Published in final edited form as:

Magn Reson Med. 2016 June ; 75(6): 2455–2463. doi:10.1002/mrm.26208.

B₀-orientation dependent magnetic susceptibility-induced white matter contrast in the human brainstem at 11.7T

Manisha Aggarwal^{1,*}, Yusuke Kageyama², Xu Li³, and Peter C. van Zijl³

¹Russell H. Morgan Department of Radiology and Radiological Science, Division of MR Research, Johns Hopkins University School of Medicine, Baltimore, MD, USA

²Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

³F. M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, USA

Abstract

Purpose—To investigate B₀-field-orientation dependent white matter contrast in the human brainstem based on R₂* and frequency difference (Δf) mapping from gradient-echo (GRE) imaging at 11.7T.

Methods—Multi-echo GRE data were acquired from two fixed human brainstem specimens at multiple orientations with respect to the static B₀-field. The B₀-orientation dependent modulation curves of R₂* and Δf measurements between short- and long- echo time regimes were used to reconstruct maps of 3D white matter orientation vectors. The results were compared with maps from diffusion MRI, susceptibility tensor imaging, and histological staining of the same specimens.

Results—R₂* and Δf maps demonstrated distinct and significant contrast modulation between the corticospinal tract (CST) and transverse pontine fibers (TPF) dependent on B₀-orientation. Interleaved fiber orientations of the CST and TPF could be sensitively resolved based on field-orientation-dependent fitting of the R₂* and Δf measurements. The fitted 3D orientation-vector maps and peak-to-peak amplitude of R₂* and Δf modulation exhibited close correspondence to primary eigenvector and anisotropy maps derived from diffusion MRI. Notably, the amplitude of B₀-orientation dependent R₂* modulation was significantly ($p < 0.005$) higher in CST compared to TPF, while fractional anisotropies were comparable.

Conclusion—Findings demonstrate the potential of B₀-orientation dependent susceptibility-induced R₂* and Δf contrasts to probe tract-specific orientation and microstructure in white matter.

Keywords

magnetic susceptibility; R₂*; frequency difference; anisotropy; white matter; microstructure

* Address correspondence to: Manisha Aggarwal, Ph.D., Johns Hopkins University School of Medicine, 217 Traylor Building, 720 Rutland Avenue, Baltimore, MD 21205, USA, Tel: (+1) 410-955-9483, maggarw2@jhu.edu.

Introduction

MR signal measured from gradient-echo (GRE) experiments combines contributions from the intrinsic spin-spin relaxation rate as well as macroscopic and mesoscopic field inhomogeneities associated with tissue-specific variations in magnetic susceptibility. Phase contrast derived from GRE images, particularly at high-field strengths, has been used to investigate the local magnetic microenvironment of gray and white matter tissue in the brain, e.g., variations in iron or myelin content [1-4]. Recent evidence indicates that GRE-based apparent transverse relaxation rate (R_2^*) and phase measurements both exhibit dependence on the local orientation of white matter fibers relative to the external B_0 -field [5-10]. While the biophysical origins of these effects are not yet completely understood, mechanisms proposed to explain the observed signal properties include anisotropy of magnetic susceptibility [8,11], the generalized Lorentzian approach for microscopic susceptibility inclusions in tissue [12,13], and a theory including both susceptibility anisotropy and structural tissue anisotropy [14].

In white matter, the microstructure of the myelin sheath has been implicated in the frequency shifts and associated phase contrast in GRE-MRI [15,16]. This notion is supported by the apparent loss of gray-white matter contrast observed in phase images of the *ex vivo* mouse brain after dysmyelination [17]. Sophisticated quantitative susceptibility mapping (QSM) methods have been introduced to calculate 3D maps of isotropic tissue susceptibilities from non-local phase data, which show excellent correlation with local tissue properties e.g., iron concentration, in deep gray matter tissue [18-23]. However, the underlying assumption of isotropic magnetic susceptibility does not necessarily hold true for white matter, which exhibits significant differences in derived QSM contrasts across different head orientations [24]. Fitting the anisotropic susceptibility in white matter with a rank-two symmetric tensor using susceptibility tensor imaging (STI) has also been proposed [25-27]. In addition to the orientation-dependence of white matter susceptibility, the evolution of GRE signal phase in white matter does not necessarily exhibit a linear behavior with echo time [7,28]. This latter effect has been attributed in part to sub-voxel contributions from white matter microstructure, particularly the contribution from water trapped in myelin lipid bilayers to signal phase (frequency) in the short-TE regime [29,30]. Recent reports [28,31] have shown that GRE-based frequency difference mapping, which measures the deviation from phase linearity between short- and long-TE regimes, can be used to extract the effects of local tissue microstructure and underlying white matter orientation on GRE signal evolution in a manner independent of non-local phase effects, which scale linearly with time.

