A novel method to measure $T_1$-relaxation times of macromolecules and quantification of the macromolecular resonances

Saipavitra Murali-Manohar$^{1,2}$ | Andrew Martin Wright$^{1,3}$ | Tamas Borbath$^{1,2}$ | Nikolai I. Avdievich$^{1}$ | Anke Henning$^{1,4}$

$^1$High-Field Magnetic Resonance, Max Planck Institute for Biological Cybernetics, Tübingen, Germany
$^2$Faculty of Science, University of Tübingen, Tübingen, Germany
$^3$IMPRS for Cognitive & Systems Neuroscience, Tübingen, Germany
$^4$Advanced Imaging Research Center, UT Southwestern Medical Center, Dallas, Texas, USA

Purpose: Macromolecular peaks underlying metabolite spectra influence the quantification of metabolites. Therefore, it is important to understand the extent of contribution from macromolecules (MMs) in metabolite quantification. However, to model MMs more accurately in spectral fitting, differences in $T_1$ relaxation times among individual MM peaks must be considered. Characterization of $T_1$-relaxation times for all individual MM peaks using a single inversion recovery technique is difficult due to eventual contributions from metabolites. On the contrary, a double inversion recovery (DIR) technique provided flexibility to acquire MM spectra spanning a range of longitudinal magnetizations with minimal metabolite influence. Thus, a novel method to determine $T_1$-relaxation times of individual MM peaks is reported in this work.

Methods: Extensive Bloch simulations were performed to determine inversion time combinations for a DIR technique that yielded adequate MM signal with varying longitudinal magnetizations while minimizing metabolite contributions. MM spectra were acquired using DIR-metabolite-cycled semi-LASER sequence. LCModel concentrations were fitted to the DIR signal equation to calculate $T_1$-relaxation times.

Results: $T_1$-relaxation times of MMs range from 204 to 510 ms and 253 to 564 ms in gray- and white-matter rich voxels respectively at 9.4T. Additionally, concentrations of 13 MM peaks are reported.

Conclusion: A novel DIR method is reported in this work to calculate $T_1$-relaxation times of MMs in the human brain. $T_1$-relaxation times and relaxation time corrected concentrations of individual MMs are reported in gray- and white-matter rich voxels for the first time at 9.4T.
1 | INTRODUCTION

Broad macromolecular resonances underlie metabolite spectra in $^1$H MR spectroscopy (MRS) at short echo times (TE). Macromolecules (MMs) between 0.5 and 4.5 ppm are attributed to mobile methyl, methylene, and methine groups of amino acids from cytosolic peptides and proteins. Several studies at field strengths between 1.5 to 3 T have highlighted the clinical relevance of MMs in aging and in pathologies such as traumatic encephalopathy, Kennedy’s disease, acute multiple sclerosis, and glioma. To understand mechanisms better in the aforementioned pathologies, it is of interest to use advantages of higher field strengths. Due to increased spectral dispersion, signal-to-noise ratio (SNR), and resolution, more MM peaks are distinguishable at ultra-high field (UHF) ($B_0 \geq 7$ T); hence, it is possible to more accurately characterize the behavior of individual MM peaks.

On the other hand, the well-resolved underlying MM spectrum at UHF often distorts the metabolite spectrum. Therefore, characterizing MM peaks additionally improves accuracy in quantifying metabolites. Various techniques have been used to handle MM signals in metabolite spectra namely: (1) opting for longer TE while measuring the metabolite spectra, (2) applying mathematical models such as a flexible spline baseline while fitting metabolite spectra, and (3) using prior information from experimentally acquired MM spectra.

The first two techniques (1, 2) do not prove to be the best solution at high field strengths ($\geq 3$ T). Using longer TEs (1) can lead to complications in quantifying metabolites due to decreased SNR and evolving J-coupling. Therefore, short TEs are preferred in studies at UHF due to the generally faster transversal relaxation times of metabolites. With a decrease in the amount of overlapping of MM peaks at UHF, a flexible spline baseline (2) may not hold to be a good solution to the problem of accounting for the MM spectrum accurately. Work done previously at 14.1 T acquired the MM spectrum by nulling metabolite resonances, and reported that the use of a prior knowledge MM spectrum in spectral fitting leads to more accurate and more reliable metabolite quantification at UHF. Hence, using prior information (3) from experimentally acquired MM spectra may prove to be a better solution.

Inversion recovery (IR) techniques are a reliable method for acquiring MM spectra due to the relatively fast $T_1$-relaxation times of MM peaks compared with those of metabolites. However, IR techniques that are used to acquire MM spectra introduce $T_1$-weighting to the MM signals which depend on the chosen inversion time (TI) and repetition time (TR). It is likely that using $T_1$-weighted MM spectra when fitting metabolite spectra, that are acquired without inversion pulses and typically a shorter TR, will influence quantitative accuracy. Indeed, a better characterization of the behavior of the relaxation times of the MM spectrum will lead to improved spectral fitting and fitting reproducibility.

Previous studies have characterized the $T_1$-relaxation times of MMs in the human brain for MM spectra as a whole or for non-overlapping MM peaks, or for groups of MM peaks. The $T_1$-relaxation times of individual MM peaks have yet to be reported for any field strength. Characterizing $T_1$-relaxation times for all individual MM peaks using a single inversion recovery technique is difficult due to eventual contributions from metabolites. More specifically, using a single-IR technique would have resulted in metabolites contaminating MM spectra at most TIs through an inversion series; consequently, making it challenging to estimate the $T_1$-relaxation times of most MM components with a sufficient range of longitudinal magnetization. Therefore, a double-IR (DIR) technique was chosen for this study because it permitted flexibility for more consistent nulling of metabolites along with observing a range of magnetizations of MM peaks. The flexibility gained with a DIR approach comes from the freedom to change the TI for both of the inversion pulses. Moreover, when using adiabatic localization sequences such as semiLASER, a DIR technique is more advantageous as this technique is shown to be insensitive to $B_0^2$ effects.

Detection of 15 MM peaks between 0.8 and 4.2 ppm has been previously reported at 9.4 T by using DIR semiLASER with metabolite cycling (MC). To measure an MM spectrum with minimal metabolite contribution an optimized combination of TIs ($T_1/T_2 = 2360/625$ ms) was required. However, for the current study, more than a single $T_1/T_2$ combination was needed; consequently, several $T_1/T_2$ combinations that yielded a range from negative to positive longitudinal magnetizations of MMs and simultaneously suppressed the metabolite signal had to be determined.

Classically, characterizing $T_1$-relaxation times of metabolites requires either an inversion recovery series or a progressive saturation approach with a TR-series to induce a sweep from negative to positive longitudinal magnetizations of the resonances of interest. The goal of the current study was to calculate the $T_1$-relaxation times of 13 MM peaks between 0.8 and 4.0 ppm in the human brain. However, simultaneously minimizing metabolite contributions to detect MM peaks while also introducing a sufficiently broad range of

**KEYWORDS**

double inversion recovery, macromolecules, MR spectroscopy, quantification, semiLASER, $T_1$-relaxation times, ultra-high magnetic field
longitudinal magnetizations to determine $T_1$-relaxation times of the MM peaks required extensive optimization on combinations of $T_{I_1}/T_{I_2}$.

To determine suitable $T_{I_1}/T_{I_2}$ combinations, exhaustive Bloch simulations were performed for an optimized DIR technique. Ultimately, from all the Bloch simulation results, 11 $T_{I_1}/T_{I_2}$ combinations were chosen for this study. Hence, a novel DIR approach with 11 different combinations of two inversion delays is described to determine the $T_1$-relaxation times of individual MM resonances. Using this approach, $T_1$-relaxation times for all MM resonances between 0.8 and 4.0 ppm are reported for the first time in the human brain at 9.4 T in both gray matter (GM) and white matter (WM).

