

Magnetization Transfer from Inhomogeneously Broadened Lines (ihMT): Experimental Optimization of Saturation Parameters for Human Brain Imaging at 1.5 Tesla

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Purpose: Recently a new MR endogenous contrast mechanism was reported. It allows specifically imaging the magnetization transfer (MT) effect arising from inhomogeneously broadened components of the NMR spectrum, and was hence dubbed ihMT. Such unique NMR lineshape properties are presumably occurring in myelin because of its specifically ordered, multilayered sheath structure. Here, optimization of a pulsed ihMT preparation module is presented to provide guidance for future studies and improve the understanding of underlying contrast mechanisms.

Methods: This study was performed at 1.5 Tesla on healthy volunteers. A pulsed ihMT preparation was implemented in combination with a HASTE readout module. The pulse width, interpulse repetition time, total saturation duration and RF saturation power were considered for optimization of the ihMT sensitivity and contrast.

Results: An optimal configuration of the preparation module was derived, leading to 10% ihMT signal in internal capsule (relative to unsaturated data) and around 200% signal increase relative to gray matter, i.e., approximately 10-fold superior contrast compared with conventional MT ratios, measured under similar experimental conditions.

Conclusion: Overall the ihMT sequence was robust, sensitive and very specific for white matter. These findings suggest great potential for assessing brain myelination and for better characterization of myelin related disorders. **Magn Reson Med** 000:000–000, 2014. © 2014 Wiley Periodicals, Inc.

Key words: magnetization transfer; ihMT; inhomogeneously broadened lines; specificity; white matter; myelin

INTRODUCTION

Magnetization Transfer (MT) imaging has been extensively used in both animal and human studies as a non-invasive means to assess tissue macromolecular content

(1,2). Although the underlying contrast mechanisms are complicated, the MT phenomenon is typically understood as a transfer of saturated-magnetization from the broad-resonance lines of motion-restricted protons (semi-solid and/or bound pool) to the MRI detectable mobile protons. In their simplest form, MT imaging results are usually presented as MT ratios (MTRs), which provide semiquantitative metric by normalizing the change in the MT prepared image to the unsaturated MR image. Although sensitive to the macromolecular content, MTRs are complex combinations of various parameters including the macromolecular fraction, the exchange rate of magnetization toward the mobile water pool as well as sequence parameters such as the frequency offset used for RF saturation and the intensity of the saturating magnetic field. To overcome this limitation and ultimately relate the observed MT signal to the underlying tissue structure, quantitative models of the MT phenomenon have been introduced (qMT) (3–5). An unbiased quantitative metric that is directly related to the macromolecular density has been extracted using advanced postprocessing algorithms. Studies performed in various tissue types, such as muscle (6) and brain (5,7,8), have shown promising results. Correlation between the motion-restricted proton fraction derived from qMT and the degree of axonal myelination has been shown for central nervous system (CNS) applications (9–11). However, although sensitive to macromolecular content, usual qMT models do not allow differentiation between the various types of macromolecules composing the spectrum. In other words, and focusing on CNS imaging, MT is sensitive to myelin but cannot be considered as a specific biomarker for it.

MT modeling can be quite complex, involving various numbers of pools and various lineshapes (4,12). The Lorentzian lineshape is suitable to describe free mobile spins whereas the Gaussian lineshape is associated with rigid systems for which the dipolar interactions between protons pairs are static (12). In contrast, the super-Lorentzian lineshape addresses the case of partially ordered materials such as biological membranes for which the homonuclear dipolar interaction is only partially averaged, giving rise to significant residual dipolar coupling (RDC) (12–14). The RDC suggests that the line can no longer be considered as homogeneously broadened (i.e., the fast mixing of absorbed RF energy across frequency cannot be assumed) and this adds an extra

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degree of freedom to the spin exchange within the semi-solid pool, that may be modeled as a dipolar order reservoir in addition to the usual Zeeman reservoir (12). Of interest, the super-Lorentzian lineshape model appears to be a particular case of partially ordered components with relatively loose constraints on proton mobility (e.g., the characteristic angle of the ordered system with respect to B_0 may have random orientation). A study focusing on molecular motion in lipid bilayers (15) has introduced a general framework for calculation of specific lineshapes corresponding to various degrees of motion restriction occurring in lipid membranes. In particular, it was found that the angular limitation of lipidic chain motion strongly influences the width of the NMR spectrum. Moreover, spin diffusion in lipid membranes has been found to be relatively slow, with a characteristic time longer than several milliseconds for a single lipid bilayer (16,17). Overall, these findings may explain some unique feature of the spectrum of myelin and its magnetization exchange properties. Because the lipid membranes composing the myelin sheath are likely more constrained than in other biological tissues and because of a multiple-layered packed structure, spin diffusion from the inner portion of the sheath to the free water would be slow, which strongly suggests that the myelin spectrum would be inhomogeneously broadened on a timescale of a few milliseconds.

The existence of the residual dipolar reservoir has been introduced and studied relatively early (12) for modeling MT properties of white matter (WM). However, it was found that its contribution to single-frequency MT saturation data was barely measurable and hence was later intentionally dismissed in CNS qMT applications (5,18). This was certainly justified in the framework of a complex fitting procedure with limited measurements and for which the number of model parameters has to remain relatively low. However, an alternative method for MT data acquisition was recently proposed (19–21). The key point of this technique relies on a quasisimultaneous dual-frequency off-resonance saturation that provides unique sensitivity to the slow exchange rate of magnetization within inhomogeneously broadened lines. Hence this technique has been named inhomogeneous MT (ihMT).

The ihMT contrast is generated by subtracting MT signal arising from dual-frequency ($+\Delta f$ and $-\Delta f$) offset saturation from signal arising from the single-frequency (Δf) offset saturation, both performed with the same total RF power. By doing so and assuming that the MT asymmetry is negligible or counterbalanced, it is expected that homogeneously broadened lines signal would be suppressed on the ihMT image because of identical saturation in the two experiments (19–21), hence revealing inhomogeneously broadened lines only. IhMT has shown tremendous specificity toward WM, in both brain and spinal cord experiments (19–26) (see Figure 1), which would be attributed to myelin because of the inhomogeneous nature of its spectrum.

Two dual-frequency offset saturation schemes have been proposed (21,23). One using amplitude modulated continuous-wave saturation pulses allowing simultaneous saturation at the dual-frequency offsets, and the

other one using a pulsed saturation scheme for which the frequency offset is switched every other pulse, allowing sequential saturation at the dual-frequency offsets. This latter approach requires fine adjustment as it introduced a delay between the $+\Delta f$ and $-\Delta f$ saturation times, which can be potentially counterproductive for the ihMT effect accumulation. Hence, in this study, we focused on optimizing the pulsed saturation scheme parameters, including the pulse width, the interpulse repetition time, the total saturation duration and the RF saturation power to increase the ihMT sensitivity and the contrast specificity. This study has two purposes: it should allow optimizing the ihMT contrast for practical clinical imaging experiments, and it should measure the underlying contrast mechanisms and parameter dependencies that will contribute to a broader understanding of a quantitative ihMT model.

