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Localized MRS Reliability of In vivo Glutamate at 3 Tesla in Shortened Scan Times: A Feasibility Study

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Abstract

Glutamate is the prime excitatory neurotransmitter in the mammalian brain and has been implicated in a wide range of psychiatric conditions. To improve the applicability and clinical reach of MRS, research is needed to develop shortened, yet reliable, MRS scanning procedures for standard 1.5-3T clinical MRI systems, particularly with youth or vulnerable populations unable to tolerate longer protocols. To this end, we evaluated the test-retest reliability of a shortened J-resolved MRS sequence in healthy adolescents ($n = 22$) aged 12-14 years. Participants underwent a series of sequential 6-minute MRS scans, with the participants remaining in situ between successive scans. Glutamate and other metabolites were acquired from the rostral anterior cingulate cortex, as glutamatergic function in this region has been implicated in a number of psychiatric illnesses. Thirteen neurochemicals were quantified as ratios to total creatine, and reliability scores were expressed as a percent difference between the two scans for each metabolite. Test-retest assessment of glutamate was reliable, as scores were less than 10% different ($7.1 \pm 4.2\%$), and glutamate values across scans were significantly correlated (Pearson $r = 0.680$, $p < 10^{-4}$). Several other neurochemicals demonstrated satisfactory reliability, including Cho ($7.4 \pm 5.6\%$), GSH ($8.6 \pm 4.1\%$), mI ($6.5 \pm 7.1\%$), and NAA ($3.5 \pm 3.6\%$), with test-retest correlations ranging from 0.747 to 0.953. A number of metabolites, however, did not demonstrate acceptable test-retest reliability using the current J-resolved MRS sequence, ranging from $13.8 \pm 13.7\%$ (Asp) to $45.9 \pm 38.3\%$ (Gly). Collectively, test-retest analyses suggest that clinically viable quantitative data can be obtained on standard MRI systems for glutamate, as well as the other metabolites, during short scan times in a traditionally challenging brain region.

Graphical abstract

Glutamate is the prime excitatory neurotransmitter in the mammalian brain and has been implicated in neuropsychiatric illness. To improve the applicability of MRS, research is needed to develop abbreviated, yet reliable, MRS scanning procedures. Here, we evaluated the test-retest

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reliability of a shortened J-resolved MRS sequence prescribed to the anterior cingulate cortex in healthy adolescents. Findings revealed satisfactory to excellent test-retest reliability for five metabolites, including glutamate.

Glutamate

N-acetylaspartate

Keywords

Glutamate; Adolescents; Spectroscopy; Reliability; Reproducibility; Imaging; Anterior Cingulate Cortex

Introduction

Glutamate is the most abundant excitatory neurotransmitter and can be non-invasively assessed using magnetic resonance spectroscopy (MRS). While earlier studies using low-field strength were unable to separate the chemical signatures of glutamate and glutamine, recent studies using advanced acquisition techniques have increasingly identified abnormal glutamate neurotransmission in mood disorders.¹ In fact, low glutamate levels may constitute a trait-like vulnerability factor for major depressive disorder (MDD). Studies have described glutamate abnormalities in children and adults with MDD as well as euthymic individuals with an MDD history.¹ While glutamate alterations have been reported in multiple brain areas, a recent meta-analysis found that decreased glutamate in the anterior cingulate cortex is a robust finding in MDD, suggesting glutamate plays a critical role in the onset and maintenance of depressive disorders.^{2,3} Research published after (or omitted from)

this meta-analysis also provides evidence of reduced glutamate concentration in the anterior cingulate cortex (ACC) of depressed subjects with effect sizes ranging from medium to large.^{4,5} Highlighting the clinical significance of these findings, increased pre-treatment glutamate levels in the ACC predicted better electroconvulsive therapy response in MDD.⁶

Despite advancements, MRS research has been marked by two primary limitations. First, prior MRS research has often probed metabolites at low field strength, particularly 1.5-2.0T, which suffers from lack of spectral and spatial resolution. More recently, the advent of higher magnetic field scanners (3T-9.4T) has vastly improved spectral and spatial resolution (due to increased peak dispersion and higher signal-to-noise ratio). Nevertheless, there are often constraints on limiting the magnetic field strength to 3T for human research—especially for studies in youth—and at times, higher field strengths are often cost prohibitive and not easily accessible. Second, given advancements in MRS, an abundance of psychiatric studies have begun probing metabolites implicated in illness onset and treatment. These populations may be poorly suited to tolerate extensive scans, which is common practice in research protocols. Collectively, there is a critical need to develop shortened and reliable MRS protocols at 3T, especially in vulnerable populations, to detect less abundant and multi-resonance metabolites (e.g., glutamate, glutamine, and GABA).

