Published in final edited form as:

NMR Biomed. 2015 August ; 28(8): 976–987. doi:10.1002/nbm.3340.

Reproducibility and optimization of *in vivo* human diffusion-weighted MRS of the corpus callosum at 3T and 7T

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Abstract

Diffusion-weighted MRS (DWS) of brain metabolites enables the study of cell-specific alterations in tissue microstructure by probing the diffusion of intracellular metabolites. In particular, the diffusion properties of neuronal *N*-acetylaspartate (NAA), typically co-measured with *N*-acetylaspartyl glutamate (NAAG) (NAA + NAAG = tNAA), have been shown to be sensitive to intraneuronal/axonal damage in pathologies such as stroke and multiple sclerosis. Lacking, so far, are empirical assessments of the reproducibility of DWS measures across time and subjects, as well as a systematic investigation of the optimal acquisition parameters for DWS experiments, both of which are sorely needed for clinical applications of the method. In this study, we acquired comprehensive single-volume DWS datasets of the human corpus callosum at 3T and 7T. We investigated the inter- and intra-subject variability of empirical and modeled diffusion properties of tNAA [$D_{\text{avg}}(\text{tNAA})$ and $D_{\text{model}}(\text{tNAA})$, respectively]. Subsequently, we used a jackknife-like resampling approach to explore the variance of these properties in partial data subsets reflecting different total scan durations. The coefficients of variation (C_V) and repeatability coefficients (C_R) for $D_{\text{avg}}(\text{tNAA})$ and $D_{\text{model}}(\text{tNAA})$ were calculated for both 3T and 7T, with overall lower variability in the 7T results. Although this work is limited to the estimation of the diffusion properties in the corpus callosum, we show that a careful choice of diffusion-weighting conditions at both field strengths allows the accurate measurement of tNAA diffusion properties in clinically relevant experimental time. Based on the resampling results, we suggest optimized acquisition schemes of 13-min duration at 3T and 10-min duration at 7T, whilst retaining low variability ($C_V \approx 8\%$) for the tNAA diffusion measures. Power calculations for the estimation of $D_{\text{model}}(\text{tNAA})$

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and $D_{\text{avg}}(\text{tNAA})$ based on the suggested schemes show that less than 21 subjects per group are sufficient for the detection of a 10% effect between two groups in case-control studies.

Keywords

diffusion-weighted MRS; inter-subject variability; intra-subject variability; power calculations; white matter

INTRODUCTION

Diffusion-weighted MRS (DWS) assesses the diffusion properties of intracellular metabolites, thus providing specific information about the microstructural properties of the compartments in which they reside (1–14). The compartmental localization of these metabolites, as well as their slow inter-compartmental exchange on the time scale of an MRI-based diffusion experiment, potentially makes them excellent compartment- and cell-specific markers for physiological and microanatomical changes in disease.

Among the metabolites accessible to *in vivo* MRS techniques, *N*-acetylaspartate (NAA) plays a central role in the evaluation of neuronal and axonal damage resulting from neurological disorders. Approximately 95% of total NAA (tNAA = NAA + NAAG; NAAG, *N*-acetylaspartyl glutamate) in the mature central nervous system is found within neurons (15), and therefore the diffusion properties of tNAA can provide a unique insight into damage to the intraneuronal/intra-axonal space, separate from confounding contributions such as the extracellular space. Early DWS studies in animal models showed that the amplitude of the diffusion-weighted tNAA peak was modulated by experimentally induced ischemic stroke (16,17). More recent studies have demonstrated the importance of compartment-specific diffusion measures derived from DWS in distinguishing disease-related changes in multiple sclerosis (18), acute cerebral ischemia (19,20), brain tumors (20,21) and psychiatric disorders (22).

The performance of *in vivo* DWS measurements on a clinical scanner and the derivation of meaningful information from these experiments remain challenging, and the robustness and reproducibility of the technique still need to be established. The low concentrations of metabolites compared with that of water, coupled with the long TEs required to accommodate the diffusion-weighting gradients, result in a relatively low signal-to-noise ratio (SNR). This, in turn, necessitates long measurement times and/or large volumes of interest, both detrimental to clinical applications. Other effects, such as strong eddy currents and inter-shot phase and amplitude fluctuations, may further affect the accuracy and reproducibility of the measurements. In addition, the diffusion properties of metabolites may require different diffusion-weighting parameters from those used in water diffusion-weighted techniques. Previous work, for example, has emphasized the importance of the choice of *b* value for an accurate estimate of the fractional anisotropy (FA) of brain metabolites from DWS experiments (23).

In order to assess the viability of DWS as a meaningful diagnostic tool, it is therefore essential to ascertain: (i) the inter- and intra-subject variability of metabolite diffusion

properties across the acquisition parameter space, e.g. the number and range of different b values used and the number of spectral averages per single diffusion-weighting condition; and (ii) the optimal parameters for a reliable DWS experiment within an experimental time that is suitable for clinical and clinical research purposes.

We chose to accomplish these goals with a set of DWS measurements performed on the anterior body of the corpus callosum (aCC). The aCC has been thoroughly studied with DWS (11,18,24,25), and the relatively straightforward organization of the cross-hemispheric fibers makes the aCC a suitable site in which to measure diffusion properties roughly parallel and perpendicular to the main fiber direction. These properties are, in turn, dictated by microstructural variables, such as, amongst others, the axonal diameter and molecular crowding in the axonal space (4). Diffusion along the callosal fibers has been linked to axonal degradation in multiple sclerosis (18), and the involvement of the aCC in several neurological disorders makes it a plausible candidate for clinically relevant measurements (26,27).

The goal of the work reported here is to help experimenters interested in obtaining robust measures for tNAA diffusion to choose the optimal combination of experimental parameters within the limitations of experimental time available. In the first part of this study, the reproducibility of tNAA DWS measurements parallel and perpendicular to the callosal fiber main direction is assessed on two MRI scanners, operating at different magnetic fields of 3T and 3T, using an almost identical protocol. The reproducibility of the derived quantities, such as the diffusion coefficients of tNAA parallel and perpendicular to the callosal fibers, is estimated across repeated measurements within subjects as well as across subjects. We also test the reproducibility of modeled quantities with potential clinical relevance, such as the cytosolic diffusion coefficient of tNAA within the callosal fibers and the orientation dispersion of axons within the volume of interest (VOI). These quantities are calculated based on a model that accounts for the subject- and position-specific macroscopic curvature of the fibers within the VOI (24). Once the impact of macroscopic curvature is removed, the cytosolic diffusion coefficient is more directly influenced by hindrances to diffusion within the intracellular space and is thus expected to be more sensitive to alterations in intracellular microstructure caused, for example, by disease processes, such as the breakdown of microtubules and neurofilaments. We also provide an estimate for the repeatability of the diffusion coefficients of two other metabolites detected within the same VOI selection: total creatine (tCr = creatine + phosphocreatine) and total choline compounds (tCho = phosphocholine + glycerophosphocholine). The second part of the study reports the dependence of the statistical properties of the diffusion parameters of tNAA obtained from direct fitting and modeling as a function of the scan parameters, such as the number and range of b values used for each diffusion-weighting direction, as well as the number of spectral averages. In addition, in order to assess the clinical viability of DWS measurements, power calculations were performed to assess the minimum number of subjects required in a case-control study in order to detect a certain effect caused by disease.

