Fiber orientation-dependent white matter contrast in gradient echo MRI

Samuel Wharton and Richard Bowtell

Recent studies have shown that there is a direct link between the orientation of the nerve fibers in white matter (WM) and the contrast observed in magnitude and phase images acquired using gradient echo MRI. Understanding the origin of this link is of great interest because it could offer access to a new diagnostic tool for investigating tissue microstructure. Since it has been suggested that myelin is the dominant source of this contrast, creating an accurate model for characterizing the effect of the myelin sheath on the evolution of the NMR signal is an essential step toward fully understanding WM contrast. In this study, we show by comparison of the results of simulations and experiments carried out on human subjects at 7T, that the magnitude and phase of signals acquired from WM in vivo can be accurately characterized by (i) modeling the myelin sheath as a hollow cylinder composed of material having an anisotropic magnetic susceptibility that is described by a tensor with a radially oriented principal axis, and (ii) adopting a two-pool model in which the water in the sheath has a reduced $T_2$ relaxation constant and effective spin density relative to its surroundings, and also undergoes exchange. The accuracy and intrinsic simplicity of the hollow cylinder model provides a versatile framework for future exploitation of the effect of WM microstructure on gradient echo contrast in clinical MRI.

Gradient echo (GE) MRI is widely used in imaging the human brain, because both the phase and magnitude of the complex NMR signal measured with GE sequences can be used to create high-resolution images that show strong contrast between different types of brain tissue (1). Recent studies have shown that there is a direct link between the orientation of the nerve fibers in white matter (WM) with respect to the magnetic field and the contrast observed in magnitude and phase images (2–6). Although the origin of this link is currently not fully understood, orientation-dependent contrast is of great interest because it could offer researchers access to a new diagnostic tool for investigating tissue microstructure using MRI. It has recently been suggested that the myelin sheaths that surround axons are the dominant source of WM contrast in GE MRI (7, 8). Creating an accurate model for characterizing the effect of the myelin sheath on the evolution of the magnitude and phase of the NMR signal is consequently an essential step toward fully understanding WM contrast and its relationship to fiber orientation. Such a model must incorporate two main features: (i) a representation of the microscopic spatial variation of resonant frequency, due to the myelin compartment— isotropic and anisotropic magnetic susceptibility effects (2, 9, 10) and chemical exchange of protons between water and macromolecules (11, 12), have been proposed as mechanisms through which myelin could perturb the resonant frequency in WM; (ii) a signal-weighting scheme to account for the reduced $T_2$ relaxation time constant of the water relative to that of water found outside the myelin sheath (13–15).

In this study, we show by comparison of the results of simulations and experiments that the fiber orientation dependence of the magnitude and phase of signals acquired from WM in vivo can be accurately characterized by (i) modeling the myelin sheath as a hollow cylinder composed of material having an anisotropic susceptibility that is described by a tensor with a radially oriented principal axis, and (ii) adopting a two-pool model in which the water in the sheath has a reduced $T_2$ relaxation constant and effective spin density relative to its surroundings, and also undergoes exchange.

Results

Frequency Difference Mapping. The excellent contrast seen in phase images means that phase mapping is a potentially useful tool for investigating the structure of WM (1, 2, 9). However, phase contrast is nonlocal, in the sense that sources of field perturbation located outside a given voxel affect the phase measured inside that voxel, making it difficult to draw clear inferences about the relationship between phase variation and the underlying tissue microstructure (16). Here, we therefore first focus on frequency difference mapping, which is a technique for creating phase-based contrast that is insensitive to nonlocal effects (SI Text). Frequency difference mapping involves generating phase maps from GE data acquired at multiple echo times, converting these to frequency maps via scaling by echo time (TE), and then calculating the difference from a reference frequency map. In a voxel that contains multiple water compartments that experience different frequency offsets and have different $T_2$ values, the phase of the average signal does not necessarily scale linearly with TE. As a consequence, the apparent frequency of the voxel is TE dependent, yielding a non-zero contribution in the frequency difference map (FDM). In contrast, frequency offsets generated by nonlocal field sources are similar in all compartments in a voxel and so produce an average phase that varies linearly with TE. Nonlocal effects are consequently eliminated in the FDM. Because the signal from myelin water decays with a time constant of less than 20 ms (13, 15), an FDM that shows the difference in frequency measured at short and long TE in the 0- to 30-ms range will be sensitive to WM microstructure.

This sensitivity is illustrated in Fig. 1, which shows high-resolution frequency maps acquired from the human brain at 7T using TE values of 5 ms (Fig. 1A) and 25 ms (Fig. 1B). It is evident from these maps and the associated FDM (Fig. 1C) that there are significant TE-dependent changes in the apparent frequency in WM regions, which result in generally negative values of the frequency difference, $Δf$. Averaging $Δf$ over the imaging volume in three healthy male subjects (20–30 y in age) gave values of $0.03 ± 0.05$ and $−1.22 ± 0.04$ Hz in grey matter (GM) and WM, respectively. Comparison with Fig. 1D, which shows a fiber orientation map created using diffusion tensor imaging (DTI), indicates that particularly negative $Δf$ values occur in the large fiber bundles oriented perpendicular to $B_0$, such as the corpus callosum and optic radiations. These preliminary results therefore suggest that the

Author contributions: S.W. and R.B. designed research, performed research, analyzed data, and wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

1To whom correspondence should be addressed. E-mail: richard.bowtell@nottingham.ac.uk.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1211075109/-/DCSupplemental.
TE-dependent frequency differences, though negligible in GM, are significant, generally negative, and fiber orientation dependent in WM.

**Orientation-Dependent WM Contrast.** To investigate further the effect of fiber orientation on the temporal evolution of magnitude and frequency difference data, five healthy male subjects aged 20–30 were imaged using a 3D multiecho, GE acquisition at 7 T, yielding 32 image datasets with TE values ranging from 2 to 33 ms. FDM were created by subtracting a reference map, formed by averaging the frequency maps associated with the first four TE values, from each phase-based frequency map. Fig. 2 shows the variation of the normalized WM magnitude signal (Fig. 2A), and of Δf (Fig. 2B), with TE. Data are divided into five groups based on the fiber orientation, defined by the angle, θ, between the local fiber direction (extracted from DTI data) and the static field, \( B_0 \). The signal magnitude decays more rapidly as the fiber orientation changes from parallel to perpendicular to \( B_0 \) as has been previously described (3–5), whereas Δf decreases monotonically from approximately zero at TE = 3 ms to generally negative values for TE = 33 ms at a rate that is strongly dependent on the local fiber orientation. The frequency difference values at long TE range from slightly positive, \( Δf = 0.01 ± 0.22 \) Hz, for fibers parallel to \( B_0 \) to strongly negative, \( Δf = −1.66 ± 0.14 \) Hz, for perpendicular fibers.

**Hollow Cylinder Fiber Model.** The fiber model adopted here (Fig. 3A) represents the myelin sheath as an infinite hollow cylinder, with an inner radius, \( r_i \), and outer radius, \( r_o \), oriented at an angle, \( θ \), to \( B_0 \). Two water pools were considered (Fig. 3B): (i) a small pool corresponding to the myelin water in the cylindrical annulus, whose signal is characterized by a transverse relaxation time constant, \( T_{2,SP} \), and a reduced relative spin density, \( ρ \); (ii) a large pool representing the water both inside and outside the myelin sheath, with a relative spin density of 1 and transverse relaxation time constant, \( T_{2,LP} \).

