Received: 1 January 2017 | Revised: 5 June 2017 | Accepted: 6 June 2017

DOI: 10.1002/nbm.3771

WILEY NMR IN BIOMEDICINE

RESEARCH ARTICLE

Localized MRS reliability of *in vivo* glutamate at 3 T in shortened scan times: a feasibility study

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Funding information
Tommy Fuss Fund; National Institute of Mental Health, Grant/Award Number: K23MH097786, R01MH101521 and R37MH068376; Klingenstein Third Generation Foundation, Grant/Award Number: Adolescent Depression Fellowship; The Dana Foundation, Grant/Award Number: Clinical Neuroscience Research Grant

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 magnetic resonance spectroscopy (MRS), research is needed to develop shortened, yet reliable, MRS scanning procedures for standard 1.5–3-T clinical magnetic resonance imaging (MRI) systems, particularly with young 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-min 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 the percentage difference between the two scans for each metabolite. Test–retest assessment of glutamate was reliable, as scores were less than 10% different (7.1 ± 4.2%), and glutamate values across scans were significantly correlated (Pearson r = 0.680, $p < 10^{-4}$). Several other neurochemicals demonstrated satisfactory reliability, including choline (Cho) (7.4 ± 5.6%), glutathione (GSH) (8.6 ± 4.1%), myo-inositol (mI) (6.5 ± 7.1%) and *N*-acetylaspartate (NAA) (3.5 ± 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 ± 13.7% (aspartate, Asp) to 45.9 ± 38.3% (glycine, 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.

KEYWORDS

adolescents, anterior cingulate cortex, glutamate, imaging, reliability, reproducibility, spectroscopy

1 | INTRODUCTION

Glutamate (Glu) is the most abundant excitatory neurotransmitter and can be non-invasively assessed using magnetic resonance spectroscopy (MRS). Although earlier studies using a low field strength were unable to separate the chemical signatures of Glu and glutamine (Gln), recent studies using advanced acquisition techniques have increasingly identified abnormal Glu neurotransmission in mood disorders.[1] Indeed, low Glu levels may constitute a trait-like vulnerability factor for major depressive disorder (MDD). Studies have described Glu abnormalities in

J. Eric Jensen and Randy P. Auerbach contributed equally to this work.

Abbreviations used: ACC, anterior cingulate cortex; Asp, aspartate; Cho, choline; Cr, total creatine; DSM, Diagnostic Statistical Manual; FID, free induction decay; GABA, γ-aminobutyric acid; Glu, glutamate; Glx, glutamate + glutamine; Gly, glycine; GSH, glutathione; K-SADS-PL, Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present; Lac, lactate; MDD, major depressive disorder; mI, myo-inositol; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartylglutamate; NEX, number of excitations; PATE, phase-adjusted TE averaging; PRESS, point-resolved spectroscopy; RMANOVA, repeated-measure analysis of variance; Scy, scyllo-inositol; STEAM, stimulated echo acquisition mode; TE, echo time; TR, repetition time; 2D–JPRESS, modified *J*-resolved point-resolved spectroscopy protocol

children and adults with MDD, as well as in euthymic individuals with an MDD history.[1] Although Glu alterations have been reported in multiple brain areas, a recent meta-analysis found that decreased Glu in the anterior cingulate cortex (ACC) is a robust finding in MDD, suggesting that Glu 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 Glu concentration in the ACC of depressed subjects with effect sizes ranging from medium to large.[4,5] Highlighting the clinical significance of these findings, increased pre-treatment Glu levels in the ACC predicted better electroconvulsive therapy response in MDD.[6]

Despite advancements, MRS research has been marked by two primary limitations. First, previous MRS research has often probed metabolites at low field strength, particularly 1.5–2.0 T, which suffers from a lack of spectral and spatial resolution. More recently, the advent of higher magnetic field scanners (3–9.4 T) has vastly improved the spectral and spatial resolution (as a result of increased peak dispersion and higher signal-to-noise ratio). Nevertheless, there are often constraints on limiting the magnetic field strength to 3 T for human research – especially for studies in youth – and, at times, higher field strengths are often cost prohibitive and not easily accessible. Second, given the 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 3 T, especially in vulnerable populations, to detect less abundant and multi-resonance metabolites [e.g. Glu, Gln and γ-aminobutyric acid (GABA)].