MATERIALS AND METHODS

Human subjects

Six healthy volunteers (three men, three women; age, 34 ± 8 years), without known neurological abnormalities, participated in this study. Each subject was scanned in five separate sessions. The study adhered to local Institutional Review Board guidelines, and informed consent was obtained from all subjects prior to the study.

MRI scanner/hardware

Three of the subjects were scanned on a 3T Achieva MRI scanner (Philips Medical Systems, Cleveland, OH, USA) at the National Institutes of Health, Bethesda, MD, USA. The scanner was equipped with an eight-channel phased array receiver head coil and gradient coils, which, in the selected mode of operation, could deliver a maximum gradient strength of 60 mT/m at a slew rate of 100 T/m/s. The other three subjects were scanned on a 7T Achieva whole-body MRI scanner (Philips Healthcare, Best, the Netherlands) at the Leiden University Medical Center, Leiden, the Netherlands. The scanner was equipped with gradient coils capable of a maximum gradient strength of 40 mT/m and a slew rate of 200 T/m/s. A head coil consisting of a quadrature birdcage transmit and 32-channel phased array receive coil (Nova Medical Inc., Wilmington, MA, USA) was used for the 7T measurements.

Anatomical imaging

For each separate scan session, a short survey scan and a sensitivity encoding (SENSE) reference scan were followed by a three-dimensional T_1 -weighted gradient-echo acquisition for positioning the VOI in the DWS experiments and for tissue segmentation in the post-processing stage. Imaging parameters for the T_1 -weighted image acquired at 3T were as follows: field of view [anterior–posterior (AP), foot–head (FH), right–left (RL)], $240 \times 240 \times 180 \text{ mm}^3$; isotropic resolution, 1 mm; TR/TE, 7.00 ms/ 3.15 ms; total scan time, 5 min 30 s. Imaging parameters for the T_1 -weighted image acquired at 7T were as follows: field of view (AP, FH, RL), $246 \times 246 \times 174 \text{ mm}^3$; resolution, $0.85 \times 0.85 \times 1 \text{ mm}^3$; TR/TE, 5.00ms/2.20ms; total scan time, 1 min 59 s.

Diffusion tensor imaging protocols

A diffusion tensor imaging (DTI) dataset was also acquired in each scan session and was used to estimate the macroscopic curvature of the axonal tracts in the modeling of the DWS data, as explained in ref. (24). Single-shot two-dimensional spin-echo echo-planar imaging was performed in both 3T and 7T scan protocols. DTI parameters for 3T acquisitions were as follows: field of view (AP, FH, RL), $224 \times 224 \times 120 \text{ mm}^3$; isotropic resolution, 2 mm; TR/TE, 7487 ms/85 ms; 32 diffusion-weighting directions with $b = 800 \text{ s/mm}^2$; total scan time, 5 min 50 s. DTI parameters for 7T acquisitions were as follows: field of view (AP, FH, RL), $224 \times 224 \times 120 \text{ mm}^3$; isotropic resolution, 2mm; TR/TE, 7209 ms/67 ms; 15 diffusion-weighting directions with $b = 1000 \text{ s/mm}^2$; total scan time, 2 min 41 s. Parallel imaging was performed for all scans with a reduction factor of three along the phase-encoding direction (AP).

Diffusion-weighted spectroscopy protocols

The point-resolved spectroscopy (PRESS) (28) sequence was chosen as the base spectroscopic sequence for the single-volume DWS experiments and was supplemented with a bipolar diffusion-weighting scheme for the minimization of eddy currents (29). The VOI was positioned at the aCC as shown in Fig. 1. The VOI dimensions were $30 \text{ (AP)} \times 15 \text{ (RL)} \times 8 \text{ (FH)} \text{ mm}^3$ for 3T and $25 \text{ (AP)} \times 15 \text{ (RL)} \times 8 \text{ (FH)} \text{ mm}^3$ for 7T experiments. For the diffusion weighting, two directions were chosen for all scans: (1) a pure RL direction in the VOI frame, which is mostly parallel to the direction of the callosal fibers; (2) a direction perpendicular to the callosal fibers, forming a 45° angle between the AP axis and the inferior–superior axis of the VOI. These gradient directions can be denoted in the VOI coordinates as $[1,0,0]$ and $[0,-1,1]$, respectively. The position of the gradient directions with respect to the VOI is shown in Fig. 1a, d. In all experiments, the center frequency was set to the tNAA singlet peak at 2.0 ppm. Water suppression was achieved using two frequency-selective excitation pulses centered at the water resonance frequency, followed by dephasing gradients. The water suppression was ‘de-optimized’ for the diffusion-weighting conditions in order to allow sufficient residual water signal for later use in the post-processing stage for zero-order phase correction of individual spectra prior to spectral averaging. A peripheral pulse unit (PPU) was used for cardiac synchronization of the DWS acquisition in order to minimize signal fluctuations caused by cardiac pulsation. Pencil-beam shimming was applied up to second order, resulting in a typical tNAA singlet linewidth of 7 Hz on the 3T scanner and 12 Hz on the 7T scanner, in agreement with 3T and 7T spectra in previous work (30). Following each scan, a shorter scan with identical VOI position and diffusion conditions was performed with the center frequency set at the water resonance frequency and without water suppression. This scan was subsequently used for eddy current correction.

The DWS parameters for the 3T acquisitions were as follows: TE = 110 ms; TR = two cardiac cycles (about 2000 ms); trigger delay, 200 ms; number of time-domain points, 1024; spectral width, 1500 Hz; gradient duration (δ), 22 ms; bipolar gap, 20 ms; diffusion time (Δ), 55 ms with seven different gradient amplitudes resulting in b values of 213, 469, 826, 1285, 1847, 2511 and 3277 s/mm^2 in the $[1,0,0]$ direction and 410, 917, 1629, 2544, 3665, 4989 and 6518 s/mm^2 in the $[0,-1,1]$ direction. Seventy-two spectra were collected for each diffusion condition (two directions, seven b values). The total DWS scan time ranged from 35 to 50 min, depending on the heart rate of the subject, typically about 60 beats per minute (BPM).

The DWS parameters for the 7T acquisitions were as follows: TE = 121 ms; TR = three cardiac cycles (about 3000 ms); trigger delay, 300 ms; number of time-domain points, 1024; spectral width, 3000 Hz; gradient duration (δ), 37 ms; bipolar gap, 16 ms; diffusion time (Δ), 60.5 ms with seven different gradient amplitudes resulting in b values of 63, 317, 664, 1278, 1912, 2885 and 3808 s/mm^2 in the $[1,0,0]$ direction and 134, 656, 1361, 2602, 3883, 5844 and 7700 s/mm^2 in the $[0,-1,1]$ direction. Forty spectra were collected for each diffusion condition. The total DWS scan time was about 40 min.

Image processing

T_1 -weighted and DTI volumes were processed in MIPAV (Medical Image Processing, Analysis and Visualization) (31,32) and JIST (Java Image Science Toolbox) (33). The T_1 -weighted image was rigidly registered to the Montreal Neurological Institute (MNI) brain with the Optimized Automatic Registration (OAR) algorithm (34), intensity inhomogeneity was corrected using N3 (35) and images were skull-stripped with the Simple Paradigm for Extra-Cerebral Tissue Removal (SPECTRE) (36) and segmented into white matter (WM), gray matter (GM) and cerebrospinal fluid (CSF) using the Topology-preserving Anatomy-Driven Segmentation (TOADS) (37,38). A three-dimensional mask in the shape of the DWS VOI was applied to the WM segmentation volume to create a mask within the spectroscopic VOI that contains only WM pixels.

