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Spectral characteristics of semi-solid protons in human brain white matter at 7 T

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Abstract

Purpose—To inform quantification of MRI magnetization transfer contrast at high field by measuring the spectral characteristics of ^1H protons in semi-solids in human brain at 7 T, while avoiding prohibitive RF tissue heating and confounding effects from chemical exchange.

Methods—Saturation-recovery type experiments were performed using brief, frequency-specific RF pulses that saturate semi-solid proton magnetization. Analysis of the subsequent recovery of water proton magnetization with a two-pool model of exchange allowed studying spectral characteristics of semi-solid protons.

Results—We show that in white matter, the semi-solid proton spectrum can be approximated with a symmetric, super-Lorentzian line at -2.58 ± 0.05 ppm from the water resonance and an average transverse relaxation time constant (T_2) of 9.6 ± 0.6 μs .

Conclusion—These results are consistent with studies at lower field that have indicated a major contribution from methylene protons to magnetization transfer contrast and will facilitate the design and quantification of magnetization transfer studies at 7 T.

Keywords

white matter; magnetization transfer; macromolecular protons; lineshape; T_2 relaxation

Introduction

In MRI of human brain, Magnetization Transfer (MT) contrast (1) has been used extensively to study tissue composition, specifically the fraction of hydrogen (^1H) protons with restricted mobility, often referred to as semi-solid protons or macromolecular protons (MPs) (2–6). These protons, having a short T_2 , are generally not directly detectable by MRI but, through MT, exert an influence on ^1H protons in water (WPs), the primary contributors to the MRI signal. Measures of the magnitude of this MT effect have been used to study MP content in brain tissue (4–6) and characterize alterations in brain myelin content in multiple sclerosis (7, 8) and other pathology (9–11).

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The mechanisms underlying MT contrast are complex (12, 13), often rendering interpretation and quantification difficult. Generally, the strongest contributions to MT come from MPs such as proteins and lipids, whose protons can affect WP magnetization through dipolar coupling and chemical exchange (2, 12, 14, 15). However, chemical exchange effects of smaller molecules may contribute as well (16, 17). And chemical exchange effects can be enhanced by using long RF irradiation as is done in so called chemical exchange saturation transfer (CEST) techniques (18). While MT generally contributes to MRI contrast to some extent, this contribution is amplified by differentially perturbing WP and MP magnetization levels from their thermal equilibrium values (2). Because of this, MT contrast is not only dependent on dipolar coupling strength and exchange rate between WP and MP, but also on specifics of the MRI pulse sequence (2). It is the latter principle that has been exploited to highlight certain contributions to MT, rendering a contrast that emphasizes either MPs (4–6, 19, 20) or exchangeable protons (16, 18) with some chemical specificity (18). On the other hand, MP magnetization levels are often not well known and difficult to control, due to the short transverse relaxation time (T_2) of MPs. Therefore, for proper quantification of MT, and avoid unintended contribution of MT in general (non-MT) MRI applications, MP magnetization levels need to be estimated.

Apart from the exchange rate between MPs and WPs, MT contrast is dependent on the characteristics of the radiofrequency (RF) irradiation, the spectral characteristics of MPs and WPs, and T_1 relaxation. Of these factors, the MP spectrum may be the most critical and most difficult to measure, because of MPs extremely short transverse relaxation time constant (T_2). Direct measurements with NMR spectrometers on *ex-vivo* brain tissue samples and various membrane model systems, including myelin extracts and lecithin, have shown that MPs have complex lineshapes with varying width and off-resonance frequency, dependent on the host molecules and strength of dipolar coupling with neighboring MPs (20–25). Typically, at field strengths up to 9.4 T, super-Lorentzian (SL) and Lorentzian lineshapes have been reported over a spectral range of about 6 ppm and T_2 values of 20–100 μ s (20–26). The general understanding is that in membrane-rich white matter, lipid methylene and methyl groups are the major contributors to these spectra (20, 24, 26, 27).

Because MP spectral characteristics may be quite different in-vivo, and at higher field strengths, various attempts have been made to infer the MP spectrum from their effect on the easier detectable WP signal using steady state MT experiments. Studies at clinical field strengths up to 3 T have reported a SL lineshape with a T_2 of in the range of 9–15 μ s for white matter (WM) in-vivo (5, 28, 29), similar to the range of 9–13 μ s found for tissue samples using this approach (6, 14, 30, 31), but below the 20–100 μ s range directly measured by NMR spectroscopy. In addition, several studies have found the MP spectrum to be shifted by about –3 ppm relative to the WP resonance (28, 32–34), consistent with the chemical shift of lipid methylene protons. However this has proven difficult to reproduce, in particular at high field, due to confounding CEST effects (35), and increased tissue heating associated with RF irradiation. Currently, most quantitative MT methods assume the MP spectrum to be centered on the water resonance.

To further investigate MP spectral characteristics, with the ultimate purpose of better quantifying MT contrast in MRI of brain tissue, we applied an indirect measurement

approach based on transient MT effects after MP saturation with a brief, RF pulse (36). Using a range of specific RF frequencies allowed us to characterize MP spectral characteristics in human brain at 7 T in an efficient manner, while minimizing effects of CEST, tissue heating, and direct WP saturation.

Methods

MRI experiments were performed on eight human subjects (ages 20–49, average 30.6, 4 female), scanned under an IRB approval on a 7 T Siemens scanner using a 32-channel receive array. In order to study MT without CEST and direct MP saturation effects, we followed a recent approach based on monitoring the saturation of WP signal following a brief, 6–12 ms MP saturation pulse (3, 37). This saturation recovery (SR) approach resembles the selective inversion recovery (IR) approach (38, 39), but sacrifices some sensitivity to obtain an improved estimate of initial MP magnetization level, and render this level insensitive to B_1 variation (3, 37). Like the IR approach, it allows sensitive determination of the parameters describing the MT process, by accounting for the direct water saturation effects (3, 37–39), and minimization of CEST effects and tissue heating. First, experiments were performed to determine parameters describing MT kinetics, assuming a two-pool model of exchange between MP and WP (defined as Experiment I), and subsequently, MP spectral characteristics were investigated by studying the magnitude of the MT effects as a function of the frequency of narrowband MP saturation pulse (defined as Experiment II).

