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Reproducibility of Phase Rotation STEAM at 3T: Focus on Glutathione

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Abstract

Purpose—The purpose of this study was to determine the reproducibility of a very short echo time (TE) phase rotation stimulated echo acquisition mode (STEAM) sequence at 3T with a focus on the detection of glutathione.

Methods—Ten healthy subjects were scanned on two separate visits. Spectra were acquired from voxels placed in the anterior and posterior cingulates. Reproducibility was assessed using mean coefficients of variation (CVs) and mean absolute differences (ADs), and reliability was assessed using standard error of measurement (SEM) and intraclass correlations (ICCs). Phantoms containing glutathione and metabolites with overlapping resonances were scanned to test the validity of glutathione quantification.

Results—Excellent reproducibility as illustrated by CVs 8.3% and ADs 11.6% for both regions was obtained for glutathione and other commonly reported metabolites. Reproducibility measures for γ -aminobutyric acid and glutamine were good overall with CVs ranging from 6.4%–10.5% and ADs ranging from 8.6%–15.5% for both regions. Glutathione absolute and relative reliability were very good (SEMs 9.9%) and fair (ICCs 0.42–0.51), respectively. Phantom studies demonstrated the ability to accurately detect glutathione from other metabolites with overlapping resonances with great precision ($R^2 = 0.09$).

Conclusion—A very short TE phase rotation STEAM sequence proved reproducible for metabolites difficult to quantify but important for the study of psychiatric and neurological illness.

Keywords

magnetic resonance spectroscopy; glutathione; glutamate; glutamine; phase rotation STEAM; reproducibility

INTRODUCTION

Recent magnetic resonance spectroscopy studies implementing very short echo times (TE <10 ms) at 3T have reported quantification of metabolites that are commonly difficult to detect, including glutamate (Glu), glutamine (Gln), γ -aminobutyric acid (GABA), and glutathione (GSH) (1–4). These techniques take advantage of the lack of signal loss due to T_2 relaxation and phase dispersion from J-coupling. A study by Wijtenburg and Knight-Scott (3) demonstrated that Glu can be more precisely detected using a very short TE of 6.5 ms using a phase rotation stimulated echo acquisition mode (PR-STEAM) sequence, then a STEAM sequence at 72-ms TE, a point-resolved spectroscopy (PRESS) sequence at 40 ms TE and TE averaging (5–7). In addition, this work suggested that GSH, a marker of oxidative stress and implicated in psychiatric and neurological conditions (8–11), could potentially be detected reliably. However, to date, there have been no studies examining the reproducibility of PR-STEAM. The purpose of this study was to determine the test-retest reproducibility of PR-STEAM spectra acquired from the anterior cingulate (AC) and the posterior cingulate (PC) in healthy participants. These brain regions were selected for their frequent implications in neuropsychiatric illnesses such as schizophrenia (12–14). Due to the growing interest in GSH measurements, phantoms containing GSH and metabolites with overlapping resonances were scanned to test the validity of detecting and quantifying GSH.

METHODS

All studies were performed under a University of Maryland Baltimore Institutional Review Board approved protocol, and informed consent was obtained from each study participant. Data were acquired on a 3T Siemens TIM Trio (Siemens Medical Solutions, Inc., Erlangen, Germany) using a 32-channel phased array head coil. Each participant (male/female, 4/6; age, 26.1 ± 9 yr) was scanned on two separate visits scheduled approximately 1 wk apart (mean duration between visits, 6.7 ± 2 d). Three-dimensional magnetization-prepared rapid gradient-echo images were prescribed so that the slices were parallel with the AC-PC line. Spectroscopic voxels were placed in two regions: the AC (Fig. 1a) and the PC (Fig. 1b). To ensure near identical voxel placement between visits, each voxel was angled to be parallel with the corpus callosum and placed so that the center of the voxel bisected either the genu or the splenium. Voxel images from visit 1 were used to assist with voxel placement during visit 2. All spectroscopic data were acquired using PR-STEAM (2,3,15–17): pulse repetition time (TR)/mixing time (TM)/TE = 2000/10/6.5 ms; volume of interest (VOI) = 6 cm³ in both the AC and PC; number of excitations (NEX) = 256; spectral width = 2.5 kHz; 2048 complex points; and phases ϕ_1 135°, ϕ_2 22.5°, ϕ_{13} 112.5°, and ϕ_{ADC} = 0°. A water reference (NEX = 16) was also acquired for phase and eddy current correction as well as quantification. A basis set of 19 metabolites was simulated using the GAVA software package (18): alanine, aspartate (Asp), creatine (Cr), GABA, glucose (Glc), Glu, Gln, GSH, glycine (Gly), glycerophosphocholine (GPC), lactate (Lac), myo-Inositol (mI), *N*-acetylaspartate (NAA), *N*-acetylaspartylglutamate (NAAG), phosphocholine (PCh), phosphocreatine (PCr), phosphoylethanolamine (PE), scyllo-Inositol (sI), and taurine (Tau). The basis set was imported into LCModel (6.3-0D) and used for quantification (19). Metabolite concentrations are reported in institutional units. Reproducibility between visits was assessed via mean coefficients of variation (CVs) and mean absolute differences (ADs).

