Published in final edited form as:

Magn Reson Med. 2019 January ; 81(1): 628–638. doi:10.1002/mrm.27398.

White matter inter-compartmental water exchange rates determined from detailed modeling of the myelin sheath

Peter van Gelderen and Jeff H Duyn

Advanced MRI Section, Laboratory of Functional and Molecular Imaging, National Institute of Neurological Disorders and Stroke, National Institutes of Health

Abstract

Purpose—Magnetization exchange (ME) between hydrogen protons of water and large molecules (semisolids) in lipid bilayers is an important factor in MRI signal generation and can be exploited to study white matter pathology. Current models used to quantify ME in white matter generally consider water to reside in one or two distinct compartments, ignoring the complexities of the myelin sheath's multi-compartment structure of alternating myelin semisolids and myelin water layers. Here, we investigated the effect of this by fitting ME data obtained from human brain at 7 T with a multi-layer model of myelin.

Methods—A multi-echo acquisition, for a T_2^* based separation of myelin water from other water signals, combined with various preparation pulses to change the (relative) state of the semisolid and water pools, analyzed by fitting with a multi-layer exchange model.

Results—The estimated lifetime within a single myelin water layer was 260 μ s, corresponding to a lipid bilayer permeability of 6.7 μ m/s. The magnetization lifetime of the aggregate of all myelin water was estimated at 13 ms, shorter than previously reported values in the range of 40–140 ms.

Conclusion—Contrary to expectations and previous reports, ME between protons in myelin semisolids and water is not limited by the myelin sheath, but rather by exchange between semisolids and water protons. The relatively short myelin water lifetime should be accounted for in the analysis of ME contrast, and affects the interpretation of tissue compartmentalization from MRI contrasts such as T_1 - and diffusion-weighting.

Keywords

exchange; magnetization transfer; myelin; myelin water; white matter; myelin permeability

Introduction

The exchange of magnetization between the hydrogen protons of water (“water protons”) and hydrogen protons of larger molecules can be an important contributor to MRI contrast. For example, it allows distinguishing between grey and white matter (1). Major contributors to magnetization exchange (ME, or “magnetization transfer”, MT) in brain tissue are

hydrogen protons of proteins and lipids, whose magnetization levels tend to differ from those of water protons because of their differing T_1 and sensitivity to saturation by RF (radio-frequency) pulses. MT effects can be enhanced by dedicated techniques that use RF irradiation pre-pulses (MT pulses) to directly and selectively saturate the magnetization of the short T_2 (and thus MRI-invisible) hydrogen protons on proteins and lipids, here referred to as “semisolid protons”.

While T_1 and MT techniques are being used widely to enhance contrast between grey and white matter, and in particular to study white matter pathology, quantitative interpretation has been hampered by the complexity of the ME processes. For example, in white matter, ME is governed by the complex structure of fiber bundles and axonal myelin sheaths, and is imperfectly described by a simple model of exchange between two homogeneous magnetization pools (2,3). A number of studies have expanded the model to include additional pools to accommodate the different properties of water and semisolids within versus outside the myelin sheath (4,5). However, uncertainty remains about the precise values of the parameters that govern ME in WM, in particular the exchange between the myelin sheath and surrounding water (4–7). To address this, we further refined the model to also distinguish between the multiple water and semisolid layers that make up the myelin sheath.

To allow extraction of the values for the increased number of parameters in this refined model, a recently developed pulsed MT approach (8) was adapted and combined with a multi-gradient-echo (MGRE) acquisition method. Performed at high field, it allowed sensitive discrimination between water within and outside the myelin sheath based on differences in magnetic environment in these pools (9,10). By using a variety of preparation pulses that differentially affected the magnetization of water and semisolids, the ME kinetics between these pools could be determined, as well as between water protons within and outside myelin.

Theory

In modeling the layer structure of the myelin sheath, we considered the ME process to be symmetric around the center of the myelin sheath, with similar magnetization flows going from the sheath towards axonal and interstitial water. This was based on the assumption that the axonal and interstitial water have similar relaxation and exchange properties and volume fractions. This allowed us to combine these water types into a single pool of magnetization termed “other water” (OW), and consider only half the thickness of the myelin sheath (Fig. 1).

The magnetization of individual water and semi-solids (SS) layers within the sheath is separately modeled. Each water layer is exchanging magnetization with its neighboring water compartments, crossing one SS layer. A semisolids compartment in the model represents the combination of half of each of the semi-solid layers neighboring an MW layer, see Figure 1A. ME also occurs between OW and a separate SS compartment. The magnetization within each compartment is considered to be well mixed on the timescale of the exchange process. Considering the aggregate magnetization of compartments with

similar NMR properties to form a “pool”, our model is referred to as the ML3 model, for ‘multilayer with three pools’, the pools being SS, MW or OW. All compartments in one pool share the same relaxation and exchange properties (and are the same size), but the magnetization in each can be different as a consequence of the exchange processes. To limit the number of parameters in the model, the SS compartment in exchange with the OW is given the same size, exchange rate and relaxation rates as the other SS compartments.

To model the magnetization in each compartment, a set of Bloch equations was formulated incorporating the effects of ME between various compartments, resulting in a coupled set of differential equations. For simplicity, here we show only the equations for the longitudinal magnetization without RF pulses; the full model included both transverse and longitudinal magnetization with rotation terms for RF and off-resonance effects:

$$\begin{aligned} \frac{f_{MW}}{N} \frac{dM_{z,i}^{MW}}{dt} = & \frac{f_{MW}}{N} (1 - M_{z,i}^{MW}) R_{1,MW} - \left(2k_{WW} + \frac{k_{WS}}{N+1} \right) M_{z,i}^{MW} + k_{WW} (M_{z,i+1}^{MW} + M_{z,i-1}^{MW}) \\ & + \frac{k_{WS}}{N+1} M_{z,i}^{SS} \end{aligned} \quad [1]$$

$$\frac{f_{SS}}{N+1} \frac{dM_{z,i}^{SS}}{dt} = \frac{f_{SS}}{N+1} (1 - M_{z,i}^{SS}) R_{1,SS} - \frac{k_{WS}}{N+1} M_{z,i}^{SS} + \frac{k_{WS}}{N+1} M_{z,i}^{MW} \quad [2]$$

$$\begin{aligned} f_{OW} \frac{dM_z^{OW}}{dt} = & f_{OW} (1 - M_z^{OW}) R_{1,OW} - \left(k_{WW} + \frac{k_{WS}}{N+1} \right) M_z^{OW} + k_{WW} M_{z,N}^{MW} + \frac{k_{WS}}{N+1} M_{z,N+1}^{SS} \end{aligned} \quad [3]$$

