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Magnetization transfer and adiabatic T1ρ MRI reveal abnormalities in normal-appearing white matter of subjects with multiple sclerosis

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Abstract

Background: Diffuse abnormalities are known to occur within the brain tissue of multiple sclerosis (MS) patients that is "normal appearing" on T1-weighted and T2-weighted magnetic resonance images.
Objectives: With the goal of exploring the sensitivity of novel MRI parameters to detect such abnormalities, we implemented an inversion-prepared magnetization transfer (MT) protocol and adiabatic T1ρ and T2ρ rotating frame relaxation methods.
Methods: Nine relapsing–remitting MS patients and seven healthy controls were recruited. Relaxation parameters were measured in a single slice just above the lateral ventricles and approximately parallel to the AC-PC line.
Results: The MT ratio of regions encompassing the normal-appearing white matter (NAWM) was different in MS patients as compared with controls ($p = 0.043$); however, the T1 measured during off-resonance irradiation (T1sat) was substantially more sensitive than the MT ratio for detecting differences between groups ($p = 0.0006$). Adiabatic T1ρ was significantly prolonged in the NAWM of MS patents as compared to controls (by 6%, $p = 0.026$), while no differences were found among groups for T2ρ. No differences among groups were observed in the cortical gray matter for any relaxation parameter.
Conclusions: The results suggest degenerative processes occurring in the NAWM of MS, likely not accompanied by significant abnormalities in iron content.

Keywords
MRI, multiple sclerosis, relapsing–remitting, rotating frame relaxation, inversion-prepared MT, adiabatic pulses

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Introduction

Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease of the central nervous system (CNS), and is the most common cause of nontraumatic neurologic disability in young adults in North America and Europe.[1] Conventional MR imaging in MS patients shows characteristic T2 bright, T1 dark, and T1 contrast-enhancing lesions. Diffuse abnormalities also occur within brain tissue that is "normal appearing" on T1-weighted and T2-weighted magnetic resonance images.

Magnetization transfer (MT) imaging generates contrast by utilizing the exchange of magnetization between bulk water protons and protons within macromolecules.[2] Several studies have shown the utility of MT imaging in MS, and have been discussed previously in a thoughtful review.[3] Changes measured in MT ratio (MTR) have been histologically

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correlated with demyelination, axonal degeneration, and microglial activation.[4–6] MTR imaging was found to detect changes in brain white matter (WM) prior to the development of conventional lesions.[7] Additionally, abnormalities in the normal-appearing white matter (NAWM) of MS patients have been observed when compared to healthy subjects, as measured by the MTR and by the bound-proton fraction.[8–10] Differences of MTR in the normal-appearing gray matter (NAGM) have also been reported but are more difficult to reproduce.[8,11–13] Quantification of T1 and T2 relaxograms has likewise revealed subtle abnormalities of NAWM and NAGM in MS.[14,15] Finally, MTR values in MS correlate with disease severity measures,[16,17] and seem to provide an independent predictor of clinical progression apart from lesion burden.[18] However, reported differences of MT and T1/T2 parameters between MS patients and controls are subtle, and the correlations of these magnetic resonance imaging (MRI) parameters with disability measures are generally weak.

Our study examines the potential of novel MRI protocols introduced by our group to provide additional contrast that cannot be appreciated with the MRI methods used to date in MS research. Specifically, we focused on rotating frame relaxation methods during adiabatic pulses, i.e. adiabatic T1ρ and T2ρ,[19–22] and on an inversion-prepared quantitative MT protocol.[23] Whereas conventional T1 and T2 parameters quantify spin relaxations in "free precession" conditions, i.e. in the absence of radiofrequency (RF) perturbations, T1ρ and T2ρ describe relaxation in the presence of RF. The rationale of using T1ρ and T2ρ relaxation parameters for tissue characterization is grounded in the fact that these methods are sensitive to a broader range of spin dynamics as compared to laboratory-frame T1 and T2 relaxations. Whereas in vivo T1 (and to a certain extent T2) depend on magnetic field fluctuations induced by tumbling dipoles (i.e. motion) that occur at frequencies near the Larmor frequency (MHz range), rotating frame relaxation parameters have additional contributions from frequencies in the range of the effective field generated by the RF pulse used for the measurements (~ kHz range).[21] Rotating frame measurements performed during adiabatic pulses are particularly advantageous for in vivo applications because of their insensitivity to B0 and B1 inhomogeneities. Adiabatic methods can also be more informative than conventional spin-lock rotating frame methods. In fact, whereas spin-lock experiments exploit only one constant effective frequency during the continuous wave irradiation, the adiabatic methods generate a time-dependent effective frequency during the application of the adiabatic pulses. The adiabatic MRI techniques have demonstrated abnormalities in multiple clinical conditions including Parkinson's disease,[24,25] CNS neoplasm,[26] and stroke.[27] Specifically, adiabatic T1ρ can detect changes in neuronal cellular density,[24,28] whereas adiabatic T2ρ has been shown to be sensitive to iron,[24] and even capable of quantifying iron levels in the brain.[29]

