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Reproducibility and optimization of \textit{in vivo} human diffusion-weighted magnetic resonance spectroscopy of the corpus callosum at 3T and 7T

This chapter was adapted from:

Abstract

Diffusion-weighted MR spectroscopy (DW-MRS) 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 DW-MRS measures across time and subjects, as well as a systematic investigation of optimal acquisition parameters for DW-MRS experiments, both of which are sorely needed for clinical applications of the method.

In this study, we acquired comprehensive single volume DW-MRS data sets 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 the 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 accurate measurement of tNAA diffusion properties in clinically relevant experimental time. Based on the resampling results we suggest optimized acquisition schemes of 13 minute duration at 3T and 10 minute duration at 7T while retaining low variability ($C_V \approx 8\%$) for the tNAA diffusion measures. Power calculations for the estimation of $D_{\text{model}}(\text{tNAA})$ and $D_{\text{avg}}(\text{tNAA})$ based on the suggested schemes show that less than 21 subjects per group are sufficient for detecting a 10\% effect between two groups in case-control studies.
3.1 Introduction

**D**iffusion-weighted MR spectroscopy (DW-MRS) assesses diffusion properties of intracellular metabolites, thus providing specific information about the microstructural properties of the compartments where 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 magnetic resonance spectroscopy (MRS) techniques, N-acetylaspartate (NAA) plays a central role in the evaluation of neuronal and axonal damage resulting from neurological disorders. Approximately 95% of tNAA in the mature CNS is found within neurons [15], and therefore tNAA diffusion properties can provide a unique insight on damage to the intraneuronal/intra-axonal space, separate from confounding contributions such as the extracellular space. Early DW-MRS 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 DW-MRS in distinguishing disease-related changes in multiple sclerosis [18], acute cerebral ischemia [19, 20], brain tumors [20, 21], and psychiatric disorders [22].

Performing in-vivo DW-MRS measurements on a clinical scanner and deriving meaningful information from these experiments remains challenging, and the robustness and reproducibility of the technique still need to be established. The low concentrations of metabolites compared to that of water, coupled with the long echo times (TE) 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. Additionally, the diffusion properties of metabolites may require different diffusion-weighting parameters than those used in water diffusion-weighted techniques. Previous work, for example, emphasized the importance of b-value choice for an accurate estimate of fractional anisotropy (FA) of brain metabolites from DW-MRS experiments [23]. In order to assess the viability of DW-MRS as a meaningful diagnostic tool, it is therefore essential to ascertain: (a) 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; (b) the optimal parameters for a reliable DW-MRS experiment within an experimental time that is suitable for clinical and clinical research purposes.

We chose to accomplish these goals with a set of DW-MRS measurements performed on the anterior body of the corpus callosum (aCC). The aCC has been thoroughly studied with DW-MRS [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, axonal diameter and molecular crowding in the axonal space [4]. Diffusion along the callosal fibers has been linked to axonal degradation in MS [18], and the involvement of the aCC in several
neurological disorders makes it a plausible candidate for clinically relevant measurements \cite{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, reproducibility of tNAA DW-MRS measurements parallel and perpendicular to the callosal fiber main direction is assessed on two MRI scanners, operating at different magnetic fields of 3T and 7T, using an almost identical protocol. The reproducibility of derived quantities, such as 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 \cite{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 breakdown of microtubules and neurofilaments. We also provide an estimate for the repeatability of the diffusion coefficients of other two metabolites detected within the same VOI selection: total creatine (creatine + phosphocreatine = tCr) and choline compounds (phosphocholine + glycerophosphocholine = tCho). The second part of the study reports the dependence of the statistical properties of the diffusion properties of tNAA obtained from the direct fit and the modeling as a function of 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. Additionally, in order to assess the clinical viability of DW-MRS measurements, power calculations are performed to assess the minimum number of subjects required in a case-control study in order to detect a certain effect due to disease.

3.2 Materials and Methods

3.2.1 Human subjects

Six healthy volunteers (three men, three women, ages: 34 ± 8 years), without known neurological abnormalities, participated in this study. Each subject was scanned in 5 separate sessions. The study adhered to local Institutional Review Board guidelines, and informed consent was obtained from all subjects prior to the study.