Where N is the number MW compartments in the model, f_{MW}/N equals the fraction of spins in one MW compartment (i.e. the fraction of the equilibrium magnetization), and f_{MW} is the total MW pool fraction. Likewise, f_{SS} is the semisolids pool fraction, f_{OW} the OW pool fraction. k_{WS} is the exchange rate between water and semisolids, k_{WW} is the exchange rate between water compartments. k_{WS} is defined as the total exchange, the sum of all $(N+1)$ SS compartments exchanging in parallel. The reader is referred to the section “Symbols & Abbreviations” for a comprehensive explanation of all symbols. Eq. 1 is only valid for $i = 2, \dots, N-1$, for $i = 1$ or N , a small modification is required; Eq. 2 is valid for $i = 1, \dots, N$, for $i = N+1$, again a modification is required, see supplementary material (SM:Theory) for details. A note on the normalization: 1) the magnetization in each compartment is normalized to its own equilibrium level ($M_z(\infty) = 1$ for every compartment) 2) the exchange rates and pool

sizes are normalized to the total number of spins. This formulation was chosen to stress that there are only two independent exchange rates in the model. Division of each equation by its respective compartment size yields a formulation with exchange rates relative to the compartment sizes, as e.g. in (3,11), see SM:Theory.

The exchange processes can also be expressed in terms of lifetimes (2,12) instead of exchange rates. The lifetime is the average time that a spin resides in one compartment (considering only exchange effects). The lifetime follows directly from the compartment size and the sum of the relevant exchange rates: $\tau \equiv \#/\Sigma k$. For example, the MW lifetime becomes:

$$\tau_{\text{MW}} = \frac{f_{\text{MW}}}{N(2k_{\text{WW}} + k_{\text{WS}}/(N+1))} \quad [4]$$

The permeability of a single myelin bilayer is related to this lifetime. The permeability is expressed as a volume per unit area per unit time. The relevant volume (V) is half the thickness of a single MW layer times the area (A), as there is exchange on both sides of the MW layers. The expression for the permeability becomes:

$$P_{\text{M}} = \frac{V}{A\tau} = \frac{Ad/2}{A\tau} = \frac{Nk_{\text{WW}}d}{f_{\text{MW}}} \quad [5]$$

where d is the thickness of a single MW layer and τ is the MW lifetime when considering only exchange across the myelin lipid bilayers (Eq. 4 with $k_{\text{ws}} = 0$).

In an MRI experiment, the signals from the individual MW compartments cannot be observed separately, only the average over the MW pool can be obtained. To facilitate the interpretation of the model and comparison to MRI data, the decay time was defined as the time the difference between a pool's average magnetization and its equilibrium level decays to 1/e of its initial value, in the absence of relaxation effects and assuming the other pools start with $M_z = 0$. For example, for OW this can be calculated using an initial condition of $M_z^{\text{OW}}(0) = 1$. The resulting equilibrium is then given by the pool size: $M_z^{\text{OW}}(\infty) = f_{\text{OW}}$, and

so the decay time is the time point where $M_z^{\text{OW}}(T_{\text{OW}}) = \frac{1 - f_{\text{OW}}}{e} + f_{\text{OW}}$. Note that even for the single compartment OW pool, this time can be different than the lifetime, as the decay time also accounts for spins that are returning after having left the compartment. The lifetime (τ) is a measure of the timescale of the exchange effects in a single compartment, in contrast, the decay time (T) serves as a measure of exchange effects on the pool averaged magnetization levels. For example, exchange between two MW compartments contributes to a shorter τ , but it does not alter T of the aggregate MW pool.

The MGRE data that was used as input for this study was modeled as three separate (non-exchanging) water pools (representing myelin water, axonal water and interstitial water), as in (9,13). The model describing the complex signal as function of echo time is given by:

$$S(t_e) = (A_1 e^{-R_{2,1}^* t_e + i\nu_1 t_e} + A_2 e^{-R_{2,2}^* t_e + i\nu_2 t_e} + A_3 e^{-R_{2,3}^* t_e} e^{i\nu_c t_e + i\varphi_c}) \quad [6]$$

Indices 1,2 and 3 refer to myelin water, axonal water and interstitial water respectively. A_i is the amplitude, $R_{2,i}^*$ the relaxation rate, ν_c is the common frequency offset, ν_1 and ν_2 are the frequency differences with respect to the common frequency offset, φ_c is the common phase.

Some assumptions of the model were tested by analyzing the data with different versions of the multilayer model, see SM. The fit of the model to the data was also compared to fitting of a simpler model without layers, the SL3 model. This model consisted of three exchanging single compartments in a linear configuration (SS-MW-OW).

Methods

MRI Acquisition

Ten subjects (8 female, age 22–49, average 30 years) were scanned on a Siemens (Erlangen, Germany) Magnetom 7 T MRI scanner, under an IRB approved protocol. To study the exchange properties of myelin water, MGRE experiments were performed with magnetization preparations that differentially affected the semisolid and water spins. The evolution of the magnetization under the effects of T_1 relaxation and ME was then followed by varying the delay between preparation and MGRE acquisition. Four different types of preparation were used: REF: no pre-pulse, IR: a single inversion pulse, 2IR: two sequential inversion pulses spaced 3 ms apart, and DIR: two inversion pulses spaced 560 ms apart. Simulation of the inversion pulse showed that the M_z of the three types spins (SS, MW and OW) immediately after the IR was 0.03, -0.68 and -0.90 respectively, for the 2IR preparation the square of these values was found; the DIR preparation aimed at minimizing the OW signal at time of the MGRE readout, inspired by previous work (14). The timing of the DIR scan was empirically optimized in preliminary testing to result in minimal signal in white matter at the first delay time. All ten subjects had one reference scan and one IR scan; 3 subjects had one 2IR scan and 6 had two (total: 15); seven subjects had one DIR scan.

