, Glu, and Gln in a single acquisition, *J*-resolved offers a good compromise, yielding superior results for Glu and Gln measures over MEGA-PRESS, with a strong ability to provide reliable GABA measures.

In addition, we examined the retest reliability of the methods over time as well as the stability of the metabolites in solution over a period of 1 month (baseline, 1 week and 1 month for Glu and GABA; baseline and 1 week for Gln – Table 3). Over the repeated measures, the *J*-resolved method seemed to yield the most stable variance with coefficient of variation (CV) values ranging from 20% to 50% (GABA), 2–11% (Glu), and 1–14% (Gln). Once again, for GABA, MEGA-PRESS displayed the most stable variance over the repeated measures (CV: 9–100%), followed by Glu (CV:

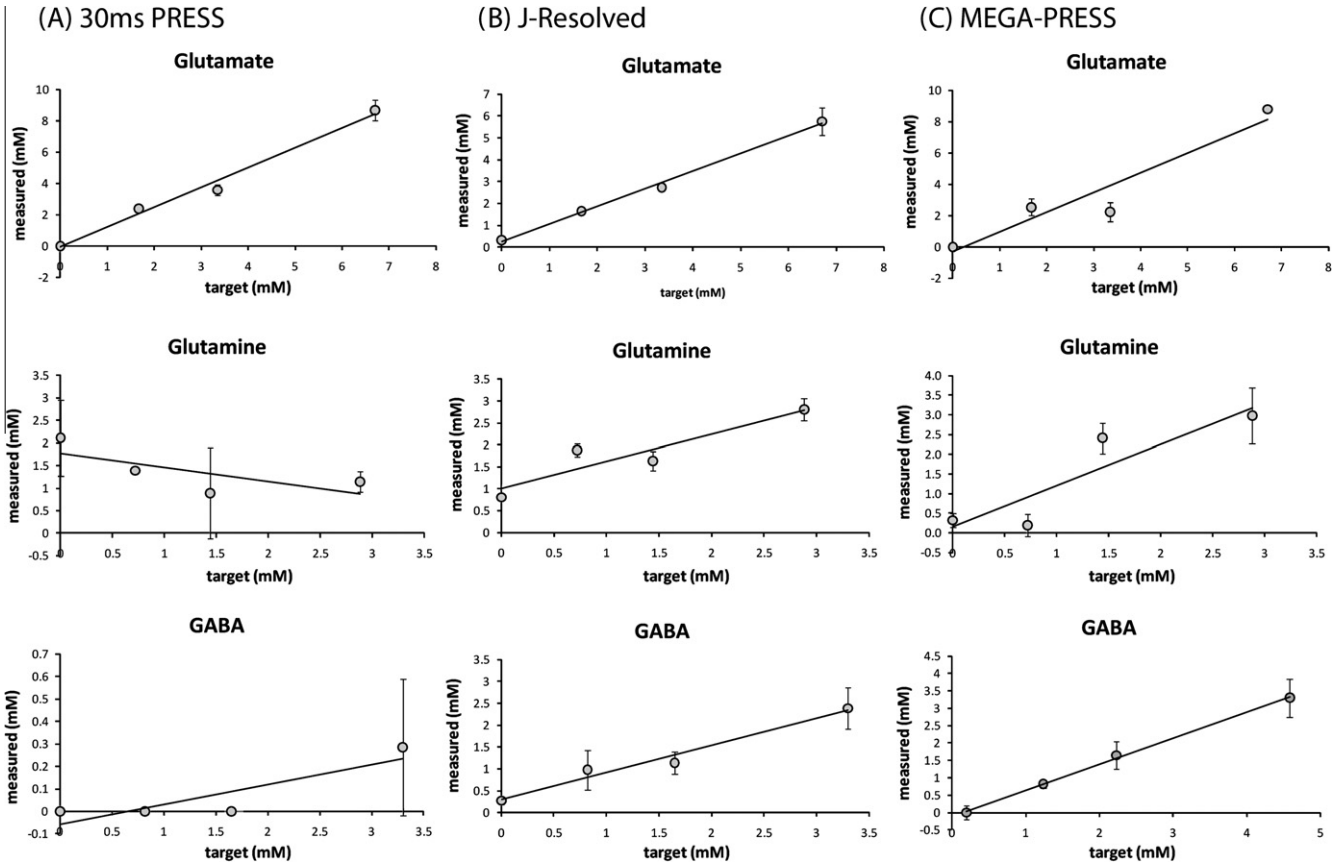

Fig. 3. Correlations between known target concentrations in the phantoms (*x*-axis) and measured values (*y*-axis) for the three methods. Points represent the mean for all three measures over the course of 1 month (Glu and GABA) and the mean of two measures over the course of 1 week (Gln). Error bars represent the standard deviation. The 10x calibration phantom is not included in the plots.

Table 2
Correlations between measured and actual concentrations of GABA, Glu, and Gln using three different methods based on linear regression analysis.

Method	Metabolite	Correlation (R^2)
J-resolved	GABA	0.973
	Glu	0.996
	Gln[b]	0.855
MEGA-PRESS	GABA	0.999
	Glu	0.910
	Gln[b]	0.815
30 ms PRESS	GABA	–
	Glu	0.987
	Gln[b,a]	0.519

[a] Correlation was negative.
[b] $n = 2$ repeated measures – baseline and 1 week.

Table 3
Reproducibility of repeated measurements as reflected by the coefficient of variation (expressed as a percentage). In cases where a metabolite could not be returned by LCModel, no CV could be reported as indicated by N/A.

	30 ms PRESS			*J*-resolved			MEGA-PRESS		
	GABA	Glu	Gln[a]	GABA	Glu	Gln[a]	GABA	Glu	Gln[a]
0.0x	N/A	N/A	39.6	32.5	36.9	1.4	96.5	N/A	53.1
0.5x	N/A	10.1	3.3	46.2	4.4	7.7	9.1	20.6	141.4
