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Comparison of reproducibility of Single Voxel Spectroscopy and Whole Brain Magnetic Resonance Spectroscopy Imaging at 3T

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Abstract

To date, single voxel spectroscopy (SVS) is the most commonly used magnetic resonance spectroscopy (MRS) technique. SVS is relatively easy to use and provides automated and immediate access to the resulting spectra. However, it is also limited in spatial coverage. A new and very promising MRS technique allows for whole-brain MR spectroscopic imaging (WB-MRSI) with much improved spatial resolution. Establishing the reproducibility of data obtained using SVS and WB-MRSI is an important first step for using these techniques to evaluate longitudinal changes in metabolite concentration. The purpose of this study was to assess and directly compare the reproducibility of metabolite quantification at 3T using SVS and WB-MRSI in 'hand-knob' areas of motor cortices and hippocampi in healthy volunteers. Ten healthy adults were scanned using both SVS and WB-MRSI on three occasions one week apart. *N*-acetyl-aspartate (NAA), creatine (Cr), choline (Cho) and myo-inositol (mI) were quantified using SVS and WB-MRSI with reference to both Cr and H₂O. The reproducibility of each technique was evaluated using the coefficient of variation (*CV*), and the correspondence between the two techniques was assessed using Pearson correlation analysis. The measured mean (range) intra-subject *CV*s for SVS were 5.90 (2.65–10.66)% for metabolites (i.e., NAA, Cho, mI) relative to Cr, and 8.46 (4.21–21.07)% for metabolites (NAA, Cr, Cho, mI) relative to H₂O. The mean (range) *CV*s for WB-MRSI were 7.56 (2.78–11.41)% for metabolites relative to Cr, and 7.79 (4.57–14.11)% for metabolites relative to H₂O. Significant positive correlations were observed between metabolites quantified using SVS and WB-MRSI techniques when the Cr but not H₂O reference was used. The results demonstrate that reproducibility of SVS and WB-MRSI is similar for quantifying the four major metabolites (NAA, Cr, Cho, mI); both SVS and WB-MRSI exhibited good reproducibility. Our findings add reference information for choosing the appropriate ¹H-MRS technique in future studies.

Keywords

magnetic resonance spectroscopy; single voxel spectroscopy; whole-brain magnetic resonance spectroscopy; metabolite; reproducibility

Introduction

Proton magnetic resonance spectroscopy (^1H -MRS) is a noninvasive method for *in vivo* quantification of brain metabolites including: *N*-acetyl-aspartate (NAA, neuronal marker), creatine (Cr, energy metabolism marker), choline (Cho, cell membrane marker), myo-inositol (mI, glia cell marker), and glutamate-glutamine (Glx, excitatory neurotransmitter and precursor amino acid).^{1–2} One major application of ^1H -MRS is the longitudinal assessment of the pathophysiological process of diseases (e.g., stroke,³ brain tumor,⁴ and schizophrenia⁵), and the evaluation of the alteration of metabolite concentration due to treatments.

Our laboratory has previously shown that Constraint-Induced Movement Therapy (CIMT) significantly improves upper extremity motor disability following stroke,^{6–11} cerebral palsy,¹² traumatic brain injury,¹³ and multiple sclerosis.¹⁴ The treatment effect is associated with substantial structural changes in grey matter (GM) in bilateral sensorimotor cortices, more anterior motor areas, and hippocampi.^{15–18} However, to determine the neuro-metabolic changes produced by CIMT or other treatments, it is important to first evaluate the normal variation and reproducibility of candidate ^1H -MRS techniques in healthy subjects.¹⁹

Two types of ^1H -MRS techniques were assessed in the current study based on their apparent advantages and limitations: 1) Single voxel spectroscopy (SVS),²⁰ and 2) whole-brain MR spectroscopic imaging (WB-MRSI).²¹ SVS is the most widely used ^1H -MRS technique for assessing in-vivo brain metabolite changes in a targeted region of interest (ROI). It has the advantage of established protocols, short acquisition time, good spectral quality, and easier data processing and interpretation compared to WB-MRSI.²² However, an SVS measurement only allows for metabolite profile evaluation within pre-defined regions (voxels) of the brain that are typically 4–8 cm³.²³ In contrast, WB-MRSI has the advantage of obtaining a simultaneous representation of the whole brain spatial distribution of metabolite concentrations²⁴ with a better spatial coverage. However, this comes with disadvantages that include longer shimming and acquisition times, lower signal-to-noise ratio (SNR), and greater range of spectral quality over the larger volume of interest (VOI).²⁵ The reproducibility of SVS and WB-MRSI in healthy subjects has been studied extensively and findings suggest that each technique reliably measures the major metabolites.^{26–29} However, no study to date has directly compared these two techniques in the same group of subjects.

The aim of this study was to assess and directly compare the reproducibility of SVS and WB-MRSI for measuring brain metabolites in the same healthy subjects. The bilateral ‘hand-knob’ motor area and hippocampus were chosen as ROIs for SVS based on our lab’s previous work.^{15–17, 30} The same ROIs extracted from SVS were mapped onto WB-MRSI to

quantify metabolite concentrations in the same areas to compare these two spectroscopic techniques carried out in the same scanning session.

Methods

Subjects

Ten healthy volunteers (five women, nine right-handed) with a mean (range) age of 25.6 (19–49) years were recruited for this study with a mean (*SD*) BMI of 27.58 (7.51). In all cases, informed written consent was obtained before the examination. The study protocol and consent form were approved by the Institutional Review Board of the University of Alabama at Birmingham.

Data Acquisition

MRI and MRS scans of the brain were completed for each participant on 3 separate occasions (Scanning Sessions 1, 2, and 3) with a mean interval of one week between sessions. Scans were obtained on a MAGNETOM Prisma 3T scanner (Siemens Healthineers, Malvern, PA) with a 20-channel head array coil. Participants were placed in the isocenter of the scanner (aligned to the nasion) to achieve consistent positioning between sessions and across participants. Foam wedges were placed on both sides of the head to minimize head motion. Each session took approximately 1 hour including: 1) **T1-weighted images**: MPRAGE (Magnetization Prepared Rapid Gradient Echo), 208 slices, 0.9mm thick, no gap, TI (inversion time) = 901ms, TR (repetition time)/TE (echo time) = 2300/2.51ms, voxel size = $0.9 \times 0.9 \times 0.9$ mm, field of view (FOV) = 230mm, flip angle = 8° , TA (acquisition time) = 5min; 2) **SVS**: four scans were acquired using spin-echo acquisition svs_se (PRESS, Point Resolved Spectroscopy) sequence, TR/TE = 2000/30ms, number of averages = 168, TA = 6min for bilateral ‘hand-knob’ motor voxels, and number of averages = 192, TA = 7min for bilateral hippocampal voxels. Water suppression was achieved using three chemical shift-selective (CHESS) water suppression pulses. The chemical shift directions were switched to reduce contamination, especially from lipids near the skull, and from the cerebrospinal fluid (CSF) near the hippocampus; 8 non-water-suppression acquisitions were acquired from the same voxel. Voxels were placed to maximize the amount of GM: a) motor voxels were defined in the axial plane, as centered on the ‘hand-knob’ area,³¹ VOI = $2 \times 2 \times 2$ cm³ (Figure 1a); b) hippocampal voxels were aligned along the anterior-posterior hippocampal axis in a reconstructed axial plane to minimize neighboring tissue including amygdala and the CSF of the ventricles,³² VOI = $9 \times 27 \times 9$ mm³ (Figure 1b). The scanner’s brain GRE (Gradient Echo) shimming option with first and second order shims was used to optimize field homogeneity across the measurement voxels, excluding outer volume tissues and lipids. Although saturation bands were not used for SVS, lipid contamination was not noticeable and did not affect spectral quality as demonstrated in the spectra shown in Figure 2. 3) **WB-MRSI** was acquired using a 3D-echo-planar spectroscopic imaging (EPSI) sequence³³ with slabs aligned along the anterior commissure - posterior commissure plane (Figure 1c). The acquisition used non-selective lipid inversion nulling³⁴ with TI = 198ms, TR/TE = 1550/17.6ms, TA = 18min, FOV = $280 \times 280 \times 180$ mm³, $50 \times 50 \times 18$ k-space points, GRAPPA factor = 1.5, flip angle = 71° , and a 135-mm-thick axial excitation slab to cover the whole brain. The effective voxel volume =

