

Magnetization transfer of water T_2 Relaxation components in human brain: implications for T_2 -based segmentation of spectroscopic volumes

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Abstract

Biexponential T_2 relaxation of the localized water signal can be used for segmentation of spectroscopic volumes. To assess the specificity of the components an iterative relaxation measurement of the localized water signal (STEAM, 12 echo times, geometric spacing from 30 ms to 2000 ms) was combined with magnetization transfer (MT) saturation (40 single lobe pulses, 12 ms duration, 1440° nominal flip angle, 1 kHz offset, repeated every 30 ms). Voxels including CSF were examined in parietal cortex and periventricular parietal white matter (10 each), as well as 13 voxels in central white matter and 16 T_1 -hypointense non-enhancing multiple sclerosis lesions without CSF inclusion. Biexponential models (excluding myelin water) were fitted to the relaxation data. In periventricular VOIs the component of long T_2 (1736 ± 168 ms) that is attributed to CSF was not affected by MT. In cortical VOIs this component had markedly shorter T_2 's (961 ± 239 ms) and showed both attenuation and prolongation with MT, indicating contributions from tissue. MS lesions and central WM showed a second tissue component of intermediate T_2 (160–410 ms). In white matter similar MT attenuation indicated strong exchange between the two tissue components, prohibiting segmentation. In MS lesions, however, markedly less MT of the intermediate component was found, which is consistent with decreased cellularity and exchange in a region that is large compared to diffusion motion. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

Inclusion of cerebrospinal fluid (CSF) in the volume of interest (VOI) may be a considerable source of error when estimating brain metabolite concentrations from localized MR spectra. Tissue and CSF fractions of the VOI may be segmented by means of high resolution MR images, or by means of a biexponential fit to the T_2 relaxation of the localized water, where the component with long T_2 is equated with CSF [1,2]. For this purpose, the amplitudes of the fitted T_2 components are quantified relative to the estimated signal of 'pure water', which represents 100% water content in the VOI. Then, the long- T_2 component can be used directly as an estimate for the partial volume, under the basic assumptions of negligible exchange, absence of T_1 losses, and 100% visibility of the signal. When setting up this method we observed, that the T_2 's of the fitted CSF

components were shorter in VOIs of cortical gray matter than in periventricular VOIs. Lacking a physiological explanation for this finding, we suspected that water is not strictly confined to compartments. In this case T_2 is altered by diffusion and exchange in a layer at the boundary between tissue and CSF. This may result in intermediate T_2 contributions, which contribute to the fitted discrete components.

The principal purpose of this study was to elucidate whether the apparent CSF component is affected by contributions from tissue. CSF should not be affected by magnetization transfer (MT) effects [3] because of its low protein content and absence of structural material. Hence, MT may be used to identify contributions from tissue signal to the slowly relaxing component. In addition, the fitted T_2 is expected to change, if a component comprises several contributions that show different degrees of MT. In this study MT attenuation was created by repetitive off-resonance irradiation of band-selective radio frequency pulses.

In principle, the T_2 -based segmentation method allows for separation of partial volumes that cannot be resolved by

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MRI, e.g., in the presence of extracellular edema. Experimental edema in brain is associated with an additional relaxation component of intermediate T_2 (200–400 ms) [4]. Such a component has also been reported for white matter (WM) of healthy subjects [5]. Biexponential T_2 relaxation is commonly observed in multiple sclerosis (MS) lesions (see [6] for a review). Regression analysis of the fitted components in contrast-enhancing MS lesions indicated that the intermediary component has a water content that is close to 100% and replaces the short component completely. Thus, the intermediate T_2 component may be used for volume correction of an edematous increase of the extracellular space [7]. In order to study the effect of changed intracellular distances and survey its applicability for VOI segmentation, this paper comprises also the component specific MT characteristics of biexponential T_2 relaxation of non-enhancing T_1 -hypointense MS lesions and of healthy WM. Extending a preliminary report [8], this study contributes initial observations of particularly low MT attenuation of the intermediate- T_2 component in MS lesions. In the present paper we also introduce a concept of estimating component specific MT ratios (MTR) after weighting the amplitudes by the corresponding T_2 values. Thus, the influence of T_2 variations arising from independent measurements or fits on the MTRs of the slowly relaxing component can be largely reduced.

2. Methods

2.1. Measurement of the localized water signal and segmentation models

The experiments were performed on a 1.5 Tesla clinical MR system (Signa Advantage, General Electric Medical Systems, Milwaukee, WI) using the standard quadrature birdcage headcoil. Nineteen healthy adult subjects (age 22–32 years) and nine patients with MS were recruited according to the ethical guidelines of the local human subject protection board.

VOIs were selected on axial T_1 -weighted 3D spoiled gradient echo (SPGR) images (TR 18 ms/TE 4.8 ms/ α 20° 38/FOV 24 cm/28 slice partitions of 4 mm thickness). The standard STEAM sequence was used for signal localization. The spectroscopic standard regions of gray matter (GM) in the parietal cortex and of subparietal white matter (WM) were studied (Fig. 1A). Parts of the lateral ventricles were deliberately included in the WM VOIs. Thirteen VOIs located in the *centrum semiovale* (without CSF inclusion, Fig. 1B) served as a control group for 16 measurements in T_1 -hypointense MS lesions, so called ‘black holes’ (Fig. 1C), which were embedded in normal appearing white matter (NAWM). No lesion showed T_1 contrast enhancement after i.v. application of Gadodiamid (Nycomed, Norway, single dose of 0.1 mM per kg body weight).