The DTI data were processed as follows: the individual diffusion-weighted volumes were rigidly registered to the $b = 0$ s/mm² image, which was then registered to the T_1 -weighted volume in MNI space using affine registration, and the same transformation was then applied to the diffusion-weighted volumes. The diffusion tensor for each voxel was then estimated and diagonalized to yield maps of the primary eigenvector ($\vec{E}_1$) and FA, which were later used in the modeling. The WM VOI mask obtained from the previous stage was then applied to the diffusion parametric maps to obtain the $\vec{E}_1$ and FA values within the VOI used for the DWS experiments. The angles between the main eigenvectors within the VOI and the applied diffusion gradients were calculated for each voxel according to the following equation:

$$\theta_{ij} = \cos^{-1} \left(\frac{\vec{E}_{1k} \cdot \vec{g}_j}{\|\vec{E}_{1k}\| \cdot \|\vec{g}_j\|} \right) \quad [1]$$

where $\vec{E}_{1k}$ is the principal eigenvector of DTI voxel k , $\vec{g}_j$ is the diffusion-weighting gradient in direction j and the $\|$ brackets denote the norms of both vectors. These angles were used later in the modeling to incorporate the macroscopic curvature of the axonal tracks within the VOI.

Spectral processing

All spectral processing was performed with custom codes in MATLAB® release R2014b (Mathworks, Natick, MA, USA). DWS data were corrected for eddy currents using the unsuppressed water data. Zero-order phase and frequency drifts were corrected for each shot using the residual water peak, and data for each condition were subsequently averaged. The number of averages was determined separately for inter- and intra-subject analysis, as described below. The residual water peak was removed with Hankel singular value decomposition (HSVD) (39), and a first-order phase correction was performed based on the tNAA peak. The spectrum from each condition was then analyzed with LCModel (40) to generate metabolite peak integrals.

Derivation of metabolite diffusion measures

Based on the LCModel data for tNAA, the following empirical quantities were calculated: $D_{\text{par}}(\text{tNAA})$, the diffusivity along the $[1,0,0]$ direction (roughly parallel to the callosal fibers); $D_{\text{perp}}(\text{tNAA})$, the diffusivity along the $[0,-1,1]$ direction (roughly perpendicular to the callosal fibers); and $D_{\text{avg}}(\text{tNAA})$, the average of the two diffusivities described above [representing the empirical apparent diffusion coefficient (ADC) of tNAA in the VOI]. Diffusivities were calculated assuming monoexponential decay of the signal as a function of b value in each direction:

$$\ln \left(\frac{s_{b,i}}{s_{b_1,i}} \right) = -b_i \cdot D_i \quad [2]$$

where $s_{b,i}$ is the measured signal in direction i , $s_{b_1,i}$ is the signal at the lowest b value for the same direction, b_i is the b value in the direction i and D_i is the calculated diffusion coefficient for direction i .

The LCModel output was also used as an input to a modeling routine that calculates the intra-axonal, or cytosolic, diffusivity of tNAA. This procedure is assumed to minimize the variability in the DWS measurements introduced by macroscopic factors, such as the position of the VOI within the WM tract, the main direction of the tract with respect to the diffusion-weighting gradients and the macroscopic curvature of the tract within the VOI. The model, thoroughly described in ref. (24), uses the angles between the main eigenvectors of the DTI data within the DWS VOI and the diffusion-weighting gradient directions. The data are fitted to the model using two fitting variables: $D_{\text{model}}(\text{tNAA})$, the cytosolic diffusion coefficient of tNAA, and σ_ϕ , the standard deviation (SD) of the axonal angular dispersion. It is important to note that $D_{\text{model}}(\text{tNAA})$ is independent of the tract geometry within the VOI and thus mostly reflects the effects of the cytosolic medium, e.g. viscosity and molecular crowding, on the diffusion of tNAA inside the axons. The empirical ADCs of tCr [$D_{\text{avg}}(\text{tCr})$] and tCho [$D_{\text{avg}}(\text{tCho})$] were calculated in a similar way to those of tNAA, based on the LCmodel data for tCr and tCho, respectively.

Inter-subject variability analysis of all diffusion measures

For inter-subject variability analysis, the entire dataset from each session was used. All spectra belonging to a single diffusion condition were averaged per session. D_{par} , D_{perp} and D_{avg} for tNAA, tCr and tCho, and $D_{\text{model}}(\text{tNAA})$, were calculated for each session for all subjects.

Intra-subject variability analysis of tNAA diffusion measures

To evaluate the dependence of the variability of the calculated diffusion measures within subjects on the number of averages, as well as on selected diffusion-weighting conditions, a jackknife-like subsampling procedure was performed on the data acquired from all subjects (41,42). In this procedure, within-session subsets of these datasets were randomly resampled without replacement prior to averaging. To evaluate the effect of the selection of specific sets of diffusion-weighting conditions, subsets of n b values ($3 \leq n \leq 7$) were selected from the

full range of seven b values. For example, g_{247} corresponds to a selection of the 2nd, 4th and 7th b values for each direction, starting from the lowest b value. For the 3T case, each subset consisted of 30, 36, 42, 48, 54 and 60 spectra per diffusion-weighting condition (the full number of spectra per condition was 72). For the 7T case, 16, 20, 24, 28 and 32 spectra were used of the 40 spectra available per condition. For each randomly selected subset, an average spectrum was obtained. These spectra were then used to calculate D_{par} , D_{perp} , D_{avg} , D_{model} and σ_{μ} . This procedure was then repeated 100 times for each subset size to obtain the jackknife averages and SDs of these quantities. A diagram of an example of the jackknife-like procedure is shown in Fig. 2, where data from one session are used to generate 100 subsampled datasets, each with three b values (of the seven available) and six averages (of the 72 available). The same procedure was applied to all sessions, resulting in five sets of diffusion properties and their SDs per subject for each jackknife subsampling. These averages and SDs were used to generate the across-session averages and SDs of the intra-session averages and SDs.

Statistical analyses

All between-group (3T *versus* 7T) analyses were performed with GraphPad Prism version 6.0b for Mac OS X (GraphPad Software, San Diego, CA, USA). Between- and within-subject analyses were accomplished with STATA release 11 (StataCorp, College Station, TX, USA). A one-way random-effects analysis of variance (ANOVA) model was used to estimate the between- and within-subject variance of the DWS measurements. Between-subject variance was used for inter-subject variability analyses, whereas within-subject variance was used for repeatability coefficient (C_R) and power/sample size calculations. C_R within the 95% confidence interval is defined as:

$$C_R = 1.96 \times \sqrt{2} \times \sigma \quad [3]$$

where σ is the within-subject SD (43–46). Power calculations were performed to estimate the sample size required to detect a difference () in tNAA diffusion measures between two groups based on the variance (σ^2) of our measurements. For this, it was assumed that the means were normally distributed and the variance was the same for both groups

($\sigma_1^2 = \sigma_2^2$). For all calculations, we used a two-sided test with significance level $\alpha = 0.05$, ($z_{1-\alpha/2} = 1.96$) and a power of 80% ($1 - \beta = 0.80$, $z_{1-\beta} = 0.84$). The sample size (n) required for each group was estimated as described in ref. (47). In addition, the coefficient of

variation ($C_v = 100 \times \frac{\sigma}{\mu}$, where μ is the mean and σ is the SD of the resampling results) is reported to allow for comparison between diffusivity measures, which have different mean values.

