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

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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 was shown, both spectroscopically and by imaging, to scale with concentration, suggesting the potential for quantitative determination of myelin density.

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 but 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

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interaction of water and myelin, and therefore may provide con-
trast specific to myelin concentration. However, direct detection is
complicated by motional restriction of the lipid chains in the mye-
lin bilayer, resulting in broad lines and, consequently, short life-
time of the MR signal.

Perhaps the first characterization of the NMR spectral prop-
erties of myelin was by Lecar et al. (16), who studied anhydrous
preparations by wide-line proton spectroscopy, concluding that
under these conditions the system is in a liquid–crystalline state.
To the best of our knowledge, the first report of myelin proton
transverse relaxation was by Ramani et al. (17). The authors
performed a multieponential fit of spin-echo decays on fixed
human WM samples from normal and multiple sclerosis patients
and reported a T2 value of ~50 μs for myelin protons. They were
also able to detect lipid signals by magic-angle spinning proton
NMR in slices of normal WM tissue but not in multiple sclero-
sis lesions. Recently, Horsch et al. (18) investigated the T2* and
T2 relaxation characteristics of myelin and reported values of
~70 μs as well as a broader distribution ranging from 50 to
1,000 μs.

The transverse relaxation properties of myelin suggest the
need for ultrashort echo time (UTE) MRI methods, which entail
collection of the free-induction decay immediately after excita-
tion. Typical implementations include either 3D radial sampling
without selective rf pulses (19) or 2D radial sampling with slice-
selective half rf pulses and ramp sampling (20) as previously
applied for the characterization of cortical bone matrix and bone
water (21, 22).

UTE MRI has been used to image the short T2* (i.e., <1 ms)
signal from human brain in vivo (23). Unlike applications to study
bone, these implementations include long T2* suppression meth-
ods to attenuate the tissue water signal. Tissue water, because
of its rotational mobility and high concentration, has an intense
long T2* signal that, without suppression, overwhelms signals from
short T2* components (Fig. S1A). Although the images indicated
the short T2* signal to be predominantly located in WM, no evi-
dence was provided to link this signal to myelin.

In this work we examine the origin and nature of the short T2*
signal of CNS tissue in freshly excised rat spinal cord (SC) in
comparison with purified myelin lipid extract with multielement
NMR. We further explore the potential for direct detection and
quantification of myelin by UTE MRI and discuss the possibili-
ties and technical hurdles associated with translating MRI-based
quantification of myelin to the clinic.

Results

High-Resolution 1H NMR Spectra of Intact SC Tissue and Bovine Myelin Extract. Fig. 1 shows a comparison of the 1H NMR spectra col-
clected for a section of excised rat thoracic SC and a deuterium
oxide (D2O) suspension of myelin lipid extract. The SC was first
immersed in 99.9% D2O for 24 h to exchange tissue water with
D2O, and hence attenuate the bulk proton signal (Fig. S1B).
The protein-free myelin lipid sample was chemically extracted from
an intact isolated myelin sample, obtained via a sucrose gradient
method (SI Materials and Methods) and quantitatively validated
with proton-decoupled 13C and 31P NMR spectroscopy (Table 1
and Fig. S2 A and B).

Both 1H spectra in Fig. 1 show a broad resonance with a su-
perimposed narrow water resonance originating from residual
HDO. Although the two broad resonances bear a high degree of
similarity, a difference spectrum (i.e., tissue – extract) (Fig. S3)
highlights the distinguishing features. First, the superimposed
fine structure apparent in the SC spectrum is consistent with mobile
proteins as they might occur in the cytoplasm, for ex-
ample. Second, the difference spectrum contains a broad re-
sonance as it might be expected from membrane proteins. The
difference spectrum was generated such that the integrated sig-
nal area is 27.9% of the total spectrum, as expected according to
the known protein fraction in myelin (details in SI Results).
Small errors in the difference spectrum could arise because we are
ignoring cytoplasmic proteins in this fraction.