Recent advances in MRS data acquisition strategies have also 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 have reported 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 point-resolved spectroscopy (PRESS) MRS method at 3 T to enhance metabolite detection.

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

2 | METHODS

2.1 | 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 because of outlier status stemming from a low signal-to-noise ratio. Thus, the final sample included 22 healthy adolescents (12 females, 10 males), aged 12–14 years (mean, 12.95 years; standard deviation, 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 min 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%, $75 000–100 000; 4.5%, $50 000–75 000; 18.2%, not reported.

2.2 | 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 (MRI) scan, during which MRS data were acquired. To test the reliability of the MRS data, two consecutive 6-min 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.

2.3 | Clinical interview

2.3.1 | 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 h 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 inter-rater reliability, and the Cohen's kappa coefficients were excellent (κ = 1.00).

2.4 | MRS acquisition

MRS data were collected on a 3-T Siemens TRIO Tim, whole-body, clinical MR system (Erlangen, Germany) using a 32-channel, phased-array design, radiofrequency (RF) head coil operating at 123 MHz for proton imaging and spectroscopy. High-resolution, T_1-weighted anatomical images were used to position a single 12-cm^3 (2 × 2 × 3-cm^3) voxel in the rostral ACC (Figure 1). Proton MRS employed a modified, *J*-resolved, point-resolved spectroscopy protocol (2D–JPRESS). Shimming of the magnetic field within the prescribed voxel was first performed 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 to 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 TE ranging from 30 to 350 ms in 15-ms increments. The acquisition parameters were as follows: repetition time (TR) = 2 s; f1 acquisition bandwidth, 67 Hz; spectral bandwidth, 2 kHz; readout duration, 512 ms; number of excitations (NEX) = 8/TE step; steady-state scans/TE-step = 1; total scan duration, 6.5 min. MRS acquisition parameters were chosen based on our previous *J*-resolved study at 4 T in which we collected 24 TE steps.[13] Our choice of NEX = 8 is based primarily on two criteria: (i) as short a scan time as feasible, whilst still obtaining usable signal-to-noise; and (ii) cycling through a complete receiver phase cycle to minimize outer-volume signal contamination and to maximize spectral quality. The MRS data acquisitions were back-to-back, with no delay or re-acquisition of anatomical images between MRS scans.

2.5 | Proton MRS processing and analysis

All spectroscopic data processing and analysis were undertaken on a LINUX workstation. In order to quantify *in vivo* brain metabolites with the JPRESS data, the 22 TE-stepped free induction decays (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 2.00 ppm using an automated phase-correcting routine developed in-house. These two pre-processing steps ensure maximum signal-to-noise in the final, *J*-resolved, two-dimensional spectra prior to LCModel fitting,[13,18,19] whilst 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 set modeled for 3 T.[13] The GAMMA-simulated model fits the *J*-resolved spectral signatures for the following metabolites: total creatine (Cr), aspartate (Asp), choline (Cho), GABA, Glu, Gln, glutathione (GSH), glycine (Gly), myo-inositol (mI), NAA, *N*-acetylaspartylglutamate (NAAG), scyllo-inositol (Scy), taurine (Tau) and lactate (Lac). The integrated area under the entire two-dimensional 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 Cr. Representative one-dimensional spectra (*J* = 0.0 Hz) are shown in Figure 1, whereas a full two-dimensional plot depicting the complex *J*-coupling patterns of the *in vivo* metabolites is shown in Figure 2.

2.6 | Statistical analysis

For all 13 neurochemicals quantified, the percentage difference (%diff) for each individual subject was calculated between the two scans using the following formula:

$$\%\text{diff} = (\text{scan 2}-\text{scan 1})/\text{mean}(\text{scan 2}, \text{scan 1})\times 100\%$$

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 scan 1 and scan 2 (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.