All inversion pulses were adiabatic hyperbolic secant pulses of 10 ms length, 750 Hz ($17.5 \mu\text{T}$) maximum amplitude and $\beta 730 \text{ s}^{-1}$ (15), giving a full width at half maximum of 3.6 ms and a total energy ($\int B_1^2$) of 1598 Hz ($0.88 \mu\text{T}^2\text{s}$). The DIR TR was 1.5 s, the others TRs 3 s. Five slices were acquired after one preparation, resulting in increasing delay times after the preparation pulse for the consecutive slices. The position of the slices was cycled to sample all five delay times at each location (16). The delay times are summarized in Table 1, the acquisition parameters in Table 2.

Multi-layer model fitting

The data were analyzed in three steps: 1) for each preparation and delay time, the signal level in each of the three water pools was determined from three-component fitting to the T_2^*

decay curve (Eq. 6) of the MGRE data according to (13); 2) the resulting values for the IR, 2IR and DIR scans were normalized to those of the REF scan and averaged over subjects and repeated scans; 3) the normalized averaged water signals were fitted with the ML3 model. Some of the model parameters were fixed, based on the REF data and (17) (see Table 4). The analysis was performed on the average signal in a region of interest in the splenium of the corpus callosum (SCC), while an area in the ventricles, adjacent to SCC, was chosen to serve as phase reference for step 1. The number of voxels in the SCC averaged 86 over all subjects (range 36–135). Please refer to SM:Methods for details of the fitting procedure.

It proved challenging to determine both the number of myelin layers and their permeability (reflected in k_{WW}) by fitting the model to the MGRE derived data, since both parameters have a very similar effect on the average signal of the MW pool. Therefore, N was fixed to 14, based on a reported average fiber size of 1.5 μm in the SCC (18,19) (with a correction for shrinkage during fixation), a g-ratio of 0.7 (20) and a myelin layer period of 8 nm (21,22). The g-ratio was defined as the ratio of the inner and outer diameter of the myelin sheath. The reported lamellar spacing ranges between 6 and 9 nm (23–25) corresponding to values for N of 12–19. The influence of the choice of N on the fitting results was investigated by fitting the model with different values of N , see SM. With N fixed, there were six parameters left to be determined: three R_1 's, two exchange rates (k_{WS} and k_{WW}) and one fraction (f_{SS}). Error analysis (see SM) showed $R_{1,MW}$ and $R_{1,SS}$ cannot be determined independently in the ML3 model, so these two rates were fitted as one average parameter.

Simulation of the ML3 model over a range of values for N was also used to predict the dependence of some of the derived parameters on thickness of the myelin sheath. The g-ratio and the single layer permeability were kept constant (equal to the value derived by fitting the ML3 model with $N=14$). The fixed g-ratio implies that the increase in myelin thickness corresponds to an increase in axonal diameter. The constant permeability requires a constant lifetime (τ_{MW}), therefore the water-to-water exchange rate k_{WW} has to scale with $1/N$, as the relative volume of a single MW compartment in the model scales with $1/N$. This scaling effect comes about because the model does not have absolute size, but is implicitly scaled to the volume of one axon (by normalization), while the permeability is based on the absolute size of the MW layer thickness. The other parameters of the model remained constant. The simulations were used to derive the axonal diameter dependence of the pool-averaged decay times, the two-pool model fitted exchange rate, and the apparent T_1 at 9.4 T (for comparison to (26), this with an adjustment of the $R_{1,SS}$ and $R_{1,MW}$ for the difference in field strength).

The programs used for the 3-component fitting of the MGRE data and the fitting of the ML3 model are available at https://amri.ninds.nih.gov/pub/software/mgre_ml3_fitting/.

Results

Multi-layer model fitting

The results of the three-component analysis of the T_2^* decay curves are given in Table 3 and Figure 2 for the experiment without preparation and the IR, 2IR and DIR experiments respectively. The signals were normalized to the corresponding signal levels in the reference

scan without preparation pulses. This normalization served both to scale the signal and to partly compensate for the saturation effects due to the finite TR. The same effects and normalization were incorporated in the ML3 model. The plots in Fig. 2 show that the three preparations give the desired results for the OW: close to full inversion for IR, a small disturbance for 2IR, and close to zero signal for DIR. The inversion of MW appears incomplete, which is due to the short T_2^* and fast exchange effects. The 2IR preparation therefore partly saturates the MW. An example of the three-compartment fitting is shown in SM:Results (Fig. S1). Figure 2 also shows the results of the ML3 model fits. Table 4 lists the values of the fixed model parameters used, as well as the results of the fitting and the compartment lifetimes, pool decay times (both defined in Theory) and compartment exchange rates as derived from these results. The differences in the values reported in Tables 3 and 4 reflect the adjustments made to account for exchange effects in the observed MGRE decays. Equivalent to Fig. 2, the pool averaged M_Z values for the experiments are plotted in Fig. 3. The plots show the M_Z values derived in the ML3 model fitting to the data, just prior to the relevant excitation giving the signals plotted in Fig. 2. The 2IR data show that the MW evolves towards the OW (going up) rather than toward SS (going down), the latter being close to saturated by the 2IR preparation (see Fig. 3). Individual compartment M_Z data is shown in SM:Results (Fig. S2).

From the compartment lifetime and an estimated MW layer thickness of 3.5 nm (23,24), the permeability of a myelin lipid layer was calculated to be 6.7 $\mu\text{m/s}$. The total of the variance weighted residue was 104. This should be compared to the expected value for a perfect model fit, which would be the number of degrees of freedom times unit variance, equal to 25 (30 data points, 5 parameters). The residue for fitting the SL3 model was 249.