RESULTS

Diffusion-weighted spectra and diffusivity calculations

Typical diffusion-weighted spectra from all b values applied in the two gradient directions are shown in Fig. 1 for 3T (Fig. 1b, e) and 7T (Fig. 1c, f) scans. Cramér–Rao lower bounds

(CRLBs) for the tNAA peak from all spectra were between 6% and 20% at 3T and between 3% and 14% at 7T. CRLBs for tCr were between 5% and 20% at 3T and 5% and 16% at 7T. CRLB for tCho was between 5% and 17% at 7T. CRLBs for tCho were mostly above 20% at 3T, even at the low b -value conditions, thus precluding an accurate calculation of $D_{\text{avg}}(\text{tCho})$ at 3T. The SNR and spectral resolution at 7T were higher than those at 3T. The highest CRLB values were typically obtained at the highest b value in the $[-1,0,0]$ diffusion-weighting direction.

In Fig. 3, the logarithm of the diffusion-weighted tNAA data is plotted as a function of b value for the two diffusion gradient directions. The data were obtained from a single session of one subject scanned at 3T (Fig. 3a, b) and one subject scanned at 7T (Fig. 3c, d). In Fig. 3a, c, the data acquired with the two gradient directions were separately fitted to two independent monoexponential decay functions [Eqn (2)]. Figure 3b, d show the modeled fit and the resulting parameters D_{model} and σ_{ϕ} for these particular datasets.

Inter-subject variability

Table 1 shows the averages, SDs and C_V values of tNAA, tCr and tCho diffusivity measures across subjects and sessions. The mean C_V values of the tNAA diffusivity measures ranged between 2% and 13% for both scanners, with the exception of $D_{\text{perp}}(\text{tNAA})$ at 3T, which was 29% across subjects and 22% across sessions. No significant differences in $D_{\text{par}}(\text{tNAA})$, $D_{\text{perp}}(\text{tNAA})$, $D_{\text{avg}}(\text{tNAA})$ and $D_{\text{model}}(\text{tNAA})$ were observed between subjects (one-way ANOVA). In Fig. 4, all measures calculated based on the full datasets from all subjects are grouped. Diffusivity measures obtained from the data acquired at 3T and 7T were not statistically significantly different (Fig. 4). $D_{\text{model}}(\text{tNAA})$ values had a smaller (not significant) SD at 7T compared with 3T: mean (SD) values were $D_{\text{model}}(\text{tNAA}) = 0.501$ (0.052) $\mu\text{m}^2/\text{ms}$ at 3T and $D_{\text{model}}(\text{tNAA}) = 0.506$ (0.035) $\mu\text{m}^2/\text{ms}$ at 7T. For $D_{\text{avg}}(\text{tNAA})$, the mean and SD were the same at 3T and 7T: mean (SD) values were $D_{\text{avg}}(\text{tNAA}) = 0.217$ (0.015) $\mu\text{m}^2/\text{ms}$ at 3T and $D_{\text{avg}}(\text{tNAA}) = 0.216$ (0.015) $\mu\text{m}^2/\text{ms}$ at 7T. Mean C_V values for the diffusion measures of tCr and tCho ranged between 3% and 22% at 7T. These values were higher for tCr at 3T (13–27%), and those of tCho at 3T are not reported.

Intra-subject variability of tNAA diffusion measures

Figure 5 shows C_V as a function of the number of spectral averages for all of the chosen b -value combinations based on data from all subjects. The relative error is observed to depend much more strongly on the highest b value used than on the number of b values. At 3T, the g_{1357} scheme demonstrates low C_V (less than 10%) for both $D_{\text{model}}(\text{tNAA})$ and $D_{\text{avg}}(\text{tNAA})$ with fewer than 400 total spectra acquired. At 7T, the g_{247} b -value scheme exhibits $C_V = 8\%$ for both $D_{\text{model}}(\text{tNAA})$ and $D_{\text{avg}}(\text{tNAA})$ using fewer than 200 total spectra.

Reproducibility and sample size analysis

Values of C_R are shown in Table 2 for $D_{\text{model}}(\text{tNAA})$ and D_{avg} of the three metabolites calculated on the basis of the entire dataset, as well as on the basis of the b -value schemes g_{1357} for 3T and g_{247} for 7T. Smaller values of C_R demonstrate greater reproducibility. The measurements from data acquired at 7T using all spectra lead to the lowest C_R values of 21%. Measurements performed at 7T using the g_{247} scheme, which utilized fewer than half

of the spectra of g_{1-7} , retained low C_R values of 25%. D_{avg} calculated using the entire dataset and D_{avg} calculated on the basis of the b -value scheme g_{1357} yielded similar C_R values of 21% and 24% at 7T and 3T, respectively. D_{model} calculated on the basis of the same schemes at 3T resulted in higher C_R (30% for the entire dataset and 32% for the g_{1357} scheme) compared with C_R values of D_{model} at 7T (21% for the entire dataset and 25% for the g_{247} scheme). The C_R values for $D_{avg}(tCr)$ and $D_{avg}(tCho)$ at 7T were similar to those calculated for $D_{avg}(tNAA)$, whereas, at 3T, the C_R values for $D_{avg}(tCr)$ were higher than those for $D_{avg}(tNAA)$.

Sample size calculations reflect the number of subjects per group required to detect a difference between two groups with a power of 80% and significance level of 5%. Sample size (n) values to detect a 10% difference in D_{model} , D_{avg} , $D_{avg}(tCr)$ and $D_{avg}(tCho)$ are shown in Table 2, and the trends in sample size values for 5%, 10%, 15% and 20% detectable differences for tNAA are depicted in Fig. 6.

DISCUSSION

In this study, we investigated the inter- and intra-subject variability of diffusivity measures of tNAA, both empirical and modeled, derived from DWS experiments performed on the human aCC with 3T and 7T scanners. We also studied the effect of scan parameters, such as the number and range of b values for each diffusion direction and number of spectral averages, in order to suggest optimal scan parameters to perform DWS experiments within a given experimental time limit.

Intra- and inter-subject variability of tNAA DWS measures

The C_V values given in Table 1 and the C_R values given in Table 2 for D_{avg} and D_{model} indicate acceptable reproducibility ($C_R = 21\%$ for D_{avg} and D_{model} at 7T and $C_R = 32\%$ for D_{model} at 3T) of the DWS measures of tNAA in the corpus callosum. At 7T, all C_V values were less than 13%, and most were in the range 2–8%. The C_V values for the diffusion measures evaluated from the data acquired at 3T were higher than those found at 7T, ranging between 3% and 29%. One should keep in mind that the variability measured in these long sessions (typically about 40 min in both scanners) also reflects patient motion and scanner-related instabilities, which are expected to be less pronounced in shorter experiments. Past DWS studies that explored the effect of disease reported substantial changes in tNAA diffusion measures hypothesized to be related to neuronal/axonal damage. In one study, an increase in $ADC(tNAA)$ of above 50% was reported in malignant brain tumors, and a decrease in $ADC(tNAA)$ of about the same magnitude was observed in ischemic stroke (20). Zheng *et al.* (19) reported an age-related drop of 27% in $ADC(tNAA)$ values. In our own study on normal-appearing WM changes in multiple sclerosis at 7T, a decrease of about 20% was observed in D_{par} of tNAA in the aCC of a small cohort of patients compared with age-matched healthy controls (18). Based on our power calculations, a difference between groups of 10% for D_{model} or D_{avg} can be detected with groups as small as 9–18 subjects each for 3T or nine subjects each for 7T. This result is particularly reassuring as it suggests the ability of DWS of tNAA to pick up subtle changes in intra-axonal structure in normal-appearing WM. It should be noted that this estimate is achieved using the full acquisition

scheme employed in our experiments. Such an acquisition would take around 35 min at 3T and 30 min at 7T, thus reaching the higher range of clinically feasible scan times.