Furthermore, quantification using internal water as reference was performed for each MM peak reported in this work by using the knowledge gathered for the $T_1$-relaxation times of MM peaks and $T_2$-relaxation results from Murali-Manohar et al.\textsuperscript{21}; thus, concentrations without correction for proton density are reported. This work is an extension of the preliminary results presented earlier as a conference abstract.\textsuperscript{22}

2 | METHODS

2.1 | Study design

Eleven healthy volunteers (6 males, 5 females, age: 27.7 ± 2.3 years) were measured on a 9.4 T Siemens Magnetom whole-body MRI scanner (Siemens Healthineers, Erlangen, Germany) using a home-built 8T/16Rx coil.\textsuperscript{23} The coil was driven in surface mode by a three-way splitter to drive power to the bottom three channel-elements as described in Giapitzakis et al.\textsuperscript{20} A $2 \times 2 \times 2$ cm$^3$ voxel was placed spanning the longitudinal fissure of the occipital lobe for GM measurements, and a voxel was placed within the left occipital-parietal transition for WM measurements (Figure 1). All eleven volunteers completed participation for GM measurements; however, only nine volunteers (4 males, 5 females, age: 28.2 ± 2.2 years) finished the second acquisition for WM measurements. Studies were performed with approval of the local ethics committee, and volunteers provided written informed consent prior to measurements.

2.2 | Bloch Simulation

A range of longitudinal magnetization values for MM signals ranging from negative to positive $M_Z$ are required to calculate $T_1$-relaxation times of MM resonances. To estimate individual MM peaks, the metabolite signals must be suppressed as much as possible. Hence, Bloch simulations were performed assuming a single proton spin to determine DIR schemes with suitable TIs $T_{I_1}$ and $T_{I_2}$. The magnetization vector ($M(x, y, z)$) was calculated for the actual inversion pulse shape implemented at the 9.4 T MRI scanner and the TIs ($T_{I_1}$ and $T_{I_2}$) as depicted in the DIR sequence scheme.

**FIGURE 1** Top, MC semi-LASER sequence preceded by double inversion recovery scheme. $T_{I_1}$ and $T_{I_2}$ were changed as given in Table 1. Bottom, Voxel were placed into GM-rich (left) and WM-rich (right) regions to measure the inversion series. Red within the highlighted region represents WM; whereas, blue within the highlighted region represents GM. The average voxel content for GM-rich and WM-rich voxels were found to be GM/WM/CSF = 72 ± 2/22 ± 3/6 ± 4% and 35 ± 6/62 ± 7/3 ± 3%, respectively.
in Figure 1; the inversion pulse profile is described in further detail by Giapitzakis et al. The available longitudinal magnetization following a DIR block is given by the M$_Z$ component, and the ratio of M$_Z$ to the initial magnetization (M$_Z$/M$_0$) was calculated for the inversion pulse profile$^{19}$ with a frequency offset range equal to the actual bandwidth of the pulse (−2000 to 2000 Hz, Supporting Information Figure S1, which is available online)$^{20}$.20

Metabolite T$_1$-relaxation times were taken from previously acquired in vivo results at 9.4 T.$^7$ MM resonances were grouped as a single MM spectrum containing all resonances with a T$_1$-relaxation time measured in vivo at 7 T.$^{13}$ In addition to effects of T$_1$-relaxation, the transmit field strength B$_1^+$ and resonance offset effects were accounted for in the Bloch simulations. M$_Z$/M$_0$ for MM spectrum and metabolite peaks were systematically simulated in increments of 50 ms for the first pass sweeping a range from 800 to 2500 and from 10 to 800 ms for TI$_1$ and TI$_2$ respectively. Following the initial simulation, tighter increments were simulated for TI$_1$/TI$_2$ combinations to find optimal inversion delays. TI$_1$/TI$_2$ combinations were chosen such that spectra had minimal metabolite residual M$_Z$ while sweeping a range of M$_Z$ for MM signals as reported in the Results section. These resultant TI$_1$/TI$_2$ combinations were then further tested in vivo to ensure acquisition of clean MM spectra with minimal contribution from metabolites. For additional information, the script used for Bloch simulations has been provided in Supporting Information Material Annex A.

| TI$_1$/TI$_2$ [ms] | M$_Z$/M$_0$ | Metabolites |
|---------------------|------------|-------------|
| 2360/625            | 0.5344     | −0.0451 to 0.0200 |
| 2150/600            | 0.5079     | −0.0020 to 0.0220 |
| 2000/575            | 0.4799     | −0.0030 to 0.0225 |
| 1900/550            | 0.4501     | 0.0056 to 0.0362 |
| 1800/525            | 0.4191     | 0.0079 to 0.0521 |
| 1050/238            | −0.0498    | −0.0146 to 0.2195 |
| 1300/80             | −0.5797    | −0.3267 to 0.0080 |
| 1300/60             | −0.6549    | −0.3528 to −0.0033 |
| 1200/20             | −0.7919    | −0.3497 to 0.0290 |
| 1250/20             | −0.8048    | −0.3787 to 0.0011 |
| 1300/20             | −0.8162    | −0.4063 to −0.0261 |

2.3 Data acquisition

High-resolution 2D FLASH images (in-plane resolution: 0.7 × 0.7 mm$^2$, slice thickness: 3.5 mm, 25 slices) were acquired in the sagittal and transversal planes to position spectroscopy voxels into GM- and WM-rich regions. Prior to spectroscopy measurements, localized FAST(EST)MAP$^{24}$ second-order shimming and power optimization$^{25,26}$ were performed to ensure that sufficient linewidths and adiabatic conditions would be fulfilled. The shim volume was set to be 150% of the volume of the voxel of interest.

A metabolite-cycled semi-LASER (MC semi-LASER) sequence$^{27}$ preceded by a novel DIR technique$^{20}$ (Figure 1) was used for spectroscopic acquisition (echo time [TE]/TR = 24/8000 ms; number of excitations [NEX] = 32) with 11 different combinations of TI$_1$ and TI$_2$ (Table 1). The TR used in all combinations of TI$_1$/TI$_2$ was given such that the following inversion pulse (TI$_1$) did not begin until 8000 ms after the position of the MC pulse (Figure 1). The transmit reference frequency was set at 2.4 ppm, and a 16-step phase cycling scheme was implemented. To avoid any influence of MC pulses on quantification based on internal water referencing, additional unsuppressed water reference signals (NEX: 16) were acquired with semi-LASER (TE: 24 ms) without MC.

MP2RAGE images$^{28}$ (resolution: 0.6 mm$^3$) were acquired with the same coil mentioned above by driving power to all eight channels. MP2RAGE data were used to calculate tissue volume fractions for each voxel to correct for the tissue composition dependence of T$_1$-relaxation as well as for MM quantification.