Model Simulations for Influence Axonal Diameter

The effect of axonal diameter on the decay times is shown in Figure 4. The exchange rate in a two-pool model fitted to ML3 simulations, had a weak quadratic dependence on N : $k \approx 16 - N + 0.02N^2$, for N between 10 and 24. Based on this two-pool exchange rate, the effective R_1 at 9.4 T (for comparison to (26)) was estimated to be $0.74 - N * 0.0027 \text{ s}^{-1}$, with the $R_{1,SS}$ equal to 1.67 s^{-1} (based on values in (27) and the expected field dependence $R_1 \propto B_0^{-0.8}$ (28)).

Discussion

General Comments

We investigated ME kinetics within and across the myelin sheath using a pulsed magnetization preparation combined with MRGE data acquisition. The latter allowed distinguishing between water pools within and outside the myelin sheath, and their respective participation in the ME process. By modeling ME as a multilayer exchange process between the individual water and semisolid layers within the myelin sheath, and with the surrounding water, both the single layer residence time as well as the behavior of the aggregate magnetization of all layers could be studied. It was found that the decay time for myelin semisolid magnetization was about 88 ms, compared to 13 ms for myelin water. This suggests that ME in white matter is primarily limited by the exchange rate between

semisolids and water, while ME between myelin water and water in axonal and interstitial spaces is relatively fast. The lifetime in a single myelin water layer was found to be 260 μ s.

Plausibility of the fitted Model

The multi-layer (ML3) model fitted the available data well ($R^2=0.997$), with an estimated χ^2 (sum of squares of residuals divided by estimated variances) four times higher than the degrees of freedom (104 versus 25). A small discrepancy between model and experimental data was observed, most notably in in DIR data presented in Figure 2. Representing a system as complex as a heterogeneous bundle of axons wrapped in myelin, complete with the abundance of supporting cells and organelles, by a few pools of uniform magnetization is bound to be inaccurate, so a small deviation of the model is not surprising. A more realistic and more complex model would potentially improve the fit, but the commensurate increase in the number of parameters would decrease the confidence that can be placed in each of the extracted parameter values.

The simpler SL3 model had a χ^2 of 249, suggesting that the additional complexity of the multi-layer model is justified. The estimated fractional error margin of the ML3 results was about 10%, and may be as high as 15% for the residence times and exchange kinetics when accounting of apparent remaining systematic errors (see SM for error analysis).

Assumptions regarding the symmetry of axonal and interstitial water pools, and the size of the SS compartment in exchange with OW were tested in simulations of models not having these assumptions. It was found that the symmetry assumption only had a minor influence on the derived results, while a model with a 3.5 times larger SS compartment connected to the OW had 30% slower water-to-water exchange rate, see SM for details.

Our choice of $N=14$ was based on literature values for the average axonal diameter in the SCC, g-ratio and myelin layer spacing. The dependence of the results on the value of N was evaluated by fitting the ML3 model with a range of N values. Only the fitted k_{ww} , and the τ_{MW} derived from it, depended (linearly) on the choice of N . The other fitted or derived parameters were found to be virtually independent of N (see SM:Results (Fig. S3 and Table S1) for details).

The single layer MW lifetime was estimated at 260 μ s. With a water layer thickness of 3.5 nm (23,24), this value translates to a permeability (Eq. 5) of 6.7 μ m/s. This is in the range of values of 5–50 μ m/s reported in literature for studies on various lipid bilayer models, with a dependence on parameters such as temperature and bilayer composition (29–33). An NMR study on optic nerve in rat (12) estimated the permeability to be 2.9 μ m/s. This somewhat lower value may be the result of the different conditions of this study: a lower sample temperature (20 versus 37° C), ex-vivo conditions, and the absence of an SS-pool in their model. On the other hand, a previous study of rat spinal cord at 9.4 T (34) found a permeability of 3.8 to 7.1 μ m/s for a single lipid bilayer, consistent with our estimate of 6.7 μ m/s.

No distinction was made between the T_1 s of SS and MW. This was not attempted based on the high correlation found in initial tests of model fitting. The difficulty in separating these two parameters can be explained by the relatively fast exchange between these pools (10–

100 s^{-1}) compared to the relaxation rates. Their combined value determined from model fitting ($\sim 1.9 \text{ s}^{-1}$) was consistent with previous results (8,27).

Link to classic two-pool MT model

Simulation of pulsed MT and inversion recovery experiments, as presented in (8,27), using the presented ML3 model and subsequent analysis with a two-pool exchange model (3,11) produced an SS pool size ($f=0.26$) and an exchange rate ($k/f=6.1 \text{ s}^{-1}$) consistent with previous results (27,35). The two-pool model fitted the simulated data well ($1-R^2$ was $2 \cdot 10^{-6}$, root mean square difference 0.0014), suggesting that the two-pool exchange model is indeed adequate for conventional MT and inversion experiments which are generally not directly sensitive to MW. This is explained by the fact that the exchange between MW and OW is fast compared to the other exchange rates, making the distinction between MW and OW less relevant. The simulations showed that the two-pool exchange rate will depend on axonal diameter, which may explain some of the contrast observed in this rate, as in e.g. in (35,36).

The two-pool model lifetimes were found to be 162 ms and 460 ms for the SS and water pools respectively. Interestingly, these times are substantially longer than the times found for the ML3 model as shown in Table 4, illustrating the complication of interpreting the fitted parameters of the more abstract two-pool model in terms of actual physical processes. Previous work based on the two-pool model (assuming $R_{1,SS}=1 \text{ s}^{-1}$) (36–38), found a smaller SS fraction ($f=0.13$), a faster exchange (equivalent of τ_{SS} 32–50 ms and τ_{OW} 217–300 ms) and faster OW relaxation ($R_{1,OW}=1.5\text{--}1.8 \text{ s}^{-1}$).

Lifetime and Decay time

The resulting lifetime of a single MW compartment was 260 μs . The majority of the exchange however is with neighboring MW compartments, indistinguishable in most MR experiment. A more relevant measure of exchange for MR purposes therefore is the decay time, i.e. the time it takes for all compartments in one pool to mix with the other pools. This time was found to be 13 ms for MW and 88 ms for SS, showing that in general the SS-MW exchange is slower than the MW-OW exchange. The SS-MW exchange does however significantly contribute to the decay of MW: the T_{MW} in a simulation without this exchange (setting $k_{ws}=0$) was 31 ms. Some of the MW in the middle layers may take substantially longer than the average 13 ms to exchange with OW, the exchange over multiple layers becomes similar to a diffusion process, the time it takes to cross multiple layers scales with the square of the number of layers.