One of the main barriers to obtaining quantitative MT data is the need for long steady-state pulses required for fitting T1 in the presence of off-resonance saturation (i.e. T1sat) and the steady-state magnetization (i.e. Mss). By utilizing an inversion-prepared protocol, we have demonstrated that it is possible to provide quantitative MT parametric maps that overcome the poor sensitivity of MTR maps and provide enhanced tissue specificity while maintaining RF exposure within specific absorption rate (SAR) limits.[23]

The overall goal of this study was to establish novel MRI protocols for detection of abnormalities in the normal-appearing brain tissue in MS. To this end, we measured adiabatic T1ρ, adiabatic T2ρ, T1sat, and MTR in the brain of nine relapsing–remitting MS (RRMS) patients and seven healthy control subjects. We specifically focused on T2 lesions, normal-appearing cortical gray matter (GM), and NAWM. We hypothesized that T1sat, as measured by our inversion-prepared MT protocol,[23] would be more robust than MTR for detecting abnormalities of NAWM in MS. In addition, we hypothesized that rotating frame relaxation parameters would also detect abnormalities in normal-appearing brain tissue of MS, thus providing complementary information to characterize brain tissue pathology.

Methods

Subject recruitment

Sixteen subjects completed the MRI study after giving informed consent using procedures approved by the Institutional Review Board: Human Subjects Committee of the University of Minnesota. Four additional subjects participated in the study, but did not provide complete data because of excessive head movements or MRI artifacts. RRMS subjects ($n = 9$) were recruited from the University of Minnesota MS Clinic and via advertisements in local MS Society publications. The control subjects ($n = 7$) were recruited via advertisements around the University of Minnesota, and from existing databases of control subjects interested in participation in research. Inclusion criteria for both groups included age 18–75 years, and ability to tolerate MRI without sedation. MS subjects had to meet 2005 revised McDonald criteria for MS,[30] and had to have at least one T2-hyperintense lesion in the region of interest (ROI). Exclusion criteria for both groups included implanted metal that might interfere with safe performance of MRI, active substance abuse, weight > 300 lbs, and CNS disorders other than MS.

Paraclinical data were obtained from each of the RRMS subjects the same day of the MRI session prior to arrival to the Center for Magnetic Resonance Research. These tests included: Expanded Disability Status Scale (EDSS), Multiple Sclerosis Functional Composite (MSFC), 9-hole Peg test (9HP), 25-foot timed walk (T25FW), Paced Auditory Serial Addition Test 3 (PASAT), Fatigue Severity

Table 1. Characteristics of MS and control subjects.

	Controls	RRMS
	Mean ± SD	Mean ± SD
N	7	9
Age (years)	37 ± 9	38 ± 10
Female/male	5/2	7/2
Disease duration (years)	–	10 ± 5
EDSS	–	2.9 ± 1.2
MSFC (*Z* score)	–	0.23 ± 0.37
9HP, left hand (seconds)	–	24.5 ± 5.2
9HP, right hand (seconds)	–	22.6 ± 5.3
T25FW (seconds)	–	4.5 ± 1.3
PASAT (correct responses)	–	47.3 ± 10.9
FSS	–	36.3 ± 15.7
CES-D	–	14.9 ± 7.5
MSQOL	–	64 ± 21

MS: multiple sclerosis; RRMS: relapsing–remitting multiple sclerosis; SD: standard deviation; EDSS: Expanded Disability Status Scale; MSFC: Multiple Sclerosis Functional Composite; 9HP: 9-hole Peg test; T25FW: timed 25-foot walk; PASAT: Paced Auditory Serial Addition Test 3; FSS: Fatigue Severity Scale; CES-D: Center for Epidemiologic Studies Depression Scale; MSQOL: Multiple Sclerosis Quality of Life-54 instrument.