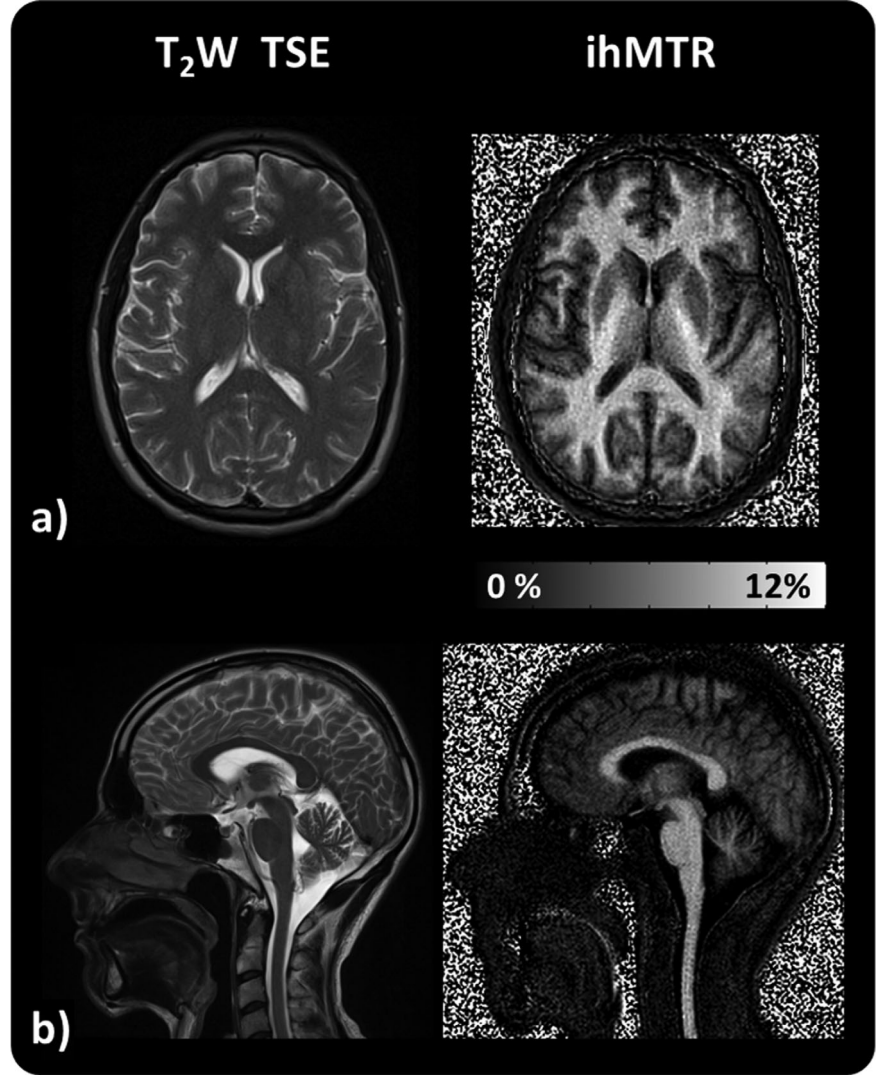
METHODS

Sequence Implementation

The pulse sequence was implemented on a Siemens MRI scanner (VB17, Siemens, Erlangen, Germany). The pulsed saturation preparation scheme was implemented in combination with a turbo spin echo readout module (HASTE product sequence, half Fourier turbo spin echo). Although such a single-shot readout module requires significant energy deposition (as compared to, for example, echoplanar imaging), it allows good image quality free of susceptibility artifacts, which was desirable in the framework of an optimization study. Furthermore, we aim to apply this technique for whole CNS investigation, thus including both brain and spinal cord imaging for which susceptibility induced artifacts occur. MT was achieved with a relatively long pulse train of frequency-shifted Hann-shaped pulses (duration pw , full width half maximum – FWHM – equal to $0.5 \times pw$), applied every Δt (Fig. 2) for a total duration τ . Following this preparation, a 0.5 ms delay was inserted to prevent eddy current effects and the HASTE readout was applied. The single frequency offset saturation was obtained by setting the frequency of the saturation pulses to $f_0 + \Delta f$ (Fig. 2a), whereas the dual-frequency saturation was achieved by switching the carrier frequency of the saturation pulses from $f_0 + \Delta f$ to $f_0 - \Delta f$, every other pulse (Fig. 2b). Hence the total energy deposited on the $+\Delta f$ side of the spectrum was halved as compared to the experiment shown in Figure 2a, and the other half energy was deposited on the negative $-\Delta f$ side.

To compensate for first order MT asymmetry effects (27), experiments depicted on Figure 2 were repeated twice, alternating the sign of the frequency offsets. Thus the ihMT sequence acquired four different MT images with (i) all saturation pulses applied at positive frequency offset $+\Delta f$; (ii) saturation pulses applied at alternating positive ($+\Delta f$) and negative ($-\Delta f$) frequency offsets; (iii) all saturation pulses applied at negative offset $-\Delta f$; and (iv) saturation pulses applied at alternating negative ($-\Delta f$) and positive ($+\Delta f$) frequency offsets. Finally, acquisition of the unsaturated free water image (S_0) was performed within the same sequence to enable calculation of quantitative ratios.

FIG. 1. Typical inhomogeneous MT (ihMT) images of CNS tissues and corresponding T_2 -weighted TSE acquisitions. IhMT images illustrate the high specificity of the technique toward myelinated tissues. Brain axial a) and sagittal b) ihMT ratio images are shown along with corresponding T_2 W images for comparison. Images were interpolated by zero-filling ($\times 2$) for improved image display. Acquisition parameters of the ihMT images were: (a) $p_w = 500 \mu s$, $\Delta t = 1 \text{ ms}$, $\tau = 0.7 \text{ s}$ and $E_{TR} \approx 39 \mu T^2.s$ for a 5 mm slice and 1.2 mm in plane resolution; and (b) $p_w = 500 \mu s$, $\Delta t = 1 \text{ ms}$, $\tau = 0.5 \text{ s}$ and $E_{TR} \approx 28 \mu T^2.s$ for an 8 mm slice and 1.3 mm in plane resolution.



The ihMT image was defined as $ihMT = MT_+ + MT_- - MT_{+-} - MT_{-+}$ where MT_+ , MT_- , MT_{+-} and MT_{-+} corresponded to the MT attenuated images acquired in conditions (i), (iii), (ii), and (iv), respectively. The ihMT ratio was defined as $ihMTR = ihMT/S_0$. Additionally, one could derive from the same dataset usual MT ratio images (one image for each saturation condition, e.g., $MTR_+ = 1 - MT_+/S_0$) as well as the usual MT asymmetry ($MTA = MT_+ - MT_-$) and the newly defined ihMT asymmetry ($ihMTA = MT_{+-} - MT_{-+}$), along with their corresponding ratios ($MTAR = MTA/S_0$ and $ihMTAR = ihMTA/S_0$). This definition of ihMTR, while consistent with prior work (21), reports a value that is twice the average difference between a single and dual frequency image. This value should be divided by two when comparing the magnitude of the effect with, for example, conventional MTRs.

Experimental Procedure

All experiments were performed on a 1.5T MRI scanner (Avanto, Siemens) on healthy volunteers, in compliance with guidelines from our institutional committee on clin-

ical investigations. A 12-channel receive-only head coil (Siemens) was used for the entire study, while RF transmission was performed with the scanner body-coil. Mid-ventricle single-slice axial imaging was realized with the following HASTE parameters: 10 mm thickness (for reduced scan time, in a context of a long optimization

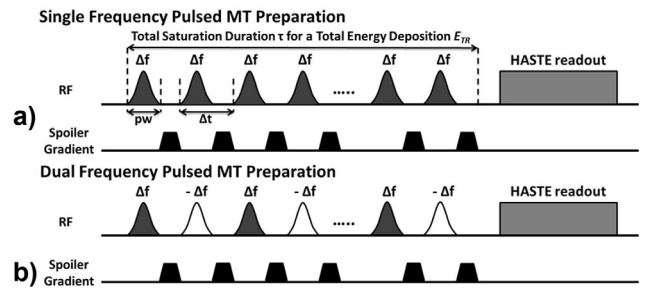


FIG. 2. ihMT sequence diagram. The ihMT contrast relies on a composite image made from the combination of single-frequency (a) and dual-frequency (b) offsets MT prepared images. E_{TR} is defined as the integrated squared B_1 , expressed in $\mu T^2.s$, and is thus directly proportional to the RF energy deposition.

