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CLINICAL INVESTIGATIVE STUDY

Single-session reproducibility of MR spectroscopy measures of glutathione in the mesial temporal lobe with MEGA-PRESS

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Abstract

Background and Purpose: Magnetic resonance spectroscopy (MRS) measures neurochemicals in vivo. Glutathione (GSH) is a neuroprotective chemical shown to vary significantly in patients with Alzheimer's disease (AD). This work investigates the reproducibility of GSH measures in the mesial temporal lobe (MTL) to identify its potential clinical utility.

Methods: MRS data were acquired from eight healthy volunteers (31.1 ± 5.2 years; 4 male/female) using Mescher-Garwood-Point Resolved Spectroscopy (MEGA-PRESS) from the MTL in the left hemisphere across two scan sessions in the same visit. Total N-acetylaspartate (tNAA), choline (tCho), creatine (tCr), and GSH were quantified. Reproducibility of quantifications of these neurochemicals were tested using coefficient of variance (CV) between scan sessions. Reproducibility of voxel placement on the left MTL was calculated by measuring the tissue overlap and percent of hippocampus within that voxel. CV measured across different scan sessions in each individual, with a CV<15% was accepted as "good" reproducibility. Paired *t*-tests were carried out to establish the significant differences between the two scans across each individual with $p<.05$ as significant.

Results: TNAA (%CV = 7.2; $p = .5$), tCr (%CV = 7.8; $p = .6$) and tCho (%CV = 9.3; $p = .4$), and GSH (%CV = 22; $p = .1$). The dice coefficient that reflects the level of overlap of hippocampal tissue in the voxel was shown to be 0.8 ± 0.1. Voxel tissue composition were: Scan 1 (cerebrospinal fluid [CSF]: 5 ± 1%, white matter [WM]: 52 ± 3%, gray matter [GM]: 43 ± 3%); Scan 2 (CSF: 5 ± 1%, WM: 52 ± 4%, GM: 44 ± 4%).

Conclusion: The data suggest measures of abundant metabolites in the MTL using the MEGA-PRESS sequence has a high reproducibility. Reproducibility of GSH in this area was poorer requiring care when interpreting measures of GSH in the MTL for clinical translational purposes.

KEYWORDS
glutathione, hippocampus, magnetic resonance spectroscopy, mesial temporal lobe, reproducibility

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INTRODUCTION

Magnetic resonance spectroscopy (MRS) is a powerful technique that can identify and quantify neurochemicals that may serve as biomarkers in the diseased brain, to inform pathophysiology.[1,2] Common commercial sequences on clinical MRI scanners, such as Point Resolved Spectroscopy (PRESS) and Stimulated Echo Acquisition Mode (STEAM), can readily measure abundant brain chemicals, such as total N-acetylaspartate (tNAA), creatine (tCr), and choline (tCho), in addition to other key neurochemicals. These sequences, however, have limited accuracy in detecting neurochemicals with less abundance, such as γ-aminobutyric acid (GABA) and glutathione (GSH). A modified version of the PRESS sequence, MEGA (Mescher-Garwood)-PRESS,[3] allows more accurate detection of lower abundant neurochemicals.[4] Also referred to as an editing sequence, MEGA-PRESS can unveil the lower abundant chemical signature by subtracting two sets of spectra: radiofrequency (RF) pulses "ON" a specific resonance frequency of a target molecule, and a spectrum with RF pulses "OFF" the resonance frequency of the target molecule.

The increased sensitivity of MEGA-PRESS to some of the lower abundant neurochemicals offers potential for clinical utility to identify neurochemical biomarkers in disease. In order to appreciate this translational potential, the reproducibility of the method needs to be established. This work is specifically focused on establishing the reproducibility of GSH measurement in the mesial temporal lobe (MTL) by MRS.

