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The effects of variations in tissue microstructure from postmortem rat brain on the asymmetry of the water proton resonance

Sean Foxley^{1,*}, Gregory S Karczmar¹, and Kazutaka Takahashi²

¹MRIRC, Department of Radiology, University of Chicago

²Department of Organismal Biology and Anatomy, University of Chicago

Abstract

Purpose—This work was performed to investigate the effects of tissue microstructure from postmortem rat brain on the shape of the water proton spectrum.

Methods—Perfusion-fixed, resected rat brains (n=4) were imaged at 9.4 Tesla. 3D DTI and 3D echo-planar spectroscopic imaging (EPSI) data were acquired with 150μm isotropic resolution. DTI data were acquired over 60 directions with b=3000 s/mm². Water spectra were produced from EPSI data acquired over 128 echoes, with 2.9Hz spectral resolution. A voxel-wise metric reflecting spectral asymmetry about the peak of the resonance was computed and compared with orientation estimates from DTI data by fitting data with the susceptibility anisotropy models.

Results—Asymmetric broadening of the water resonance was computed for mixed populations of grey/white matter as determined by thresholding the fractional anisotropy. Asymmetry was shown to be differentially affected by tract orientation relative to B₀ in high FA voxels, while low FA voxels exhibited little sensitivity. Anatomical structures in the hippocampus were also found to produce distinct changes in the water resonance.

Conclusion—Present results demonstrate that structural variations in tissue architecture cause characteristic, reproducible changes in the water resonance shape. This suggests that water spectra are sensitive to cytoarchitectural variations in brain tissue.

Keywords

MRI; EPSI; postmortem rat brain; white matter; microstructure; water proton spectroscopy

INTRODUCTION

Many neurodegenerative diseases, such as ALS and MS, are characterized by microstructural changes in the white matter (1,2). While these changes occur anatomically at a scale that is much smaller than typical imaging resolutions used in clinical MRI, their effects on the measured MR signal have been widely reported (3,4). The underlying

* Address correspondence to: Sean Foxley, University of Chicago Magnetic Resonance Imaging Research Center, 5841 S Maryland Ave, MC 2026, Chicago, IL 60637, Tel: 1-773-834-5119, foxley@uchicago.edu.

mechanisms driving these observations have been attributed to microstructural changes including axonal loss (5), demyelination (6), and gliosis (7); however, the effect that each specific microstructural change has on the reported MRI contrast is less clear. This has motivated a large body of work attempting to understand microstructural effects on the MRI signal, and how changes in microstructure can be used to improve MRI specificity to neurodegenerative disease related tissue changes.

As one focus of this effort, extensive work has been performed to both model and characterize the MRI correlates of the microstructural properties of white matter (8–17). Continued progress in this area would have a profound effect on the clinical applicability of MRI techniques to detection/diagnosis of neurodegenerative diseases. Both signal magnitude and phase of data collected from white matter have been demonstrated to change with fiber orientation relative to B₀ (8–11,13,14,17–24) and undergo non-linear evolution with time (8–11,17). These findings suggest that there are effects on the MRI signal that are due to the constituent magnetic susceptibilities of the different microstructural components that make up the tissue. Of particular interest is myelin, due its specific contribution to the local magnetic field microenvironment (10,25,26). Increasing evidence indicates that myelin exhibits susceptibility anisotropy (11,27–29) due to the anisotropic, diamagnetic lipid chains that form the lipid bilayer.

In this work, multi-gradient echo data were acquired using echo-planar spectroscopic imaging (EPSI), producing voxel-wise, high spectral resolution water spectra from postmortem fixed rat brain. This technique has been shown to be extremely sensitive to subtle variations in local magnetic susceptibility in both fixed (13) and unfixed (30–35) tissue samples. The effects of structural variability were investigated; specifically, asymmetric changes in the water proton resonance (36) were quantified and compared with diffusion data to determine whether microstructural variations, including cytoarchitecture and orientation relative to B₀, produce measureable changes in the water resonance.

METHODS

Sample Preparation

All procedures performed on animals followed protocols approved by the Institutional Animal Care and Use Committee and were in compliance with the Animal Welfare Act and the NIH Guide for the Care and Use of Laboratory Animals. Adult rats were deeply anesthetized with 60 mg/kg pentobarbital and sacrificed by intercardial perfusion with 0.01M phosphate buffer saline solution (PBS), followed by 4% paraformaldehyde (pH 7.4)/PBS fixative solution. Brains were carefully removed from the skulls and post-fixed in the same fixative overnight at 4°C. Brains were washed in PBS prior to imaging for at least 72h to remove fixative.

MR Imaging

Resected rat brains (n=4) were dried of excess PBS and placed in 10 ml falcon tubes. Tubes were filled with Fluorinert (FC-3283, 3M Electronics) for susceptibility matching and to improve shimming.

Data were acquired at 9.4 Tesla (20 cm internal diameter, horizontal bore, Bruker BioSpec Small Animal MR System, Bruker Biospin, Billerica, MA) using a 35mm internal diameter quadrature volume coil (Rapid MR International, Columbus, Ohio). Brains were aligned such that the anterior/posterior portion of the olfactory limb of the anterior commissure (AC) was approximately parallel to B0 and the hemispheric midline was parallel to the scanner YZ plane. This ensured consistency of position relative to B0 across samples.

Third order shimming was iteratively performed over an ellipse that encompassed the entire brain, but did not extend beyond the boundaries of the falcon tube/Fluorinert interface, using the Paravision mapshim protocol. B0 maps were produced by recording the voxel-wise frequency of the peak of the resonance, including additional sub-spectral resolution frequency produced by estimating the maximum peak amplitude of the resonance, described below. The L^2 norm of the gradient of the 3D B0 map was calculated (∇B_0) across all subjects; the average voxel-wise gradient ($\pm$ the 95% confidence interval) was 63 ± 184 $\mu\text{G}/\text{cm}$. This is equivalent to 9.5 ± 27 $\mu\text{G}/\text{voxel}$ or 0.4 ± 1.2 mHz/voxel . This suggests that shimming was quite good and that any additional gradient across the voxel due to B0 would impart little effect on the spectrum.