Two-pool model

Following an initial RF pulse that differentially saturates the WP and MP pools, the fractional saturations of the two pools, FS_{WP} and FS_{MP} , experience bi-exponential evolutions as follows (3, 37, 40):

$$FS_{WP}(t) = 1 - \frac{M_{WP}(t)}{M_{WP}(\infty)} = a_1 e^{-\lambda_1 t} + a_2 e^{-\lambda_2 t} \quad [1]$$

$$FS_{MP}(t) = 1 - \frac{M_{MP}(t)}{M_{MP}(\infty)} = \frac{a_1(-\lambda_1 + k_{WM} + R_{1,WP})}{k_{WM}} e^{-\lambda_1 t} + \frac{a_2(-\lambda_2 + k_{WM} + R_{1,WP})}{k_{WM}} e^{-\lambda_2 t} \quad [2]$$

$$2\lambda_{1,2} = R_{1,WP} + R_{1,MP} + k_{MW} + k_{WM} \pm \sqrt{(R_{1,MP} - R_{1,WP} + k_{MW} - k_{WM})^2 + 4k_{MW}k_{WM}} \quad [3]$$

$$a_{1,2} = \pm \frac{FS_{WP}(0)(R_{1,WP} + k_{WM} - \lambda_{2,1}) - FS_{MP}(0)k_{WM}}{\lambda_1 - \lambda_2} \quad [4]$$

$$(1 - f)k_{WM} = f k_{MW} \quad [5]$$

In these equations, M_{WP} and M_{MP} are the longitudinal magnetizations of the two pools, $R_{1,WP}$ and $R_{1,MP}$ are their relaxation rates, λ_1 and λ_2 are fast and slow rate constants of the saturation recovery, and a_1 and a_2 are the corresponding amplitudes. Parameters k_{WM} and k_{MW} represent the MT exchange rate constants relative to WP and MP pool sizes respectively.

Experiment I: Determination of MT parameters—To measure the parameters related to the two-pool exchange model, two types of preparation pulses, namely a WP IR pulse and a composite broadband MP SR pulse were used to saturate the two pools to different extents. After variable delay t , EPI image acquisition was performed to sample $FS_{WP}(t)$. The use of two different preparation pulses facilitated fitting λ_1 and λ_2 , and furthermore allowed extraction of k_{MW} , k_{WM} , f , and $R_{1,WP}$ by assuming $R_{1,MP} = 2 \text{ s}^{-1}$ and $FS_{MP}(0) = 0.93$ for the broadband SR experiment as determined previously (3). The inversion pulse was adiabatic, with a hyperbolic secant envelope, duration of 5.12 ms, energy of $0.51 (\mu\text{T})^2\text{s}$, maximum B_1 (amplitude of the RF field) of 833 Hz (1 Hz equals $0.0235 \mu\text{T}$) and β of 1400 s^{-1} (41). The broadband SR pulse had a duration of 6 ms and consisted of a train of 16 hard pulses with angles $60^\circ, -120^\circ, 120^\circ, -120^\circ, \dots, 120^\circ, -60^\circ$, with a B_1 amplitude of 833 Hz (3).

Experiment II: Determination of MP spectral characteristics—To determine MP spectral characteristics, the broadband SR pulse was replaced by a frequency-specific SR pulse and applied at 12 different frequency offsets (F) with respect to the water resonance frequency, ranging from -16 kHz to 16 kHz ($-16, -8, -4, -2, -1, -0.5, 0.5, 1, 2, 4, 8, 16 \text{ kHz}$). The order of scans for different values of F was randomized for each subject. The pulse had a duration of 12 ms, and a hyperbolic secant envelope for its amplitude, identical to that used for the inversion pulse described above, with a β of 600 s^{-1} (41). The full width at half maximum (FWHM) of its power spectral density is 119 Hz. A relatively low B_1 amplitude of 200 Hz was used in order to avoid saturation and the non-linear relationship between $FS_{MP}(0)$ and the integral of squared B_1 amplitude over the pulse duration for all values of F (42, 43). This simplified the derivation of the MP spectrum from the experimental data.

Image acquisition—For both IR and SR experiments, image data were acquired using single-shot EPI, sampling 5 slices consecutively after the preparation pulse; cycling the slice order over 5 repetitions thus resulted in acquisition of 5 delay times for each slice (44). Axial-oblique slices of 2 mm thickness were placed, with 3.4 mm inter-slice gap, parallel to AC-PC line and encompassed the central part of the corpus callosum. The delay times for the IR experiment were 6, 63, 144, 282 and 1200 ms (defined as the time from the center of the inversion pulse to the center of the EPI excitation pulse). The delay times for the broadband SR experiments were: 7, 127, 258, 401 and 559 ms and for the frequency-specific SR experiments were: 10, 130, 260, 402 and 559 ms. The IR and SR delay times were chosen to sample the signal recovery dynamics, within the constraint of the minimal slice

repetition time (TR) set by the duration of the EPI readout. The image resolution was 144×108 with SENSE rate-2 acceleration, the field-of-view was 240×180 mm. The echo time (TE) was 24 ms, TRs were 6 and 3 s for IR and SR experiments respectively. In order to suppress signals from scalp lipids, the TE was increased by 0.49 ms on even numbered repetitions (3). A multi gradient echo (MGRE) sequence with TR of 0.2 s, TE of 2.5 ms, 7 echoes with echo spacing of 0.74 ms, the same resolution and field-of-view as the EPI scans and total scan time of 21.6 s, was scanned before all IR and SR experiments. This MGRE sequence served as reference to reconstruct the accelerated EPI scans and was also used to estimate the B_0 (amplitude of the static field) inhomogeneity. For IR experiments, six repeat measurements were performed, the first two of which omitted the inversion pulse and were used to provide a reference signal to estimate $M_{WP}(\infty)$ in Eq. [1], and allow conversion of the measured signals to $FS_{WP}(t)$. Similarly, ten repetitions (including two serving as reference) and six repetitions (including one reference) were acquired for the broadband and frequency-specific SR experiments respectively.

Data Analysis

Pre-processing—Pre-processing included motion correction, signal polarity correction, averaging, and calculation of $FS_{WP}(t)$. Prior to averaging repetitions, complex images were spatially registered to correct for motion. Only in-plane registration was performed, as the small number of slices did not support through-plane motion correction. Polarity correction was needed only for IR (magnitude) data, because of signal rectification during the complex-to-magnitude conversion. It was performed based on the phase difference between the IR images with the (un-inverted) reference image. The $FS_{WP}(t)$ level expressed in Eq. [1] was determined by dividing each IR image by the corresponding reference image (i.e. data acquired without inversion pulse). Analogous analysis was performed for the SR data, however without performing the signal polarity adjustment. All processing was done in IDL (Exelsis Visual Information Solutions, Boulder, CO, USA).