Recent advances in MRS data acquisition strategies also have sought to improve data quality—particularly in MRS protocols with a shorter acquisition period at lower field strengths. J-resolved MRS is a method that exploits the J-coupling properties of many brain metabolites, and provides traditional one-dimensional spectral information and resolves the spectral resonances of highly overlapping and low-abundant metabolites.⁷⁻¹⁴ J-resolved MRS studies report improved detection accuracy and precision of most brain metabolites, in particular those that are highly overlapped and J-coupled. In this reliability study, we utilized an optimized J-resolved PRESS MRS method at 3T to enhance metabolite detection.

To address important limitations of prior work, we conducted a test-retest reliability and feasibility study of 6-minute J-resolved MRS scans with participants remaining in situ between successive scans. Given prior work implicating glutamatergic dysfunction in MDD,¹ data were acquired from a single 12 cm³ (2×2×3 cm) voxel in the rostral ACC. Data were collected in healthy adolescents, aged 12-14 years, to minimize neurodevelopmental and pubertal differences. We hypothesized that glutamate would show appropriate test-rest reliability. In secondary analyses, we evaluated test-retest reliability for 12 other neurochemicals.

Methods

Participants

Participants included 23 healthy adolescents recruited from the greater Boston area through online advertisements, posted flyers, and direct mailings. One participant was excluded from the analyses due to outlier status stemming from a low signal-to-noise ratio. Thus, the final sample included 22 healthy adolescents (12 female, 10 male) aged 12-14 years ($M = 12.95$, $SD = 0.72$). Inclusion criteria included English fluency and right-handedness. Exclusion criteria included lifetime diagnosis of any psychopathology, mental retardation, organic

brain syndrome, head injury resulting in loss of consciousness for 5 minutes, or seizures. Participants endorsed the following races: 77.3% White, 4.5% Asian, 4.5% Black or African American, and 13.6% multiple races. The annual parental income distribution of the study participants included the following: 63.6% = more than \$100,000, 13.6% = \$75,000 to \$100,000, 4.5% = \$50,000 to \$75,000, and 18.2% = not reported.

Procedure

The Partners Institutional Review Board provided approval for the study. Adolescents provided assent, and legal guardians provided written consent. The research study included two study visits. On the first visit, adolescents completed a semi-structured diagnostic interview of lifetime mental illness. During the second study visit, participants completed a magnetic resonance imaging scan, during which MRS data were acquired. To test the reliability of the MRS data, two consecutive 6-minute MRS acquisition scans were completed with the participants remaining in situ between successive scans. The MRS data acquisitions were back-to-back, with no delay or re-acquisition of anatomical images between MRS scans. The average length between the first (clinical characterization) and second (MRS acquisition) visit was 9.41 ± 6.38 days. Participants were remunerated for their participation.

Clinical Interview

Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present (K-SADS-PL).¹⁵—The K-SADS-PL is a semi-structured clinical interview used to assess current and past psychiatric disorders according to the Diagnostic Statistical Manual (DSM; version: IV-TR),¹⁶ and past research has demonstrated excellent reliability and validity.¹⁷ Graduate students and bachelor's-level research assistants administered the clinical interview after receiving 40 hours of training, which included didactics, mock interviews, and direct supervision. A clinically licensed psychologist (RPA) reviewed 20% of the interviews selected at random to assess interrater reliability, and the Cohen's kappa coefficients were excellent ($\kappa = 1.00$).

Magnetic Resonance Spectroscopy Acquisition—MRS data were collected on a 3T Siemens TRIO Tim, whole-body, clinical MR system (Erlangen, Germany) using a 32-channel phased-array design RF head coil operating at 123 MHz for proton imaging and spectroscopy. High-resolution T1-weighted anatomical images were used to position a single 12 cm^3 ($2 \times 2 \times 3 \text{ cm}$) voxel in the rostral ACC (Figure 1). Proton MRS employed a modified J-resolved PRESS protocol (2D-JPRESS). Shimming of the magnetic field within the prescribed voxel was first done automatically using an automated shimming routine. Afterwards, the voxel shim was further refined manually using the interactive shim function to achieve unsuppressed water linewidths ranging from 7-10 Hz. Following the additional automated optimization of water suppression power, carrier-frequency, tip angles and coil tuning, the 2D-JPRESS sequence collected 22 echo time (TE)-stepped spectra with the echo-time ranging from 30 ms to 350 ms in 15 ms increments. Acquisition parameters were: repetition time (TR) = 2s, f1 acquisition bandwidth = 67 Hz, spectral bandwidth = 2 kHz, readout duration = 512 ms, NEX = 8/TE-step, steady-state scans/TE-step = 1, total scan duration = 6.5 min. MRS acquisition parameters were chosen based on our prior J-resolved