GSH is a tri-peptide molecule consisting of cysteine, glycine, and glutamate. It serves in a variety of cellular functions, including as the principal antioxidant in the brain. GSH is relevant to neurodegenerative diseases,[5] where oxidative stress is implicated. GSH depletion has been observed in Alzheimer's disease (AD)[6] and mild cognitive impairment (MCI).[7] GSH quantification using standard MRS methods is complicated because several peaks in the spectrum are in close proximity with spectral peaks of more abundant neurochemicals, such as creatine and glutamate. With spectral editing, MEGA-PRESS can selectively localize peaks, such as GSH and GABA. There are studies focusing on the reproducibility of GSH at 3 Tesla (T)[8] using both editing and nonediting sequences, and at 7T[9] using the STEAM sequence. At 3T, voxel placement in the cortical region of the medial temporal lobe, including the bilateral anterior cingulate, gave a coefficient of variance (CV) of 13.1% for GSH measures acquired with MEGA-PRESS. More recently, MEGA-PRESS measures of GSH at 3T from the dorsal anterior cingulate cortex was shown to measure GSH at a CV of 7.3%.[10] A 7T study showed good CV (9.3%) with single voxels placed over the precuneus and posterior cingulate. These studies focused primarily on cortical areas which are generally easier to acquire a better shim, and, therefore, a smaller CV would be expected. It was shown that reproducibility of GSH measures using all these methods in cortical areas was relatively good across different acquisition sequences and scanner strengths; however, the reproducibility of these measures in the harder to shim, subcortical areas, are still to be determined.

The hippocampus is one of the first anatomical regions to demonstrate tissue loss in AD.[11,12] Recently,[6] MEGA-PRESS GSH measures in the hippocampal structures were shown to be significantly reduced in AD patients compared to age-matched controls. These reductions correlated to declining cognitive function. This suggests that obtaining reliable measures of GSH within the MTL encompassing the hippocampus could be used in AD diagnosis and understanding AD pathophysiology. However, the MTL is an area that is generally difficult to shim and obtain defined resonances due to its size and proximity to sinuses and bone, leading to poorer reproducibility. This was shown in a study carried out on a 1.5T scanner with a STEAM sequence reporting a poorer reproducibility and precision in measure of proton (1H) MRS in the hippocampus and thalamus for key metabolites (not GSH) with CVs ranging from 7% to 54% when compared to the cortical region 5-22%, with the glutamine resonances having the worst reproducibility in these regions.[13] Another 3T study,[14] using a variant of the semi-adiabatic localization by adiabatic selective refocusing (semi-LASER) sequence, measured metabolites from the hippocampus including GSH with a CV <20%. The more abundant metabolites, such as NAA, Cr, Cho, glutamate, glutamine, and myo-inositol (which can be challenging to detect at specific echo times), had CVs of <10%. The signal-to-noise is generally poorer for lower-strength scanners, therefore, an improvement in CV would be expected at higher-strength scanners. A 7T study using a modification of the MEGA pulses with the semi-LASER sequence looked at the reproducibility of GABA within the hippocampus and reported a relatively good reproducibility (CV <15%).[15] To our knowledge, there has yet to be a study that investigates the reproducibility of MEGA-PRESS measures of GSH on the MTL specifically encompassing the hippocampus at 3T, which we have undertaken here.

We chose to focus on three key factors that can be considered to impact reproducibility in a single voxel MRS study: (1) the placement of the MRS voxel that encompasses the region of interest and, therefore, the concentration of the different tissue types within the voxel (ie, white matter [WM], gray matter [GM], and cerebrospinal fluid [CSF]); (2) the shim of the area as measured through the linewidth of the more abundant peaks (ie, unsuppressed water and creatine); and (3) the relative changes of GSH to tNAA within the region, in addition to the changes of the more abundant peaks, tCr/tNAA, and tCho/tNAA. In this work, we focused our measures of reproducibility within the MTL encompassing the hippocampus of young healthy individuals to ascertain the reliability of measures in the absence of pathology and neuronal loss.

METHODS

Eight healthy volunteers (mean age: 31.1 $\pm$ 5.2 years, gender: 4 men/4 women) were recruited within the general university and research institute population and for medical conditions and MRI contraindications. This study was carried out in accordance with ethics approved by the local human ethics committee.