DTI was performed using a standard diffusion-weighted 3D spin echo sequence (TR = 400ms, TE = 18.5ms, b value = 3000 s/mm^2 , $\delta = 5\text{ms}$ = 11.04ms, spatial resolution = 150 μm isotropic, number of b0's = 16, number of directions = 60, receiver bandwidth = 200kHz, partial Fourier along first phase encoding direction = 7/8, duration = 55hrs 19min 40sec).

3D-EPSI data were acquired using a multi-gradient echo sequence with an oscillating readout gradient train. '3D' in this context refers to the number of spatial dimensions; data were in fact acquired in 4D with the inclusion of time. Sequence parameters were chosen so that the entire voxel-wise free induction decay was sampled to the noise floor with sufficiently high temporal resolution. This ensured that resultant spectra did not have FID truncation-related ringing artifacts, and that they had sufficient bandwidth, respectively (TR = 1000 ms, TE of first echo = 2.65ms, echo spacing = 2.65ms, number of echoes = 128, receiver bandwidth = 75kHz, flip angle = 68° , 150 μm isotropic resolution, 4 averages, duration = 9 hours).

EPSI data processing

Data were processed and analyzed with IDL (ITT Visual Information Solutions, Boulder CO), Matlab (The MathWorks Inc., Natick, MA, 2012), and FSL (FMRIB Software Library, FMRIB, Oxford, UK).

3D multiple-gradient echo data were processed to produce voxel-wise water spectra. Each 4D complex array ($k_x \times k_y \times k_z \times t$) was Fourier transformed in all dimensions to produce three spatial dimensions and one spectral dimension ($x \times y \times z \times \nu$). For the complex spectrum, ν , spectral ghosting was corrected at each point in space (x, y, z) (37).

The maximum peak magnitude is a robust and easily identifiable feature of the resonance to use as a reference point for voxel-wise spectral analyses. However, this peak magnitude can

be ‘missed’ if it does not reside at any of the specific Fourier components that compose the spectrum (i.e. it lies at a frequency between two points). In order to maintain consistency in subsequent voxel-wise spectral analyses, it was necessary to estimate the maximum peak magnitude in voxels where the peak was between Fourier components. This was achieved by applying the Fourier shift theorem to the complex data in the time domain; by adding a linear phase terms in the temporal domain, sub-spectral resolution shifts can be performed in the frequency domain and the voxel-wise maximum signal magnitude in the peak identified (38).

To account for phase accrual after excitation, real and imaginary components were phased on a voxel-by-voxel basis with a zeroth order phase correction to produce pure absorption spectra (39). The maximum peak magnitude estimate and phasing were alternately iterated until the maximum signal amplitude was identified in the absorption spectrum.

The relative frequency of the voxel-wise maximum peak amplitude of the resonance is dependent on and used for the construction of B0 maps. This includes the small additional sub-spectral resolution frequency shift estimate produced from implementing the Fourier shift method (describe above). Without this additional source of frequency information, the B0 maps are inherently discretized by the spectral resolution of the acquisition.

Water peak height (PH) images were constructed, where image contrast was produced by the maximum voxel-wise signal amplitude of the water spectrum (40). This was achieved by shifting the position of the maximum peak amplitude of the water resonance to the central Fourier component of the frequency axis (i.e. the 64th point). This step has no effect on the shape of the resonance or related metrics (e.g. asymmetry, described below). However, since the details of the microstructural magnetic field environment are specifically contained in the shape of the water resonance, this step does serve to eliminate any relative background field information from each spectrum; this is analogous to implementing a background field removal technique, such as the PDF (41) or the SHARP (42) filter, to T2* weighted gradient echo data processed in the temporal domain.

Data analysis

Voxel-wise spectral asymmetry (*ASYM*) was computed by subtracting the integral of the high field half of the spectrum (relative to the main water peak, identified as 0Hz for simplicity) from the integral of the low field half of a spectrum, and normalized by the total spectrum integral. This produces a unitless asymmetry metric equivalent to the fraction of the total resonance. Integration was performed to $\pm 58\text{Hz}$ (20 points) from the peak to ensure that the details of the resonance were captured while still reaching the spectral baseline.

$$ASYM(x, y, z) = \frac{\int_0^{58} f(x, y, z, v) dv - \int_{-58}^0 f(x, y, z, v) dv}{\int_{-58}^{58} f(x, y, z, v) dv} \quad \text{Eq.1}$$

The mean $b = 0 \text{ s/mm}^2$ dataset from each diffusion acquisition was registered to the respective PH image via affine transformation using the FSL linear registration tool (FLIRT)

(43). Subsequent diffusion and tractography results were registered using the resultant transformation matrix.

Fractional anisotropy (FA) was computed from diffusion data fit to a standard tensor model using DTIFIT (FSL, Oxford) and thresholded to produce masks. Threshold values included $FA < 0.3$, $0.3 \leq FA < 0.45$, $0.45 \leq FA < 0.6$, and $FA \geq 0.6$. These values represent mixed populations of grey and white matter across the whole brain, with the volume fraction of grey matter decreasing and white matter increasing with increasing FA threshold values. Visual inspection of the different masks compared with the PH image suggest that $FA < 0.3$ and $FA \geq 0.6$ were predominantly grey and white matter, respectively, while the other two masks were composed of highly mixed populations. The respective mask sizes for both FA threshold and Γ (described below) are shown in Figure 1.