Reliability was assessed using standard error of measurement (SEM) and intraclass correlations (ICCs). ICCs are a measure of relative consistency and are impacted by the variability between subjects, whereas SEM is a measure of absolute consistency and enables assessment of reliability within individual subjects regardless of the between-subject variability (20–22). SEMs may be a better assessment of reliability in this study that examined only healthy controls with little intersubject variance (20,23,24).

To determine the validity of quantifying GSH at a very short TE, five phantoms with increasing concentrations of GSH (0 mM, 1.0 mM, 2.5 mM, 5 mM, and 10 mM) were created. In addition to GSH, each phantom contained the following metabolites at neurobiological concentrations per Govindaraju et al. (25): mI, Cr, Glc, Glu, GLn, and GABA. Phantoms were scanned using the following parameters: TR/TM/TE = 10,000/10/6.5 ms; VOI = 6 cm³; NEX = 64; spectral width = 2.5 kHz; 2048 complex points; and phases $\phi_1 = 135^\circ$, $\phi_2 = 22.5^\circ$, $\phi_{13} = 112.5^\circ$, and $\phi_{ADC} = 0^\circ$. Spectroscopic data were quantified with LCModel. Linear regression was computed to determine how well the LCModel quantified GSH concentration corresponded to the known GSH concentration.

RESULTS

Figure 2 shows a representative spectrum from the AC and PC with LCModel fits and residual. In the AC (Table 1), the following metabolites were detected in all participants with Cramer-Rao lower bounds (CRLB) $\leq 20\%$: Asp, Cr, GABA, GSH, Glu, GPC, GSH, mI, NAA, and PCr as well as the combined metabolites total Cho (tCho), total Cr (tCr), and total NAA (tNAA), and Glx. For Gln, CRLB were all $\leq 22\%$, while PE and sI had CRLBs of $<21\%$ and $<24\%$, respectively. The following metabolites could not be consistently identified as indicated with a CRLB $>25\%$: Glc, Gly, Lac, NAAG, PCh, and Tau. For all metabolites with CRLB $\leq 20\%$, including GSH, mean CVs were 7% or below and mean ADs were all below 11% for the same metabolites, showing excellent reproducibility. Specifically, tCho, tCr, tNAA, mI, Glx, and Glu all had mean CVs below 4.5% and mean ADs of $<6.2\%$. GSH reproducibility measures were only slightly greater than those reported for tCho. SEM for AC GSH showed excellent reliability at 0.19, which when scaled relative to the mean GSH concentration was 6.9%. The ICCs for GSH are lower than other metabolites, but this may be due to the low variability among healthy subjects (23). For metabolites fitting CRLB $\leq 25\%$, Gln, PE, and sI had slightly elevated mean CVs of 9.4–12.4% and mean ADs of 15.5%–20.6%. Mean line widths and signal-to-noise ratio (SNR) reported by LCModel were 0.02 Hz and 36.6 for visit 1 and 0.03 Hz and 33.5 for visit 2, respectively. These values were not significantly different ($P > 0.05$).

Similar to the AC, Asp, Cr, GABA, Glu, GPC, GSH, mI, NAA, and PCr could be identified with CRLB $\leq 20\%$ in the PC (Table 2). However, unlike the AC, Gln could be identified with CRLB $\leq 20\%$, whereas PE and sI had CRLBs $\leq 35\%$. Again, Gly, Lac, NAAG, PCh, and Tau could not be consistently identified with CRLBs exceeding 35%. The reproducibility in the PC was very good but slightly worse than the AC, with all mean CVs being $<11\%$ and all mean ADs being $<15\%$. The commonly reported metabolites tNAA, tCr, tCho, Glu, and mI had mean CVs and mean ADs below 7.5% and 11.2%, respectively. In the PC, GSH reproducibility measures were slightly larger than tCho but less than Gln. SEM

for PC GSH showed good reliability at 0.21, which when scaled relative to the mean GSH concentration was 9.9%. Similar to the AC, the ICCs for GSH are smaller than most metabolites, but this may be due to the homogeneous subject sample. Mean line widths and SNR reported by LCModel were 0.02 Hz and 32.3 for visit 1 and 0.02 Hz and 31.1 for visit 2, respectively. These values were not significantly different ($P > 0.05$). Overall, GSH measures in both regions were excellent and comparable to the reproducibility measures of tCho.