Here, we investigate B_0 -orientation dependent R_2^* and signal frequency contrasts in the fixed human brainstem, which has a complex structure with orthogonally-interleaving fiber populations [32]. Quantitative R_2^* and frequency difference maps derived from 3D multi-orientation GRE data at 11.7T were used to probe field-orientation-dependent neuroanatomical contrast. Further, we investigated 3D estimation of voxel-wise white matter orientation maps based on the B_0 -orientation dependent R_2^* and frequency difference modulation curves, and compared the results with maps from diffusion tensor imaging (DTI), STI, and histology to evaluate the structural basis of susceptibility-induced contrasts. Our results reveal distinct tissue contrasts in the brainstem modulated by B_0 -orientation, and

3D estimates of white matter orientation maps that exhibit close correspondence to maps from DTI, and demonstrate the potential to probe tract-specific microstructure in white matter.

Methods

Specimen preparation

Axial slabs (~1.5 cm thick) at the level of the pons were dissected from the left half of two immersion-fixed (10% formalin) adult human brainstems in the teaching collection in the Department of Neurology (obtained from the Maryland State Anatomy Board). Prior to MRI, specimens were transferred to phosphate buffered saline for 24 hrs, and embedded in a thin layer of agarose (4% weight/volume) gel to minimize tissue deformation. For MRI, specimens were placed in 30-mm-diameter tubes and held in place via cotton gauze inserted into the tubes. The tubes were filled with Fomblin® (Solvay Solexis, NJ, USA) to minimize susceptibility distortion near the specimen surface.

MR data acquisition

MR experiments were conducted on an 11.7T vertical-bore NMR spectrometer (Bruker Biospin, MA, USA) with an actively-shielded micro2.5 gradient system (maximum strength=1000 mT/m), using a 30-mm sawtooth RF transceiver coil. Specimens were first positioned with the long-axis of the brainstem aligned parallel to the B_0 -field (denoted for reference as the 0° position). This corresponds to the conventional orientation of the brainstem for human subjects scanned in the supine position on horizontal-bore clinical scanners. 3D data were acquired using a multi-echo GRE sequence with flip angle= 45° , 8 echoes, TE1/inter-echo interval= $2.8/4$ ms, TR=100 ms, and receiver bandwidth 100 kHz. Acquisition time was ~38 min per orientation at an isotropic resolution of $170 \times 170 \times 170$ (μm)³. During imaging, specimen temperature was maintained at 36°C via thermostatically-controlled airflow. Following the first scan, specimens were manually rotated about the anterior-posterior (x -) axis of the brainstem, and data acquired at 11 orientations spanning angles between 0° to 90° relative to B_0 (angular variation about medial-lateral (y -) axis $< \pm 6^\circ$). 7 additional images were acquired with orientations of the brainstem long-axis approximately uniformly distributed over a hemisphere about the B_0 -axis. First and second order shimming was performed after localizer scans at each orientation to minimize field distortions. For reference, co-registered DTI data were acquired at the same resolution with the specimen in the 0° position, using a 3D diffusion-weighted gradient-and-spin-echo (DW-GRASE) sequence [33] with twin-navigator echoes for phase correction (TE/TR= $30/700$ ms, turbo factor/EPI factor= $4/3$, diffusion gradient duration/separation= $3.6/12$ ms, 2 averages). For fitting of diffusion tensors, one minimally diffusion-weighted (b_0) image and six diffusion-encoding directions were acquired with b -value= 2000 s/mm².