Voxel-wise determination of MP saturation—To determine λ_1 , λ_2 , k_{WM} , k_{MW} , and $R_{1,WP}$, both the IR and the broadband SR data from Experiment I were analyzed on a voxel-wise basis. Defining $FS_{MP}(0, F)$ as the $FS_{MP}(0)$ created by a frequency-specific SR pulse applied at frequency F , $FS_{MP}(0, F)$ can be then calculated based on analysis of the frequency-specific SR data in Experiment II. The following steps were involved:

- a. Fit Eq. [1] to the IR and broadband SR data jointly, yielding one pair of decay rates (λ_1 , λ_2) and two pairs of amplitudes (a_1 , a_2) for each voxel.
- b. Assuming $R_{1,MP}=2 \text{ s}^{-1}$ and $FS_{MP}(0)$ for the broadband SR experiment to be 0.93 as determined previously (3), calculate k_{WM} , k_{MW} , $R_{1,WP}$, using Eq. [3–4], based on the decay rates and amplitudes found under Step (a).
- c. Fit Eq. [1] to the frequency-specific SR data, to find a pair of amplitudes ($a_1(F)$, $a_2(F)$) for every voxel at each F , using decay rates (λ_1 , λ_2) found under Step (a). Then calculate $FS_{MP}(0, F)$ using these ($a_1(F)$, $a_2(F)$) pairs, according to Eq. [2] by setting $t=0$ s, with all other parameters known from Steps (a) and (b), as shown in Eq. [6].

$$FS_{MP}(0, F) = \frac{a_1(F)(-\lambda_1 + k_{WM} + R_{1,WP})}{k_{WM}} + \frac{a_2(F)(-\lambda_2 + k_{WM} + R_{1,WP})}{k_{WM}} \quad [6]$$

Calculation of B_0 shift for frequency-specific SR scans— B_0 inhomogeneity and its drift over the course of the experiments can potentially change the effective F for each of the frequency-specific SR pulses. B_0 measurement with methods like WASSR (45) can be used to correct for these effects. Here, B_0 inhomogeneity for each scan was calculated voxel-wise as follows:

- a. The phase of even echoes of the MGRE scan was fitted to a linear model to estimate spatial variations (inhomogeneity) in B_0 .
- b. The EPI (either IR or SR) scan following the MGRE was assumed to suffer from the same B_0 inhomogeneity as the MGRE scan.
- c. The relative B_0 shift between successive EPI scans (temporal drift) was determined from their relative phase.
- d. The absolute B_0 shift, B_0 , for a specific EPI scan was calculated by summing over all its previous relative B_0 shifts and the B_0 inhomogeneity of the first EPI scan, as determined in Step (c) and Step (b) respectively.

MP spectral characteristics in a WM region of interest—To infer MP spectral characteristics in WM in human brain, we performed region of interest (ROI) analysis. Since the total scan time for each subject took up to ~1.35 hours and only motion within the axial plane was corrected for by in-plane registration, care was taken to select the ROI well within WM. This was done from images of the MP fraction f thresholded at 0.2, and Gaussian smoothed over a kernel of 7 voxels. Fig. 1 shows an example of WM ROI's on five slices for one of the 7 subjects studied.

The ROI averaged $FS_{MP}(0, F)$ was calculated for each subject. Following previous work (14, 30), a SL lineshape (Eq. [7]) and Lorentzian (L) lineshape (Eq. [8]) were fit to this data.

$$g_{SL}((F - \Delta f_0(F) - \Delta F_{SL}), T_{2,SL}, A_{SL}) = A_{SL} \sqrt{\frac{2}{\pi}} \int_0^{\frac{\pi}{2}} \frac{T_{2,SL}}{|3\cos^2\theta - 1|} \exp\left(-2\left(\frac{2\pi(F - \Delta f_0(F) - \Delta F_{SL})T_{2,SL}}{3\cos^2\theta - 1}\right)^2\right) \sin\theta d\theta \quad [7]$$

$$g_L((F - \Delta f_0(F) - \Delta F_L), T_{2,L}, A_L) = A_L \frac{T_{2,L}}{\pi \left(1 + \left(2\pi(F - \Delta f_0(F) - \Delta F_L)T_{2,L}\right)^2\right)} \quad [8]$$

In Eqs. [7] and [8], A is a scaling factor, F is the frequency of the applied frequency-specific SR pulse as mentioned above, and F is the resonance frequency of MPs. $f_0(F)$ is the WM

ROI averaged frequency offset caused by the B_0 shift for a frequency-specific SR scan applied at frequency F , and is equal to $\gamma B_0/2\pi$, with B_0 determined above and γ being the gyromagnetic ratio. The averages and standard deviations were calculated for T_2 and F over subjects for both lineshapes. Fitting using the sum of two Lorentzians to individual subject $FS_{MP}(0,F)$ was also performed, using a common F_L determined from the single Lorentzian fit for both Lorentzians, to account for the potential contribution of two MP pools with distinct spectral characteristics (31, 46–48). Finally, fitting SL, Lorentzian and sum of two Lorentzians to $FS_{MP}(0,F)$ data combined from all subjects was also performed.

Potential effects of B_1 amplitude on the MP spectrum—The validity of our approach to infer the MP spectrum implicitly assumes a linear relationship between $FS_{MP}(0,F)$ and the power of off resonance MT pulses, which holds true only when the B_1 amplitude is low enough to avoid substantial saturation (42, 43). To study the effect of B_1 amplitude of the frequency-specific SR pulse on the inferred MP spectrum, we fitted a Lorentzian lineshape to simulated $FS_{MP}(0,F)$'s using Bloch equation. First, $FS_{MP}(0,F)$ for the MPs with a $T_{2,input}$ of 65 μ s is simulated, under the influence of frequency-specific hyperbolic secant SR pulse with a duration of 12 ms and a B_1 of 200 Hz (matching the preparation pulses used in the frequency-specific SR experiments), applied at several different frequency offsets, ranging from -16 kHz to 16 kHz. A Lorentzian fit yielded a $T_{2,output}$. Then, dependence of $T_{2,output}$ on $T_{2,input}$ and B_1 were studied by varying $T_{2,input}$ while keeping B_1 fixed as 200 Hz, and varying B_1 while keeping $T_{2,input}$ fixed as 65 μ s, respectively. A Lorentzian rather than a SL lineshape was used in all these simulations, due to the lack of a rigorous way to simulate Bloch equations incorporating a SL lineshape, under influence of a shaped MT pulse (49).