UTE MRI of Intact SC. Fig. 2 shows a set of images of freshly ex-
cised rat SC, obtained with a 3D radial, ramp-sampled, dual-
echo inversion recovery UTE (de-IR-UTE) pulse sequence
(Fig. S4). Long T2* tissue water signal was attenuated via adi-
batic inversion and complex echo subtraction. Adiabatic in-
version was used to significantly reduce the signal intensity from
tissue water, which would then be further attenuated with echo
subtraction. We empirically selected TI to achieve the greatest
WM intensity while minimizing gray matter (GM) intensity signal
in the complex echo difference image in accordance with the
expectation of low signal in GM considering its very low
myelin content. Images were collected at both short (20 μs; Fig.
2A) and long (1,200 μs; Fig. 2B) TE. The magnitude of the
complex difference image and signal profile (Fig. 2 C and D)

Table 1. Lipids of myelin with abbreviations used in the text

<table>
<thead>
<tr>
<th>Myelin lipid</th>
<th>ID</th>
<th>Norton*</th>
<th>NMR†</th>
<th>% labile 1H ± σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>CHOL</td>
<td>44.8</td>
<td>43.1</td>
<td>0.94 ± 0.04</td>
</tr>
<tr>
<td>Galactocerebroside</td>
<td>GC</td>
<td>17.5</td>
<td>19.6</td>
<td>2.20 ± 0.39</td>
</tr>
<tr>
<td>Galactosulfatide</td>
<td>GS</td>
<td>2.5</td>
<td>NA‡</td>
<td>0.28 ± 0.05†</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>PE</td>
<td>3.4</td>
<td>3.9</td>
<td>0.24 ± 0.05</td>
</tr>
<tr>
<td>PE plasmalogen</td>
<td>PEPl</td>
<td>11.3</td>
<td>11.8</td>
<td>0.70 ± 0.13</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>PC</td>
<td>8.0</td>
<td>7.8</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>PC plasmalogen</td>
<td>PCpl</td>
<td>0.3</td>
<td>2.0</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>Sph</td>
<td>5.2</td>
<td>5.9</td>
<td>0.37 ± 0.08</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>PI</td>
<td>0.7</td>
<td>2.0</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>Phosphatidyserine</td>
<td>PS</td>
<td>0.2</td>
<td>3.9</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>5.05 ± 0.79</td>
</tr>
</tbody>
</table>

Comparison of average bovine myelin lipid molar ratios reported by Nor-
ton et al. (25) and quantitative multi-NMR methods. Also shown are average
percentages of labile protons pertaining to each lipid component. PC, phos-
phatidylcholine; PE, phosphatidylethanolamine.

*From Norton et al. (23).
†Present study. 13C and 31P NMR.
‡Variability (σ, SD) due to lipid chain length [CHn(CH2)n; n = 10–25].
§Not measured owing to a lack of an unambiguous resonance.
†Assuming a GS molar percentage of 2.5%.
highlight the short T2* 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-D2O 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. 3A shows the results from fitting of the proton NMR spectrum of purified myelin suspended in D2O. 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 T2* distributions of the four SL components, were combined into a myelin T2* histogram (Fig. 3B). At 20 °C, 26.4% of the total signal has an effective lifetime of <25 μs, 51.8% of <100 μs, and 91.6% of <1,000 μs. 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 D2O to form HDO. The calculated average percentage of labile protons (5.05% ± 0.79%) agreed well with the experimental HDO peak areas (5.13% ± 2.00%). Given the excess D2O used 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. 5A, B, and C, respectively. 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.
shown is the calculated signal fraction of labile myelin protons (green shaded).

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second moment ($M_2 = 1.06 \times 10^5 \text{s}^2$), a large fourth moment ($M_4$) to $M_2$ ratio [$M_4/(M_2)^2 = 6.57$], and a full width at 1/10

maximum ($\Delta \nu_{1/10}$) to full width at 1/2 maximum ($\Delta \nu_{1/2}$) ratio $> 3$

($\Delta \nu_{1/10}: \Delta \nu_{1/2} \approx 7$). Following previous work (32), we modeled the myelin lipid extract spectrum as a sum of SLs representing

protons from general alkyl chain methylenes, cholesterol alkyl

chain methylenes, terminal methyls, and choline. Although the $T_2^*$

distribution showed a wide range of values (0.0008–26 ms), the distribution was dominated by that of the alkyl chain methylenes because they make up $\sim 75%$ of the four myelin lipid proton moieties under consideration. One notes a bimodal distribution (Fig. S5) with the first peak significantly attenuated as expected for an SL lineshape determined by the angular dependence of $T_2^*$ (details in SI Results).