T_2 relaxation of the unsuppressed localized water signal

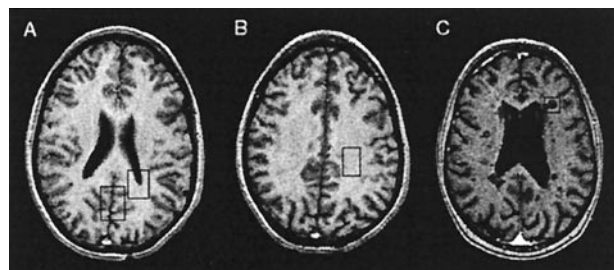


Fig. 1. A: Typical VOI locations in parame-dian-parietal GM and subparietal WM including parts of the lateral ventricles. B: VOI location in the *centrum semiovale*. C: The T_1 -hypointense lesions (‘black holes’) appear well delineated on 3D SPGR images. Confounding contributions from CSF were excluded from the VOIs of central WM and lesions.

was studied by $n = 12$ consecutive single acquisitions at echo times (TE) spaced geometrically between $TE_{\min} = 30$ ms and $TE_{\max} = 2000$ ms as in [7]:

$$TE_i = TE_{\min} \left(\frac{TE_{\max}}{TE_{\min}} \right)^{\frac{i-1}{n-1}}, \quad i = 1, 2, \dots, n. \quad (1)$$

Measurements with $n = 32$ TE points were performed in one subject, in order to overcome possible limitations in detecting signal of intermediate T_2 when both tissue and CSF are present (Fig. 2). Diffusion weighting by the sinusoidal crusher gradients (10 mTesla/m peak amplitude) was independent of TE, since their duration (10 ms) and spacing (13.7 ms TM interval) was kept constant. In order to avoid TE dependent signal variations due to residual eddy currents, control measurements were regularly performed on a phantom with a T_2 of 270 ms. If false T_2 components were detected, the pre-emphasis settings of the gradient amplifiers were re-adjusted.

Integration of the water resonance suffered from phasing difficulties and TE dependent line-shapes due to changes in the proportion between CSF and tissue signal. For practical reasons the water signal was measured as the maximum of the acquired half echo, which is displayed as the fraction of the dynamic range of the analog-to-digital-converter. For a reliable, but rather time-consuming determination of the water signal we used LCMoDel (Vs. 5.1, S. W. Provencher, Oakville, Ontario, Canada [9]) to fit an experimental singlet resonance with automated phase correction and a non-analytic line-shape to the water resonance. Although receiver noise gives rise to a positive offset in the echo amplitude, there was excellent correspondence with the area of the water resonance as determined by LCMoDel (differences < 0.5%, Pearson’s coefficient of correlation $r > 0.998$). In order to avoid potentially biased initial guesses, the appropriate number of components and the necessity of a noise baseline were determined from single experiments by means of the DISCRETE program [10] (courtesy of S. W. Provencher). For reasons of convenience, however, the model functions were routinely fitted to the relaxation decay using the Marquardt-Levenberg algorithm written in IDL (IDL 4.1, Research Systems Inc., Boulder, CO). For a given

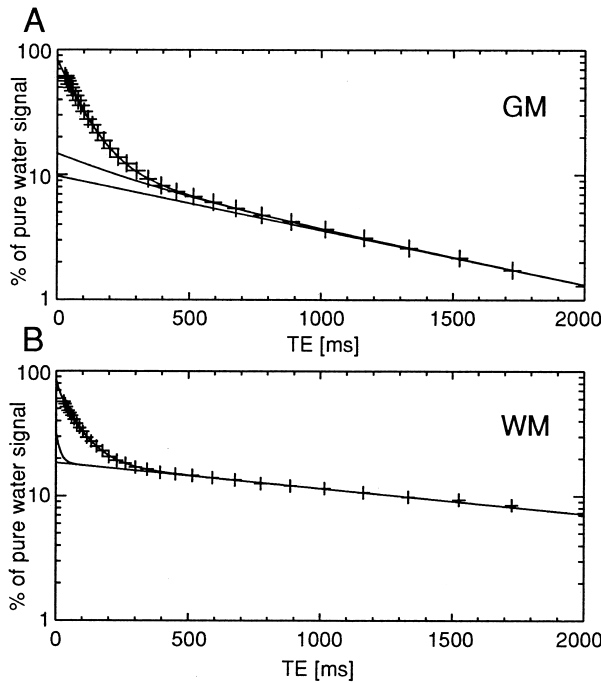


Fig. 2. Semilogarithmic plots of 32 TE point relaxation measurements of the localized water signal. In extension of the biexponential tissue/CSF model used in the paper, *three* exponentials were fitted. The curves indicate the sum of all three components, the long- T_2 component (attributed to CSF), and the third component added to the long- T_2 component. A: The intermediate component (5%, $T_{2int} = 273$ ms) in GM could not be detected consistently with the 12 point standard protocol. Biexponential fitting increased CSF from 10% ($T_{2long} = 998$ ms) to 12% ($T_{2long} = 852$ ms). B: In periventricular WM the intermediate component was not detected, while remnants of myelin water gave rise to a large erroneous component of 5.5 ms T_2 . Shown here is a fit where this T_2 was fixed to 15 ms. This yielded 14.3% myelin water, 18.4% CSF ($T_{2long} = 2110$ ms) and 53.6% brain water ($T_{2short} = 79$ ms). The biexponential model resulted in 3% more CSF through decrease of T_{2short} (to 76 ms) and T_{2long} (to 2050 ms).

model both algorithms yielded identical results within the error of the fit.