Principal and secondary diffusion directions estimated were computed using BEDPOSTX (44). Results of these analyses were then used to perform probabilistic tractography with PROBTRACKX2 (45) of the AC. The resultant AC 'tract' is populated by voxel-wise connectivity values, indicating the number of streamlines that pass through each voxel and the seed mask. Larger values indicate increased connectivity. Tractography results were thresholded by a minimum streamline count to isolate the respective tract to areas of only high streamline content and binarized to create a tract specific mask.

The voxel-wise solid angle (Γ) of orientation about B0 was found by computing:

$$\Gamma = \arccos (| PDD(x, y, z, B0) |) \quad \text{Eq.2}$$

where $PDD(x, y, z, B0)$ is the component of the mean principal diffusion direction (PDD) vector estimate along the axis that is parallel to B0. Since anti-parallel directions can be equivalently treated as parallel, the absolute value was taken to ensure that values were between 0° and 90° .

All statistical analyses performed involved comparisons of 4 sample populations, and as such, a one-way ANOVA test was performed. Post-hoc analysis of the data was performed in order to determine the statistical power given the reported sample population size. An estimate was made of two 'worst case scenarios' by using an FA threshold value of either below or above 0.5. In doing this, both ROIs were composed of mixed populations of both white and grey matter. Using the standard deviation of asymmetry values across all angles relative to B0 (as reported in Table 1) for each of the two test ROIs, a confidence level (alpha) of 5%, and a sample population of 4 predicted a statistical power of 98%. It was assumed that if a comparison of means across samples of mixed populations produced high statistical power, then increasingly high volume fraction populations of white and grey matter produced from FA threshold values larger or smaller than 0.5 would as well. As such, $n=4$ was considered sufficient for this study.

RESULTS

Asymmetry vs. orientation

Asymmetric broadening of water spectra was differentially affected by white matter orientation relative to B0, as demonstrated by results specific to the AC shown in Figure 2. A typical water peak-height image of a coronal cross-section including the AC is shown (Fig 2a), with Γ overlain on the magnified AC (Fig 2b). The AC was selected as a representative white matter tract because it is an easily tracked area of the rat brain with high FA (i.e. limited crossing fibers that could potentially confound the effect of orientation on the water spectrum). Moreover, when placed in the magnet with the rostral/caudal axis of the brain parallel to B0, it is composed of white matter that spans the range of possible solid angles relative to B0 (ranging from parallel to perpendicular). Typical water spectra from individual voxels representing a range of different orientations are shown in Figures 2c–f. There is a clear secondary peak in at 0° and 30° (Figs 2c and 2d, respectively). As the orientation becomes closer to perpendicular to B0 (Figs 2e and 2f), the two peaks increasingly overlap and become less distinguishable from one another.

Orientation dependencies of the spectral line shape are demonstrated in scatter plots shown in Figure 3. These plots show the mean change in spectral asymmetry as a function of Γ across the entire brain, as per the indicated FA threshold. Specifically, the mean asymmetry was computed for spectra within incremental bins of 5°. Results are shown for each sample (shown with different colors, Figs 3a–d) together with the respective fit using the susceptibility anisotropy model (29):

$$\text{Asym}(\Gamma) = c_0 + c_1 \cdot \sin(2\Gamma + \phi_0) + c_2 \cdot \sin(\Gamma + \phi_1) \quad \text{Eq. 3}$$

As the FA threshold value increases, the effect of orientation on spectral asymmetry increases as well. The standard deviation of the constituent asymmetry values pooled across Γ for each sample was computed to evaluate the amount of variability in the asymmetry as function of each FA thresholded ROI. Pooled across datasets, this metric shows a trend of increasing variability with increasing FA threshold value (Table 1); the range of asymmetry values at FA = 0.6 being statistically significantly larger than the others ($p < 0.01$). This suggests that as the volume fraction of voxels becomes increasingly dominated by white matter, and the tracts become more angularly homogenous relative to B0 (as determined by increasing the FA threshold), the asymmetric broadening of the water spectra becomes increasingly sensitive to, and dependent on, that orientation. The goodness-of-fit of the anisotropic susceptibility model was estimated using an adjusted R^2 for each sample across threshold values (Table 2). None of the pooled group-wise adjusted R^2 values were statistically significantly different from one another; the mean value across subjects indicates variable degrees of goodness-of-fit of the model to the data regardless of FA threshold range.

Figure 4 shows the voxel-wise R_2^* values computed using a monoexponential fit to the magnitude FID plotted as a function of Γ across the same FA threshold masks produced for Figure 3. Data were, again, fit with the susceptibility anisotropy model. Adjusted R^2 values for each fit are reported in Table 3. As with the model fit to the asymmetry vs. Γ , none of the

pooled group-wise adjusted R^2 values were found to be statistically different from one another. However, the means suggest that the adjusted R^2 value increases with increased FA threshold value. Moreover, the mean value for FA = 0.6 was 0.92, which is consistent with previously reported values (29).

Pairs of FIDs and spectra from three typical voxels are shown in Figure 5. Voxels were selected because the FIDs had very similar $R2^*$ and very high R^2 values, based on a monoexponential fit. The corresponding pure-absorption spectra (blue lines in Figs 5b, 5d, and 5f) however, have very different shapes from one another. Whereas the highly monoexponential FID in Figure 5a produces a very symmetric Lorentzian water line, as the FIDs increasingly deviate from monoexponentiality, as seen by the increasing normalized residuals (Figs 5c and 5e), the spectra become more asymmetric (Figs 5d and 5f). Moreover, the spectra appear to increasingly deviate from the Lorentzian produced from the fits to the respective FIDs (red-dashed lines in Figs 5b, 5d, and 5f) as the normalized residuals increase.