3.2.2 MRI Scanner/Hardware

Three of the subjects were scanned on a 3 Tesla Achieva MRI scanner (Philips Medical Systems, Cleveland, OH, USA) at the National Institutes of Health, Bethesda, MD. The scanner was equipped with an 8-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 7 Tesla Achieva whole-body MRI scanner (Philips Healthcare, Best, The Netherlands) at the Leiden University Medical Center, 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 (Nova Medical Inc., Wilmington, MA, USA) was used for the 7T measurements.

3.2.3 Scan Protocol

3.2.3.1 Anatomical imaging

For each separate scan session, a short survey scan and a sensitivity encoding (SENSE) reference scan were followed by a 3D T<sub>1</sub>-weighted gradient-echo acquisition for positioning the VOI in the DW-MRS experiments and for tissue segmentation in the post-processing stage. Imaging parameters for the T<sub>1</sub>-weighted image acquired at 3T were: field of view (anterior-posterior (AP), foot-head (FH), right-left (RL)): 240×240×180 mm<sup>3</sup>, 1 mm isotropic resolution, TR/TE: 7.00 / 3.15 ms, total scan time: 5.30 min. Imaging parameters for the T<sub>1</sub>-weighted image acquired at 7T were: field of view (AP, FH, RL): 246×246×174 mm<sup>3</sup>, resolution: 0.85×0.85×1 mm<sup>3</sup>, TR/TE: 5.00 / 2.20 ms, total scan time of 1.59 min.

3.2.3.2 Diffusion tensor imaging protocols

A DTI data set was also acquired in each scan session and was used to estimate macroscopic curvature of the axonal tracts in the modeling of the DW-MRS data, as explained in [24]. Single-shot 2D spin-echo echo-planar imaging was performed in both 3T and 7T scan protocols. DTI parameters for 3T acquisitions were: field of view (AP, FH, RL): 224×224×120 mm<sup>3</sup>, 2 mm isotropic resolution, TR/TE: 7487 / 85 ms, 32 diffusion weighting directions with b = 800 s/mm<sup>2</sup>, total scan time: 5.50 min. DTI parameters for 7T acquisitions were: field of view (AP, FH, RL): 224×224×120 mm<sup>3</sup>, 2 mm isotropic resolution, TR/TE: 7209/67 ms, 15 diffusion weighting directions with b = 1000 s/mm<sup>2</sup>, total scan time: 2.41 min. Parallel imaging was performed for all scans with a reduction factor of 3 along the phase-encoding direction (AP).

3.2.3.3 Diffusion-weighted spectroscopy protocols

The PRESS (Point Resolved Spectroscopy) [28] sequence was chosen as the base spectroscopic sequence for the single volume DW-MRS experiments and was supplemented with a bipolar diffusion-weighting scheme for minimization of eddy currents [29]. The VOI was positioned at the anterior body of the corpus callosum as shown in Figure 3.1 VOI dimensions were 30 (AP) × 15 (RL) × 8 (FH) mm<sup>3</sup> for 3T and 25 (AP) × 15 (RL) × 8 (FH) mm<sup>3</sup> for 7T experiments. For the diffusion weighting, two directions were chosen for all scans: (1) a pure right-left 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 anterior-posterior 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]. The position of the gradient directions with respect to the VOI is shown in Figure 3.1 (panels a and 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 DW-MRS acquisition in order to
FIGURE 3.1: Planning of the single volume DW-MRS 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 solid 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]} direction and in the g_{[-1,0,0]} directions are shown in panels (b) and (e). Spectra acquired at 7T with the same gradient directions are shown in panels (c) and (f). A line broadening of 5 Hz was applied to all of the spectra for display purposes.

minimize signal fluctuations due to 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 DW-MRS parameters for the 3T acquisitions were: TE = 110 ms, TR = 2 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 7 different gradient amplitudes resulting in b-values of 213, 469, 826, 1285, 1847, 2511, 3277 s/mm^2 in the [1,0,0] direction and 410, 917, 1629, 2544, 3665, 4989, 6518 s/mm^2 in the [0,-1,1] direction. 72 spectra were collected for each diffusion condition (2 directions, 7 b-values). Total DW-MRS scan time ranged from 35-50
minutes, depending upon heart rate of the subject, typically about 60 beats per minute (BPM).