Potential effects of assumptions for the values of $R_{1,MP}$ and $FS_{MP}(0)$ —A potential source for inaccuracies in the extracted parameters are incorrect assumptions for the values of $R_{1,MP}$ and $FS_{MP}(0)$. For the broadband SR experiment (Experiment I), $R_{1,MP}=2.0$ s $^{-1}$ and $FS_{MP}(0)=0.93$ were assumed, and these may not be entirely accurate. We investigated the effect of changes in these values on the MP T_2 determined in Experiment II, based on subject and ROI averaged values of decay rates (λ_1 , λ_2) and amplitudes (a_1 , a_2) for all IR and SR experiments, which were fitted without using any assumption. By varying $R_{1,MP}$ from 1 to 3 s $^{-1}$ and $FS_{MP}(0)$ from 0.8 to 1.0 respectively, and going through procedures described above, new T_2 values were found for both SL and Lorentzian lineshapes, and these were compared with the original values.

Quantification of the symmetry of the MP spectrum—The effect of CEST on our spectrum measurements is expected to be small because of the short, 12 ms duration of the RF pulses used for the SR experiment. To investigate the presence of any remaining CEST effect, we analyzed spectral symmetry with the notion that any asymmetries would point to a potential CEST contribution (28, 33, 42, 43). Symmetry was shown by inspecting the residuals after subtracting the fits from ROI averaged $FS_{MP}(0,F)$ of all subject, done for all types of lineshapes, including SL, Lorentzian and sum of two Lorentzians.

Results

Voxel-wise fitting of the 2-pool model (Eq. [1]) to the IR and broadband SR data allowed robust extraction of the model parameters. ROI averaged results are shown in Fig. 2 and Table 1. A single set of rate constants (λ_1 and λ_2) fitted both IR and SR data well, as judged from the close fit to the data apparent in Fig. 2. The ROI-averaged values for $R_{1,WP}$, f , and k_{MW} were similar to those found in a previous study based on IR and SR measurements at 7 T (37).

With the voxel-wise fast (λ_1) and slow (λ_2) rate constants known, voxel-wise two-pool model fitting of $FS_{WP}(t)$ to the frequency-specific SR data yielded maps of coefficients $a_1(F)$ and $a_2(F)$, as exemplified in Fig. 3 (top two rows). Next, using Eq. [6], voxel-wise values for $a_1(F)$ and $a_2(F)$ were used to calculate $FS_{MP}(0,F)$, whose maps is shown as the bottom row of Fig. 3, and further ROI-averaged values for $FS_{MP}(0,F)$ were determined for each subject. Before proceeding with fitting line shapes to these data, small adjustments to the data were made to correct for potential shifts in F due to instrument drift. These correction values $f_0(F)$ were small relative to the spectral range.

Table 2 summarizes the results of fitting a SL (Eq. [7]), Lorentzian (Eq. [8]), and sum of two Lorentzians to subject-wise ROI-averaged $FS_{MP}(0,F)$. $T_{2,SL}$ of $9.6 \pm 0.6 \mu s$ and F_{SL} of $-773 \pm 14 \text{ Hz}$ ($-2.58 \pm 0.05 \text{ ppm}$), and $T_{2,L}$ of $65 \pm 2 \mu s$ and F_L of $-727 \pm 28 \text{ Hz}$ ($-2.42 \pm 0.09 \text{ ppm}$) were found for SL and Lorentzian fitting respectively. For the two-Lorentzian fit, a common F_L of -727 Hz (-2.42 ppm) determined from the single Lorentzian fit was used. This fitting resulted in a $74 \pm 3\%$ fraction of MPs with a T_2 of $23 \pm 5 \mu s$, and a $26 \pm 3\%$ fraction with a T_2 of $124 \pm 13 \mu s$. Judging from the R^2 (adjusted for degrees of freedoms) values, the SL and sum of two Lorentzians had similar performance in the fitting, and both provided better fit than Lorentzian.

SL and Lorentzian fits to ROI averaged $FS_{MP}(0,F)$ data were also performed on all subjects jointly (but without averaging over subjects, since $f_0(F)$ is different across subjects). This is shown in Fig. 4a, yielding $T_{2,SL}$ of $9.5 \mu s$ and F_{SL} of -781 Hz (-2.60 ppm) for the former, and $T_{2,L}$ of $65 \mu s$ and F_L of -726 Hz (-2.42 ppm) for the later. Fitting to the same data using sum of two Lorentzians, resulted in $T_{2,L}$'s of $23 \mu s$ (73%) and $122 \mu s$ (27%), as shown in Fig. 4b.

After subtraction of the fitted (symmetric) lineshapes from the spectral data, residual signal appears small for the SL fit (Fig. 5a), and the fit of two Lorentzians (Fig. 5c). The small residuals and the absence of any clear asymmetry in their distribution is consistent with expected suppression of CEST effects, and associated asymmetries, with the measurement approach followed here. However, deviation of the residuals from zero is obvious for the Lorentzian fit (Fig. 5b), confirming previous steady state MT studies (4–6, 19) where SL provided a fit superior over a Lorentzian lineshape.

Results of the simulations performed to investigate the influence of the B_1 amplitude on inferred T_2 are shown in Fig. 6. As expected, for an actual MP T_2 ($T_{2,input}$) of $65 \mu s$, the inferred T_2 ($T_{2,output}$) drops precipitously with RF power (Fig. 6b). However, at the experimental B_1 amplitude of 200 Hz , this drop remains limited: the $T_{2,output}$ of $60 \mu s$

underestimates $T_{2,\text{input}}$ by 5 μs , i.e. less than 10%. According to Fig. 6b, variation of B_1 by 25 Hz results in an error in the estimation of $T_{2,\text{output}}$ on the order of 1 μs , which suggest a low sensitivity to B_1 inhomogeneity. Similarly, at other values of $T_{2,\text{input}}$, and $B_1 = 200$ Hz, the difference between $T_{2,\text{input}}$ and $T_{2,\text{output}}$ remains modest (Fig. 6c).