2.4 Data preprocessing

The raw data were reconstructed with an in-house MATLAB (version 2016a, MathWorks, Natick, MA) tool and were processed with a similar method as described in Giapitzakis et al.$^{27}$

Firstly, the FIDs were truncated to 250 ms so that data with better SNR were available for the following data preprocessing steps. Then they were frequency and phase-aligned in the time domain. This was followed by metabolite-cycling subtraction and the data were then averaged. After this, the data were zero-order phase and eddy current corrected using the phase information from the MC water signal. Signals from all 16 receive channels were then combined using an SVD method. The residual water in the spectra was removed using a HSVD method, and finally, the FIDs were truncated to 150 ms.
2.5 Spectral fitting

Spectra were fitted with LCModel (v6.3-1L) using simulated Voigt lines (CHSIMU) to fit 13 MM peaks. MM data were fit from 0.8 to 4.0 ppm. Initial fitting of MM spectra was performed using the subject-wise summed results for each TI1/TI2 combination. Metabolites predicted to contribute by Bloch simulations were initially included as characteristic spectral pattern in the basis sets, but only those metabolites which were fit in the initial trial (fitting subject-wise summed spectra) was kept in the final fit settings to avoid over-parameterization (Supporting Information Table S1). Complete basis vectors were not suitable for fitting metabolite residuals for the NAA(CH3) singlet and tCr resonances at most TI1/TI2 combinations due to the minor contributions to spectra and different relaxation of different moieties of the same metabolites. Thus, the contributions from these metabolite signals were simulated using narrow Voigt line shapes. Following determination of metabolite contributions from summed spectra, optimized basis sets were defined for fitting of the individual data sets (Supporting Information Material Annex B).

The chosen combinations of simulated Voigt lines were used to represent residual singlets in the spectra: N-acetyl aspartate (NAA(CH3) – 2.008 ppm), and creatine (tCr(CH3) – 3.027 ppm and tCr(CH2) – 3.925 ppm) across the inversion series to handle residual metabolite signals (Supporting Information Material Annex B). Myo-Inositol (mI) was simulated using VeSPA. 30 Aspartate (NAA(CH3) – 2.008 ppm), and creatine (tCr(CH3) – 3.925 ppm) across the inversion series to handle residual metabolite signals (Supporting Information Material Annex B). Glycero-phosphocholine (GPC), mI, glutamate (Glu), mI and aspartate moiety of NAA (NAAasp) were simulated in VesPA, and were included in the basis set only for TI1/TI2 combinations that had negative M2 (i.e., inverted peaks with negative amplitudes). The chemical shifts of MM peaks were chosen from Murali-Manohar et al. 21 Fit settings files are given in Supporting Information Material Annex B.

To fit the MM null point data (TI1/TI2 = 1050/238 ms), a metabolite basis set including NAA(CH3), tCr(CH2), tCr(CH3), GPC, glutamine (Gln), Glu, gamma-aminobutyric acid (GABA), glycine (Glycin), Aspartate (Asp), mI, NAAasp, and taurine (Tau) were simulated using VeSPA. 30 Metabolite basis vectors were chosen to be given here because of the low MM signals and the strong metabolite contributions. The simulated basis set was created with a modified semiLASER protocol with sequence parameters and pulses adjusted to match those in the present study.

Individual phases of metabolite residuals were adjusted in the LCModel basis sets as necessary depending on the magnetization achieved from a TI1/TI2 combination. Furthermore, the observed phase for DIR combinations was adjusted individually for GM- and WM-rich voxels to improve the accuracy of fits. DKNTMN parameter was set to 99 to keep the baseline stiff to allow for a more accurate fit of the MM data, and water scaling was performed to more accurately quantify the MM resonances.

LCModel water scaling was performed with respect to the downfield NAA amide resonance at 7.79 ppm (wsppm = 7.79, n1met = 1) rather than the LCModel default water scaling peak (tCr(CH3) taking three protons contribution). In particular, there were no peaks in the upfield range of the spectrum that were not affected by the DIR scheme. Thus, the downfield NAA amide resonance was chosen as its amplitude was stable and was not affected by the DIR scheme. Water scaling with the stable NAA-NH resonance not only allowed shifting of the spectrum with respect to a known resonance frequency, but also was included to account for variations in coil loading across subjects. Automated LCModel phase correction was also constrained by the downfield NAA peak. This approach provided a stable reference for quality control of LCModel fitting results.

2.6 Segmentation

MP2RAGE images were segmented into GM, WM, and CSF probability maps with SPM12. 2D FLASH images used for voxel placement were co-registered to the MP2RAGE image using SPM12, which returned an affine transformation between the image spaces. The affine matrix relating the two images was used in a home-built Python (v3.7) tool to determine the tissue fraction in spectroscopy voxels.

2.7 T1 calculation

Following spectral fitting in LCModel, all data were sorted by TI1/TI2 combination and fitted to the DIR signal equation.

\[
S = \frac{a}{2} \left( 1 - 2e^{-\frac{\text{T}2}{\text{T}1}} + e^{-\left(\frac{\text{T}1 + \text{T}2}{\text{T}1}\right)} \right).
\]

The optimization was done as a four-parameter bi-exponential model on the signal curve. In this model, TI1 and TI2 were both known parameters while a and T1 were both unknown variables which were optimized on. Optimizing for T1 from Equation (1) was performed in Python using the SciPy, NumPy, Matplotlib, and Pandas tool kits with a non-linear least squares, Levenberg-Marquardt algorithm; where a was assumed as a constant. Individual LCModel concentrations from all subjects were used in the curve fitting to calculate MM T1-relaxation times.

T1-relaxation has been shown to vary predominantly due to tissue type for water in contrast to T2-relaxation which also varies spatially depending on microscopic susceptibility differences. Hence, an assumption to further estimate...
the relaxation of theoretically pure GM and WM voxels, $T_1$, GM and $T_1$, WM respectively, was used. The following two linear equations were concurrently solved by assuming a linear relationship of relaxation time to the contribution of tissue type:

$$f_{\text{GM, vol}} \cdot T^\text{pure voxel}_{1,\text{GM}} + f_{\text{WM, vol}} \cdot T^\text{pure voxel}_{1,\text{WM}} = T^\text{rich voxel}_{1,\text{GM}} \cdot (1 - f_{\text{CSF, vol}})$$  \hspace{1cm} (2)

$$f'_{\text{GM, vol}} \cdot T^\text{pure voxel}_{1,\text{GM}} + f'_{\text{WM, vol}} \cdot T^\text{pure voxel}_{1,\text{WM}} = T^\text{rich voxel}_{1,\text{WM}} \cdot (1 - f'_{\text{CSF, vol}})$$  \hspace{1cm} (3)

where $f_y$ represents the tissue fraction in measures from GM-rich voxels and $f'_y$ represents the tissue fraction measures from WM-rich voxels. More specifically, by solving Equations (2) and (3) as a linear system, it allows us to then approximate the $T_1$-relaxation time of voxels with arbitrary ratios of GM and WM.

### 2.8 Quantification of MM peaks

MM concentrations were quantified in protons mmol/kg by using spectra acquired with a $T_1$/$T_2$ combination of 2360/625 ms from all subjects in both GM- and WM-rich voxels. Spectra corresponding to $T_1$/$T_2$ combination of 2360/625 ms were chosen for quantification because it offered maximal MM signal retention as well as minimal metabolite residuals.

For internal water referencing, the concentrations resulting from LCModel fitting were corrected for tissue water fractions and relaxation times as follows:

$$[M] = \frac{S_{\text{MM}} \times (f_{\text{GM}} \times R_{H2O,GM} + f_{\text{WM}} \times R_{H2O,WM} + f_{\text{CSF}} \times R_{H2O,CSF})}{S_{H2O} \times T_1 \times T_2 \times R_{\text{MM}} \times \#H_{\text{MM}} \times (1 - f_{\text{CSF}}) \times \frac{2}{(1 + F_s)} \times \frac{[\text{H}_2\text{O}]}{[\text{metabolite}]} \times [\text{water}]}$$

where $f_y = \frac{f_y \times a_y}{f_{\text{GM, vol}} \times a_{\text{GM}} + f_{\text{WM, vol}} \times a_{\text{WM}} + f_{\text{CSF, vol}} \times a_{\text{CSF}}}$.