Discussion

In this work we explored the feasibility of direct imaging and

quantification of myelin by magnetic resonance as an alternative to indirect detection techniques such as MT or $T_2$ relaxometry. Direct detection of myelin would remove any ambiguities in analysis and provide a contrast specific to myelin concentration.

To explore the feasibility of direct myelin imaging, we first investigated the origins of the short $T_2^*$ signal in intact SC and myelin extracts. We then presented preliminary UTE with long $T_2^*$ suppression images of myelin in WM.

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 isolated human myelin (28). In that study a synthetic $^{13}C$ spectrum of myelin was generated as a weighted sum of individual lipid spectra, based upon the known ratios of the individual lipid components.

The synthetic spectrum was found to be in good agreement with the observed MAS spectrum, which in turn closely matches our solution spectrum (Fig. S2B), except for the absence of signals assigned to protein resonances in the MAS spectrum.

Any conclusions from extracted or synthetically produced myelin beg the question whether the resulting material retains the biophysical properties of native myelin. It is known that in aqueous suspensions myelin lipids spontaneously self-assemble into bilayer structures (i.e., vesicles). In this enthalpy-driven process (29) the myelin lipid suspension regains a structural order reminiscent of physiological myelin minus the protein component.

The spectrum of the isolated, reconstructed myelin exhibited a very broad line with relatively narrow components centered at $\sim 3.5$ ppm upfield from water, consistent with methylene protons of alkyl chains, the main constituent of myelin. Further, this spectrum bears a high degree of similarity with that of neural tissue, which supports the notion that, upon aqueous suspension of the extract, a bilayer structure analogous to that for native myelin is reconstituted. The SC spectrum is expected to contain no myelin contributions such as from proteins. The difference spectrum (Fig. S3) suggests the presence of motionally restricted membrane proteins, along with a small fraction of mobile (perhaps cytoplasmic) proteins yielding narrow resonances.

The myelin resonance is consistent with the SL lineshape of a dipolar-broadened liquid–crystalline lipid system (24, 30) as suggested by several lineshape properties (31), including a small second moment ($M_2 = 1.06 \times 10^5 \text{s}^2$), a large fourth moment ($M_4$) to $M_2$ ratio [$M_4/(M_2)^2 = 6.57$], and a full width at 1/10 maximum ($\Delta \nu_{1/10}$) to full width at 1/2 maximum ($\Delta \nu_{1/2}$) ratio $> 3$ ($\Delta \nu_{1/10}: \Delta \nu_{1/2} \approx 7$). Following previous work (32), we modeled the myelin lipid extract spectrum as a sum of SLs representing protons from general alkyl chain methylenes, cholesterol alkyl chain methylenes, terminal methyls, and choline. Although the $T_2^*$ distribution showed a wide range of values (0.0008–26 ms), the distribution was dominated by that of the alkyl chain methylenes because they make up $\sim 75%$ of the four myelin lipid proton moieties under consideration. One notes a bimodal distribution (Fig. S5) with the first peak significantly attenuated as expected for an SL lineshape determined by the angular dependence of $T_2^*$ (details in SI Results).

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

![Fig. 4](image_url) Fig. 4. (A) Fitted myelin peaks for myelin suspensions of various concentrations. (B) Linear correlation plot of MR signal as a function of myelin concentration for the total (triangle), myelin (circle), and HDO (square) signal components with calculated $R^2$ values. All two-tailed $P$ values $< 0.01$. Also shown is the calculated signal fraction of labile myelin protons (green shaded).
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 T2* values of 20 and 70 μs 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 T2* components are due to membrane proteins, which cannot contribute a short T2* component, as suggested by the broad resonance in the difference spectrum (Fig. S4) and in other reports (24, 33).

