

AD _____

Award Number: W81XWH-10-1-0714

TITLE: Magnetic Resonance Characterization of Axonal Response to Spinal Cord Injury

PRINCIPAL INVESTIGATOR: Felix W. Wehrli, Ph.D.

CONTRACTING ORGANIZATION: University of Pennsylvania
Philadelphia, PA 19104

REPORT DATE: October, 2012

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

☒ Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) 26-10-2012		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 09/27/2011 – 09/26/2012	
4. TITLE AND SUBTITLE Magnetic Resonance Characterization of Axonal Response to Spinal Cord Injury				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-10-1-0714	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Felix W. Wehrli, Ph.D.				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Pennsylvania Philadelphia PA 19104-6205				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S Army Medical Research and Material Command Fort Detrick, MD 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Assessment of axon health in spinal cord injury (SCI) is vital for proper diagnosis and treatment. Magnetic resonance imaging (MRI) is routinely performed in patients and provides valuable information about cord edema and hemorrhage. However, comprehensive prediction of axonal changes from in vivo MR imaging remains elusive. At the U. Penn site, we are applying two novel MRI methods to the problem of assessment of axonal loss, axonal diameter distribution, and myelin loss (q-space imaging (QSI) and ultra-short echo-time (UTE) MRI) first on animal specimens and then on human subjects. During the reporting period we have further developed and published the UTE MRI method for myelin quantification. Our results suggest that UTE MRI will be able to quantify myelin content. Direct quantification of myelin content would remove ambiguities that exist in indirect methods leading to a more accurate assessment of myelin health. We have all injured and perfusion fixed spinal cords representing the different post-injury periods in our possession (supplied by the Drexel performance site). However, we have not been able to execute the QSI due to problems related to the scanner upgrade involving matching of our custom gradients to the instrument (detailed in the report below). However, we anticipate resumption of scanning before 12/12 and completion of the work in 2013.					
15. SUBJECT TERMS Axon Architecture, Spinal Cord Injury, Axon Loss, Myelin, Q-Space Imaging, UTE, MRI					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 14	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	1
Body.....	1
Key Research Accomplishments.....	2
Reportable Outcomes.....	3
Conclusion.....	3
References.....	3
Appendices.....	4

INTRODUCTION

Spinal cord injuries (SCI) produce direct mechanical disruption with subsequent severe degeneration of axons, and are the processes underlying the associated neurologic deficits observed in such injuries. Histological studies of fixed tissue in animal models of SCI have described axonal loss and demyelination occurring after trauma. Research at the University of Pennsylvania site brings novel magnetic resonance methodology to bear with the objective of obtaining quantitative information on axonal degeneration and myelin loss following spinal cord injury in a mouse model by pursuing the following Specific Aims per the work statement:

1. *We will perform q-space MR imaging (QSI) and simulations of QSI to quantify axonal architecture in healthy and injured mouse spinal cords.*
2. *We will quantify myelin content with three quantitative MRI techniques in healthy and injured mouse spinal cords and compare the results with histology.*

Specific Aim 1:

Subsequent to the upgrade of the Bruker NMR/MRI system, hardware modifications were needed to connect our previous custom gradient coil to the new system. The gradient coil also had to be optimized and recalibrated for the new system. This included a systematic and thorough analysis of RF noise sources in the hardware, resulting in the implementation of new shielding and a new filter box on the gradient leads to isolate the receive chain from both room-ambient RF signals and RF noise leakage from the gradient amplifier. Our implementations were similar in design to those used by Bruker for their gradient coils. In addition, we discovered a crack in the epoxy of our custom gradient coil that produced a vibrational phase instability in the data, and we corrected this by applying a new layer of epoxy resin to the gradient coil windings. After these modifications to the gradient coil hardware had been accomplished, we performed a new calibration of our custom gradient coil as this was needed to accurately calculate axon diameters from the q-space data. To ensure a reliable calibration we used three independent methods that agreed with each other: 1) a high-resolution transverse image of a capillary tube of known inner diameter (measured by micro-CT) filled with PEG in water was acquired using our custom gradient for the frequency axis. The Bruker gradient for the phase axis and the frequency-axis scale factor was adjusted to give equal frequency/phase tube diameters; 2) projections of the same PEG/water tube were acquired orthogonal to the tube at varying gradient amplitudes using our custom coil and the resulting NMR line widths were plotted versus actual current applied to the coil; and 3) the ADC of deionized water was measured using our custom coil and compared to the literature value at the same temperature. Finally, as the old QSI pulse sequence program did not run on the new system, a new QSI pulse sequence program had to be developed.

Once the new pulse sequence program has been tested, QSI experiments will be performed, followed by histologic analysis and QSI simulations.

Progress has been made toward translation of the QSI methodology to the clinic (as reported in part previously). The pulse sequence designed toward the end of the prior reporting year has since yielded QSI displacement maps on 1.5T on a clinical imager (Siemens Sonata) on fixed pig spinal cords. Since the pig spinal cord is similar in size to the human spinal cord and the experiments were performed with standard imaging gradients the results suggest feasibility of performing studies in humans with spinal cord injury (even though this was not a specific objective of the current project). This research was presented as a poster at the 2012 Annual Meeting of the International Society for Magnetic Resonance in Medicine in Melbourne, Australia. Notably, we have been able to distinguish white matter tracts differing in axon diameter and density. Figure 1a shows analyzed white matter regions. Figure 1b shows the mean displacements in the three regions, commensurate with the different axon sizes (1).

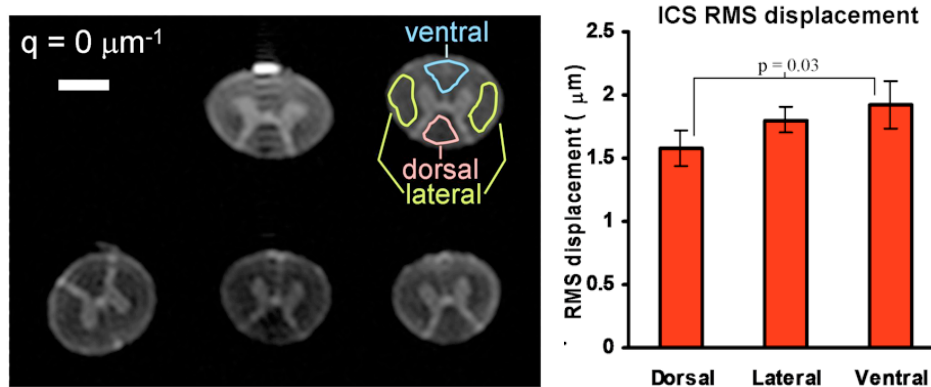


Figure 1 a) Sample transverse images of pig spinal cord acquired at 1.5T with regions of interest indicated (white bar = 5mm); b) RMS displacement obtained from QSI displacement maps consistent with known axon diameters in the three WM tracts examined.