1.0x	N/A	9.6	113.9	22.4	2.1	13.4	17.4	26.6	16.2
2.0x	107.2	7.6	19.8	19.8	10.8	8.9	11.9	1.0	23.8

[a] $n = 2$ repeated measures – baseline and 1 week.

1–27%) and Gln (CV: 16–141%). The 30 ms PRESS technique fared well with Glu with a CV range of 7–10%, but showed substantial measurement instability for Gln (CV: 3–114%) over the three time-points. The only 30 ms PRESS GABA measurement obtained (2x phantom) showed a CV of 107%. Overall, these results confirm that for measurement of GABA, MEGA-PRESS offers the most reliability and stability, but for simultaneous measurement of GABA, Glu, and Gln, *J*-resolved provides superior stability for Glu and much better stability for Gln in comparison. The 30 ms PRESS acquisition is suitable for making stable measures of Glu but not GABA or Gln.

3.2. Analysis II: Monte-Carlo simulation at physiologic concentration

This analysis was undertaken to assess the measurement reliability of each technique across a typical range of spectral quality at typical in vivo concentration values. Since the 30 ms GABA dataset was only able to fit 17 of the 200 noise-added and line-broadened spectra, we did not calculate a standard deviation in the mean for this measure. Of the three MRS methods, *J*-resolved proved the most stable with SDm values of 0.023 (GABA), 0.003 (Glu), and 0.068 (Gln) (Fig. 4). These values indicate that *J*-resolved offers more robust measurement stability in the presence of widely varying spectral quality compared to MEGA-PRESS, which yielded comparative SDm values of 0.131 (GABA), 0.810 (Glu), and 1.412 (Gln), and 30 ms PRESS which had SDm values of 0.134 (Glu) and 1.66 (Gln). The error bars in the *J*-resolved data for each binned data point ($n = 20$) are very small compared to those for MEGA-PRESS and 30 ms PRESS (Fig. 4). In all, this analysis strongly illustrates the robust measures of GABA, Glu, and Gln that the *J*-resolved

technique can offer and that these measures seem relatively immune to widely varying spectral quality compared to MEGA-PRESS and 30 ms PRESS.