The average inter-subject variability values in D_{avg} and in D_{model} at 7T were 5.7% and 6.3%, respectively, and thus comparable. At 3T, the variability of D_{avg} was $C_V = 6\%$, whereas the C_V value of D_{model} was higher at 10%. D_{avg} is roughly equivalent to the ADC of tNAA within the VOI, i.e. it includes the effect of restrictions on the diffusion of tNAA imposed by axonal membranes. As the typical DWS measurement is performed on a large volume, these geometric factors introduce a confound that varies across subjects and VOI locations. The model presented in ref. (24) provides a method to remove the impact of macroscopic curvature of WM tracts within the volume, and yields a diffusion measure, D_{model} , which represents the cytosolic diffusion coefficient and thus includes the impact of tortuous diffusion within the axonal medium. The stability of the resulting D_{model} depends greatly on the SNR of the single-volume DWS measurements that generate the dataset needed for the fitting procedure. This hypothesis is also supported by the significantly higher variance at 3T in the second fitting parameter of the model, i.e. the SD of the axonal microscopic misalignment σ_ϕ (Table 1). It can be appreciated that, at 7T, where the CRLB values for the individual measurements were relatively low, the variability in D_{model} was comparable with that of D_{avg} . At 3T, where the CRLBs were higher, in particular at the high b -value range, D_{model} is found to be less stable.

Effect of b -value scheme on intra-subject variability of tNAA diffusion measures

Figure 5 can serve as a guideline for the choice of a combination of b values and number of averages in order to reach a desired variability in both D_{model} and D_{avg} . It is clearly seen that sampling the upper range of b values is critical to obtain low variability, e. g. schemes g_{247} , g_{1357} and g_{1-7} , which all include a balanced sampling of the entire b -value range with three, four and seven b values, respectively, quickly converge to low variability values. However, the scheme g_{123} has almost twice the variability of g_{247} for the same number of acquisitions. There is a substantial difference between 3T and 7T in the number of averages needed to reach the same variability, stemming from the intrinsically higher SNR of spectroscopic measurements at 7T. For example, for the scheme g_{247} , about 200 acquisitions are needed to reach 8% variability in both diffusion measures at 7T, whereas a similar number of acquisitions at 3T will result in $C_V = 18\%$. Such an acquisition protocol (three b values applied in two gradient directions and 32 averages per diffusion-weighting condition) will result in an acquisition time of 9.6 min at 7T, when a TR of three heart beats is used. A similar variability ($\sim 8\%$) can be reached at 3T when the scheme g_{1357} is used with 432 acquisitions (four b values applied in two gradient directions with 48 averages), with a resulting acquisition time of 14.4 min when a TR of two heart beats is used. Power calculations based on these schemes suggest that a minimum of 11–13 subjects per group could suffice to detect a subtle difference of 10% in $D_{\text{model}}(\text{tNAA})$ at 7T and $D_{\text{avg}}(\text{tNAA})$ at both 3T and 7T in case-control studies. $D_{\text{model}}(\text{tNAA})$ at 3T will necessitate a minimum of 21 subjects per group in order to detect the same effect.

In Fig. 5, the differences in C_V of $D_{\text{model}}(\text{tNAA})$ and $D_{\text{avg}}(\text{tNAA})$ are shown to be rather minor, and can only be seen when the variability is high, especially at 7T. Power and C_R

calculations shown in Table 2 imply that $D_{\text{model}}(\text{tNAA})$ is more stable at 7T compared with 3T. $D_{\text{model}}(\text{tNAA})$ successfully accounts for the inter-subject differences in macroscopic and microscopic distributions of axonal directions within the VOI, and thus we believe that it is a clinically relevant measure for the cytosolic diffusion coefficient of tNAA. However, the non-linear nature of the fitting procedure implies a higher sensitivity to SNR, mainly as a result of error propagation properties. Thus, at the reduced SNR at 3T, the variability of $D_{\text{model}}(\text{tNAA})$ is markedly higher than at 7T.

Inter-subject variability of tCr and tCho DWS measures

At 7T, both C_V and C_R for $D_{\text{avg}}(\text{tCr})$ and $D_{\text{avg}}(\text{tCho})$ were similar to those obtained for tNAA, whereas, at 3T, the C_R values for $D_{\text{avg}}(\text{tCr})$ were higher than those for $D_{\text{avg}}(\text{tNAA})$, and low SNR for the tCho resonances precluded the reliable calculation of $D_{\text{avg}}(\text{tCho})$. As the experimental protocol was dictated by the goal of characterizing the diffusion properties of tNAA in a well-defined WM tract, the small size of the resulting VOI resulted in low SNR for the tCho resonances. This, of course, does not preclude the possibility of robustly measuring the diffusion properties of tCho and other metabolites, as has been shown in several previous studies, but it does emphasize the sensitivity of the calculation of diffusion coefficients to SNR, through the propagation of error. In addition, the low bandwidth of the refocusing pulses used in the PRESS sequence resulted in a large chemical shift displacement for the metabolites other than tNAA, especially at 7T, causing the effective VOI for tCr and tCho to be significantly shifted from the medial part of the corpus callosum (Fig. S2). As our measurements of D_{avg} are based on an assumption of gradient directions that are roughly parallel and perpendicular to the callosal fibers in the medial region of the corpus callosum, and not on three mutually orthogonal directions, this shift may have affected the values of $D_{\text{avg}}(\text{tCr})$ and $D_{\text{avg}}(\text{tCho})$ reported here.

Limitations of the study

Our study was subject to several challenges and limitations. The five repeated scans per subject allowed the examination of the long-term repeatability of the metabolite diffusion measures, but the small number of subjects used in our study severely limited the evaluation of across-subject variability. The choice of different subjects for the 3T and 7T measurements, which were performed on different continents, prevented us from performing a more complete comparison of DWS quantities of tNAA across these two field strengths. Moreover, the larger VOI size at 3T is an additional limiting factor for such a comparison. This choice was dictated by the lower SNR at 3T and the goal of devising protocols with a clinically relevant scan time. There were several sources of variability that were not properly quantified. As the experiment was performed using cardiac triggering based on PPU, differences in trigger delays, as well as in heart rate and circulation, can introduce variability to TR, subsequently affecting T_1 saturation. Subject motion across sessions, as well as small differences in the positioning of the VOI in different sessions, can also affect the variability observed in the study, especially in our experimental set-up, where the VOI is positioned on the corpus callosum. Our 3T data were acquired with a gradient setting that allowed relatively high gradient amplitude (at the expense of a lower slew rate). More conventional gradient systems may necessitate a different choice of diffusion-weighting parameters to reach the desired b values recommended in this work. Finally, we restricted this study to

focus on the diffusion properties of tNAA in a specific WM pathway, the corpus callosum. This choice was guided by the apparent simplicity of the fiber structure in the corpus callosum, its easy identification and the simplicity of repositioning the DWS VOI in subsequent sessions. The corpus callosum also allows a relatively straightforward implementation of our model for extracting $D_{\text{model}}(\text{tNAA})$ from the two gradient directions. In the future, we plan to provide a more general model that will allow an arbitrary choice of DWS VOI with a variety of fiber orientations within the VOI. Lastly, it should be noted that the empirical diffusion coefficients in this work were calculated based on the fitting of the metabolite signal to a single exponential. The diffusion of metabolites in the intracellular space is not expected to be Gaussian, and thus the signal decay is not monoexponential. This directly affects the metabolite diffusion coefficient, as can be appreciated in Fig. S1. In Fig. S1, $D_{\text{avg}}(\text{tNAA})$ shows a clear dependence on the maximum b value used. The choice of a monoexponential fit is simple and practical, especially in the b -value range used in this work, but does not reflect the true nature of the diffusion of metabolites in their microenvironments.