Figure 3a shows spectra from each of the five phantoms as a function of known GSH concentration. The red bars highlight the visible alterations in the spectrum as a function of increasing GSH concentration at 3.77 ppm, 2.97 ppm, and 2.56 ppm. Figure 3b shows detected LCModel concentration as a function of known GSH concentration. Finally, GSH data were fit to a linear regression, which resulted in an $R^2 = 0.994$.

DISCUSSION AND CONCLUSIONS

To our knowledge, this is the first reproducibility study performed on a very short TE STEAM sequence at 3T. Spectroscopic data using PR-STEAM at a TE of 6.5 ms were acquired from the AC and PC in healthy participants scanned during two separate sessions approximately 1 wk apart. Data were quantified with LCModel, and reproducibility was assessed by mean CVs and mean ADs between visits. Even though the metabolite concentrations in this study are in institutional units, the regional relationships between concentrations are similar to those from a previously published study (26). Excellent reproducibility was established for commonly reported metabolites as well as other metabolites such as GABA, Gln, and GSH in both the AC and PC. In addition, the validity of detecting GSH at a very short TE was verified using multiple phantoms with varying GSH concentrations and neurobiologically relevant concentrations of metabolites with overlapping resonances. A major advantage of this technique is that it enables acquisition of multiple metabolites reflecting neuronal and glial integrity, neurotransmission, cellular membrane health, and oxidative stress in a single acquisition, thereby reducing scan time, which is appealing for use in clinical populations.

Compared with other 3T reproducibility studies that used PRESS, our reproducibility results were superior for difficult-to-measure metabolites such as Glu, Gln, and GABA. When calculated according to Mullins et al.'s definition of CV (6), the PR-STEAM technique had significantly better CVs for mI, Glu, Gln, and GABA and comparable CVs for NAA, Cho, and tCr in data from the AC. With reproducibility measures calculated according to Wellard et al. (27), measures of reliability for commonly reported metabolites of NAA, Cho, Cr, and mI were comparable between their study and this study. However, the reliability of Glx was substantially better at a very short TE, with a mean Glx relative variability of -0.12 versus 0.7 and 1.0 reported in the right and left frontal lobes (27). Mullins et al. and Wellard et al. did not list the number of metabolites incorporated into their basis set, making exact comparisons of reproducibility measures difficult. A more comprehensive metabolite basis set may improve CRLBs and potentially improve reliability. Both of these studies acquired PRESS data at 40 ms and 30 ms, respectively, from the frontal lobes. One caveat about data acquisition using STEAM is the reduction in signal by half compared with PRESS, leading

to reduced SNR; however, PRESS cannot achieve very short TEs similar to STEAM and SPECIAL. By acquiring data at a TE of 6.5 ms, the reduction in signal loss due to T₂ relaxation and phase dispersion due to J-coupling has a significant positive impact on the reproducibility of metabolites with lower SNR peaks, making it easier to detect metabolites such as GABA, Glu, and Gln. It is important to note that reproducibility is impacted by several factors, including SNR and voxel location, such that the measures presented here may not generalize across all brain regions of interest in neurological and psychiatric research.

The reproducibility of GSH was excellent and comparable to the reproducibility of tCho. All CRLBs for GSH were 8.2% or less for both regions. While in institutional units, the GSH concentrations reported are within range of those reported by Govindaraju et al. (25) and Cooper and Kristal (28). Comparatively, the 95% confidence intervals for GSH/tCr between this study and our previous study (2) overlap. Subtle differences in metabolite values may be explained by differing head coils, voxel sizes, participants, and LCModel versions. In terms of reproducibility, there are no other studies that report reproducibility measures for GSH in vivo. Previous GSH reproducibility studies in phantoms by Terpstra et al. (29) and Lagopoulos et al. (8) influenced the range of concentration values used in phantom experiments in this study; however, we included more metabolites with resonances that overlap with GSH to mimic conditions in vivo. The phantom experiments presented here further validate the capability to precisely quantify GSH from other metabolites in vitro; however, the accuracy of fits to GSH in vitro may not translate directly to accuracy of GSH measurements in vivo. Overall, this study shows that it is possible to quantify GSH in a reliable and valid manner without the use of spectral editing techniques.