Seven studies were selected from the literature for comparison to the current results (4,5,7,12,34,39,40). (39) is a study on fixed rat spinal cord samples reporting a two-pool analysis of MT experiments as the equivalent of $k_{ws}=4.7 \text{ s}^{-1}$, $f_{SS}=0.19$ for an SS lifetime of 40 ms, substantially shorter than the two-pool lifetime derived from the ML3 model (previous paragraph). The study also suggests, based on two-pool T_2 analysis, that the MW-OW exchange is slow compared to the MW T_2 (~ 13 ms) for thicker axons ($4\text{--}5 \mu\text{m}$), but has a significant effect for smaller axons ($1\text{--}2 \mu\text{m}$); however, the exchange was not quantified in this study. In (34), with multi-component T_2 measurements on rat spinal cord in-vivo at 9.4

T, it was concluded that the exchange between MW and OW is an important factor in determining the resulting T_2 values and their observed dependence on myelin thickness. The MW lifetime was estimated to be 43 to 150 ms, depending on this thickness. A prior study at 9.4 T of freshly excised rat optic nerve samples (12) analyzed ME with a three-pool model, for axonal-, myelin-, and interstitial water based on T_1 and T_2 differences. Not including an SS pool, the authors found MW lifetime to be 138 ms. The equivalent of $R_{1,OW}$ and $R_{1,MW}$ values were found to be 0.63 s^{-1} , and $R_{1,MW}$ 1.82 s^{-1} respectively. Omission of the SS pool, the lower temperature, the ex-vivo condition, and the thickness of the myelin sheath may all have contributed to the longer lifetime.

The remaining three studies (4,5,7,40) all used a combination of T_2 measurements with MT or inversion preparation, with a four-pool model analysis. (5) used freshly dissected bovine optic nerve samples, scanned at 1.5 T at 20°C ; (7,40) used freshly dissected bovine brain samples at 37°C and at 2.1 T and 4.7 T respectively; (4) was an human (in-vivo) study at 1.5 T (results from SCC, scenario II are quoted here). The resulting lifetimes and decay times for these studies are printed in Table 5. The study in (5) did not resolve the fraction of the SS pools, therefore only two of the lifetimes could be calculated from their results. In general, the exchange between the SS compartments and the neighboring water was substantially faster than what was found for the ML3 model, especially the SS compartment in exchange with OW, while the MW-OW exchange was slower than the ML3 results. Some of these differences may be due to the difference between tissues (the number of myelin layers in particular), the difference between in-vivo versus ex-vivo measurements, and perhaps the difficulty in quantifying MT effects at lower field strength. At 1.5 T, the $R_{1,SS}$ can be estimated to be about 7 s^{-1} , based on values in (27) and expected field dependence ($B_0^{-0.8}$ (28)), which means the $T_{1,SS}$ is on the same time scale as the SS lifetime, making it difficult to separate their influence on the observable (water) signals. Variations in the assumed size of the SS compartment in exchange with OW may also contribute to some of the differences, see SM.

Because of the complexity of the multi-layer exchange process, the exchange induced decay of MW is not a mono-exponential evolution. The process is further complicated by concurrent exchange with the SS-compartments. While the decay time is a good indication of the time scale of the process, it is not possible to capture the consequences of all the exchange processes in a single number. This also complicates the comparison to previously published results based on simpler models, the exchange related signal evolution in those models has a different shape and not all parameters have a one-to-one correspondence to values in the ML3 model, as is illustrated by the different lifetimes found when fitting a two-pool model to ML3 simulations (see previous section).

Some possible mechanisms contributing to the SS lifetime are: spin-diffusion along the methylene protons of the carbon chain of the lipids and/or membrane proteins, direct contact between parts of the lipid chains (1,41–44), as well as the exchange at the interface between the semisolids and water. As most of the lipid chain does not have direct contact with water, ME between SS and MW becomes a 2-step process: ME within the SS pool itself, and ME on the interface, mostly through ME at the hydrophilic head groups of the lipids. While the

exchange time at the interface may be as short as 1 ms (45), the sparsity of exchange sites compared to the number of spins in the SS pool likely limit the combined exchange rate. In effect, a small and fast exchanging pool would need to be inserted between every SS compartment and its neighboring water compartment in the ML3 model, but the parameters for this additional refinement could likely not be estimated with any precision from our experiments.

Implications

Our findings confirm the earlier conclusion that longitudinal relaxation of water is slow compared to the exchange rates involved, meaning that exchange between SS and water and relaxation in SS dominate overall water relaxation rates (27), consistent with a high degree of correlation between T_1 and f data (46). The dependence of the exchange time between SS and OW on the number of myelin layers as predicted by the ML3 model is consistent with the measured effective R_1 at 9.4 T for different sizes of axons in rat spinal cord (26). Both experiments and the model show that using T_1 contrast to suppress non-myelin water inherently suppresses most of the myelin water as well: the exchange between MW and OW is too fast to separate the two efficiently. Furthermore, the exchange is fast enough to influence the apparent transverse relaxation rates of both MW and OW. The T_2 of brain tissue is similar to the estimated decay time of the OW, so relaxation in the MW and exchange with OW can have a significant effect (34,39).

Finally, the exchange across myelin layers is important for diffusion experiments. The axonal and interstitial pools have different diffusion properties, the time scale of the exchange between these pools determines if they can be distinguished in a diffusion experiment (see e.g. (47,48)). This time is much longer than T_{MW} because the MW spins on average have to travel only about one quarter of the myelin sheath to get to OW, while for the axonal to interstitial exchange the entire sheath needs to be traversed. We estimate this exchange time to be around 300 ms (for SSC axons), which is slow on the time scale of most diffusion experiments.