In bovine myelin suspended in D2O, 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 T2* components. Under these conditions (i.e., selective half-sinc pulses of 400-600-μs duration (34)), all but the longest T2* components of the myelin protons would elude detection.

Our results indicate approximately 20% of the protons in myelin lipids to have an effective T2* less than 25 μs. Even under the more favorable conditions of our imaging experiments, the majority of these short T2* 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 μm, which is approximately one pixel with our current imaging parameters (more discussion in SI Materials and Methods). It should be noted that despite the shortness of these components, it is possible to detect them, although their impact in clinical settings is minimal because these signals do not affect the PSF; they themselves are not detectable.

A limitation that needs to be noted for this method is that it detects myelin solely on the basis of its T2* properties. Thus, errors in long T2* suppression may lead to signal misclassification as short T2* 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 T2* 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 μs 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 T2* values increase by ca. 30% (Fig. 3B), raising the minimum T2* value from 8 to 10.5 μs. At body temperature the scan parameters used in our current experiments at 9.4 T (except for a 20-μs hard pulse of 7.6° flip angle to match the peak B1 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 MRSI 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 T2* 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 T2*-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 T2* 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 Micros.2 gradients (100 Gcm maximum strength) and BAPM40 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 13C 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 2H (SW = 3.2 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 μs. 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 SL fitting was done bovine myelin extract, crystalline lipid system with an SL lineshape that can be written as:

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

where $\omega_0$ is the chemical shift, $\theta$ 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 \( \theta \) is uniformly sampled and setting \( f \) (in radians) 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 line shapes (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 HDD. 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): \( SW = 200 \text{ kHz}, TE = 20/1,200 \mu s, TI = 500 \text{ ms, TR} = 1 \text{ s, field of view} = 15 \text{ mm, matrix size} = 128 \times 128 \times 128, \text{ number of views} = 5,342, \text{ pulse duration} = 20 \text{ ms}. \) 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 \( TE = 1,200 \mu s \). A 3D image of the short \( T_2^* \) components was obtained as the complex difference of the two echo images (i.e., \( TE_1 - TE_2 \)). 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/\( \text{O}_2 \) 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.

A 3D 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).

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Supporting Information

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SI Results

High-Resolution NMR Spectra of Intact Spinal Cord (SC). Fig. SI/4 depicts the $^1$H NMR spectrum of freshly excised rat thoracic SC immersed in Fomblin oil. The full spectrum highlights the dominance of the tissue water resonance at 4.8 ppm. Increasing the vertical scale by a factor of 500 shows the presence of a much weaker broad peak. This peak, centered near the methylene (-CH$_2$) proton resonance frequency (i.e., shifted ca. 3.5 ppm upfield from water) is consistent with the chemical shift of lipid protons.

Fig. S1B is a comparison of the $^1$H NMR spectra of freshly dissected rat thoracic SC, before and after 24-h immersion in D$_2$O to remove exchangeable protons, including bulk tissue and myelin water. The two spectra are qualitatively similar in that they both exhibit a dominant water resonance coupled with a much weaker broad envelope. However, the spectrum for the D$_2$O exchanged SC clearly shows a dramatic reduction in water signal intensity, resulting in a cleaner representation of the broad peak.

Validation of the Composition of Bovine Myelin Extract. Of the 10 primary myelin lipids, seven are phospholipids (i.e., each containing a single, uniquely chemically shifted phosphate group) and can therefore be readily characterized quantitatively with $^{31}$P NMR spectroscopy (1). Fig. S2A shows the proton-decoupled $^{31}$P NMR spectrum of purified bovine myelin. In addition to a resonance at approximately 0.42 ppm (phosphoric acid calibration standard), separate resonances for each of the seven phospholipids of myelin were observed. As shown in Table 1, integration over the individual lipid resonances yielded a relative myelin phospholipid molar ratio in good agreement with the literature (2). Fig. S2B shows the proton-decoupled $^{13}$C spectrum for purified bovine myelin dissolved in the ternary solvent mixture of chloroform, methanol, and water to remove residual sucrose contaminants. Dissolution in the ternary mixture inverts the membrane proteins, such as PLP and MBP (see, e.g., refs 5 and 6).