Each volunteer attended a single scan visit. At this single visit, variability between scan sessions was assessed by conducting two scan sessions for each volunteer. This involved setting up the volunteer in the scanner and conducting a first scan to acquire the first set of

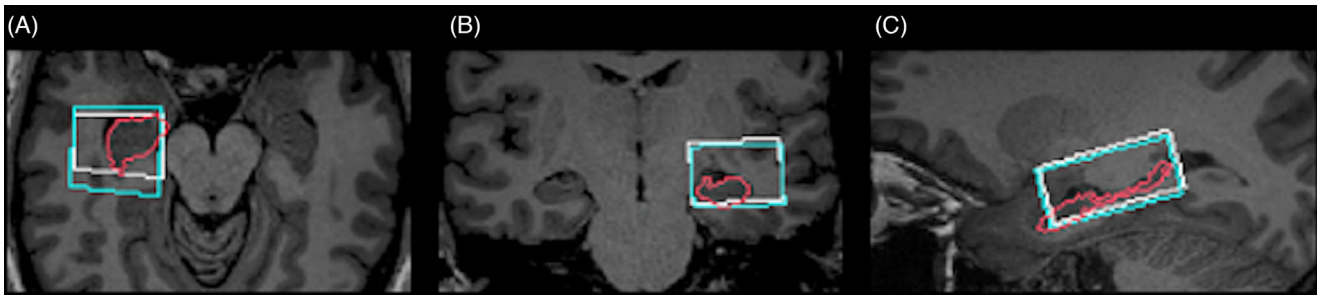

FIGURE 1 Example from a single subject of the overlay of the voxel placements for Scan 1 (blue box) and Scan 2 (white box) encompassing the left hippocampus in the (A) axial, (B) coronal, and (C) sagittal planes. The hippocampus structure is shown in the red outline within the boxes.

data (Scan 1). The scanner bed was then moved out of the scanner so that the planning profile from the first scan was voided on the scanner system and the volunteer vacated the scanner bed. The volunteer was then set up for a second scan with a new planning session to acquire the second set of data (Scan 2). Both scans used the exact same protocol.

All scans were conducted on a 3T Siemens Prisma system, using a 1H, 64-channel head coil. Sequence protocols consisted of a T1 high-resolution structural scan and a MEGA-PRESS work in progress (WIP) (https://www.cmrr.umn.edu/spectro) for acquisition of GSH MRS measures. T1 Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) parameters were repetition time (TR): 2300 milliseconds, echo time (TE): 2.98 milliseconds, resolution: 1 mm isotropic. MEGA-PRESS parameters were optimized to be most sensitive to signals from the GSH cysteine β-proton as according to Sanaei Nezhad et al.[16] These parameters were TE: 130 milliseconds, TR: 2500 milliseconds, 256 dynamics in total for OFF/ON spectral groups. These two groups were separated into four averages of 32 dynamics (ie, 128 dynamics ON/128 dynamics OFF) and 2048 data points. MEGA editing pulses (with bandwidth of 50 Hz) were set to the sensitivity of the cysteine resonances at 4.56 ppm and off resonance at 7 ppm (an appropriate distance away from the water peak as guided by the creators of the sequence WIP). This enabled the detection of the GSH peak at 2.99 ppm. Water suppression was applied using variable power and optimized relaxation delays. An unsuppressed water peak was also acquired within the same region following each scan with the same parameters (TE: 130 milliseconds, TR: 2500, voxel: 45 mm $\times$ 30 mm $\times$ 30 mm, 32 averages) but without water suppression for use as an internal reference in processing and determination of spectral quality. Flip angle optimization was carried out on each individual prior to data acquisition. Spectra measures were carried out on a single, oblong-shaped voxel (45 mm $\times$ 30 mm $\times$ 30 mm) placed on the left MTL. Voxel planning was carried out on T1 weighted MPRAGE scans that were reconstructed to encompass the left MTL in the three planes to ensure the voxel was moved within tissue as much as possible and away from bone and sinus areas. An example of this plan is shown in Figure 1. Planning was carried out to ensure the majority of the hippocampus was encompassed. The total time for the sequence was just under 12 minutes. It should be noted that time did extend by an extra 2-5 minutes if manual shimming was required to improve the shim quality.

Shimming within the MTL can be challenging and the shimming processes often involved manual higher-order shims with careful planning of the voxel to encompass mainly tissue with minimization of ventricle CSF and sinus areas. Average linewidths measured from the tCr peaks for Scan 1 was 11.4 $\pm$ 0.9 Hz and Scan 2 was 13.4 $\pm$ 2.9 Hz. Unedited MEGA-PRESS (ie, the "off") measures were fitted for tCr, tCho, and tNAA peaks, and the "on" MEGA signal for the GSH peak at 2.99 ppm. In the majority of cases, manual shimming was not required. Manual shimming was required when there were larger ventricle volumes within the voxel. The requirement for extra manual shimming processes may vary across scanner manufacturers. It is anticipated that this could be a real challenge in an older population with degenerated hippocampi.