The signal evolution was modeled by simulating the spatially varying frequency perturbation due to the myelin sheath, taking account of three different contrast mechanisms: (i) isotropic magnetic susceptibility; (ii) chemical exchange; and (iii) anisotropic magnetic susceptibility. Expressions for the frequency variation were formed for the different mechanisms (SI Text).

Because the model uses an infinite cylinder approximation, the frequency perturbations are axially invariant and can be fully represented by their variation in a plane perpendicular to the axis of the hollow cylinder. Chemical exchange of protons between water and exchange sites on macromolecules, such as those found at the surface of myelin bilayers (17), can result in a small shift, \( δ_{OE} \), of the water resonant frequency (12). The effect of this exchange is included in the model by introducing a parameter, \( E = δ_{OE}/ω_0 \), representing the fractional exchange-induced frequency shift in the myelin sheath relative to the surrounding spaces. Calculation of the spatially varying frequency perturbation due to a hollow cylinder of isotropic susceptibility is straightforward (10), but the frequency perturbation due to anisotropic susceptibility is more complex to evaluate.

The highly ordered lipid molecules within the myelin sheath form the most likely source of anisotropic magnetic susceptibility (18), since similar lipid structures have been shown to exhibit anisotropic magnetic properties (19, 20). The lipids are packed together in bilayers, which spiral around the axon to form the myelin sheath. The long, aliphatic lipid chains are consequently radially aligned, leading to the expectation that the susceptibility tensor at each location is cylindrically symmetrical with a radially oriented principal axis (18). In the reference frame in which its principal axis is aligned with the x-direction, the susceptibility tensor, \( χ \), can be written as shown in Fig. 3C, where \( χ_x \) and \( χ_A \) define the magnitudes of the isotropic and anisotropic susceptibility. The calculation of the frequency perturbation due to this form of anisotropy in the susceptibility is detailed in SI Text. The calculation involves forming the gradient of the magnetic scalar potential, followed by the addition of an (isotropic) sphere of Lorentz correction (21). Expressions describing the frequency perturbation due to the different mechanisms are listed in Table 1.

Fig. 4 shows maps of the frequency perturbations produced by the hollow cylinder, with its principal axis parallel (\( θ = 0° \) and...
Hollow Cylinder Fiber Model

perpendicular (θ = 90°) to \(B_0\), as a result of the three different mechanisms: isotropic susceptibility (\(\chi_I\)), exchange (\(E\)), and radial anisotropic susceptibility (\(\chi_A\)). Simulations were carried out for a g-ratio (14), \(r_i/r_o\), of 0.8. Inspection of the maps and Table 1 makes evident a few key points: exchange generates a frequency offset that is independent of orientation and position (i.e., homogeneous) and confined to the material of the hollow cylinder (Fig. 4 B and E). When the hollow cylinder is parallel to \(B_0\), anisotropic and isotropic susceptibility generate homogeneous frequency offsets within the material, whose sizes depend on the magnitude of the effective susceptibility along the cylinder’s axis (Fig. 4 A and C). When the hollow cylinder is perpendicular to \(B_0\), isotropic susceptibility generates no frequency offset inside the hollow cylinder, but produces a spatially varying (inhomogeneous) frequency offset outside the cylinder and a similar sort of variation in the material of the cylinder, superimposed upon a uniform (homogeneous) frequency offset (Fig. 4D). The frequency variation generated by the radial anisotropic susceptibility for the perpendicular case is rather different, with a nonzero, homogeneous frequency offset inside the hollow cylinder and an average offset of the opposite sign produced in the material of the cylinder, along with a weak inhomogeneous field, also present outside the cylinder (Fig. 4F). When the cylinder is oriented at intermediate angles to \(B_0\), the frequency perturbation is a simple weighted superposition of the parallel and perpendicular maps.

### Table 1. Expressions describing the frequency perturbation in the hollow cylinder model

<table>
<thead>
<tr>
<th>Mechanism/region</th>
<th>(r &lt; r_i)</th>
<th>(r_i &lt; r &lt; r_o)</th>
<th>(r &gt; r_o)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\chi_I) ×</td>
<td>0</td>
<td>(\frac{1}{2} \left( c^2 - \frac{1}{2} - s^2 \text{cos}^2 \phi \left( \frac{r}{r_o} \right) \right))</td>
<td>(\frac{s^2 \text{cos}^2 \theta \left( \frac{r}{r_o} \right)}{r})</td>
</tr>
<tr>
<td>(E) ×</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(\chi_A) ×</td>
<td>(\frac{\mu_0}{4\pi} \ln \left( \frac{r_i}{r} \right))</td>
<td>(s^2 \left( \frac{r}{r_o} - \text{cos}^2 \phi \left( 1 + \frac{1}{2} \ln \left( \frac{r_i}{r} \right) \right) - \frac{r}{r_o} \right))</td>
<td>(\frac{s^2 \text{cos}^2 \theta \left( \frac{r}{r_o} \right)}{r})</td>
</tr>
</tbody>
</table>

Frequency perturbation, \(\Delta \omega (r)/c_0\), in the internal \((r < r_i)\), annular \((r_i < r < r_o)\), and external \((r > r_o)\) compartments of the hollow cylinder model, are listed for the three different contrast mechanisms (isotropic magnetic susceptibility, \(\chi_I\), exchange, \(E\), and anisotropic magnetic susceptibility, \(\chi_A\)). In these expressions \(\text{sin}^2 \theta\) and \(\text{cos}^2 \theta\) have been shortened to \(s^2\) and \(c^2\) respectively, and \(\phi\) is the azimuthal angle.

### Fitting the Hollow Cylinder Model to Frequency Difference Data.

Simulations were carried out to characterize the effect of microstructure on the GE signal evolution in WM. The signal was calculated for four different combinations of the mechanisms by which myelin could perturb the frequency in the hollow cylinder model, and fitted to the magnitude and frequency difference data shown in Fig. 2. These combinations were (i) \(\chi_I\), (ii) \(\chi_I + E\), (iii) \(\chi_I + \chi_A\), and (iv) \(\chi_I + E + \chi_A\). The frequency perturbations due to the different mechanisms (Fig. 4) were linearly superposed for simulations involving more than one source of contrast, and the signal was formed by summing weighted contributions from a 2D grid in the standard manner (22), including points within a circular region outside the myelin annulus whose radius was dictated by the fiber volume fraction (FVF). The phase of the signal at each time point was converted into a frequency value using the same method as was applied to the experimental data. In addition to \(\gamma_I, E\), and \(\chi_A\), other variable parameters in the simulations were (i) the g-ratio; (ii) the T2 values of the two water pools, \(T_2\); and (iii) the relative proton density, \(\rho\), in the myelin sheath. To simplify the fitting procedure, the FVF was fixed as 0.5, based on literature values (14).