Specific Aim 2:

We previously reported preliminary results on our endeavors toward direct imaging of myelin in the spinal cord of rodents by 3D ultra-short echo-time (UTE) MRI. This work has now been published in the Proceedings of the National Academy of Sciences (PNAS) (2). In brief: We identified the spectrum of myelin in the spinal cord *in situ* as well as in myelin lipids extracted via a sucrose gradient method, and investigated its spectral properties. High-resolution solution NMR spectroscopy showed the extract composition to be in agreement with myelin's known chemical make-up. The 400-MHz ^1H spectrum of the myelin extract, at 20°C (room temperature) and 37°C, consists of a narrow water resonance superimposed on a broad envelope shifted approximately 3.5 ppm upfield, suggestive of long-chain methylene protons. Superimposed on this signal are narrow components resulting from functional groups matching the chemical shifts of the constituents making up myelin lipids. The spectrum could be modeled as a sum of super-Lorentzians with a T_2^* distribution covering a wide range of values (0.008-26 ms). Overall, there was a high degree of similarity between the spectral properties of extracted myelin lipids and those found in neural tissue. The normalized difference spectrum had the hallmarks of membrane proteins not present in the myelin extract. Using 3D radially ramp-sampled proton MRI, with a combination of adiabatic inversion and echo subtraction, the feasibility of direct myelin imaging *in situ* is demonstrated. Last, the integrated signal from myelin suspensions is shown, both spectroscopically and by imaging, to scale with concentration, suggesting the potential for quantitative determination of myelin density in both healthy and injured spinal cords.

KEY RESEARCH ACCOMPLISHMENTS

- Demonstrated feasibility of translation of the Q-Space mapping technique of axon diameter, intra- and extra-axonal space, from the laboratory to the clinic.
- Isolated myelin and showed that its NMR properties match those of myelin *in situ* in the spinal cord.
- Demonstrated a path toward quantitative myelin imaging in humans.
- Identified the magnetic resonance signal of myelin and showed feasibility of direct imaging of neural myelin as a new metric for the evaluation of spinal cord injury.
- Fully characterized the relaxation properties of myelin toward a more complete understanding of the MRI signal in quantitative myelin imaging (Wilhelm et al, 20th Annual ISMRM Scientific Meeting, Melbourne, Australia (3))
- Published major paper in the Proceedings of the National Academy of Sciences (Wilhelm, et al, PNAS 2012).

- Performed experiments on the upgraded Bruker Instruments micro-imaging system after interfacing custom-built gradients for high-resolution q-space imaging and achieved performance goals.

OUTCOMES

The new myelin imaging technique developed under this grant has shown feasibility for quantitative assessment of myelin content in the CNS of the injured rat spinal cord.

CONCLUSION

While substantial progress has been made toward achievement of the goals, the main objective to obtain data in the injured cord and the hypothesized temporal changes has not been attained yet, largely due to the technical problems that have now been solved. Our partners at Drexel University have provided the injured, fixed spinal cords and we are confident we will complete the project during year 3 of this award.

REFERENCES

1. Ong HH, Bhagat YA, Magland JF, Wehrli FW. Feasibility of low q-space diffusion MRI at 1.5T. Proc. ISMRM, 2012; Melbourne, Australia. p. 349.
2. Wilhelm MJ, Ong HH, Wehrli SL, Li C, Tsai PH, Hackney DB, Wehrli FW. Direct magnetic resonance detection of myelin and prospects for quantitative imaging of myelin density. *Proc Natl Acad Sci, USA* 109(24): 9605-9610; 2012.
3. Wilhelm MJ, Ong HH, Wehrli FW. Super-Lorentzian framework for investigation of T2* distribution in myelin. Proc. ISMRM, 2012; Melbourne, Australia. p. 2394.

Feasibility of low q-space diffusion MRI at 1.5T

Henry H. Ong¹, Yusuf Bhagat¹, Jeremy Magland¹, and Felix W. Wehrli¹

¹Laboratory for Structural NMR Imaging, Department of Radiology, University of Pennsylvania School of Medicine, Philadelphia, PA, United States

Introduction

By exploiting the regularity of molecular diffusion restrictions such as axon membranes and myelin sheaths¹, q-space imaging^{2,3} (QSI) offers potential for indirect assessment of white matter (WM) axonal architecture. For example, QSI can accurately estimate mean axon diameter (MAD) and intracellular volume fraction (ICF)^{4,5}. Unfortunately, the application of QSI on a clinical scanner is severely constrained by the low gradient strengths available, which limits the maximum achievable q-value ($q = (2\pi)^{-1}\gamma G\delta$, G = gradient amplitude, and δ = gradient duration). Low maximum q-value leads to insufficient displacement resolution to accurately study axons, which have an MAD of 1-3 μm . Low q-value diffusion MRI⁵, in which axonal architecture information is extracted by fitting the q-space signal decay ($E(q)$) at low q-values ($q^{-1} \gg \text{MAD}$) under the short gradient pulse approximation (SGPA), does not require high gradient amplitudes. However, low clinical gradient strengths lead to violation of SGPA. Here, we test the feasibility of implementing low q-value diffusion MRI on a 1.5T system by assessing axonal architecture in excised fixed pig spinal cords.

Methods

As described in [5], at low q-values ($q^{-1} \gg \text{MAD}$), the signal decay is given by $E(q) = \exp(-2\pi^2 q^2 Z^2)$ (Eq. 1), where Z is the root mean squared (RMS) displacement of diffusing molecules during a diffusion time Δ ^{6,7}. As $E(q)$ contains signal from extra- and intra-cellular spaces (ECS and ICS), a two-compartment version of Eq. 1 can be defined: $E(q) = f_E \cdot \exp(-2\pi^2 q^2 Z_E^2) + f_I \cdot \exp(-2\pi^2 q^2 Z_I^2)$ (Eq. 2), where f_E and f_I are the relaxation-weighted ECS and ICS volume fractions and Z_E and Z_I are the RMS displacement of diffusing molecules in the ECS and ICS. From Eq. 2, MAD and ICF can be estimated from Z_I and f_I , respectively.

For validation of this method, five fixed cervical spinal cords (SC) harvested from five skeletally mature Yucatan mini-pigs were used. Before experiments, the SCs were placed in tubes filled with Fomblin (Sigma-Aldrich) to keep the specimens hydrated and to remove any background signal. The low q-value diffusion MRI method was implemented on a 1.5T Siemens Sonata MRI scanner (Erlangen, Germany) with 40 mT/m gradients using a custom single-slice PGSE with multi-shot fly-back EPI readout pulse sequence. The body coil was used for transmit and a custom-built 4-channel phased array coil (Insight MRI) was used for receive. The imaging parameters were: $\Delta/\delta/\text{TE} = 98.7/55/257\text{ms}$, 128×128 , $\text{FOV} = 64 \times 64\text{ mm}$, slice thickness = 10 mm, number of shots = 8, $\text{NA} = 36$, and $\text{TR} = 2\text{ s}$. The diffusion gradient was applied perpendicular to the SCs in 32 increments ($q_{\text{max}} = 0.08\text{ }\mu\text{m}^{-1}$) and the scan time ~5 hours. Note that these values for δ and Δ violate SGPA. All five SCs were imaged simultaneously. After Fourier transform, a 3D matrix of 32 2D images at various q-values was obtained. An average $E(q)$ was measured in ROIs within the dorsal, ventral, and lateral columns of the SCs (Fig. 1). This average $E(q)$ was fit with Eq. 2 under the constraint $f_E + f_I = 1$.