The DW-MRS parameters for the 7T acquisitions were: TE = 121 ms, TR = 3 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 (A) = 60.5 ms with 7 different gradient amplitudes resulting in b-values of 63, 317, 664, 1278, 1912, 2885, 3808 s/mm$^2$ in the [1,0,0] direction and 134, 656, 1361, 2602, 3883, 5844, 7700 s/mm$^2$ in the [0,-1,1] direction. 40 spectra were collected for each diffusion condition. Total DW-MRS scan time was about 40 minutes.

### 3.2.4 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 3D mask in the shape of the DW-MRS 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/mm$^2$ 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 (E$_1$) and the fractional anisotropy (FA), which are later used in the modeling. The white matter VOI mask obtained from the previous stage was then applied to the diffusion parametric maps to obtain the E$_1$ and FA values within the VOI used for the DW-MRS experiments. The angles between the main eigenvectors within VOI and the applied diffusion gradients were calculated for each voxel according to the following equation:

$$\theta_{ij} = \cos^{-1}\left(\frac{E_{1k} \cdot g_j}{\|E_{1k}\| \|g_j\|}\right)$$

(3.1)

where E$_{1k}$ is the principal eigenvector of DTI voxel $k$, $g_j$ is the diffusion-weighting gradient in direction $j$, and $\| \cdot \|$ denotes the norm of a vector. These angles were used later in the modeling to incorporate the macroscopic curvature of the axonal tracks within the VOI.

### 3.2.5 Spectral processing

All spectral processing was done with custom codes in MATLAB® (release R2014b, Mathworks, Natick, MA, USA). DW-MRS 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.

### 3.2.6 Derivation of metabolite diffusion measures

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

$$\ln\left(\frac{S_{b,i}}{S_{b_1,i}}\right) = -b_i \cdot D_i$$

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 DW-MRS measurements introduced by macroscopic factors such as the position of the VOI within the white matter tract, the main direction of the tract with respect to the DW gradients, and the macroscopic curvature of the tract within the VOI. The model, thoroughly described in \([24]\), uses the angles between the main eigenvectors of the DTI data within the DW-MRS 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 $\sigma_\phi$, the standard deviation 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 apparent diffusion coefficients 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.

### 3.2.7 Inter-subject variability analysis of all diffusion measures

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

### 3.2.8 Intra-subject variability analysis of tNAA diffusion measures

For evaluating the dependence of the variability of the calculated diffusion measures within subject 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 data sets were randomly resampled without replacement prior to averaging. For evaluating the effect of 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 7 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 out of the 40 spectra available per condition. For each randomly selected subset, an average spectrum was obtained. These spectra for were then used to calculate $D_{\text{par}}$, $D_{\text{perp}}$, $D_{\text{avg}}$, $D_{\text{model}}$, and $\sigma_{\phi}$. This procedure was then repeated 100 times for each subset size to obtain the jackknife averages and standard deviations of these quantities.

A diagram of an example of the jackknife-like procedure is shown in Figure 3.2, where data from one session is used to generate 100 subsampled data sets, each with 3 b-values (out of the 7 available) and 6 averages (out of the 72 available). The same procedure was applied to all sessions, resulting in 5 sets of diffusion properties and their standard deviations per subject for each jackknife subsampling. These averages and standard deviations are used to generate the across-session averages and standard deviations of the intra-session averages and standard deviations.

3.2.9 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, California, USA. Between and within subject analyses were accomplished with STATA release 11, StataCorp, College Station, Texas, USA. A one-way random-effects ANOVA model was used to estimate the between and within subject variance of the DW-MRS measurements. Between-subject variance was used for inter-subject variability analyses whereas within-subjects variance was used for repeatability coefficient and power/sample size calculations. The repeatability coefficient ($C_R$) within the 95% confidence interval is defined as:

$$C_R = 1.96 \times \sqrt{2} \times \sigma$$

(3.3)

Where $\sigma$ is the within-subject standard deviation \cite{43,46}. Power calculations were done to estimate the sample size required to detect a difference ($\Delta$) in tNAA diffusion measures between two groups based on the variance ($\sigma^2$) 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 \cite{47}. Additionally, the coefficient of variation ($C_V = 100 \times \frac{\sigma}{\mu}$, where $\mu$ is the mean and $\sigma$ is the standard deviation of the resampling results) is reported to allow for comparison between diffusivity measures, which have different mean values.