study at 4T where we collected 24 TE-steps.¹³ Our choice of an NEX of 8 is based primarily on two criteria: (i) as short a scan time as feasible while still obtaining usable signal-to-noise and (ii) cycling through a complete receiver phase-cycle to minimize outer-volume signal contamination and maximize spectral quality. The MRS data acquisitions were back-to-back, with no delay or re-acquisition of anatomical images between MRS scans.

Proton MRS Processing and Analysis—All spectroscopic data processing and analysis was undertaken on a LINUX workstation. In order to quantify in vivo brain metabolites with the JPRESS data, the 22 TE-stepped free-induction decay (FIDs) were first zero-filled out to 64 points, Gaussian-filtered, and Fourier transformed, consistent with our previously published methods.¹³ Prior to Fourier Transform in f1, every TE-stepped spectrum was first truncated at 256 points to eliminate the Gaussian noise from the tail end of the FID. Then, each TE-stepped spectrum was phase-corrected based on the prominent N-acetylaspartate (NAA) resonance at 2.00ppm using an automated phase-correcting routine developed in house. These two pre-processing steps ensure maximum signal-to-noise in the final, J-resolved 2D spectra prior to LCModel fitting^{13,18,19}, while maintaining spectral resolution. Every J-resolved spectral extraction within a bandwidth of 67 Hz was fitted with LCModel and its theoretically-correct template, which used an optimized GAMMA-simulated J-resolved basis sets modeled for 3T.¹³ The GAMMA-simulated model fits the J-resolved spectral signatures for the following metabolites: total creatine (Cr), aspartate (Asp), choline (Cho), γ -aminobutyric acid (GABA), glutamate (Glu), glutamine (Gln), glutathione (GSH), glycine (gly), myo-inositol (mI), N-acetylaspartate (NAA), N-acetyl-aspartyl-glutamate (NAAG), scyllo-inositol (Scy), taurine (Tau), and lactate (Lac). The integrated area under the entire 2D surface for each metabolite was calculated by summing the raw peak areas across all 64 J-resolved extractions for each metabolite. All metabolites were expressed as ratios to total creatine (Cr). Representative 1D spectra ($J=0.0$ Hz) are shown in Figure 1 whereas a full 2D plot depicting the complex J-coupling patterns of the in vivo metabolites is shown in Figure 2.

Statistical Analysis—For all thirteen neurochemicals quantified, the percent difference (%diff) for each individual subject was calculated between the two scans using the following formula:

$$\%diff = (\text{scan2} - \text{scan1}) / \text{mean}(\text{scan2}, \text{scan1}) \times 100\%$$

Then, a group average of %diff reliability scores was calculated for each metabolite. In addition, for each metabolite, Pearson correlations were computed between the two scans to test reliability. Correlation results were interpreted exclusively for metabolites showing satisfactory variance between scan1 and scan2 (operationalized as <10% difference). Finally, a repeated measure analysis of variance (RMANOVA) tested main effects for *Time* (Time 1, Time 2) for each metabolite that demonstrated <10% difference and showed sufficient test-retest reliability. To demonstrate stability, we anticipated that the main effect for *Time* would be non-significant.

Results

Table 1 summarizes the reliability scores for each participant and for all thirteen metabolites as well as the group mean and standard deviation of reliability scores. Cho ($7.4 \pm 5.6\%$), Glu ($7.1 \pm 4.2\%$), GSH ($8.6 \pm 4.1\%$), mI ($6.5 \pm 7.1\%$), and NAA ($3.5 \pm 3.6\%$) had group reliability scores under 10%, which is indicative of clinically viable reliability. The remaining metabolites did not show sufficient reliability, ranging from $13.8 \pm 13.7\%$ (Asp) to $45.9 \pm 38.3\%$ (Gly) (see Table 2). As shown in Table 2 and Figure 3A-E, Pearson correlations for all metabolites showing satisfactory variance ($<10\%$ difference) were all highly significant; Cho: $r = 0.747$, $p < 10^{-5}$; Glu: $r = 0.680$, $p < 10^{-4}$; GSH: $r = 0.821$, $p < 10^{-5}$; mI: $r = 0.748$, $p < 10^{-4}$; NAA: $r = 0.953$, $p < 10^{-11}$.