4. Discussion

This study compared the accuracy and reproducibility over repeated measures of three commonly used methods of proton MRS to measure GABA, Glu, and Gln. All three methods perform quite well for the measurement of Glu in the physiologic concentration range, with *J*-resolved yielding the most accurate measures (R^2 = 0.996), followed by 30 ms PRESS (R^2 = 0.987) and MEGA-PRESS (R^2 = 0.910). However, for the measurement of Gln, 30 ms PRESS failed to track the target concentrations (Fig. 3). Again, *J*-resolved appears to provide the most accurate and stable measure of Gln (R^2 = 0.855), followed by MEGA-PRESS (R^2 = 0.815). For the measurement of GABA, MEGA-PRESS is clearly optimal (R^2 = 0.999), displaying the highest level of accuracy and reliability across all measurement time-points. This result is consistent with the in vivo findings of GABA reproducibility in the literature where fitted GABA/Cr test–retest reliability is <15% [26]. As with Gln, 30 ms PRESS is inadequate to measure GABA.

We have reported previously on the use of the *J*-resolved methodology for measuring Glu and Gln in comparison to 30 ms PRESS [17], concluding that the *J*-resolved technique is superior in accurately and precisely measuring Glu and Gln compared to 30 ms PRESS, which tended to overestimate Glu and Gln concentrations with higher variance between repeated measures. Our previous claim is strongly supported by the current study. However, in this study we extend the comparison to include GABA measurements as well as Glu and Gln and have implemented a GABA-optimized MEGA-PRESS difference-editing technique for this purpose. The potential of the difference-edited method is to provide not only an edited GABA spectrum, but also to derive measures of Glu and Gln from the 68 ms sub-spectrum. Although our results clearly indicate that MEGA-PRESS is the best way to measure GABA, the 68 ms sub-spectra did not fare well in the measurement of Gln (Fig. 3). This technique does, however, suggest that one could potentially obtain a reasonable measure of Glu and optimal GABA in one MEGA-PRESS scan.

Past studies have assessed the efficacy of obtaining optimal and simultaneous measures of Glu and Gln from the TE-averaged spectrum [27]. In the TE-averaged spectrum, which is equivalent to the *J* = 0.0 Hz spectral extraction in the *J*-resolved method, both Glu and Gln have a "pseudo-singlet" at 2.35 ppm and 3.75 ppm respectively, which is the result of the coupled sidebands of these former multiplets canceling out [27]. However, although the reliable quantification of Glu benefits greatly from this method, manifested as a near-solitary single resonance at 2.35 ppm, the Gln "pseudo-singlet" at 3.75 ppm is much more hindered due to less abundance and severe overlap from residual bleed from myo-inositol (mI) and Glu. A recent method to iteratively and automatically fit the entire topological surface of the 2D *J*-resolved spectrum has been proposed, which uses prior spectral knowledge of in vivo metabolites to quantify all coupled and uncoupled resonances in the 2D-spectrum [28,29]. Unlike our LCModel serial fitting approach of JPRESS spectra presented here, this topological method represents a truly two-dimensional approach as the entire *J*-resolved real spectral