MRS preprocessing and quantification

All MRS preprocessing was carried out on jMRUI (V6.0; www.jmrui.eu, EU).[17] All eight "on/off" spectra for each acquisition were visually inspected to account for any contamination of the signal such as movement or scanner-related noise. Across all eight volunteers, no spectra needed to be excluded for artifacts. All four unedited and edited spectra were summed together to create one edited and unedited spectra each. Hankel Lanczos singular value decomposition algorithm[18] from jMRUI was used to remove the residual water peak. Phase estimation of the tNAA peak was carried out using advanced method for accurate, robust, and efficient spectral fitting (AMARES toolbox from JMRUI), and this phase was set individually for all of the edited and unedited spectra prior to summing spectra together for each group. The tNAA peak was then referenced at 2.01 ppm for all spectra. A difference spectra was produced by subtracting the summed edited ("on") spectra from the summed unedited ("off") spectra. This unveiled the GSH peak at 2.99 ppm. Peaks were identified and quantified using AMARES with defined prior knowledge for GSH including frequency shift. Cramer-Rao lower bounds of less than 20% was used to identify spectra with a "good" fit. The "autopick" option in AMARES was used on the unedited spectra to identify and quantify the tNAA, tCr, and tCho peaks and was

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visually checked to ensure it was accurate. Peaks were fitted using a Lorentizan line shape spectra. An unsuppressed water reference acquisition was incorporated for normalization. Final quantified values were tissue corrected to assume no metabolite was present in CSF using the tissue segmentation fractions within the voxel.

T1 segmentation for tissue correction

Tissue segmentation was carried out on the T1 MPRAGE using the GANNET software's[19] (https://github.com/markmikkelsen/Gannet, John Hopkins University, Baltimore, USA) GannetCoregister and GannetSegment tools in conjunction with SPM 12 (fil.ion.ucl.ac.uk/spm, University London College, London, UK). A mask of the voxel was created and subsequently coregistered to the T1 image. Tissue within the voxel area was segmented and percentage values relating to the fraction of GM, WM, and CSF were reported. It was assumed that there were insignificant amounts of metabolites present in CSF. Final concentrations were corrected to be present in GM and WM to remove the contribution of CSF to the final measure (Equation 1)[20] by dividing the measured concentration of the metabolite ($Conc_{metab}$) and the sum of the fraction of tissue volume of GM and WM within the voxel.

$$Conc_{CSF\ Corr} = \frac{Conc_{Metab}}{Tissue\ Fraction_{GM} + Tissue\ Fraction_{WM}} \quad (1)$$

Calculation of coefficient of variance and statistics

In this work, single session reproducibility is defined as consistency between Scan 1 and Scan 2 of each subject (ie, "intra-scan" reproducibility). Measures to ascertain reproducibility were defined as mean CVs between scans (Equation 2) for the (1) % overlap of hippocampal structures; (2) % of GM, WM, and CSF within the voxel; and (3) the ratio of the signal of the GSH peak in the subtracted data, tCho, tCr, and tNAA from the unedited spectra. CV was calculated using the formula (StDev, Standard Deviation):

$$CV\,(\%) = \left(mean_{across\ all\ subjects} \left(\frac{StDev_{Scan1\ and\ Scan\ 2}}{mean_{Scan1\ and\ Scan\ 2}} \right) \right) \times 100\% \quad (2)$$

Paired *t*-tests were carried out to look at between-session differences in tNAA, tCr, tCho, and GSH concentrations, and their ratios to NAA. Paired *t*-tests were also carried out to test for statistically significant differences between-session for hippocampal volume overlap and tissue (GM, CSF, WM) fractions within the voxel, as well as linewidths. CVs were calculated using the mean and the standard deviation between the two sessions.