Results and Discussion

Fig. 2 shows a sample $E(q)$ with the fit from Eq. 2. The fit shows good agreement with $E(q)$ ($R^2 > 0.98$). For display purposes, a one-compartment fit (Eq. 1) is also shown to illustrate its poor agreement. Fig. 3 shows bar graphs of f_E , Z_E , f_I , and Z_I fitting results for each ROI averaged over all five SCs. Z_I falls

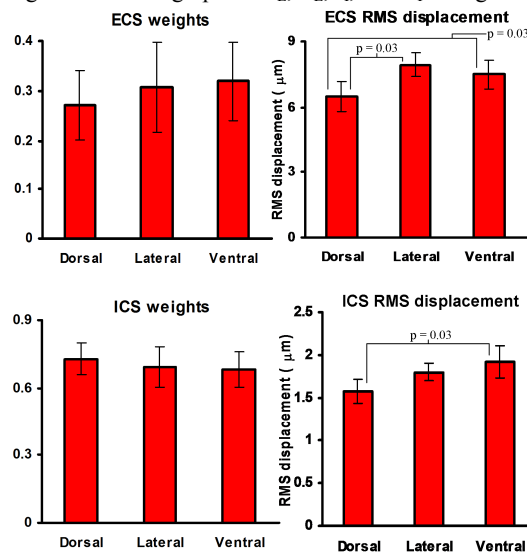


Fig. 3. Bar graphs of f_E (ECS weighting), Z_E (ECS RMS displacement), f_I (ICS weighting), and Z_I (ICS RMS displacement) fitting results. Standard deviation bars are shown. Significant p-values (< 0.05) of paired t-tests between the different ROIs are shown.

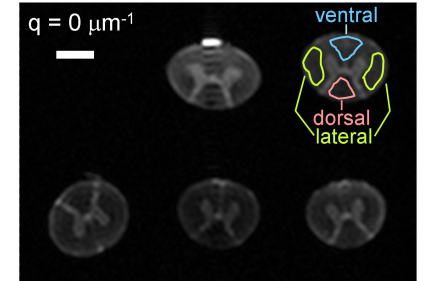


Fig. 1. Sample image at $q = 0\text{ }\mu\text{m}^{-1}$. White bar = 5 mm. ROI locations are shown for the dorsal, ventral and lateral WM columns. The bright spot above the center top SC is residual surface PBS.

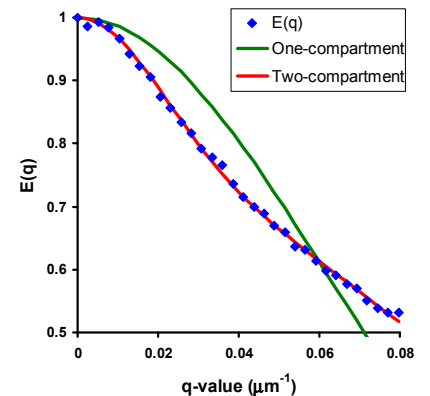


Fig. 2. Sample $E(q)$ for a lateral WM column ROI (blue diamonds) with one-compartment (green line) and two-compartment (red line) fits.

within 1-2 μm , which is the expected range of axon diameters in mammals⁸. Z_E is lower than that expected for free water (~20 μm for $\Delta = 98.7\text{ms}$). The ADC calculated from Z_E ($\sim 0.25 \times 10^{-3}\text{ mm}^2/\text{s}$) agrees with literature values for fixed spinal cord WM tissue⁹, which provides further evidence that ADCs measured at low b-values ($< 2500\text{ s/mm}^2$) primarily reflect diffusion in ECS¹⁰. The average f_I was ~0.7, which falls within the expected ICF for WM¹¹. An ANOVA analysis indicated no significant differences in f_I among the WM columns as previously seen in mouse SP⁵. Paired t-tests indicated that the dorsal column Z_E and Z_I are significantly smaller than those of the ventral WM column, which matches previous observations of smaller MAD and increased axon density in the dorsal compared with ventral columns^{4,8,9}.

Conclusion

This work demonstrates the feasibility of implementing low q-value diffusion MRI on a 1.5T scanner. The results show that despite violating SGPA, this method has the potential to accurately assess regional axonal architecture with metrics such as MAD and ICF.

References: 1. Beaulieu, C, *NMR Biomed*, **15**:435 (2002). 2. Callaghan, PT, *Principles of NMR Microscopy*, Oxford University Press (1991). 3. Cohen, Y, et al., *NMR Biomed*, **15**:516 (2002). 4. Ong, HH, *Neuroimage*, **40**:1619 (2008). 5. Ong, HH, *Neuroimage*, **51**:1360 (2010). 6. Price W, *Concepts Magn Res*, **10**:299 (1997). 7. Kimmich R, *NMR: Tomography, Diffusometry, Relaxometry*, Springer-Verlag (1997). 8. Williams, PL, et al., *Gray's anatomy*, Churchill Livingstone (1995). 9. Schwartz, ED, et al., *AJNR*, **26**:390 (2005). 10. Schwartz, E.D., et al., *Neuroreport*, **16**:73 (2005). 11. Sykova, E, et al., *Physiol Rev*, **88**:1277 (2008). **Acknowledgements:** NIH R21 EB003951

Direct magnetic resonance detection of myelin and prospects for quantitative imaging of myelin density

Michael J. Wilhelm^{a,1}, Henry H. Ong^a, Suzanne L. Wehrli^b, Cheng Li^a, Ping-Huei Tsai^{a,2}, David B. Hackney^c, and Felix W. Wehrli^{a,3}

^aLaboratory for Structural NMR Imaging, Department of Radiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104; ^bNMR Core Facility, Joseph Stokes Jr. Research Institute, Children's Hospital of Philadelphia, Philadelphia, PA 19104; and ^cDepartment of Radiology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115

Edited by Alan P. Koretsky, National Institutes of Health, Bethesda, MD, and accepted by the Editorial Board April 27, 2012 (received for review September 13, 2011)

Magnetic resonance imaging has previously demonstrated its potential for indirectly mapping myelin density, either by relaxometric detection of myelin water or magnetization transfer. Here, we investigated whether myelin can be detected and possibly quantified directly. We identified the spectrum of myelin in the spinal cord *in situ* as well as in myelin lipids extracted via a sucrose gradient method, and investigated its spectral properties. High-resolution solution NMR spectroscopy showed the extract composition to be in agreement with myelin's known chemical make-up. The 400-MHz ¹H spectrum of the myelin extract, at 20 °C (room temperature) and 37 °C, consists of a narrow water resonance superimposed on a broad envelope shifted ~3.5 ppm upfield, suggestive of long-chain methylene protons. Superimposed on this signal are narrow components resulting from functional groups matching the chemical shifts of the constituents making up myelin lipids. The spectrum could be modeled as a sum of super-Lorentzians with a T₂* distribution covering a wide range of values (0.008–26 ms). Overall, there was a high degree of similarity between the spectral properties of extracted myelin lipids and those found in neural tissue. The normalized difference spectrum had the hallmarks of membrane proteins, not present in the myelin extract. Using 3D radially ramp-sampled proton MRI, with a combination of adiabatic inversion and echo subtraction, the feasibility of direct myelin imaging *in situ* is demonstrated. Last, the integrated signal from myelin suspensions is shown, both spectroscopically and by imaging, to scale with concentration, suggesting the potential for quantitative determination of myelin density.

myelin *in situ* | myelin NMR spectrum | super-Lorentzian fitting | ultrashort echo time

Myelin is a critical feature of nervous system white matter (WM) and accounts for 14% of the wet mass of WM (1). It is a lipid–protein bilayer that extends from the outer membrane of glial cells (i.e., oligodendrocytes in the CNS) and discretely winds around individual axonal fibers, leading to an increase in conduction velocity (1). By speeding conduction and reducing axonal energy requirements, myelin makes large and complex organisms possible. Myelin also contributes to the mechanical and functional structure of the axon. In addition, some oligodendrocytic cells and precursors can support action potentials themselves (2). Deficiencies of myelin lay at the core of numerous neurodegenerative disorders, such as multiple sclerosis and schizophrenia (1). These deficiencies may result from developmental or acquired abnormalities in oligodendrocyte function, which also leads to axonal degeneration. Assessment of myelin may reveal CNS abnormalities far beyond those associated with classic demyelinating diseases. MRI of myelin has the potential to characterize not only loss of this important component of the CNS but also to reveal axonal and supporting glial integrity and function.