Since we cannot discriminate the signals from the individual water layers, it is not possible to determine both the number of layers and the permeability from our experiments. Nevertheless, if the layer permeability can be established independently, the presented model could potentially be used to estimate the number of myelin layers in areas where myelin thickness is relatively uniform. Or without an external value for the permeability, analysis based on the ML3 model could be used to derive a relative measure of myelin thickness.

Conclusion

We studied ME in human brain white matter with a model that incorporated individual myelin water layers as separate magnetization compartments. Fitting this model to 7 T MT-MGRE data resulted in a plausible permeability estimate of 6.7 $\mu\text{m/s}$ for the myelin lipid bilayers. In addition, the model was found to be consistent with the acquired data, previous MT results and the observed dependence of relaxation parameters on axonal diameter (26,34). The single myelin water layer lifetime was estimated at 260 μs . The decay time of the pooled magnetization of all myelin water layers was estimated at 13 ms, substantially

shorter than previously concluded (4) and short compared to the lifetime of lipids exchanging with water, which was estimated at 88 ms. The results suggest that exchange across myelin lipid bilayers is an important factor in T_1 , T_2 and MT contrasts in the brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations & Symbols

2IR	double inversion
DIR	double inversion for OW suppression
IR	inversion recovery
ME	magnetization exchange
MGRE	multi-gradient-echo
ML3	multilayer model with three pools, combining axonal and interstitial space
MT	magnetization transfer
MW	myelin water (water in between myelin lipid bilayers)
OW	other water (water outside myelin sheath)
REF	reference scan (without magnetization preparation)
RF	radio frequency
SCC	splenium of the corpus callosum
SD	standard deviation
SE	standard error
SL3	exchange model with three pools, no layers
SS	semi-solids

Glossary

In the following list, 'P' represents one of the three pool types: $P \in [SS, MW, OW]$.

f	fraction of spins in semisolids in the two-pool MT model
f_p	fraction of spins in the sum of all compartments in pool P
k_{ws}	exchange rate between water and semisolid pools, relative to total number of spins

k_{ww}	exchange rate between water compartments, relative to total number of spins
$M_{z,i}^P$	longitudinal magnetization of compartment i in pool P
N	number of MW compartments (layers) in the model
$R_{1,P}$	longitudinal relaxation rate of pool P
$R_{2,P}$	transverse relaxation rate of pool P
τ_P	single compartment lifetime in pool P
T_P	decay time of aggregate magnetization of pool P, as defined in Theory
ν_P	frequency offset in pool P

References

1. Koenig SH, Brown RD 3rd, Spiller M, Lundbom N. Relaxometry of brain: why white matter appears bright in MRI. *Magnetic resonance in medicine*. 1990; 14:482–495. [PubMed: 2355830]
2. Forsen S, Hoffman RA. Study of Moderately Rapid Chemical Exchange Reactions by Means of Nuclear Magnetic Double Resonance. *J Chem Phys*. 1963; 39:2892–2901.
3. Edzes HT, Samulski ET. Cross relaxation and spin diffusion in the proton NMR of hydrated collagen. *Nature*. 1977; 265:521–523. [PubMed: 834303]
4. Kalantari S, Laule C, Bjarnason TA, Vavasour IM, MacKay AL. Insight into in vivo magnetization exchange in human white matter regions. *Magnetic resonance in medicine*. 2011; 66:1142–1151. [PubMed: 21381107]
5. Stanisz GJ, Kecojevic A, Bronskill MJ, Henkelman RM. Characterizing white matter with magnetization transfer and T(2). *Magnetic resonance in medicine*. 1999; 42:1128–1136. [PubMed: 10571935]
6. Dortch RD, Moore J, Li K, Jankiewicz M, Gochberg DF, Hirtle JA, Gore JC, Smith SA. Quantitative magnetization transfer imaging of human brain at 7 T. *NeuroImage*. 2013; 64:640–649. [PubMed: 22940589]
7. Bjarnason TA, Vavasour IM, Chia CL, MacKay AL. Characterization of the NMR behavior of white matter in bovine brain. *Magnetic resonance in medicine*. 2005; 54:1072–1081. [PubMed: 16200557]
8. van Gelderen P, Jiang X, Duyn JH. Rapid measurement of brain macromolecular proton fraction with transient saturation transfer MRI. *Magnetic resonance in medicine*. 2017; 77:2174–2185. [PubMed: 27342121]
9. Sati P, van Gelderen P, Silva AC, Reich DS, Merkle H, de Zwart JA, Duyn JH. Micro-compartment specific T2* relaxation in the brain. *NeuroImage*. 2013; 77:268–278. [PubMed: 23528924]
10. Wharton S, Bowtell R. Fiber orientation-dependent white matter contrast in gradient echo MRI. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:18559–18564. [PubMed: 23091011]
11. Gochberg DF, Kennan RP, Gore JC. Quantitative studies of magnetization transfer by selective excitation and T1 recovery. *Magnetic resonance in medicine*. 1997; 38:224–231. [PubMed: 9256101]
12. Dortch RD, Harkins KD, Juttukonda MR, Gore JC, Does MD. Characterizing inter-compartmental water exchange in myelinated tissue using relaxation exchange spectroscopy. *Magnetic resonance in medicine*. 2013; 70:1450–1459. [PubMed: 23233414]