Here $y$ corresponds to either GM, WM, or CSF; $f_y$ is the fraction of the respective tissue type determined by segmentation; $a_y$ are the relative densities of MR-visible water for the given tissue types (78%, 65%, 97% for GM, WM and CSF respectively); these $a_y$ remain uncorrected for the relative densities of GM and WM tissue (1.04 g/ml)$^{33-45}$ and were taken to be 1.0 g/ml. $\#H_{\text{MM}}$ is the number of protons that contribute to the signal of an MM peak, and was not accounted in this work. To arrive at proton mmol/kg concentrations, the concentration of water within a voxel, [H$_2$O], was assumed to be that of pure water (55,510 mmol/kg). $S_{\text{MM}}$ is the signal from an MM peak:

$$R_{H2O,y} = \exp\left[-\frac{\text{TE}}{T_{2H2O}}\right] \left[1 - \exp\left[-\frac{\text{TR}}{T_{1H2O}}\right]\right]$$

is the relaxation correction factor for each water compartment $y$. $T_{1\text{H2O}}$ and $T_{2\text{H2O}}$ are the $T_1$- and $T_2$-relaxation times of water in the compartment $y$; in particular, the relaxation times of water in GM are $T_{1\text{H2O,GM}} = 2120$ ms, $T_{2\text{H2O,GM}} = 37$ ms; in WM are $T_{1\text{H2O,WM}} = 1400$ ms, $T_{2\text{H2O,WM}} = 30$ ms; and in CSF are $T_{1\text{H2O,CSF}} = 4800$ ms, $T_{2\text{H2O,CSF}} = 181$ ms at 9.4 T.$^{28}$

$R_{\text{MM}} = (1 - 2e^{-\frac{\text{TE}}{T_{2\text{MM}}}} + 2e^{-\frac{\text{TE} + \text{TR}}{T_{1\text{MM}}}}) \left[1 - \exp\left[-\frac{\text{TR}}{T_{1\text{MM}}}\right]\right]$ is the relaxation correction term for macromolecules. $T_{2\text{MM}}$ were considered from Murali-Manohar et al.$^{21}$ and $T_{1\text{MM}}$ determined from this work were taken. The denominator $1 - f_{\text{CSF}}$ was implemented for partial-volume correction arising from contributions of CSF to the voxel volume. The factor $\frac{2}{1 + F_s}$ was introduced to correct for the multiplication of even numbered acquisitions with the scaling factor ($F_s$) from metabolite cycling.

### 3 RESULTS

#### 3.1 Bloch simulations

Bloch simulation results for $T_1$/$T_2 = 2000/575$ and 2150/600 ms are shown in Supporting Information Figure S1. Simulations estimated similar metabolite nulling efficiency for the all chosen TIs retaining the MM signal at different observable magnetizations except for $T_1$/$T_2 = 1050/238$ ms. The MM null spectrum resulted in a maximum $M_z/M_0$ for each MM spectrum. The $M_z/M_0$ for MM spectrum after DIR range block from −0.82 to 0.58 for the 11 chosen TIs and the corresponding $M_z/M_0$ for each MM spectrum. The $M_z/M_0$ for MM spectrum after DIR range block from −0.82 to 0.58 for the 11 chosen $T_1$/$T_2$ combinations.

#### 3.2 Inversion series of spectra and voxel content

Metabolite-nulled spectra were obtained with the 11 chosen $T_1$/$T_2$ combinations encompassing a range of magnetizations. Summed spectra from the inversion series for GM and WM MM spectra are displayed in Figure 2.
The subject-wise summed spectra display good spectral quality with 13 resolved MM peaks across the 11 T1/T2 combinations. However, there was noticeable noise and sometimes slight lipid contamination in the individual, non-summed spectra. Nevertheless, 13 MM peaks could be observed in a majority of spectra in both GM-rich and WM-rich voxels. Therefore, all data sets were included in the analysis. On average, the tissue content for GM-rich and WM-rich voxels were found to be GM/WM/CSF = 72 ± 2/22 ± 3/6 ± 4% and 35 ± 6/62 ± 7/3 ± 3% respectively. Indeed, residual metabolite signals were observed in several DIR spectra at various T1/T2 combinations, and the residual metabolite resonances corresponding to each T1/T2 combination is given in Supporting Information Table S1.

3.3 | Spectral fitting

Figure 3 shows fits and residues from LCModel for T1/T2 = 2360/625, 1300/20, and 1050/238 ms subject-wise summed spectra from GM-rich voxels. Fits corresponding to the same T1/T2 combinations from WM-rich voxels are given in Supporting Information Figure S2. Supporting Information Figure S3 shows fits for the remaining eight T1/T2 combinations for subject-wise summed spectra from GM-rich voxels. The tailored basis sets, for each T1/T2 combination and according to tissue type, fit the pre-processed data well. Furthermore, the residual for summed spectra fit from both GM-rich and WM-rich voxels showed minimized metabolite residuals. The fit residuals lacked structure in individual per subject fits (Figure 4). Metabolite residual fitting using basis vectors and fitted Voigt lines (Supporting Information Table S1) for residual peaks was sufficient for 10 T1/T2 combinations spectra; for the T1/T2 = 1050/238 ms combination yielding almost MM nulled spectra, a metabolite basis set was included, and it accurately accounted for metabolite contributions to spectra.

3.4 | T1 relaxation times

Figure 5 and Supporting Information Figure S4 show all MM peak data fitted to Equation (1) to calculate the T1-relaxation times of MM peaks in 3D signal plot diagrams. Black crosses represent individual data points from the T1/T2 series. The dashed, blue line represents the result from fitting the data to Equation (1) with a Levenberg-Marquardt algorithm. Resultant T1-relaxation times of 13 MM peaks are reported for GM-rich and WM-rich voxels in Table 2 and shown in Figure 6. T1-relaxation times of MM peaks range from 204 to 510 ms and 253 to 564 ms in GM-rich and WM-rich voxels respectively. M3.62 had the longest T1-relaxation time in both GM- and WM-rich voxels. The results from GM-rich and WM-rich voxels were used to solve for the T1-relaxations of theoretically pure GM and WM voxels with a linear system of two equations and are reported in Table 2. The goodness of fit for each MM T1-relaxation time calculated was assessed by the coefficient of determination (R2). The differences in T1-relaxation times between GM- and WM-rich voxels were evaluated using Welch’s t-test (α = 0.05) for all MM peaks. Adjusted P-values were calculated using the Bonferroni correction (\( \frac{N}{N} \)) to correct for multiple comparisons. Significant differences in T1-relaxation times between GM- and WM-rich voxels are denoted in Figure 6 and Table 2 by an asterisk: *P < .0038. Results from Welch’s t-test and P-values are reported in Supporting Information Table 3.

3.5 | Quantification of MM peaks

The T1/T2 combination of 2360/625 ms was chosen for quantification of MM peaks by means of internal water referencing. The unsuppressed water spectrum used for internal water referencing. The concentration results are thus reported in Figure 7 and Supporting Information Table S4. The concentration values were corrected for T1-relaxation
times of MMs from this work and for $T_2$-relaxation times from Murali-Manohar et al.,\textsuperscript{21} with corrections as described in the Methods section. $M_{2.04}$ has the highest concentration in both GM- and WM-tissue types with 78.4 ± 10.7 and 76.6 ± 10.9 protons mmol/kg respectively. $M_{0.92}$ has similar concentrations in GM- and WM-voxels, 21.1 ± 3.3 and 21.0 ± 2.6 protons mmol/kg, respectively. Wilcoxon rank-sum tests ($\alpha = 0.05$) were performed to assess for differences in concentrations between tissue types for all MM peaks. After correcting for multiple comparisons, using a Bonferroni correction ($\frac{\alpha}{N_{\text{tests}}} = 0.0038$), a significant difference for the concentration of $M_{3.75}$ ($P = .0009$) between GM and WM was found (Figure 7 and Supporting Information Table S4).