A diverse assortment of experimental techniques has been applied toward the goal of observing and quantifying myelin. The common methods rely on optical microscopy of histologically

stained tissue samples (3). X-ray diffraction (4) and nonlinear optical techniques (5, 6) also provide insight into myelin ultrastructure. Unfortunately, all these techniques are destructive and thus applicable only to animal studies.

More recently, myelin-specific chemical contrast markers that selectively bind to myelin have emerged. Such agents are currently under development for both MRI (7) and positron-emission tomography (8). Although these techniques are potentially promising, concerns over toxicity may pose significant hurdles to their clinical implementation.

So far, MRI has had the greatest impact toward nondestructive myelin assessment in both laboratory animals and humans. Further, MRI has the added benefit that signal contrast originates from endogenous protons and hence is not reliant upon injectable chemical probes nor limited by contrast-related temporal delays.

To date, two indirect MR techniques applicable to studies *in vivo* have demonstrated histologically correlated sensitivity to myelin: magnetization transfer (MT) and T₂ relaxometry. In MT, cross-relaxation between myelin protons and tissue water is exploited (9). The signal attenuation resulting from off-resonance saturation (MT ratio) has been found to scale with myelin concentration (10). T₂ relaxometry yields T₂ spectra, typically by inversion of the Carr–Purcell echo decay using an inverse Laplace transformation (11). Spectral components with T₂ values ranging from 10 to 50 ms have been assigned to motionally restricted myelin water (12, 13) and have demonstrated strong correlation with myelin-specific histologic staining (12, 14).

Although MT and T₂ relaxometry have shown promise, they both rely on indirect detection of myelin through the interaction of water with myelin. This complex interaction is affected by nonmyelin loss-related changes, which can lead to ambiguities in data interpretation. For example, MT is sensitive not only to myelin content but also to axon density (15). Therefore, even though both techniques may distinguish normal from abnormal WM, they rely on the invariance of the myelin–water interaction.

Direct detection of myelin with MR would remove some complications in the analysis from the intermediate effects of the

Author contributions: M.J.W., H.H.O., and F.W.W. designed research; M.J.W., H.H.O., and S.L.W. performed research; M.J.W., H.H.O., S.L.W., C.L., P.-H.T., and F.W.W. analyzed data; and M.J.W., H.H.O., C.L., D.B.H., and F.W.W. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. A.P.K. is a guest editor invited by the Editorial Board.

Freely available online through the PNAS open access option.

¹Present address: Department of Chemistry, Temple University, Philadelphia, PA 19122.

²Present address: Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei 10617, Taiwan, Republic of China.

³To whom correspondence should be addressed. E-mail: wehrli@mail.med.upenn.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1115107109/-DCSupplemental.

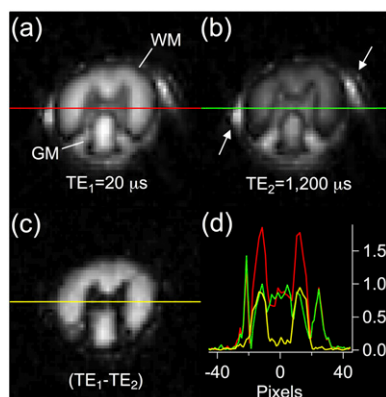


Fig. 2. The 3D de-IR-UTE images from rat thoracic SC averaged over five central slices. Magnitude images obtained for (A) TE = 20 μ s, (B) TE = 1,200 μ s, and (C) complex difference (A – B) (maximum-intensity range decreased by a factor of two to highlight myelin signal). (D) Intensity profiles across the three images (delineated as red, green, and yellow lines in A, B, and C, respectively) to show relative WM, GM, and background intensity. The most intense signal, present in the short- and long-echo profiles, originates from residual surface water. WM and GM are indicated in A, and arrows highlight residual surface water in B. The dark boundary observed at the GM/WM and WM/surface water interfaces in both echo images stems from partial voluming of adjacent regions with different T₁ resulting in destructive interference.

highlight the short T_2^* signal, which predominantly results from myelin protons.

MR Signal Dependence on Myelin Concentration. To separate the myelin and water peaks in the ^1H spectra of the myelin- D_2O suspensions, the spectra were modeled as a weighted sum of four super-Lorentzians (SL) for myelin resonances representing protons from general alkyl chain methylenes (noncholesterol, mostly from fatty acid residues), cholesterol alkyl chain methylenes, terminal methyls, and choline, and a Lorentzian for the HDO peak (details in *Materials and Methods*). Fig. 3*A* shows the results from fitting of the proton NMR spectrum of purified myelin suspended in D_2O . The fitting results were virtually identical for all myelin concentrations. Even though the signal envelope is very broad, relatively narrow resonances are also observed, likely due to proton pairs aligned with an average orientation at the magic angle relative to the static field (24).

Relative signal fractions, accounting for losses during excitation and acquisition, along with associated T_2^* distributions of the four SL components, were combined into a myelin T_2^* histogram (Fig. 3B). At 20 °C, 26.4% of the total signal has an effective lifetime of <25 μ s, 51.8% of <100 μ s, and 91.6% of <1,000 μ s. At 37 °C these values are 16.9%, 44.8%, and 86.3%, respectively.

Fig. 4A shows a series of fitted myelin signals as a function of decreasing myelin concentration. The NMR signal areas for the total and separate spectral components (i.e., HDO and myelin) are plotted in Fig. 4B, indicating linear scaling with myelin concentration ($R^2 = 0.99$). We attribute the positive correlation of the water peak area with myelin concentration as resulting from labile protons from myelin constituents exchanging with D_2O to form HDO. The calculated average percentage of labile protons, for each of the 10 myelin lipid components, is listed in Table 1. The average signal contribution from the 0.1% impurity of the D_2O solvent, calculated as the y-intercept from the line of best fit (Fig. 4B), was subtracted from all of the HDO points, yielding an estimate of the labile myelin proton signal contribution. The predicted range of signal contributions from labile protons ($5.05\% \pm 0.79\%$) agreed well with the experimental HDO peak areas ($5.13\% \pm 2.00\%$). Given the excess D_2O used

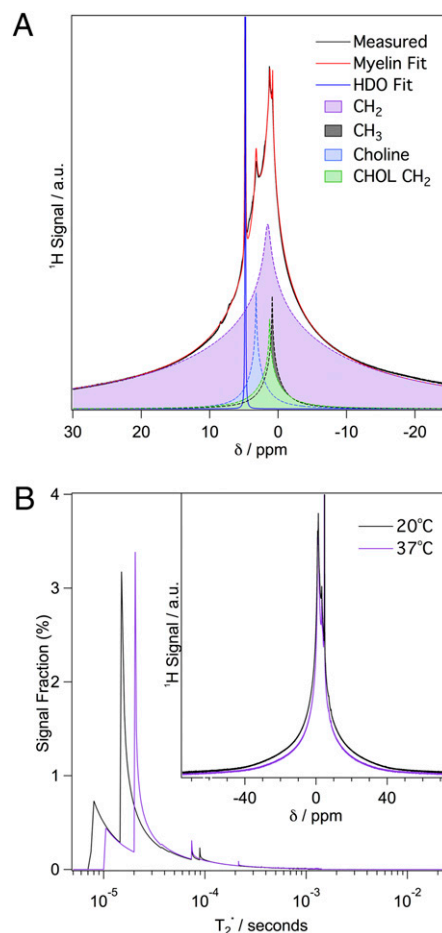
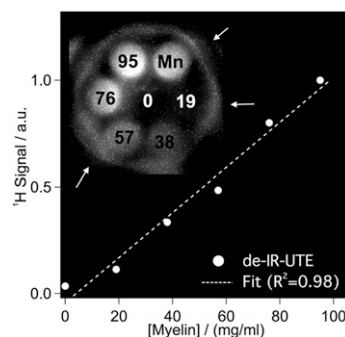