Table 1
Timing Parameter Sets Investigated for ihMT Optimization

	pw	Δt	τ	Volunteers	Comments
Pulse width study*	250, 500, 750 μ s	1 ms	0.7 s	2 ♀, 1 ♂, 22 y.o. average	Results displayed on Figs. 5a, S1a and 8a
Interpulse repetition time study*	500 μ s	1, 1.5, 2 ms	0.7 s	1 ♀, 2 ♂, 23 y.o. average	Results displayed on Figs. 5b, S1b and 8b
Total saturation duration study*	500 μ s	1 ms	0.3, 0.5, 0.7, 1, 1.3s	1 ♀, 2 ♂, 23 y.o. average	Results displayed on Figs. [6 and 7], S2, S3 and 8c
Additional study: highly-sampled energy protocol	500 μ s	1.5 ms	0.5 s	2 ♂, 36 y.o. average	Results displayed on Fig. 4

*All parameter sets (pw, Δt , τ) were tested with four different energy deposition E_{TR} values, up to the SAR limited value. E_{TR} values of approximately 2, 15, 30 and 42 μ T².s were selected. In order to keep E_{TR} values constant when varying the parameter to be investigated, different B_{1RMS} values were used. Rules for conversion from the saturation-pulse flip angle to E_{TR} are detailed in the Methods section. For each configuration, the total number of saturation pulses can be calculated as $\tau/\Delta t$.

study performed in vivo), 220 mm field of view, 192×192 image matrix, 789 Hz/pixel readout bandwidth, 21 ms effective TE, 3 s TR, and 120° spin-echo refocusing angle for SAR (Specific Absorption Rate) considerations.

Optimization of the Pulsed Saturation Scheme Parameters

The current study focused on optimizing the main free parameters of the saturation scheme, which drive the ihMT contrast mechanism: the pulse width pw, the interpulse repetition time Δt , the total saturation duration τ and the RF saturation power. The criteria for optimization were the ihMT sensitivity (i.e., ihMTR value) as well as the contrast between highly myelinated WM and cortical gray matter (GM). Saturation frequency offsets of $\pm \Delta f = \pm 7$ kHz, were chosen based on previous optimization (20,21). Moreover, and according to coarse preliminary adjustments (data not shown), the investigation ranges of timing parameters were limited to $250 \mu s \leq pw \leq 750 \mu s$, $1 ms \leq \Delta t \leq 2 ms$ and $0.3 s \leq \tau \leq 1.3 s$. Each timing parameter value was varied independently keeping the two other timing parameters constant (Table 1), and four different values of energy deposition (E_{TR}) were investigated. The 4 MT-prepared images required to generate the ihMT contrast were averaged 20 times (20 NEX) and S_0 was averaged three times, overall corresponding to 4'15" of acquisition time for each parameter set (pw, Δt , τ , E_{TR}). Three healthy volunteers underwent each study (Table 1).

Adjustment of the energy deposition was achieved by changing the flip angle (FA) of the individual RF saturation pulses: $FA = \gamma \langle B_1 \rangle pw$, where $\langle B_1 \rangle$ is the average B_1 amplitude of a saturation pulse. For the Hann-shaped pulses used in this work, the normalized amplitude integral was 0.5 (i.e., $\langle B_1 \rangle = 0.5 \times B_1^{peak}$ where B_1^{peak} is the peak B_1 amplitude) and the normalized power integral was 0.375, so that $B_{1RMS} = \sqrt{0.375 \times B_1^{peak}}$, where B_{1RMS} is the root-mean-square B_1 calculated over a single pulse. Then the total energy deposited during the pulse train of one ihMT preparation module was directly proportional to E_{TR} , calculated as $E_{TR} = B_{1RMS}^2 \tau pw / \Delta t$, and expressed in μ T².s. Note that if B_{1RMS} is an indicator of the instantaneous power deposition occurring during pw, E_{TR} represents an index of the average RF energy absorbed during the whole saturation period τ (i.e., per TR), and is thus directly related to the SAR level, providing that the additional RF energy used for the

readout module is accounted for. The maximum investigated E_{TR} value, which corresponded to the maximum allowed SAR level, slightly differed with volunteers as it typically depends on the body shape (e.g., size and weight) and patient positioning within the body RF transmit coil¹. Hence, E_{TR} used as variable allowed comparing various configurations obtained at identical SAR level (within the limitation of the above patient dependency). In our experiments, the SAR limitations typically limited E_{TR} to be less than 45 μ T².s with the proposed readout.

Additionally, and to refine the ihMT contrast regimes involved, two extra volunteers (Table 1) underwent experiments with high sampling of saturation energy (nine E_{TR} values), higher Signal-to-Noise ratio - SNR (30 NEX) and the following timing parameters: pw = 500 μ s, $\Delta t = 1.5$ ms, $\tau = 0.5$ s. The acquisition time was 6'15" for each tested E_{TR} value.

Postprocessing

Magnitude data were exported as DICOM files and further processed using Matlab (vR2010b, The MathWorks Inc., Natick, MA) custom routines. The ihMT, MT, MTA and ihMTA signals and ratios were computed as defined above and measured in Regions-Of-Interest (ROIs) selected in both brain hemispheres in WM and GM areas, including frontal white matter (FWM), internal capsule (IC), cortical gray matter (cGM), splenium of corpus callosum (CC s), major forceps (MF), thalamus (Ta), putamen (Pu), genu of corpus callosum (CC g), and cingulate gyrus (CG) (see Figure 3). Identical ROIs were used for all metrics analyses. As an index of specificity to myelinated tissue, the IC-to-cGM ratio of the MTR and ihMTR values were also calculated. SNR of the ihMT image was calculated as the mean ROI signal over the standard deviation of noise measured in a signal-free region of the image. Though accurate quantification of SNR may require more refined modeling depending on coil configuration and k-space acquisition strategy, such a calculation provides a fair SNR estimation. Finally, mean ROI values and standard errors of the mean were calculated across volunteers for each brain area, and are reported in all presented graphs.

¹Note that switching the supervision mode from normal to first controlled level did not allow higher energy to be investigated, because the limiting factor was the head SAR, whose limits are identical (3.2 W/Kg) for both operating mode (IEC guidelines (28)).