CONCLUSIONS

In this study, we evaluated the reproducibility of empirical and modeled diffusion properties of tNAA in the corpus callosum based on DWS data acquired at two MRI scanners operating at 3T and 7T. Statistical assessment of the intra-subject variability shows that DWS experiments can be performed at both field strengths within clinically relevant scan times of about 10–13 min whilst retaining low variance (~8%) for the estimated diffusion properties of tNAA. These measurements provide ample power to detect group mean differences with groups of 13 or fewer subjects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to thank the Intramural Research Program of the National Institute of Neurological Disorders and Stroke (NINDS), and the NINDS Neuroimmunology Clinic and the National Institutes of Health (NIH) Clinical Center Radiology and Imaging Sciences MRI technologists for their support and expertise. Emily Wood was supported by a Medical Scientist Training Program (MSTP) grant and by an NINDS Competitive Intramural Graduate Fellowship.

Abbreviations used

aCC	anterior body of the corpus callosum
ADC	apparent diffusion coefficient
ANOVA	analysis of variance
AP	anterior–posterior
BPM	beats per minute

C_R	repeatability coefficient
CRLB	Cramér–Rao lower bound
CSF	cerebrospinal fluid
C_V	coefficient of variation
D_{avg}	empirical diffusion coefficient
D_{model}	cytosolic model-based diffusion coefficient of tNAA
D_{par}	diffusivity along the [1,0,0] direction (roughly parallel to the callosal fibers)
D_{perp}	diffusivity along the [0,−1,1] direction (roughly perpendicular to the callosal fibers)
DTI	diffusion tensor imaging
DWS	diffusion-weighted MRS
FA	fractional anisotropy
FH	foot–head
GM	gray matter
HSVD	Hankel singular value decomposition
JIST	Java Image Science Toolbox
MIPAV	Medical Image Processing, Analysis and Visualization
MNI	Montreal Neurological Institute
NAA	N-acetylaspartate
NAAG	N-acetylaspartyl glutamate
OAR	Optimized Automatic Registration
PPU	peripheral pulse unit
PRESS	point-resolved spectroscopy
RL	right–left
SD	standard deviation
SENSE	sensitivity encoding
SNR	signal-to-noise ratio
SPECTRE	Simple Paradigm for Extra-Cerebral Tissue Removal
tCho	total choline compounds

tCr	total creatine
tNAA	total N-acetylaspartate
TOADS	Topology-preserving Anatomy-Driven Segmentation
VOI	volume of interest
WM	white matter
σ	standard deviation of the axonal angular dispersion

REFERENCES

1. Posse S, Cuenod CA, Le Bihan D. Human brain: proton diffusion MR spectroscopy. *Radiology*. 1993; 188(3):719–725. [PubMed: 8351339]
2. Nicolay K, Braun KP, Graaf RA, Dijkhuizen RM, Kruiskamp MJ. Diffusion NMR spectroscopy. *NMR Biomed*. 2001; 14(2):94–111. [PubMed: 11320536]
3. Valette J, Guillermier M, Besret L, Boumezeur F, Hantraye P, Lebon V. Optimized diffusion-weighted spectroscopy for measuring brain glutamate apparent diffusion coefficient on a whole-body MR system. *NMR Biomed*. 2005; 18(8):527–533. [PubMed: 16177956]
4. Marchadour C, Brouillet E, Hantraye P, Lebon V, Valette J. Anomalous diffusion of brain metabolites evidenced by diffusion-weighted magnetic resonance spectroscopy in vivo. *J. Cereb. Blood Flow Metab*. 2012; 32(12):2153–2160.
5. Valette J, Giraudeau C, Marchadour C, Djemai B, Geffroy F, Ghaly MA, Le Bihan D, Hantraye P, Lebon V, Lethimonnier F. A new sequence for single-shot diffusion-weighted NMR spectroscopy by the trace of the diffusion tensor. *Magn. Reson. Med*. 2012; 68(6):1705–1712. [PubMed: 22302673]
6. Upadhyay J, Hallock K, Erb K, Kim DS, Ronen I. Diffusion properties of NAA in human corpus callosum as studied with diffusion tensor spectroscopy. *Magn. Reson. Med*. 2007; 58(5):1045–1053. [PubMed: 17969098]
7. Upadhyay J, Hallock K, Ducros M, Kim DS, Ronen I. Diffusion tensor spectroscopy and imaging of the arcuate fasciculus. *Neuroimage*. 2008; 39(1):1–9. [PubMed: 17928238]
8. Ellegood J, Hanstock CC, Beaulieu C. Trace apparent diffusion coefficients of metabolites in human brain using diffusion weighted magnetic resonance spectroscopy. *Magn. Reson. Med*. 2005; 53(5):1025–1032. [PubMed: 15844150]
9. Ellegood J, Hanstock CC, Beaulieu C. Diffusion tensor spectroscopy (DTS) of human brain. *Magn. Reson. Med*. 2006; 55(1):1–8. [PubMed: 16342152]
10. Kan HE, Techawiboonwong A, van Osch MJ, Versluis MJ, Deelchand DK, Henry PG, Marja ska M, van Buchem MA, Webb AG, Ronen I. Differences in apparent diffusion coefficients of brain metabolites between grey and white matter in the human brain measured at 7T. *Magn. Reson. Med*. 2012; 67(5):1203–1209. [PubMed: 22083562]
11. Kroenke CD, Ackerman JJ, Yablonskiy DA. On the nature of the NAA diffusion attenuated MR signal in the central nervous system. *Magn. Reson. Med*. 2004; 52(5):1052–1059. [PubMed: 15508157]
12. Pfeuffer J, Tkac I, Gruetter R. Extracellular–intracellular distribution of glucose and lactate in the rat brain assessed noninvasively by diffusion-weighted ¹H nuclear magnetic resonance spectroscopy in vivo. *J. Cereb. Blood Flow Metab*. 2000; 20(4):736–746. [PubMed: 10779018]
13. Branzoli F, Techawiboonwong A, Kan H, Webb A, Ronen I. Functional diffusion-weighted magnetic resonance spectroscopy of the human primary visual cortex at 7T. *Magn. Reson. Med*. 2013; 69(2):303–309. [PubMed: 23165888]
14. Branzoli F, Ercan E, Webb A, Ronen I. The interaction between apparent diffusion coefficients and transverse relaxation rates of human brain metabolites and water studied by diffusion-weighted spectroscopy at 7T. *NMR Biomed*. 2014; 27(5):495–506. [PubMed: 24706330]