We also conducted a RMANOVA to test the stability over time of metabolites demonstrating $<10\%$ concentration difference between scan 1 and scan 2 and showed sufficient test-retest reliability. With the exception of Cho, $F(1,21) = 4.78$, $p = 0.04$, $\eta^2 = 0.19$, all other metabolites showed no significant change from Time 1 to Time 2 (all p s $> .10$).

Discussion

The current 2D J-resolved approach on a 3T clinical MRS system provided clinically acceptable test-retest reliability in several key metabolites, including Glu, GSH, mI and NAA with short scan times (~6 minute) and in a traditionally challenging region of the brain, the rostral ACC. This high degree of test-retest reliability and stability with the subject in situ between repeated scans indicates that Glu (as well as other selected metabolites) can be reliably probed using a short scan. Establishing reproducibility opens up many possibilities for high temporal resolution MRS studies on a 3T clinical scanner, especially in populations with low tolerance to the scanning process (e.g., neuropsychiatric samples and young children with normal or atypical development). Short, reliable, and stable scans also will provide an ideal platform for functional MRS scans (e.g., exposing participants to emotional elicitations during the MRS scans). With improvements in scanner hardware and processing methods, further improvements in temporal resolution are expected.

Although Glu was the primary measure of interest in this particular study, the ability to reliably measure other important metabolites such as GSH and mI paves the way for short and/or high temporal resolution MRS studies at 3T that focus on oxidative stress and neuroinflammation in which GSH and mI have been implicated.²⁰⁻²² Other highly coupled and temporally dynamic metabolites such as GABA and Gln, however, were characterized by poor test-retest reliability indicating that the current acquisition sequence does not provide a reliable platform for detecting time- and stimulus-dependent fluctuations in such metabolites. Nevertheless, metabolite-specific editing MRS techniques such as MEGAPRESS²³ may prove useful in obtaining reliable measures of GABA in a temporally-sensitive paradigm, yet may come at the expense of other important metabolites. More generally, with the improvements in scanner hardware at 3T as well as MRS acquisition methodology and processing/analysis, it is likely that the temporal resolution of clinical MRS will markedly improve.

As with any investigation, the current study is not without limitations. First, it was assumed that the participants did not move between each 6-minute successive MRS scans. Unfortunately, we did not acquire any localizer images between MRS scans so we cannot evaluate the potential confounding effect of participant motion.

Second, only a single methodology was tested with respect to both data acquisition and processing/analysis. Ideally, from a data acquisition standpoint, we would have quantitatively compared several, more advanced MRS techniques with our current uniformly-sampled J-PRESS sequence. Other MRS methods include advanced 1-dimensional techniques (e.g., ultra short-echo STEAM,²⁴ LASER/SEMI-LASER²⁵) and more advanced 2-dimensional techniques (e.g., maximum-echo based J-PRESS²⁶ or non-uniformly-sampled J-PRESS^{27,28})—with both sets of techniques likely offering increased sensitivity in the detection of glutamate, glutamine, and GABA. In addition, even more advanced spectral acquisition techniques such as compressed sensing, can greatly reduce MRS data acquisition times while still preserving the detailed spectral information of highly coupled metabolites²⁹ as well as alternative spectral analysis techniques such as maximum entropy reconstruction.³⁰ However, given the limitations of scanning young children with relatively low tolerance, as well as difficulty implementing these advanced acquisition schemes on a clinical system, these comparisons were not feasible.

Third, in terms of post-processing, our serial LCModel approach is but one of many strategies that have been employed in 2D MRS quantification. Although our LCModel technique has been shown to work well, it does not exploit the full essence of the 2D-J-resolved spectrum such as ProFit.^{31,32} In particular, our serial LCModel technique treats each J-resolved spectral extraction as a separate entity, focusing primarily on the ppm axis, whereas ProFit models consider both the directly- and indirectly-detected dimensions, thus harnessing the full wealth of 2-dimensional spectral information of the coupled metabolite signals. Alternative and/or more advanced post-processing methods such as straight TE-averaging for optimal Glu detection³³ or phase-adjusted TE-averaging (PATE)³⁴ for improved Glu-Gln discrimination could have been employed for a more advanced comparative study.