Image reconstruction and processing

Real and imaginary data were reconstructed in IDL6.4 (ITT Visual Information Solutions) to generate magnitude and phase images. Prior to 3D Fourier transformation, k -space data were apodized by a 10% trapezoidal function to reduce noise and zero-filled to twice the matrix size. R_2^* maps were computed for each orientation via linear least-squares fitting of the

natural logarithm of multi-echo GRE magnitude data versus TE. Phase maps at odd-numbered echoes were unwrapped using a Laplacian-based unwrapping algorithm [24], and spatially filtered using V-SHARP [19,34] with variable spherical deconvolution kernel size (maximum radius=9 voxels, regularization parameter=0.05) to remove low-frequency background fields. The filtered phase data were normalized by $2\pi\text{TE}$ to yield maps of the frequency shift referenced to the water center frequency. Frequency difference (Δf) maps at each orientation were calculated by taking the difference between the high-pass filtered frequency shift maps from the 1st and 7th echoes (TE=2.8 ms and TE=26.8 ms, respectively). The Δf maps encode the TE-dependent evolution of signal frequency (deviation from linearity of signal phase with TE) at each voxel [7]. Diffusion tensors were reconstructed via multivariate linear fitting in IDL [33] and diagonalized to obtain primary diffusion eigenvector, fractional anisotropy (FA), and direction-encoded color (DEC) maps.

GRE data at multiple specimen orientations were spatially registered to the 0° data space using 6-parameter rigid-body mapping in the automated image registration tool [35] implemented in DiffeoMap (www.mristudio.org), followed by affine registration to minimize residual misalignment. The intensity-based registration was driven by the 3rd echo magnitude images for optimal contrast, and resulting transformation matrices were applied to the R_2^* , frequency shift, and Δf maps using tri-linear interpolation for resampling. The 3D transformation matrices were used to calculate the rotation angles and $\hat{H}_{0i}$ vectors (i.e., unit vector along the applied main magnetic field in the specimen frame of reference at the i^{th} orientation).

Data analysis

The R_2^* and Δf measurements at the sampled specimen orientations were fit voxel-wise to generalized models [31]:

$$R_2^* = A_{R_2^*} g_1(\theta_i) + B_{R_2^*} \quad (1)$$

$$\Delta f = A_{\Delta f} g_2(\theta_i) + B_{\Delta f} \quad (2)$$

where $\cos \theta_i = |\mathbf{V} \cdot \hat{H}_{0i}|$; θ_i denotes the angle between the local fiber orientation vector $\mathbf{V} = [v_1, v_2, v_3]$ in 3D space (to be estimated) and $\hat{H}_{0i}$; $g_1(\theta)$ and $g_2(\theta)$ in Eqs. [1] and [2] were modeled as $a_1 \sin^2 \theta + a_2 \sin^4 \theta$ and $\sin^2 \theta$, respectively. The $g_2(\theta)$ fitting is based on the assumption that resonance frequency shifts induced by the presence of cylindrically-shaped myelinated nerve fibers exhibit $\sin^2 \theta$ dependence on B_0 -orientation [12,16,30]. The relationship for R_2^* dependence on fiber orientation is relatively less straightforward [6,16,31,36], and was modeled by incorporating a linear combination of $\sin^2 \theta$ and $\sin^4 \theta$ terms in Eq. [1] with $a_1 \sin^2 \theta + a_2 \sin^4 \theta$ normalized to lie in the interval [0,1]. The coefficients A and B represent the peak-to-peak amplitudes of orientation-dependent modulations of R_2^* and Δf and corresponding θ -independent baseline offsets, respectively [31]. 5- (or 6-) parameter Levenberg-Marquardt nonlinear least-squares optimization was used to fit the models independently using MATLAB (Mathworks Inc.) to derive estimates of coefficients A , B , and respective 3D fiber orientation vectors at each voxel based on R_2^*

and f measurements. Similar to DTI, where color maps are scaled by FA, R_2^* and f modulation based fiber orientation maps were scaled by their fitted peak-to-peak amplitudes.

Additionally, the frequency shift data were fit to the six-element susceptibility tensor model [11] at each voxel, by computing the least-squares solution to:

$$\frac{(\Delta B_z)_i}{\mu_0 H_0} = FT^{-1} \left[\frac{1}{3} \hat{H}_{0i} \cdot FT \{ \bar{\chi} \} \cdot \hat{H}_{0i} - \hat{H}_{0i} \cdot \bar{k} \cdot FT \{ \bar{\chi} \} \cdot \hat{H}_{0i} \right] \quad 3$$