Asymmetry and structure

Previous work reported PH images are particularly sensitive to the cytoarchitecture of the mouse brain (13). Specifically, different layers of the mouse hippocampus were well resolved. Similarly, this work demonstrates that the asymmetric broadening of the water resonance is also sensitive to structural variation. Figure 6 shows a typical axial cross-section of a rat brain acquired with modest spatial resolution. The water peak-height image (Fig 6a) is used as a reference. The green box indicates the region magnified in Figure 6a, with the hippocampus segmented for clarity. The asymmetry map (Fig 6b) clearly indicates characteristic layered architecture of the hippocampus, where contiguous laminar structures are differentiated by the sign of the asymmetry metric. Principal and secondary diffusion direction estimates are shown in Figure 6c overlain on the gray-scale asymmetry map. The positive asymmetry layer (pink arrows in Figs 6b and 6c) corresponds with a region of crossing fibers containing in-plane fibers running left right (red cylinders). This is suggestive of the stratum lacunosum moleculare, which contains Schaffer collaterals from CA1 neurons and perforant fibers from the entorhinal cortex that run parallel to this diffusion direction estimate (46). Surrounding this are areas of negative asymmetry; in these areas, diffusion direction estimates show fibers that are perpendicular to those of the stratum lacunosum moleculare (blue/purple cylinders). Both the stratum radiatum and the molecular layer of the dentate gyrus contain apical dendrites from the granule cells and pyramidal neurons, respectively, that parallel these diffusion direction estimates as well.

DISCUSSION

This study investigates the effect that microstructural variation in postmortem rat brain tissue has on the symmetry of the water resonance about the peak, as measured using echo-planar spectroscopic imaging. Changes in both tissue orientation and composition were shown to produce measurable and variable asymmetrical broadening of the spectrum. Work from other labs has used data in the temporal domain to study the effects of both the axon orientation relative to B0 and the presence of myelin on the microscopic magnetic field

distribution. Experimental measurements have demonstrated voxel-wise non-linear phase evolution as a function of time in white matter, indicating that the underlying field distribution across different microscopic tissue compartments is producing a measurable effect. However, when data are analyzed in the temporal domain (i.e. on the proton free induction decay), magnetic susceptibility dependent variations in the water proton frequency distribution require parameterized fitting algorithms (47–51). Therefore, the model used to fit the data mediates the estimated contribution of the various tissue microstructure compartments on the evolution of the water signal with time. Further, these data have generally been acquired out to a fraction of the entire FID, thereby potentially missing important features of the FID that occur at later echo times. As an alternative to data fitting, this work proposes a model free approach. The complete FID data (as collected with EPSI) can be Fourier transformed along the time dimension, thereby transforming the voxel-wise FIDs to water proton spectra; this approach explicitly resolves individual frequency components with high spectral resolution and provides direct access to information regarding the effects of the underlying magnetic field distribution on the water proton population.

Results from this work demonstrate that spectral asymmetry in what is predominantly white matter (FA = 0.6) as a function of orientation relative to the main magnetic field produce measurable effects on the water proton resonance line-shape. Compared with results from predominately grey matter (FA < 0.3), the range of variability in sample-wise pooled asymmetry values across orientations was significantly larger (Table 1). As the threshold value of the FA used to produce the white matter mask increased, there was also increasing variability in the asymmetry across orientations. This suggests that as the population of axons become more homogeneously oriented relative to B₀ within a voxel, the effect of this orientation on resonance line-shape became more pronounced. This makes intuitive sense; if the resonance shape is dependent on orientation of axons relative to B₀, as the distribution of orientations of axons within a voxel increases (as would be the case with lower FA values in white matter due to crossing fibers), the effect that orientation has on the resonance would be increasingly obscured.

Empirical results vs. modeling

The present results are consistent with work produced using both phantoms and simulations. Chu *et al.* (52) demonstrated the effect that orientation has on spectra produced from cylindrically compartmentalized paramagnetic hyperfine shift-reagent phantoms. By relegating the different reagents to cylinders and surrounding coaxial tubes, they produced spectra that changed solely due to sample orientation relative to B₀. This ‘nested-cylinder’ approach was adapted to model white matter, where the outer coaxial tube represents myelin, the inner cylinder represents the axon, and the model is placed in a non-cylindrical extra-axonal space (8–11). Each of the three constituent compartments of the model was assigned literature-appropriate magnetic susceptibility values, a field distribution was calculated, and the MRI signal computed. Resultant spectra produced by simulating the signal of both single axon and packed axon models demonstrate that the different constituent compartments generate distinct frequency distributions. The superposition of these, which constitute a spectrum of the entire model (as would effectively be measured in an EPSI

experiment), are non-symmetric water spectra with multiple well-resolved peaks. Each peak within the spectrum is specific to the underlying individual compartments, and their frequency relative to one another changes with orientation relative to B₀.

These modeled time-dependent signals are based on T₂'; compartmental T₂ values were not explicitly factored in to these models and, therefore, had no impact on the signal decay rate. Had they been included, the different T₂ values would further serve to differentially broaden the constituent compartments of the water resonance since the modulation of the signal magnitude with time would become T₂* dependent; and T₂* is shorter than either T₂ or T₂' (assuming neither is infinite). Results reported in this work are from T₂*-dependent FIDs; as such, spectra are undoubtedly broadened relative to the model counterparts. Yet, even with this extra source of compartmentally dependent peak broadening (as compared with models dependent on T₂'), spectra collected in the present work from white matter consistently produce multiple individually resolved peaks. Both the trend in asymmetry as a function of orientation relative to B₀ and the difference in asymmetry between different layers within the hippocampus suggest that the multiple peaks resolved in the present data are consistent on a voxel-by-voxel basis.