Scale (FSS), Center for Epidemiologic Studies Depression Scale (CES-D), and the Multiple Sclerosis Quality of Life-54 instrument (MSQOL). A summary of subject characteristics is reported in Table 1. All 16 subjects included in the study completed the MT and adiabatic T1ρ and T2ρ measurements, except two RRMS patients who did not complete the adiabatic T2ρ measurement.

MRI parameters and settings

The study was performed using a 4 T, 90 cm bore magnet interfaced to a Varian DirectDrive™ console (Varian is now Agilent, Santa Clara, CA, USA). A volume coil was used for signal transmission and reception from the human brain, and proper padding was utilized in order to minimize head movements. After initial scout images, we selected a single axial oblique brain slice just above the lateral ventricles and parallel to the AC-PC line for the various relaxation measurements. Images were acquired using fast spin echo readout, repetition time (TR) = 5–8 s (depending on the relaxation measurement performed), echo time (TE) = 0.073 s, matrix 256 × 256, field of view (FOV) = 25.6 × 25.6 cm^2, echo train=16, and slice thickness = 4 mm. The adiabatic T1ρ/T2ρ and MT measurements were obtained as described previously (Figure 1)[22,23] with adiabatic full passage (AFP) hyperbolic secant (HS1) pulses having RF peak amplitude $\omega_1^{max}/(2\pi)$ = 0.88 kHz, pulse duration = 0.006 s, and inversion bandwidth = ~1.6 kHz. For the MT experiment, a 6 kHz off-resonance continuous-wave (CW) irradiation was implemented with incremental duration (0, 0.3, 0.6, 0.9, 1.2 s) and amplitude $\omega_1^{max}/(2\pi)$ = 0.15 kHz. Separate MT measurements were performed with and without an initial global inversion achieved by an adiabatic HS1 pulse (pulse length = 6ms, $\omega_1^{max}/(2\pi)$ = 1.2 kHz, bandwidth = ~3.3 kHz). The acquisition time for T1ρ/T2ρ and for MT measurements was ~6.7 min and 21.3 min, respectively. The raw images were inspected during the experimental session in order to identify movements during and between relaxation measurements, so that the acquisition could be restarted and the slice repositioned if needed. In three experimental sessions, the slice of interest needed to be repositioned to ensure spatial congruency among the various measurements. The duration of the entire experimental session was ~40–60 min, and the estimated specific absorption rate was always below the Food and Drug Administration (FDA) limit of 3 W/kg averaged over the head for 10 minutes (http://www.fda.gov/cdrh/ode/mri340.pdf).

Post-processing

The relaxation time constants T1ρ and T2ρ were estimated by mono-exponential fitting of the signal intensity decays on a pixel-by-pixel basis. The relaxation time constant T1sat was estimated using a nonlinear regression algorithm that took into account data sets acquired with and without the inversion preparation.[23] The fitting procedures additionally provided maps of signal intensity in the absence of AFP trains or CW irradiation (i.e. S_0), which resembled the contrast generated by the readout only. Finally, MTR was calculated based on the ratio of the signal intensity (SI) obtained with and without a 600 ms-long CW irradiation ($SI_{(MT=600ms)}$ and SI_0, respectively), according to $(1–(SI_{(MT=600ms)}/SI_0)*100$. Relaxation analyses were performed using custom-built functions within Aedes software (Kuopio, Finland) operating on a Matlab R2009b platform (The Mathworks Inc, Natick, MA, USA).