An iterative fitting procedure was then carried out for each of the four combinations. In each iteration, separate field maps were produced for each of the five \(\theta\)-ranges used to group the in vivo data shown in Fig. 2. A reduced chi-squared residual was then formed by comparing the evolution of the simulated and measured magnitude and frequency-difference data. The parameter values that yielded the lowest residual for each combination are shown in Table 2. Simulations based on isotropic susceptibility effects only \(\chi_I\) failed to yield a successful fit to the measured data and are therefore not described in the table. The best fit (solid lines in Fig. 2) was achieved by including the effect of exchange, isotropic susceptibility, and radial anisotropic susceptibility \(\chi_I + E + \chi_A\). The plots in Fig. 2 indicate that the orientation-dependent evolution of the magnitude and frequency difference can be well characterized by the hollow cylinder model. However, because several mechanism combinations can produce a good fit to the experimental data (Table 2), further information is needed to identify which of the proposed effects underlies the experimentally measured contrast.

### Calculating the Nonlocal Field.

The nonlocal field perturbation, resulting from the anatomical distribution of gray and white matter, potentially provides such information, because it is dependent on the magnetic susceptibility of the myelin sheath and in particular upon the type of susceptibility anisotropy that is present (18). By subtracting the TE-dependent local frequency offset, calculated using the hollow cylinder model (solid lines in Fig. 2), on a voxel-by-voxel basis from the frequency maps measured at different TE values, and then averaging the differences, a single frequency map that represents the effects of
The results presented here show that the fiber orientation-dependent GE contrast (phase and magnitude) measured in vivo in WM can be explained using a simple model in which the myelin sheath is represented as a hollow cylinder composed of material with a small $T_1$ and anisotropic magnetic susceptibility. The fiber orientation-dependent change in apparent frequency with TE results from the combination of two effects: the loss of the signal from the myelin compartment at long TE and the dependence of the frequency offset in the myelin sheath on its orientation to the applied field. The dependence of the rate of decay of the magnitude signal on fiber orientation is a consequence of the orientation-dependent change in the spatial variation of frequency, and consequent signal dephasing, which is produced by the myelin sheath. The spatially varying fields increase monotonically in strength as fiber orientation varies from parallel to perpendicular, producing a corresponding increase in $R_2^\parallel$.

### Failure of the Model in Which Myelin Has Purely Isotropic Susceptibility

It was necessary to include anisotropic susceptibility or exchange in the model of the myelin compartment to explain the fiber orientation-dependent variation of frequency difference and signal magnitude with TE, because the average relative frequency offset inside the material of a hollow cylinder of purely isotropic susceptibility varies from $\chi_I/3$ to $-\chi_I/6$ as the fiber orientation varies from parallel to perpendicular (Fig. 4 and Table 1), thus producing frequency differences that range over negative and positive values. In contrast, the measured frequency differences are negative at long TE values for all fiber orientations (Fig. 2B). Adding the effect of either radial anisotropic susceptibility and/or exchange can produce a frequency difference that is negative for all fiber orientations. However, it was not possible to explain the measured frequency evolution, when considering nonlocal effects (Fig. 5), without incorporating the effect of anisotropic susceptibility (i.e., the $\chi_I + E$ model failed to fit the data). The fit including all three mechanisms ($\chi_I + E + \chi_A$) had a significantly lower residual ($P < 0.05$ via F test on residuals in Table 3) than the fit excluding an exchange component ($\chi_I + \chi_A$), suggesting that an exchange contribution should be included in an accurate myelin model. In the following, we consider the implications of the hollow cylinder model and the parameter values that yield the best overall fit to the experimental data ($\chi_I + E + \chi_A$ in Table 3).

### Discussion

#### Hollow Cylinder Model

The field perturbations due to the hollow cylinder model populated with isotropic susceptibility (A and D), exchange-related field offsets (B and E), and radial orientation anisotropic susceptibility (C and F). The field perturbations are simulated with the cylinder axis (fiber orientation) parallel to $B_0$, $\theta = 0^\circ$ (A–C), and with the cylinder axis perpendicular to $B_0$, $\theta = 90^\circ$ (D–F). The fields are simulated for a g-ratio ($r_r/\rho$) of 0.8. For ease of comparison, the results are shown for the relevant perturbation ($\chi_I$, E, or $\chi_A$) set equal to 1 ppb.

nonlocal fields can be formed. The resulting map is model specific, because it depends on the values of $\chi_I$, $E$, and $\chi_A$ used in estimating the local frequency offset (i.e., the frequency offset in a voxel due to the myelin in that voxel); comparing it to an appropriate simulation of the nonlocal field distribution due to the anatomical distribution of WM and GM provides a further test of the model’s validity. For such simulations, forward field calculations (9, 10) were carried out using susceptibility distributions based on segmented GM/WM tissue masks generated from $T_2$-weighted images of each subject. The nonlocal field offsets due to the difference in both the isotropic and anisotropic susceptibility of WM compared with GM were calculated and fitted to the measurements (SI Text). Because we assumed that the susceptibility of GM is isotropic, this approach required the fitting of only one further variable parameter, representing the average isotropic susceptibility offset, $\chi_{I-L-P}$ of the large (non-myelin) pool of WM, relative to GM.

Fitting was carried out for the mechanism combinations and associated parameter sets that had yielded good fits to the local frequency variation (Fig. 2). However, the g-ratio was fixed at a value of 0.8 (the best fit value in Table 2) to reduce the computational time. The average difference between the simulated and estimated nonlocal fields was calculated over WM voxels for each subject. If the average difference over the five subjects was significantly different from zero, the underlying model was rejected. Fig. 5 shows results from one subject, calculated using the parameters that gave the smallest differences for the different mechanism combinations. For the $\chi_I + \chi_A$ and $\chi_I + E + \chi_A$ combinations, the simulated nonlocal field offsets in WM (Fig. 5 E and F) are in reasonable agreement with the model-based estimates from the experimental data (Fig. 5 B and C). However, for the $\chi_I + E$ combination, the generally negative model-based offsets (Fig. 5A) do not match the simulated data (Fig. 5D). These results show that only the fiber model combinations including susceptibility anisotropy produce nonlocal fields that are consistent with simulations based on the anatomical distribution of WM and GM (Fig. S1). Table 3 details the parameter values for each mechanism combination that produced the best fit to the local data (Fig. 2), as well as yielding a nonlocal field distribution that was in good agreement with the simulated offsets (Fig. 5).