Fig. 3. ^1H NMR spectra and analysis of purified bovine myelin extract suspended in D_2O . (A) NMR spectrum (black) and SL fitting showing the resulting myelin (red) and HDO (blue) fits, as well as the four individual SL components of myelin (shaded). The four fitted SL components consisted of (i) SL containing 74.3% of the intensity, centered at 1.5 ppm, corresponding to the general alkyl chain methylene protons (CH_2), (ii) SL containing 12.4% of the intensity, centered at 0.9 ppm, corresponding to the terminal methyl protons (CH_3), (iii) SL containing 11.1% of the intensity, centered at 3.2 ppm, corresponding to the choline methyl protons (Choline), and (iv) SL containing 2.1% of the intensity, centered at 1.3 ppm, corresponding to the cholesterol alkyl chain methylene protons (CHOL CH_2). (B) T_2^* histogram of myelin components at 20 and 37 °C derived from the SL fitting. There are small T_2^* components that extend up to 26 ms, but these cannot be observed at the displayed scale. *Inset:* Myelin extract spectra collected at the two temperatures.

in the suspensions, it is reasonable to assume that all labile myelin protons had exchanged with deuterium.

Fig. 5 *Inset* shows the complex difference 2D projection de-IR-UTE image for a series of myelin suspensions of increasing concentration. Region of interest (ROI) average intensities from each of the myelin samples in the image are plotted in Fig. 5 and, analogous to the spectral data, are linearly correlated with myelin concentration ($R^2 = 0.98$).

On the basis of the relaxation characteristics of myelin (notably the lifetime of the various T_2^* components), Bloch equation simulations (details in *SI Materials and Methods*) accounting for losses sustained during the rf pulse sampling suggest that, under the spectral recording conditions, 81.7% of the total spectral signal is recovered, where the shorter T_2^* components account for the majority of the signal lost. The depletion of the imaging signal is more severe because it entails coherence losses during both



In contrast to the SL fitting performed in this work, Horch et al. performed multiexponential fitting of the time-domain signal decay (18). Although the SL lineshape theoretically

The purity of our myelin extract was validated by 9.4 T high-resolution proton-decoupled ^{31}P (Fig. S2A) and ^{13}C (Fig. S2B) NMR, showing good agreement with previously determined molar ratios (25) (Table 1). The slight variations are reasonable given the differences in the anatomical origin of the sample (brain vs. SC) (26), inherent variability of the measurement (27), and the developmentally immature nature of the tissue examined (26). Our ^{13}C spectra also agreed well with Husted et al.'s reported proton-decoupled magic-angle spinning (MAS) ^{13}C NMR spectra of

cannot be described with a basis set of exponential functions, the authors suggested that the resulting errors would be small for the case of myelin. Horch et al. analyzed free-induction decay signals of myelinated mammalian and amphibian nerves, as well as synthetic myelin at 4.7 T, yielding histograms of relaxation times. The authors detected significant components with T_2^* values of 20 and 70 μ s in frog sciatic nerve, which they conjectured to arise from protein and methylene protons of myelin, respectively. In contrast, because our myelin extract was free of protein, the present data alternatively suggest the short T_2^* components (<25 μ s) to arise from myelin lipids. This is not to imply that membrane proteins cannot contribute a short T_2^* component, as suggested by the broad resonance in the difference spectrum (Fig. S4) and in other reports (24, 33).

In bovine myelin suspended in D_2O , we found the integrated spectral areas to scale linearly with myelin concentration (Fig. 4), as did ROI intensities of the 2D projection de-IR-UTE images (Fig. 5), thus suggesting that quantitative myelin imaging may be feasible. Direct 3D de-IR-UTE imaging of a rat SC in situ at 400 MHz highlights the potential of such an approach, as demonstrated with images showing signal from the WM regions only. Absolute quantification would require a reference sample, ideally with relaxation and density properties matching myelin. The reference sample should also be chemically stable.

Previously, Waldman et al. (23) obtained images of the human brain using a slice-selective UTE along with soft-tissue suppression, essentially showing intense signal from WM regions of the brain, which they attributed to short T_2^* components. Under these conditions [i.e., selective half-sinc pulses of 400- to 600- μ s duration (34)], all but the longest T_2^* components of the myelin protons would elude detection.

Our results indicate approximately 20% of the protons in myelin lipids to have an effective T_2^* less than 25 μ s. Even under the more favorable conditions of our imaging experiments, the majority of these short T_2^* components remains undetectable. The very short lifetime of the signal has potentially adverse effects on the point-spread function (PSF) manifesting as blurring. A simple estimation based on the FWHM of the spectra (Fig. 3) predicts an intrinsic resolution (defined as the minimum achievable pixel size) on the order of 100 μ m, which is approximately one pixel with our current imaging parameters (more details in *SI Materials and Methods*). It should be noted that although the blurring from the shortest T_2^* component would be greater, its impact on the PSF is negligible because this signal fraction remains virtually undetected.

A limitation that needs to be noted for this method is that it detects myelin solely on the basis of its T_2^* properties. Thus, errors in long T_2^* suppression may lead to signal misclassified as short T_2^* and hence falsely identified as myelin. Such errors could be accounted for and perhaps mitigated by tailoring a reference sample so as to contain water of comparable concentration and relaxation times to those of neural tissue. Last, there are other possible nonmyelin short T_2^* sources that could contribute to the UTE image intensity, including glial cell membranes, calcifications, tissue scars, vasculature, and hemorrhage (23), that would be indistinguishable from myelin.

Another potential problem could arise from saturation of the myelin signal via cross-relaxation (35). Even though adiabatic inversion of tissue water has minimal direct effect on the myelin lipid proton signal, transfer of magnetization from the water to the myelin proton pool could occur via chemical exchange or dipolar coupling. This mechanism would result in a reduction in the detected myelin signal, an effect that requires further scrutiny.

The potential for translation of the method to the clinic will be challenging. Nevertheless, it is encouraging to note that with dedicated hardware rf pulses of 20 μ s or less have already been shown to be feasible on clinical equipment at 3 T field strength, as in recent work by Wu et al., who imaged the collagen matrix

of cortical bone (36). Further, at 37 °C the measured T_2^* values increase by ca. 30% (Fig. 3B), raising the minimum T_2^* value from 8 to 10.5 μ s. At body temperature the scan parameters used in our current experiments at 9.4 T (except for a 20- μ s hard pulse of 7.6° flip angle to match the peak B_1 amplitudes of clinical head coils) predict 4.9% of the total myelin proton signal [i.e., 0.7% of the total proton signal given that myelin accounts for 14% of WM (1)] to be recoverable on a 3 T clinical MRI system (for calculation details see *SI Materials and Methods*).