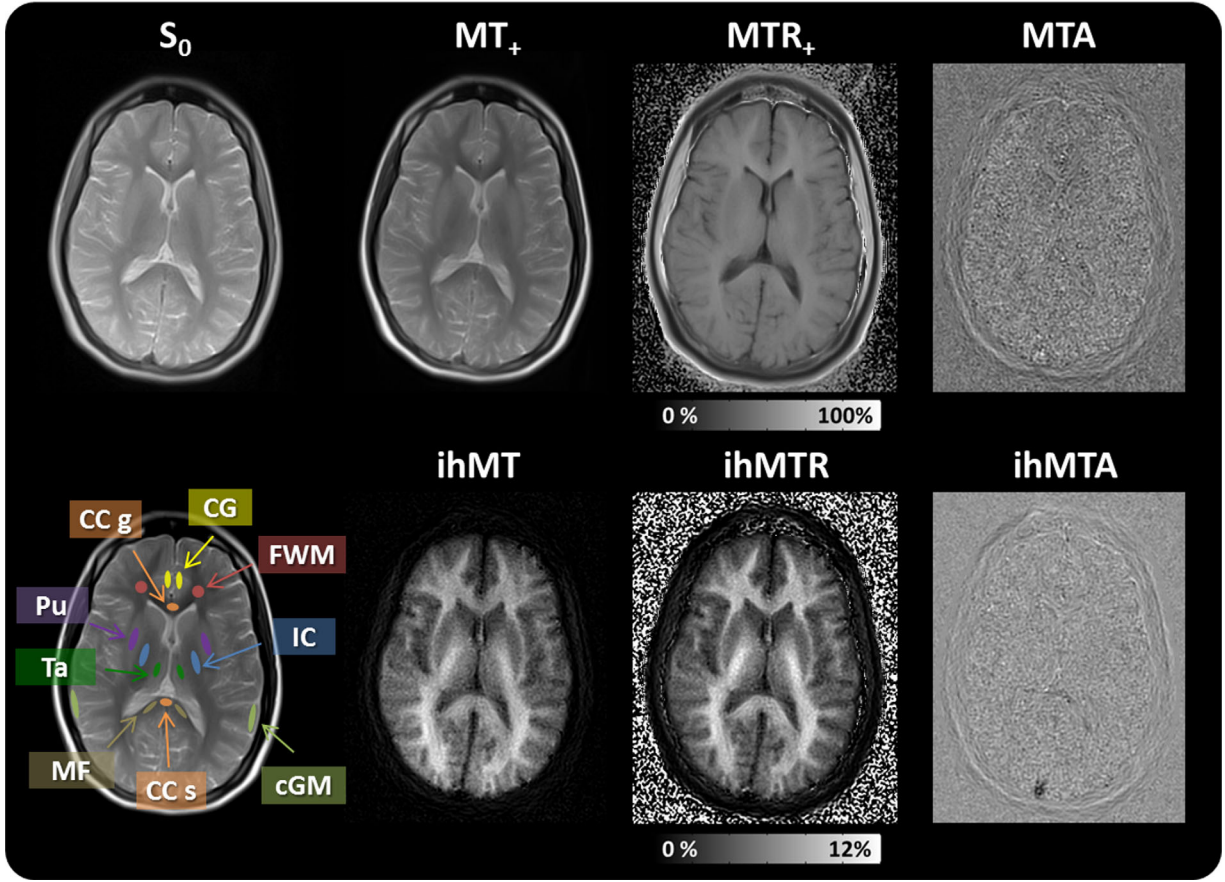


FIG. 3. Illustration of a representative ihMT dataset obtained with $p_w=500 \mu\text{s}$, $\Delta t=1 \text{ ms}$, $\tau=0.7 \text{ s}$ and $E_{TR} \approx 44 \mu\text{T}^2\text{s}$. All displayed images were obtained within the same acquisition. Both MT_+ and ihMT images as well as corresponding ratio images are shown for visual comparison. The MT asymmetry was close to the noise level. In addition the very low ihMT asymmetry evidenced negligible physiologic noise. Images were interpolated by zero-filling ($\times 2$) for improved image display. Typical ROIs used for data processing are indicated here for reference. FWM: frontal white matter, IC: internal capsule, cGM: cortical gray matter, CC s: splenium of corpus callosum, MF: major forceps, Ta: thalamus, Pu: putamen, CC g: genu of corpus callosum, CG: cingulate gyrus.

RESULTS

General Behavior of the ihMT Effect

Typical images obtained with the proposed ihMT sequence are shown in Figures 1 and 3. Enhanced specificity of the ihMT signal for myelinated tissue is evidenced on Figure 1 as intense signal observed in highly myelinated WM structures with little signal present in cGM and almost no signal in skin, subcutaneous fat, muscle, and vertebrae. In contrast, the lack of specificity of conventional MT (Fig. 3) was clearly evidenced as MT images exhibited strong signal in these areas. A maximum SNR around 32 was measured on the ihMT image (Fig. 3, IC area) using $p_w=500 \mu\text{s}$, $\Delta t=1 \text{ ms}$, $\tau=0.7 \text{ s}$, $E_{TR} \approx 44 \mu\text{T}^2\text{s}$ and 20 NEX.

Figure 4 shows the variations of ihMTR, MTR_{+} , MTR_{+-} , MTAR, and ihMTAR values measured in IC as a function of RF energy deposition (expressed as E_{TR} , Figures 4a,c, and as $B_{1\text{RMS}}$, Figures 4b,d) for the highly-sampled energy protocol. This dual representation is helpful to comprehend the ihMT contrast regimes relative to the RF energy level. The general trend of the ihMTR curves was similar although their shapes differed whether plotted as a function of E_{TR} or $B_{1\text{RMS}}$. The

ihMTR value rose quickly (from 1% to $\sim 9\%$) with increasing energy deposition ($E_{TR} < 20 \mu\text{T}^2\text{s}$, $B_{1\text{RMS}} < 10 \mu\text{T}$), then tended to saturate at higher levels. Of interest, according to Figure 4b, for $B_{1\text{RMS}} < 3 \mu\text{T}$, the ihMTR value was lower than 1%, suggesting inefficient building of the effect in this regime. The ihMTR reached a maximum asymptotic value of $\sim 9.5\%$ in the IC for $E_{TR} \geq 35 \mu\text{T}^2\text{s}$. From Figures 4c,d, MTR_{+-} and MTR_{+} showed monotonic increase with E_{TR} and $B_{1\text{RMS}}$, reaching approximately 49% and 44%, respectively, for $E_{TR} \approx 41 \mu\text{T}^2\text{s}$ or $B_{1\text{RMS}} \approx 16 \mu\text{T}$. Unlike ihMTR, no evidence of a plateau over the investigated energy range was seen. Note that the difference between MTR_{+-} and MTR_{+} datasets intrinsically evidences the build-up of the ihMT effect. The MT asymmetry was low with MTAR values remaining smaller than 0.6%. Moreover the ihMT asymmetry provided ihMTAR values within $\pm 0.2\%$ of the S_0 signal, i.e., close to noise level, thus demonstrating data consistency. Indeed, because both dual-frequency saturation schemes should yield identical saturation (i.e., MT_{+-} should be equal to MT_{-+}), only physiologic noise (e.g., motion, perfusion...) should appear on such dataset because of repeated acquisitions. For all studied parameter sets and brain areas, MTAR and ihMTAR