### Table 2. Mechanism combinations and associated parameter values that best fitted the measured magnitude and frequency difference data shown in Fig. 2

<table>
<thead>
<tr>
<th>Mechanism combination</th>
<th>$T_{2,SP}, ms$</th>
<th>$T_{2,LP}, ms$</th>
<th>$\chi_I, ppb$</th>
<th>$E, ppb$</th>
<th>$\chi_A, ppb$</th>
<th>$\rho$</th>
<th>g-ratio</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\chi_I + E$</td>
<td>8 ± 2</td>
<td>36 ± 2</td>
<td>$-160 \pm 20$</td>
<td>50 ± 10</td>
<td>—</td>
<td>0.8</td>
<td>0.1</td>
<td>0.37</td>
</tr>
<tr>
<td>$\chi_I + \chi_A$</td>
<td>8 ± 2</td>
<td>38 ± 2</td>
<td>$-60 \pm 20$</td>
<td>—</td>
<td>$-140 \pm 20$</td>
<td>0.8</td>
<td>0.1</td>
<td>0.38</td>
</tr>
<tr>
<td>$\chi_I + E + \chi_A$</td>
<td>8 ± 2</td>
<td>36 ± 2</td>
<td>$-100 \pm 20$</td>
<td>20 ± 10</td>
<td>$-100 \pm 20$</td>
<td>0.7</td>
<td>0.1</td>
<td>0.25</td>
</tr>
</tbody>
</table>

The quoted error is the step length of the fitting algorithm. Also shown is the reduced chi-squared residual for each fit. If the residual was higher than 1.14 ($P < 0.05$), the fit was deemed unsuccessful.
Magnetic Susceptibility of Myelin and WM. The anisotropy of the magnetic susceptibility of the myelin can be characterized by the absolute difference in the magnitudes of the principal components of the cylindrically symmetric susceptibility tensor, which takes a value of $3\chi_A/2 = -180 \pm 30$ parts per billion (ppb). This value is consistent in magnitude and sign with the value of $\sim-200$ ppb, which was estimated by Lounia et al. (19) for the oriented lipids in lipoprotein shells. We can also estimate the difference in the average WM volume susceptibility when lipids in lipoprotein shells. We can also estimate the difference in magnitude between the myelin water compartment due to the low $T_2$ value of 6 ms recently estimated by van Gelderen et al. (22). This discrepancy is not surprising, because we have estimated $T_2$ rather than $T_2^*$ by separating off the effects of myelin sheath-induced frequency dispersion. The fitted exchange value of 10 ppb (Table 3) yields an average WM exchange-related frequency offset, relative to GM, of $\sim1$ ppb, which is lower than, but of the same sign as, the 6- to 13-ppb exchange-induced frequency offset range measured by Shmueli et al. (11) on postmortem brain tissue samples.

Implications for Phase Contrast. Dramatic differences are observed in phase-based frequency maps acquired at short TE relative to similar maps acquired at longer TE (Fig. 1). These results represent in vivo evidence for a significant local frequency offset in WM at long TE, which is dependent on microstructure, as predicted by He and Yablonsky (25). However, the theoretical framework used by these authors, which assumed purely isotropic susceptibility and a cylindrical Lorentz cavity with its axis parallel to the fiber direction, does not explain the orientation-dependent frequency differences that we measured in WM. In this study, we have shown that radial susceptibility anisotropy in the myelin sheath is needed to explain these effects and must therefore be included in an accurate myelin model. In particular, this anisotropy generates an average frequency offset that is positive in the myelin, but negative inside the lumen. Reduction of the signal from the myelin compartment consequently leaves a negative local frequency offset in WM relative to GM, which is strongest in fibers that are perpendicular to the field. This local frequency difference may in part explain the fact that the boundaries between GM and WM in phase images acquired at long TE appear sharper than from simulations of the frequency offset due to isotropic susceptibility differences (16). The work presented here clearly demonstrates that frequency maps created from GE phase images acquired at long TE do not fully represent the frequency perturbations due to the myelin compartment. The implications of this finding need to be carefully considered in studies using phase data to calculate quantitative information about differences in WM/GM tissue composition (18, 23).

Limitations of the Hollow Cylinder Model. Although the hollow cylinder model successfully describes the orientation-dependent contrast observed in WM, it is clearly a simplistic representation of the myelin sheath. The fitting yields g-ratio values of $\sim0.8$ (Table 2), which are larger than the expected average value of $\sim0.6$ (14), suggesting that the hollow cylinder model underestimates the thickness of the myelin sheath. A possible explanation for this underestimated is the laminae nature of the myelin sheath, which consists of repeating units formed from extracellular and cytoplasmic layers, separated by a lipid bilayer (17). It is likely that the water content and magnetic properties of each sublayer of the myelin sheath unit are very different from one another. An improvement on the model used here would be to break up the homogeneous cylindrical sheath into a series of layers.

Table 3. Mechanism combinations and associated parameter values that best fitted the local frequency variation with TE (Fig. 2), as well as yielding a nonlocal field in good agreement with simulated nonlocal offsets due to the WM/GM anatomy (Fig. 5)

<table>
<thead>
<tr>
<th>Mechanism combination</th>
<th>$T_{2\text{-GM}},$ ms</th>
<th>$T_{2\text{-LP}},$ ms</th>
<th>$\chi_A,$ ppb</th>
<th>$E,$ ppb</th>
<th>$\chi_A,$ ppb</th>
<th>$\rho$</th>
<th>$X_{1\text{-LP}},$ ppb</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_1 + \chi_A$</td>
<td>$10 \pm 2$</td>
<td>$38 \pm 2$</td>
<td>$-60 \pm 20$</td>
<td>$-120 \pm 0.1$</td>
<td>$20 \pm 0.70$</td>
<td>$X_1 + E + \chi_A$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$X_1 + \chi_A$</td>
<td>$10 \pm 2$</td>
<td>$36 \pm 2$</td>
<td>$-60 \pm 20$</td>
<td>$10 \pm 120 \pm 0.7$</td>
<td>$0.1 \pm 10.59$</td>
<td>$10 \pm 120 \pm 0.7$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The errors and residuals are also displayed, as described in Table 2. The g-ratio was fixed at 0.8 (best-fit value from Table 2) to reduce simulation time.
layers representing different water pools. Another extension to the model would be to include the effects of water diffusion in and between compartments. Diffusion in the inhomogeneous field outside the myelin sheath could lead to a reduction in dephasing, thus affecting the rate of decay of the signal magnitude, but the low diffusion coefficient of myelin water (26) means that the frequency difference values are unlikely to be significantly affected by diffusion.

**Applications of Frequency Difference Mapping.** Frequency difference mapping forms a powerful method for investigating WM microstructure; unlike phase mapping (1), it is insensitive to nonlocal frequency offsets produced by large anatomical structures and by external sources of field inhomogeneity, and in contrast to susceptibility mapping (23) and susceptibility tensor imaging (9), it does not require the solution of an ill-posed inverse problem. Data acquisition for frequency difference mapping is very simple, relying only on the use of multiecho gradient echo sequences, which are available on all modern scanners, and useful FDM can be generated from images acquired at just two echo times (Fig. 1). Because the frequency difference in WM is dependent on the sheath geometry (g-ratio and FVF) and orientation (11) of the fiber length with respect to the magnetic field, FDM may potentially be used to identify local fiber orientation. Because such measurements are based on standard GE MRI, it may be possible to generate high-resolution fiber orientation maps in this manner.

**Materials and Methods**

**Data Acquisition.** Data were acquired in vivo with the approval of the University of Nottingham’s Medical School Ethics Committee and all subjects gave informed consent. The GE data were acquired using a Philips Achieva 7T scanner. High-resolution data were acquired from three subjects using a 32-channel receiver coil with a 3D GE sequence [repetition time (TR) 36 ms; flip angle 14°; field of view (FOV) 224 × 224 × 50 mm3; in-plane resolution 0.6 mm; slice thickness 1.2 mm; scan time 5 min]. For the main data acquisition, subjects were imaged using a 3D GE sequence involving the acquisition of a train of 15 echoes (ΔTE 2 ms; TR 38 ms; flip angle 14°; FOV 224 × 224 × 50 mm3; isotropic resolution 0.6 mm; 256 × 256 × 160 mm3; isotropic resolution 1 mm) data were acquired on all subjects at 3 T, also using a 32-channel head coil.