After scaling by volume and correction for variations of sensitivity, the component amplitudes (s_{short} , s_{int} , s_{long}) were expressed relative the T_2 -corrected water signal obtained from the calibration experiment, which served as a reference for 100% water content in the VOI [11]. Comparability of s_{short} requires correction for individual proportions of tissue and CSF. Division by $1 - s_{long}$ yields the ‘MR water content’ of tissue as introduced in (2):

$$\beta_{MR} = s_{short} / (1 - s_{long}). \quad (2)$$

Equating s_{long} with its volume fraction assumes 100% water content, i.e., that the signal observed *in vivo* corresponds to the density of ‘pure’ water. Upon the assumption that the groups to exhibit distinct features, we used a two-sided heteroscedastic t test throughout this paper, and a two-sided paired t test for statistical comparison within one group, e.g., for MT effects or for different models.

2.2. Magnetization transfer

Attenuation of the observable water signal by magnetization transfer was stimulated by saturating the motion-restricted magnetization using single lobe radio frequency pulses (hamming-filtered sinc mainlobe) of 1440° nominal flip angle, which were applied 1 kHz off-resonance with a repetition period of 30 ms. These parameters were chosen in order to achieve a high degree of saturation, but they were still in the range of values reported for clinical MT protocols [12]. Forty saturation pulses were applied, which was the maximum number compatible with system restrictions, yielding a total saturation time of 1.2 s. By this saturation scheme 96% of the steady state attenuation was achieved for the total water signal in central WM at 30 ms TE [13]. To account for a possible saturation of the water signal through radio-frequency noise or leakage, the transmitter was kept unblanked when no off-resonance pulses were applied in the reference experiment.

Because change of TE required a sequence download, the time between two acquisitions was 15 s and the total measuring time for one VOI amounted to 6 min. To reduce the influence of subject motion, relaxation with and without MT was measured interleaved. In patients, however, the relaxation measurements were run consecutively to meet increased constraints in time and compliance. The difference spectrum of two gradient broadened scans with and without MT preparation (on resonance) did not reveal spikes of direct saturation at frequencies differing more than 250 Hz from the pulse offset. This is in agreement with a spectroscopy study where pulses of similar shape and duration were used [14]. In addition, direct saturation of the visible signal components by a single off resonance pulse was estimated by numerical integration of the Bloch equations using the measured T_2 values and neglecting T_1 . Cumulative saturation of the longitudinal magnetization was then calculated neglecting exchange between compartments and with the motion-restricted magnetization.

Magnetization transfer ratios (MTR) were calculated from the amplitudes of the fitted relaxation components, which are denoted by the subscript index = ‘short’, ‘int’, or ‘long’:

$$MTR_{index} = 1 - \frac{s_{index}^{MT}}{s_{index}^{ref}}. \quad (3)$$

Since there is a lower limit for TE, the amplitudes can be regarded as exponential extrapolations to zero TE by the corresponding T_2 values. In particular, this will affect the long and the intermediate T_2 components, because they are mainly determined by the data points that do not have major contributions from the fast relaxing component, i.e., at TE longer than 300 ms. Variation in T_2 may thus impose an error onto the MTR values, which are estimated from two independent fits. In order to study the influence of T_2 on the MTRs, the amplitudes were weighted by

$$s_{\text{index}} \rightarrow s_{\text{index}} \cdot \exp(-TE/T_{2\text{index}}) \quad (4)$$

before applying Eq. (3).

3. Results

3.1. Choice of model functions

The ability to detect short and long T_2 times is limited by TE_{min} and TE_{max} . Water that is trapped in myelin sheaths (so called ‘myelin water’ [15]) could not be fitted reliably to our data, because its T_2 is about 15 ms and thus much shorter than TE_{min} . On the other hand, the signal did not decay completely into the receiver noise at TE_{max} , when the VOI contained CSF. A strong inverse correlation between baseline and long- T_2 component (as detected by DISCRETE) indicated that the long- T_2 component could not be distinguished from the noise baseline. For this reason, CSF and noise were represented by a single component. Owing to the exclusion of CSF from the VOIs, a separation of two exponential components and the baseline was possible in MS lesions and central WM, because the second component decayed into the noise with T_2 times intermediate to tissue and CSF (Fig. 3A and 3B). We shall call this the ‘biexponential tissue model’ to distinguish it from the tissue/CSF model of Ernst et al. [2].

For an average VOI size of 6.1 mL in central WM the average amplitude of the noise baseline was 0.4% of the reference water signal. Such small contributions may safely be neglected in the tissue/CSF segmentation of spectroscopic VOIs. The baseline amplitudes were not changed by MT effects. Not accounting for receiver noise in the biexponential tissue model prolonged the T_2 of the intermediate component to values between 250 ms and 500 ms (see dashed curves in Fig. 3C). This model will not be considered further, because the intermediate T_2 increased with MT, as proportion between noise and the attenuated signal changed.