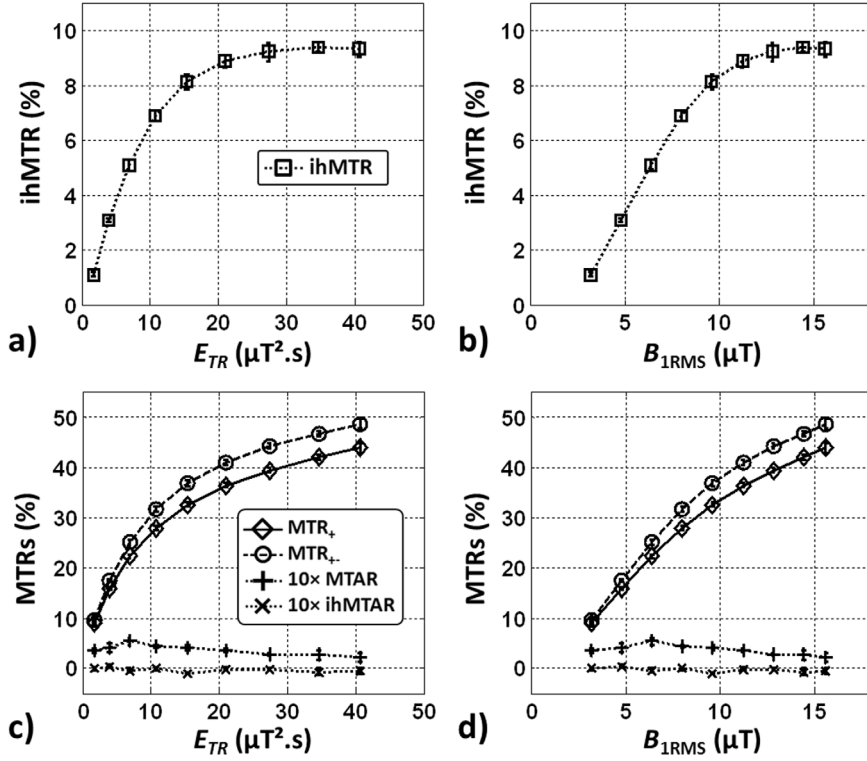


FIG. 4. Highly B_1 -sampled ihMT dataset as measured in IC. General trend of ihMT and MTR behaviors are shown as a function of total energy deposition (E_{TR} , a) and (c), and instantaneous power (B_{1RMS} , b) and (d).

values remained very low, as observed here, and are, therefore, not presented in the following. Finally, the low standard errors of all measurements (small error bars) illustrated weak variations across volunteers and a strong robustness of the ihMT technique. Similar general behaviors were obtained in the other investigated brain areas although with lower maximum ihMTR.

Parameter Optimization

Figures 5 shows the variations of ihMTR measured in IC and cGM as a function of E_{TR} for various pulse width and interpulse repetition time values. Other brain areas are not presented here for the sake of clarity. Corresponding MTR datasets are reported as supplemental figures. For all brain areas, pulse widths of 500 μs and 750 μs yielded very close ihMTR values for all energy levels (Fig. 5a, blue and red curves). Conversely for $pw=250$ μs , ihMTR values were significantly lower in WM areas for $E_{TR} \geq 14.4$ $\mu T^2.s$, whereas no distinct difference could be observed in GM. In contrast, MTR values obtained for pw of 500 μs and 750 μs were very close for all energy levels, and significantly lower than those obtained with pw of 250 μs (Fig. S1a).

Figure 5b shows the effect of the interpulse repetition time on the ihMTR, demonstrating similar behavior in all investigated brain areas, although with different ratio values (see Table 2). For moderate energy levels ($E_{TR} \leq 14.4$ $\mu T^2.s$) no significant variation of the ihMTR with Δt was obtained, whereas a trend for higher ihMTR values for shorter Δt appeared from 29.3 $\mu T^2.s$, becoming more pronounced for 42 $\mu T^2.s$. Regarding MTR data, no sign of complete saturation was evidenced across the range of investigated RF energy levels and similar MTR

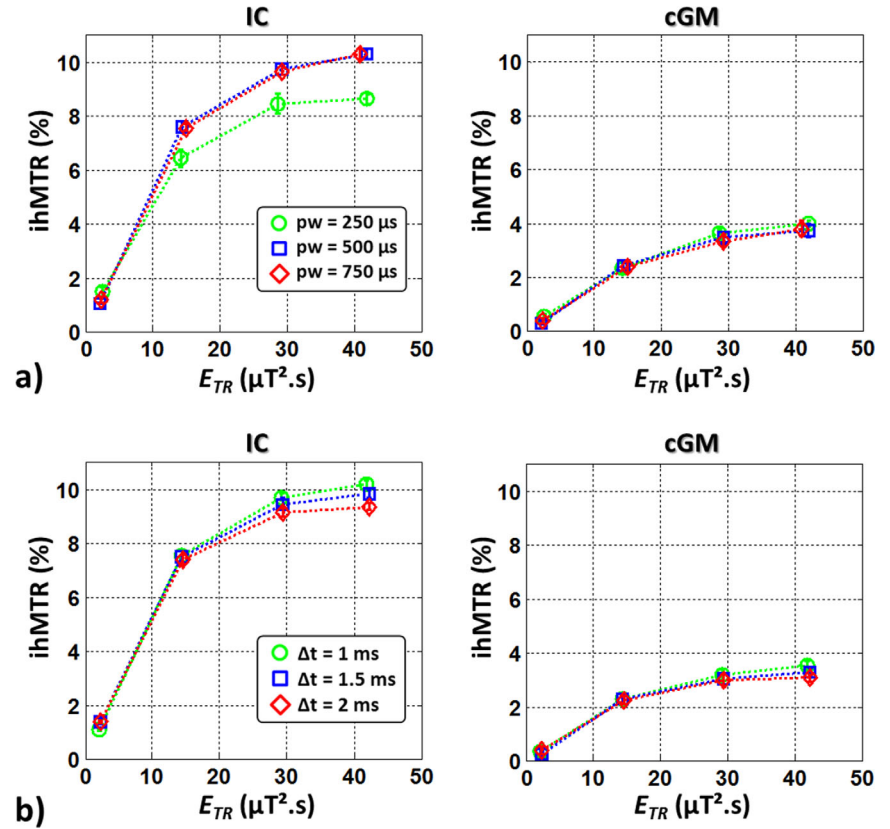
values were obtained regardless of the actual Δt (Supporting Fig. S1b, which is available online).

Figures 6 and 7 show the effect of the total saturation time on ihMTR. Here, two WM areas (IC and FWM) are displayed to evidence slightly different behavior of the ihMT signal dynamic. Results are displayed using the dual energy representation on Figure 6 [i.e., versus E_{TR} in (a) and vs. B_{1RMS} in (b)] to provide insight into the ihMT signal buildup at high instantaneous power. In contrast, Figure 7 directly shows the ihMTR as a function of the total saturation time for better assessment of the optimal setting. At high energy levels ($E_{TR} \geq 29.4$ $\mu T^2.s$), highest ihMTR values were obtained for $\tau=0.5$ s to 0.7 s (Fig. 6, blue curve and Fig. 7). Short ($\tau=0.3$ s) and long ($\tau=1.3$ s) saturations times yielded loss of ihMT effect efficiency and resulted in lower ihMTR values (Fig. 6, green and red curves, and Fig. 7). For low energy values ($E_{TR} \leq 14.7$ $\mu T^2.s$), the shorter the saturation duration the stronger the ihMT effect. Regarding the MTR data, there was no evidence of complete saturation across the investigated energy range but short τ values tended to weaken the intensity of MT effects at high energy (Supporting Fig. S2). More generally, for all investigated brain areas, the higher the energy deposition, the longer the optimal τ to maximize MTR (Supporting Fig. S3) and ihMTR (Fig. 7). Of interest, the optimal τ value was longer for MTR than that for ihMT (e.g., τ of 1 s was optimal for MTR₊ as compared to 0.7 s for ihMTR, in IC).

ihMT Contrast Specificity for WM

Overall, the following parameter set was found optimal for ihMT sensitivity at 1.5T: $500 \leq pw \leq 750$ μs , $\Delta t = 1$ ms