3.3 Results

3.3.1 Diffusion-weighted spectra and diffusivity calculations

Typical diffusion-weighted spectra from all b-values applied in the two gradient directions are shown in Figure 3.1 for 3T (Panels b and e) and 7T (Panels c and f) scans. Cramér-Rao lower bounds (CRLB) for the tNAA peak from all spectra were between 6% and 20% at 3T and between 3% and 14% at 7T. CRLB 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. CRLB values for tCho were mostly above 20% at 3T, even at the low b-value conditions and thus precluding an
Figure 3.2: Schematic description of the method in which data subsets are randomly selected from the full data set of a single session of a specific subject.

Accurate calculation of $D_{avg}(t\text{Cho})$ at 3T. 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 Figure 3.3, 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 (panels a and b) and one subject scanned at 7T (panels c and d). In panels (a) and (c), the data acquired with the two gradient directions were separately fitted to two independent monoexponential decay functions (equation 3.2). Panels (b) and (d) show the modeled fit and the resulting parameters $D_{model}$ and $\sigma_{\phi}$ for these particular data sets.

3.3.2 Inter-subject variability

Table 3.1 shows the averages, standard deviations, and coefficients of variation of tNAA, tCr and tCho diffusivity measures across subjects and sessions. The mean coefficients of
Figure 3.3: Logarithm of the diffusion-weighted tNAA signal measured with the diffusion weighting applied along the [0, −1, 1] and [−1, 0, 0] directions as a function of b-value (measured in s/mm²). Panels 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 data set acquired at 3T (a) and one acquired at 7T (c). Panels 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 $\sigma_{\phi}$. 

\[ D_{\text{par}} = 0.36 \, \mu\text{m}^2/\text{ms} \] 
\[ D_{\text{perp}} = 0.08 \, \mu\text{m}^2/\text{ms} \] 
\[ D_{\text{model}} = 0.52 \, \mu\text{m}^2/\text{ms} \] 
\[ \sigma_{\phi} = 26.7494 \, \text{deg.} \] 
\[ D_{\text{par}} = 0.39 \, \mu\text{m}^2/\text{ms} \] 
\[ D_{\text{perp}} = 0.06 \, \mu\text{m}^2/\text{ms} \] 
\[ D_{\text{model}} = 0.50 \, \mu\text{m}^2/\text{ms} \] 
\[ \sigma_{\phi} = 22.7294 \, \text{deg.} \]
variation of the tNAA diffusivity measures ranged between 2% and 13% for both scanners, with the exception of that of $D_{\text{perp}}(t\text{NAA})$ at 3T, which was 29% across subjects and 22% across sessions. No significant differences in $D_{\text{par}}(t\text{NAA})$, $D_{\text{perp}}(t\text{NAA})$, $D_{\text{avg}}(t\text{NAA})$, $D_{\text{model}}(t\text{NAA})$ were observed between subjects (one-way ANOVA). In Figure 3.4, all measures calculated based on the full data sets from all subjects were grouped. Diffusivity measures obtained from the data acquired at 3T and 7T were not statistically different (Figure 3.4). $D_{\text{model}}(t\text{NAA})$ values had a smaller (not significant) standard deviation (SD) at 7T compared to 3T: mean (SD) values were $D_{\text{model}}(t\text{NAA}) = 0.501 (0.052)$ at 3T and $D_{\text{model}}(t\text{NAA}) = 0.506 (0.035) \mu m^2/\text{ms}$ at 7T. For $D_{\text{avg}}(t\text{NAA})$, the mean and SD were the same at 3T and 7T: mean (SD) values were $D_{\text{avg}}(t\text{NAA}) = 0.217 (0.015)$ at 3T and $D_{\text{avg}}(t\text{NAA}) = 0.216 (0.015) \mu m^2/\text{ms}$ at 7T. Mean coefficients of variation 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.
Table 3.1: Average, standard deviation and coefficient of variation for $D_{\text{model}}$, $D_{\text{par}}$, $D_{\text{avg}}$, $D_{\text{perp}}$ and $\sigma_\phi$ values calculated based on complete datasets acquired from 3 subjects at 3T and 3 subjects at 7T.