1.55ml and the nominal voxel volume = 0.313ml. Interleaved non-water-suppressed acquisition was collected within the same grid size as the water-suppressed metabolite acquisition using a gradient echo technique with 20° excitation pulse. Frequency alignment was applied during EPSI acquisition: each k-space acquisition was aligned using a frequency estimate based on a linear extrapolation of a measurement of the frequency drift at the center $k = 0$ acquisition. In addition, correction for spatially variant B_0 shifts was implemented using an eddy-current correction scheme based on the phase of the water reference dataset.³⁵ Shimming was performed with an automated 3D mapshim procedure, followed by manual shimming if needed to obtain a magnitude of real water peak linewidth below 30Hz across the whole brain. When time permitted, localizer scans were acquired at the end of the scan session to check for gross head motion.

Data Processing

Tissue segmentation: T1 images were segmented into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) based on the probability maps in FSL (FAST, FMRIB's Automated Segmentation Tool, <http://www.fmrib.ox.ac.uk/fsl/>).³⁶ SVS raw data headers were exported to form ROIs for masking each of the tissue classes³⁷ and spectra fitting. The same ROI files were also imported into MIDAS (WB-MRSI analyzing package, metabolic imaging and data analysis system) for WB-MRSI ROI mapping.

Spectral Quantification

Spectral data were quantified using jMRUI (SVS data) and MIDAS (WB-MRSI data). For practical reasons, 5 metabolites (NAA, Cho, Cr, mI and Glx) were targeted because they are most relevant to our future clinical research, and could be quantified most accurately.

Detailed processing steps are described below:

- **SVS:** For SVS acquired data, images were processed in jMRUI³⁸ (version 5.2, <http://www.jmrui.eu/2015/05/jmrui-5-2/>). The residual water peak was removed using the HLSVDPro Filter (Hankel Lanczos Singular Values Decomposition Filter based on the Propack Library).³⁹ Spectra were then quantified in the time domain using the AMARES (advanced method for accurate, robust, and efficient spectral fitting)⁴⁰ algorithm. A prior knowledge file of metabolite spectra was created based on an in-house MRS phantom⁴¹ spectra profile consisting of peaks for NAA, Cho, Cr, mI and Glx. This profile was automatically applied to all SVS scans. Cramer-Rao lower bounds (CRLB) and metabolite linewidths, serving as an estimation of uncertainty and assessment of spectra quality, were calculated for each peak.
- **WB-MRSI:** For WB-MRSI acquired data, images were processed in the MIDAS^{35,42} package. First, whole-brain metabolite reconstruction was completed using a fully automatic batch processing pipeline in the MIDAS package, which included: data resampling, segmentation, spatial reconstruction (spatial FDFT, Four Dimensional Fourier Transform), B_0 map correction and spatial registration, spectral reconstruction (spectral FDFT), brain and scalp mask formation (MRMask), smoothing, spectral fitting (FITT2, automated spectral fitting), and normalization. Metabolite data were reconstructed into $64 \times$

64 × 32 voxels with an individual voxel volume of approximately 0.1ml. Voxel data with metabolite linewidth greater than 12Hz or CSF > 30% were excluded from further analysis. Second, ROIs were mapped by extracting the location of voxel locations from the raw data headers from the SVS acquisitions. Voxel locations were then imported into MIDAS for the WB-MRSI dataset in subject space using the inverse spatial transformation in the MINT (Map INTEgrated spectrum) program⁴³ and spectral fitting was carried out across the whole brain. Finally, a second regional spectral fitting was conducted within the 'hand-knob' motor area and hippocampal ROIs to quantify the regional metabolites with improved SNR. Metabolites that were quantified in MINT included: NAA, Cho, Cr, Glx, glutamate, glutamine, gamma-aminobutyric acid (GABA), mI, scy-inositol, taurine, acetate, and lactate. However, only the 5 targeted metabolites (NAA, Cho, Cr, mI and Glx) were included for analysis.

All metabolites in both programs (jMRUI and MIDAS) were quantified with respect to both internal references (Cr and H₂O). In jMRUI, metabolite concentrations to H₂O were calculated using the equation:⁴⁴

$$[\text{Met}] = \frac{\text{Signal}(\text{met})}{\text{Signal}(\text{H}_2\text{O})} \times \frac{2}{n(\text{met})} \times \{ [\text{H}_2\text{O}(\text{GM})] \times f(\text{GM}) + [\text{H}_2\text{O}(\text{WM})] \times f(\text{WM}) + [\text{H}_2\text{O}(\text{CSF})] \times f(\text{CSF}) \}$$

[Met] is the corrected metabolite concentration with reference to H₂O; Signal (met) and Signal (H₂O) are the integral values of the measured metabolite peaks and H₂O peak; 2 for the number of water protons, n(met) is the number of protons that give rise to the metabolite peak (that is, n_{NAA} = 3; n_{Cho} = 9; n_{Cr} = 3; n_{mI} = 4; and n_{Glx} = 4); H₂O (GM), H₂O (WM), and H₂O (CSF) are the water concentrations in these tissues (specifically, H₂O in GM is 43.3M, H₂O in WM is 35.8M, H₂O in CSF is 55.5M);⁴⁵ f(GM), f(WM), and f(CSF) are the fractions of tissue (GM, WM, CSF) volume in the ROI voxel.

In MIDAS, metabolite ratios to H₂O were calculated using the equation:

$$[\text{Met}] = \frac{\text{Met}(\text{area})}{\text{H}_2\text{O}(\text{area})} \times \frac{1}{1 - \text{Percent}(\text{CSF})}$$

[Met] is the corrected metabolite concentration with reference to H₂O; Met(area) is the integral value of the measured metabolite peak after correction for GM/WM fractions and proton numbers; H₂O (area) is the H₂O signal equivalent to that of 100% water at the same voxel location; Percent (CSF) is the percentage of CSF volume in the ROI voxel. In the automatic processing package, MIDAS applied the same tissue and proton number correction strategies as shown in the jMRUI equation for metabolites quantification.