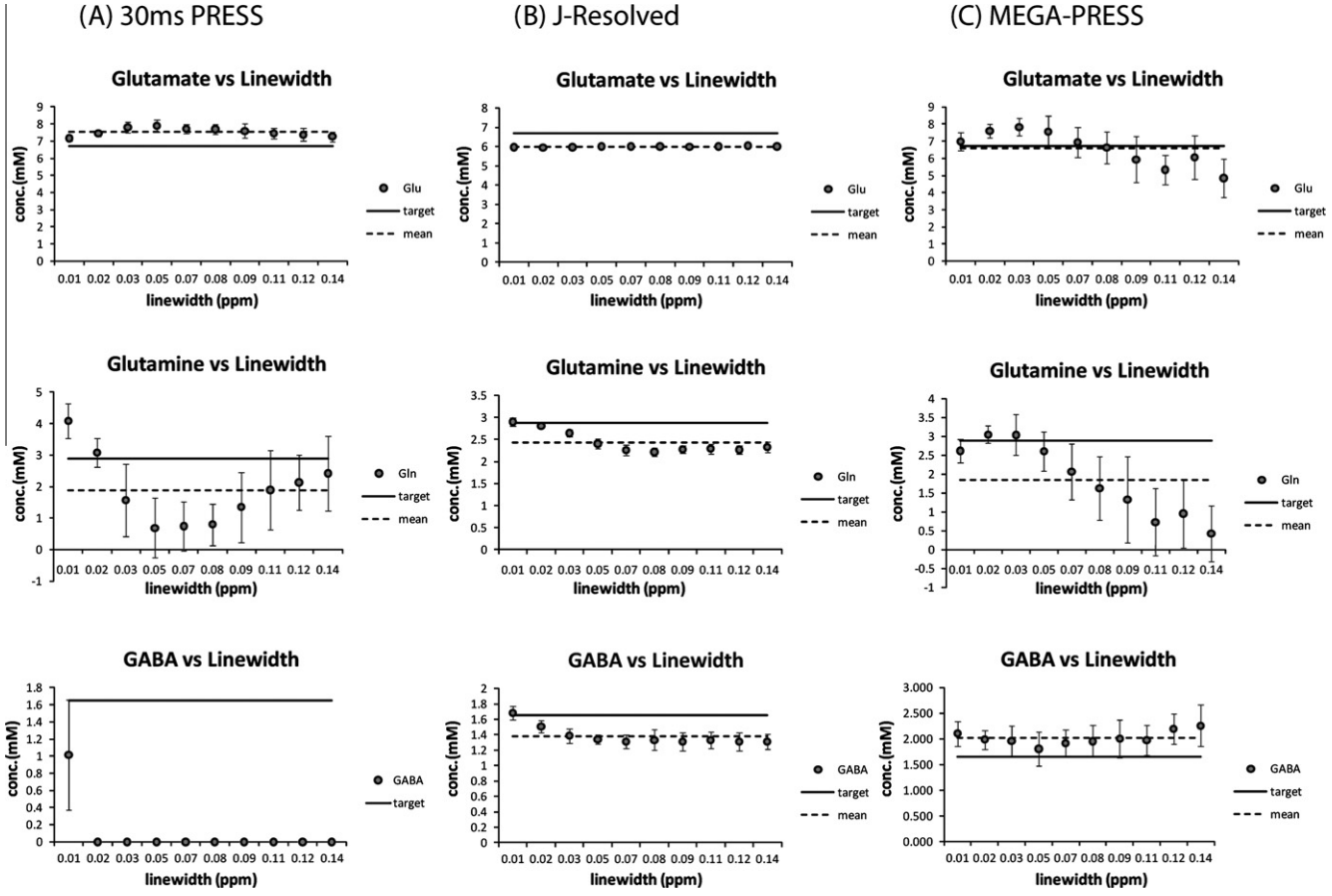

Fig. 4. Monte-Carlo simulation plots of measured concentrations of GABA, Glu, and Gln versus Gaussian linewidth and signal-to-noise for the three methods. Each point represents the mean measurement over 20 repeated measures with each simulated line-broadening level. The NAA signal-to-noise (from LCModel) for each point ranges from 18 to 30 (30 ms PRESS), 15–24 (*J*-resolved 0.0 Hz), 13–22 (68 ms MEGA-PRESS "OFF" spectrum). Error bars represent the standard deviation over 20 repeated measures.

surface is simultaneously fitted in both the directly-detected dimension (chemical-shift) and indirectly-detected dimension (f1). This would theoretically offer an advantage over our own LCModel method as it is actively performing a multi-parameter fit in both the chemical-shift and *J*-frequency domains simultaneously, which makes more efficient use of the available spectral data. Nonetheless, when compared to our own approach to fitting the entire *J*-resolved spectrum using serial LCModel fits [17], both methods show considerable improvements in quantifying Glu and Gln compared to 30 ms PRESS. Both methods also suggest a stable GABA measurement, which would indicate that the *J*-resolved technique could potentially offer the best compromise in yielding quality and simultaneous measures of GABA, Glu, and Gln.