RESULTS

To confirm the level of accuracy in voxel placement, hippocampal tissue segmentation volumes and Sorenson dice coefficients were calculated for both Scan 1 and Scan 2 within the voxel. The mean hippocampal tissue volume for Scan 1 was 2770.4 ± 232.6 mm and Scan 2 was 2793.1 ± 250.9 mm. The fraction of hippocampus within the voxel was on average 87.8±1.8% of the total hippocampus structure for Scan 1 and 93.9±1.9% for Scan 2. There was no significant difference in the amount of hippocampal tissue in the voxels between Scan 1 and Scan 2 ($p = .2$). The average difference in the amount of hippocampal tissue that did not overlap between the two measures was 169.2 mm (standard deviation = 78.3 mm; CV = 4.4%), which was not significant ($p = .8$). The overlays for an example of hippocampal voxel placements across the two measures are shown in Figure 1. The mean dice coefficient (a value that reflects the overlap between two spatial measures)[21] was 0.8 ± 0.1 across all volunteers, suggesting a good overlap of voxel placements between the two measures. Tissue volume fractions inside voxels between both measures showed high reproducibility (nonsignificantly different) of CSF (%CV = 12.5%; $p = .8$), GM (%CV = 2.6%; $p = .99$), and WM (%CV = 1.8%; $p = .9$; Table 1).

TABLE 1 Tissue segmentations in voxel.

	Scan 1	Scan 2	%CV	*p*-value
WM	0.52 ± 0.03	0.52 ± 0.04	1.76	.89
GM	0.43 ± 0.03	0.43 ± 0.05	2.57	.99
CSF	0.05 ± 0.01	0.05 ± 0.01	12.53	.78

Note: Characteristics of the volume segmented for the three different tissue types within the voxel displayed as a fraction of tissue within the voxel (with total of all tissue equivalent to 1). Coefficient of variance (%) between the two scans for each tissue type from each individual. *p*-values obtained from paired *t*-tests between the two scans. All the data represent mean±standard deviation unless otherwise indicated.
Abbreviations: CSF, cerebrospinal fluid; CV, coefficient of variance; GM, gray matter; WM, white matter.

Spectral quality was comparable between the two scans (Figure 2—examples from a single volunteer), with clean baselines and sharp linewidths in the unedited spectra (Figure 2a,c). The Cramer-Rao lower bounds of all metabolites in these volunteers was <20%. The CV between Scans 1 and 2 was acceptable for quantification of the tNAA (7.2%; $p = .5$), tCr (7.8%; $p = .6$), and tCho (9.3%; $p = .4$) peaks. The GSH resonance (Figure 2b,d) was smaller and less prominent compared to the other metabolites in the unedited spectra. This may explain the poorer CV observed for GSH (22%, $p = .11$; Table 2). However, the quantification of the two measures were not significantly different ($p = .11$). The CV of GSH did not materially improve when expressed as a ratio of tNAA (21.6%), and neither did tCr (9.5%) and tCho (10%). Scatter plots reflecting individual volunteer values for the different metabolites across the different scans are shown in Figure 3. These plots confirm that for the most part, most of the volunteers had better reproducible values for the more prominent metabolites, except for GSH. It should be noted one individual seemed to have consistently higher values for their second visit measures for these metabolites.

The mean linewidth for the water peak was 14.6 ± 1.0 Hz for Scan 1 and 15.4 ± 2.7 Hz for Scan 2, with a CV of 8.0%. The mean linewidths of