The exclusion criteria for spectra were: 1) inappropriate voxel placement, 2) CRLB peaks >20%, 3) metabolite linewidth > 20Hz for SVS, and > 12Hz for WB-MRSI; for whole brain spectra, 4) full width at half maximum (FWHM) during shimming > 25Hz, and 5) percentage of number of accepted voxels < 30%. Scanning parameters were not optimized for acquiring Glx data; it was therefore excluded from analysis.

Data Analysis

The percentage of GM, WM, and CSF in each ROI were compared across the three longitudinal scans using an ANOVA in SPSS12; a two-tailed p -value of < 0.05 was considered statistically significant. Coefficients of variation (CV) values^{46–47} were used to assess the reproducibility of each of the two spectroscopic techniques for each metabolite concentration ratio by ROI. Intra-subject CV values were used here as an index of change over repeated measurements within a single subject. Intra-subject CV values were calculated for each spectroscopic technique by expressing the standard deviation (SD) of the measurements from Scanning Sessions 1, 2, and 3 of a given metabolite concentration ratio in a given ROI from a single subject as percentage of the mean of these measures. The mean of the intra-subject CV values from each of the 10 subjects was used to represent a summary value for the entire sample. Sensitivity of the two spectroscopic techniques was indexed by a variant of the Minimal Detectable Change (MDC) statistic,⁴⁸ which provides an estimate of the minimum change required to be confident that changes observed are not due *only* to error or natural variation or some combination of both. The equation^{49–50} used to determine the minimum percentage change in metabolite concentration observed in a longitudinal study that could be considered with at least 95% confidence to be larger than a change due to error or natural variation is:

$$\text{Sensitivity} = 1.96 \times \sqrt{n} \times \frac{CV}{\sqrt{N}}$$

CV is the median intra-subject CV value from the ten healthy subjects; N is the number of subjects in the study sample (here we used $N = 20$ individuals, a common number of subjects in MRS clinical studies⁴⁶); 1.96 is the z -statistic value that corresponds with a two-tailed, 95% confidence interval; it is multiplied by the $\sqrt{2}$ to adjust for the number of measurements (n), in this case two, e.g., to detect metabolite changes from before to after treatment.

The correspondence between the two spectroscopic techniques for metabolite quantification was quantified using Pearson correlation coefficients; a p -value of < 0.05 (one-tailed given our directional hypothesis) was considered statistically significant.

Results

Spectral quality

A total of 120 SVS scans ($4 \text{ voxels} \times 3 \text{ sessions} \times 10 \text{ subjects}$), and 30 WB-MRSI scans ($3 \text{ sessions} \times 10 \text{ subjects}$) were collected. With four extracted SVS ROIs mapped onto each individual WB-MRSI scan, a total of 120 WB-MRSI volumes ($30 \text{ WB-MRSI scans} \times 4 \text{ ROIs}$) were generated. After excluding poor quality spectra, the resultant spectra number was 115 for SVS and 117 for WB-MRSI. Example spectra from ‘hand-knob’ motor and hippocampus voxels in the same subject acquired using both techniques are shown in Figure 2.

Linewidth is an important indicator of spectral quality. Prior research indicates the variation in metabolite quantification accuracy obtained using ^1H -MRS is dependent on linewidth.

^{51–53} The overall averaged linewidths for all metabolites were 6.7 (range: 5.4–9.3) and 10.5 (range: 8.1–15.1) for ‘hand-knob’ motor and hippocampal voxels respectively using SVS; compared to 6.0 (range: 5.3–11.1) and 7.9 (range: 3.3–9.7) using WB-MRSI for included spectra. More specifically, the mean metabolite linewidths were 6.8 vs. 6.6 using SVS compared to 6.1 vs. 5.8 using WB-MRSI for left vs. right ‘hand-knob’ motor voxels; and 10.1 vs. 10.9 using SVS compared to 8.1 vs. 7.8 using WB-MRSI for left vs. right hippocampal voxels. There was no clear relationship between intra-subject *CV* and metabolite linewidth for either SVS or WB-MRSI (Supplementary Table 1 and Supplementary Table 2).

Voxel segmentation

The SVS voxels were consistently placed in the same anatomical locations in bilateral ‘hand-knob’ motor cortex and hippocampus. Voxels, on average across participants and testing sessions, were composed of $38.30 \pm 5.86\%$ (mean $\pm$ *SD*%) GM, $52.29 \pm 7.49\%$ WM, and $9.42 \pm 4.12\%$ CSF in ‘hand-knob’ motor voxels; and $77.51 \pm 6.04\%$ GM, $20.89 \pm 5.70\%$ WM, and $1.60 \pm 1.60\%$ CSF in hippocampal voxels. There was no significant statistical difference ($p > 0.05$) in tissue fractions for GM, WM, and CSF among the three scanning sessions (Table 1).

Reproducibility

Figure 3 displays intra-subject *CV* values, which were used to index the reproducibility of SVS and WB-MRSI techniques, for the metabolites studied. Mean intra-subject *CV* values are reported separately for the metabolite concentration ratios with reference to Cr and to H₂O by ROI. Generally speaking, the intra-subject *CV*s for all metabolites (Table 2) were: 1) similar between SVS (11% for motor ROIs, 13% for hippocampal ROIs-except for mI/H₂O in right hippocampus) and WB-MRSI (11% for motor ROIs, 14% for hippocampal ROIs); 2) consistently higher in hippocampal ROIs than in motor ROIs using either Cr or H₂O references; 3) generally higher when using ratios to H₂O than to Cr. Moreover, a slight superiority of SVS over WB-MRSI was associated with 1) voxel location and 2) internal reference for quantification. More specifically, 1) the mean *CV* over all metabolites in hippocampal ROIs was 8.7% for SVS, which was slightly better than the mean *CV* for WB-MRSI (9.3%); in motor ROIs, the mean *CV* for SVS (6.2%) and for WB-MRSI (6.1%) were similar. 2) When using an internal reference to Cr, SVS (mean *CV*s of 4.5, 7, 7% for NAA, Cho, mI respectively across all ROIs) showed less variability than WB-MRSI (mean *CV*s of 6, 6.5, 10.5% for NAA, Cho, mI respectively across all ROIs). On the contrary, when using a reference to H₂O, SVS (mean *CV*s of 7, 7.5, 7.5, 12% for NAA, Cr, Cho, mI respectively across all ROIs) showed more variability than WB-MRSI (mean *CV*s of 6.5, 6.5, 7, 11% for NAA, Cr, Cho, mI respectively across all ROIs).

Sensitivity

Table 3 lists the minimum percent changes in metabolite ratios that can be detected for the two spectroscopic techniques studied. That is, Table 3 lists the maximum percent change in metabolite ratios that could be expected due *only* to measurement error, natural variation, or some combination of both. Overall, a variation of 9% in concentration ratios could be expected for either SVS or WB-MRSI in the absence of an intervention or other type of

systematic change between scanning sessions. In addition, a variation of 5% could be expected in all motor ROIs (mean variation = 3%), while larger variations were generally expected for hippocampal voxels (mean variation = 5%). There were only minimal differences in sensitivity between the two techniques, with a mean variation for SVS of 7% (range = 2–9%) in hippocampal and 4% (range = 2–5%) in motor ROIs, and a mean variation for WB-MRSI of 8% (range = 4–9%) in hippocampal and 4% (range = 2–5%) in motor ROIs.