13. Li X, van Gelderen P, Sati P, de Zwart JA, Reich DS, Duyn JH. Detection of demyelination in multiple sclerosis by analysis of T2* relaxation at 7 T. *NeuroImage Clinical*. 2015; 7:709–714. [PubMed: 26594617]
14. Oh SH, Bilello M, Schindler M, Markowitz CE, Detre JA, Lee J. Direct visualization of short transverse relaxation time component (ViSta). *NeuroImage*. 2013; 83:485–492. [PubMed: 23796545]
15. Tannus A, Garwood M. Adiabatic pulses. *NMR in biomedicine*. 1997; 10:423–434. [PubMed: 9542739]
16. Ordidge RJ, Gibbs P, Chapman B, Stehling MK, Mansfield P. High-speed multislice T1 mapping using inversion-recovery echo-planar imaging. *Magnetic resonance in medicine*. 1990; 16:238–245. [PubMed: 2266843]
17. Jiang X, van Gelderen P, Duyn JH. Spectral characteristics of semisolid protons in human brain white matter at 7 T. *Magnetic resonance in medicine*. 2017; 78:1950–1958. [PubMed: 28150877]
18. Aboitiz F, Scheibel AB, Fisher RS, Zaidel E. Fiber composition of the human corpus callosum. *Brain Res*. 1992; 598:143–153. [PubMed: 1486477]
19. Tomasch J. Size, distribution, and number of fibres in the human corpus callosum. *Anat Rec*. 1954; 119:119–135. [PubMed: 13181005]
20. Stikov N, Campbell JS, Stroh T, Lavelee M, Frey S, Novek J, Nuara S, Ho MK, Bedell BJ, Dougherty RF, Leppert IR, Boudreau M, Narayanan S, Duval T, Cohen-Adad J, Picard PA, Gasecka A, Cote D, Pike GB. Quantitative analysis of the myelin g-ratio from electron microscopy images of the macaque corpus callosum. *Data Brief*. 2015; 4:368–373. [PubMed: 26217818]
21. Kirschner DA, Caspar DL. Comparative diffraction studies on myelin membranes. *Ann N Y Acad Sci*. 1972; 195:309–320.
22. Worthington CR. Structural parameters of nerve myelin. *Proceedings of the National Academy of Sciences of the United States of America*. 1969; 63:604–611. [PubMed: 5259752]
23. Franks NP, Melchior V, Kirschner DA, Caspar DL. Structure of myelin lipid bilayers. Changes during maturation. *J Mol Biol*. 1982; 155:133–153. [PubMed: 7077673]
24. Laursen RA, Samiullah M, Lees MB. The structure of bovine brain myelin proteolipid and its organization in myelin. *Proceedings of the National Academy of Sciences of the United States of America*. 1984; 81:2912–2916. [PubMed: 6201866]
25. Vandenheuvel FA. Structural Studies of Biological Membranes: The Structure of Myelin. *Ann N Y Acad Sci*. 1965; 122:57–76. [PubMed: 14313514]
26. Harkins KD, Xu J, Dula AN, Li K, Valentine WM, Gochberg DF, Gore JC, Does MD. The microstructural correlates of T1 in white matter. *Magnetic resonance in medicine*. 2016; 75:1341–1345. [PubMed: 25920491]
27. van Gelderen P, Jiang X, Duyn JH. Effects of magnetization transfer on T1 contrast in human brain white matter. *NeuroImage*. 2016; 128:85–95. [PubMed: 26724780]
28. Korb JP, Bryant RG. The physical basis for the magnetic field dependence of proton spin-lattice relaxation rates in proteins. *J Chem Phys*. 2001; 115:10964–10974.
29. Conlon T, Outhred R. The temperature dependence of erythrocyte water diffusion permeability. *Biochimica et biophysica acta*. 1978; 511:408–418. [PubMed: 687621]
30. Cass A, Finkelstein A. Water Permeability of Thin Lipid Membranes. *J Gen Physiol*. 1967; 50:1765–1784. [PubMed: 6034767]
31. Finkelstein A. Water and nonelectrolyte permeability of lipid bilayer membranes. *J Gen Physiol*. 1976; 68:127–135. [PubMed: 956767]
32. Franks NP, Lieb WR. Rapid movement of molecules across membranes. Measurement of the permeability coefficient of water using neutron diffraction. *J Mol Biol*. 1980; 141:43–61. [PubMed: 7431400]
33. Finkelstein A, Cass A. Effect of cholesterol on the water permeability of thin lipid membranes. *Nature*. 1967; 216:717–718. [PubMed: 6082492]
34. Harkins KD, Dula AN, Does MD. Effect of intercompartmental water exchange on the apparent myelin water fraction in multiexponential T2 measurements of rat spinal cord. *Magnetic resonance in medicine*. 2012; 67:793–800. [PubMed: 21713984]

35. van Gelderen P, Jiang X, Duyn JH. Rapid measurement of brain macromolecular proton fraction with transient saturation transfer MRI. *Magnetic resonance in medicine*. 2016
36. Yarnykh VL, Yuan C. Cross-relaxation imaging reveals detailed anatomy of white matter fiber tracts in the human brain. *NeuroImage*. 2004; 23:409–424. [PubMed: 15325389]
37. Sled JG, Pike GB. Quantitative imaging of magnetization transfer exchange and relaxation properties in vivo using MRI. *Magnetic resonance in medicine*. 2001; 46:923–931. [PubMed: 11675644]
38. Levesque IR, Sled JG, Narayanan S, Giacomini PS, Ribeiro LT, Arnold DL, Pike GB. Reproducibility of quantitative magnetization-transfer imaging parameters from repeated measurements. *Magnetic resonance in medicine*. 2010; 64:391–400. [PubMed: 20665783]
39. Dula AN, Gochberg DF, Valentine HL, Valentine WM, Does MD. Multiexponential T2, magnetization transfer, and quantitative histology in white matter tracts of rat spinal cord. *Magnetic resonance in medicine*. 2010; 63:902–909. [PubMed: 20373391]
40. Barta R, Kalantari S, Laule C, Vavasour IM, ALM, CAM. Modeling T1 and T2 relaxation in bovine white matter. *J Magn Reson*. 2015; 259:56–67. [PubMed: 26295169]
41. Seiter CHA, Chan SI. Molecular Motion in Lipid Bilayers - Nuclear Magnetic-Resonance Line-Width Study. *Journal of the American Chemical Society*. 1973; 95:7541–7553.
42. Huster D, Feller SE, Gawrisch K. Nocsy NMR crosspeaks between lipid headgroups and hydrocarbon chains - Spin diffusion or molecular disorder? *Biophysical journal*. 1999; 76:A354–A354.
43. Ellena JF, Hutton WC, Cafiso DS. Elucidation of cross-relaxation pathways in phospholipid vesicles utilizing two-dimensional ¹H NMR spectroscopy. *J Am Chem Soc*. 1985; 107:1530–1537.
44. Huster D, Yao XL, Hong M. Membrane protein topology probed by H-1 spin diffusion from lipids using solid-state NMR spectroscopy. *Journal of the American Chemical Society*. 2002; 124:874–883. [PubMed: 11817963]
45. Kumashiro KK, Schmidt-Rohr K, Murphy OJ, Ouellette KL, Cramer WA, Thompson LK. A novel tool for probing membrane protein structure: Solid-state NMR with proton spin diffusion and X-nucleus detection. *Journal of the American Chemical Society*. 1998; 120:5043–5051.
46. Fatouros PP, Marmarou A. Use of magnetic resonance imaging for in vivo measurements of water content in human brain: method and normal values. *Journal of neurosurgery*. 1999; 90:109–115.
47. Nilsson M, van Westen D, Stahlberg F, Sundgren PC, Latt J. The role of tissue microstructure and water exchange in biophysical modelling of diffusion in white matter. *Magn Reson Mater Phy*. 2013; 26:345–370.
48. Harkins KD, Does MD. Simulations on the influence of myelin water in diffusion-weighted imaging. *Physics in medicine and biology*. 2016; 61:4729–4745. [PubMed: 27271991]