A secondary analysis to assess the effects of variable spectral quality on the measurement of GABA, Glu, and Gln at in vivo concentrations provides further insight into the relative performance of these techniques. In our Monte-Carlo based simulation, both 30 ms PRESS and especially *J*-resolved techniques indicate stable measures of Glu over a range of spectral qualities typically encountered in vivo (Fig. 4) with 30 ms PRESS slightly overshooting and *J*-resolved slightly undershooting the Glu target value of 6.7 mM. However upon closer inspection of the 30 ms PRESS Glu data, one can see a distinct and opposite response of the Glu estimates with linewidth, compared to the 30 ms PRESS Gln response. As linewidth increases, Glu estimates show an initial rise while the Gln estimates show a corresponding decrease. In the highly overlapped spectral region of Glu and Gln, it appears that with 30 ms PRESS, the Glu and Gln measures seem highly interdependent and correlated in a negative manner that is solely reliant on linewidth. This observation really brings into question the ability of 30 ms PRESS to reliably and separately measure Glu and Gln in vivo where conditions can vary widely. Also of note is the seemingly high fluctuation and dependence of Gln with smaller fluctuations of Glu. With linewidth variation, Glu ranges between approximately 7 and 8 mM, whereas Gln ranges between 0.8 and 4 mM. Hence, it seems that with 30 ms PRESS, any small deviation in fitting Glu will have a large, direct and opposite effect on Gln estimation. In stark contrast are the results for JPRESS which show virtually no dependence of Glu estimation with linewidth, and thus no sign of interdependence of Gln estimates on Glu values. Although JPRESS Gln estimation does display a dependence on linewidth, this dependence seems to level off and stabilize at a certain point.

MEGA-PRESS Glu measures (derived from the 68 ms sub-spectrum) display considerable instability, indicating a sensitivity to spectral quality that would potentially render this method inadequate to obtain reliable in vivo Glu data (Fig. 4). Both 68 ms MEGA-PRESS (sub-spectral measures) and 30 ms PRESS display extreme dependence of Gln measures on spectral quality whereas *J*-resolved shows drastically less instability over the spectral quality range (Fig. 4). For GABA measurements, MEGA-PRESS shows the least dependence on spectral quality whereas the *J*-resolved technique seems to suggest some sensitivity especially in the well-shimmed range, with stability achieved with the broader linewidths. The 30 ms PRESS technique is simply unable to make any reliable measures of GABA as seen in Fig. 4. Taken together, both the repeated variable concentration phantom tests (Fig. 3) and the variable spectral quality tests at physiologic levels (Fig. 4) support the idea that the *J*-resolved method may offer the best chance of obtaining reliable and simultaneous measures of GABA, Glu, and Gln with, of course, the compromise of less stable GABA measurements compared to MEGA-PRESS.

4.1. Limitations and future directions

This phantom study provides valuable information pertaining to the relative performance of accurately, precisely and simultaneously measuring GABA, Glu, and Gln under simulated in vivo conditions at high field (4 Tesla). However, most clinical systems operate at 3 Tesla field strength, which yields less spectral resolution; thus, our 4 Tesla data presented here may not be directly applicable to 3 Tesla studies. Nonetheless, we feel that this study serves as a basic starting point for future studies which seek to quantitatively determine the optimal MRS technique to answer the appropriate clinical questions. Hence, it should serve as a valuable guide to clinicians interested in these three brain compounds which have been implicated in neuropsychiatric disorders. However, the current study does have some limitations which may have affected the results and are thus worthy of review and discussion.