Correlation

When using Cr as a reference, medium to high positive correlations (Pearson's r ranging from 0.6–0.9) were observed between the two spectroscopic techniques for all metabolites, except for NAA/Cr in hippocampal ROIs, and mI/Cr in left hippocampus (Table 4). When using H₂O as a reference, the correlations between the two techniques were relatively low and inconsistent.

Discussion

SVS is currently the most commonly used ¹H-MRS technique to measure the concentration of brain metabolites. Although simple to use and readily available on standard MR scanners, SVS has limited coverage and limited spatial resolution. The relatively new WB-MRSI technique that is currently used at a limited number of institutions, offers promising advantages of greater spatial coverage and higher spatial resolution. These techniques have the potential to offer important new insight into neuro-metabolic changes that develop due to clinical interventions (e.g., CIMT). Therefore, it is important to assess the reproducibility and normal variation in healthy participants before utilizing them to evaluate treatment-induced neuro-metabolic changes in pathological conditions. This study provides information regarding intra-subject variability of neuro-metabolic changes for SVS and WB-MRSI. Moreover, it is the first study to directly compare the reproducibility of these two different spectroscopic techniques in the same group of healthy participants.

In the present study, we observed similar variability in longitudinal measurements of neuro-metabolites for SVS and WB-MRSI techniques. There was a slight superiority in the reproducibility of SVS over WB-MRSI for motor ROIs and for quantification with reference to Cr. However, if one is interested in multiple regions across the brain, considering the medium to high correlation (r range 0.6–0.9) between SVS and WB-MRSI for quantifying metabolites using the Cr reference, it would seem advisable to use the WB-MRSI technique, since it has the self-evident advantage of providing a comprehensive view of the metabolite profile over the whole brain within one measurement session. SVS is useful where reduced spatial resolution is not crucial for a given investigation, and where the assessment of specific brain areas is technically challenging to evaluate. For example, poor homogeneity and low SNR may be observed within the hippocampus due to its small anatomical size and adjacent structures of the skull, which include bone and air filled sinuses.⁴⁶ The negative correlations observed when taking H₂O as reference were surprising, one possible explanation was that when taking H₂O as the reference, the inherent differences in the SVS and MRSI acquisition protocols, and differences in quantification methods in the processing

programs (jMRUI vs. MIDAS) were more pronounced compared with using the Cr reference. However, since there were equivalent numbers of negative and positive correlations between SVS and MRSI, we've chosen not to interpret these values in this paper.

The *CVs* reported in the present study are largely consistent with previous studies. For WB-MRSI, the mean *CVs* of NAA, Cho, and Cr were 7.1–12.1% for hippocampus²⁹ and 1.9–4.4% for the frontal lobe²² (including the 'hand-knob' area in the posterior frontal lobe) using 3T acquisition methods. For SVS, our observed *CVs* in the hippocampus (ranges from 3.4–10.7%) were much lower than in previously reported studies (ranges from 6.1–17.8%).^{32,54–55} The higher variability in the previous studies could be due to lower magnetic field strength (1.5T³² vs. 3T in this study), or fewer averages (excitation average = 64^{51–52} vs. 192 in this study). Comparison to previous studies is not possible for metabolite concentration estimates from the 'hand-knob' motor area because no prior research has investigated the reproducibility of SVS within this specific region. Based on the sensitivity index used here, a change in all metabolites of > 9% in the hippocampus, and a change of > 5% in motor ROIs could be expected to be due to treatment rather than normal variation in a group of 20 subjects in the context of our study. However, different studies may result in different sensitivity criteria. This finding is in line with a previous study that found a minimum detectable change in metabolites to be 10%.⁴⁶ In addition, we observed 1) higher variability in hippocampi than motor voxels, and 2) greater variability using H₂O than Cr as a reference, which is consistent with previous studies using both SVS^{46,54} and WB-MRSI^{28–29} techniques.

The reproducibility of ¹H-MRS is influenced by many factors that can be generally categorized as: 1) acquisition factors including instrument variability, the magnetic field strength,⁵⁶ spectroscopic techniques,²³ spectroscopic sequences with different acquisition parameters,⁵⁷ subject motion, and biological variability;⁴⁸ 2) processing factors, including water removal strategy,⁵⁸ and spectral fitting method⁵⁹ (such as time-domain vs. frequency-domain methods based on different processing programs,⁶⁰ e.g., jMRUI vs. LC Model vs. MIDAS); and 3) quantification factor,⁶¹ including absolute quantification vs. relative quantification, external vs. internal reference quantification, prior knowledge used in data analysis, and simulated vs measured model spectra.

In our current study, the many factors mentioned above could have contributed to the observed differences in reproducibility, and the relatively low correlations between SVS and WB-MRSI for quantification using H₂O as a reference. For example, our dataset came from two sources acquired using different spectroscopic techniques--SVS (TR/TE = 2000/30ms) vs. WB-MRSI (TR/TE = 1550/17.6ms). Moreover, the data were processed independently in two programs (jMRUI for SVS vs. MIDAS for WB-MRSI) that have various differences in spectral fitting and quantification methods.

SVS has been the most widely used technique for measuring metabolites in targeted ROIs because of its short acquisition time and the resulting good spectral quality it provides. WB-MRSI has mainly been utilized for clinical purposes, such as monitoring the progress of tumor growth,⁶² where it is important to determine the effect of a disease process on the

whole brain within an acceptable measuring time (approximately 18 min). In the present study, we report relatively similar variability between SVS and WB-MRSI, with medium to high positive correlations between these two techniques when using Cr as a reference for metabolite quantification.

Limitations

Due to scanner availability and subject tolerance, we were not able to acquire an end of scan localizer after each SVS scan for every subject. These additional scans would be of value to assess subject movement. However, we did acquire some localizer scans at the end of the session in the first few subjects, and no gross head motion was detected. Subjects were always positioned/stabilized comfortably in the scanner, and the scan quality was comparable to other studies.^{27,41,54} We were able to obtain three weekly sessions of scans in the same group of volunteers. However, additional scanning sessions could have improved the evaluation of reproducibility and sensitivity of the ¹H-MRS techniques. Moreover, since brain metabolite levels vary with age,⁶³ the values obtained from the subjects within this study (mean age = 25.6, range = 19–49) may underestimate the variability occurring in the general population. The scanning protocols were specific for optimum performance of each technique providing good spectral quality in a reasonable measurement time for typical MRS clinical examination. Matching acquisition parameters (for example, using the same TR and TE for SVS and WB-MRSI) may be desirable for better comparison of the two techniques. However, this was not possible due to differences in the structure and timing of the measurement sequences. For example, the minimum available TE was not the same for both techniques. Minimum TEs were 30ms for SVS and 17.6ms for WB-MRSI. Minimum TEs were used in the study for optimized SNR, reduced J-coupling evolution and better detection of the shorter T2 metabolites. Improved reproducibility may be obtained with SVS methods that have been designed and optimized for high and ultra-high field scanners at some research institutions. Two such techniques are the ultra-short TE STEAM (Simulated Echo-Acquisition-Mode) sequence⁶⁴ and the sLASER (semiadiabatic Localization by Adiabatic Selective Refocusing) sequence.⁶⁵ Furthermore, the current study selected the ‘hand-knob’ motor area and hippocampus as the ROIs based on prior work and research interests of this laboratory. A comprehensive comparison of these two spectroscopic techniques will require evaluating other areas throughout the brain. In addition, investigating other brain metabolites with each technique will be of great importance for the future application of these spectroscopic techniques.