FIG. 5. Pulse width and interpulse repetition time optimization results. IhMT ratios measured in IC and cGM are shown as a function of the energy deposition E_{TR} for different pulsewidth values in (a), and for different interpulse repetition time values in (b). All data were acquired using $\Delta t = 1$ ms and $\tau = 0.7$ s in a), and using $pw = 500$ μ s and $\tau = 0.7$ s in b). Optimal pw lies in the range 500–750 μ s for all investigated brain areas. Optimal Δt value is 1 ms for all investigated brain areas. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



and $\tau = 0.7$ s. Corresponding ihMTR values measured in different brain areas at the highest energy level and averaged over six independent volunteers are reported in Table 2. The IC-to-cGM ihMTR and MTR ratios are shown as a function of E_{TR} in Figure 8, for all tested parameter sets. Similar curve shapes were obtained for other WM areas, although with relatively lower ratios. At low energy levels, cGM ihMTR values were relatively low ($<0.5\%$, Figs. 5–7) leading to subsequent noise amplification on the IC-to-cGM ratio values and therefore to unreliable measurements for ratios of ihMTR (Fig. 8, large error bars for $E_{TR} \approx 2 \mu T^2.s$). For higher energy levels, the evolution of IC-to-cGM ihMTR and MTR ratios with E_{TR} demonstrated a good match (within error bars) for all configurations of timing parameters, except for $pw = 250 \mu$ s. Although a slight and progressive loss of IC-to-cGM contrast occurred with increasing E_{TR} values for both ihMTR and MTR, one major observation can be made regarding Figure 8: ihMT provided tremendous specificity toward WM as compared to standard MT measured under similar experimental conditions. For instance, the IC ihMTR was approximately 200% higher than that of cGM for $E_{TR} \approx 30 \mu T^2.s$, whereas an increase of only 20% was obtained for standard MTR between these two brain structures.

DISCUSSION

This study focused on the optimization of a pulsed ihMT preparation scheme. The key feature of the ihMT method relies on the dual-frequency saturation that sensitizes MT contrast to the exchange of magnetization

within inhomogeneously broadened lines of semisolid macromolecules. This sensitization is expected to be efficient as long as the dual saturation is realized faster than the magnetization exchange time scale within the inhomogeneous line and for adequate RF energy deposition. Hence optimizing the timing parameters of the pulsed saturation scheme was important.

Table 2
MT and ihMT Ratios Measured in Various Brain Areas^a

	MTR ₊		ihMTR	
	Mean	SE (CV)	Mean	SE (CV)
Gray Matter				
Cortical GM	41.6	±1.8 (4.4%)	3.68	±0.41 (11.2%)
Cyngulate Gyrus	40.7	±1.7 (4.2%)	3.47	±0.41 (11.7%)
Thalamus	42.0	±3.1 (7.5%)	4.91	±0.55 (11.2%)
Putamen	46.1	±1.2 (2.7%)	5.62	±0.59 (10.5%)
White Matter				
Internal Capsule	48.1	±0.8 (1.7%)	10.15	±0.24 (2.4%)
Frontal WM	52.3	±1.0 (1.9%)	8.69	±0.25 (2.9%)
Major Forceps	51.8	±1.3 (2.5%)	8.62	±0.43 (5.0%)
CC Genu	51.4	±1.9 (3.7%)	7.27	±0.59 (8.1%)
CC Splenium	49.7	±1.9 (3.9%)	7.35	±0.20 (2.7%)

^aCC = corpus callosum. Reported brain areas refer to those defined on Figure 3. The mean, standard error (SE) and corresponding coefficient of variation (CV = SE/mean) were calculated for both MTR and ihMTR over 6 independent volunteers (3♀, 3♂, 23 y.o. average), for the highest energy level, $E_{TR} \approx 42 \mu T^2.s$, and using optimal parameter set: $pw = 500 \mu$ s, $\Delta t = 1$ ms and $\tau = 0.7$ s.

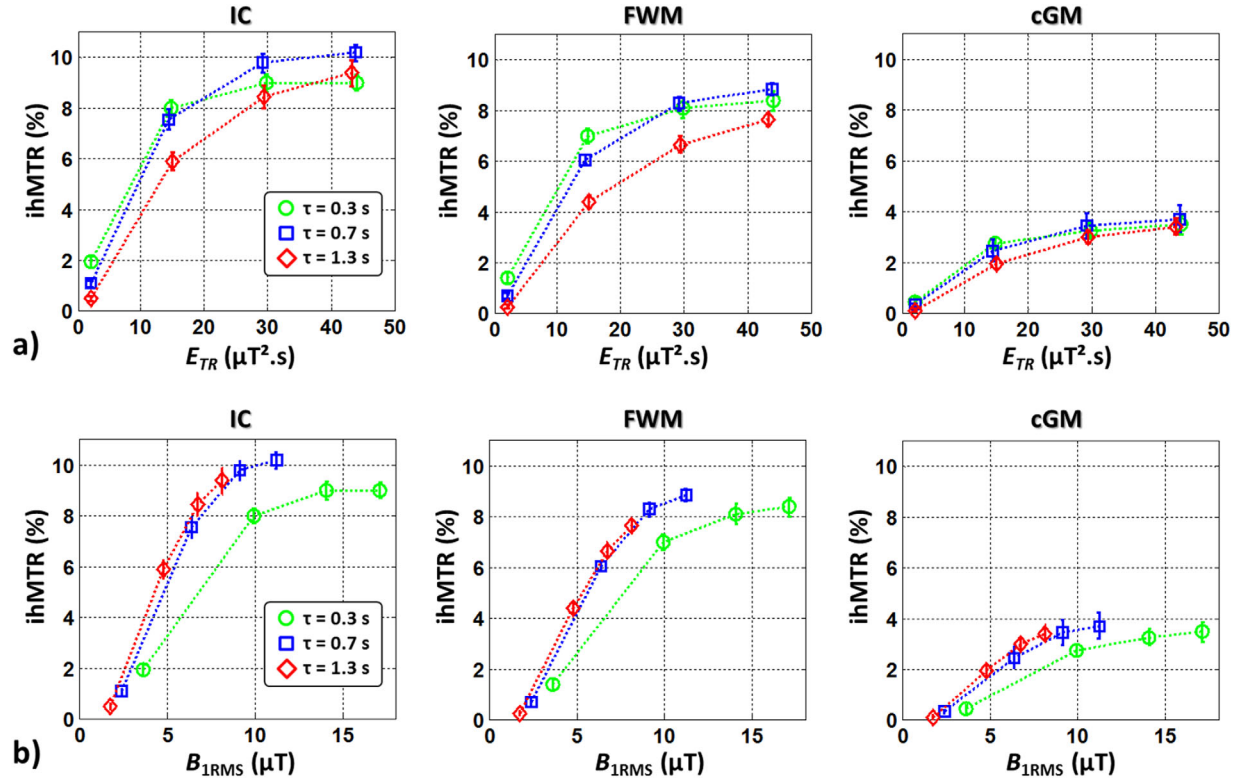


FIG. 6. Total saturation time optimization study. IhMT ratios measured in IC, FWM and cGM are shown as a function of the energy deposition E_{TR} in (a), and as a function of the instantaneous power deposition B_{1RMS} in (b). For purpose of clarity only results obtained for τ of 0.3 s, 0.7 s, and 1.3 s are presented here (see Figure 7 for full optimization). All data were acquired using $p_w = 500 \mu s$ and $\Delta t = 1$ ms. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ihMT Is a Robust, Sensitive, and Specific Method for WM Imaging

Overall, the ihMT sequence used in this study appeared robust, relatively sensitive and highly specific to WM and myelinated GM (e.g., thalamus and putamen), suggesting strong specificity for myelin content. Robustness was illustrated by low standard error calculated over specific ROIs from independent volunteers (Figs. 5–7 and Table 2). IhMT ratios on the order of 7–10% of the unsaturated water signal were obtained in WM areas (Table 2), corresponding to a relatively good sensitivity, compatible with

relatively short scan times. Finally the specificity of ihMT for myelinated structures, quantified by a relative ihMT signal increase of around 200% in IC with respect to cortical GM, was tremendously higher than that of standard MT measured in our experimental conditions (relative signal increase of around 20%). These findings are very promising in the context of increasing effort of the whole MRI community to develop new methods for assessing brain myelination for better characterization of WM pathologies.