Our variable phantom concentration repeated measures study measured the reproducibility of GABA, Glu, and Gln acquisition and quantification over time. These results should provide an estimate of scanner machine performance as well as operator performance over time. These factors, combined with the base noise level of the measurements would be expected to result in some variance. However, an additional source of variance in this study is likely related to the lack of stability of some of the more labile metabolites in our phantom solutions [30,31]. Such compounds as Gln and N-acetylaspartylglutamate (NAAG) are apparently less stable in solution compared to Glu, Cr, and NAA. Although based on our experience in making LCModel phantom stock solutions as well as Provencher's LCModel guide, we were aware that some compounds have a relatively short half-life in solution, we initially assumed that any degradation of these less stable compounds would be minimal over the course of a month. Nonetheless, to err on the side of caution we only included the first two time-points (baseline and 1 week) for Gln to minimize the chances of our repeated measures variance being significantly affected by Gln degradation. In future studies this variance could be eliminated by making the aqueous phantom solutions from dry, pre-measured chemical mixtures immediately before each measurement time-point.

Another possible caveat concerns the LCModel template design and construction. Our 30 ms PRESS and *J*-resolved template were the result of GAMMA-based simulations. Simulated templates do have definite advantages over phantom-based templates such as increased spectral resolution, absence of baseline noise and no spectral or baseline artifact. Thus, LCModel fits with simulated templates tend to yield lower Cramer-Rao standard deviations compared to phantom-based templates. Simulated templates become especially important in the construction of T2-dependent models such as in our featured *J*-resolved fitting template [17], where the decay for each modeled metabolite can be simulated and precisely controlled. Conversely, phantom-acquired fitting templates do provide the advantage of including acquisition-specific features for a given pulse sequence and parameter set such as eddy-current and shim effects which would affect lineshape and phase. Thus, one could argue that phantom-based templates would more closely approximate the actual acquired spectrum. Although a rigorous comparison of simulated versus phantom-acquired LCModel templates is beyond the scope of this work, it is noteworthy to point out that our combination of simulated and phantom-based templates may have introduced a possible bias in our results, although it is likely minimal. A future comparative study would endeavor to utilize either all simulated or all phantom-based templates.

The original aim of this study was to provide the most optimal measures of GABA, Glu and Gln in phantoms that are free of spectral baseline features such as macromolecules and lipids which can vary widely in vivo and hinder quantification. Also, phantoms provide the most stable and controlled biochemical measures as they are not subject to the biological variance sources encountered in vivo. While we did strive to simulate noise and linewidth

conditions that exist in vivo, we did not undertake an in vivo study to complement our phantom results. Although an in vivo comparison of these MRS methods would be helpful, it was not our aim to do so. While it is likely that some investigators will move toward using numerical simulations instead of phantom data for basis sets as described by Kaiser and colleagues [32], solutions of metabolites still offer the advantage of more closely replicating experimental conditions. Clearly, reproducibility in human subjects is key to evaluating different editing sequences. However, given the variability of the human brain across time, fundamental questions about the accuracy of these methods can be answered only with phantoms where the actual concentration of each metabolite is known.

Finally, there are technical issues concerning the acquisition of spectral data that would need to be addressed in future studies for further refinement of technique comparison. Although we did use an optimal WET water-suppression technique [19], we observed some degree of water-suppression variability in our phantom data, likely due to the fact that our WET sequence is optimized to in vivo water and not phantoms. This fact, combined with the variable levels of residual water-to-metabolite level, which is acquisition and processing-dependent, would introduce a possible additional source of variance into our data due to differing spectral baselines. Future studies would optimize the water suppression for phantom work. For the MEGA-PRESS sequence, it has been shown that an increase in GABA-editing efficiency of ~24% can be achieved through the use of the inner-volume saturation (IVS) technique to account for the spatially-dependent variance of the *J*-coupled evolution of the GABA spin system, a significant source of signal loss in the standard MEGA-PRESS experiment [33]. Future studies would make use of this technology to improve results.

In summary, this phantom study reveals that the *J*-resolved technique offers the most potential for obtaining optimal and simultaneous measures of GABA, Glu, and Gln in a single acquisition. Although the specialized GABA-editing difference technique of MEGA-PRESS provides the most accurate and stable measure of GABA, it falls short in its ability to obtain reliable and stable measures of Glu and Gln. The commonly used and widely available single-echo 30 ms PRESS acquisition seems only useful for obtaining reliable measures of Glu as well as the dominant resonances as choline, Cr, and NAA and does not allow for the realistic measurement of GABA and Gln.