Given that tissue proton concentration is ~100 M, the concentration of detectable myelin protons is approximately 700 mM. In comparison with proton MR spectroscopic imaging (MRSI) of brain metabolites, where the metabolite concentrations are on the order of 10 mM, detectable myelin proton concentration, and hence intrinsic SNR, should be a factor of approximately 70 greater than that of typical metabolites. However, this gain in SNR compared with MRSI is mitigated by the reduced sampling time imposed by the much shorter T_2^* of the protons in myelin compared with those in brain metabolites. We estimate reduced overall sampling time to result in a loss on the order of a factor of 10. Given a reported resolution for MRSI of 5–10 mm (37), we project the resolution achievable with our method to be roughly on the order of 2.5–5 mm with T_2^* -induced PSF blurring not exceeding 0.6 mm (*SI Results*).

Conclusions

We have characterized the spectral properties of the myelin proton signal in situ, as well as in reconstituted suspensions of myelin lipid extract. Our results show that the short T_2^* component of WM originates primarily from myelin lipid protons and further that direct imaging of these protons is possible even though the shortest components are not detectable. Last, our analysis suggests that translation from the laboratory to clinical MRI will be challenging.

Materials and Methods

All MR spectroscopy and imaging experiments were performed on a 9.4 T vertical-bore spectrometer/microimaging system (Bruker DMX 400) with Micro2.5 gradients (100 G/cm maximum strength) and BAFFA40 amplifiers.

Neural Tissue Preparation. SC samples were harvested from healthy adult Sprague-Dawley rats (Charles River Laboratories) and bovine spinal columns (Bierig Brothers Veal and Lamb Products). The rats were killed by carbon dioxide asphyxiation in accordance with an Institutional Animal Care and Use Committee-approved protocol. After killing, rat spinal columns were removed, and the SC was dissected out.

NMR Spectroscopy. High-resolution proton-decoupled ^{13}C NMR [Sweep width (SW) = 24 kHz, number of scans (NS) = 36,768, number of real and imaginary data points (TD) = 65,536, repetition time (TR) = 1.36 s, α = 30°] and proton-decoupled 31P (SW = 3.23 kHz, NS = 8,536, TD = 8,192, TR = 1.27 s, α = 30°) spectra were collected for samples of purified bovine myelin extract, dissolved in a (5:4:2) ternary mixture of deuterated chloroform (99.8 atom % D; Acros Organics), methanol (99.8 atom % D; Acros Organics), and 0.2 M EDTA/water (99.9 atom % D; Sigma-Aldrich).

All 1H NMR spectra were collected with the following parameters: SW = 100 kHz, NS = 256, TD = 262,144, TR = 3.6 s, α = 90°, pulse duration = 9.6 μ s. Freshly excised SC sections (<2-h postmortem interval) were immersed in a perfluorinated oil (Fomblin-Y; Sigma-Aldrich) before experiments.

SL Fitting of Proton Spectrum. As described by Wennerström (30), only partial averaging of dipolar coupling via translational and rotational diffusion occurs, resulting in a dipolar-broadened liquid-crystalline lipid system with an SL lineshape that can be written as:

$$L(\omega) = \int_0^{\pi/2} \frac{\sin(\theta)}{[3\cos^2(\theta) - 1]} f\left[\frac{\omega - \omega_0}{[3\cos^2(\theta) - 1]}\right] d\theta \quad [1]$$

where ω_0 is the chemical shift, θ is the angle of the lipid bilayer surface normal with respect to B_0 , and $f(\omega - \omega_0)$ is any highly peaked lineshape such as

a Gaussian or Lorentzian. Assuming θ is uniformly sampled and setting $f(\omega - \omega_0)$ to be a Lorentzian, it can be seen from Eq. 1 that an SL is composed of a series of scaled Lorentzians. From the widths and intensities of these Lorentzians, the T_2^* distribution of a single SL can be calculated. Protons at different chemical shifts (e.g., alkyl chain methylenes, terminal methyls, and choline) are each expected to give rise to SL lineshapes (32).

Spectral fitting was performed in Matlab (Mathworks). Four SLs were used to represent general alkyl chain methylenes (noncholesterol, mostly from fatty acid residues), cholesterol alkyl chain methylenes, terminal methyls, and choline, whereas a single Lorentzian was used to model residual HDO. Because cholesterol alkyl chain methylenes sit deep within the lipid bilayer, it is reasonable to expect them to be more mobile than the general alkyl chain methylenes, therefore resulting in a narrower SL. The chemical shifts of each SL were set to the known isotropic shift of the various moieties, and the width and relative intensities were free parameters. The R^2 of the fit was greater than 0.99.

UTE MR Imaging. The 3D de-IR-UTE imaging (Fig. S4): SW = 200 kHz, TE = 20/1,200 μ s, TI = 500 ms, TR = 1 s, field of view = 15 mm, matrix size = 128 $\times$ 128 $\times$ 128, number of views = 5,342, pulse duration = 20 μ s. The sequence was based on that used by Anumula et al. (38). TI was determined empirically as the duration yielding optimal GM suppression (because GM is expected to have negligible myelin concentration) in a complex difference

image. A refocusing gradient was applied immediately after the first readout gradient, after which a second gradient echo was collected at TE = 1,200 μ s. A 3D image of the short T_2^* components was obtained as the complex difference of the two echo images (i.e., TE₁ – TE₂). A complex difference is necessary to distinguish the possible presence of both inverted and non-inverted voxel signals.

A 2D projection de-IR-UTE sequence was used to image the series of myelin/D₂O suspensions to avoid signal losses resulting from settling of myelin during scanning. The Mn doped water phantom served to identify the locations of the samples in the image. All experimental parameters were identical to those used in the 3D de-IR-UTE experiments.

All image reconstruction was done in Matlab (Mathworks) using a fast gridding algorithm (39) and incorporating k-space trajectory correction (40). All images were smoothed via bilinear interpolation with Image J (National Institutes of Health).

ACKNOWLEDGMENTS. We thank Joseph J. Sarver and Louis J. Soslowsky for providing a source of fresh rat CNS tissue and Jim Delikatny and Jeremy Magland for their help with the super-Lorentzian analysis. M.J.W. thanks Mary A. Selak for patient guidance and assistance perfecting the sucrose gradient-based isolation of myelin. This work was supported by National Institutes of Health Grant T32 EB00814 and US Department of Defense Award W81XWH-10-1-0714.