Beyond line broadening, another potential confound that could further obfuscate the microstructural contribution to the measured spectrum as compared with simulations is within-voxel microstructural heterogeneity. Xu *et al.* (11) demonstrated the effect that varying the axon geometry away from circular cross-sectioned cylinders has on the simulated 'frequency distribution'; increased geometric irregularity in the model produced spectral broadening that increasingly obscured the individual microstructural dependent components of the spectrum. Additionally, reported simulations neglect voxel depth; models were designed to represent a cross-section of idealized white matter, where all axons are parallel to one another and the simulated cross-section is perpendicular to the axons long axis. Segmented serial scanning electron microscopy results of small volumes of brain tissue clearly show that white matter is quite heterogeneous over the axon length (53). Finally, white matter is composed of more constituent components than just axons, such as neuroglia, and different regions of the brain vary in both axon density and g-ratio. All of these potential heterogeneities in white matter would occur within individual voxels of the images reported here, and are additional sources of microstructural variation that could further perturb the local magnetic field distribution and broaden the spectrum. Since these additional aspects have not been included in any models, it is difficult to predict how the spectra and spectral asymmetry should behave in that context.

Susceptibility anisotropy

Fitting the change in asymmetry across orientations with the susceptibility anisotropy model produced modest results. Regardless of threshold value, adjusted R² values were typically moderately high. Of the 16 fits across all subjects and threshold values, 12 of them were greater than 0.75 (Table 2). This means that in all of these cases, the model can explain at least 75% of the variance in the data. This result stands in slight contrast with the results of fitting R₂* vs. Γ . While the adjusted R² values for ROIs of predominantly grey matter (FA < 0.3) suggest that the susceptibility anisotropy model was not necessarily the right model to

fit the data, all values for ROIs composed of predominately white matter (FA = 0.6) were greater than 0.9. These high values are consistent with previous work (29).

The discrepancy between the reported effects of orientation on asymmetry and $R2^*$ is likely because increasing asymmetry is indicative of increasing deviation from monoexponentiality in the FID, as shown in Figure 5. Even with the most asymmetric spectrum (Fig 5f) the R^2 value of the fit to the FID is still quite high. This indicates that while a monoexponential produces good fits to FID data, even those collected well out to the noise floor, it may not necessarily be the correct model. As such, the effect of orientation on the water resonance asymmetry may be more challenging to interpret in the context of the susceptibility anisotropy model and will be a continued area of investigation. Of particular interest will be deviations of analyses of spectral data from current models developed from data simulated and collected in the temporal domain.

Asymmetry and structure

The cytoarchitecture in hippocampus shown in PH images (Fig 6) is very different than that of pure white matter. However, the resolved layered structures in the PH image are potentially modified, at least in part, by differing neuronal components, as demonstrated by the strong correlation of peak asymmetry with the underlying diffusion direction estimates. This result suggests that water peak height images may be an additional source of information specific to brain microstructure.

Use of the maximum peak amplitude as a reference point

The choice to use the maximum peak amplitude as the point of reference for computing asymmetry has potential drawbacks. As indicated in the Methods section, the effect of both shifting each voxel-wise peak of the spectrum to a common Fourier component as well as implementing the Fourier shift theorem to improve the estimate of the maximum peak amplitude is analogous to implementing advanced background field removal techniques on data in the temporal domain (41,42). While these versions of advanced filtering are quite good at estimating and removing the background, they still suffer from imprecisions due to noise and other fitting variables. Yet even in some idealized case where perfect background removal is achieved, the subsequent remaining phase that is dependent on the tissue microenvironment is an average of the phase accrual of the magnetization vector from each microstructural compartment. As the heterogeneity of the microstructural environment increases and/or compartmentally specific magnetic susceptibility values diverge, the phase that is measured becomes less and less reflective of any one compartment, but is rather a weighted mean of phases across the voxel. Similar to this, the voxel-wise frequency about which the asymmetry is computed is difficult to optimize. Where it might be more desirable to specify a reference compartment within each voxel (for example, the Fourier component specific to the extra-axonal space), identifying that frequency is extremely challenging. While this choice to use the peak may not be specific to some underlying microstructural property, it's an easily identifiable, robust, and objective feature of the resonance shape. It is quite likely that the temporal and frequency domain approaches could ultimately complement one another. For example, the use of a background filter on gradient echo data

could help inform the choice of the optimal frequency about which the asymmetry is computed. Optimizing this will be a focus of future development of this approach.

CONCLUSION

Substantial work has been performed to investigate the effects of white matter microstructure on the local magnetic field distribution and the effects this has on the time evolution of the MRI signal. The present work compliments these previous results, demonstrating that structural variations in tissue architecture, with an emphasis placed particularly on the orientation of white matter relative to B₀, cause characteristic, reproducible changes in the water resonance shape. This suggests that water spectra measured using EPSI are sensitive to cytoarchitectural variations in brain tissue and may indicate a possible source of MRI biomarkers for pathological changes in the microstructural environment.