The signal from central WM showed the least distinct components. However, two components were found to be either the most probable solution or a significant alternative to one or three components by the DISCRETE program. Central WM was evaluated using both models: The biexponential tissue model was used for comparison to MS lesions. In the tissue/CSF model the long T_2 was fixed to 2000 ms (see drawn curves in Fig. 2C), which is often used as an approximation for residual CSF signal [16,17]. When the tissue/CSF model was applied to central WM, however, we observed an increase of $T_{2\text{short}}$ ($p = 0.003$) and 27% average MT of the 2000 ms component. This strongly suggests that slowly relaxing signal from tissue contributes to this component, and that the approximation by the tissue/CSF model is not fully suitable to describe central WM.

The long- T_2 components showed rather large variations, especially in T_2 . Since the long- T_2 component is mainly determined by the data points that do not have major con-

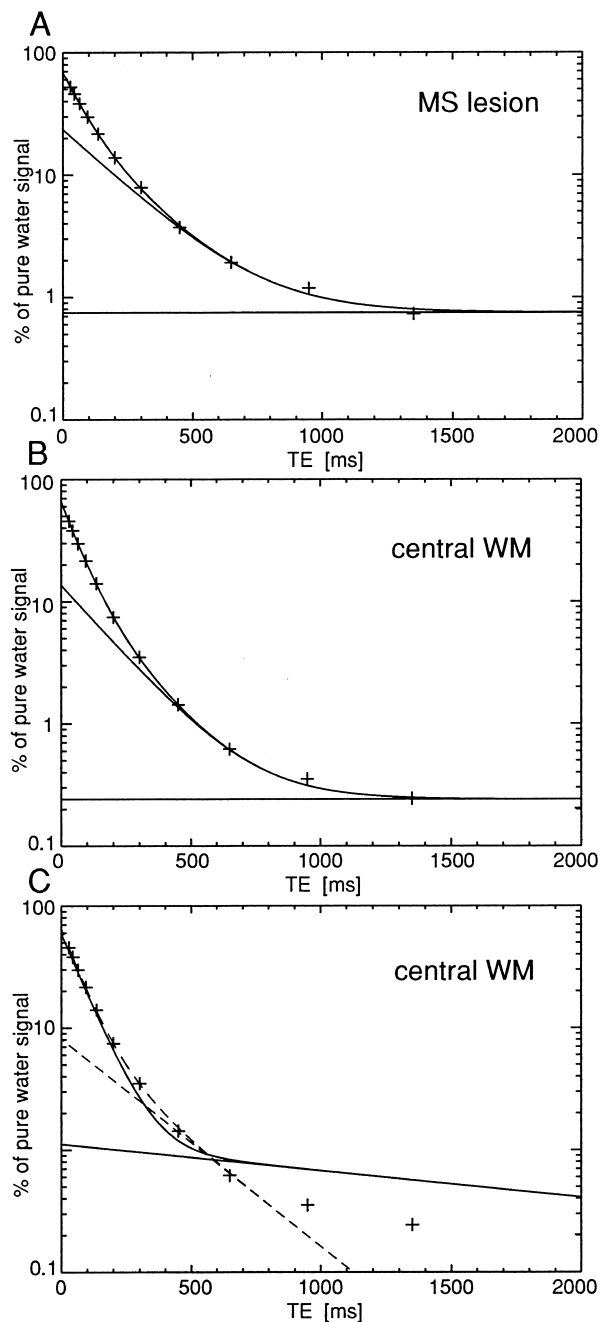


Fig. 3. Semilogarithmic plots of the 12 TE point standard relaxation measurement A: in the MS lesion VOI shown in Fig. 1C, and B: in the white matter VOI of Fig. 1B. The curves indicate the fitted biexponential tissue model, the constant baseline, and the intermediate T_2 component added to the baseline. Complete decay of the water signal into the noise at $TE \approx 1300$ ms increased the reliability of the baseline. The baseline appears lower in B than in A because the WM VOI was larger. C: Typical deviation of the fit when using the tissue/CSF model with $T_{2\text{long}}$ fixed to 2000 ms (drawn), and when omitting the baseline (dashed) (Same data as in B).

tributions from the fast relaxing component (i.e., at $TE > 300$ ms), the variation in T_2 may also affect the corresponding amplitude, and hence the MTR. Weighting of s_{long} according to Eq. [4] yielded a convergence of the corre-

sponding MTR_{long} values, the ‘focus’ for GM being at TE = 1250 ms (Fig. 4A) and at TE = 750 ms for WM (Fig. 4B). At these TE values the difference in T_2 between MT and reference measurement is largely reduced. Thus, the standard deviation (SD) was reduced by 58% in GM, and by 48% in WM. The tilt of the mean MTR reflects the difference in the mean relaxation rates with and without MT. In MS lesions T_2 -weighting of s_{int} ‘focused’ MTR_{int} at TE = 300 ms, where the SD was reduced by 56%. (Fig. 4C). Control WM also had a minimum of SD at TE = 300 ms, but with a minor reduction of 6% (Fig. 4D). In stark contrast, the values of MTR_{short} diverged in all regions after T_2 -weighting (not shown), the focus being close to TE = 0.