<table>
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<th>3T Sub 1</th>
<th>3T Sub 2</th>
<th>3T Sub 3</th>
<th>7T Sub 1</th>
<th>7T Sub 2</th>
<th>7T Sub 3</th>
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<tr>
<td>tNAA $D_{\text{model}}$</td>
<td>Mean (SD) ($\mu$m$^2$/ms)</td>
<td>0.52 (0.05)</td>
<td>0.51 (0.03)</td>
<td>0.48 (0.07)</td>
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<tr>
<td>tNAA $D_{\text{avg}}$</td>
<td>Mean (SD) ($\mu$m$^2$/ms)</td>
<td>0.22 (0.01)</td>
<td>0.22 (0.01)</td>
<td>0.21 (0.02)</td>
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<tr>
<td></td>
<td>$C_V$ (%)</td>
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<td>3</td>
<td>11</td>
<td>12</td>
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</tr>
<tr>
<td>tNAA $D_{\text{par}}$</td>
<td>Mean (SD) ($\mu$m$^2$/ms)</td>
<td>0.36 (0.03)</td>
<td>0.38 (0.02)</td>
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<td></td>
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<td>5</td>
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</tr>
<tr>
<td>tNAA $D_{\text{perp}}$</td>
<td>Mean (SD) ($\mu$m$^2$/ms)</td>
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<td>0.07 (0.02)</td>
<td>0.08 (0.00)</td>
<td>0.07 (0.00)</td>
<td>0.06 (0.00)</td>
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<tr>
<td></td>
<td>$C_V$ (%)</td>
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<td>22</td>
<td>2</td>
<td>6</td>
<td>7</td>
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<tr>
<td>tNAA $\sigma_\phi$</td>
<td>Mean (SD) ($\mu$m$^2$/ms)</td>
<td>26.78 (12.96)</td>
<td>22.57 (4.68)</td>
<td>36.41 (15.37)</td>
<td>23.81 (1.79)</td>
<td>22.02 (1.66)</td>
</tr>
<tr>
<td></td>
<td>$C_V$ (%)</td>
<td>48</td>
<td>21</td>
<td>42</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>tCr $D_{\text{avg}}$</td>
<td>Mean (SD) ($\mu$m$^2$/ms)</td>
<td>0.16 (0.02)</td>
<td>0.16 (0.03)</td>
<td>0.16 (0.02)</td>
<td>0.17 (0.01)</td>
<td>0.16 (0.02)</td>
</tr>
<tr>
<td></td>
<td>$C_V$ (%)</td>
<td>13</td>
<td>19</td>
<td>14</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>tCr $D_{\text{par}}$</td>
<td>Mean (SD) ($\mu$m$^2$/ms)</td>
<td>0.23 (0.03)</td>
<td>0.23 (0.06)</td>
<td>0.20 (0.04)</td>
<td>0.24 (0.03)</td>
<td>0.24 (0.04)</td>
</tr>
<tr>
<td></td>
<td>$C_V$ (%)</td>
<td>13</td>
<td>27</td>
<td>18</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>tCr $D_{\text{perp}}$</td>
<td>Mean (SD) ($\mu$m$^2$/ms)</td>
<td>0.10 (0.02)</td>
<td>0.09 (0.02)</td>
<td>0.12 (0.02)</td>
<td>0.09 (0.01)</td>
<td>0.09 (0.01)</td>
</tr>
<tr>
<td></td>
<td>$C_V$ (%)</td>
<td>17</td>
<td>22</td>
<td>17</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>tCho $D_{\text{avg}}$</td>
<td>Mean (SD) ($\mu$m$^2$/ms)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.10 (0.01)</td>
<td>0.12 (0.01)</td>
</tr>
<tr>
<td></td>
<td>$C_V$ (%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>tCho $D_{\text{par}}$</td>
<td>Mean (SD) ($\mu$m$^2$/ms)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.13 (0.03)</td>
<td>0.15 (0.02)</td>
</tr>
<tr>
<td></td>
<td>$C_V$ (%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>tCho $D_{\text{perp}}$</td>
<td>Mean (SD) ($\mu$m$^2$/ms)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.07 (0.01)</td>
<td>0.08 (0.01)</td>
</tr>
<tr>
<td></td>
<td>$C_V$ (%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>17</td>
<td>9</td>
</tr>
</tbody>
</table>
3.3.3 Intra-subject variability of tNAA diffusion measures