Conclusions

This study demonstrates similarly reliable quantification of NAA, Cr, Cho, and mI using SVS and WB-MRSI; both have good reproducibility. However, the degree of variability in the metabolite concentration ratios varied with ROI and quantification method. Specifically, more variability can be expected in hippocampal than in motor areas, and more variability using H₂O than Cr as a reference. This is a preliminary study providing information for the application of these two spectroscopic techniques. There is an ongoing investigation into the factors that may affect the reproducibility of each technique.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

SVS	single voxel spectroscopy
WB-MRSI	whole-brain MR spectroscopic imaging
NAA	<i>N</i> -acetyl-aspartate
Cr	creatine
Cho	choline
mI	myo-inositol
Glx	glutamate-glutamine
GABA	gamma-aminobutyric acid
CV	coefficient of variation
CIMT	Constraint-Induced Movement Therapy
ROI	region of interest
SNR	signal-to-noise ratio
VOI	volume of interest
FOV	field of view
CHESS	chemical shift-selective
PRESS	Point Resolved Spectroscopy
STEAM	STimulated Echo Acquisition Mode
EPSI	echo-planar spectroscopic imaging
MIDAS	metabolic imaging and data analysis system
FDFT	Four Dimensional Fourier Transform
MINT	Map INTEgrated
FWHM	full width at half maximum

CRLB	Cramer-Rao lower bounds
MDC	Minimal Detectable Change
sLASER	semiadiabatic Localization by Adiabatic Selective Refocusing

References

- [1]. Eliassen JC, Boespflug EL, Lamy M, Allendorfer J, Chu WJ, Szaflarski JP. Brain-mapping techniques for evaluating poststroke recovery and rehabilitation: a review. *Top Stroke Rehabil.* 2008;15(5):427–450. doi:10.1310/tsr1505-427. [PubMed: 19008203]
- [2]. McKay J, Tká I. Quantitative in vivo neurochemical profiling in humans: where are we now? *Int J Epidemiol.* 2016;45(5):1339–1350. doi:10.1093/ije/dyw235. [PubMed: 27794521]
- [3]. Mountz JM. Nuclear medicine in the rehabilitative treatment evaluation in stroke recovery. Role of diaschisis resolution and cerebral reorganization. *Eura Medicophys.* 2007;43(2):221–239. [PubMed: 17268387]
- [4]. Martínez-Bisbal MC, Celda B. Proton magnetic resonance spectroscopy imaging in the study of human brain cancer. *Q J Nucl Med Mol Imaging.* 2009;53(6):618–630. [PubMed: 20016453]
- [5]. Wijtenburg SA, Yang S, Fischer BA, Rowland LM. In vivo assessment of neurotransmitters and modulators with magnetic resonance spectroscopy: application to schizophrenia. *Neurosci Biobehav Rev.* 4 2015;51:276–295. doi:10.1016/j.neubiorev.2015.01.007. [PubMed: 25614132]
- [6]. Taub E, Miller NE, Novack TA, et al. Technique to improve chronic motor deficit after stroke. *Arch Phys Med Rehabil.* 1993;74(4):347–354. [PubMed: 8466415]
- [7]. Taub E, Uswatte G, King DK, Morris D, Crago J, Chatterjee A. A placebo-controlled trial of Constraint-Induced Movement therapy for upper extremity after stroke. *Stroke.* 2006;37(4):1045–1049. doi:10.1161/01.STR.0000206463.66461.97. [PubMed: 16514097]
- [8]. Taub E, Uswatte G, Mark VM, et al. Method for enhancing real-world use of a more-affected arm in chronic stroke: the transfer package of CI therapy. *Stroke.* 2013;44(5):1383–1388. doi:10.1161/STROKEAHA.111.000559. [PubMed: 23520237]
- [9]. Wolf SL, Winstein CJ, Miller JP, et al. Effect of Constraint-Induced Movement therapy on upper extremity function 3–9 months after stroke: the EXCITE randomized clinical trial. *JAMA.* 2006;296(17):2095–2104. doi:10.1001/jama.296.17.2141. [PubMed: 17077374]
- [10]. Wolf SL, Winstein CJ, Miller JP, et al. Retention of upper limb function in stroke survivors who have received Constraint-induced Movement therapy: the EXCITE randomized trial. *Lancet Neurol.* 2008;7(1):33–40. doi:10.1016/S1474-4422(07)70294-6. [PubMed: 18077218]
- [11]. Uswatte G, Taub E, Mark VW, Perkins C, Gauthier L. CNS plasticity and rehabilitation In Frank RG, Rosenthal M, & Caplan B (Eds.), *Handbook of rehabilitation psychology*. 2nd ed. Washington, DC: American Psychological Association; 2010:391–406.
- [12]. Taub E, Griffin A, Nick J, Gammons K, Uswatte G, Law CR. Pediatric CI therapy for stroke-induced hemiparesis in young children. *Dev Neurorehabil.* 2007;10(1):3–18. [PubMed: 17608322]
- [13]. Taub E, Uswatte G. Use of CI therapy for improving motor ability after chronic CNS damage: a development prefigured by Paul Bach-y-Rita. *J Integr Neurosci.* 2005;4(4):465–477. [PubMed: 16385641]
- [14]. Mark VW, Taub E, Uswatte G, et al. Constraint-Induced Movement therapy for the lower extremities in multiple sclerosis: case series with 4-year follow-up. *Arch Phys Med Rehabil.* 2013;94(4):753–760. doi:10.1016/j.apmr.2012.09.032. [PubMed: 23111280]
- [15]. Gauthier LV, Taub E, Perkins C, Ortmann M, Mark VW, Uswatte G. Remodeling the brain: plastic structural brain changes produced by different motor therapies after stroke. *Stroke.* 2008;39(5):1520–1525. doi:10.1161/STROKEAHA.107.502229. [PubMed: 18323492]
- [16]. Liepert J, Bauder H, Miltner WHR, Taub E, Weiller C. Treatment-Induced cortical reorganization after stroke in humans. *Stroke.* 2000;31(6):1210–1216. doi:10.1161/01.STR.31.6.1210. [PubMed: 10835434]