4  |  DISCUSSION

While it is possible to estimate $T_1$-relaxation time of the MM spectrum when using a single inversion recovery approach by simultaneously fitting metabolites, SIR techniques have been used to measure $T_1$-relaxation times of non-overlapping MM resonances as well as the whole MM spectrum. However, this could lead to misestimation of the MM peaks underlying metabolites and to ambiguities with respect to distinguishing metabolite and MM signals during spectral fitting.\textsuperscript{46,47} Although there were also metabolite residuals present in the proposed DIR technique, this approach reduced metabolite residuals better at the expense of $T_1$-weighting. Bloch simulations were used to predict where metabolites would be contributing to the MM spectra. Supporting Information Table S1 shows in detail where metabolite vectors or singlets were included in the MM spectral fitting. Albeit, $T_1$-relaxation time estimates for individual MM peaks were possible.

Careful evaluation of Bloch simulation results helped determine valid sets of $T_1$/$T_{1\text{r}}$ combinations. While there were many simulated $T_1$/$T_{1\text{r}}$ combination results that maintained high $M_z$ of MMs, the contributions of metabolites in many of these cases led to heavy contamination in MM spectra or noisy MM spectra. While the chosen $T_1$/$T_{1\text{r}}$ combinations provided good MM spectra there were still metabolite residuals that required fitting.

All 11 $T_1$/$T_{1\text{r}}$ combinations were used in the calculation of $T_1$-relaxation times for MM peaks. The five $T_1$/$T_{1\text{r}}$ combinations with negative $M_z/M_0$ covered a range from −0.82 to −0.58, and the five $T_1$/$T_{1\text{r}}$ combinations with positive $M_z/M_0$ covered a range from 0.42 to 0.53 as can be seen from Table 1. Although the entire range of MMs $M_z/M_0$ could not
be sampled due to the additional need to suppress metabolite signal, the chosen approach appears to be adequate for modeling the $T_1$-relaxation of MM peaks evidenced by the low standard deviation and $R^2$ of the curve fit for $T_1$-relaxation values which imply good confidence. Previous results\textsuperscript{48} suggested a TR of 8000 ms to be the best bet for a comfortable scan duration and to stay within specific absorption rate limits.

It was expected that WM-rich voxels may be slightly noisier and lower in SNR. This arose from the fact that WM-rich voxels were typically further away from the transmit coil elements; thus, the achieved $B_1$ was lower for WM-rich voxels compared to GM-rich voxels. As was evidenced by the data in this study, WM data tended to be slightly noisier (Figure 2), but the metabolite residuals in MM spectra for GM-rich and WM-rich voxels were similar. However, MM peaks were $T_1$-weighted slightly differently in GM-rich and WM-rich voxels due to the differences in their $T_1$-relaxation times.

Reliable HSVD for residual metabolite suppression was not achievable for particular $T_1$/$T_2$ combinations potentially due to the broad linewidths encountered and low SNR. A detailed list of all the metabolite residuals present in the corresponding $T_1$/$T_2$ combination spectra that were fitted is given in Supporting Information Table S1. $M_{4.03}$ and $M_{4.20}$ were excluded from analysis due to water contamination in the spectra in multiple $T_1$/$T_2$ combinations.

NAAasp, mI, Glu, and GPC were predicted to be metabolite residuals in the negative $M_z$ MM spectra due to their $T_1$-relaxation times\textsuperscript{49} and were furthermore visible when fitting spectra. To correct for the contribution these metabolites, they were simulated and added to all basis sets corresponding to $T_1$/$T_2$ combinations resulting in negative magnetization.

From Supporting Information Figure S4, it can be seen that the negative $M_z$ points had larger amounts of signal for $M_{2.99}$, $M_{3.62}$, and $M_{3.75}$ which skewed the signal fit resulting in a longer calculated $T_1$-relaxation time. It could be that lower weight metabolites contributed to the resonances within these peaks, which led to the increased estimation of the $T_1$-relaxation time. Attempts to account for metabolite resonances at these peaks were performed by including metabolite basis vectors; however, it was not possible to reliably fit all potential contributions from lower weight metabolites with this scheme. The increased $T_1$-relaxation times for these peaks at 3T have been reported by Hoefemann et al\textsuperscript{17}; in their study the observed $T_1$-relaxation times reached the “predefined borders of … 400 ms for $T_1$.”

$T_1$-relaxation times of MM peaks in GM- and WM-rich voxels range from 204 to 510 ms and from 253 to 564 ms, respectively. For pure GM and WM voxels, $T_1$-relaxation times are between 189 and 478 ms, and between 220 and 612 ms respectively. Indeed, the linear relationship used to calculate $T_1$-relaxation times likely simplifies the complex nature of the MM spectrum. However, it could be useful in modelling MM spectra voxel-wise where $T_1$-weighting of MMs is present such as FID-MRSI\textsuperscript{50}.
The broad range of MM peak T1-relaxation times suggests that approximating the T1-relaxation time of the MM spectrum as single value may not be ideal for quantifying MM peaks with high accuracy. Furthermore, when a measured IR MM spectrum is included to fit metabolites, T1-weighting within the MM spectrum could lead to misestimates of metabolite concentrations. For example, the MM spectrum corresponding to TI1/TI2 = 2360/625 ms was used in fitting metabolites in previous works.21,27 However, as seen from Table 3, T1-weighting for MM peaks following this DIR scheme range from 42% to 87% in a GM-rich voxel and 35% to 83% in a WM-rich voxel. Hence, quantifying metabolites with this particular MM spectrum could lead to over- or under-fitting of metabolites amplitudes depending on the T1-weighting of different MM peaks.

While comparing differences between GM- and WM-rich T1-relaxation times, significant differences (P < .0038) between tissue types were found for all MM peaks except M0.92, M1.21, M1.67, M2.04, and M2.26 as shown in Figure 6 and Supporting Information Table S3. Based on T1-relaxation differences between GM- and WM-rich voxels, tissue type specific MM models could be more appropriate than using averaged MM spectra to fit MM signals especially in metabolite spectra. The relaxation times of MM peaks do not follow a clear trend of increased T1-relaxation time of resonances as field strength increase.51 Behar et al52 measured the T1-relaxation times of 13 MM peaks in both GM-rich and WM-rich voxels at 9.4T are presented in the table with accompanying R2 values for the fit of the data to Equation (1). Furthermore, T1-relaxation times of pure GM and WM voxels for these peaks are also reported as solved by Equations (3) and (4). MM peaks that have significant differences between GM- and WM-rich voxels are indicated by an asterisk *P < .0038. Full statistical results are in Supporting Information Table S3.