The extracted MP T_2 values proved robust against small changes in assumed values for $R_{1,\text{MP}}$ and $FS_{\text{MP}}(0)$. Changing $R_{1,\text{MP}}$ from 1 to 3 s^{-1} resulted in very little change in $FS_{\text{MP}}(0, F)$. For the SL lineshape, $T_{2,\text{SL}}$ changed from 9.4 to 9.3 μs , while for the Lorentzian, $T_{2,\text{L}}$ changed from 65 to 64 μs . Changes of $FS_{\text{MP}}(0)$ from 0.8 to 1.0 resulted in a similar $T_{2,\text{SL}}$ in the range of 9.3–9.4 μs , and $T_{2,\text{L}}$ remained at a constant value of 64 μs , although increasing $FS_{\text{MP}}(0)$ of the broadband SR experiment results in increased $FS_{\text{MP}}(0, F)$.

Discussion

In order to aid interpretation of MT contrast at high field, we investigated MP spectral characteristics in human brain WM at 7 T. For this purpose, we used a dedicated indirect measurement approach that minimized direct saturation of WPs, CEST effects, and tissue heating, problems often associated with the generation of MT contrast in-vivo. We found that at 7 T, the MP spectrum is well represented by a symmetric, SL lineshape with a time constant $T_{2,\text{SL}}$ of 9.6 ± 0.6 μs , and shifted by -2.58 ± 0.5 ppm relative to the water resonance. As will be discussed in the following, these results are consistent with previous findings and mechanistic interpretations of MT from studies at lower field and point to dipolar coupling between methylene protons as a major determinant of the MP spectral characteristics. We anticipate that these values for the MP spectral characteristics will help design and quantification of future MT studies at 7T.

As has been found with previous indirect measurement of MP spectral characteristics at 1.5 T and 3 T, (4–6, 14) the 7 T data could be reliably fit with a SL lineshape. This is consistent with a dipolar coupling mechanism in semi-solids such as membrane proteins and lipids (14, 25, 29, 50). In addition, it was found that the 9.6 μs $T_{2,\text{SL}}$ at 7 T was similar to the 9.2 μs – 13.1 μs range of values found in previous studies at lower fields between 0.5 T and 3 T (4–6, 28, 51). Like the SL lineshape, the field independence of the linewidth is suggestive of a dominant contribution of dipolar coupling, rather than width being determined by chemical shift differences between the ^1H species contributing to MT (26). Previous direct measurements of MP spectral characteristics on fixed human WM tissue samples have found 50–65 μs for $T_{2,\text{L}}$ (20, 23) at 7 T and 4.7 T. These values were based on Lorentzian lineshapes, and translate into $T_{2,\text{SL}}$ values of 16–21 μs for SL lines of the same FWHM. These ex-vivo Lorentzian linewidths therefore are quite consistent with the in-vivo values, especially when considering the differences in measurement conditions.

Our MT study at 7 T found that the center of the MPs spectrum is shifted upfield relative to the resonance frequency of WPs by -2.58 ppm. This result agrees well with values of -2.34 ppm previously reported at 3 T (28), and the -2.55 ppm reported in WM of cat brain at 4.7 T (32). Combined, these studies show that the absolute spectral frequency shift is proportional to field strength, an observation consistent with a chemically shifted proton species contributing to MT. The value of the shift is somewhat smaller than the range of -3.2 to -3.9

ppm measured for methylene protons, the dominant proton species in myelin (24). While methylene protons are most abundant in WM (20), a not insignificant contribution to our MT signal may have come from, more downfield shifted proton species (e.g. in proteins and smaller molecules). These protons, while measured together with the methylene protons, may shift the combined spectral line downfield from the -3.2 to -3.9 ppm range.

To the extent that MT is caused by methylene protons in myelin lipids, their substantial orientational order may introduce a dependence of spectral linewidth on the direction of white matter fibers relative to the magnetic field. In fact, a recent study found that $T_{2,SL}$ may vary between 12 and 15 μs , dependent on fiber orientation (29). Due to lack of information about fiber orientation, we could not confirm this dependence in our own data. Nevertheless, if confirmed, a fiber-orientation dependent MP T_2 would further underscore the importance of the dipolar coupling mechanism for the apparent MP T_2 , and furthermore predict an orientation dependence of MT effects. Similarly, since the effect of an inversion RF pulse on MP magnetization depends on MP spectral characteristics (3), orientation dependence of MP T_2 may also render apparent T_1 (as measured from an IR experiment (3)) orientation dependent.

An advantage of the proposed method for the measurement of MP characteristic is the insensitivity to CEST effects, which can substantially contribute to so called “z-spectra” typically acquired to quantify MT effects (12, 28, 52), and thus confound interpretation. When generating MT contrast with seconds-long RF irradiation, MT from exchangeable protons can reduce the amplitude of the WP signal by as much of 2–4%, which is substantial when considering that typical asymmetry in z-spectra due to non-exchanging protons is only on the order of 1–2% (28). Although currently several approaches exist to distinguish CEST from other (non-exchange) MT effects (43, 52, 53), CEST effects were not explicitly excluded from z-spectrum asymmetry quantifications reported previously (28, 32). Nevertheless, it appears that the quantitative shifts characterizing this asymmetry are quite similar between the proposed and previous methods and amount to about -2.6 ppm.

A potential source for inaccurate quantification of MP spectral characteristics with the proposed method is the potential dependence on RF irradiation strength (B_1 amplitude). Preliminary experimental study of effects of B_1 on the spectrum measurement was performed on two subjects in a similar manner as described above. Experiment I was done in exactly the same way as described in Method for both subjects. In Experiment II, multiple B_1 's were used for the frequency-specific SR experiment, to quantify the B_1 dependence of ROI averaged $FS_{MP}(0, F)$. In lack of a rigorous way to do the simulation based on a SL lineshape (49) as mentioned above, attempts were made to simulate these data using single Lorentzian and a combination of multiple Lorentzians respectively, however no close fit was found. This may reflect the intrinsic insufficiency of a Lorentzian lineshape to represent the underlying interaction between the immobile MPs. The situation may be further complicated by the existence of multiple MP species, each with a SL lineshape centered on a different resonance frequency (24). Thus, while our spectral measurements provide a measure of linewidth and chemical shift, both of which provide insights into the molecular source of MT in white matter, uncertainty remains about the precise lineshape.