**Data Processing.** Image data were coregistered using FSL FLIRT (27). Phase maps were unwrapped using a 3D algorithm (23), and frequency maps were then created from the high-resolution data by scaling the resulting phase data by 2πTE. For the main study, the phase map measured at TE 2 ms was subtracted from the other phase images before dividing by an effective echo time of (TE-2ms) to yield frequency maps with reduced sensitivity to any transmit-rf phase effects (23). All frequency maps were high-pass filtered using the SHARP method (23). The filtered frequency offsets that varied slowly with spatial position, and these were removed by subtraction of a sixth-order 3D polynomial fit. The magnitude data were normalized relative to the first TE data. The DTI data were processed using the FSL DTIFIT software (28) to yield eigenvectors and fractional anisotropy (FA) maps. The primary eigenvectors were then used to produce the fiber orientation maps. For analysis of the orientation-dependent frequency maps in WM, FSL FAST (29) was used to segment white matter in the high-resolution data with FA < 0.25 were not used in further analysis to ensure reliability of the fiber orientation information. The process for fitting the parameters of the hollow cylinder model involved iteratively varying each parameter over a sensible range, using a step length chosen to give a compromise between accuracy and fitting time. A detailed description of the fitting methods, including parameter ranges, is listed in SI Text. The quoted errors for all fitted parameters are equal to the relevant step lengths.

**ACKNOWLEDGMENTS.** This research was supported by Medical Research Council Programme Grant G0901321 and Engineering and Physical Sciences Research Council Fellowship Grant EP/I026924/1 (to S.W.).

References

Supporting Information

Wharton and Bowtell 10.1073/pnas.1211075109

SI Text

Frequency Perturbation Due to a Hollow Cylinder. Here we describe the spatially varying NMR frequency perturbation due to a long hollow cylinder of inner radius, \( r_0 \), and outer radius, \( r_o \), whose axis makes an angle, \( \theta \), with the applied magnetic field, \( B_0 \), listing the perturbations due to isotropic susceptibility, anisotropic susceptibility, and exchange effects resulting from the material of the hollow cylinder. In each case, the perturbation is described in cylindrical polar coordinates, and it is assumed that the \( B_0 \) field vector lies in the x-z plane. The ratio of the frequency offset to \( \omega_0 = \gamma B_0 \) is quoted, where \( \gamma \) is the magnetogyric ratio.

Frequency perturbation due to a hollow cylinder formed from material with isotropic magnetic susceptibility, \( \chi_I \):

\[
\Delta \omega_I(r) / \omega_0 = \begin{cases} 
\frac{\chi_I}{2} \sin^2 \theta \cos 2\phi \left( \frac{r_0^2 - r_1^2}{r_0^2} \right) & : r > r_0 \\
\frac{\chi_I}{2} \left( \cos^2 \theta - \frac{1}{3} \sin^2 \theta \cos 2\phi \left( \frac{r_1^2}{r_0^2} \right) \right) & : r_0 > r > r_1 \\
0 & : r < r_1
\end{cases}
\]  

\[ \text{[S1]} \]

The average frequency offset (averaged over \( r \) and \( \phi \)) in each compartment is given by

\[
\frac{\langle \Delta \omega_I \rangle}{\omega_0} = \begin{cases} 
0 & : r > r_0 \\
\frac{\chi_I}{2} \left( \cos^2 \theta - \frac{1}{3} \sin^2 \theta \cos 2\phi \left( \frac{r_1^2}{r_0^2} \right) \right) & : r_0 > r > r_1 \\
0 & : r < r_1
\end{cases}
\]  

\[ \text{[S2]} \]

Frequency perturbation due to a hollow cylinder composed of material in which exchange, \( E \), occurs.

\[
\Delta \omega_E(r) / \omega_0 = \begin{cases} 
0 & : r > r_0 \\
E & : r_0 > r > r_1 \\
0 & : r < r_1
\end{cases}
\]  

\[ \text{[S3]} \]

The average frequency offset in each compartment follows the same form.

Frequency perturbation due to a hollow cylinder formed from material with anisotropic magnetic susceptibility, such that the principal axis of the cylindrically symmetric susceptibility tensor is radially directed.

The average frequency offset in each compartment is given by

\[
\frac{\langle \Delta \omega_A \rangle}{\omega_0} = \begin{cases} 
0 & : r > r_0 \\
\frac{\chi_A}{2} \left( -\frac{1}{3} \sin^2 \theta \left( 1 - \frac{3}{2} \frac{r_1^2}{r_0^2} \ln \left( \frac{r_0}{r_1} \right) \right) \right) & : r_0 > r > r_1 \\
\frac{3\chi_A \sin^2 \theta}{4} \ln \left( \frac{r_0}{r_1} \right) & : r < r_1
\end{cases}
\]  

\[ \text{[S5]} \]

Derivation of Frequency Perturbation Due to a Hollow Cylinder with Radial Anisotropic Magnetic Susceptibility. In the reference frame in which its principal component is aligned with the x-axis, the anisotropic part of the cylindrically symmetric susceptibility tensor can be written as

\[
\chi_{\text{a}} = \begin{bmatrix} 
0 & 0 & \chi_A/2 \\
0 & 0 & -\chi_A/2 \\
-\chi_A/2 & \chi_A/2 & 0
\end{bmatrix}
\]  

\[ \text{[S6]} \]

where \( \chi_A \) characterizes the anisotropic magnetic susceptibility. We assume that this anisotropy results from the radially oriented lipid chains in the hollow cylinder formed by the myelin sheath. Transforming to a common reference frame that is defined with respect to the hollow cylinder and using standard cylindrical coordinates, we can write the susceptibility tensor at locations defined by azimuthal angle, \( \phi \), as

\[
R_z(\phi) \chi R_z^{-1}(\phi) = \begin{bmatrix} 
\cos \phi & \sin \phi & 0 \\
-\sin \phi & \cos \phi & 0 \\
0 & 0 & 1
\end{bmatrix} \times \begin{bmatrix} 
0 & -\sin \phi & \cos \phi \\
\cos \phi & -\sin \phi & 0 \\
0 & 0 & 1
\end{bmatrix}
\]  

\[ \text{[S7]} \]

This expression can be simplified to

\[
R_z(\phi) \chi R_z^{-1}(\phi) = \frac{3\chi_A}{4} \begin{bmatrix} 
\cos 2\phi + 1/3 & -\sin 2\phi & 0 \\
-\sin 2\phi & 1/3 - \cos 2\phi & 0 \\
0 & 0 & -2/3
\end{bmatrix}
\]  

\[ \text{[S8]} \]

If we assume that the z-axis of the hollow cylinder makes an angle, \( \theta \), with the applied magnetic induction, \( B_0 \), and lies in the...
where $H_0 = B_0 / \mu_0$ and $\mu_0$ is the vacuum magnetic permittivity.