### T1 Relaxation times of Macromolecules

| MM     | GM-rich T1 [ms] | R2  | WM-rich T1 [ms] | R2  | Pure-GM T1 [ms] | Pure-WM T1 [ms] |
|--------|----------------|-----|----------------|-----|----------------|-----------------|
| M0.92  | 280 ± 10       | 0.959 | 284 ± 10       | 0.966 | 278 ± 26       | 288 ± 23        |
| M1.21  | 273 ± 27       | 0.752 | 280 ± 28       | 0.782 | 269 ± 71       | 286 ± 62        |
| M1.39  | 326 ± 21       | 0.867 | 270 ± 24       | 0.828 | 358 ± 57       | 220 ± 50        |
| M1.67  | 231 ± 13       | 0.903 | 253 ± 17       | 0.894 | 218 ± 37       | 273 ± 32        |
| M2.04  | 279 ± 8        | 0.971 | 319 ± 9        | 0.974 | 256 ± 22       | 355 ± 19        |
| M2.26  | 281 ± 7        | 0.977 | 313 ± 9        | 0.972 | 263 ± 20       | 342 ± 17        |
| M2.56  | 309 ± 20       | 0.866 | 327 ± 18       | 0.915 | 299 ± 50       | 343 ± 44        |
| M2.70  | 306 ± 19       | 0.879 | 383 ± 22       | 0.902 | 262 ± 52       | 452 ± 45        |
| M2.99  | 463 ± 15       | 0.953 | 518 ± 15       | 0.966 | 431 ± 39       | 567 ± 34        |
| M3.21  | 204 ± 20       | 0.785 | 379 ± 29       | 0.836 | 103 ± 59       | 535 ± 51        |
| M3.62  | 510 ± 30       | 0.846 | 564 ± 35       | 0.853 | 479 ± 82       | 612 ± 71        |
| M3.75  | 280 ± 24       | 0.805 | 434 ± 25       | 0.887 | 191 ± 63       | 571 ± 55        |
| M3.86  | 280 ± 27       | 0.764 | 307 ± 22       | 0.871 | 264 ± 66       | 331 ± 58        |

**FIGURE 5** A signal scatter plot and fitted solution (blue dashed line) from the inversion series for M0.92 fitted by Equation (1). Black crosses represent data points from the 11 volunteers acquired in GM-rich voxels, and the blue line is the fitted solution to Equation (1). T1-relaxation calculation plots for the other MM peaks are reported in Supporting Information Figure S4. Points were excluded when the CRLB was equal to 999 because these points were not fitted in LCModel. In the present fit of M0.92, no points are excluded.
of the M_{0.93} peak as 250 ms at 4.0T, which is slightly lower compared to the T_1-relaxation time measured in this work at 9.4 T confirming the field strength trend for this non-overlapping MM peak. However, recent work at 3 T found the T_1-relaxation time of M_{0.92} to be about 290 ms.17

For the first time, concentrations of 13 MM peaks are reported (Supporting Information Table S4) for both GM- and WM-rich voxels after correcting for T_1- and T_2-relaxation times. Previous works^5,20,53,54 have reported concentrations for some or all peaks without correcting for T_1- or T_2-relaxation times. Inversion recovery preceding the localization scheme will lead to strong T_1-weighting of the MM spectrum, and not correcting for the relaxation times will result in discrepancies in concentrations across sites while using different inversion recovery techniques. Due to severe overlap of MM peaks, the number of protons contributing to each peak is not easily accountable; hence, the concentration values were not corrected for the number of contributing protons. The concentration of M_{0.92} agrees with the values reported by Hofmann et al^{55} and Snoussi et al^{53} and are slightly less than the recent results from Landheer et al^{54} However, concentrations for other MM peaks or groups of MM peaks vary among literature.^20,53-55 The results from Landheer et al^{54} appear that they could be in agreement with the current work at 9.4 T; however, the uncorrected T_1-weighting could be a cause of discrepancy between results.

Statistical tests highlighted a significant difference for elevated concentration of M_{3.75} (P = .0009) in WM. Furthermore, the results suggest that there could be slight variation between tissue types for M_{1.39}, M_{2.99}, and M_{3.21}; whereas the differences between GM and WM tissue signal for M_{2.26} have been reported in MRSI results.\cite{56} Figure 7 and Supporting Information Table S4 suggest that there are trends toward elevated concentrations of M_{3.75} in GM and for M_{2.99}, and M_{3.21} in WM; however, this study was not able to claim with certainty that these concentrations vary in a significant manner. Further work focusing on specific MM peaks is necessary to assess tissue concentration differences appropriately.

FIGURE 6  T_1-relaxation times for 13 MM peaks are reported. Results are shown for GM- and WM-rich voxels as well as for GM- and WM-pure voxels. The T_1-relaxation estimates for pure voxels were extrapolated by means of Equations (2) and (3). Additionally, these values are reported in Table 2. MM peaks that have significant differences following Welch’s t-test and Bonferroni correction between GM- and WM-rich voxels are indicated by an asterisk *P < .0038. Full statistical results are in Supporting Information Table S3

TABLE 3  Calculated T_1-weighting in percent of MM peaks following DIR scheme (T_1/T_2 = 2360/625 ms; TR = 10 s) using T_1-relaxation times from measured GM- and WM-rich voxels in this work

| T_1-weighting [%] | M_{0.92} | M_{1.21} | M_{1.39} | M_{1.67} | M_{2.04} | M_{2.26} | M_{2.56} | M_{2.70} | M_{2.99} | M_{3.21} | M_{3.62} | M_{3.75} | M_{3.86} |
|-----------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| GM              | 78      | 80      | 71      | 87      | 79      | 78      | 74      | 74      | 48      | 91      | 42      | 79      | 79      |
| WM              | 78      | 78      | 80      | 83      | 72      | 73      | 70      | 61      | 41      | 62      | 35      | 53      | 74      |
Finally, it has been previously reported by Giapitzakis et al.\textsuperscript{57} that significant differences arise in some of the metabolite concentrations while using MM spectrum from different brain region to account for MM contribution underlying the metabolite spectrum. Based on the differences in T1-relaxation times of MM peaks in GM- and WM-rich voxels reported in this work, we believe that it could be beneficial to account for underlying MM spectra with tissue content considered in order to maintain quantitative accuracy in spectral fitting of metabolites.

5 | CONCLUSIONS

In this work, we report a novel DIR technique to measure T1-relaxation times of individual macromolecular peaks. The study here is performed at 9.4 T; however, this technique is extendible to all field strengths. T1-relaxation times for 13 MM resonances in vivo at 9.4 T are reported for the first time. The differences in the T1-relaxation times between the MM resonances suggest that T1-weighting from inversion pulses and short TRs should be considered while using experimentally acquired MM spectra in fitting metabolite spectra. Alternatively, simulation models which account for relaxation effects of MMs could also provide a solution for estimating MM contributions to metabolite spectra where overlapping peaks are difficult to distinguish. This will allow us to produce more accurate quantitative results by correcting for the influence of MMs in short TE spectra. Furthermore, concentrations of 13 MM peaks after correcting for both T1- and T2-weighting are reported.

ACKNOWLEDGMENTS

The authors thank Wolfgang Bogner, Cristina Cudalbu, Malgorzata Marjańska, Jim Murdoch, Kye Stachowski, and David Edmondson for the constructive discussions and valuable suggestions to this work. Open access funding enabled and organized by Projekt DEAL.