- [17]. Sterling C, Taub E, Davis D, et al. Structural neuroplastic change following Constraint-Induced Movement therapy in children with cerebral palsy. *Pediatrics* 2013;131(5):e1664–e1669. [PubMed: 23610209]
- [18]. Taub E, Uswatte G, Mark VM. The functional significance of cortical reorganization and the parallel development of CI therapy. *Front Hum Neurosci.* 6 2014;8:396. doi:10.3389/fnhum.2014.00396. [PubMed: 25018720]
- [19]. Li BS, Babb JS, Soher BJ, Maudsley AA, Gonen O. Reproducibility of 3D proton spectroscopy in the human brain. *Magn Reson Med.* 2002;47(3):439–446. doi:10.1002/mrm.10081. [PubMed: 11870829]
- [20]. Bertholdo D, Watcharakorn A, Castillo M. Brain proton magnetic resonance spectroscopy: introduction and overview. *Neuroimaging Clin N Am.* 2013;23(3):359–380. doi:10.1016/j.nic.2012.10.002. [PubMed: 23928194]
- [21]. Ding XQ, Lanfermann H. Whole brain 1H-Spectroscopy: A developing technique for advanced analysis of cerebral metabolism. *Clin Neuroradiol.* 2015;25 (Suppl 2):245–250. doi:10.1007/s00062-015-0428-5. [PubMed: 26156124]
- [22]. Currie S, Hadjivassiliou M, Craven IJ, Wilkinson ID, Griffiths PD, Hoggard N. Magnetic resonance spectroscopy of the brain. *Postgrad Med J.* 2013;89(1048):94–106. doi:10.1136/postgradmedj-2011-130471. [PubMed: 23014671]
- [23]. Ding XQ, Lanfermann H. Whole Brain 1H-Spectroscopy: A Developing Technique for Advanced Analysis of Cerebral Metabolism. *Clin Neuroradiol.* 2015;25(Suppl 2):245–250. doi: 10.1007/s00062-015-0428-5. [PubMed: 26156124]
- [24]. Lecocq A, Le Fur Y, Maudsley AA, et al. Whole-brain quantitative mapping of metabolites using short echo three-dimensional proton MRSI. *J Magn Reson Imaging.* 2015;42(2):280–289. doi: 10.1002/jmri.24809. [PubMed: 25431032]
- [25]. Hsu SH, Chou MC, Ko CW, et al. Proton MR spectroscopy in patients with pyogenic brain abscess: MR spectroscopic imaging versus single-voxel spectroscopy. *Eur J Radiol.* 2013;82(8):1299–1307. doi:10.1016/j.ejrad.2013.01.032. [PubMed: 23453705]
- [26]. Wijtenburg SA, Rowland LM, Edden RA, Barker PB. Reproducibility of brain dpectroscopy at 7T using conventional localization and spectral editing techniques. *J Magn Reson Imaging.* 2013;38(2):460–467. doi:10.1002/jmri.23997. [PubMed: 23292856]
- [27]. Nacewicz BM, Angelos L, Dalton KM, et al. Reliable non-invasive measurement of human neurochemistry using proton spectroscopy with an anatomically defined amygdala-specific voxel. *Neuroimage.* 2012;59(3):2548–2559. doi:10.1016/j.neuroimage.2011.08.090. [PubMed: 21924361]
- [28]. Maudsley AA, Domenig C, Sheriff S. Reproducibility of serial whole-brain MR spectroscopic imaging. *NMR Biomed.* 2010;23(3):251–256. doi:10.1002/nbm.1445. [PubMed: 19777506]
- [29]. Veenith TV, Mada M, Carter E, et al. Comparison of inter subject variability and reproducibility of whole brain proton spectroscopy. *PLoS One.* 2014;9(12):e115304. doi:10.1371/journal.pone.0115304. [PubMed: 25517503]
- [30]. Mark V, Taub E, Uswatte G, et al. Randomized controlled trial of CI therapy for progressive MS: increased real-world function and neuroplasticity on MRI. (2014). *Neurology*, 82 (Meeting Abstracts 1): I7.1.008.
- [31]. Yousry TA, Schmid UD, Alkadhi H, et al. Localization of the motor hand area to a knob on the precentral gyrus. A new landmark. *Brain.* 1997;120 (Pt 1):141–157. [PubMed: 9055804]
- [32]. Hammen T, Stadlbauer A, Tomandl B, et al. Short TE single-voxel 1H-MR spectroscopy of hippocampal structures in healthy adults at 1.5 Tesla--how reproducible are the results? *NMR Biomed.* 2005;18(3):195–201. doi:10.1002/nbm.958. [PubMed: 15884101]
- [33]. Ebel A, Maudsley AA. Improved spectral quality for 3D MR spectroscopic imaging using a high spatial resolution acquisition strategy. *Magn Reson Imaging.* 2003;21(2):113–120. [PubMed: 12670597]
- [34]. Spielman DM, Pauly JM, Macovski A, Glover GH, Enzmann DR. Lipid-suppressed single- and multisection proton spectroscopic imaging of the human brain. *J Magn Reson Imaging.* 1992;2(3):225–262. [PubMed: 1562775]