The reproducibility and goodness of fit measures for Gln proved to be surprisingly good. All CRLBs for Gln were 22%, and the mean CRLBs were approximately 14%–15% for both regions. In addition, the ratio of Gln to Glu in both regions falls within the expected range of 22%–33% (25), also providing support for accurate detection of Gln. As mentioned previously, the reproducibility of Gln with this technique is superior to another 3T study (6). Simulations by Hancu and Port (30) showed a low CV but high absolute fitting error for fits to Gln using a very short TE STEAM sequence (TE = 5 ms). However, a very short TE STEAM sequence was not tested in vivo, and the LCModel basis set in that simulation lacked GSH and NAAG, which could contribute to the high absolute fitting error for Gln. Overall, the ability to reliably measure Gln, in conjunction with Glu, at 3T provides a more comprehensive picture of glutamatergic function in the brain. The Glu signal provided with ¹H-MRS reflects the total amount of Glu and does not simply reflect Glu neurotransmission, but most likely reflects multiple mechanisms. Gln, the major metabolite of synaptic Glu, may provide additional information for indexing glutamatergic neurotransmission (31). Therefore, the ability to measure Gln is vitally important to a better understanding of glutamatergic alterations in brain conditions.

Another interesting result of this study was the ability to reliably detect GABA. There have been several reproducibility studies using spectral editing for detection of GABA at 3T (32–35), which reported reproducibility measures on fits from a combination of the GABA C-2 resonance at 3.01 ppm and macromolecules denoted GABA+ versus reproducibility

measures derived from fits to the entire GABA spectrum in this study. In anterior regions of the brain, Geramita et al. (32) reported a within-subject CV of 8.59% for GABA+ from an 18-cm³ AC voxel using 776 acquisitions, whereas O'Gorman et al. (33) reported a range of within-session CVs of 7%–12% depending on the fitting software from a 30-cm³ left dorsolateral prefrontal cortex voxel using 320 averages. Comparatively, the GABA CV for the AC of 6.4% from a smaller voxel (6 cm³) in this study is superior. In posterior regions of the brain, Bogner et al. (34) reported inter-individual CVs of 19.2% or 21.7% depending on the postprocessing procedures from 22.5 cm³ voxel averaged 128 times in the left occipital lobe, and Evans et al. (35) showed group CVs for occipital and sensorimotor cortices of 9.1% and 12%, respectively, for data acquired from 27-cm³ voxels using 80 averages. The PC GABA CV of 10.5% from a smaller voxel (6 cm³) in this study was comparable or better than these posterior GABA studies. In addition, a recent reproducibility study by Near et al. (4) utilized SPECIAL to detect GABA in the occipital lobe. SPECIAL is an unedited localization technique that can achieve very short TEs, acquire the entire GABA spectrum, and does not lose half of its signal like STEAM. CVs for GABA quantified using LCModel were 8.7%, similar to the CV reported here. Although LCModel provides handling of the macromolecule background, it has yet to be determined whether the GABA concentrations reported here are valid; therefore, additional phantom studies are needed to validate our GABA measurements.

The focus of this study was on determining the reproducibility of the technique, but absolute and relative reliability were also assessed with SEMs and ICCs, respectively. Overall, for GSH and commonly reported metabolites, the SEMs were very good in both regions (2.2%–9.9%), whereas the ICCs were fair to good (0.37–0.92). According to Weir and colleagues, SEM may be a better indicator for assessing reliability in a homogeneous sample since it is independent of inter-subject variability (20,23,24).

One limiting factor of this study is that at very short TEs, the macromolecule background is prominent due to its short T₂ nature. For quantification, we used LCModel, which parameterizes the macromolecule background to remove its influence from concentration outputs. However, Cudalbu et al. (36) have shown that inclusion of a metabolite-nulled spectrum in the basis set leads to more reliable and accurate metabolite quantification. Future studies will incorporate a metabolite-nulled experiment, and the result will be incorporated into LCModel to potentially further improve macromolecule baseline identification.

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FIG. 1.
T₁-weighted images showing voxel placement in the AC (a) and PC (b).

FIG. 2.

Representative LCMoel fits (black lines) from the AC (**a**) and the PC (**b**) acquired from the same participant. The original spectra are shown as gray lines, and the fit residual is shown above each spectrum. Examination of the residual reveals that all metabolites and macromolecules are accounted for in the fit.

FIG. 3.

a: Spectra acquired from phantoms containing increasing concentrations of GSH (red boxes). Each phantom had the same concentration of Glu, Gln, Cr, mI, Glc, and GABA. **b:** Linear regression on the resultant LCMoDel concentration as a function of known GSH concentration was excellent at $R^2 = 0.994$, demonstrating the validity of GSH quantification at a very short TE.