ORCID

Saipavitra Murali-Manohar \(\text{https://orcid.org/0000-0002-4978-0736}\)
Andrew Martin Wright \(\text{https://orcid.org/0000-0002-7182-7270}\)
Tamas Borbath \(\text{https://orcid.org/0000-0003-3679-2380}\)
Nikolai I. Avdievich \(\text{https://orcid.org/0000-0001-7608-0869}\)

REFERENCES

1. Behar KL, Rothman DL, Spencer DD, Petroff OAC. Analysis of macromolecule resonances in 1H NMR spectra of human brain. Magn Reson Med. 1994;32:294-302.
2. Marjańska M, Deelchand DK, Hodges JS, et al. Altered macromolecular pattern and content in the aging human brain. NMR Biomed. 2018;31:e3865.
3. Louis MS, Alosco M, Rowland B, et al. Using machine learning techniques for identification of chronic traumatic encephalopathy related spectroscopic biomarkers. In IEEE Applied Imagery Pattern Recognition Workshop (AIPR), Washington, DC, 2017:1-5. https://doi.org/10.1109/AIPR.2017.8457949.
4. Mader I, Kritzkj J, Klose U, et al. Proton MRS in Kennedy disease: Absolute metabolite and macromolecular concentrations. J Magn Reson Imaging. 2002;16:160-167.
5. Mader I, Seeger U, Weissert R, et al. Proton MR spectroscopy with metabolite-nulling reveals elevated macromolecules in acute multiple sclerosis. *Brain*. 2001;124:953-961.

6. Mlynárík V, Cudalbu C, Clément V, Marino D, Radovanovic I, Gruetter R. In vivo metabolic profiling of glioma-initiating cells using proton magnetic resonance spectroscopy at 14.1 Tesla. *NMR Biomed*. 2012;25:506-513.

7. Deelchand DK, Van De Moortele PF, Adriany G, et al. In vivo 1H NMR spectroscopy of the human brain at 9.4 T: Initial results. *J Magn Reson*. 2010;206:74-80.

8. Soher BJ, Young K, Maudsley AA. Representation of strong baseline contributions in 1H MR spectra. *Magn Reson Med*. 2001;45:966-972.

9. Cudalbu C, Mlynárík V, Gruetter R. Handling macromolecule signals in the quantification of the neurochemical profile. *J Alzheimer’s Dis*. 2012;31(SUPPL. 3):S101-S115.

10. Cudalbu C, Mlynárík V, Xin L, Gruetter R. Quantification of in vivo short echo-time proton magnetic resonance spectra at 14.1 T using two different approaches of modelling the macromolecule spectrum. *Meas Sci Technol*. 2009;20:104034-104040.

11. Lopez-Kolkovsky AL, Mériaux S, Boumezbeur F. Metabolite and macromolecule T1 and T2 relaxation times in the rat brain in vivo at 17.2T. *Magn Reson Med*. 2016;75:503-514.

12. Cudalbu C, Mlynárík V, Xin L, Gruetter R. Comparison of T1 relaxation times of the neurochemical profile in rat brain at 9.4 Tesla and 14.1 Tesla. *Magn Reson Med*. 2009;62:862-867.

13. Xin L, Schaller B, Mlynárík V, Lu H, Gruetter R. Proton T1 relaxation times of metabolites in human occipital white and gray matter at 7 T. *Magn Reson Med*. 2013;69:931-936.

14. Pfeuffer J, Tkac I, Provencher SW, Gruetter R. Towards an in vivo neurochemical profile: Quantification of 18 metabolites in 1H NMR spectra of rat brain at TE = 2 ms. *J Magn Reson*. 1999;120:5545.

15. De Graaf RA, Brown PB, McIntyre S, Nixon TW, Behar KL, Rothman DL. High magnetic field water and metabolite proton T1 and T2 relaxation in rat brain in vivo. *Magn Reson Med*. 2006;56:386-394.

16. Kreis R, Slotboom J, Hofmann L, Boesch C. Integrated data acquisition and processing to determine metabolite content, relaxation times, and macromolecule baseline in single examinations of individual subjects. *Magn Reson Med*. 2005;54:761-768.

17. Hoefemann M, Bolliger CS, Chong DQG, Veen JW, Kreis R. Parameterization of metabolite and macromolecule contributions in interrelated MR spectra of human brain using multidimensional modeling. *NMR Biomed*. 2020;33:e4328-e4345.

18. Hwang J-H, Graham GD, Behar KL, Alger JR, Prichard JW, Rothman DL. Short echo time proton magnetic resonance spectroscopic imaging of macromolecule and metabolite signal intensities in the human brain. *Magn Reson Med*. 1996;35:633-639.

19. de Graaf RA, Brown PB, McIntyre S, Nixon TW, Behar KL, Rothman DL. High magnetic field water and metabolite proton T1 and T2 relaxation in rat brain in vivo. *Magn Reson Med*. 2006;56:386-394.

20. Giapitzakis I, Avdievich N, Henning A. Characterization of macromolecular baseline of human brain using metabolite cycled semi-LASER at 9.4T. *Magn Reson Med*. 2018;80:462-473.

21. Murali-Manohar S, Borbath T, Wright AM, Soher B, Mekle R, Henning A. T2 relaxation times of macromolecules and metabolites in the human brain at 9.4 T. *Magn Reson Med*. 2020;84:542-558.

22. Murali-Manohar S, Wright A, Borbath T, Henning A. Longitudinal Relaxation times of Macromolecular Resonances at 9.4 T in Human Brain. In: 27th Annu Meet Exhib Int Soc Magn Reson Med (ISMRM 2019). Montréal, QC, Canada; 2019.

23. Avdievich NI, Giapitzakis I-A, Pfommer A, Henning A. Decoupling of a tight-fit transceiver phased array for human brain imaging at 9.4T: Loop overlapping rediscovered. *Magn Reson Med*. 2018;79:1200-1211.

24. Gruetter R, Tkáč I. Field mapping without reference scan using asymmetric echo-planar techniques. *Magn Reson Med*. 2000;43:319-323.

25. Versluis MJ, Kan HE, Van Buchem MA, Webb AG. Improved signal to noise in proton spectroscopy of the human calf muscle at 7 T using localized B1 calibration. *Magn Reson Med*. 2010;63:207-211.

26. Mekle R, Mlynárík V, Gambarota G, Hergt M, Krueger G, Gruetter R. MR spectroscopy of the human brain with enhanced signal intensity at ultra-short echo times on a clinical platform at 3T and 7T. *Magn Reson Med*. 2009;61:1279-1285.

27. Giapitzakis I-A, Shao T, Avdievich N, Mekle R, Kreis R, Henning A. Metabolite-cycled STEAM and semi-LASER localization for MR spectroscopy of the human brain at 9.4T. *Magn Reson Med*. 2018;79:1841-1850.

28. Hagberg GE, Bause J, Ethofer T, et al. Whole brain MP2RAGE-based mapping of the longitudinal relaxation time at 9.4T. *NeuroImage*. 2017;144:203-216.

29. Provenceher SW. Automatic quantitation of localized in vivo 1H spectra with LCMR. *NMR Biomed*. 2001;14:260-264.

30. Soher B, Semanchuk P, Todd D, Steinberg J, Young K. VeSPA: Integrated applications for RF pulse design, spectral simulation and MRS data analysis. In *Proceedings of the ISMRM 19th Annual Meeting & Exhibition*, Montréal, Québec, Canada, 2011. p. 1410.

31. Soher B. Versatile Simulation Pulses and Analysis. *User Code Contributions – semi-LASER*. https://scion.duhs.duke.edu/vespa/contrib/wiki/i103e57e-1d27-4d36-8b31-d9c5e2f73e82013.

32. Ashburner J, Barnes G, Chen C, et al. *SPM12 Manual*. London, UK: Wellcome Trust Centre for Neuroimaging; 2014.

33. van Rossum G. Python Tutorial, Technical Report CS-R9526. Amsterdam: Centrum voor wiskunde en informatica (CWI); 1995.

34. Oliphant T. *A Guide to NumPy*. Vol. 1. Trelgol Publishing USA; 2006.

35. Redpath TW, Smith FW. Technical note: Use of a double inversion recovery pulse sequence to image selectively grey or white brain matter. *Br J Radiol*. 1994;67:1258-1263.