3.2. Tissue/CSF model

The apparent T_2 of the slowly relaxing component attributed to CSF was almost twice as high in the ventricles as in the sulci adjacent to cortical gray matter (see Table 1 for numerical results in the absence of MT). Off-resonance saturation of the bound water significantly attenuated the long- T_2 component in gray matter ($p = 0.0007$). Both T_{2long} and T_{2short} increased ($p = 0.0005$ and $p = 0.001$). Average MTR was $9.7\% \pm 4.8\%$, which was larger than the calculated direct saturation (4.3%). This clearly demonstrates contributions from tissue signal to the slowly relaxing component in VOIs of cortical GM. The numerical results for T_2 and the MT ratios are given in Table 2. In contrast to GM, the CSF component in periventricular WM did not show MT effects that were significantly different from zero. There were no significant differences in T_{2short} between GM and the WM regions ($p > 0.1$), but MTR_{short} was smaller in GM than in WM ($p = 0.0015$). Although the tissue water contents in periventricular and central WM were consistent ($p = 0.87$), the MTRs of the tissue component s_{short} tended to be smaller in the latter group, but this did not reach significance.

3.3. Biexponential tissue model

Central WM showed an intermediate T_2 component, which was in the range between 150 ms and 410 ms. In MS lesions, both T_2 times were longer than in WM ($p < 0.001$). In addition, s_{int} and the total water content showed a parallel increase and were on average significantly higher than in control WM. Here the intermediate component was on average only slightly less attenuated by MT than the tissue component with short T_2 . In MS lesions, both MTR_{short} and MTR_{int} were significantly smaller than in control WM ($p < 0.004$). The intermediate component was significantly more attenuated by MT than the one with short T_2 ($p = 0.01$). The difference became highly significant ($p = 6.4 \cdot 10^{-7}$) at the focus (TE \sim 300 ms) of the T_2 -weighted MTR_{int} (Fig. 3C). In central WM, ($0.4\% \pm 0.1\%$) a significant prolongation of T_{2int} was observed after MT ($p = 0.0018$). Like the long- T_2 component VOIs of cortical GM, this indicates that in WM

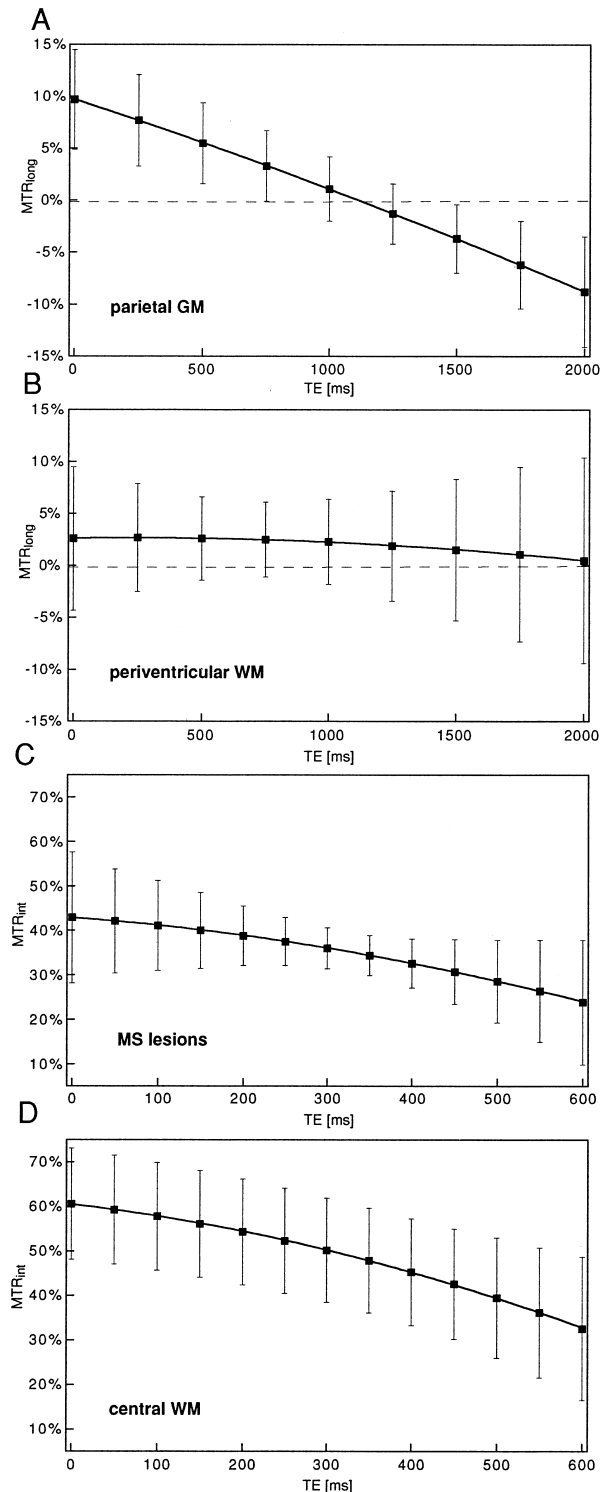


Fig. 4. Mean and SD of the MTR_{long} and MTR_{int} calculated after exponential weighting of the amplitudes (Eq. (4)). The SD is larger at TE = 0 because the component amplitudes are influenced by their T_2 . The TE dependence of the mean MTR was fitted by a second order polynomial yielding $r > 0.999$ in all cases. The tilt is mainly caused by the change in T_2 (negative slope if T_2 increases with MT). Note the qualitatively different behavior in A and B, and in C and D. In GM, the fitted polynomial yielded zero MTR_{long} at TE = 1130 ms.