Using $\mathbf{M} = \frac{1}{2} \mathbf{H}$ and Eqs. S8 and S9, we then find the magnetization within the body of the hollow cylinder (i.e., in the region where $r_o > r > r_i$) is given by

$$
\mathbf{M} = \frac{3\chi_A H_0}{4} \left[ \begin{array}{c}
(\cos 2\phi + 1/3) \sin \theta \\
-\sin 2\phi \sin \theta \\
-2 \cos \theta/3
\end{array} \right].
$$

S10

We now evaluate the magnetic scalar potential, $\Phi(r)$, that is produced by this magnetization distribution. This potential, which is related to the field perturbation, $H(r) \times \mathbf{H} = -\nabla \Phi(r)$, is given by (1)

$$
\Phi(r) = -\frac{1}{4\pi} \int \frac{\nabla \cdot \mathbf{M}_S (\mathbf{r})}{|\mathbf{r} - \mathbf{r}'|} d^3 r' + \frac{1}{4\pi} \oint \mathbf{n} \cdot \mathbf{M}_S (\mathbf{r}') \frac{dS'}{|\mathbf{r} - \mathbf{r}'|}.
$$

S11

where $\mathbf{n}$ is the unit vector that is locally normal to the surface of the magnetization distribution. First, let us consider the surface integral term in Eq. S11 that comprises contributions from the outer ($r = r_o$) and inner ($r = r_i$) surfaces of the hollow cylinder. At the outer surface, $\mathbf{n}$ points away from the axis of the cylinder, and by using Eqs. S10 and S11 we find that the integral over the outer surface can be written as

$$
\Phi_S(r) = \frac{\chi_A H_0 r_o \sin \theta}{4\pi} \int_0^\infty \frac{d\phi}{2\pi} \int_0^{\infty} \frac{dz \cos \phi}{|z - r|}.
$$

S12

Using the Green’s function expansion of $|z - r|^{-1}$ in cylindrical coordinates and integrating over $\phi'$ and $z'$ then gives

$$
\Phi_S(r) = \frac{\chi_A H_0 r_o \sin \theta \cos \phi}{4\pi} \left\{ \begin{array}{ll}
\frac{r_o - r_i}{2r_o} & : r > r_o \\
\frac{1}{2} \frac{r - r_i}{r} & : r_o > r > r_i \\
0 & : r < r_i
\end{array} \right. 
$$

S13

where $r_o$ and $r_i$ represent the larger and smaller of the two terms $r$ and $r_i$ (i.e., $r_o = r$ and $r_i = r_i$ for $r > r_o$, and $r_o = r_i$ and $r_i = r$ for $r < r_i$). Following a similar procedure for the surface integral at the inner surface of the hollow cylinder, while taking account of the fact that the surface normal here points radially toward the axis of the cylinder, we find that the total potential generated by the surface integrals in the three regions—(i) outside the hollow cylinder ($r > r_o$); (ii) in the body of the hollow cylinder ($r_o > r > r_i$); (iii) inside the hollow cylinder ($r < r_i$)—is

$$
\Phi_S(r) = \frac{\chi_A H_0 r_o \sin \theta \cos \phi}{4\pi} \left\{ \begin{array}{ll}
\frac{r_o - r_i}{2r_o} & : r > r_o \\
\frac{1}{2} \frac{r - r_i}{r} & : r_o > r > r_i \\
0 & : r < r_i
\end{array} \right. 
$$

S14

To calculate the full scalar potential, we must also include the contribution of the volume integral in Eq. S11. When considering material of isotropic magnetic susceptibility, this integral can be neglected because the divergence of the magnetization is always zero; however, for the arrangement of anisotropic material considered here, this is not the case and we find

$$
\nabla \cdot \mathbf{M}_A = \frac{3}{2} \chi_A H_0 \sin \theta \frac{\cos \phi}{r}.
$$

S15

Substituting Eq. S15 into Eq. S11, and using a similar approach to that used in the calculation of the surface integral, we find that the volume integral’s contribution to the scalar potential is given by

$$
\Phi_V(r) = \left\{ \begin{array}{ll}
\frac{3}{8} \chi_A H_0 r_o \sin \theta \cos \phi \left( \frac{r_o - r_i}{r} \right) & : r > r_o \\
\frac{3}{8} \chi_A H_0 r_o \sin \theta \cos \phi \left( \frac{1}{2} \frac{1 - r_i}{r} \right) + \ln \left( \frac{r_o}{r_i} \right) & : r_o > r > r_i \\
\frac{3}{8} \chi_A H_0 r_o \sin \theta \cos \phi \ln \left( \frac{r_o}{r_i} \right) & : r < r_i
\end{array} \right.
$$

S16

We are only interested in the component of the magnetic field, $\mathbf{H}(r)$, generated by this potential, which is aligned with the main magnetic field. If we calculate $-\nabla \Phi$ in the frame of reference defined by the fiber nerve, the relevant field contribution is

$$
\Delta H_A = -\frac{\chi}{\mu_0} \sin \theta \cos \theta - \frac{\chi}{\mu_0} \cos \theta \sin \theta,
$$

S17

which reduces to $-\frac{\chi}{\mu_0} \sin \theta$ because the potential is invariant with $z$-coordinate. Evaluating this expression using the total potential formed from the sum of $\Phi_S$ and $\Phi_V$ yields

$$
\Delta H_A(r) = \left\{ \begin{array}{ll}
\frac{\chi_A H_0 r_o \sin \theta \cos \phi}{8} \left( \frac{r_o - r_i}{r} \right) & : r > r_o \\
\frac{\chi_A H_0 r_o \sin \theta}{8} \left( -4 - \cos 2\theta \left( \frac{r_i - r_o}{r} \right)^2 - 3 \right) + 6 \ln \left( \frac{r_o}{r_i} \right) & : r_o > r > r_i \\
\frac{3\chi_A H_0 r_o \sin \theta}{4} \ln \left( \frac{r_o}{r_i} \right) & : r < r_i
\end{array} \right.
$$

S18

Because $\mathbf{B} = \mu_0 (\mathbf{H} + \mathbf{M})$, we must add in the direct contribution of the magnetization, to find the perturbation of the magnetic induction, $\Delta \mathbf{B}_A$, which dictates the change in NMR frequency. However, in doing this it is important to apply the sphere of Lorentz correction (2) that is needed to yield the field actually experienced by the resonant nuclei. In this case, we must add an additional contribution to the magnetic field, $\delta \mathbf{B}_k$, in the region ($r_o > r > r_i$), which can be calculated from Eq. S10 using

$$
\delta \mathbf{B}_k = \frac{\mu_0}{3} \left( \sin \theta \mathbf{\hat{x}} \mathbf{M}_A + \cos \theta \mathbf{\hat{z}} \mathbf{M}_A \right)
$$

where $\mathbf{\hat{x}}$ and $\mathbf{\hat{z}}$ are unit vectors in the reference frame that is defined with respect to the fiber nerve. The final expression for the field perturbation then becomes

$$
\delta \mathbf{B}_A = \frac{\mu_0}{3} \left( \sin \theta \mathbf{\hat{x}} \mathbf{M}_A + \cos \theta \mathbf{\hat{z}} \mathbf{M}_A \right)
$$