36. Jones E, Oliphant E, Peterson P, et al. *SciPy: Open Source Scientific Tools for Python*. 2001.

37. Hunter JD. Matplotlib: A 2D graphics environment. *Comput Sci Eng*. 2007;9:90-95.

38. McKinney W. *pandas: A Foundational Python Library for Data Analysis and Statistics*. 2010.

39. Ethofer T, Mader I, Seeger U, et al. Comparison of longitudinal metabolite relaxation times in different regions of the human brain at 1.5 and 3 Tesla. *Magn Reson Med*. 2003;50:1296-1301.

40. Hasan KM, Walimuni IS, Kramer LA, Narayana PA. Human brain iron mapping using atlas-based T2 relaxometry. *Magn Reson Med*. 2012;67:731-739.

41. Gasparovic C, Song T, Devier D, et al. Use of tissue water as a concentration reference for proton spectroscopic imaging. *Magn Reson Med*. 2006;55:1219-1226.

42. Ernst T, Kreis R, Ross B. Absolute quantitation of water and metabolites in the human brain. I. Compartments and water. *J Magn Reson Ser B*. 1993;102:1-8.
614

Magnetic Resonance in Medicine

43. Rieth KG, Fujiwara K, Di Chiro G, et al. Serial measurements of CT attenuation and specific gravity in experimental cerebral edema. Radiology. 1980;135:343-348.

44. Torack RM, Alcalá H, Gado M, Burton R. Correlative assay of computerized cranial tomography (CCT), water content and specific gravity in normal and pathological postmortem brain. J Neuropathol Exp Neurol. 1976;35:385-392.

45. Brooks R, Di Chiro G, Keller M. Explanation of cerebral white-gray contrast in computed tomography. J Comput Assist Tomogr. 1980;4:489-491.

46. Nassirpour S, Chang P, Henning A. High resolution maps of individual macromolecule components in the human brain at 9.4 T. In: The ISMRM 25th Annual Meeting and Exhibition. Honolulu, HI, USA; 2017. p. 619.

47. Heckova E, Považán M, Strasser B, et al. Effects of different macromolecular models on reproducibility of FID-MRSI at 7T. Magn Reson Med. 2020;83:12-21.

48. Murali-Manohar S, Wright A, Henning A. Challenges in estimating T1 Relaxation Times of Macromolecules in the Human Brain at 9.4 T. In: MRS Workshop 2018 Metabolic Imaging. Utrecht, Netherlands; 2018.

49. Wright A, Murali-Manohar S, Borbath T, Henning A. Longitudinal relaxation times of metabolites in vivo at 9.4 T. In: 27th Annual Meet Exhib Int Soc Magn Reson Med (ISMRM 2019).Montréal, QC, Canada; 2019. p. 0514.

50. Nassirpour S, Chang P, Henning A. High and ultra-high resolution metabolite mapping of the human brain using 1H FID MRSI at 9.4T. NeuroImage. 2016;(December):211-221.

51. Cudalbu C, Behar KL, Bhattacharyya PK, et al. Contribution of macromolecules to brain 1H MR spectra: Experts’ consensus recommendations. NMR Biomed. 2020. https://doi.org/10.1002/nbm.4393.

52. Behar KL, Rothman DL, Spencer DD, Petroff OAC. Analysis of macromolecule resonances in 1H NMR spectra of human brain preparation of brain tissue. Isis. 1994;32:294-302.

53. Snuossi K, Gillen JS, Horska A, et al. Comparison of brain gray and white matter macromolecule resonances at 3 and 7 Tesla. Magn Reson Med. 2015;74:607-613.

54. Landheer K, Gajdošík M, Treacy M, Juchem C. Concentration and effective T2 relaxation times of macromolecules at 3T. Magn Reson Med. 2020;84:2327–2337.

55. Hofmann L, Slotboom J, Boesch C, Kreis R. Characterization of the macromolecule baseline in localized1H-MR spectra of human brain. Magn Reson Med. 2001;46:855-863.

56. Považán M, Strasser B, Hangel G, et al. Simultaneous mapping of metabolites and individual macromolecular components via ultra-short acquisition delay 1H MRSI in the brain at 7T. Magn Reson Med. 2017;79:1231-1240.

57. Giapitzakis IA, Borbath T, Murali-Manohar S, Avdievich N, Henning A. Investigation of the influence of macromolecules and spline baseline in the fitting model of human brain spectra at 9.4T. Magn Reson Med. 2019;81:746-758.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

FIGURE S1 Bloch Simulation results for an inversion pulse profile20 (pulse duration: 15 ms) after a DIR scheme with TIs (A) T11/T12 = 2000/575 ms and (B) T11/T12 = 2150/600. The resulting inversion bandwidth is represented by the frequency axis (x-axis); while the y-axis depicts the resulting M2/M0 of MMs and metabolites at the end of the DIR sequence block. M2/M0 of MM spectrum after DIR block are 0.48 and 0.51 in (A) and (B) respectively with almost nulled metabolites.

FIGURE S2 Fit of WM subject-wise summed spectra of T11/T12 combinations 2360/625, 1300/20 and 1050/238 ms

FIGURE S3 Fits of subject-wise summed GM spectra of T11/T12 combinations 2150/575, 1900/550, 1800/525, 1300/60, 1300/80, 1200/20, and 1250/20 ms

FIGURE S4 Surface Curve fitted to T11/T12 series for M1, M1.39, M1.67, M2.04, M2.26, M2.56, M2.70, M2.99, M3.21, M3.62, M3.75, and M3.96 from GM-rich voxel to calculate T1 relaxation times. For better visualization of the 3D plots a cine is provided as Supporting Information Video S1.

TABLE S1 Residual peaks present corresponding to each T11/T12 are given in the table. tCr(CH2), tCr(CH3), and NAA(CH3) residual peaks were added as singlets using simulated Voigt lines in LCModel for fitting spectra. NAAsupp, ml, GPC, and Glu were added to handle the residuals in negative M1 spectra. For T11/T12 = 1050/238 ms, a complete basis set was provided which was simulated for a semi-LASER sequence using VeSPA; specifically without singlets used in the basis set for any metabolite residuals.

TABLE S2 Bloch simulation considering DIR scheme for m1 has a negative contribution in T11/T12 = 2360/625, 2150/575, 2000/5505, 2000/575, 1900/550, 1800/525, 1600/20, and 1250/20 ms spectra (considering T1-relaxation times from Wright et al.46).

TABLE S3 Differences in T1-relaxation times between GM- and WM-rich tissue types were analyzed using Welch’s t-test with Bonferroni adjusted p-values to account for multiple comparisons (a = 0.0038). Significant differences are denoted by an asterisk, and p-values are given for all analyses.

TABLE S4 Concentrations (protons mmol / kg) of 13 MM peaks are given in the table below after correcting for T1 and T2 relaxation times. Mann-Whitney U-tests were performed with multiple comparisons being accounted using Bonferroni corrections (a = 0.0038). A significant difference for M3.75 being elevated in WM was found when assessing MM concentrations.

Video S1 The cine representation of Figure 5 shows a rotation of the 3D space of the T1-relaxation time curve fitting for M0.92. T11 and T12 are given in the x- and y- axes respectively in seconds; z-axis represents the normalized signal [a.u.]. The black crosses are individual data points from eleven volunteers and the blue line is the projected fitting to equation 1.

How to cite this article: Murali-Manohar S, Wright AM, Borbath T, Avdievich NI, Henning A. A novel method to measure T1-relaxation times of macromolecules and quantification of the macromolecular resonances. Magn Reson Med. 2021;85:601–614. https://doi.org/10.1002/mrm.28484