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References

[1] G. Sanacora, G.F. Mason, D.L. Rothman, K.L. Behar, F. Hyder, O.A. Petroff, R.M. Berman, D.S. Charney, J.H. Krystal, Reduced cortical gamma-aminobutyric acid levels in depressed patients determined by proton magnetic resonance spectroscopy, Arch. Gen. Psychiatry 56 (1999) 1043–1047.
[2] G. Sanacora, R. Gueorguieva, C.N. Epperson, Y.T. Wu, M. Appel, D.L. Rothman, J.H. Krystal, G.F. Mason, Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression, Arch. Gen. Psychiatry 61 (2004) 705–713.
[3] G. Hasler, J.W. van der Veen, T. Tumonis, N. Meyers, J. Shen, W.C. Drevets, Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy, Arch. Gen. Psychiatry 64 (2007) 193–200.
[4] J.R. Bustillo, L.M. Rowland, P. Mullins, R. Jung, H. Chen, C. Qualls, R. Hammond, W.M. Brooks, J. Lauriello, (1)H-MRS at 4 Tesla in minimally treated early schizophrenia, Mol. Psychiatry (2009).
[5] J. Theberge, R. Bartha, D.J. Drost, R.S. Menon, A. Malla, J. Takhar, R.W. Neufeld, J. Rogers, W. Pavlosky, B. Schaefer, M. Densmore, Y. Al-Semaan, P.C. Williamson, Glutamate and glutamine measured with 4.0 T proton MRS in never-treated patients with schizophrenia and healthy volunteers, Am. J. Psychiatry 159 (2002) 1944–1946.
[6] G. Sanacora, A. Saricicek, GABAergic contributions to the pathophysiology of depression and the mechanism of antidepressant action, CNS Neurol. Disord. Drug Targets 6 (2007) 127–140.
[7] F.M. Benes, S. Berretta, GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder, Neuropsychopharmacology 25 (2001) 1–27.
[8] G. Sperk, S. Furtinger, C. Schwarzer, S. Pirker, GABA and its receptors in epilepsy, Adv. Exp. Med. Biol. 548 (2004) 92–103.
[9] A. Wassef, J. Baker, L.D. Kochan, GABA and schizophrenia: a review of basic science and clinical studies, J. Clin. Psychopharmacol. 23 (2003) 601–640.
[10] K. Domschke, P. Zwanzger, GABAergic and endocannabinoid dysfunction in anxiety – future therapeutic targets?, Curr Pharm. Des. 14 (2008) 3508–3517.
[11] J.R. Keltner, L.L. Wald, B.D. Frederick, P.F. Renshaw, In vivo detection of GABA in human brain using a localized double-quantum filter technique, Magn. Reson. Med. 37 (1997) 366–371.
[12] J. Shen, D.L. Rothman, P. Brown, In vivo GABA editing using a novel doubly selective multiple quantum filter, Magn. Reson. Med. 47 (2002) 447–454.
[13] R. Gruetter, E.J. Novotny, S.D. Boulware, D.L. Rothman, G.F. Mason, G.I. Shulman, W.V. Tamborlane, R.G. Shulman, Non-invasive measurements of the cerebral steady-state glucose concentration and transport in humans by 13C nuclear magnetic resonance, Adv. Exp. Med. Biol. 331 (1993) 35–40.
[14] I. Tkac, P. Andersen, G. Adriany, H. Merkle, K. Ugurbil, R. Gruetter, In vivo 1H NMR spectroscopy of the human brain at 7 T, Magn. Reson. Med. 46 (2001) 451–456.
[15] I. Tkac, Z. Starcuk, I.Y. Choi, R. Gruetter, In vivo 1H NMR spectroscopy of rat brain at 1 ms echo time, Magn. Reson. Med. 41 (1999) 649–656.