Table 1

Metabolite Concentrations with CRLB 25% in the AC

	Visit No.	Mean (SD), IU	Mean CRLB, %	Mean CV, %	Mean AD Between Visits, %	ICC	SEM, % ^a
Asp	1	3.73 (0.29)	11.4	7.1	10.8	-0.11	9.2
	2	3.96 (0.37)	11.3				
Cr	1	5.18 (0.61)	7.7	5.2	7.7	0.74	6.5
	2	5.34 (0.48)	8.9				
GABA	1	3.16 (0.20)	11.7	6.4	8.6	0.15	7.3
	2	2.99 (0.24)	13.3				
Gln	1	2.96 (0.49)	13.8	9.4	15.5	0.24	14.6
	2	3.02 (0.47)	14.7				
Glu	1	13.31 (0.62)	3.3	1.9	2.7	0.85	2.6
	2	13.37 (0.79)	3.4				
GPC	1	2.48 (0.31)	3.4	4.4	6.2	0.89	5.5
	2	2.43 (0.31)	3.7				
GSH	1	2.69 (0.13)	6.4	5.7	8.3	0.42	6.8
	2	2.78 (0.28)	6.4				
NAA	1	11.97 (0.31)	2.5	2.7	3.9	0.37	3.5
	2	12.15 (0.62)	2.6				
PCr	1	4.94 (0.71)	8.2	6.2	8.4	0.72	8.7
	2	4.77 (0.58)	10.2				
PE	1	3.44 (0.69)	12.7	12.4	20.6	0.36	16.1
	2	3.81 (0.61)	12.5				
sI	1	0.42 (0.07)	18.1	10.4	15.9	0.75	11.1
	2	0.45 (0.08)	18.1				
tCho	1	2.48 (0.31)	3.4	4.4	6.2	0.89	5.5
	2	2.43 (0.31)	3.7				
tNAA	1	12.67 (0.31)	2.0	2.2	3.1	0.44	2.9
	2	12.78 (0.56)	2.3				
mI	1	9.06 (0.69)	3.0	2.7	3.9	0.92	3.2
	2	9.33 (0.82)	3.0				
tCr	1	10.12 (0.38)	2.0	1.8	2.6	0.82	2.2
	2	10.12 (0.44)	2.1				
Glx	1	16.27 (0.97)	3.7	2.0	2.8	0.79	2.9
	2	16.39 (0.63)	3.6				

^a Scaled relative to the mean metabolite concentration.

Table 2

Metabolite Concentrations with CRLB 20% in the PC

	Visit No.	Mean (SD), IU	Mean CRLB, %	Mean CV, %	Mean AD Between Visits, %	ICC	SEM, % ^a
Asp	1	3.40 (0.35)	12.0	6.8	9.1	0.75	7.9
	2	3.22 (0.47)	13.0				
Cr	1	4.67 (1.00)	8.2	13.1	17.8	0.43	17.3
	2	4.30 (0.82)	9.1				
GABA	1	2.75 (0.43)	13.0	10.5	14.2	0.35	13.3
	2	2.63 (0.39)	13.8				
Gln	1	2.68 (0.40)	14.6	8.9	12.4	0.52	10.8
	2	2.62 (0.32)	15.2				
Glu	1	11.27 (1.28)	3.9	7.2	11.2	0.59	8.2
	2	10.83 (1.11)	4.1				
GPC	1	1.56 (0.17)	6.1	7.6	10.9	0.66	8.8
	2	1.57 (0.22)	5.6				
GSH	1	2.16 (0.23)	7.6	8.6	11.6	0.51	9.9
	2	2.01 (0.27)	8.2				
NAA	1	10.78 (1.10)	2.7	4.5	6.3	0.70	6.5
	2	10.58 (1.00)	2.9				
PCr	1	3.86 (0.59)	9.8	9.9	13.2	0.47	9.9
	2	4.13 (0.30)	9.3				
tCho	1	1.61 (0.19)	4.7	7.4	10.5	0.70	8.6
	2	1.58 (0.22)	4.6				
tNAA	1	11.42 (1.15)	2.2	4.3	6.1	0.67	6.9
	2	11.29 (1.13)	2.3				
mI	1	7.25 (0.61)	3.8	5.3	7.5	0.62	7.4
	2	7.32 (0.87)	3.7				
tCr	1	8.53 (0.92)	2.2	4.4	6.3	0.64	7.3
	2	8.43 (0.81)	2.1				
Glx	1	13.95 (1.46)	4.3	5.9	8.0	0.51	8.1
	2	13.45 (1.32)	4.2				

^aScaled relative to the mean metabolite concentration.