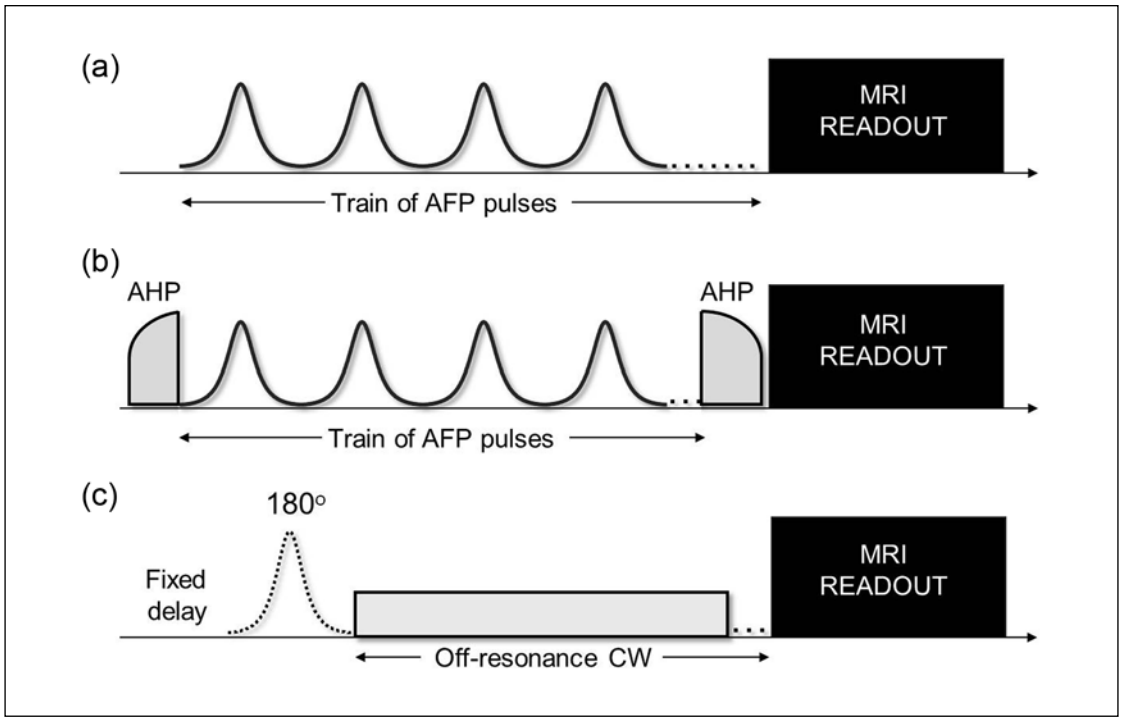

Figure 1. Pulse sequences used to measure adiabatic T1ρ (a), T2ρ (b) and magnetization transfer (c). In the adiabatic T1ρ configuration (a) a train of 0, 4, 8, 12 and 16 adiabatic full passage (AFP) pulses is placed prior to the imaging readout. In the adiabatic T2ρ configuration (b) the AFP train is embedded between two adiabatic half passage (AHP) pulses that first bring magnetization to the transverse plane and then bring it back to the z-axis before the readout. The AFP pulses are delivered with phases according to MLEV-4. In the inversion-prepared MT protocol (c), a global on-resonance inversion by an AFP pulse is either turned off or on prior to the off-resonance continuous-wave (CW) irradiation. In the present study, the MRI readout was fast spin echo.
MT: magnetization transfer; MRI: magnetic resonance imaging.

ROIs

T2-hyperintense lesions (T2L) in MS patients were carefully identified manually (by A.C.) from T2-weighted images (those obtained with the S_0 maps), whereas both in patients and controls cortical GM and NAWM regions were selected based on raw magnetization transfer images. In the MS brains, the NAWM regions were specifically selected to avoid "dirty-appearing" white matter. In our patient population there was no indication of cortical GM lesions. However, cortical GM lesions cannot be ruled out, as the MRI acquisition protocol was not optimized for their detection. The ROIs were then saved as ROI masks (Figure 2). The ROI masks were applied to the various maps to generate the mean of each MRI parameter in each tissue type and each subject. Only in a few cases were the ROIs masks individually adjusted to take into account left-over slice misalignments between relaxation measurements.

Statistical analysis

Means and standard deviations (SD) were calculated for each MRI parameter, grouped by tissue type and subject population. Coefficients of variation among subjects (cv) were calculated as SD/mean from the population of healthy controls, while correlations of MRI parameters with clinical scales were estimated from the MS subjects. MRI parameters in NAWM and GM were compared between MS and control subjects by two-tailed unpaired *t* tests, while within-subjects comparisons between ROIs in each of the two groups of subjects were performed with two-tailed paired *t* tests. The level of significance was set at $p = 0.05$.