- [35]. Maudsley AA, Darkazanli A, Alger JR, et al. Comprehensive processing, display and analysis for in vivo MR spectroscopic imaging. *NMR Biomed.* 2006;19(4):492–503. doi:10.1002/nbm.1025. [PubMed: 16763967]
- [36]. Zhang Y, Brady M, Smith S. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *IEEE Trans Med Imag.* 2001;20(1):45–57. doi:10.1109/42.906424.
- [37]. Kraguljac NV, Reid MA, White DM, den Hollander J, Lahti AC. Regional decoupling of N-acetyl-aspartate and glutamate in schizophrenia. *Neuropsychopharmacology.* 2012;37(12):26352642. doi:10.1038/npp.2012.126.
- [38]. Stefan D, Di Cesare F, Andrasescu A, et al. Quantitation of magnetic resonance spectroscopy signals: the jMRUI software package. *Meas Sci Technol.* 2009;20(10):104035. doi:10.1088/0957-0233/20/10/104035.
- [39]. Laudadio T, Mastronardi N, Vanhamme L, Van Hecke P, Van Huffel S. Improved Lanczos Algorithms for Blackbox MRS Data Quantitation. *J Magn Reson.* 2002;157(2):292–297. [PubMed: 12323148]
- [40]. Vanhamme L, van den Boogaart A, Van Huffela S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson.* 1997;129(1):35–43. [PubMed: 9405214]
- [41]. Sabati M, Sheriff S, Gu M, et al. Multivendor implementation and comparison of volumetric whole-brain echo-planar MR spectroscopic imaging. *Magn Reson Med.* 2015;74(5):1209–1220. doi:10.1002/mrm.25510. [PubMed: 25354190]
- [42]. Maudsley AA, Domenig C, Govind V, et al. Mapping of brain metabolite distributions by volumetric proton MR spectroscopic imaging (MRSI). *Magn Reson Med.* 2009;61(3):548–559. doi:10.1002/mrm.21875. [PubMed: 19111009]
- [43]. Goryawala MZ, Sheriff S, Maudsley AA. Regional distributions of brain glutamate and glutamine in normal subjects. *NMR Biomed.* 2016;29(8):1108–1116. doi:10.1002/nbm.3575. [PubMed: 27351339]
- [44]. Bustillo JR, Rowland LM, Mullins P, et al. 1H-MRS at 4 tesla in minimally treated early schizophrenia. *Mol Psychiatry.* 2010;15(6):629–636. doi:10.1038/mp.2009.121. [PubMed: 19918243]
- [45]. Ernst T, Kreis R, Ross BD. Absolute quantitation of water and metabolites in the human brain. I. Compartments and water. *J Magn Reson B.* 1993;102(1):1–8.
- [46]. Allaili N, Valabregue R, Auerbach EJ, et al. Single-voxel 1H spectroscopy in the human hippocampus at 3T using LASER sequence: characterization of neurochemical profile and reproducibility. *NMR Biomed.* 2015;28(10):1209–1217. doi:10.1002/nbm.3364. [PubMed: 26282328]
- [47]. Saleh MG, Near J, Alhamud A, Robertson F, van der Kouwe AJ, Meintjes EM. Reproducibility of macromolecule suppressed GABA measurement using motion and shim navigated MEGA-SPECIAL with LCModel, jMRUI and GANNET. *MAGMA.* 2016;29(6):863–874. doi:10.1007/s10334-016-0578-8. [PubMed: 27393351]
- [48]. Vellozo CA, Lehman LA. Ability to detect change in patient function: responsiveness designs and methods of calculation. *J Hand Ther.* 2010;23(4):361–370. doi:10.1016/j.jht.2010.05.003. [PubMed: 20638823]
- [49]. Brooks WM, Friedman SD, Stidley CA. Reproducibility of 1H-MRS in vivo. *Magn Reson Med.* 1999;41(1):193–197. [PubMed: 10025629]
- [50]. Mullins PG, Rowland L, Bustillo J, Bedrick EJ, Lauriello J, Brooks WM. Reproducibility of 1H-MRS measurements in schizophrenic patients. *Magn Reson Med.* 2003;50(4):704–707. doi:10.1002/mrm.10598. [PubMed: 14523955]
- [51]. Bartha R Effect of signal-to-noise ratio and spectral linewidth on metabolite quantification at 4T. *NMR Biomed.* 2007;20(5):512–521. doi:10.1002/nbm.1122. [PubMed: 17205487]
- [52]. Kanowski M, Kaufmann J, Braun J, Bernarding J, Tempelmann C. Quantitation of simulated short echo time ¹H human brain spectra by LCModel and AMARES. *Magn Reson Med.* 2004;51(5):904–912. [PubMed: 15122672]

- [53]. Pfeuffer J, Tkac I, Provencher SW, Gruetter R. Toward an in vivo neurochemical profile: quantification of 18 metabolites in short-echo-time (1)H NMR spectra of the rat brain. *J Magn Reson.* 1999;141(1):104–120. [PubMed: 10527748]
- [54]. Venkatraman TN, Hamer RM, Perkins DO, Song AW, Lieberman JA, Steen RG. Single-voxel 1H PRESS at 4.0 T: precision and variability of measurements in anterior cingulate and hippocampus. *NMR Biomed.* 2006;19(4):484–491. doi:10.1002/nbm.1055. [PubMed: 16763968]
- [55]. Geurts JJ, Barkhof F, Castelijns JA, Uitdehaag BM, Polman CH, Pouwels PJ. Quantitative 1H-MRS of healthy human cortex, hippocampus, and thalamus: metabolite concentrations, quantification precision, and reproducibility. *J Magn Reson Imaging.* 2004;20(3):366–371. doi: 10.1002/jmri.20138. [PubMed: 15332241]
- [56]. Bartha R, Drost DJ, Menon RS, Williamson PC. Comparison of quantification precision of human short echo time 1H spectroscopy at 1.5 and 4.0 Tesla. *Magn Reson Med.* 2000;44:185–192. [PubMed: 10918316]
- [57]. Knight-Scott J, Brennan P, Palasis S, Zhong X. Effect of repetition time on metabolite quantification in the human brain in 1 H MR spectroscopy at 3 tesla. *J Magn Reson Imaging.* 2017;45(3):710–721. doi:10.1002/jmri.25403. [PubMed: 27474158]
- [58]. van den Boogaart A, van Ormondt D, Pijnappel WWF, de Beer R, Ala-Korpela M. In: *Mathematics in Signal Processing III*. McWhirter JG, Ed. Oxford: Clarendon Press; 1994:175–195.
- [59]. Mandal PK. In vivo proton magnetic resonance spectroscopic signal processing for the absolute quantitation of brain metabolites. *Eur J Radiol.* 2012;81(4):e653–664. doi:10.1016/j.ejrad.2011.03.076. [PubMed: 21515011]
- [60]. Brix MK, Ersland L, Hugdahl K, et al. Within- and between-session reproducibility of GABA measurements with MR spectroscopy. *J Magn Reson Imaging.* 2017; doi:10.1002/jmri.25588.
- [61]. Mosconi E, Sima DM, Osorio Garcia MI, et al. Different quantification algorithms may lead to different results: a comparison using proton MRS lipid signals. *NMR Biomed.* 2014;27(4):431–443. doi:10.1002/nbm.3079. [PubMed: 24493129]
- [62]. Morita N, Harada M, Otsuka H, Melhem ER, Nishitani H. Clinical Application of MR Spectroscopy and Imaging of Brain Tumor. *Magn Reson Med Sci.* 2010;9(4):167–175. [PubMed: 21187685]
- [63]. Maudsley AA, Govind V, Arheart. Associations of age, gender and body mass with 1H MR-observed brain metabolites and tissue distributions. *NMR Biomed.* 2012;25(4):580–593. doi: 10.1002/nbm.1775. [PubMed: 21858879]
- [64]. Tkáč I, Gruetter R. Methodology of 1H NMR Spectroscopy of the Human Brain at Very High Magnetic Fields. *Appl Magn Reson.* 2005;29(1):139–57. doi: 10.1007/BF03166960 [PubMed: 20179773]
- [65]. Öz G, Tkáč I. Short Echo, single-Shot, Full-Intensity Proton Resonance Spectroscopy for Neurochemical Profiling at 4T Validation in the cerebellum and Brainstem. *Magn Reson Med.* 2011;65:901–10. doi: 10.1002/mrm.22708. [PubMed: 21413056]

Figure 1. Single voxel spectroscopy (SVS) and whole-brain MR spectroscopic imaging (WB-MRSI) placement.

1a) 'hand-knob' motor voxel placement (black box); **1b)** hippocampal voxel placement (black box); **1c)** whole brain MRSI acquisition box (solid-line), shimming box (break-line), and saturation band placement.

Figure 2. Example spectra from left motor cortex and hippocampus in one healthy subject processed using SVS and WB-MRSI.

2a, b) Spectra from SVS of motor cortex and hippocampus; **2c, d)** Spectra from WB-MRSI of motor cortex and hippocampus. WB-MRSI data was acquired and SVS ROIs were mapped onto the whole brain to extract the spectra profiles. SVS: single voxel spectroscopy; WB-MRSI: whole-brain MR spectroscopic imaging; NAA: N-acetyl-aspartate; Cr: creatine; Cho: choline; ml: myo-inositol; I.U.: institutional unit.