Intrinsic Point-Spread Function (PSF). Intrinsic resolution expected for proton imaging of myelin, at 20 and 37 °C, under the experimental conditions of the present work at 9.4 T, as well as under conditions achievable on a 3 T clinical scanner are presented in the table below. The FWHM of the PSF for a 3D radial pulse (Fig. S4) was computed as described below from Eq. S1 on the basis of effective T$_2^*$ values estimated from the myelin lipid spectra in Fig. 3 and acquisition times used at 9.4 T and values currently feasible at 3 T on a clinical scanner (Discussion).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>20</th>
<th>37</th>
</tr>
</thead>
<tbody>
<tr>
<td>FWHM (Hz) @ 9.4T</td>
<td>2,059</td>
<td>1,525</td>
</tr>
<tr>
<td>$\Delta x_0$ (mm) @ 9.4T</td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>$\Delta x_0$ (mm) @ 3T</td>
<td>0.77</td>
<td>0.57</td>
</tr>
</tbody>
</table>

$T_2^*$ Distribution of a Super-Lorentzian (SL) Lineshape. Fig. S5 shows the calculated $T_2^*$ distribution for the SL representing the alkyl chain methylene protons as derived from the fit of the myelin lipid extract $^1$H NMR spectrum at 20 °C. It is representative of the general $T_2^*$ distribution characteristics for all SLs. The $T_2^*$ distribution is bimodal, with the first peak starting at 8 μs and the second peak starting at 16 μs. These two peaks arise from protons with an effective angle of $\theta = 0$ and $\pi/2$ relative to the main magnetic field. The factor of two difference in $T_2^*$ between the peaks results from the $|3\cos^2\theta - 1|^{-1}$ angular dependence of $T_2^*$ for an SL lineshape (7). The large difference in peak intensities is due to the much lower weights ($\sin 0 \times |3\cos^2\theta - 1|^{-1}$) of $T_2^*$ components at $\theta = 0$ compared with those at $\theta = \pi/2$.

SI Materials and Methods

Myelin Extract Preparation. A sucrose gradient-based centrifugation method was used to isolate intact myelin, in which the lipid bilayer structure has been shown to be largely preserved (8). After isolation, the crude myelin was dissolved in a (4:2:1) ternary mixture of chloroform, methanol, and water to remove residual sucrose contaminants. Dissolution in the ternary mixture inverts the bilayer, thereby releasing imbedded proteins, yielding myelin lipids (9). Purified myelin lipids were removed from the chloroform phase after evaporation under a continuous flow of nitrogen gas. The remaining myelin lipid residue was then resuspended in distilled water, frozen, and lyophilized to remove all remaining traces of solvent. When not in use, the purified myelin extract was relyophilized and stored frozen at 258 K.

Effect of $T_2^*$ Decay on Point-Spread Function (PSF). The PSF is governed by the ratio of acquisition time to $T_2^*$, which determines the extent to which the highest spatial-frequency signals are at-
tenuated during readout. For 3D radial sampling the FWHM of the PSF in units of voxel length is given as (10):

$$FWHM = 0.41 \frac{T_{acq}}{T_{2}^*}$$  \[S1\]

An effective $T_{2}^*$ was estimated from the FWHM of the spectrum, and $T_{acq}$ is given as $N_s/BW$, with $N_s$ and $BW$ representing the number of samples along each radial trajectory, and $BW$ is the sampling frequency bandwidth.