Results

Figure 2 shows representative relaxation maps from control and RRMS subjects. The range of adiabatic T1ρ (~170–220 ms) and T2ρ (~80–90 ms) both from GM and WM tissues were in good agreement with previous studies at 4T.[22,24,25] Variations of adiabatic T1ρ and T2ρ among healthy controls (Table 2) were below 15% in cortical GM (cv = 10% and 13% for T1ρ and T2ρ, respectively) and below 5% in WM (2% and 4%, respectively). In RRMS patients, T1ρ and T2ρ were significantly prolonged in T2 lesions as compared to NAWM ($p = 0.0041$ and $p = 0.01$, respectively) and to cortical GM ($p = 0.033$ and $p = 0.031$, respectively). T1ρ was significantly prolonged by ~6% in the NAWM of RRMS patients as compared to healthy controls ($p = 0.026$), while

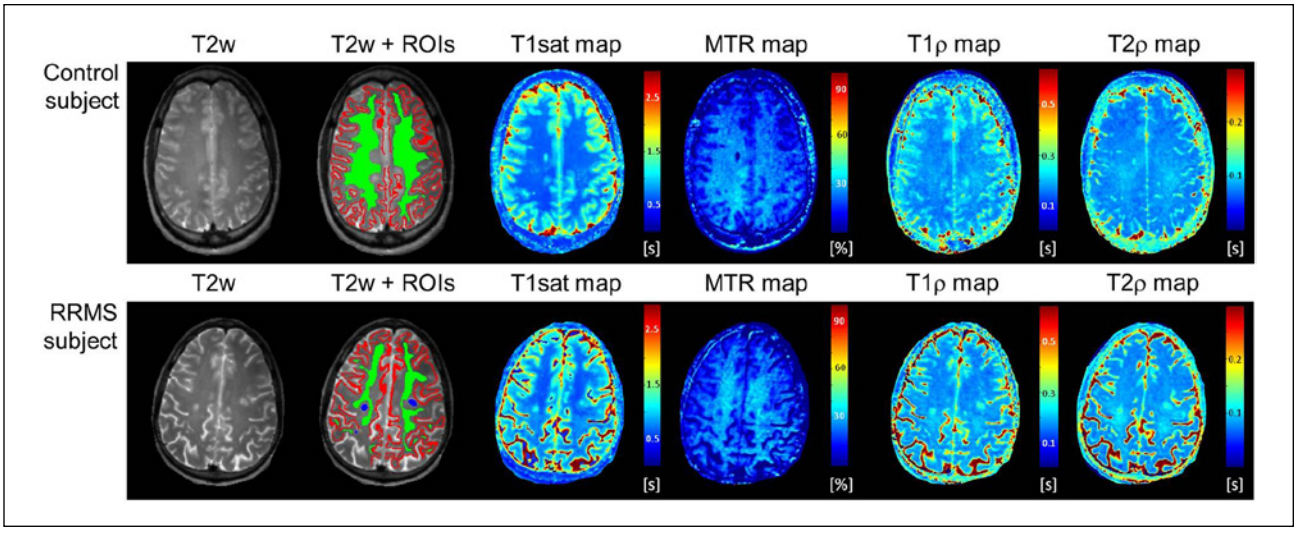

Figure 2. Examples of T2-weighted (T2w) images, regions of interest (normal-appearing white matter = green, cortical gray matter = red, T2-lesions = blue), T1sat, MTR, T1ρ and T2ρ maps from one healthy control subject (top row) and one RRMS subject (bottom row). T1sat: T1 measured during off-resonance irradiation; MTR: magnetization transfer ratio; RRMS: relapsing–remitting multiple sclerosis.

Table 2. Comparisons of MRI parameters by tissue type between groups.