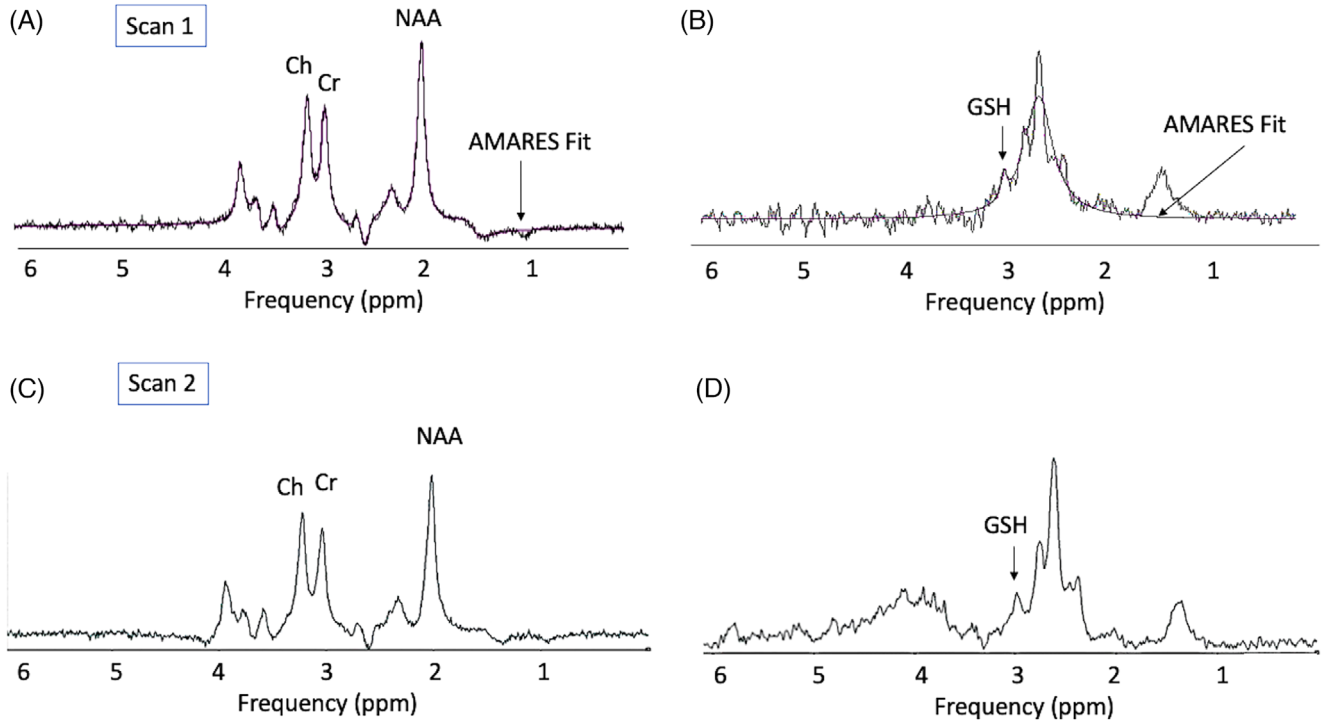

FIGURE 2 Example of an unedited spectra from a single volunteer from Scan 1 (A, B) and Scan 2 (C, D). Unedited spectra (A, C) show N-acetyl aspartate (NAA), creatine (Cr), choline (Ch) peaks, with difference spectra (B, D) showing the glutathione (GSH) peak. The advanced method for accurate, robust, and efficient spectral fitting (AMARES) spectral fit line for the peaks is shown in a separate overlaying line. PPM, parts per million.

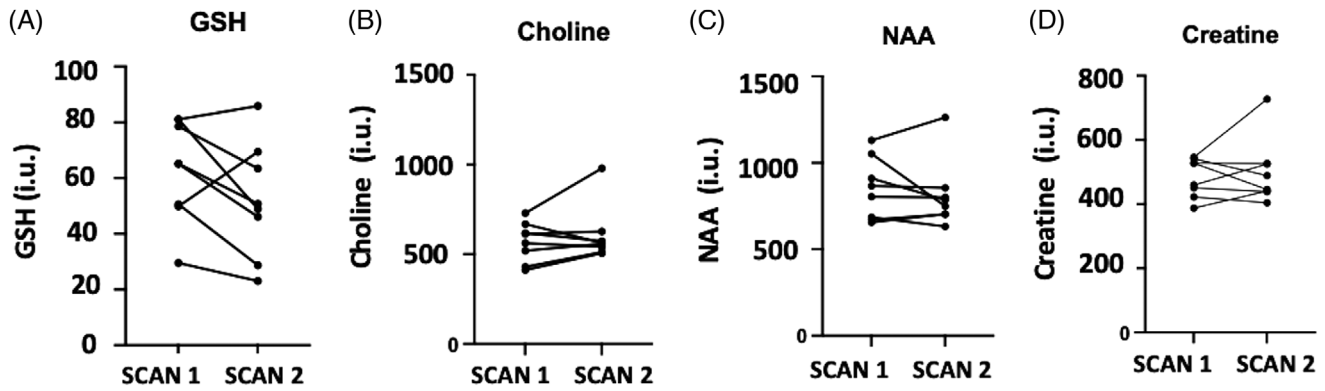

FIGURE 3 Plots showing raw data for individual participants across both Scans 1 and 2 for (A) glutathione (GSH), (B) choline, (C) N-acetyl aspartate (NAA), and (D) creatine. i.u., International Unit.

the creatine peak was 11.4 ± 0.9 Hz for Scan 1 and 13.4 ± 3 Hz for Scan 2, with a CV of 13.2%.