3 | RESULTS

Table 1 summarizes the reliability scores for each participant and for all 13 metabolites, as well as the group mean and standard deviation of the reliability scores. Cho (7.4 ± 5.6%), Glu (7.1 ± 4.2%), GSH (8.6 ± 4.1%), mI (6.5 ± 7.1%) and NAA (3.5 ± 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 ± 13.7% (Asp) to 45.9 ± 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, $r = 0.953$, $p < 10^{-11}$.

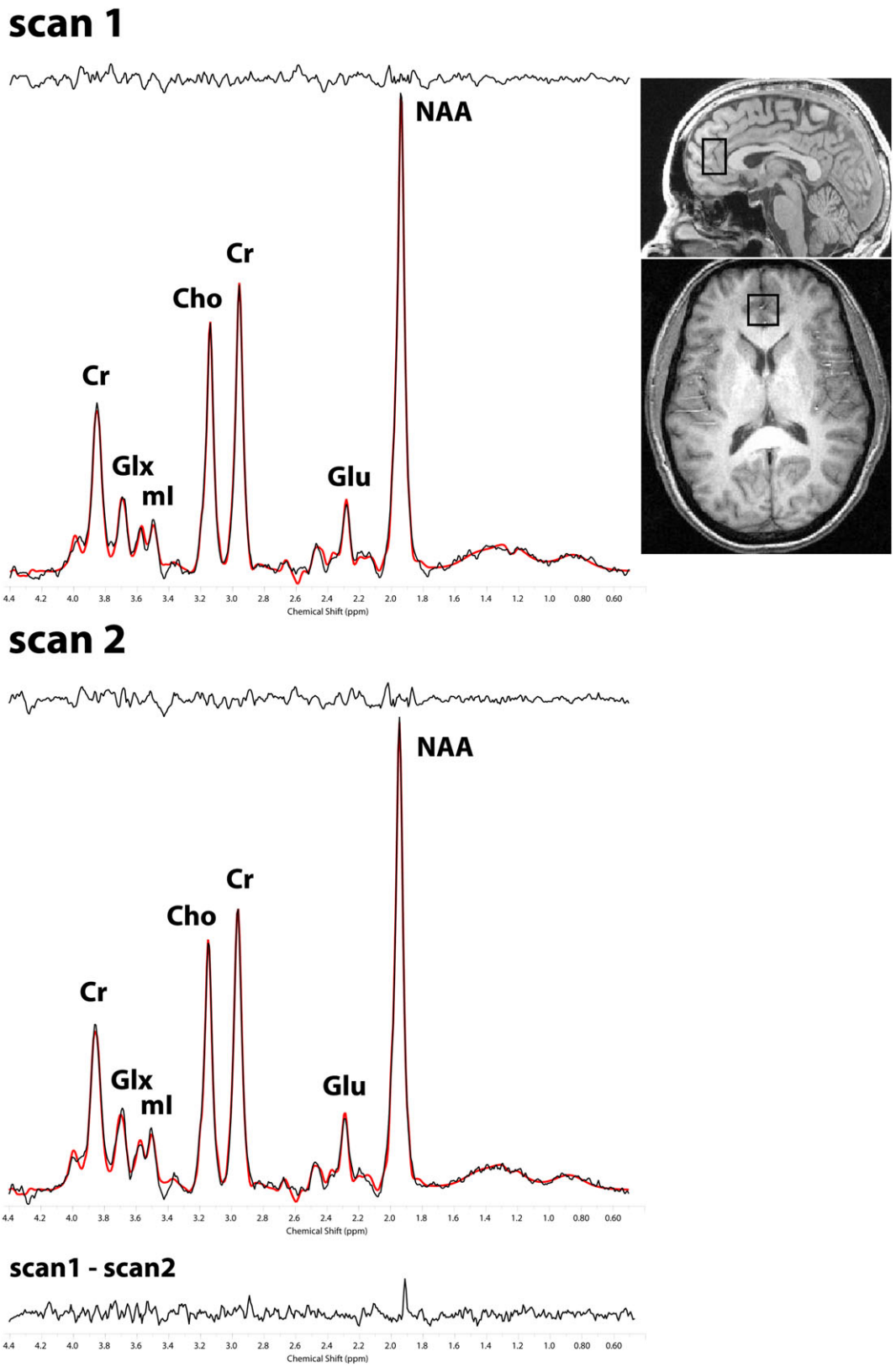

FIGURE 1 Representative *J*-resolved spectra from a 2 × 2 × 3-cm^3 voxel in the rostral anterior cingulate cortex (ACC) for successive scans in the same participant. Spectra are from the *J* = 0.0 Hz extraction (out of 64 extractions) and displayed with 1 Hz exponential filtering, LCModel fit and residual. The