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References

1. Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, Burrell JR, Zoing MC. Amyotrophic lateral sclerosis. *Lancet*. 2011; 377(9769):942–955. [PubMed: 21296405]
2. Steinman L. Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. *Cell*. 1996; 85(3):299–302. [PubMed: 8616884]
3. Rovira A, Wattjes MP, Tintore M, Tur C, Yousry TA, Sormani MP, De Stefano N, Filippi M, Auger C, Rocca MA, Barkhof F, Fazekas F, Kappos L, Polman C, Miller D, Montalban X. group Ms. Evidence-based guidelines: MAGNIMS consensus guidelines on the use of MRI in multiple sclerosis-clinical implementation in the diagnostic process. *Nature reviews Neurology*. 2015; 11(8): 471–482. [PubMed: 26149978]
4. Turner MR, Agosta F, Bede P, Govind V, Lule D, Verstraete E. Neuroimaging in amyotrophic lateral sclerosis. *Biomarkers in medicine*. 2012; 6(3):319–337. [PubMed: 22731907]
5. DeLuca GC, Ebers GC, Esiri MM. Axonal loss in multiple sclerosis: a pathological survey of the corticospinal and sensory tracts. *Brain: a journal of neurology*. 2004; 127(Pt 5):1009–1018. [PubMed: 15047586]
6. Thiessen JD, Zhang Y, Zhang H, Wang L, Buist R, Del Bigio MR, Kong J, Li XM, Martin M. Quantitative MRI and ultrastructural examination of the cuprizone mouse model of demyelination. *NMR in biomedicine*. 2013; 26(11):1562–1581. [PubMed: 23943390]
7. Meadowcroft MD, Mutic NJ, Bigler DC, Wang JL, Simmons Z, Connor JR, Yang QX. Histological-MRI correlation in the primary motor cortex of patients with amyotrophic lateral sclerosis. *Journal of magnetic resonance imaging: JMIR*. 2015; 41(3):665–675. [PubMed: 24615949]
8. Chen WC, Foxley S, Miller KL. Detecting microstructural properties of white matter based on compartmentalization of magnetic susceptibility. *NeuroImage*. 2013; 70:1–9. [PubMed: 23268785]
9. Sati P, van Gelderen P, Silva AC, Reich DS, Merkle H, de Zwart JA, Duyn JH. Micro-compartment specific T2* relaxation in the brain. *NeuroImage*. 2013; 77:268–278. [PubMed: 23528924]
10. Wharton S, Bowtell R. Fiber orientation-dependent white matter contrast in gradient echo MRI. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109(45): 18559–18564. [PubMed: 23091011]

11. Xu T, Foxley S, Kleinnijenhuis M, Chen WC, Miller KL. The effect of realistic geometries on the susceptibility-weighted MR signal in white matter. *Magnetic resonance in medicine*. 2017
12. Duyn JH, van Gelderen P, Li TQ, de Zwart JA, Koretsky AP, Fukunaga M. High-field MRI of brain cortical substructure based on signal phase. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(28):11796–11801. [PubMed: 17586684]
13. Foxley S, Domowicz M, Karczmar GS, Schwartz N. 3D high spectral and spatial resolution imaging of ex vivo mouse brain. *Medical physics*. 2015; 42(3):1463–1472. [PubMed: 25735299]
14. Lee J, Shmueli K, Fukunaga M, van Gelderen P, Merkle H, Silva AC, Duyn JH. Sensitivity of MRI resonance frequency to the orientation of brain tissue microstructure. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(11):5130–5135. [PubMed: 20202922]
15. Liu C. Susceptibility tensor imaging. *Magnetic resonance in medicine*. 2010; 63(6):1471–1477. [PubMed: 20512849]
16. MacKay A, Laule C, Vavasour I, Bjarnason T, Kolind S, Madler B. Insights into brain microstructure from the T2 distribution. *Magnetic resonance imaging*. 2006; 24(4):515–525. [PubMed: 16677958]
17. Wharton S, Bowtell R. Gradient echo based fiber orientation mapping using R2* and frequency difference measurements. *NeuroImage*. 2013; 83:1011–1023. [PubMed: 23906549]
18. Bender B, Klose U. The in vivo influence of white matter fiber orientation towards B(0) on T2* in the human brain. *NMR in biomedicine*. 2010; 23(9):1071–1076. [PubMed: 20665897]
19. Chappell KE, Robson MD, Stonebridge-Foster A, Glover A, Allsop JM, Williams AD, Herlihy AH, Moss J, Gishen P, Bydder GM. Magic angle effects in MR neurography. *AJNR American journal of neuroradiology*. 2004; 25(3):431–440. [PubMed: 15037469]
20. Cherubini A, Peran P, Hagberg GE, Varsi AE, Luccichenti G, Caltagirone C, Sabatini U, Spalletta G. Characterization of white matter fiber bundles with T2* relaxometry and diffusion tensor imaging. *Magnetic resonance in medicine*. 2009; 61(5):1066–1072. [PubMed: 19253372]
21. Sukstanskii AL, Yablonskiy DA. On the role of neuronal magnetic susceptibility and structure symmetry on gradient echo MR signal formation. *Magnetic resonance in medicine*. 2014; 71(1): 345–353. [PubMed: 23382087]
22. Wiggins C, Gudmundsdottir V, LeBihan D, Lebon V, Chaumeil M. Orientation dependence of white matter T2* contrast at 7T: a direct demonstration; *Proceedings of the 16th Annual Meeting of ISMRM*; Toronto, Canada. 2008. 237
23. Schafer A, Bowtell R, Turner R. Orientation dependence of grey/white matter contrast in ultra high fields; *Proceedings of the 17th Annual Meeting of ISMRM*; Honolulu, HI, USA. 2009. 2921
24. Denk C, Hernandez Torres E, MacKay A, Rauscher A. The influence of white matter fibre orientation on MR signal phase and decay. *NMR in biomedicine*. 2011; 24(3):246–252. [PubMed: 21404336]
25. Lee J, Shmueli K, Kang BT, Yao B, Fukunaga M, van Gelderen P, Palumbo S, Bosetti F, Silva AC, Duyn JH. The contribution of myelin to magnetic susceptibility-weighted contrasts in high-field MRI of the brain. *NeuroImage*. 2012; 59(4):3967–3975. [PubMed: 22056461]
26. Liu C, Li W, Johnson GA, Wu B. High-field (9.4 T) MRI of brain dysmyelination by quantitative mapping of magnetic susceptibility. *NeuroImage*. 2011; 56(3):930–938. [PubMed: 21320606]
27. Li W, Wu B, Avram AV, Liu C. Magnetic susceptibility anisotropy of human brain in vivo and its molecular underpinnings. *NeuroImage*. 2012; 59(3):2088–2097. [PubMed: 22036681]
28. Oh SH, Kim YB, Cho ZH, Lee J. Origin of B0 orientation dependent R2*(*) (=1/T2(*)) in white matter. *NeuroImage*. 2013; 73:71–79. [PubMed: 23376494]
29. Lee J, van Gelderen P, Kuo LW, Merkle H, Silva AC, Duyn JH. T2*-based fiber orientation mapping. *NeuroImage*. 2011; 57(1):225–234. [PubMed: 21549203]
30. Du W, Fan X, Foxley S, Zamora M, River JN, Culp RM, Karczmar GS. Comparison of high-resolution echo-planar spectroscopic imaging with conventional MR imaging of prostate tumors in mice. *NMR in biomedicine*. 2005; 18(5):285–292. [PubMed: 15973657]
31. Du W, Karczmar GS, Uftring SJ, Du YP. Anatomical and functional brain imaging using high-resolution echo-planar spectroscopic imaging at 1.5 Tesla. *NMR in biomedicine*. 2005; 18(4): 235–241. [PubMed: 15759296]