- van der Knaap MS, Valk J (2005) *Magnetic Resonance of Myelination and Myelin Disorders*, eds Heilmann U, Mennecke-Buhler D (Springer, Berlin), pp 1–19.
- Kárádóttir R, Hamilton NB, Bakiri Y, Attwell D (2008) Spiking and nonspiking classes of oligodendrocyte precursor glia in CNS white matter. *Nat Neurosci* 11:450–456.
- Laule C, et al. (2006) Myelin water imaging in multiple sclerosis: Quantitative correlations with histopathology. *Mult Scler* 12:747–753.
- Avila RL, et al. (2005) Structure and stability of internodal myelin in mouse models of hereditary neuropathy. *J Neuropathol Exp Neurol* 64:976–990.
- Wang H, Fu Y, Zickmund P, Shi R, Cheng JX (2005) Coherent anti-Stokes Raman scattering imaging of axonal myelin in live spinal tissues. *Biophys J* 89:581–591.
- Fu Y, Huff TB, Wang HW, Wang H, Cheng JX (2008) Ex vivo and in vivo imaging of myelin fibers in mouse brain by coherent anti-Stokes Raman scattering microscopy. *Opt Express* 16:19396–19409.
- Frullano L, Wang C, Miller RH, Wang Y (2011) A myelin-specific contrast agent for magnetic resonance imaging of myelination. *J Am Chem Soc* 133:1611–1613.
- Stankoff B, et al. (2006) Imaging of CNS myelin by positron-emission tomography. *Proc Natl Acad Sci USA* 103:9304–9309.
- Doussset V, et al. (1992) Experimental allergic encephalomyelitis and multiple sclerosis: Lesion characterization with magnetization transfer imaging. *Radiology* 182:483–491.
- Mottershead JP, et al. (2003) High field MRI correlates of myelin content and axonal density in multiple sclerosis—a post-mortem study of the spinal cord. *J Neurol* 250:1293–1301.
- Whittall K, Mackay A (1989) Quantitative interpretation of NMR relaxation data. *Magn Reson Med* 84:134–152.
- MacKay A, et al. (1994) In vivo visualization of myelin water in brain by magnetic resonance. *Magn Reson Med* 31:673–677.
- Gulani V, Webb AG, Duncan ID, Lauterbur PC (2001) Apparent diffusion tensor measurements in myelin-deficient rat spinal cords. *Magn Reson Med* 45:191–195.
- Laule C, et al. (2008) Myelin water imaging of multiple sclerosis at 7 T: Correlations with histopathology. *Neuroimage* 40:1575–1580.
- Schmierer K, Scaravilli F, Altmann DR, Barker GJ, Miller DH (2004) Magnetization transfer ratio and myelin in postmortem multiple sclerosis brain. *Ann Neurol* 56:407–415.
- Lecar H, Ehrenstein G, Stillman I (1971) Detection of molecular motion in lyophilized myelin by nuclear magnetic resonance. *Biophys J* 11:140–145.
- Ramani A, Aliev AE, Barker GJ, Tofts PS (2003) Another approach to protons with constricted mobility in white matter: Pilot studies using wide-line and high-resolution NMR spectroscopy. *Magn Reson Imaging* 21:1039–1043.
- Horch RA, Gore JC, Does MD (2011) Origins of the ultrashort-T2 1H NMR signals in myelinated nerve: A direct measure of myelin content? *Magn Reson Med* 66:24–31.
- Wu Y, et al. (1998) Evaluation of bone mineral density using three-dimensional solid state phosphorus-31 NMR projection imaging. *Calcif Tissue Int* 62:512–518.
- Robson MD, Gatehouse PD, Bydder M, Bydder GM (2003) Magnetic resonance: An introduction to ultrashort TE (UTE) imaging. *J Comput Assist Tomogr* 27:825–846.
- Techawiboonwong A, Song HK, Wehrli FW (2008) In vivo MRI of submillisecond T(2) species with two-dimensional and three-dimensional radial sequences and applications to the measurement of cortical bone water. *NMR Biomed* 21:59–70.
- Wu Y, et al. (1999) Multinuclear solid-state three-dimensional MRI of bone and synthetic calcium phosphates. *Proc Natl Acad Sci USA* 96:1574–1578.
- Waldman A, et al. (2003) MRI of the brain with ultra-short echo-time pulse sequences. *Neuroradiology* 45:887–892.
- Bloom M, Holmes K, Mountford C, Williams P (1986) Complete proton magnetic resonance in whole cells. *J Magn Reson* 69:73–91.
- Norton WT, Autilio LA (1966) The lipid composition of purified bovine brain myelin. *J Neurochem* 13:213–222.
- Norton WT (1974) Isolation of myelin from nerve tissue. *Methods Enzymol* 31(Pt A):435–444.
- Norton WT, Autilio LA (1965) The chemical composition of bovine CNS myelin. *Ann N Y Acad Sci* 122:77–85.
- Husted C, Montez B, Le C, Moscarello MA, Oldfield E (1993) Carbon-13 “magic-angle” sample-spinning nuclear magnetic resonance studies of human myelin, and model membrane systems. *Magn Reson Med* 29:168–178.
- Wimley WC, White SH (1993) Membrane partitioning: Distinguishing bilayer effects from the hydrophobic effect. *Biochemistry* 32:6307–6312.
- Wennerström H (1973) Proton nuclear magnetic resonance lineshapes in lamellar liquid crystals. *Chem Phys Lett* 18:41–44.
- MacKay AL (1981) A proton NMR moment study of the gel and liquid-crystalline phases of dipalmitoyl phosphatidylcholine. *Biophys J* 35:301–313.
- Ulmus J, Wennerström H, Lindblom G, Arvidson G (1975) Proton NMR bandshape studies of lamellar liquid crystals and gel phases containing lecithins and cholesterol. *Biochim Biophys Acta* 389:197–202.
- MacKay AL, Burnell EE, Bienvenue A, Devaux PF, Bloom M (1983) Flexibility of membrane proteins by broad-line proton magnetic resonance. *Biochim Biophys Acta* 728:460–462.
- Sussman MS, Pauly JM, Wright GA (1998) Design of practical T2-selective RF excitation (TELEX) pulses. *Magn Reson Med* 40:890–899.
- Edzes HT, Samulski ET (1977) Cross relaxation and spin diffusion in the proton NMR or hydrated collagen. *Nature* 265:521–523.
- Wu Y, et al. (2010) Bone matrix imaged in vivo by water- and fat-suppressed proton projection MRI (WASPI) of animal and human subjects. *J Magn Reson Imaging* 31:954–963.
- Gruber S, Mlynárik V, Moser E (2003) High-resolution 3D proton spectroscopic imaging of the human brain at 3 T: SNR issues and application for anatomy-matched voxel sizes. *Magn Reson Med* 49:299–306.
- Anumula S, et al. (2006) Measurement of phosphorus content in normal and osteomalacic rabbit bone by solid-state 3D radial imaging. *Magn Reson Med* 56:946–952.
- Greengard L, Lee JY (2004) Accelerating the nonuniform fast fourier transform. *SIAM Rev* 46:443–454.
- Rad HS, et al. (2011) Quantifying cortical bone water in vivo by three-dimensional ultra-short echo-time MRI. *NMR Biomed* 24:855–864.

Super-Lorentzian framework for investigation of T_2^* distribution in myelin

Michael J. Wilhelm¹, Henry H. Ong², and Felix W. Wehrli²

¹Department of Chemistry, Temple University, Philadelphia, PA, United States, ²Laboratory for Structural NMR Imaging, Department of Radiology, University of Pennsylvania School of Medicine, Philadelphia, PA, United States

Introduction

Deficiencies of myelin, a lipid bilayer sheath critical for normal function of white matter (WM), lay at the core of numerous neurodegenerative disorders such as multiple sclerosis and schizophrenia (1). At present, there are few alternatives to destructive histologic methods to directly assess myelin. The short T_2^* of myelin protons make ultra-short echo time (UTE) MRI a potential imaging modality to directly detect myelin (2). In contrast, indirect MRI methods such as magnetization transfer and T_2 relaxometry are based on complex interactions between water and myelin, which can lead to ambiguities in data analysis. Characterizing the T_2^* distribution of myelin is key to developing optimal UTE methods for myelin imaging. Previous attempts have used multi-exponential fitting of the FID (3), which is not only an ill-posed problem (4), but also theoretically incorrect. Myelin is a liquid crystalline lipid system that is described by a sum of super-Lorentzians (SL) rather than a multi-Lorentzian lineshape (5, 6). Here, we use this SL framework to calculate T_2^* distributions by fitting ^1H NMR spectra of myelin lipid extract and intact rat spinal cord (SC).