Table 1
Fitted parameters of models for T_2 relaxation of the localized water signal

	Cortical GM	Periventricular WM	Central WM		MS lesions
No. of VOIs	10	10	13		16
VOI [ml]	11.7 (9.6–13.5)	7.6 (4.8–10.6)	6.1 (3.7–10.0)		3.8 (1.8–7.3)
model	tissue/CSF	tissue/CSF	tissue/CSF	tissue/tissue	tissue/tissue
$T_{2\text{long}}$ [ms]	961 ± 239	1736 ± 168	—	—	—
s_{long} [%]	9.5 ± 3.7	13.7 ± 6.2	1.2 ± 0.2	—	—
$T_{2\text{short}}$ [ms]	76.6 ± 1.8	78.4 ± 2.4	80.9 ± 3.0	69.1 ± 3.7	78.6 ± 3.8 ⁺⁺
s_{short} [%]	68.0 ± 3.3	55.3 ± 4.8	63.0 ± 2.2	54.7 ± 6.6	55.4 ± 5.7
$T_{2\text{int}}$ [ms]	—	—	—	186.7 ± 25.3	257.0 ± 52.9 ⁺
s_{int} [%]	—	—	—	11.2 ± 4.9	19.2 ± 5.7 ⁺
$s_{\text{short}} + s_{\text{int}}$ [%]	—	—	64.2 ± 2.2 ¹	65.9 ± 2.8	74.6 ± 3.1 ⁺⁺
β_{MR} [%]	75.2 ± 2.2	64.0 ± 2.4	63.8 ± 2.2	61.3 ± 4.5 ²	68.4 ± 4.2 ⁺

Values given as mean ± SD. Ranges are given in parenthesis.

Significance levels of MS lesions vs. central WM (unpaired heteroscedastic t -test) are marked by ⁺ $p < 0.01$, ⁺⁺ $p < 0.001$.

100% amplitude refers to the T_2 -corrected signal of pure water.

¹ Calculated using s_{long} instead of s_{int} .

² Calculated using s_{int} instead of s_{long} .

this component is not specific with respect to MT. In lesions, however, the changes in $T_{2\text{int}}$ were not significant and $T_{2\text{short}}$ was consistent with and without MT ($p = 0.80$). This indicates a more homogeneous composition of the components regarding MT effects for the lesion group.

4. Discussion

4.1. Methodological aspects

The ‘MR visibility’ of the in vivo water signal depends strongly on TE_{min} , which limits the detection of short- T_2 moieties. Thus, the numerical results for the ‘tissue water content’, β_{MR} , and the ‘total water content’, $s_{\text{short}} + s_{\text{int}}$, in WM are subject to TE_{min} being 30 ms as discussed in [2] and [7]. The difference between 100% and the β_{MR} (or s_{short}

+ s_{int} for the biexponential tissue model) represents the ‘MR invisible’ rest of solid material, bound water responsible for MT, and myelin water. The implications of demyelination for the segmentation, within the biexponential tissue model have been discussed in [7]. Although myelin water could not be fitted to our data, it may systematically contribute to the signal at TE_{min} . Exclusion of the 30 ms data point in central WM yielded reduced s_{int} ($p = 0.042$ two-sided paired t test) and an insignificant prolongation of the relaxation times, and hence better agreement with a three-compartment model [5].

It should be noted, that in the context of MT the term ‘biexponential model of WM’ generally denotes an exchange model of myelin water and a second component of tissue water as introduced by [18]. Since the attenuation of myelin water could not be determined and the temporal behavior of exchange is not known for our pulsed saturation

Table 2
 T_2 times and MT ratios of the attenuated water signal

With MT	Cortical GM	Periventricular WM	Central WM	Central WM	MS lesions
$T_{2\text{long}}$ [ms]	1050 ± 267**	1768 ± 184	—	—	—
MTR_{long} [%]	9.7 ± 4.8	2.6 ± 6.9	27.0 ± 1.1	—	—
$T_{2\text{w}} \text{MTR}_{\text{long}}^1$ [%]	1.1 ± 3.1	2.3 ± 4.1	—	—	—
$T_{2\text{short}}$ [ms]	80.4 ± 2.3**	77.1 ± 5.0	84.0 ± 4.0*	71.4 ± 5.8	79.2 ± 4.8 ⁺⁺
$\text{MTR}_{\text{short}}$ [%]	61.5 ± 0.7	66.4 ± 2.5	64.8 ± 0.5	64.5 ± 1.6	58.3 ± 6.0 ⁺
$T_{2\text{int}}$ [ms]	—	—	—	233.4 ± 39.8*	289.0 ± 44.5 ⁺⁺
MTR_{int} [%]	—	—	—	60.6 ± 12.5	42.9 ± 13.3 ⁺
$T_{2\text{w}} \text{MTR}_{\text{int}}^2$ [%]	—	—	—	52.4 ± 11.8	34.8 ± 5.9 ⁺⁺
MTR_{sum} [%] ³	—	—	—	64.3 ± 0.6	54.6 ± 3.6 ⁺⁺

Values given as means ± SD.