Figure 3.5 shows the coefficient of variation as a function of 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.

3.3.4 Reproducibility and sample size analysis

Repeatability coefficients \( (C_R) \) are shown in Table 3.2 for \( D_{\text{model}}(\text{tNAA}) \) and \( D_{\text{avg}} \) of the three metabolites calculated based on the entire dataset as well as based on the b-value scheme \( g_{1357} \) 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 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%. \( D_{\text{avg}} \) calculated using the entire dataset and \( D_{\text{avg}} \) calculated based on the b-value scheme \( g_{1357} \) yielded similar \( C_R \) values of 21% and 24% at 7T and 3T, respectively. \( D_{\text{model}} \) calculated based on the same schemes at 3T resulted in higher \( C_R \) (30% for the entire dataset and 32% for \( g_{1357} \) scheme) compared to \( C_R \) values of \( D_{\text{model}} \) at 7T (21% for the entire dataset and 25% for \( g_{247} \) scheme). The \( C_R \) values for \( D_{\text{avg}}(\text{tCr}) \) and \( D_{\text{avg}}(\text{tCho}) \) at 7T were similar to those calculated for \( D_{\text{avg}}(\text{tNAA}) \), whereas at 3T the \( C_R \) values for \( D_{\text{avg}}(\text{tCr}) \) were higher than those for \( D_{\text{avg}}(\text{tNAA}) \).

Sample size calculations reflect the number of subjects per group required to detect a difference between two groups with power of 80% and significance level of 5%. Sample size \( (n) \) values to detect a 10% difference in both \( D_{\text{model}}, D_{\text{avg}}, D_{\text{avg}}(\text{tCr}) \) and \( D_{\text{avg}}(\text{tCho}) \) are shown in Table 3.2 while the trends in sample size values for 5, 10, 15 and 20% detectable differences for tNAA are depicted in Figure 3.6.

3.4 Discussion

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

3.4.1 Intra- and inter-subject variability of tNAA DW-MRS measures

The coefficients of variation \( (C_V) \) given in Table 3.1 and the reproducibility coefficients \( (C_R) \) given in Table 3.2 for \( D_{\text{avg}} \) and \( D_{\text{model}} \) indicate acceptable reproducibility \( (C_R = 21\% \) for \( D_{\text{avg}} \) and \( C_R = 32\% \) for \( D_{\text{model}} \) at 3T) of the DW-MRS measures of tNAA in the corpus callosum. At 7T, all \( C_V \) values were less than 13%, and most were in the range of 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 minutes in both scanners) also reflects patient motion and scanner-related instabilities, which are expected to be less pronounced in shorter experiments. Past
DW-MRS 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 white matter changes in MS at 7T, a decrease of about 20% was observed in the $D_{\text{par}}$ of tNAA in the aCC of a small cohort of MS patients compared to age-matched healthy controls [18]. Based on our power calculations, a difference between groups of 10% for $D_{\text{model}}$ or $D_{\text{avg}}$ can be detected with groups as small as 9-18 subjects each for 3T or 9 subjects each for 7T. This result is particularly reassuring as it suggests the ability of DW-MRS of tNAA to pick up subtle changes in intra-axonal structure in normal appearing white matter. It should be noted that this estimate is achieved using the full acquisition scheme used in our experiments. Such an acquisition would take around 35 minutes at 3T and 30 minutes at 7T, thus reaching the higher range of clinically-feasible scan times.