S19
To gain further insight into the effect of these field perturbations on the NMR signal, it is useful to calculate the average field offset in each compartment, which is given by

\[
\langle \Delta B_A \rangle = \left\{ \frac{X_A}{2} \left( -\frac{1}{3} \sin^2 \theta \left( 1 - \frac{3}{2} \frac{r^2}{R^2 - r^2} \ln \left( \frac{r}{R} \right) \right) \right) : r > r_o \\
- \frac{3X_A \sin^2 \theta}{4} \ln \left( \frac{r}{R} \right) : r < r_i \\
\frac{X_A}{2} \left( -\frac{1}{3} \sin^2 \theta \left( 1 - \frac{3}{2} \frac{r^2}{R^2 - r^2} \ln \left( \frac{r}{R} \right) \right) \right) : r_o > r > r_i 
\]

[S20]

The frequency offsets described in Eqs. S3 and S4 can be simply derived from Eqs. S19 and S20 using the Larmor equation.

**Fitting the Hollow Cylinder Model to Frequency Difference and Magnitude Data.** The fit was carried out by iteratively stepping through parameter values over a chosen range. The range and step-length values for each parameter were chosen to give a good compromise between accuracy and fitting time, taking values (min – max: step length) of g-ratio (0.5–0.9: 0.1); T2,SP (2–20 ms: 2 ms); T2,LP (22–50: 2 ms); ρSP (0.1–1.5: 0.1); γI (−0.24–0.24 ppm: 0.02 ppm); E (−0.12–0.12 ppm: 0.01 ppm); γA (−0.24–0.24 ppm: 0.02 ppm).

For simulating the frequency offsets, a hollow cylinder with an outer radius r_o = 20 pixels was placed at the center of a 2D matrix of 69 × 69 pixels in size. The g-ratio was adjusted by varying the internal radius, r_i. The spatially varying frequency offsets for each of the contrast mechanisms were simulated in the 2D matrix using the analytical expressions listed above. In each iteration, frequency offset maps were calculated for the five θ values corresponding to the mean values of the θ ranges used in analyzing the in vivo data (9°, 27°, 45°, 63°, and 81°). For each of the four mechanism combinations (γI; γI + E; γI + γA; and γI + E + γA), a composite map was created by superposing the simulated offsets due to each mechanism after appropriate modulation by the values of γI, E, and γA. The NMR signal, S, at echo time, TE, was simulated via

\[
S(TE) = \sum_i \rho_i \exp \left( iTE \Delta B_i - \frac{TE}{T_2} \right),
\]

where the index i denotes the pixel number in the 2D matrix of ΔB values, ρ is the relative proton density (which takes a value of 1 for pixels in the external and internal compartments and ρSP for pixels within the cylindrical annulus), γ is the magnetogenic ratio, and T2 is the decay constant (T2 = T2,LP for pixels in the external and internal compartments and T2 = T2,SP for pixels within the cylindrical annulus). In this study, we defined the sign of the frequency by f = +gΔB. The fiber volume fraction (FVF) was set at 0.5 by only summing signal contributions from within a central, circular region of interest of radius 28.3 pixels (r_e/FVF)^2. The phase and magnitude of the complex signal, S, were then calculated. The phase was processed in an identical manner to that applied to the experimental data to yield frequency difference values.

For each parameter set and mechanism combination, the simulated magnitude and frequency difference values were compared with the measured data. A chi-squared residual was formed by summing the square of the residual difference between simulated and measured data scaled by the reciprocal of the square of the SD over subjects for each measurement. A reduced chi-squared residual was then calculated using the hollow cylinder model on a voxel-by-voxel basis from the frequency maps measured at different TE values, and then averaging the differences over TE;

\[
\chi^2 = \sum_i \left( \frac{S_i(\text{sim}) - S_i(\text{meas})}{\text{SD}_i} \right)^2
\]

If the reduced residual was higher than 1.14 (P < 0.05), the fit was deemed unsuccessful.

**Nonlocal Fields: Comparing Model-Based Estimates Derived from Experimental Measurements to Forward Field Calculations.** A comparison of two different model-dependent estimates of the nonlocal field perturbations generated by the differences in susceptibility of white matter (WM) and grey matter (GM) was carried out to test which combinations of the three different contrast mechanisms could explain the experimental data when used in conjunction with the hollow cylinder model. Two nonlocal field maps were produced for each mechanism combination and parameter set that successfully characterized the local signal behavior: (i) an estimate was derived from the experimental data by subtracting off the effect of the TE-dependent local frequency offset, calculated using the hollow cylinder model on a voxel-by-voxel basis from the frequency maps measured at different TE values, and then averaging the differences over TE; (ii) a simulated map was produced by a forward field calculation based on the anatomical distribution of GM and WM derived from T1-weighted image data.

**Field simulations.** To calculate the nonlocal field perturbations produced by differences in the average susceptibility of WM and GM, forward field calculations were carried out using susceptibility distributions based on segmented tissue masks generated from the magnetization-prepared rapid gradient echo (MPRAGE) data of each subject. We calculated the field offset, ΔB(\text{WM-WM}) produced by a susceptibility distribution in which WM and GM were given isotropic susceptibility values of 1 and 0 ppm, respectively, and also calculated the offset, ΔB(\text{GM-CSF}) generated when WM regions with fractional anisotropy (FA) >0.25 were allocated an anisotropic susceptibility of magnitude of 1 ppm, with the direction of the principal axis of the cylindrically symmetric susceptibility tensor defined using fiber orientation information from the diffusion tensor imaging (DTI) data. In these simulations it was assumed that the susceptibility of gray matter is purely isotropic and that the susceptibilities of GM and CSF are the same. The steps followed in calculating ΔB(\text{WM-WM}) and ΔB(\text{GM-CSF}) are described below. The simulated offsets were then transformed into the same space as the GE data using spatial transformations identified from coregistration of the GE magnitude and MPRAGE images.

**Simulations of the nonlocal field due to isotropic and anisotropic susceptibility.** For simulating ΔB(\text{WM-WM}), the binary susceptibility distribution described above was convolved with the dipole field kernel to create a field map for each subject (3, 4). To be consistent with the experimentally measured data, this field map was then high-pass filtered using the SHARP method (5), with a kernel of radius 2 mm and a truncation value of 1 × 10^{-6} followed by the subtraction of a sixth-order 3D polynomial fit.