Conclusions

A novel approach was introduced to study MPs spectral characteristics while minimizing effects of RF-related tissue heating, direct saturation of WPs and CEST effects. Applied to WM in human brain at 7 T, MPs were found to have a symmetric, SL lineshape with a $T_{2,SL}$ of 9.6 μ s and an resonance frequency F_{SL} of -773 Hz (-2.58 ppm), consistent with previous results at low field, and consistent with a previously suggested major contribution of dipolarly-coupled lipid methylene protons. These results, as well as the proposed measurement approach, are expected to facilitate the use and interpretation of quantitative MT approaches at high field.

References

1. Wolff SD, Balaban RS. Assessing contrast on MR images. *Radiology*. 1997; 202:25–9. DOI: 10.1148/radiology.202.1.8988186 [PubMed: 8988186]
2. Henkelman RM, Huang X, Xiang Q-SS, Stanisz GJ, Swanson SD, Bronskill MJ. Quantitative interpretation of magnetization transfer. *Magn Reson Med* [Internet]. 1993; 29:759–766. DOI: 10.1002/mrm.1910290607
3. van Gelderen P, Jiang X, Duyn JH. Effects of magnetization transfer on T1 contrast in human brain white matter. *Neuroimage* [Internet]. 2015; 128:85–95. DOI: 10.1016/j.neuroimage.2015.12.032
4. Yarnykh VL. Pulsed Z-spectroscopic imaging of cross-relaxation parameters in tissues for human MRI: Theory and clinical applications. *Magn. Reson. Med.* [Internet]. 2002; 47:929–939. DOI: 10.1002/mrm.10120
5. Sled JG, Pike GB, Bruce Pike G. Quantitative imaging of magnetization transfer exchange and relaxation properties in vivo using MRI. *Magn. Reson. Med.* [Internet]. 2001; 46:923–931. DOI: 10.1002/mrm.1278
6. Ramani A, Dalton C, Miller DH, Tofts PS, Barker GJ. Precise estimate of fundamental in-vivo MT parameters in human brain in clinically feasible times. *Magn. Reson. Imaging* [Internet]. 2002; 20:721–731. DOI: 10.1016/S0730-725X(02)00598-2
7. Davies GR, Tozer DJ, Cercignani M, Ramani a, Dalton CM, Thompson aJ, Barker GJ, Tofts PS, Miller DH. Estimation of the macromolecular proton fraction and bound pool T2 in multiple sclerosis. *Mult. Scler.* [Internet]. 2004; 10:607–13. DOI: 10.1191/1352458504ms1105oa
8. Levesque IR, Giacomini PS, Narayanan S, Ribeiro LT, Sled JG, Arnold DL, Pike GB. Quantitative magnetization transfer and myelin water imaging of the evolution of acute multiple sclerosis lesions. *Magn. Reson. Med.* 2010; 63:633–640. DOI: 10.1002/mrm.22244 [PubMed: 20146232]
9. Love S. Demyelinating diseases. *J. Clin. Pathol.* 2006; 59:1151–1159. DOI: 10.1136/jcp.2005.031195 [PubMed: 17071802]
10. Alizadeh A, Dyck SM, Karimi-Abdolrezaee S. Myelin damage and repair in pathologic CNS: challenges and prospects. *Front. Mol. Neurosci.* [Internet]. 2015; 8:35.doi: 10.3389/fnmol.2015.00035
11. Popescu BF, Lucchinetti CF. Pathology of demyelinating diseases. *Annu Rev Pathol* [Internet]. 2012; 7:185–217. DOI: 10.1146/annurev-pathol-011811-132443
12. Van Zijl PCM, Zhou J, Mori N, Payen JF, Wilson D, Mori S. Mechanism of magnetization transfer during on-resonance water saturation. A new approach to detect mobile proteins, peptides, and lipids. *Magn. Reson. Med.* 2003; 49:440–449. DOI: 10.1002/mrm.10398 [PubMed: 12594746]
13. Chen JH, Sambol EB, DeCarolis P, O'Connor R, Geha RC, Wu YV, Singer S. High-resolution MAS NMR spectroscopy detection of the spin magnetization exchange by cross-relaxation and chemical exchange in intact cell lines and human tissue specimens. *Magn. Reson. Med.* 2006; 55:1246–1256. DOI: 10.1002/mrm.20889 [PubMed: 16676334]
14. Morrison C, Henkelman RM. A model for magnetization transfer in tissues. *Magn. Reson. Med.* [Internet]. 1995; 33:475–482.