The average inter-subject variability values in $D_{\text{avg}}$ and in $D_{\text{model}}$ at 7T were 5.7% and 6.3%, respectively, and thus comparable. At 3T, the variability of $D_{\text{avg}}$ was $C_V = 6\%$,
### Table 3.2: Repeatability and sample size values

<table>
<thead>
<tr>
<th>Scanner</th>
<th>Measure</th>
<th>$\sigma^2a$ (variance)</th>
<th>n (samples$^b$)</th>
<th>$C_R^b$ (% mean)</th>
<th>Scan time$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3T $g_{1-7}$</td>
<td>tNAA $D_{model}$</td>
<td>$2.87 \times 10^{-3}$</td>
<td>18</td>
<td>0.15 (30%)</td>
<td>33.6 min</td>
</tr>
<tr>
<td></td>
<td>tNAA $D_{avg}$</td>
<td>$0.22 \times 10^{-3}$</td>
<td>9</td>
<td>0.04 (21%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tCr $D_{avg}$</td>
<td>$0.61 \times 10^{-3}$</td>
<td>24</td>
<td>0.07 (34%)</td>
<td></td>
</tr>
<tr>
<td>7T $g_{1-7}$</td>
<td>tNAA $D_{model}$</td>
<td>$1.42 \times 10^{-3}$</td>
<td>9</td>
<td>0.10 (21%)</td>
<td>28.0 min</td>
</tr>
<tr>
<td></td>
<td>tNAA $D_{avg}$</td>
<td>$0.22 \times 10^{-3}$</td>
<td>9</td>
<td>0.04 (21%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tCr $D_{avg}$</td>
<td>$0.11 \times 10^{-3}$</td>
<td>4</td>
<td>0.03 (15%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tCho $D_{avg}$</td>
<td>$0.30 \times 10^{-3}$</td>
<td>12</td>
<td>0.05 (24%)</td>
<td></td>
</tr>
<tr>
<td>3T $g_{1357}$</td>
<td>tNAA $D_{model}$</td>
<td>$3.30 \times 10^{-3}$</td>
<td>21</td>
<td>0.16 (32%)</td>
<td>19.2 min</td>
</tr>
<tr>
<td></td>
<td>tNAA $D_{avg}$</td>
<td>$0.29 \times 10^{-3}$</td>
<td>11</td>
<td>0.05 (24%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tCr $D_{avg}$</td>
<td>$0.71 \times 10^{-3}$</td>
<td>28</td>
<td>0.07 (37%)</td>
<td></td>
</tr>
<tr>
<td>7T $g_{247}$</td>
<td>tNAA $D_{model}$</td>
<td>$2.01 \times 10^{-3}$</td>
<td>13</td>
<td>0.12 (25%)</td>
<td>33.6 min</td>
</tr>
<tr>
<td></td>
<td>tNAA $D_{avg}$</td>
<td>$0.32 \times 10^{-3}$</td>
<td>12</td>
<td>0.05 (25%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tCr $D_{avg}$</td>
<td>$0.18 \times 10^{-3}$</td>
<td>7</td>
<td>0.04 (18%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tCho $D_{avg}$</td>
<td>$0.27 \times 10^{-3}$</td>
<td>11</td>
<td>0.05 (23%)</td>
<td></td>
</tr>
</tbody>
</table>

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

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

$^b$Significance level ($\alpha$) = 0.05, power ($1 - \beta$) = 0.80, detectable difference ($\Delta$) = 10%.

$^c$NSA=72 at 3T and NSA=40 at 7T.