32. Fan X, Abe H, Medved M, Foxley S, Arkani S, Zamora MA, Olopade OI, Newstead GM, Karczmar GS. Fat suppression with spectrally selective inversion vs. high spectral and spatial resolution MRI of breast lesions: qualitative and quantitative comparisons. *Journal of magnetic resonance imaging: JMRI*. 2006; 24(6):1311–1315. [PubMed: 17096393]
33. Foxley S, Fan X, Jansen SA, Zamora M, Markiewicz E, Al-Ahmadie H, Karczmar GS. High spectral and spatial resolution MRI of age-related changes in murine prostate. *Magnetic resonance in medicine*. 2008; 60(3):575–581. [PubMed: 18727042]
34. Medved M, Fan X, Abe H, Newstead GM, Wood AM, Shimauchi A, Kulkarni K, Ivancevic MK, Pesce LL, Olopade OI, Karczmar GS. Non-contrast enhanced MRI for evaluation of breast lesions: comparison of non-contrast enhanced high spectral and spatial resolution (HiSS) images versus contrast enhanced fat-suppressed images. *Academic radiology*. 2011; 18(12):1467–1474. [PubMed: 21962476]
35. Spees WM, Yablonskiy DA, Oswood MC, Ackerman JJ. Water proton MR properties of human blood at 1.5 Tesla: magnetic susceptibility, $T(1)$, $T(2)$, $T^*(2)$, and non-Lorentzian signal behavior. *Magnetic resonance in medicine*. 2001; 45(4):533–542. [PubMed: 11283978]
36. Foxley S, Fan X, Mustafi D, Haney C, Zamora M, Markiewicz E, Medved M, Wood AM, Karczmar GS. Sensitivity to tumor microvasculature without contrast agents in high spectral and spatial resolution MR images. *Magnetic resonance in medicine*. 2009; 61(2):291–298. [PubMed: 19165878]
37. Du W, Du YP, Fan X, Zamora MA, Karczmar GS. Reduction of spectral ghost artifacts in high-resolution echo-planar spectroscopic imaging of water and fat resonances. *Magn Reson Med*. 2003; 49(6):1113–1120. [PubMed: 12768590]
38. Fan X, Du W, MacEneaney P, Zamora M, Karczmar G. Structure of the water resonance in small voxels in rat brain detected with high spectral and spatial resolution MRI. *J Magn Reson Imaging*. 2002; 16(5):547–552. [PubMed: 12412031]
39. de Brouwer H. Evaluation of algorithms for automated phase correction of NMR spectra. *Journal of magnetic resonance*. 2009; 201(2):230–238. [PubMed: 19836281]
40. Al-Hallaq HA, Fan X, Zamora M, River JN, Moulder JE, Karczmar GS. Spectrally inhomogeneous BOLD contrast changes detected in rodent tumors with high spectral and spatial resolution MRI. *NMR Biomed*. 2002; 15(1):28–36. [PubMed: 11840550]
41. Liu T, Khalidov I, de Rochefort L, Spincemaille P, Liu J, Tsiouris AJ, Wang Y. A novel background field removal method for MRI using projection onto dipole fields (PDF). *NMR in biomedicine*. 2011; 24(9):1129–1136. [PubMed: 21387445]
42. Schweser F, Deistung A, Lehr BW, Reichenbach JR. Quantitative imaging of intrinsic magnetic tissue properties using MRI signal phase: an approach to in vivo brain iron metabolism? *NeuroImage*. 2011; 54(4):2789–2807. [PubMed: 21040794]
43. Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. *Medical image analysis*. 2001; 5(2):143–156. [PubMed: 11516708]
44. Behrens TE, Woolrich MW, Jenkinson M, Johansen-Berg H, Nunes RG, Clare S, Matthews PM, Brady JM, Smith SM. Characterization and propagation of uncertainty in diffusion-weighted MR imaging. *Magnetic resonance in medicine*. 2003; 50(5):1077–1088. [PubMed: 14587019]
45. Behrens TE, Berg HJ, Jbabdi S, Rushworth MF, Woolrich MW. Probabilistic diffusion tractography with multiple fibre orientations: What can we gain? *NeuroImage*. 2007; 34(1):144–155. [PubMed: 17070705]
46. Shepherd TM, Ozarslan E, King MA, Mareci TH, Blackband SJ. Structural insights from high-resolution diffusion tensor imaging and tractography of the isolated rat hippocampus. *NeuroImage*. 2006; 32(4):1499–1509. [PubMed: 16806988]
47. Du YP, Chu R, Hwang D, Brown MS, Kleinschmidt-DeMasters BK, Singel D, Simon JH. Fast multislice mapping of the myelin water fraction using multicompartiment analysis of $T2^*$ decay at 3T: a preliminary postmortem study. *Magnetic resonance in medicine*. 2007; 58(5):865–870. [PubMed: 17969125]
48. Hwang D, Kim DH, Du YP. In vivo multi-slice mapping of myelin water content using $T2^*$ decay. *NeuroImage*. 2010; 52(1):198–204. [PubMed: 20398770]