**Estimation of Fraction of Detected Signal.** The fraction of the total myelin signal detected by imaging, was estimated as follows. Including the effect of the adiabatic inversion pulse and assuming the magnetization to have reached a steady state, the acquired myelin signal was calculated as:

$$S = f_{xy} \frac{1 - (1 - f_{z,inv}) \exp(- TI/T_1) - f_{z,inv} \exp(-(TR - TI)/T_1)}{(1 - f_{z,inv} f_{z} \exp(-(TR - TI)/T_1))} \times \exp(-TE/T_2^*),$$  \[S2\]

where $f_{z,inv}$ is the normalized longitudinal magnetization of the myelin protons in response to the adiabatic inversion pulse. $T_{inv}$ is the adiabatic inversion pulse duration, $TI$ is the inversion time, defined as the interval between the end of the adiabatic pulse and the start of the rectangular (hard) pulse. Further, $f_{xy}$ and $f_{z}$ represent the normalized transverse and longitudinal magnetizations in response to the hard pulse. $TR$ and $TE$ are the repetition time and echo time (defined as the interval between the end of hard pulse and the start of signal acquisition, i.e., the first readout point), and $T_1$ and $T_{2}^*$ are the relaxation times.

The fractions of myelin proton components and their corresponding $T_{2}^*$ relaxation times were retrieved from the myelin spectroscopic data by SL curve fitting (Fig. 3). $T_1$ was obtained by standard inversion recovery of the myelin lipid extract, yielding a value of 0.66 ± 0.03 s. The response of the magnetization to the adiabatic and rectangular pulses due to coherence losses during nutation of the magnetization were computed numerically from the Bloch equations. A matrix form-based Bloch equation simulation algorithm was used to accelerate the computation. Specifically, the pulses were approximated by a series of rectangular subpulses of 1-μs duration, and the effect of each pulse on the magnetization was evaluated by multiplication with rotation and relaxation matrices. The same approach was also used for computing losses during the RF pulse in the spectroscopic experiments. All computations were performed in Matlab (MathWorks).

Fig. S1. The $^1$H NMR spectrum of freshly excised rat thoracic SC. (A) In the full-scale spectrum the tissue water resonance only is observable. The magnified (500×) view shows the presence of a broad envelope centered on the methylene proton resonance frequency with narrower resonances superimposed. (B) Same sample spectrum before (blue) and after (black) 24-h immersion in D$_2$O. Inset: Reduction of tissue water signal after D$_2$O exchange.

Fig. S2. High-resolution multinuclear NMR spectra of myelin in solution. (A) Proton-decoupled $^{31}$P spectrum. In addition to the phospholipids of myelin, resonances from phosphatidic acid (PA), cardiolipin (CL), and phosphoric acid (H$_3$PO$_4$) are visible at 0.091, 0.003, and −0.417 ppm, respectively. (B) Proton-decoupled solution-phase $^{13}$C NMR spectrum of purified bovine myelin extract (red) and a condensed-phase $^{13}$C MAS NMR spectrum of human myelin extract (black, inverted; modified from ref. 3). A line broadening of 50 Hz was applied to match the apparent resolution of the MAS spectrum. The organic solvent resonances for deutero-chloroform and methanol are denoted with blue asterisks (*) at 77.23 and 49.15 ppm, respectively. The non-line broadened CHO, L:C1, GC:C6, and trimethylamine resonances, used for quantification, have been magnified to show detail. The MBP resonance, located at 157.8 ppm in the MAS spectrum, has been denoted with a vertical blue arrow.

Fig. S3. The $^1$H NMR spectra of D$_2$O exchanged rat thoracic SC (black), fit of the non-HDO components of the purified bovine myelin suspended in D$_2$O (red), and difference spectrum generated by constraining the total integrated area to 27.9% of the SC spectrum, which is suggestive of protein resonances. Inset: Full spectral view, highlighting the residual presence of a broad component in the difference spectrum.
Fig. S4. Pulse sequence timing diagram of the radial ramp-sampled dual-echo inversion recovery ultrashort echo time imaging pulse sequence used for myelin detection. K-space was sampled along the surface of 65 radially concentric cones for a total of 5,342 views. Water suppression was achieved by the combination of a long adiabatic inversion preparation pulse and echo subtraction (diagram not drawn to scale).

Fig. S5. Calculated $T_2^*$ distribution for the SL representing the alkyl chain methylene protons as derived from the fit of the myelin lipid extract $^1$H NMR spectrum at 20 °C.