	Control (n = 7)	cv	RRMS (n = 9)	p value	Difference
T1ρ cortical GM (s)	0.221 ± 0.029	13%	0.225 ± 0.009	p = 0.695	1%
T1ρ NAWM (s)	**0.172 ± 0.003**	**2%**	**0.182 ± 0.011**	***p* = 0.026**	**6%**
T1ρ T2L (s)	n.a.	n.a.	0.312 ± 0.029	n.a.	n.a.
T2ρ cortical GM (s)	0.092 ± 0.009	10%	0.097 ± 0.012[a]	p = 0.396	5%
T2ρ NAWM (s)	0.084 ± 0.003	4%	0.087 ± 0.007[a]	p = 0.328	4%
T2ρ T2L (s)	n.a.	n.a.	0.140 ± 0.035[a]	n.a.	n.a.
T1sat cortical GM (s)	1.101 ± 0.055	5%	1.165 ± 0.086	p = 0.11	6%
T1sat NAWM (s)	**0.630 ± 0.030**	**5%**	**0.738 ± 0.059**	***p* = 0.0006**	**17%**
T1sat T2L (s)	n.a.	n.a.	1.216 ± 0.358	n.a.	n.a.
MTR cortical GM (%)	17.69 ± 2.02	11%	17.21 ± 2.27	p = 0.671	–3%
MTR NAWM (%)	**29.34 ± 2.74**	**9%**	**25.95 ± 3.23**	***p* = 0.043**	**–12%**
MTR T2L (%)	n.a.	n.a.	16.48 ± 7.06	n.a.	n.a.

MRI: magnetic resonance imaging; T1sat: T1 measured during off-resonance irradiation; GM: gray matter; NAWM: normal-appearing white matter; T2L: T2-hyperintense lesions; MTR: magnetization transfer ratio; RRMS: relapsing–remitting multiple sclerosis; n.a.: not applicable. Values of MRI parameters are shown as mean ± standard deviation (SD) for different tissue type and subject populations. Intersubject coefficient of variation (cv = SD/mean) were calculated in healthy control subjects. *P* values refer to unpaired two-sided *t* test between multiple sclerosis (MS) subjects and healthy control subjects. Difference of MRI parameters between groups (MS minus controls) are also shown. Parameters that show statistically significant differences between groups are displayed in bold. [a]n = 7 rather than 9.

T2ρ values were not significantly different between groups. No significant differences among groups were observed for either T1ρ or T2ρ in cortical GM. In MS subjects, cortical GM T1ρ was highly correlated with MSFC (r = 0.688, p = 0.041), PASAT (r = 0.71, p = 0.032), and to a lesser extent to EDSS (r =−0.652, p = 0.057), while cortical GM T2ρ was correlated with T25FW (r = −0.759, p = 0.048). No other significant correlations were observed between adiabatic relaxation time constants and paraclinical measures.

The range of measured T1sat (~650–1200 ms) was in good agreement with previous studies at 4T,[23] and the cv of T1sat was 5% both in cortical GM and WM, which is significantly less than the cv of MTR in the same regions (11% and 9%). In RRMS patients, T1sat values were significantly prolonged in T2 lesions as compared to NAWM (p = 0.0045), but were not different from cortical GM values (p = 0.724). On the other hand, MTR values were significantly smaller in T2 lesions than in NAWM (p = 0.0071) but not different from cortical GM (p = 0.776). T1sat relaxation time was significantly prolonged by ~17% in the NAWM of RRMS patients as compared to healthy controls (p = 0.0006), while MTR was 12% smaller (p = 0.043). No significant correlations were observed between MT parameters and any of the paraclinical measures.

Discussion

In this study we obtained quantitative measures of adiabatic rotating frame relaxations and MT parameters in the brain of RRMS subjects and healthy controls, with the goal of addressing the feasibility and sensitivity of these MRI measures for characterizing normal-appearing brain tissue in MS. Based on theoretical considerations and on our previous experience in other tissue types and subject populations,[21,22,24–27] we anticipated that adiabatic relaxation and quantitative MT parameters would identify abnormalities in the normal-appearing brain tissue of MS. The results of the present study demonstrate that adiabatic relaxation methods, along with the inversion-prepared quantitative MT protocol, hold great potential for the study of MS. Adiabatic relaxations were highly reproducible among the healthy controls investigated here, who were relatively similar in age (37 ± 9 years). The intersubject reproducibility was especially striking for T1ρ, allowing reliable detection of small differences between groups. T1sat values measured with the inversion-prepared MT protocol were also found to be tightly distributed among healthy subjects, with cvs that were about half of those observed for the MTR. However, the higher reproducibility of T1sat as compared to MTR measurements was achieved at the expense of substantially longer acquisition times.