Voxel placement had an average tissue composition for Scan 1 of 5 ± 1% (CSF), 52 ± 3% (WM), and 43 ± 3% (GM) and for Scan 2 composition values were 5 ± 1% (CSF), 52 ± 4% (WM), and 44 ± 4% (GM).

DISCUSSION

This is the first study focusing on estimating the reliability of MEGA-PRESS GSH measures in the MTL encompassing the hippocampus, as an important structure for AD pathophysiology, but a challenging area to obtain well-separated peaks for low-concentration metabolites. In the current context, there are two important factors affecting the reproducibility of measures: (1) obtaining a measure from the MTL with its impact on MRS quality using the MEGA PRESS sequence and (2) measuring GSH using this sequence in the MTL.

We have shown that it is possible to measure tNAA, tCr, and tCho with a high level of single-session reproducibility (CV<10%) using a voxel that encompassed a significant amount of the hippocampus. By using a specific shaped voxel, we aimed to reduce CSF and WM contamination and also achieve linewidths that were reflective of good

TABLE 2 Metabolites quantified in both scans.

	Scan 1	Scan 2	%CV	*p*-value
tNAA	847 ± 178	812 ± 195	7.16	.47
tCr	483 ± 61	500 ± 102	7.84	.58
tCh	569 ± 111	607 ± 155	9.26	.35
GSH	62.7 ± 18.3	52.1 ± 20.8	21.97	.11
GSH/tNAA	0.075 ± 0.020	0.065 ± 0.025	21.63	.25
tCr/tNAA	0.58 ± 0.08	0.62 ± 0.05	9.47	.25
tCh/tNAA	0.68 ± 0.11	0.75 ± 0.07	10.00	.07

Note: Metabolites from the unedited total N-acetyl aspartate (tNAA), total creatine (tCr), and total choline (tCh) and the difference glutathione (GSH) spectrum from voxel encompassing the hippocampus. "Mean" is the mean quantities (normalized to internal water – institutional units) of each metabolite measured across Scan 1 and Scan 2 for all individuals. All metabolites are corrected for CSF. Coefficient of variance (%) between the two measures for each tissue type from each individual. *p*-values obtained from paired *t*-tests between the two measures. All the data represent mean±standard deviation unless otherwise indicated.
Abbreviation: CSF, cerebrospinal fluid; CV, coefficient of variance.

shimming in a difficult shim region. Reproducibility of voxel placement was also achievable over multiple sessions (reflected by a dice coefficient of 0.83), translating to good reproducibility of metabolites from the unedited spectra. The reproducibility of GSH within this cohort was poorer (CV 21.97%) to that of the more abundant metabolites (CV<10%), which could be attributed to a reduced signal-to-noise of GSH within the tissue due to a smaller concentration of the metabolite. This low level of reproducibility suggests further improvements need to be considered before the method can be utilized for clinical applications.

The CV of GSH in the MTL in the present study is poorer than that from another study that looked at GSH in the frontal lobe,[8] which reported a CV of 13.1%. Recently, GSH reproducibility was measured in the occipital and primary cortices reporting CVs of <10%.[22] Lower CVs in these cortical regions are not surprising given the reduced volume of CSF in cortical regions compared to the MTL, and that the MTL is proximal to the sinuses and bone which impacts the B0 and magnetic susceptibility of this area resulting in large linewidths. It is often the case that advanced shimming is required, incorporating second-order shims and manual intervention to obtain well-separated resonances. This, in combination with low-concentration metabolites, such as GSH being dominated by larger concentration resonances, can be challenging to acquire reproducible measures of GSH with the standard PRESS/STEAM sequences. MEGA-PRESS is likely the most sensitive method to detect and quantify GSH in vivo.[6,23] To date, clinical applications[24] of MEGA-PRESS have focused on measuring GABA, another neurochemical that is low in concentration with a resonance overlapping more prominent resonances in non-MEGA-PRESS MRS sequences. It should be noted that the appearance of the water peak is close to the GSH peak at 4.56 ppm. There is a possibility that water suppression pulses may also play a part in affecting the final GSH signal that is measured. This perhaps warrants a clearer understanding of the impact of the water suppression pulses; however, it can be argued that this effect could be consistent across all individuals and, therefore, when investigating differences between groups, this effect may be negated.