Figure 3. Intra-subject variability of all metabolites with reference to Cr and H₂O in the four regions of interests (ROIs) (bilateral ‘hand-knob’ motor areas and hippocampus).

The spread of data within each ROI reflects inter-subject variation. The box and whisker plot uses Tukey’s method: the central lines in each box denote median values, the lower and upper boundaries are the 25th and 75th percentile, the error bars are the 10th and 90th percentile, and the closed circles are outlying data points. *CV*: coefficient of variation; SVS: single voxel spectroscopy; WB-MRSI: whole-brain MR spectroscopic imaging; NAA: N-acetyl-aspartate; Cr: creatine; Cho: choline; mI: myo-inositol; H₂O: water; L: left; R: right. Motor = ‘hand-knob’ area; Hippo = hippocampus.

Table 1.

Comparison of mean regional tissue fraction (%) across the three scanning sessions.

	L_Motor			R_Motor			L_Hippo			R_Hippo		
	GM	WM	CSF	GM	WM	CSF	GM	WM	CSF	GM	WM	CSF
Session#1	36.52 ±5.74	54.49 ±8.40	8.99 ±4.70	40.78 ±4.08	49.23 ±6.86	9.99 ±4.81	77.22 ±3.85	20.14 ±3.81	2.64 ±1.63	76.73 ±9.26	22.76 ±8.86	0.51 ±0.61
Session#2	35.90 ±5.77	55.42 ±6.64	8.68 ±3.58	41.28 ±6.91	48.50 ±8.22	10.23 ±4.73	74.14 ±6.37	23.61 ±6.31	2.25 ±1.68	79.60 ±4.41	19.65 ±4.41	0.75 ±0.81
Session#3	34.93 ±4.56	54.72 ±6.32	8.65 ±3.68	40.44 ±5.35	51.28 ±7.13	9.97 ±3.94	76.92 ±5.10	20.31 ±4.57	2.77 ±1.87	80.46 ±4.90	18.86 ±4.78	0.68 ±0.79
p-value	0.811	0.957	0.980	0.946	0.692	0.990	0.360	0.236	0.785	0.422	0.354	0.770

Note. Values are mean $\pm$ *SD*. P-value reported is for repeated-measures ANOVAs. GM: grey matter; WM: white matter; CSF: cerebrospinal fluid; L: left; R: right; *SD*: standard deviation. Motor = 'hand-knob' area; Hippo = hippocampus.

Table 2.

Comparison of reproducibility (*CV%*) in measuring regional metabolite concentration between SVS and WB-MRSI.

	Mean (Median) Intra-Subject <i>CV%</i>							
	SVS				WB-MRSI			
	L_Motor	R_Motor	L_Hippo	R_Hippo	L_Motor	R_Motor	L_Hippo	R_Hippo
NAA/Cr	2.97 (2.56)	4.47 (4.25)	3.36 (2.54)	6.09 (5.69)	2.78 (2.69)	2.99 (2.47)	8.70 (6.97)	9.53 (7.11)
Cho/Cr	4.62 (3.86)	6.60 (6.50)	6.82 (6.38)	9.45 (9.67)	6.52 (5.95)	5.16 (4.83)	6.40 (5.71)	7.69 (6.01)
mI/Cr	5.69 (3.81)	6.24 (3.57)	5.67 (4.99)	10.66 (6.85)	11.04 (8.53)	8.05 (7.78)	11.41 (10.95)	10.43 (8.87)
NAA/H₂O	4.90 (3.43)	4.34 (3.23)	7.37 (7.66)	10.81 (11.20)	4.57 (3.43)	4.81 (4.83)	7.56 (6.07)	9.25 (7.56)
Cr/H₂O	8.38 (7.42)	7.42 (6.37)	5.00 (4.25)	9.60 (9.33)	5.66 (5.53)	5.31 (5.34)	8.04 (5.70)	7.76 (6.43)
Cho/H₂O	6.11 (3.61)	4.90 (4.84)	6.38 (5.45)	12.36 (11.88)	6.68 (7.08)	5.38 (5.82)	7.48 (7.85)	9.38 (7.24)
mI/H₂O	10.97 (7.76)	6.62 (6.47)	9.35 (8.69)	20.55 (14.91)	9.50 (8.22)	5.90 (5.15)	14.11 (14.89)	13.32 (10.49)

CV: coefficient of variation; SVS: single voxel spectroscopy; WB-MRSI: whole-brain MR spectroscopic imaging; NAA: N-acetyl-aspartate; Cr: creatine; Cho: choline; mI: myo-inositol; H₂O: water; L: left; R: right. Motor = 'hand-knob' area; Hippo = hippocampus.

Table 3.

Comparison of sensitivity for detecting metabolite concentration changes between SVS and WB-MRSI.

	Sensitivity%							
	SVS				WB-MRSI			
	L_Motor	R_Motor	L_Hippo	R_Hippo	L_Motor	R_Motor	L_Hippo	R_Hippo
NAA/Cr	2	3	2	4	2	2	4	4
Cho/Cr	2	4	4	6	4	3	4	4
mI/Cr	2	2	3	4	5	5	7	5
NAA/H ₂ O	2	2	5	7	2	3	4	5
Cr/H ₂ O	5	4	3	6	3	3	4	4
Cho/H ₂ O	2	3	3	7	4	4	5	4
mI/H ₂ O	5	4	5	9	5	3	9	7

Sensitivity was indexed using a coefficient of variation (*CV*) based equation to determine the minimum percentage change in metabolites that could be observed with at least 95% confidence in a study. SVS: single voxel spectroscopy; WB-MRSI: whole-brain MR spectroscopic imaging; NAA: N-acetyl-aspartate; Cr: creatine; Cho: choline; mI: myo-inositol; H₂O: water; L: left; R: right. Motor = 'hand-knob' area; Hippo = hippocampus.

Table 4.

Correlations between SVS and WB-MRSI for regional metabolite concentration quantification.

	L_Motor		R_Motor		L_Hippo		R_Hippo	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
NAA/Cr	0.576	0.041 *	0.603	0.032 *	0.541	0.053	0.042	0.454
Cho/Cr	0.772	0.004 *	0.926	0.000 *	0.730	0.008 *	0.637	0.024 *
mI/Cr	0.749	0.006 *	0.712	0.011 *	0.188	0.301	0.713	0.010 *
NAA/H₂O	-0.530	0.058	-0.153	0.337	0.077	0.416	0.304	0.197
Cr/H₂O	-0.420	0.113	-0.668	0.017 *	0.012	0.478	-0.063	0.431
Cho/H₂O	0.200	0.290	-0.232	0.260	-0.381	0.139	-0.579	0.040 *
mI/H₂O	0.592	0.036 *	0.622	0.028 *	0.164	0.326	0.232	0.260

SVS: single voxel spectroscopy; WB-MRSI: whole-brain MR spectroscopic imaging; NAA: N-acetyl-aspartate; Cr: creatine; Cho: choline; mI: myo-inositol; H₂O: water; L: left; R: right. Motor = 'hand-knob' area; Hippo = hippocampus.

* $p < 0.05$.