Significance levels for the fitted T_2 (two-sided paired t -test with vs. without MT) are marked by * $p < 0.01$, ** $p < 0.001$.

Significance levels of MS lesions vs. central WM (unpaired heteroscedastic t -test) are marked by ⁺ $p < 0.01$, ⁺⁺ $p < 0.001$.

¹ MTR calculated from amplitudes corrected by $\exp(-1000 \text{ ms}/T_{2\text{long}})$.

² MTR calculated from amplitudes corrected by $\exp(-300 \text{ ms}/T_{2\text{int}})$.

³ MTR_{sum} calculated from $s_{\text{short}} + s_{\text{int}}$.

technique, the MTRs cannot be discussed quantitatively. The purpose of this study, however, was to assess the specificity of T_2 components qualitatively by their MT behavior. Nevertheless, we found that in lesions the intermediate T_2 component was distinctly less attenuated by MT than the component with short- T_2 . Thus, reduced MT may not be generally interpreted as myelin loss, but only if the intermediate component is not increased, i.e., if WM appears normal on T_2 -weighted MRI. This further suggests that the intermediate component has to be taken into account to model MT in multiple sclerosis and edematous WM. A four-component model with T_2 times fixed at 20 ms, 80 ms, 120 ms and 2000 ms has recently been introduced for the evaluation of component specific MT in WM [17]. The systematic deviations we saw for the fit with $T_{2\text{long}} = 2\text{ s}$ (Fig. 3C) support this approach, which constitutes a combination of myelin water and the two models applied in this study. Owing to their ‘single shot’ capability multi-echo sequences (as used in [15–17]) are superior to the time consuming approach of increasing TE in repetitive scans for T_2 relaxation measurements, especially if the sampling of more TE points and the detection of myelin water is desired.

Direct saturation was estimated to be about 2% (1.9% - 2.4%) for the short T_2 's, somewhat smaller for intermediate T_2 (0.84% in WM, 0.58% in lesions), and merely 0.08% for $T_{2\text{long}}$ in GM (0.07% in WM). Apparently, the high B_1 amplitude causes a nutation even outside the measured saturation profile, thus creating transient transverse magnetization that is subject to relaxation. After 40 pulses this accumulated to considerable saturations of about 30% for the short- T_2 components. The intermediate component would appear saturated by 22.0% in WM, and by 15.5% in MS lesions (for $T_1 = 1.89\text{ s}$ taken from [5]). Assuming a T_1 of 3s for CSF, the tiny losses accumulated to 4.3% (GM) and 2.2% (WM). Although these are quite rough estimates, this may be taken as *caveat* that part of the observed MTR is due to direct saturation.

In healthy subjects the parameters of the dominating short- T_2 component - in particular the MTRs - could be determined with rather small coefficients of variation. However, the variation of $\text{MTR}_{\text{short}}$ was much larger in periventricular WM than in GM and in central WM. In addition, the SD of β_{MR} was much larger than in a previous study [11]. This was mainly due to flow related phase errors of the rather large CSF signal, by which the echo amplitudes in some acquisitions were arbitrarily reduced. Contrary to the ventricles, no flow effects were observed in the *sulci*.

In MS lesions and central WM $T_{2\text{long}}$ was only three times longer than $T_{2\text{short}}$, which is at the brink where a reliable detection of the two components is possible. Rapid loss of the signal increases the experimental error of the intermediate component in the biexponential tissue model when compared to the tissue/CSF model, especially when only a few TE points are sampled like in this study. In control WM, the rather large SD of MTR_{int} did hardly improve by T_2 -weighting (Fig. 3D). Hence, the genuine

variation of s_{int} due to low signal was much larger than the influence of $T_{2\text{int}}$. It is worth noting, that the apparent decrease in MTR_{int} at TE-300 ms, by which it became significantly different from $\text{MTR}_{\text{short}}$ ($p = 0.02$), was due to the MT related increase in $T_{2\text{int}}$. This problematic behavior is not unexpected, as s_{int} and $T_{2\text{int}}$ were smaller than in lesion VOIs. In MS lesions, the large standard deviations of the estimated parameters (especially in $T_{2\text{int}}$) may be in part explained by VOI heterogeneity with respect to partial lesion volume, edema and demyelination. In addition, MTR values may have suffered from subject motion between reference and MT experiment as indicated by the strong focus in Fig. 3C.

4.2. Gray and white matter

The linear dimensions of the CSF and tissue sub-spaces are larger than the diffusion radius, even if calculated for 1.2 s, the duration of MT preparation. Exchange with tissue water will hence take place in a layer at the tissue boundary and result in signal contributions of intermediate T_2 . Absence of MT on the CSF component and consistent T_2 times in periventricular WM show that the biexponential model is appropriate to differentiate between tissue and CSF. Since the sub-spaces are compact and clearly separated, exchange at the ventricular boundary may be neglected. In cortical VOIs, however, a more complex geometrical structure is found, and this study shows that the assumption of two separate compartments is not strictly valid. The small but systematic MT effect on the long- T_2 component can be explained by local exchange with the tissue signal or by contributions from an intermediate tissue component showing MT. This is also consistent with the observation that MTR vanishes when weighting the amplitudes with TE = 1100 ms (Fig. 3A). Moreover, a component of 270 ms T_2 was detected in cortical GM by the 32-point relaxation measurement. This component was not seen in periventricular WM, which may indicate structural differences between the WM regions. There were also differences in mean MTR, though hampered by the large error in periventricular WM.