49. Nam Y, Lee J, Hwang D, Kim DH. Improved estimation of myelin water fraction using complex model fitting. *NeuroImage*. 2015; 116:214–221. [PubMed: 25858448]
50. van Gelderen P, de Zwart JA, Lee J, Sati P, Reich DS, Duyn JH. Nonexponential T(2) decay in white matter. *Magnetic resonance in medicine*. 2012; 67(1):110–117. [PubMed: 21630352]
51. Wu Z, He H, Sun Y, Du Y, Zhong J. High resolution myelin water imaging incorporating local tissue susceptibility analysis. *Magnetic resonance imaging*. 2017; 42:107–113. [PubMed: 28629954]
52. Chu SC, Xu Y, Balschi JA, Springer CS Jr. Bulk magnetic susceptibility shifts in NMR studies of compartmentalized samples: use of paramagnetic reagents. *Magnetic resonance in medicine*. 1990; 13(2):239–262. [PubMed: 2156125]
53. Kasthuri N, Hayworth KJ, Berger DR, Schalek RL, Conchello JA, Knowles-Barley S, Lee D, Vazquez-Reina A, Kaynig V, Jones TR, Roberts M, Morgan JL, Tapia JC, Seung HS, Roncal WG, Vogelstein JT, Burns R, Sussman DL, Priebe CE, Pfister H, Lichtman JW. Saturated Reconstruction of a Volume of Neocortex. *Cell*. 2015; 162(3):648–661. [PubMed: 26232230]

Figure 1.

ROI sizes for each FA threshold range and each angle of principal diffusion direction relative to B0. Plotted values are the mean $\pm$ the standard error across samples.

Figure 2.

(a) Typical coronal water PH image. The blue box indicates the expanded region in the inset (b) showing the AC. The color overlay indicates the angle (Γ) of the principal diffusion direction relative to B_0 . Typical absorption spectra from voxels in the AC (c–f) are shown for angles ranging from parallel (c) to perpendicular (f) to B_0 . A secondary peak is clearly resolved when axons are parallel to B_0 (c). As Γ increases towards 90° , the peak shifts towards the main water peak, becoming increasingly less distinguishable. This suggests that the underlying microstructural susceptibility distribution is changing with angle and that this can be detected in the spectrum.

Figure 3.

Plots of mean spectral asymmetry from the entire brain computed in 5° increments as a function of Γ , the angle of the principal diffusion direction relative to B0, for each of the 4 samples. Data were fit with the susceptibility anisotropy model (Eq. 3) to investigate the modulation of spectral asymmetry with field orientation (solid lines). The increasing FA threshold range suggests increasing populations of white matter and decreasing population of grey matter. Goodness-of-fit was estimated with an adjusted R^2 , results of which are reported in Table 2.

Figure 4.

Plots of mean $R2^*$ values from the entire brain computed in 5° increments as a function of Γ , the angle of the principal diffusion direction relative to B_0 , for each of the 4 samples (note that the sample specific colors are consistent with figure 3). Data were fit with the susceptibility anisotropy model (Eq. 3) to investigate the modulation of $R2^*$ with field orientation (solid lines). Goodness-of-fit was estimated with an adjusted R^2 , results of which are reported in Table 3.

Figure 5.

Plot of FID data collected out to 339.2 ms (blue dots, a, c, e) with monoexponential fits (red lines) and the respective absorption water spectra (b, d, f). R_2^* values between the FIDs are the same and R^2 values for each are quite high (see insets in a, c, and e. All subsequent values are also reported in these insets). However, deviations from monoexponentiality become evident at higher echo times, as seen in the reported residual values, normalized by the area under the respective fit. These deviations are reflected in the increased asymmetric broadening of the respective spectra (blue solid lines) compared with the corresponding spectra produced from the fit (red dashed lines). Each voxel that the data were collected from has a similar very small gradient magnitude across it.

Figure 6.

(a) Typical axial PH image. The green box indicates the expanded region in the inset (b and c) showing the hippocampus. Layered structure is resolved in the asymmetry map (b) as indicated by a change in sign (white arrows). Principal and secondary diffusion direction vectors are shown in figure (c). A region of contiguous orientation estimates (red cylinders) in the stratum lacunosum moleculare (white arrows) correlate with the asymmetry sign change, suggesting that underlying white matter could be contributing to this.

Table 1Standard deviation of asymmetry as a function of Γ

SUBJ	FA < 0.3	0.3	FA < 0.45	0.45	FA < 0.6	FA	0.6
1	0.0023		0.0032		0.0061		0.0137
2	0.0016		0.0052		0.0053		0.0114
3	0.0039		0.0025		0.0030		0.0096
4	0.0009		0.0038		0.0044		0.0111
Mean	0.0022		0.0037		0.0047		0.0115*

*
p << 0.01 by one-way ANOVA

Table 2Adjusted R^2 for fit to asymmetry as a function of Γ

SUBJ	FA < 0.3	0.3	FA < 0.45	0.45	FA < 0.6	FA	0.6
1	0.9244		0.6760		0.8597		0.9511
2	0.9453		0.9534		0.9450		0.9290
3	0.9709		0.6567		0.5368		0.7604
4	0.7691		0.9361		0.4403		0.8724
Mean	0.9024		0.8055		0.6955		0.8782

No groups are statistically different by one-way ANOVA

Table 3Adjusted R^2 for fit to R_2^* as a function of Γ

SUBJ	FA < 0.3	0.3	FA < 0.45	0.45	FA < 0.6	FA	0.6
1	-0.2031		0.9574		0.9904		0.9168
2	-0.0547		-0.2137		0.9764		0.9128
3	0.8454		-0.5256		0.6324		0.9275
4	-1.7377		0.9422		0.9059		0.9475
Mean	-0.2875		0.2901		0.8965		0.9248

No groups are statistically different by one-way ANOVA