difference spectrum (scan 1 – Scan 2) is displayed at the bottom. Cho, choline; Cr, total creatine; Glu, glutamate; Glx, glutamate + glutamine; mI, myo-inositol; NAA, *N*-acetylaspartate

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 $p > 0.10$).

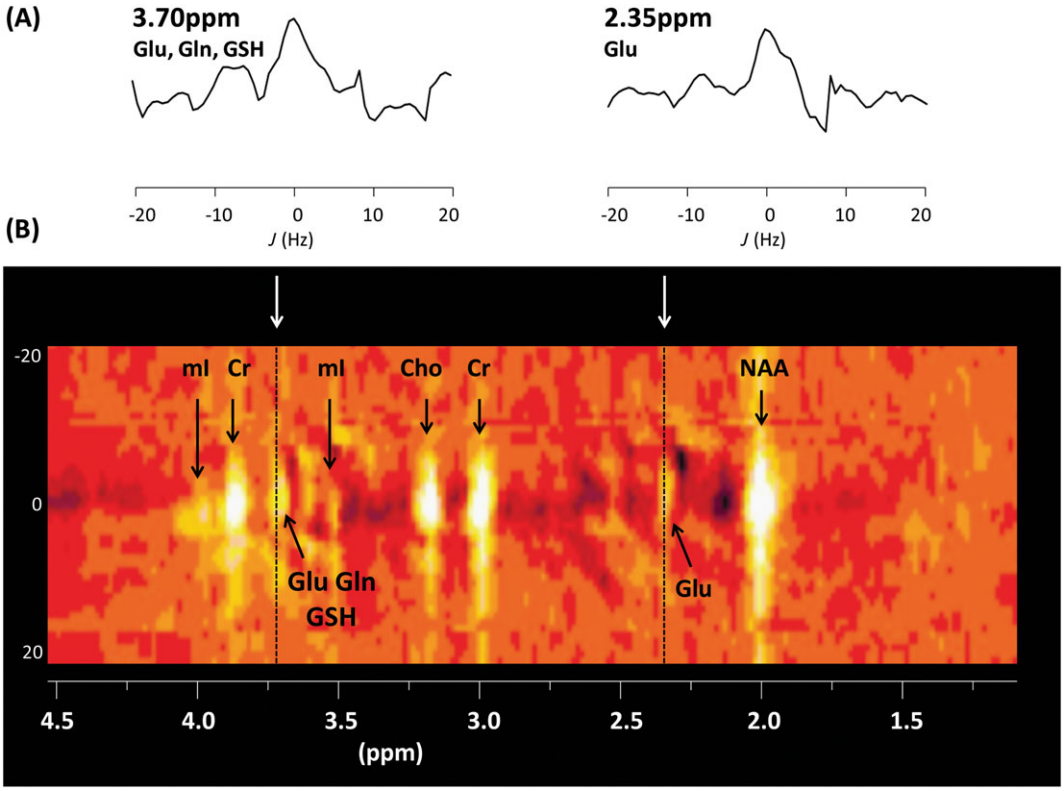

FIGURE 2 Two-dimensional spectral plot of a rostral anterior cingulate cortex (ACC) spectrum B, showing the *J*-coupling patterns of the coupled metabolites across *J* space. Selected *J* extractions along the f1 dimension A, are plotted for 3.70 and 2.35 ppm, depicting the coupling pattern of glutamate (Glu) (2.35 ppm), as well as the interplay between Glu, glutamine (Gln) and glutathione (GSH) (3.70 ppm). Cho, choline; Cr, total creatine; mI, myo-inositol; NAA, *N*-acetylaspartate