15. Liepinsh E, Otting G. Proton exchange rates from amino acid side chains--implications for image contrast. *Magn. Reson. Med.* 1996; 35:30–42. DOI: 10.1002/mrm.1910350106 [PubMed: 8771020]
16. Zhou J, Zijl PCM van. Chemical exchange saturation transfer imaging and spectroscopy. *Prog. Nucl. Magn. Reson. Spectrosc.* 2006; 48:109–136. DOI: 10.1016/j.pnmrs.2006.01.001
17. Liu G, Song X, Chan K W Y, McMahon MT. Nuts and bolts of chemical exchange saturation transfer MRI. *NMR Biomed.* 2013; 26:810–828. DOI: 10.1002/nbm.2899 [PubMed: 23303716]
18. Van Zijl PCM, Yadav NN. Chemical exchange saturation transfer (CEST): What is in a name and what isn't? *Magn. Reson. Med.* 2011; 65:927–948. DOI: 10.1002/mrm.22761 [PubMed: 21337419]
19. Morrison C, Stanis G, Henkelman RM. Modeling magnetization transfer for biological-like systems using a semi-solid pool with a super-Lorentzian lineshape and dipolar reservoir. *J Magn Reson B* [Internet]. 1995; 108:103–113. DOI: 10.1006/jmrb.1995.1111
20. Horch RA, Gore JC, Does MD. Origins of the ultrashort-T2 1H NMR signals in myelinated nerve: A direct measure of myelin content? *Magn. Reson. Med.* [Internet]. 2011; 66:24–31. DOI: 10.1002/mrm.22980
21. Chan SI, Feigenson GW, Serter CHA. Nuclear Relaxation Studies of Lecithin Bilayers. *Nature* [Internet]. 1971; 231:110–112.
22. Finer EG, Darke A. Phospholipid hydration studied by deuterium magnetic resonance spectroscopy. *Chem. Phys. Lipids.* 1974; 12:1–16. DOI: 10.1016/0009-3084(74)90064-4 [PubMed: 4856625]
23. Ramani A, Aliev AE, Barkera GJ, Tofts PS. Another approach to protons with constricted mobility in white matter: Pilot studies using wide-line and high-resolution NMR spectroscopy. *Magn. Reson. Imaging.* 2003; 21:1039–1043. DOI: 10.1016/S0730-725X(03)00207-8 [PubMed: 14684209]
24. Wilhelm MJ, Ong HH, Wehrli SL, Li C, Tsai P-H, Hackney DB, Wehrli FW. Direct magnetic resonance detection of myelin and prospects for quantitative imaging of myelin density. *Proc. Natl. Acad. Sci.* 2012; 109:9605–9610. DOI: 10.1073/pnas.1115107109 [PubMed: 22628562]
25. Malyarenko DI, Zimmermann EM, Adler J, Swanson SD. Magnetization transfer in lamellar liquid crystals. *Magn. Reson. Med.* [Internet]. 2013; 72:1427–1434. DOI: 10.1002/mrm.25034
26. Feigenson GW, Chan SI. Nuclear magnetic relaxation behavior of lecithin multilayers. *J. Am. Chem. Soc.* [Internet]. 1974; 96:1312–1319. DOI: 10.1021/ja00812a009
27. Koenig SH. Cholesterol of myelin is the determinant of gray-white contrast in MRI of brain. *Magn. Reson. Med.* [Internet]. 1991; 20:285–91. DOI: 10.1002/mrm.1910200210
28. Hua J, Jones CK, Blakeley J, Smith SA, van Zijl PCM, Zhou J. Quantitative description of the asymmetry in magnetization transfer effects around the water resonance in the human brain. *Magn. Reson. Med.* [Internet]. 2007; 58:786–793. DOI: 10.1002/mrm.21387
29. Pampel A, Müller DK, Anwender A, Marschner H, Möller HE. Orientation dependence of magnetization transfer parameters in human white matter. *Neuroimage.* 2015; 114:136–146. DOI: 10.1016/j.neuroimage.2015.03.068 [PubMed: 25862261]
30. Li JG, Graham SJ, Henkelman RM. A flexible magnetization transfer line shape derived from tissue experimental data. *Magn. Reson. Med.* 1997; 37:866–871. DOI: 10.1002/mrm.1910370610 [PubMed: 9178237]
31. Stanis GJ, Kecojovic A, Bronskill MJ, Henkelman RM. Characterizing white matter with magnetization transfer and T2. *Magn. Reson. Med.* 1999; 42:1128–1136. DOI: 10.1002/(SICI)1522-2594(199912)42:6<1128::AID-MRM18>3.0.CO;2-9 [PubMed: 10571935]
32. Pekar J, Jezzard P, Roberts DA, Leigh JS, Frank JA, McLaughlin AGC. Perfusion imaging with compensation for asymmetric magnetization transfer effects. *Magn. Reson. Med.* [Internet]. 1996; 35:70–79. DOI: 10.1002/mrm.1910350110
33. Ng MC, Hua J, Hu Y, Luk KD, Lam EY. Magnetization transfer (MT) asymmetry around the water resonance in human cervical spinal cord. *J. Magn. Reson. Imaging.* 2009; 29:523–528. DOI: 10.1002/jmri.21610 [PubMed: 19243033]
34. Swanson SD, Pang Y. MT is Symmetric but Shifted with Respect to Water. *Proc. Intl. Soc. Mag. Reson. Med.* 2003; 11:660.

35. Mougin OE, Coxon RC, Pitiot a, Gowland Pa. Magnetization transfer phenomenon in the human brain at 7 T. *Neuroimage* [Internet]. 2010; 49:272–81. DOI: 10.1016/j.neuroimage.2009.08.022
36. Goldman M, Shen L. Spin-spin relaxation in LaF₃. *Phys. Rev.* 1966; 144:321–331. DOI: 10.1103/PhysRev.144.321
37. van Gelderen P, Jiang X, Duyn JH. Rapid measurement of brain macromolecular proton fraction with transient saturation transfer MRI. *Magn. Reson. Med.* [Internet]. 2016; n/a–n/a. doi: 10.1002/mrm.26304
38. Gochberg DF, Gore JC. Quantitative magnetization transfer imaging via selective inversion recovery with short repetition times. *Magn. Reson. Med.* 2007; 57:437–441. DOI: 10.1002/mrm.21143 [PubMed: 17260381]
39. Dortch RD, Moore J, Li K, Jankiewicz M, Gochberg DF, Hirtle JA, Gore JC, Smith SA. Quantitative magnetization transfer imaging of human brain at 7T. *Neuroimage*. 2013; 64:640–649. DOI: 10.1016/j.neuroimage.2012.08.047 [PubMed: 22940589]
40. Forsén S, Hoffman R. Study of moderately rapid chemical exchange reactions by means of nuclear magnetic double resonance. *J. Chem. Phys.* [Internet]. 2004; 39:2892–2901. DOI: 10.1063/1.1734121
41. Tannús A, Garwood M, Tannus A, Garwood M. Adiabatic pulses. *NMR in Biomedicine*. 1997; 10:423–434. DOI: 10.1002/(SICI)1099-1492(199712)10:8<423::AID-NBM488>3.0.CO;2-X [PubMed: 9542739]
42. Scheidegger R, Vinogradov E, Alsop DC. Amide proton transfer imaging with improved robustness to magnetic field inhomogeneity and magnetization transfer asymmetry using saturation with frequency alternating RF irradiation. *Magn. Reson. Med.* 2011; 66:1275–1285. DOI: 10.1002/mrm.22912 [PubMed: 21608029]
43. Lee JS, Regatte RR, Jerschow A. Isolating chemical exchange saturation transfer contrast from magnetization transfer asymmetry under two-frequency rf irradiation. *J. Magn. Reson.* 2011; 215:56–63. DOI: 10.1016/j.jmr.2011.12.012 [PubMed: 22237631]
44. Ordidge RJ, Gibbs P, Chapman B, Stehling MK, Mansfield P. High-speed multislice T1 mapping using inversion-recovery echo-planar imaging. *Magn. Reson. Med.* 1990; 16:238–245. DOI: 10.1002/mrm.1910160205 [PubMed: 2266843]
45. Kim M, Gillen J, Landman BA, Zhou J, Van Zijl PCM. Water saturation shift referencing (WASSR) for chemical exchange saturation transfer (CEST) experiments. *Magn. Reson. Med.* 2009; 61:1441–1450. DOI: 10.1002/mrm.21873 [PubMed: 19358232]
46. Levesque IR, Pike GB. Characterizing healthy and diseased white matter using quantitative magnetization transfer and multicomponent T2 relaxometry: A unified view via a four-pool model. *Magn. Reson. Med.* 2009; 62:1487–1496. DOI: 10.1002/mrm.22131 [PubMed: 19859946]
47. Kalantari S, Laule C, Bjarnason TA, Vavasour IM, MacKay AL. Insight into in vivo magnetization exchange in human white matter regions. *Magn. Reson. Med.* [Internet]. 2011; 66:1142–1151. DOI: 10.1002/mrm.22873
48. Charvolin J, Rigny P. Pulsed NMR in dynamically heterogeneous systems. *J. Magn. Reson.* 1971; 4:40–46. DOI: 10.1016/0022-2364(71)90022-9
49. Graham SJ, Henkelman RM. Understanding pulsed magnetization transfer. *J. Magn. Reson. Imaging.* 1997; 7:903–912. DOI: 10.1002/jmri.1880070520 [PubMed: 9307918]
50. Wennerstrom H. Proton nuclear magnetic resonance lineshapes in lamellar liquid crystals. *Chem. Phys. Lett.* 1973; 18:41–44. DOI: 10.1016/0009-2614(73)80333-1
51. Stanisz GJ, Odobina EE, Pun J, Escaravage M, Graham SJ, Bronskill MJ, Henkelman RM. T1, T2 relaxation and magnetization transfer in tissue at 3T. *Magn. Reson. Med.* [Internet]. 2005; 54:507–512. DOI: 10.1002/mrm.20605
52. Yadav NN, Chan KWY, Jones CK, McMahon MT, Van Zijl PCM. Time domain removal of irrelevant magnetization in chemical exchange saturation transfer Z-spectra. *Magn. Reson. Med.* 2013; 70:547–555. DOI: 10.1002/mrm.24812 [PubMed: 23798323]
53. Zaiss M, Schmitt B, Bachert P. Quantitative separation of CEST effect from magnetization transfer and spillover effects by Lorentzian-line-fit analysis of z-spectra. *J. Magn. Reson.* 2011; 211:149–155. DOI: 10.1016/j.jmr.2011.05.001 [PubMed: 21641247]