Figure 1.

A) To derive the model, the semisolid layers in between the individual water layers of the myelin sheath are split in two and combined into one compartment. B) The ML3 model, illustrated for $N=5$ (actual model fitting used higher N), showing the two types of exchanging water compartments (MW and OW) and the semisolids (SS) in exchange with the water compartments.

Figure 2.

The averages and SE over subjects as derived in the fitting of step 2, plotted as error bars, and the fitting of the ML3 exchange model plotted as lines. Data are normalized to the amplitudes derived from the reference data.

Figure 3.

The longitudinal magnetization for the three experiments with preparation, as derived from the fitting of the ML3 exchange model. The values shown reflect the magnetization just prior to the excitation pulses generating the signal shown in Fig 2.

Figure 4.

Decay times as function of the number of myelin compartments (N), reflecting the slowing down of the exchange with increasing axon diameter. Values were estimated based on simulation of the ML3 model with parameters fitted for $N=14$ and assuming the permeability of single myelin lipid bilayer is constant.

Table 1

Delay times from the middle of the last inversion pulse to the middle of the excitation pulse.

IR	ms	9	199	448	812	1500
2IR	ms	9	87	193	357	750
DIR	ms	154	203	252	301	350

Table 2

Parameters of the MGRE acquisition following the various preparation pulses.

Slice thickness	mm	2
Slice gap	mm	2.4
Field of view (AP, LR)	mm	240, 160
Resolution (AP, LR)		90, 60
Echo Spacing	ms	0.53
Echo Times	ms	2.16–44.6
Flip angle		$\pi/2$
Pulse Length	ms	2.5
Peak B_1	μT	6.5
$\int B_1^2 dt$	$\mu\text{T}^2\text{s}$	0.026
Navigator TE	ms	19.6

Table 3

Averages, SE and relative (percentage) SE over 10 subjects for the parameters in the three-component model (Eq. 6) fitted to the reference MGRE data; the three components are myelin-, axonal- and interstitial-water (subscripts 1–3). The frequency offset of the last component is zero by definition; its frequency offset is absorbed in the common offset parameter.

		Average	SE	SE %
A_1		0.132	0.007	5.3
A_2		0.411	0.005	1.2
A_3		0.572	0.006	1.0
$R_{2,1}^*$	s^{-1}	152	4	2.6
$R_{2,2}^*$	s^{-1}	25.7	0.9	3.5
$R_{2,3}^*$	s^{-1}	38.5	1.2	3.1
ν_1	Hz	31.4	1.3	4.1
ν_2	Hz	−5.7	0.1	1.8

Table 4

Parameters of the ML3 model; only the five ‘fitted’ parameters are free variables in the model, the others are fixed before fitting, or derived from the results; the compartmental exchange rates are the rates scaled by the respective compartment sizes (as in k/f), see SM:Theory.

Fixed parameters	$R_{2,SS}^{\dagger}$	ms^{-1}	16
	$R_{2,MW}$	s^{-1}	130
	$R_{2,OW}$	s^{-1}	33
	$v_{SS}^{\dagger}$		700
	v_{MW}		35
	v_{OW}		0
	f_{MW}/f_{OW}		0.136
	N		14
Fitted parameters	f_{SS}		0.24
	$R_{1,MW}, R_{1,SS}$	s^{-1}	1.84
	$R_{1,OW}$	s^{-1}	0.36
	k_{WS}	s^{-1}	3.5
	k_{WW}	s^{-1}	12.6
Derived parameters	f_{MW}		0.09
	f_{OW}		0.67
	τ_{SS}	ms	67
	τ_{MW}	ms	0.26
	τ_{OW}	ms	52
	T_{SS}	ms	88
	T_{MW}	ms	13
	T_{OW}	ms	106
Compartmental exchange rates	k'_{MS}	s^{-1}	37
	k'_{MM}, k'_{MO}	s^{-1}	1938
	k'_{SM}, k'_{SO}	s^{-1}	15
	k'_{SO}	s^{-1}	0.35
	k'_{OM}	s^{-1}	19

† : values taken from SS-spectrum measurements (17).

Table 5

Pool fractions, lifetimes and decay times as derived from five studies in the literature, 'MS' refers to SS pool exchanging with the MW, 'OS' refers to the SS-pool exchanging with OW.

	(5)	(7)	(4)	(12)	(40) [‡]
<i>f</i>	MS	0.09 [*]	0.09		0.11
	MW	0.11	0.12	0.16	0.056–0.12
	OW	0.67	0.71	0.84 [*]	0.66–0.72
	OS	0.13 [*]	0.09		0.11
<i>τ</i>	MS	73	40		63–124
	MW	53	46	138	25–90
	OW	278	127	832 [*]	208–353
<i>T</i>	OS	29	17		52–79
	MS	115	90		150–208
	MW	61	165	117	25–105
	OW	77	327	621 [*]	78–208
	OS	26	16		47–72

^{*} as derived from reported k-values

^{*} combination of axonal and interstitial pools

[‡] a range of values is reported, covering the three samples in the study