TABLE 1 Individual glutamate to creatine (Glu/Cr) ratios and calculated percentage difference [(scan 2 − scan 1)/mean(scan 2, scan 1) × 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 percentage difference for all metabolites and Pearson correlation across scans for healthy adolescents (n = 22)

Metabolites	Difference (%)	Pearson r	p
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

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

4 | DISCUSSION

The current two-dimensional *J*-resolved approach on a 3-T clinical MRS system provided clinically acceptable test–retest reliability in several key metabolites, including Glu, GSH, mI and NAA, with short scan times (~6 min) 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. The establishment of reproducibility opens up many possibilities for high-temporal-resolution MRS studies on a 3-T clinical scanner, especially in populations with low tolerance to the scanning process (e.g. neuropsychiatric patients and young children with normal or atypical development). Short, reliable and stable scans will also 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 3 T 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 the detection of time- and stimulus-dependent fluctuations in such metabolites. Nevertheless, metabolite-specific editing MRS techniques, such as MEGAPRESS,[23] 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 3 T, 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-min successive MRS scan. Unfortunately, we did not acquire any localizer images between MRS scans, and 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 should have quantitatively compared several, more advanced, MRS techniques with our current uniformly sampled J-PRESS sequence. Other MRS methods include advanced one-dimensional techniques [e.g. ultrashort-echo stimulated echo acquisition mode (STEAM),[24] LASER/SEMI-LASER[25]] and more advanced two-dimensional techniques (e.g. maximum echo-based J-PRESS[26] or non-uniformly sampled J-PRESS[27,28]), with both sets of techniques probably offering increased sensitivity in the detection of Glu, Gln and GABA. In addition, even more advanced spectral acquisition techniques, such as compressed sensing, can greatly reduce MRS data acquisition times, whilst still preserving the detailed spectral information of highly coupled metabolites,[29] 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 the difficulty in 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 two-dimensional MRS quantification. Although our LCModel technique has been shown to work well, it does not exploit the full essence of the two-dimensional *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 two-dimensional spectral information of the coupled metabolite signals. Alternative and/or more advanced post-processing methods,