When fitting a biexponential model this intermediate component will contribute to both tissue and CSF, thus increasing $T_{2\text{short}}$ and decreasing $T_{2\text{long}}$. In the example of Fig. 3A the associated error was about 3%, which is small enough to justify the biexponential model for quantification of MR spectra. Higher transverse relaxation rates $1/T_{2\text{long}}$ were observed for smaller fractions of CSF in the VOI (as expressed by s_{long}). The coefficient of linear correlation was 0.96 ($p < 0.001$), although the functional relation is more complicated and probably difficult to model theoretically. The contribution of blood to the intermediate signal remains to be elucidated as well. Accounting for the unknown intermediate component is likely to be more important for smaller voxels than typically used in single volume MR spectroscopy. While its use for segmentation is yet to be

established, it may provide additional structural information, e.g., in cortical atrophy.

4.3. MS lesions and white matter

In long-standing MS lesions the intermediate- T_2 component is associated with widening of the extra-cellular space [19]. The extracellular space resembles CSF in protein content, but embodies meshes of the neuropil matrix. T_1 -hypointense lesions are characterized by severely reduced cellularity, matrix destruction, and myelin loss [20]. Reduction of MTR_{short} when compared to healthy WM may be due to myelin loss in the lesion and surrounding NAWM. The increase in T_{2short} and MR water content have been discussed in the context of ‘acute’ MS lesions where we suggested the use of s_{int} to correct for the increase of the extracellular space [7]. In this study, the absence of contrast enhancement implied that there was no protein evasion of proteins into the extracellular space. Since increased macromolecular content could alter the MT effect, we have chosen ‘chronic’, rather than ‘acute’ MS lesions. In contrast to CSF, however, no statement can be made a priori about MTR_{int} . Comparing 34.5% MTR (the T_2 -weighted estimate) and 15.5% direct saturation leaves on average about 20% MTR due to solid material and exchange. The significantly different MT effects suggest that the different components represent sub-spaces that are large compared to diffusion motion, and may thus be used for segmentation. It should be noted, that the duration of the MT saturation of is much longer as the typical observation time, which is limited by the T_2 decay of the components. Thus, our protocol imposed a rather conservative criterion for exchange in tissue. Of different types of MS lesions, ‘black holes’ were found to have the largest mean diffusivity [21]. This corresponds to a diffusion length of 47 μm for the typical TE of 300 ms and to 94 μm for 1.2s respectively (calculated from the mean diffusivity as measured in [21]). Even in ‘open’ MS lesions this radius comprises a number of cells [19], which explains the moderate MT attenuation of s_{int} . Because cellularity may vary within the lesion, this component should be regarded an average of local contributions. However, on 3D SPGR images most T_1 -hypointense lesions were clearly delineated from NAWM (see Fig. 1C). Consistency of the T_2 times with/out MT demonstrated the absence of inhomogeneous contributions and thus supports the specificity of the T_2 component in WM. Further evidence is drawn from the regression analysis shown in [8], which suggests that s_{int} replaces s_{short} with almost 100% water content.

Validity of the biexponential model in homogeneous WM is doubtful, as mainly indicated by the similarity of the MTRs. In healthy brain tissue the extra-cellular space has a sponge-like structure with narrow intercellular distances that favor diffusion to and exchange across cell membranes. The extra-cellular volume fraction is about 20%, and hence largely underestimated by the average s_{int} . The signal at TE > 300 ms may be due to incomplete averaging in larger

vessels or locally widened spaces, e.g., in vascular cuffs, which in young subjects not yet show on MRI. Assuming primarily different MT properties of the components (as seen in MS lesions), this indicates that both tissue components undergo almost complete exchange during the 1.2 s saturation period. Although elimination of T_2 variation yielded MTR_{int} at TE = 300 ms significantly lower than MTR_{short} , the difference was almost exactly matched by the difference in cumulative direct saturation. The parameters, which could be determined most accurately in WM, were the total water content and the corresponding total MTR (Table 2). Though s_{short} and s_{int} are inversely correlated, they have about the same water content. This is a qualitatively different situation from the replacement shown in [7] and [8], where extrapolation of s_{short} and total water content indicated a water content of s_{int} that is close to 100%.

5. Conclusion

In contrast to ventricular CSF, the relaxation component attributed to CSF in cortical GM contains contributions from an intermediate- T_2 component subject to MT, but this does not devalue its use for segmentation of spectroscopic VOI. Although a second component improved the fit in central white matter, similar MT indicates severe averaging of both components, which can thus be regarded unsuitable for segmentation purposes. In MS lesions, however, the significantly different MT effects suggest that the different components represent sub-spaces that are wide compared to diffusion motion, and may thus be useful for segmentation. With decreasing dimensions and increasing spatial heterogeneity of the anatomic structures, sulcal CSF and MS lesions become more prone to exchange and averaging. Hence, a comparison between regions of different anatomic structures must be judged with caution.

This initial in vivo study may demonstrate the use of combining T_2 measurement with weighting by other mechanisms to study compartmentation and exchange processes. Our results on T_1 -hypointense MS lesions suggest that the intermediate- T_2 component should to be taken into account when interpreting or modeling decreased MT attenuation in MS.

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