---

**Figure 3.6:** Number of subjects required to detect difference (in percent of mean) with significance level of $\alpha = 0.05$ and power of $1 - \beta = 0.80$ using the suggested $g_{1357}$ b-value scheme for 3T and the $g_{247}$ scheme for 7T.
whereas the \( C_V \) of \( D_{\text{model}} \) was higher at 10\%. \( D_{\text{avg}} \) 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. Since the typical DW-MRS measurement is performed on a large volume, these geometric factors introduce a confound that varies across subjects and VOI locations. The model presented in [24] provides a way to remove the impact of macroscopic curvature of white matter tracts within the volume and yields a diffusion measure, \( D_{\text{model}} \), which represents the cytosolic diffusion coefficient, and thus includes the impact of tortuous diffusion within the axonal medium. The stability of the resulting \( D_{\text{model}} \) depends greatly on the SNR of the single DW-MRS measurements that generate the data set 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 standard deviation of the axonal microscopic misalignment, \( \sigma_\phi \) (Table 3.1). It can be appreciated that at 7T, where the CRLB values for the individual measurements were relatively low, the variability in \( D_{\text{model}} \) was comparable to that of \( D_{\text{avg}} \). At 3T, where the CRLB were higher, in particular at the high b-value range, \( D_{\text{model}} \) is found to be less stable.

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

Figure 3.5 can serve as a guideline for choosing a combination of b-values and number of averages in order to reach a desired variability in both \( D_{\text{model}} \) and \( D_{\text{avg}} \). It is clearly seen that sampling the upper range of b-values is critical for obtaining 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 3, 4, and 7 b-values, respectively, quickly converge to low variability values. On the other hand, 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 (3 b-values applied in 2 gradient directions and 32 averages per diffusion weighting condition) will result in an acquisition time of 9.6 minutes at 7T, when a TR of 3 heart beats is used. A similar variability (\( \sim 8\% \)) can be reached at 3T when the scheme \( g_{1357} \) is used with 432 acquisitions (4 b-values applied in 2 gradient directions with 48 averages), with a resulting acquisition time of 14.4 minutes when a TR of 2 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 \( D_{\text{avg}}(\text{tNAA}) \) both at 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 Figure 3.5 the differences in the coefficient of variation 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 repeatability coefficient calculations shown in Table 3.2 imply that \( D_{\text{model}}(\text{tNAA}) \) is more stable at 7T compared to 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 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 due to error propagation properties. Thus, at the reduced SNR at 3T, the variability of \( D_{\text{model}}(\text{tNAA}) \) is markedly higher than
3.4.3 Inter-subject variability of tCr and tCho DW-MRS 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, while 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 $D_{\text{avg}}(\text{tCho})$. Since the experimental protocol was dictated by the goal of characterizing the diffusion properties of tNAA in a well-defined white matter 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 propagation of error. Additionally, the low bandwidth of the refocusing pulses used in the PRESS sequence resulted in a large chemical shift displacement for the metabolites other than the tNAA, especially at 7T, causing the effective VOI for tCr and tCho to be significantly shifted from the medial part of the corpus callosum (Figure 3.8 in the supplement). Since our measurement of $D_{\text{avg}}$ are based on an assumption of gradient directions that are roughly parallel and perpendicular to the callosal fibers in the medial region of the CC, 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.

3.4.4 Limitations of the study

Our study was subject to several challenges and limitations. The five repeated scans per subject allowed examine 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, prevents us from performing a more complete comparison of DW-MRS quantities of tNAA across these two field strengths. Moreover, the bigger 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. Since the experiment was performed using cardiac triggering based on PPU, differences trigger delays, as well as in heart rate and circulation can introduce variability to the TR, and 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 setup, 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 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 white matter 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 DW-MRS 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 on providing a more general model that will allow an arbitrary choice of DW-MRS VOI with a variety of fiber orientations.
within the VOI. Lastly, one should note that the empirical diffusion coefficients in this work were calculated based on fitting 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 metabolites’ diffusion coefficient, as can be appreciated in Figure 3.7 in the supplementary material. In this figure the $D_{\text{avg}}(\text{tNAA})$ shows a clear dependency 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.

3.5 Conclusion

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

Figure 3.7: $D_{\text{avg}}(\text{tNAA})$ as a function of the highest $b$-value used in the experiment. The total number of acquisitions was kept constant for all conditions.
Figure 3.8: The effective VOI for the tNAA (yellow) and tCho (white) at 7T. Number of subjects required to detect difference (in percent of mean) with significance level of $\alpha = 0.05$ and power of $1 - \beta = 0.80$ in tCr and tCh using the suggested $g_{1357}$ b-value scheme for 3T and the $g_{247}$ scheme for 7T.