In our group of RRMS patients, we observed a slightly but significantly longer adiabatic T1ρ in NAWM regions as compared to healthy controls (by 6%, $p = 0.0026$). Within T2 lesions T1ρ was more strikingly prolonged, and was >80% longer than in the WM of healthy controls (Table 2). It is tempting to speculate that the longer T1ρ observed in the NAWM and in the T2L of MS brains might reflect the same pathologic processes in each tissue type. Pathologic abnormalities within T2 lesions include myelin loss, axonal damage, and (depending on the stage or age of the lesion) inflammation.[1] Whereas only post-mortem studies can confirm the pathophysiological processes that underlie the T1ρ findings, prior histopathologic studies in animals have shown a relation between T1ρ and neuronal density.[24,28] The present results thus seem to suggest the presence of ongoing axonal degeneration in NAWM tissue of RRMS, and that adiabatic T1ρ is a sensitive measure for detecting it. However, other phenomena, such as inflammation, might explain T1ρ lengthening in such regions. Because no T2ρ differences were observed above our detection threshold (~5%) in the NAWM of MS, the degenerative process occurring in NAWM does not seem to be accompanied by abnormal iron levels. While several studies have demonstrated a correlation between disease progression and the levels of iron deposition in deep GM structures,[31] no experimental evidence has so far shown abnormal iron levels in the NAWM of MS.

Although it is known that cortical GM is involved early in the disease process of MS, we did not observe detectable differences in T1ρ or T2ρ that suggest neurodegeneration or abnormal iron levels, respectively, at this stage of the disease. Yet, we observed that GM T1ρ was correlated with MSFC and PASAT disability scores, and that GM T2ρ was correlated with T25FW. The small sample size used for the present study nonetheless warrants caution in interpreting these findings and indicates the need for further investigations with larger cohorts.

Small decreases of MTR values in MS NAWM are well documented in the literature,[4,32] and our MTR findings are in agreement with previous findings. However, the present study additionally demonstrates that the T1sat measured with the inversion-prepared MT protocol is a more robust and sensitive parameter than the MTR for detection of NAWM abnormalities in MS patients (i.e. bigger changes and higher levels of significance when comparing MS with healthy controls). Some groups have reported correlations of MTR parameters in NAWM with disability scores,[33–35] but the correlations are generally weak and not always reproducible. Despite the higher sensitivity of T1sat in detecting differences in NAWM, we did not observe here any correlation between T1sat and clinical scores. Future investigations on a larger group of subjects are needed to corroborate the potential usage of MT parameters as a marker of disease severity.

The MRI protocol used in this study was based on a single slice acquisition, with the various relaxation measurements taking several minutes to be completed. Under these conditions, head movements can seriously compromise the robustness of the quantitative estimates of the relaxation parameters, since the acquired brain slice might differ during data acquisition. The present results were not compromised by head movements, since we carefully controlled for them during both data acquisition and data processing. Yet, the inherent sensitivity to head motion and the limited brain coverage of the current MRI protocol are clear obstacles to clinical application. Possible strategies for reducing scan time and increasing brain coverage include giving up full relaxation conditions by using shorter TR, using parallel acquisition and using alternative, faster readout schemes, although such approaches might inherently introduce lower signal-to-noise ratios.

In conclusion, we demonstrated the feasibility of using new MRI parameters to identify abnormalities in the normal-appearing brain tissue in MS. In agreement with previous findings, MT parameters could detect changes in NAWM of MS vs controls, however T1sat was found to be substantially more sensitive than MTR in separating MS subjects from controls. Adiabatic T1ρ, but not T2ρ, also demonstrated abnormalities in NAWM of RRMS vs controls, suggesting neurodegenerative processes likely not accompanied by significant abnormalities in iron content.

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Conflicts of interest

Dr Carpenter has received research support from Biogen Idec, Celgene, Roche, Sanofi-Aventis, EMD Serono and Teva. Drs Mangia, Eberly, Garwood, Michaeli and Tyan have nothing to declare.

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