15. Choi J-K, Dedeoglu A, Jenkins BG. Application of MRS to mouse models of neurodegenerative illness. *NMR Biomed.* 2007; 20(3):216–237. [PubMed: 17451183]
16. Wick M, Nagatomo Y, Prielmeier F, Frahm J. Alteration of intracellular metabolite diffusion in rat brain in vivo during ischemia and reperfusion. *Stroke.* 1995; 26(10):1930–1933. discussion 1934. [PubMed: 7570750]
17. van der Toorn A, Dijkhuizen RM, Tulleken CA, Nicolay K. Diffusion of metabolites in normal and ischemic rat brain measured by localized ^1H MRS. *Magn. Reson. Med.* 1996; 36(6):914–922. [PubMed: 8946357]
18. Wood ET, Ronen I, Techawiboonwong A, Jones CK, Barker PB, Calabresi P, Harrison D, Reich DS. Investigating axonal damage in multiple sclerosis by diffusion tensor spectroscopy. *J. Neurosci.* 2012; 32(19):6665–6669. [PubMed: 22573688]
19. Zheng DD, Liu ZH, Fang J, Wang XY, Zhang J. The effect of age and cerebral ischemia on diffusion-weighted proton MR spectroscopy of the human brain. *Am. J. Neuroradiol.* 2012; 33(3):563–568. [PubMed: 22081680]
20. Harada M, Uno M, Hong F, Hisaoka S, Nishitani H, Matsuda T. Diffusion-weighted in vivo localized proton MR spectroscopy of human cerebral ischemia and tumor. *NMR Biomed.* 2002; 15(1):69–74. [PubMed: 11840555]
21. Colvin DC, Yankeeelov TE, Does MD, Yue Z, Quarles C, Gore JC. New insights into tumor microstructure using temporal diffusion spectroscopy. *Cancer Res.* 2008; 68(14):5941–5947. [PubMed: 18632649]
22. Du F, Cooper AJ, Thida T, Shinn AK, Cohen BM, Ongur D. Myelin and axon abnormalities in schizophrenia measured with magnetic resonance imaging techniques. *Biol. Psychiatry.* 2013; 74(6):451–457. [PubMed: 23571010]
23. Ellegood J, Hanstock CC, Beaulieu C. Considerations for measuring the fractional anisotropy of metabolites with diffusion tensor spectroscopy. *NMR Biomed.* 2011; 24(3):270–280. [PubMed: 20925126]
24. Ronen I, Budde M, Ercan E, Annese J, Techawiboonwong A, Webb A. Microstructural organization of axons in the human corpus callosum quantified by diffusion-weighted magnetic resonance spectroscopy of N-acetylaspartate and post-mortem histology. *Brain Struct. Funct.* 2014; 219(5):1773–1785. [PubMed: 23794120]
25. Ronen I, Ercan E, Webb A. Axonal and glial microstructural information obtained with diffusion-weighted magnetic resonance spectroscopy at 7T. *Front. Integr. Neurosci.* 2013; 7:13. [PubMed: 23493316]
26. Roosendaal SD, Geurts JJ, Vrenken H, Hulst HE, Cover KS, Castelijns JA, Pouwels PJ, Barkhof F. Regional DTI differences in multiple sclerosis patients. *Neuroimage.* 2009; 44(4):1397–1403. [PubMed: 19027076]
27. Zhang L, Harrison M, Heier LA, Zimmerman RD, Ravdin L, Lockshin M, Ulu AM. Diffusion changes in patients with systemic lupus erythematosus. *Magn. Reson. Imaging.* 2007; 25(3):399–405. [PubMed: 17371731]
28. Bottomley PA. Spatial localization in NMR spectroscopy in vivo. *Ann. NY Acad. Sci.* 1987; 508:333–348. [PubMed: 3326459]
29. Reese T, Heid O. Reduction of eddy-current-induced distortion in diffusion MRI using a twice-refocused spin echo. *Magn. Reson. Med.* 2002; 182:177–182.
30. Li Y, Xu D, Ozturk-Isik E, Lupo JM, Chen AP, Vigneron DB, Nelson SJ. T1 and T2 metabolite relaxation times in normal brain at 3T and 7T. *J. Mol. Imaging Dynamic.* 2012; S(1):1–5.
31. McAuliffe MJ, Lalonde FM, McGarry D, Gandler W, Csaky K, Trus BL. Medical image processing, analysis and visualization in clinical research. *Proceedings of the 14th IEEE Symposium on Computer-Based Medical Systems, Bethesda, MD, USA.* 2001:381–386.
32. Bazin PL, Cuzzocreo JL, Yassa MA, Gandler W, McAuliffe MJ, Bassett SS, Pham DL. Volumetric neuroimage analysis extensions for the MIPAV software package. *J. Neurosci. Methods.* 2007; 165(1):111–121. [PubMed: 17604116]
33. Lucas BC, Bogovic JA, Carass A, Bazin PL, Prince JL, Pham DL, Landman BA. The Java Image Science Toolkit (JIST) for rapid prototyping and publishing of neuroimaging software. *Neuroinformatics.* 2010; 8(1):5–17. [PubMed: 20077162]

34. Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. *Med. Image Anal.* 2001; 5(2):143–156. [PubMed: 11516708]
35. Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans. Med. Imaging.* 1998; 17(1):87–97. [PubMed: 9617910]
36. Carass A, Cuzzocreo J, Wheeler MB, Bazin PL, Resnick SM, Prince JL. Simple paradigm for extra-cerebral tissue removal: algorithm and analysis. *Neuroimage.* 2011; 56(4):1982–1992. [PubMed: 21458576]
37. Bazin PL, Pham DL. Homeomorphic brain image segmentation with topological and statistical atlases. *Med. Image Anal.* 2008; 12(5):616–625. [PubMed: 18640069]
38. Shiee N, Bazin PL, Ozturk A, Reich DS, Calabresi PA, Pham DL. A topology-preserving approach to the segmentation of brain images with multiple sclerosis lesions. *Neuroimage.* 2010; 49(2): 1524–1535. [PubMed: 19766196]
39. de Beer R, van den Boogaart A, van Ormondt D, Pijnappel WW, den Hollander JA, Marien AJ, Luyten PR. Application of time-domain fitting in the quantification of in vivo ^1H spectroscopic imaging data sets. *NMR Biomed.* 1992; 5(4):171–178. [PubMed: 1449952]
40. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn. Reson. Med.* 1993; 30(6):672–679. [PubMed: 8139448]
41. Shao, J.; Tu, D. *Springer Series in Statistics. Vol. XVII.* Springer; New York: 1995. *The Jackknife and Bootstrap.*
42. Efron, B.; Tibshirani, RJ. *An Introduction to the Bootstrap.* CRC Press; Boca Raton, FL: 1994.
43. Bartlett JW, Frost C. Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables. *Ultrasound Obstet. Gynecol.* 2008; 31(4):466–475. [PubMed: 18306169]
44. Vaz S, Falkmer T, Passmore AE, Parsons R, Andreou P. The case for using the repeatability coefficient when calculating test–retest reliability. *PLoS One.* 2013; 8(9):e73990. [PubMed: 24040139]
45. Beckerman H, Roebroek ME, Lankhorst GJ, Becher JG, Bezemer PD, Verbeek AL. Smallest real difference, a link between reproducibility and responsiveness. *Qual. Life Res.* 2001; 10(7):571–578. [PubMed: 11822790]
46. Myles PS, Cui J. Using the Bland–Altman method to measure agreement with repeated measures. *Br. J. Anaesth.* 2007; 99(3):309–311. [PubMed: 17702826]
47. Rosner, B. *Fundamentals of Biostatistics.* 6th. Thomson-Brooks/Cole; Boston MA, USA: 2006.