For comparison purpose, the standard MTR values derived from the ihMT preparation and corresponding

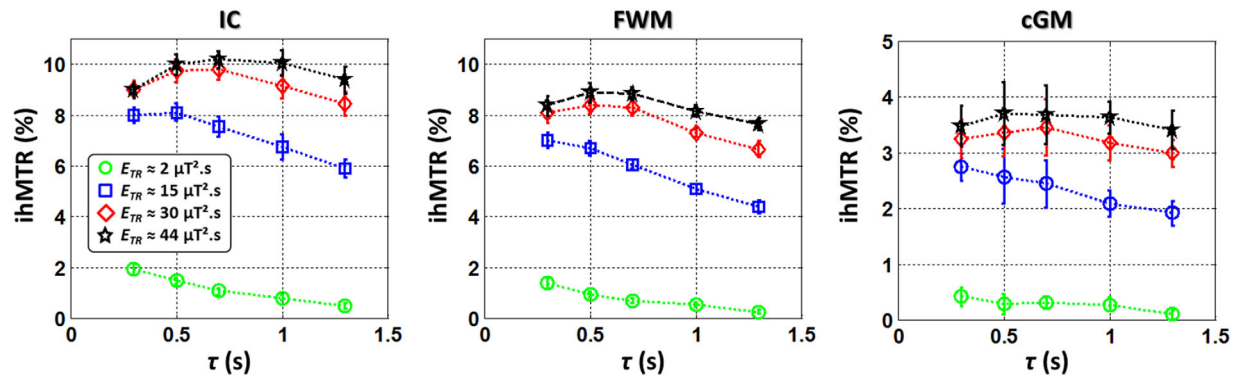


FIG. 7. Total saturation time optimization study. In contrast with Figure 6, the ihMT ratios measured in IC, FWM, and cGM are directly shown as a function of τ here, for four different energy deposition values. The optimal τ value depends on the energy level and slightly varies with investigated brain area. All data were acquired using $p_w = 500 \mu s$ and $\Delta t = 1$ ms. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

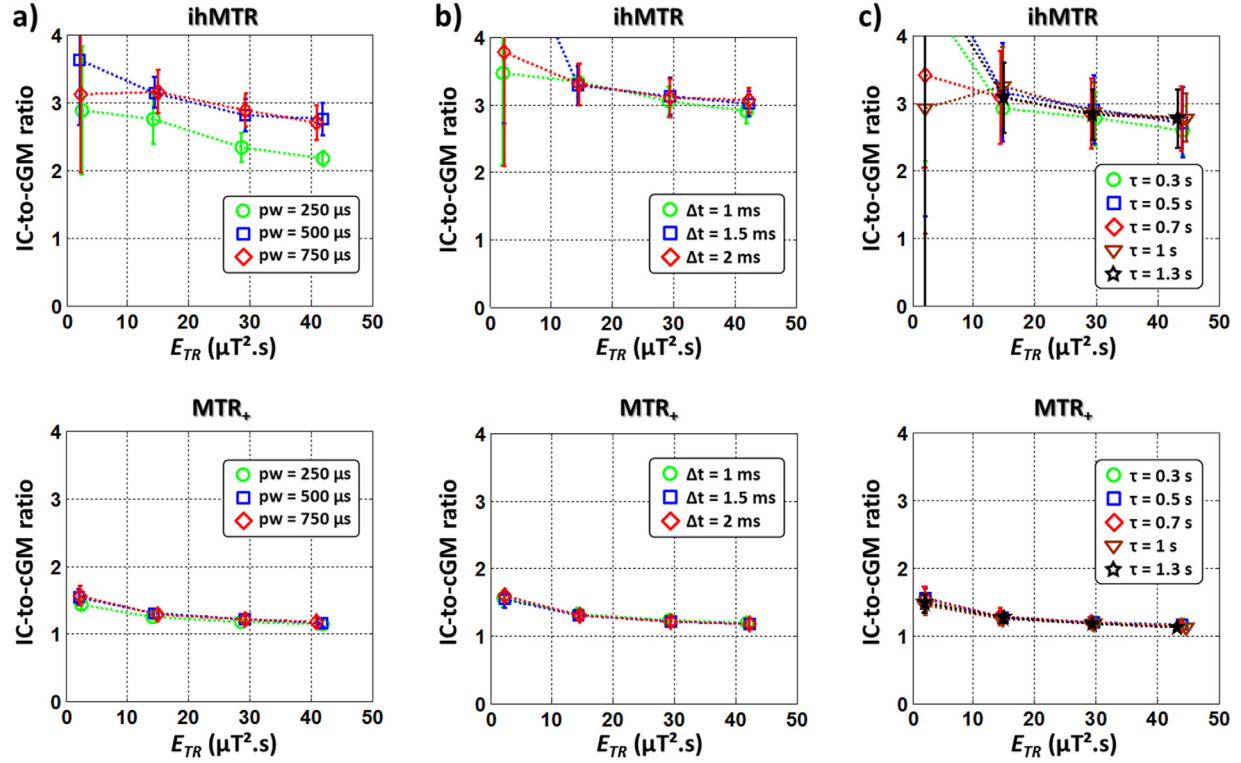


FIG. 8. Comparison of ihMT and regular MT specificity toward WM. The specificity for WM is shown as the IC-to-cGM ratios of the ihMTR and MTR for the pulse width study in (a), the interpulse repetition time study in (b), and the total saturation time study in (c). Lower values of the IC-to-cGM ratio for $pw = 250 \mu s$ (a) green curve) as compared to all other settings demonstrate a loss of specificity of ihMT toward WM, which could be attributed to direct saturation effects. Besides this particular setting, the IC ihMT ratio was typically 200% more intense than that of cGM for $E_{TR} \sim 30 \mu T^2.s$ whereas the corresponding MTR_+ was only 20% stronger, demonstrating superior WM specificity of the ihMT technique. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

WM-to-GM MTR ratios were found fairly consistent with the range of values measured by others, for the same frequency offset and using a different sequence (5), although higher WM-to-GM contrast has also been measured in other studies for both MTR and qMT (29,30). Note however, that the relatively thick slice used in the current study likely induced significant partial volume effects, especially for cGM ROIs, which would yield to underestimation of IC-to-cGM ratios for both ihMTR and MTR. Overall, this does not undermine presented results as the ihMT and MT comparison was performed using the same experimental settings.

Due to the novel character of ihMT, the literature describing it is sparse and hence, providing a complete characterization of the pulsed preparation scheme will provide guidance for future studies. In the original ihMT studies (20,21), the pulsed preparation was achieved using Gaussian shaped pulses of $336 \mu s$ FWHM. Assuming a normalized power integral of 0.25, the 80 mG peak RF saturation applied at $\Delta f = 7$ kHz with a 1 ms interpulse and 1.02 s total saturation time yielded $E_{TR} \approx 16 \mu T^2.s$. Using these settings an ihMTR of $\sim 6\%$ was measured in IC at 3T, consistent with our findings at 1.5T for a similar configuration (Fig. 7). This coarse comparison reinforce confidence in the ihMTR values obtained in our study, and further illustrates the good robustness of the technique, as it demonstrates cross-vendor and cross-platform operability and suggests little dependency, if any, to the magnetic

field strength (31). Increase of the B_0 field strength will limit the maximum allowed value of B_{1RMS} for MT preparation as a consequence of higher energy deposition. Consequently lower maximum ihMT ratios would be expected using this sequence configuration. In this perspective, the actual optimal parameter set derived hereby may not be strictly transferable to higher field strength.