For the simulation of the nonlocal field, ΔB(\text{WM-WM}) due to the anisotropic susceptibility of WM, a cylindrically symmetric anisotropic susceptibility tensor, given by

\[
\chi = \begin{bmatrix}
X_{\text{WM}} & 0 & 0 \\
0 & -X_{\text{WM}}/2 & 0 \\
0 & 0 & -X_{\text{WM}}/2
\end{bmatrix}
\]

[S22]

was assigned to each voxel in WM regions where the FA value was greater than 0.25, and X_{\text{WM}} was set to a value of 1 ppm. The orientation of the tensor for each WM voxel was aligned with the corresponding principal fiber orientation vector calculated from the DTI data. A Fourier method was then used to simulate the field perturbation due to this anisotropic susceptibility distribution. For each voxel, the orientation of the principal axis of the susceptibility tensor relative to B_0 can be expressed using the spherical polar angles ϑ and φ. The magnetization induced
in the sample reference frame due to this susceptibility tensor is then given by
\[
M = \frac{\chi_{A-WM}}{4} \begin{bmatrix} 3 \sin 2\theta \cos \phi_T \\ 3 \sin 2\theta \sin \phi_T \\ 1 + 3 \cos 2\theta_T \end{bmatrix}.
\] [S23]

The z-component of the field perturbation, \( \Delta B_z \), produced by this magnetization can be derived by transforming the problem into the Fourier domain and using a spherical harmonic expansion, yielding
\[
\Delta B_z = \frac{B_0}{4} \text{IFT} \left\{ \text{FT} \left[ \chi_{A-WM} \sin 2\theta \cos \phi_T \right] \cdot \left( \sin 2\theta_0 \cos \phi_T / 2 \right) \right\} + \text{IFT} \left\{ \text{FT} \left[ \chi_{A-WM} \sin 2\theta \sin \phi_T \right] \cdot \left( \sin 2\theta_0 \sin \phi_T / 2 \right) \right\} + \text{IFT} \left\{ \text{FT} \left[ \chi_{A-WM} \left( 1 + 3 \cos 2\theta_T \right) \right] \cdot \left( \cos^2 \theta_0 - 1/3 \right) \right\}.
\] [S24]

Here, \( \theta_0 \) and \( \phi_T \) are the spherical polar coordinates measured relative to the \( B_0 \) direction, whereas \( \text{IFT} \{ \} \) denotes a 3D inverse-Fourier transform. It is worth noting that the contributions to \( \Delta B_z \) of the \( x \)- and \( y \)-components of the magnetization have been accounted for in the derivation of Eq. [S24] (6). Following calculation of \( \theta_T \) and \( \phi_T \) for relevant WM voxels using the associated DTI fiber orientation map from each subject, \( \Delta B(\chi_{A-WM}) \) can be calculated using Eq. [S24].

The total nonlocal field, \( \Delta B(\chi_{WM}) \), was formed from the weighted combination of \( \Delta B(\chi_{A-WM}) \) and \( \Delta B(\chi_{A-WM}) \):
\[
\Delta B(\chi_{WM}) = \left( \chi_I + \chi_{1-LP} \right) \Delta B(\chi_{A-WM}) - \frac{1}{2} \chi_A \Delta B(\chi_{A-WM}).
\] [S25]

Here, \( \nu \) is the volume fraction of WM occupied by myelin, \( \chi_I \) is the difference in the isotropic susceptibility of myelin relative to the general matrix of WM tissue in which the myelin sheaths are embedded, and \( \chi_{1-LP} \) is the difference in the isotropic susceptibility of this general WM relative to GM and CSF, whereas \( \chi_A \) characterizes the radially oriented anisotropic susceptibility in the myelin sheath. The values of \( \chi_I \) and \( \chi_A \) are predefined by fitting to the local frequency data shown in Fig. 2, leaving \( \chi_{1-LP} \) as the single variable parameter in fitting to the model-based estimate of the nonlocal field measurements.

The factor of \(-1/2\) used in weighting in the \( \Delta B(\chi_{A-WM}) \) term in Eq. [S25] is needed to properly represent the average contribution of the radially oriented anisotropic susceptibility in the myelin sheaths to the nonlocal field. When the hollow cylinder formed by a myelin sheath is oriented parallel to the \( B_0 \) field, the induced magnetization is directed along the field direction and takes a value of \(-H_{0z_{A}}/2\) at all positions in the sheath. However, when the sheath is perpendicular to the field, the magnetization directed along the applied field direction varies in magnitude from \( H_{0z_{A}} \) at azimuthal positions where the field is radially directed, to \(-H_{0z_{A}}/2\), at positions where the field is azimuthally directed. Averaging over the entire sheath yields an average magnetization of \( H_{0z_{A}}/4 \) along the field direction, whereas the magnetization perpendicular to the field averages to zero. This behavior can be represented by using a cylindrically symmetric susceptibility tensor of the form shown in Eq. S22 with \( \chi_{A-WM} = -\chi_A/2 \), as has recently been described by Li et al. (7).

**Estimating the nonlocal fields from the experimental data.** For generating the model-dependent estimates of the nonlocal fields from the experimental data, the variation of the local frequency (rather than the frequency difference) with TE was simulated using the hollow cylinder fiber model on a voxel-by-voxel basis for WM voxels identified from the DTI data as having FA >0.25. The average orientation of fibers with respect to the field at each WM voxel, which was required for calculating the local fields, was then subtracted from the measured frequency at each TE to yield the effective nonlocal field at each relevant voxel. However, through subtraction of the local frequency variation, the contribution of the annulus of the hollow cylinder (myelin sheath) to the nonlocal field has been removed and must be reintroduced to ensure the myelin component is properly accounted for. The myelin contribution was reintroduced by adding a field of magnitude, \( \nu B_0 \left( \chi_I + 1 + \chi_{1-LP} \right) \times \left( \cos^2 \theta - 1/3 \right) \), to the nonlocal field maps at each WM voxel for each TE. Finally, these adjusted nonlocal field maps were averaged over each TE value to give a single model-dependent nonlocal field map; this was done for each mechanism combination and parameter set that had yielded a successful fit to the frequency difference and magnitude data shown in Fig. 2.

**Comparison of nonlocal field estimates.** The model-specific nonlocal field maps generated from the experimental data were compared with simulated maps produced using Eq. S25, with \( \chi_{1-LP} \) values ranging from \(-0.1\) to \(0.1\) ppm in \(0.01\)-ppm increments, by taking the difference and summing the squares of the difference over WM voxels. The \( \chi_{1-LP} \) value that yielded the lowest least-squares residual was then used for further comparisons and average difference calculations, and is listed in Table 3. As described in the main text, average difference values were formed for each subject and for each mechanism combination. Fig. S1 shows the variation of the average difference values over WM voxels in each subject for each mechanism combination (in each case using the parameters that gave the smallest differences). The plot includes the average differences for the representative images from subject 1 that are shown in Fig. 5. The error bars represent the SD of the average difference over TE values. The plot clearly shows that only models incorporating anisotropic susceptibility \( (\chi_I + \chi_A) \) yield a low average difference, whereas the \( \chi_I + \chi_{1-LP} \) combination produces a significant average difference of \(-1.5\) Hz.

**Previous Work on Frequency Difference Mapping.** Frequency difference mapping was first described in an abstract by Schweser et al. (8) titled “Nonlinear Evolution of GRE Phase as a Means to Investigate Tissue Microstructure,” which was presented at the 19th Meeting of the International Society for Magnetic Resonance in Medicine. The authors reported measurement of a significant fiber orientation-dependent frequency difference in the optic radiations of a healthy subject at 3 T.

**Fig. S1.** Plot of the average difference values over WM voxels in each subject, for each mechanism combination. The plot shows that only models incorporating anisotropic susceptibility have a low average difference. Error bars represent the SD of the average difference over TE values.