Theory and Methods

According to Wennerström (6), due to averaging effects from translational and rotational diffusion, for a given orientation of a lipid bilayer, the lineshape can be expressed as: $L(\delta - \delta_0, \theta) = [3\cos^2\theta - 1]^{-1} f[(\delta - \delta_0)/|3\cos^2\theta - 1|]$ (Eq.1), where δ is the chemical shift centered at δ_0 , θ is the angle of the lipid bilayer surface normal with B_0 , and $f(x)$ is any highly peaked lineshape such as a Gaussian or Lorentzian. The SL lineshape, $L_{SL}(\delta - \delta_0)$, results from a uniform sampling of θ from 0 to $\pi/2$: $L_{SL}(\delta - \delta_0) = \int L(\delta - \delta_0, \theta) \sin\theta d\theta$ (Eq.2). By assuming $f(x)$ to be a Lorentzian, it can be seen from Eqs. 1 and 2 that a SL is composed of a series of scaled Lorentzians. From the widths and intensities of these Lorentzians, the T_2^* distribution of a single SL can be calculated. Multiple SLs have been used to fit NMR spectra of model membrane systems in which the SLs arise from protons at different chemical shifts, e.g. alkyl methylenes, terminal methyls, and choline (7). Therefore, it is possible in theory to perform a multi-SL fit of a ^1H NMR spectrum of myelin and calculate a T_2^* distribution.

Rat and bovine SC samples were harvested from Sprague-Dawley rats (Charles River Labs) and a local butcher. Myelin lipids were extracted from bovine SC tissue with a sucrose gradient method (8), dissolution in a ternary mixture (chloroform/methanol/water), and lyophilization. Previous work has shown that this protocol extracts myelin lipids with little to no protein (2). Dehydrated myelin lipid extract was then re-suspended in 99.9% D_2O (Sigma-Aldrich) to regenerate a bilayer structure. ^1H NMR spectra at 9.4T (DMX-400, Bruker Instruments) were obtained for a freshly excised rat thoracic SC immersed in Fomblin (Sigma-Aldrich), as well as the myelin lipid extract. Rat SC was immersed in D_2O for 24 hrs prior to experiments to reduce the dominant tissue water peak.

Spectral fitting was performed in Matlab (Mathworks). Four SLs were used to represent general alkyl chain methylenes, cholesterol alkyl chain methylenes (as they have shorter chain lengths), terminal methyls, and choline, while a single Lorentzian was used to model residual HDO. The chemical shifts of each SL were set to the known isotropic shift of the various moieties, and the width and relative intensities were free parameters.

Results and Discussion

Fig. 1 shows myelin lipid extract and rat SC ^1H NMR spectra with the results of the four-component SL fits. Both spectra are comprised of a narrow peak from residual HDO and a broad resonance (linewidth ca. 1700Hz) from myelin. A large 4^{th} to 2^{nd} moment ratio (ca. 6.6), suggests that this broad resonance has a SL lineshape. The SC ^1H NMR spectrum had additional minor peaks from intracellular proteins and other non-myelin protons. Despite the additional complexity of the SC spectrum, both SL fits agree well with the ^1H NMR spectra ($R^2 > 0.99$). Fig. 2 shows the relative fractions (theoretical and fitted) of the four SL myelin components in myelin extract and intact rat SC. As expected, the signal is dominated (>70%) by alkyl methylene protons. Deviations from theoretical values may result from inaccuracies of the SL framework to describe non-chain alkyl protons, e.g. choline and terminal methyls.

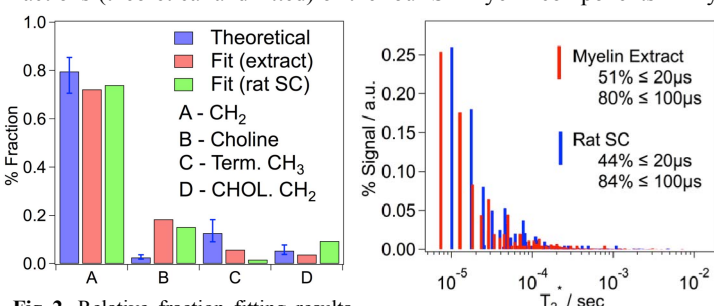


Fig 2. Relative fraction fitting results of the four SL components with expected theoretical fractions. Error bars account for variation in alkyl chain length.

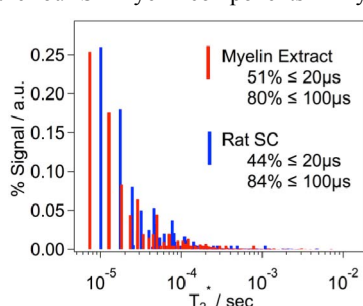


Fig 3. Calculated T_2^* distributions for myelin lipid extract (red) and rat SC (blue). Signal fractions with $T_2^* < 20$ and $< 100 \mu\text{s}$ are reported.

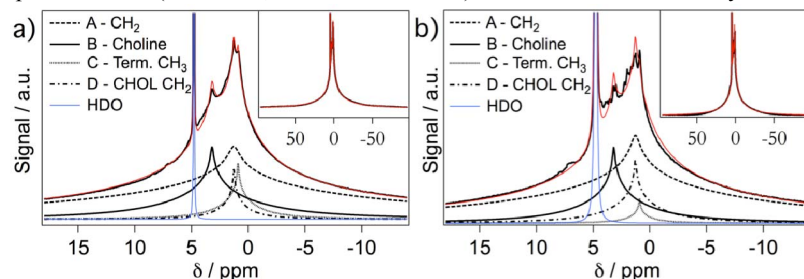


Fig 1. ^1H NMR spectra at $\sim 20^\circ\text{C}$ (black) and SL based fit (red) for a) myelin extract ($R^2=0.999$) and b) rat SC ($R^2=0.997$). The four SL and Lorentzian peaks of the fit are shown. Full spectral width is shown in inset with HDO peak truncated.

by alkyl methylene protons. Deviations from theoretical values may result from inaccuracies of the SL framework to describe non-chain alkyl protons, e.g. choline and terminal methyls.

Fig. 3 shows the T_2^* distributions derived from the SL fits for the myelin lipid extract and SC. The T_2^* distributions are highly skewed with a wide range (10 μs to 10ms). Despite this range, roughly 50% (80%) of the signal has a T_2^* less than 20 μs (100 μs). This result highlights the difficulty of direct myelin imaging even with UTE MRI. Further investigation is needed to study the system at body temperature as increased molecular motion is likely to result in longer effective T_2^* s.

Conclusion

This work uses a SL framework to characterize the T_2^* distribution of myelin, which would provide guidance toward developing UTE methods for myelin imaging. The results indicate that at ambient temperature $\sim 50\%$ of the myelin lipid proton signal has $T_2^* < 20 \mu\text{s}$.

References: 1. van der Knaap, MS et al, *Magnetic Resonance of Myelin, Myelination, and Myelin Disorders*, Springer-Verlag (1995). 2. Wilhelm, MJ et al, *Proc. of the ISMRM* (2011). 3. Horch et al, *Mag. Res. Med.*, **66**:24 (2011). 4. Epstein, CL et al, *SIAM Rev.*, **50**:504 (2008). 5. Bloom, M et al, *Chem. Phys. Lipids*, **4**:107 (1975). 6. Wennerström, H et al, *Chem. Phys. Lett.*, **18**:41 (1973). 7. Ulmuis, J et al, *Biochim. Biophys. Acta*, **389**:197 (1975). 8. Larocca, JN et al, *Curr. Protoc. Cell Biol.*, **3.25**:1 (2006). **Acknowledgements:** NIH T32 EB00814