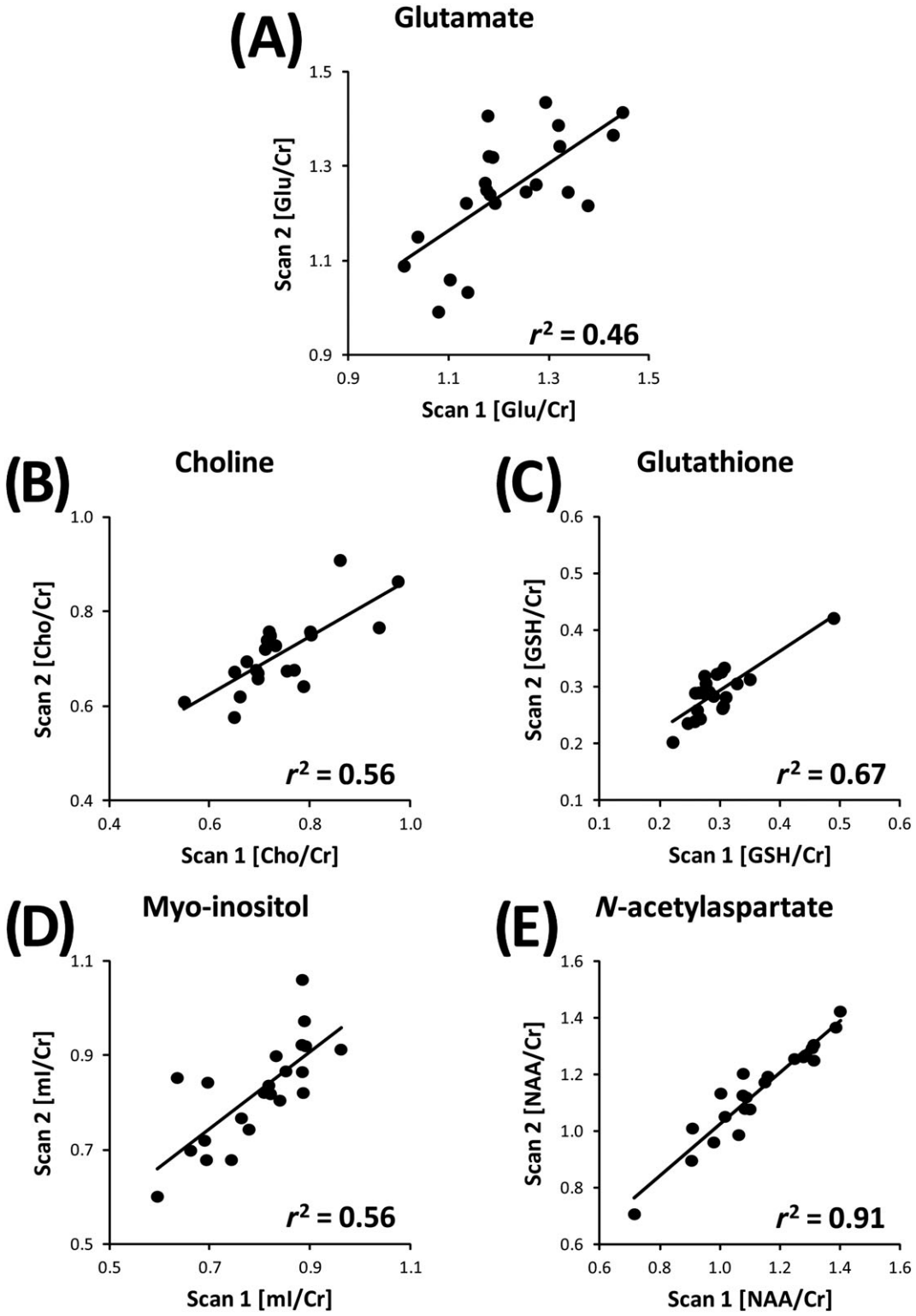

FIGURE 3 Scatterplots and regression lines for glutamate (Glu) A, choline (Cho) B, glutathione (GSH) C, myo-inositol (mI) D, and *N*-acetylaspartate (NAA) E, for the two scans. Metabolite ratios are normalized to creatine (Cr)

such as straight TE averaging for optimal Glu detection,[33] 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 cerebrospinal fluid (CSF) content variability and minor variations in fitting of the large water peak adding to potential errors in metabolite quantification. Although Cr 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 Cr 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 3-T 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 up intriguing avenues for the application of high temporal, short MRS data acquisition times in health and disease.

ACKNOWLEDGEMENTS

The project was supported through funding from the National Institute of Mental Health (NIMH) R01MH101521 (DAP), R37MH068376 (DAP), The Dana Foundation: Clinical Neuroscience Research Grant (DAP, RPA), Tommy Fuss Fund (RPA, DAP) and the Klingenstein Third Generation Foundation Adolescent Depression Fellowship (RPA). RPA was further supported by NIMH K23MH097786. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or NIMH.

DISCLOSURES OF INTEREST

Over the past 3 years, DAP has received consulting fees from Akili Interactive Laboratories, BlackThorn Therapeutics, Boehringer Ingelheim, Pfizer and Posit Science for activities unrelated to the current research. No other authors report any conflicts of interest.

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How to cite this article: Jensen JE, Auerbach RP, Pisoni A, Pizzagalli DA. Localized MRS reliability of *in vivo* glutamate at 3 T in shortened scan times: a feasibility study. *NMR in Biomedicine*. 2017;30:e3771. https://doi.org/10.1002/nbm.3771