There are a limited number of papers investigating the reproducibility of metabolite measurements using MRS in the hippocampus. One study[14] showed a strong (<10%) CV of within-session scans of the hippocampus for glutamine, glutamate, tNAA, tCr, and tCho in young healthy individuals using a non-MEGA PRESS (semi-LASER) sequence at 3T with a shorter TE of 28 milliseconds. Reproducibility of tNAA, tCr, and tCho was 3%, 2%, and 3.3%, respectively, which was an improvement upon the present study (<10%). It should be noted that differences in the sequence parameters (eg, TE differences, not optimal for GSH) would also impact the relaxivity of the metabolites impacting overall measures. Linewidth reproducibilities were comparable to our study (<5%), suggesting that shim profiles were overall similar in the region. Voxel planning encompassing the hippocampus length in the sagittal plane using a rectangular voxel in the semi-LASER sequence study was in line with the planning that was done in our study. Reproducibility of tissue fractions within this voxel was slightly worse with a CSF CV (17.2%) compared to our CSF CV (10.2%). Hippocampal overlap reproducibility was good in both studies with agreed finding[14] that poorer CSF reproducibility within the voxel, suggesting the importance of CSF correction. A previous study[25] using a longer TE of 120 milliseconds and the semi-LASER sequence showed similar tissue composition in a rectangular voxel to our values, with a large proportion attributed to the presence of WM. CV values for tNAA, tCr, and tCho were shown to be <10% using this method of measures; however, the authors could not quantify GSH. It has previously been demonstrated[26] that regional metabolite differences within the hippocampus structure can be significantly different when measured with a PRESS-MRSI sequence at 1.5T, suggesting that accurate voxel placement in longitudinal studies is imperative. This work also looked at the impact of increased averages on reproducibility and found that 512 averages led to lower (ie, better) CVs. Our current work looked at using a clinically feasible sequence which would need to be kept within a time limit (<10 minutes) for patient compliance and comfort thus limiting the number averages. In line with this, recent work by Gajdošík et al.[27] showed at long TEs, CVs within the hippocampus between sessions for tCr and tCho <7% and NAA <5%, again with GSH not quantified in this work. It should also be highlighted, that in our current study, a water reference with the same parameters (including TE) was acquired within the same voxel location; however, recently, it has been proposed that acquisitions with a long TE should endeavor to acquire unsuppressed water references with both the same TE and a short TE[20] to control for T2 relaxation effects, potentially important when investigating pathologies.

It is noteworthy that this investigation was on a healthy population with minimal tissue loss in the hippocampus. In AD patients, it is expected that involved manual shimming and voxel planning would be required due to tissue loss. This may increase the scan time, which may challenge patients. Evidence to date[28] suggests that GSH changes in AD as measured by MRS at 3T (~30%) are greater than that of

the CV we report here. Consistent with reported changes in GSH in AD, MRS has unveiled differences in other neurochemicals within the hippocampus in patients with MCI and AD. Using 1H[6,29–31] and 31P[32–34] MRS, studies have shown changes in high energy metabolites, pH, and metabolites relating to cellular function within the hippocampus, suggesting potential utility for early diagnosis, disease progression, and drug response monitoring. It is practical to incorporate MEGA-PRESS on clinical scanners with multi-channel head coils commonly used in clinic. While it is feasible to acquire GSH measures in the MTL using MEGA-PRESS, care in understanding the constraints of the interpretation of the measures should be acknowledged. Additionally, it is also important to note that to improve signal to noise ratio and shimming, the voxel needs to be relatively big and planned appropriately to minimize the inclusion of CSF and sinuses. This large-sized voxel will include considerable partial voluming with other tissue types and structures which is unavoidable. There is significant value in investing more in sequence development to address the precision and reliability of neurochemical measures within the MTL especially for structures such as the hippocampus for potential clinical utility.

This work investigated the reproducibility of MRS measures in the hippocampus using the MEGA-PRESS sequence. MEGA-PRESS has the ability to separate GSH spectra from other metabolites in the human brain. We have shown that it is possible to measure key metabolites including GSH in the MTL with the reproducibility for key metabolites such as NAA, Cr, and Cho being higher than that of GSH in this region.

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