On the Optimal Parameter Settings

Little influence of the pulse width has been observed, as long as direct saturation (DS) effects were limited ($pw > 250 \mu s$). The excitation bandwidth (BW) of the $250 \mu s$ Hann pulse (8 kHz FWHM and 16 kHz total BW, excluding secondary lobes) was actually very broad, and hence strong DS effects were expected when applied at 7 kHz offset frequency. For other settings DS would be reduced because of narrower pulses. In general, the overall DS effects are a complex combination of T_1 , T_2 , and BW (2,32), however, studying these effects in details is outside the scope of the present study. In presented experiments, strong DS is consistent with the higher MTR values observed on all brain locations with the $250 \mu s$ pulse, and likely explains lower ihMTR values in WM areas (Fig. 5). The different pattern observed for GM was probably due to a measurement bias, characterized by strong error bars and resulting from difficulty to avoid partial volume effects and motion in selecting ROIs in such small areas.

The slight dependency of the ihMT effect with the interpulse repetition time over the presented range ($\Delta t < 2$ ms) may be explained by a mixing time effect. It is actually expected from theoretical considerations that inhomogeneous components of the spectrum would tend to homogenize for long exchange times and hence, short Δt (i.e. fast dual-frequency saturation switch) would be advantageous because of reduced mixing time. Using the presented sequence, a progressive loss of the ihMT effect has been observed, leading to complete vanishing of the ihMT signal for long pulses and Δt of 40ms (data not shown). In addition, a similar effect has also been reported with a slightly modified version of the proposed sequence (33), allowing for the estimation of a characteristic exchange time of the inhomogeneously broadened lines. In this regards it is worth noting that MTR datasets were similar regardless of the actual Δt (Supporting Fig. S1b), consistent with a dominantly homogeneously broadened MT phenomenon for which the mixing time of spin exchange within the line should not affect the MT signal.

The dependency of ihMT on the total saturation time was more pronounced, and an interesting limiting factor was found for the shortest τ value of 0.3 s. For low levels of energy deposition ($E_{TR} \leq 15 \mu T^2.s$) the buildup of the ihMT effect was more efficient when the instantaneous power (i.e., B_{1RMS}) was high (Fig. 6, green curves). However the effect tended to saturate prematurely for higher deposited energy ($E_{TR} \geq 29 \mu T^2.s$, corresponding to $B_{1RMS} \geq 14 \mu T$). Conversely, increasing the saturation duration allowed the use of lower B_{1RMS} , hence distributing the RF energy over time. No saturation of the ihMT effect was obtained for these parameters, producing a significant boost of the ihMT effect for high energy levels (Fig. 6, blue curves). For instance in IC, saturation of the ihMTR at $\sim 9\%$ was obtained for $\tau = 0.3$ s, whereas for $\tau = 0.7$ s, no complete saturation occurred and the ihMTR reached more than 10%. Long saturation durations ($\tau = 1.3$ s) appeared inefficient, independently of the energy level, because of T_1 effects counterbalancing the build-up of the ihMT contrast and leading to lower ihMTR (Fig. 6, red curves). Although slightly dependent on the actual brain area (Fig. 7), an optimal τ value of ~ 0.7 s was found for the total saturation duration in WM, consistent with the range of T_1 measured at 1.5T (34). Moreover, the MTR data showed a trend for longer optimal τ values for cGM as compared to WM areas (Fig. S3), consistent with longer T_1 s in GM (34). Finally, for all investigated brain areas, the optimal τ values were shorter for ihMTRs than for the corresponding MTRs, suggesting an additional mechanism leading to a shorter effective longitudinal relaxation for the ihMT effect.

Ultimately, the optimization results could be summarized as follows: 1/ direct saturation effects must be reduced, 2/ the interpulse interval should be as short as possible 3/ the RF energy should be distributed over time and 4/ T_1 recovery effects have to be minimized; these rules of thumb being challenged by a global SAR constraint (note that a fairly optimized configuration is obtained from $E_{TR} \approx 30 \mu T^2.s$). Overall the optimal parameter set was obtained for a configuration, which tended to be close to continuous saturation, i.e., with short interpulse and relatively long pulses. Note how-

ever, that for an RF duty cycle of 75% ($p_w = 750 \mu s$ and $\Delta t = 1$ ms) the RF amplifier was close to its maximum load for the highest RF energy tested here; a p_w of 500 μs (RF duty cycle of 50%) is recommended with this system, especially as the ihMTR value loss was negligible compared with 750 μs (Fig. 5).

Limitations of the Current Study

A 2D single-slice readout module was used for parameter optimization purposes. In theory, the HASTE sequence may be used for multislice and/or 3D acquisitions, although slice “cross-talk” and T_2 blurring may produce artifacts. For full brain-volume coverage adapted to clinical routine alternative strategies should eventually be considered. For example a 3D gradient echo implementation based on pulse amplitude modulation was recently proposed (23). Moreover, due to the large slice thickness used in this study, quantitative values obtained here are likely affected by significant partial volume effects, especially for GM structures. It is expected that higher resolution would lead to refined measurements.

Effects of nondipolar origin, such as CEST (35), could potentially bias the ihMT effect as they contribute small asymmetries to the Z-spectrum; however such effects are anticipated to be small here because of very large frequency offsets (7 kHz corresponding to approximately 110 ppm here). A careful study of such confounding effects may be required to isolate and identify potential sources of systematic error when using broadband saturation pulses or smaller frequency offsets.

Previous work has suggested that 7 kHz is at a broad maximum of the ihMT effect (21). Though there is some additional room for optimization of the frequency, major changes in frequency and ihMT values are not anticipated, especially when short pulses with broad bandwidths are used for the ihMT saturation as in this work. It has been suggested that ihMTR differences between IC and FWM are frequency dependent, and may partially arise from anisotropy effects related to the orientation of WM fibers with respect to B_0 (21). Such an effect is actually anticipated to influence the partial averaging of the RDC (15) that is hypothesized to give rise to the broad myelin spectrum. In these circumstances the optimal frequency offset for efficient probing of the ihMT effect may be WM-orientation dependent, which may challenge the extraction of quantitative metrics related to myelin density. However the overall anisotropy effect should be small because the cylindrical symmetry of myelin sheaths reduces its degrees of freedom by spatial averaging effects. Quantitative models of the ihMT phenomenon will be required to address the full potential of the technique as a quantitative tool to assess myelin content.

Finally, although remarkably sensitive to myelinated structures, the ihMT technique will need to be validated against gold standard immunohistology and compared with existing techniques (36) (e.g., qMT, myelin water fraction, DTI) to demonstrate its myelin specificity and to reach acceptance of the community as a myelin-specific imaging tool. Then studies of WM diseases will be required to ultimately promote ihMT as a clinical biomarker for myelin.

CONCLUSIONS

We have presented an optimization of a new endogenous contrast mechanism, ihMT, which specifically addresses the inhomogeneous broadening of partially ordered materials such as biological membranes, and which presumably occurs in myelin. This study was performed at 1.5T on healthy volunteers, and demonstrated strong robustness, good sensitivity and high specificity of the ihMT signal for myelinated tissues. An optimal configuration of the pulsed preparation module was derived, leading to 10% ihMT signal in IC (relative to unsaturated data) and 200% relative signal increase with respect to cGM, i.e., approximately 10-fold superior contrast than standard MTR, as measured under similar experimental conditions. In the future, the ihMT technique could prove to be a valuable tool for studying various WM pathologies, especially myelin-related disorders.

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