FIG. 1.
Example of WM ROI selection. ROI location is shown superimposed on EPI scan (top row), together with corresponding f maps. Selection was based on thresholding f at 0.2, followed by Gaussian smoothing with a 7 voxel kernel.

FIG. 2.

Two-pool model fitting (shown in lines) to subject and WM ROI averaged $FS_{WP}(t)$ (shown in dots) of IR (a) and broadband SR (b) experiments, with the error bars representing the standard deviations over subjects.

FIG. 3.

Examples of maps of $-a_I(F)$ (first row) and $a_2(F)$ (second row), and $FS_{MP}(0,F)$ (third row), for the frequency-specific SR data fit, at different frequency F (from left to right: $-16, -8, -4, -2, -1, -0.5, 0.5, 1, 2, 4, 8, 16$ kHz)

FIG. 4.

A SL lineshape (shown as the red dashed curve in a) and a Lorentzian lineshape (shown as the black solid curve in a) fitting to the WM ROI averaged $FS_{MP}(0,F)$ of all subjects jointly (shown in blue dots); sum of two Lorentzians (shown as the curve in b) fits to the same data (shown in blue dots).

FIG. 5.

Residuals for the SL fit (a), single Lorentzian fit (b), and sum of two Lorentzians fit (c) subtracted from the WM ROI averaged $FS_{MP}(0, F)$ of all subjects (shown in dots), with data in range of $0 \text{ Hz} < F < 1500 \text{ Hz}$ marked in red and $-1500 \text{ Hz} < F < 0 \text{ Hz}$ marked in blue.

FIG. 6.

Simulations of influence of B_1 amplitude on inferred T_2 : (a) Lorentzian fit (the curve) to simulated $FS_{MP}(0,F)$ (the dots) of MP pool with $T_{2,input}$ of 65 μ s, using Bloch equation, under the influence of frequency-specific hyperbolic secant pulses with B_1 of 200 Hz, a common pulse duration of 12 ms, resulting in a $T_{2,output}$ of 60 μ s; (b) B_1 dependence of $T_{2,output}$ by varying B_1 while keeping $T_{2,input}$ fixed as 65 μ s; (c) $T_{2,input}$ dependence of $T_{2,output}$ by varying $T_{2,input}$ while keeping B_1 fixed as 200 Hz.

Average (standard deviation) of the extracted two-pool model parameters in WM ROI's over subjects from Experiment I, with $R_{1, MP}$ assumed as 2.0 s^{-1} uniform across the brain for all subjects, as determined previously by van Gelderen et al (3, 37); all rates are reported in s^{-1} ; R^2 was adjusted for degrees of freedoms.

Table 1

$a_1(\text{IR})$	$a_2(\text{IR})$	$a_1(\text{SR})$	$a_2(\text{SR})$	λ_1	λ_2	$R_{1, WP}$	$R_{1, MP}$	f	k_{WM}	k_{MW}	R^2
0.216	1.75	-0.197	0.254	9.95	0.716	0.363	2.0	0.250	2.07	6.21	0.99996
(0.009)	(0.01)	(0.010)	(0.010)	(0.32)	(0.015)	(0.010)		(0.012)	(0.13)	(0.25)	(1×10^{-5})

Table 2

Results of T_2 , F and R^2 (adjusted for degrees of freedoms) for fitting of SL, Lorentzian and sum of two Lorentzians lineshapes to $FS_{MP}(0, F)$ data, report in the form of subject average $\pm$ (standard deviation over subjects); for sum of two Lorentzians fit, a common F_L for both Lorentzians was assumed to be -727 Hz (-2.42 ppm), identical to that obtained from the single Lorentzian fit. The component with T_2 of $23\pm 5\mu s$, composes $74\pm 3\%$ of the total MPs, and the other component with T_2 of $124\pm 13\mu s$ composing the rest.

	SL	Lorentzian	Sum of 2 Lorentzians
T_2 (μs)	9.6 ± 0.6	65 ± 2	$23\pm 5/124\pm 13$
F (Hz)	-773 ± 14	-727 ± 28	-727
R^2	0.982 ± 0.005	0.939 ± 0.013	0.989 ± 0.003