[16] R. Gruetter, S.A. Weisdorf, V. Rajanayagan, M. Terpstra, H. Merkle, C.L. Truwit, M. Garwood, S.L. Nyberg, K. Ugurbil, Resolution improvements in in vivo 1H NMR spectra with increased magnetic field strength, J. Magn. Reson. 135 (1998) 260–264.
[17] J.E. Jensen, S.C. Licata, D. Ongur, S.D. Friedman, A.P. Prescot, M.E. Henry, P.F. Renshaw, Quantification of *J*-resolved proton spectra in two-dimensions with LCModel using GAMMA-simulated basis sets at 4 Tesla, NMR Biomed. 22 (2009) 762–769.
[18] V. Govindaraju, K. Young, A.A. Maudsley, Proton NMR chemical shifts and coupling constants for brain metabolites, NMR Biomed. 13 (2000) 129–153.
[19] R.J. Ogg, P.B. Kingsley, J.S. Taylor, WET, a T1- and B1-insensitive water-suppression method for in vivo localized 1H NMR spectroscopy, J. Magn. Reson. B 104 (1994) 1–10.
[20] P.A. Bottomley, Spatial localization in NMR spectroscopy in vivo, Ann. N. Y. Acad. Sci. 508 (1987) 333–348.
[21] M. Mescher, H. Merkle, J. Kirsch, M. Garwood, R. Gruetter, Simultaneous in vivo spectral editing and water suppression, NMR Biomed. 11 (1998) 266–272.
[22] D.L. Rothman, O.A. Petroff, K.L. Behar, R.H. Mattson, Localized 1H NMR measurements of gamma-aminobutyric acid in human brain in vivo, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 5662–5666.
[23] K. Young, V. Govindaraju, B.J. Soher, A.A. Maudsley, Automated spectral analysis I: formation of a priori information by spectral simulation, Magn. Reson. Med. 40 (1998) 812–815.
[24] S.A. Smith, T.O. Levante, B.H. Meier, R.R. Ernst, Computer simulations in magnetic resonance. an object-oriented approach, J. Magn. Reson. A 106 (1994) 75–105.
[25] S.W. Provencher, Estimation of metabolite concentrations from localized in vivo proton NMR spectra, Magn. Reson. Med. 30 (1993) 672–679.
[26] W. Bogner, S. Gruber, M. Doelken, A. Stadlbauer, O. Ganslandt, U. Boettcher, S. Trattnig, A. Doerfler, H. Stefan, T. Hammen, In vivo quantification of intracerebral GABA by single-voxel (1)H-MRS-How reproducible are the results?, Eur J. Radiol. 73 (2010) 526–531.
[27] R. Hurd, N. Sailasuta, R. Srinivasan, D.B. Vigneron, D. Pelletier, S.J. Nelson, Measurement of brain glutamate using TE-averaged PRESS at 3 T, Magn. Reson. Med. 51 (2004) 435–440.
[28] R.F. Schulte, P. Boesiger, ProFit: two-dimensional prior-knowledge fitting of *J*-resolved spectra, NMR Biomed. 19 (2006) 255–263.
[29] R.F. Schulte, T. Lange, J. Beck, D. Meier, P. Boesiger, Improved two-dimensional *J*-resolved spectroscopy, NMR Biomed. 19 (2006) 264–270.
[30] K. Arii, H. Kobayashi, T. Kai, Y. Kokuba, Degradation kinetics of L-glutamine in aqueous solution, Eur. J. Pharm. Sci. 9 (1999) 75–78.
[31] J. Coster, R. McCauley, J. Hall, Glutamine: metabolism and application in nutrition support, Asia Pac. J. Clin. Nutr. 13 (2004) 25–31.
[32] L.G. Kaiser, K. Young, G.B. Matson, Numerical simulations of localized high field 1H MR spectroscopy, J. Magn. Reson. 195 (2008) 67–75.
[33] R.A. Edden, P.B. Barker, Spatial effects in the detection of gamma-aminobutyric acid: improved sensitivity at high fields using inner volume saturation, Magn. Reson. Med. 58 (2007) 1276–1282.