Figure 1.

Planning of the single-volume diffusion-weighted MRS (DWS) experiment on the anterior body of the corpus callosum as seen on sagittal (a) and coronal (d) T_1 -weighted image slices from a 3T scan. The full arrows show the diffusion gradient directions perpendicular (a) and parallel (d) to the callosal fibers applied in all scans. Typical spectra acquired at 3T with diffusion weighting in the $g_{[0,-1,1]}$ and $g_{[-1,0,0]}$ directions are shown in (b) and (e). Spectra acquired at 7T with the same gradient directions are shown in (c) and (f). A line broadening of 5 Hz was applied to all of the spectra for display purposes.

Figure 2.
Schematic description of the method in which data subsets are randomly selected from the full dataset of a single session of a specific subject.

Figure 3.

Logarithm of the diffusion-weighted total *N*-acetylaspartate (tNAA) signal measured with diffusion weighting applied along the $[0,-1,1]$ and $[-1,0,0]$ directions as a function of b value (measured in s/mm^2). (a) and (c) show the monoexponential fits used to calculate the parallel $[D_{\text{par}}(\text{tNAA})]$ and perpendicular $[D_{\text{perp}}(\text{tNAA})]$ diffusivity values from one dataset acquired at 3T (a) and one acquired at 7T (c). (b) and (d) show the same data fitted to the model described in the text, which yields the cytosolic diffusivity $D_{\text{model}}(\text{tNAA})$ and the standard deviation of the axonal angular dispersion σ_{ϕ} .

Figure 4. Diffusivity measures from all subjects and all sessions obtained from the experiments performed at 3T and 7T. tCho, total choline compounds; tCr, total creatine; tNAA, total *N*-acetylaspartate.

Figure 5.

Coefficients of variation for D_{model} (a, c) and D_{avg} (b, d) obtained from all datasets acquired at 3T and 7T, respectively, shown as a function of the number of acquisitions for different b -value combinations.

Figure 6. Number of subjects required to detect a difference (as a percentage of the mean) with a significance level of $\alpha = 0.05$ and power of $1 - \beta = 0.80$ using the suggested g_{1357} b -value scheme for 3T and g_{247} scheme for 7T.

Table 1

Average, standard deviation (SD) and coefficient of variation (C_V) for D_{model} , D_{par} , D_{avg} , D_{perp} and σ_ϕ values calculated on the basis of the complete datasets acquired from three subjects at 3T and three subjects at 7T

		3T Sub1	3T Sub2	3T Sub3	7T Sub1	7T Sub2	7T Sub3
tNAA D_{model}	Mean (SD) ($\mu\text{m}^2/\text{ms}$) C_V (%)	0.52 (0.05) 10	0.51 (0.03) 5	0.48 (0.07) 15	0.50 (0.06) 12	0.51 (0.01) 2	0.50 (0.02) 5
tNAA D_{avg}	Mean (SD) ($\mu\text{m}^2/\text{ms}$) C_V (%)	0.22 (0.01) 5	0.22 (0.01) 3	0.21 (0.02) 11	0.21 (0.02) 12	0.22 (0.01) 2	0.22 (0.01) 3
tNAA D_{par}	Mean (SD) ($\mu\text{m}^2/\text{ms}$) C_V (%)	0.36 (0.03) 7	0.38 (0.02) 5	0.34 (0.05) 14	0.35 (0.04) 13	0.38 (0.01) 2	0.36 (0.02) 5
tNAA D_{perp}	Mean (SD) ($\mu\text{m}^2/\text{ms}$) C_V (%)	0.08 (0.02) 29	0.07 (0.02) 22	0.08 (0.00) 2	0.07 (0.00) 6	0.06 (0.00) 7	0.07 (0.01) 11
tNAA σ_ϕ	Mean (SD) C_V (%)	26.78 (12.96) 48	22.57 (4.68) 21	36.41 (15.37) 42	23.81 (1.79) 8	22.02 (1.66) 8	29.05 (3.59) 12
tCr D_{avg}	Mean (SD) ($\mu\text{m}^2/\text{ms}$) C_V (%)	0.16 (0.02) 13	0.16 (0.03) 19	0.16 (0.02) 14	0.10 (0.01) 9	0.12 (0.01) 11	0.13 (0.01) 8
tCr D_{par}	Mean (SD) ($\mu\text{m}^2/\text{ms}$) C_V (%)	0.23 (0.03) 13	0.23 (0.06) 27	0.20 (0.04) 18	0.13 (0.03) 21	0.15 (0.02) 16	0.17 (0.00) 3
tCr D_{perp}	Mean (SD) ($\mu\text{m}^2/\text{ms}$) C_V (%)	0.10 (0.02) 17	0.09 (0.02) 22	0.12 (0.02) 17	0.07 (0.01) 17	0.08 (0.01) 9	0.09 (0.02) 21
tCho D_{avg}	Mean (SD) ($\mu\text{m}^2/\text{ms}$) C_V (%)	-	-	-	0.17 (0.01) 9	0.16 (0.02) 13	0.17 (0.02) 10
tCho D_{par}	Mean (SD) ($\mu\text{m}^2/\text{ms}$) C_V (%)	-	-	-	0.24 (0.03) 14	0.24 (0.04) 17	0.25 (0.03) 11
tCho D_{perp}	Mean (SD) ($\mu\text{m}^2/\text{ms}$) C_V (%)	-	-	-	0.09 (0.01) 12	0.09 (0.01) 13	0.09 (0.02) 22

tCho, total choline compounds; tCr, total creatine; tNAA, total *N*-acetylaspartate.

Table 2

Repeatability and sample size values

Scanner	Measure	σ^2 ^a (variance)	n (sample size) ^b	C_R ^a (% mean)	Scan time (NSA)
3T g ₁₋₇	tNAA D_{model}	2.87×10^{-3}	18	0.15 (30%)	33.6 min (NSA=72)
	tNAA D_{avg}	0.22×10^{-3}	9	0.04 (21%)	
	tCr D_{avg}	0.61×10^{-3}	24	0.07 (34%)	
7T g ₁₋₇	tNAA D_{model}	1.42×10^{-3}	9	0.10 (21%)	28 min (NSA=40)
	tNAA D_{avg}	0.22×10^{-3}	9	0.04 (21%)	
	tCr D_{avg}	0.11×10^{-3}	4	0.03 (15%)	
	tCho D_{avg}	0.30×10^{-3}	12	0.05 (24%)	
3T g ₁₃₅₇	tNAA D_{model}	3.30×10^{-3}	21	0.16 (32%)	19.2 min (NSA=72)
	tNAA D_{avg}	0.29×10^{-3}	11	0.05 (24%)	
	tCr D_{avg}	0.71×10^{-3}	28	0.07 (37%)	
7T g ₂₄₇	tNAA D_{model}	2.01×10^{-3}	13	0.12 (25%)	12 min (NSA=40)
	tNAA D_{avg}	0.32×10^{-3}	12	0.05 (25%)	
	tCr D_{avg}	0.18×10^{-3}	7	0.04 (18%)	
	tCho D_{avg}	0.27×10^{-3}	11	0.05 (23%)	

C_R , repeatability coefficient; NSA, number of spectral averages; tCho, total choline compounds; tCr, total creatine; tNAA, total *N*-acetylaspartate.

^aUnit: $\mu\text{m}^4/\text{ms}^2$.

^bSignificance level (α) = 0.05, power ($1 - \beta$) = 0.80, detectable difference () = 10%.