Last, a water unsuppressed scan was not acquired in this study. Although there are definite benefits to obtaining a water-unsuppressed reference signal, this does have its drawbacks, namely CSF content variability and minor variations in fitting of the large water peak adding to potential errors in metabolite quantification. While creatine referencing does have some disadvantages, this study was focused on the immediate test-retest and stability of our MRS methodology and it was assumed that total creatine did not change. Future studies exploring high temporal resolution MRS and/or shortened studies should focus on a quantitative comparable analysis of all the current and available MRS acquisition and analysis techniques offered on a 3T clinical system.

In spite of these limitations, the current findings indicate that a widely available and easily implemented J-resolved technique yields reliable data for various important metabolites, including Glu, which opens intriguing avenues for the application of high temporal, short MRS data acquisition times in health and disease.

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Abbreviations used

ACC	Anterior Cingulate Cortex
DSM	Diagnostic Statistical Manual
FID	Free-induction decay
MDD	Major Depressive Disorder
K-SADS-PL	Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present

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Figure 1.

Representative J-resolved spectra from a 2×2×3 cm voxel in the rostral ACC for successive scans in the same participant. Spectra are from the J=0.0 Hz extraction (out of 64 extractions) and displayed with 1 Hz exponential filtering, LCModel fit and residual. The difference spectrum (scan 1 – scan 2) is displayed at the bottom.

Figure 2.

Two dimensional spectral plot of a rACC spectrum (B) showing the J-coupling patterns of the coupled metabolites across J-space. Select J-extractions along the f1 dimension (A) are plotted for 3.70ppm and 2.35ppm depicting the coupling pattern of Glu (2.35ppm), as well as the interplay between Glu, Gln and GSH (3.70ppm).

Figure 3.

Scatterplots and regression lines for (A) glutamate (Glu), (B) choline (Cho), (C) glutathione (GSH), (D) myo-inositol (ml), and (E) N-acetylaspartate (NAA) for the two scans.

Metabolite ratios are normalized to creatine (Cr).

Table 1

Individual Glu/Cr ratios and calculated % difference $[(\text{scan2} - \text{scan1})/\text{mean}(\text{scan2}, \text{scan1}) \times 100\%]$ for healthy adolescents (n = 22)

Subject #	Glu(scan 1)	Glu(scan 2)	% Difference
1	1.27	1.26	1%
2	1.14	1.22	7%
3	1.38	1.22	12%
4	1.43	1.37	5%
5	1.17	1.26	8%
6	1.18	1.41	18%
7	1.29	1.44	10%
8	1.34	1.24	7%
9	1.14	1.03	10%
10	1.25	1.25	1%
11	1.32	1.39	5%
12	1.18	1.32	11%
13	1.04	1.15	10%
14	1.18	1.24	5%
15	1.45	1.41	2%
16	1.32	1.34	2%
17	1.18	1.25	6%
18	1.19	1.32	10%
19	1.10	1.06	4%
20	1.08	0.99	9%
21	1.01	1.09	7%
22	1.19	1.22	2%

Table 2

Group average % difference for all metabolites and Pearson correlation across scans for healthy adolescents (n = 22).

Metabolites	% Difference	Pearson <i>r</i>	<i>p</i> -value
Asp	13.28	−0.130	0.564
Cho	7.29	0.747	<10 ^{−5}
GABA	43.98	−0.246	0.269
Glu	6.93	0.680	<10 ^{−4}
Gln	21.34	0.286	0.197
GSH	8.60	0.821	<10 ^{−5}
Gly	45.05	−0.041	0.857
mI	6.48	0.748	<10 ^{−4}
NAA	3.47	0.953	<10 ^{−11}
NAAG	19.53	0.774	<10 ^{−4}
Scy	26.96	0.738	<10 ^{−4}
Tau	30.21	0.530	0.011
Lac	35.77	−0.048	0.832

Note: aspartate (Asp), choline (Cho), γ -aminobutyric acid (GABA), glutamate (Glu), glutamine (Gln), glutathione (GSH), glycine (Gly), myo-inositol (mI), N-acetylaspartate (NAA), N-acetyl-aspartyl-glutamate (NAAG), scyllo-inositol (Scy), taurine (Tau), and lactate (Lac). *P*-values refer to Pearson *r* correlations.