in k -space; where $(\Delta B_z)_i$ denotes the measured magnetic field shift at orientation i , μ_0 is the vacuum permeability, k the spatial frequency vector, H_0 the magnitude of the applied magnetic field, $\chi(x0033F)$ denotes the rank-2 susceptibility tensor. B_z in Eq. [3] was calculated from GRE phase data measured at TE=26.8 ms for each orientation. The eigenvalues of the susceptibility tensor (χ_1, χ_2, χ_3), magnetic susceptibility anisotropy (MSA) given by $\chi_{\text{ani}} = \chi_1 - (\chi_2 + \chi_3)/2$ [26,27], and maps of the primary susceptibility eigenvector corresponding to the most paramagnetic eigenvalue were calculated via eigenvalue decomposition of the voxel-wise fitted tensors using MATLAB.

Regions of interest (ROIs) for quantitative analysis were defined using ROEditor (www.mristudio.org), and were based on co-registered DEC maps reconstructed from DTI to delineate specific fiber tracts. Statistical comparisons of R_2^* and f measurements were performed based on Wilcoxon rank-sum tests using IDL6.4.

Histological processing

Following MR experiments, specimens were paraffin-embedded and sectioned serially into 10- μm thick sections. Select alternate slides were stained using Luxol fast blue (LFB, for myelin) and Bielschowsky's silver impregnation method (for labeling axons and neurofibrils) as described previously [37]. Histological slides were imaged under a Zeiss AxioObserver.Z1 microscope (Carl Zeiss Microscopy, NY, USA) using a 5 $\times$ objective.

Results

Fig. 1 shows R_2^* and frequency shift maps for two representative orientations of the brainstem axis (0° and 89°). In the pons (DEC map in Fig. 1a), longitudinal fascicles of the corticospinal tract (CST) interweave orthogonally through medio-laterally running transverse pontine fibers (TPF). At the 0° specimen orientation, CST fibers were oriented parallel to B_0 and exhibited significantly ($p < 0.001$) lower R_2^* values compared to pontine fibers perpendicular to B_0 . At 89° , this contrast was found to be reversed, with a drastic increase ($p < 0.001$) in R_2^* measurements observed in the CST ($R_2^* = 23.1 \pm 3.2 \text{ s}^{-1}$) relative to TPF, which in turn showed a corresponding decrease in R_2^* ($R_2^* = -17.3 \pm 2.2 \text{ s}^{-1}$) compared to the 0° position (Fig. 1b). Frequency shift maps at the 0° specimen orientation revealed negative (diamagnetic) frequency shifts for TPF with respect to the CST, with a reversal of the relative polarity observed at 89° (Fig. 1c). This inversion of contrast between the two fiber groups can be distinctly seen in Fig. 1, which shows specific tracts highlighted at each orientation.

Fig. 2 shows results of frequency difference (Δf) mapping for one representative specimen orientation (long-axis of the brainstem parallel to B_0). The B_0 -field is oriented perpendicular to the plane of the sections for the sampling orientation shown. The Δf maps exhibited local contrast specific to white matter regions (Fig. 2d), which showed significant dependence on fiber orientation relative to B_0 . Comparison with the LFB-stained histological section (Fig. 2e) reveals relatively large frequency differences specific to myelinated fibers perpendicular to B_0 , as evidenced by close correspondence of the resulting Δf contrasts to LFB staining of in-plane myelinated TPF fibers (Fig. 2d-e). Plots of R_2^* and Δf measurements in the CST and TPF as a function of angle between the brainstem axis and B_0 are shown in Fig. 3, and demonstrate distinct orientation-dependent modulation of both R_2^* and Δf contrasts. The relative contrast between the two fiber groups was found to reach extrema at specimen angles close to 0° or 90° with respect to B_0 , with progressive minimization of the contrast observed at angles approaching 45° (Fig. 3a-b). Scatter plots of voxel-wise R_2^* and Δf measurements at acquired specimen orientations versus angle made by the corresponding DTI primary eigenvector with B_0 (Fig. 3c-d) illustrate strong dependences of R_2^* and Δf on local fiber orientation.

Fig. 4 shows representative results of 3D estimation of orientation vector and $A_{R_2^*}$ maps from voxel-wise fitting of the multi-orientation R_2^* data to Eq. [1], along with corresponding FA and DEC maps from DTI. Fitting of the experimental R_2^* measurements to Eq. [1] with $\sin^4\theta$ and sum of $\sin^2\theta$, $\sin^4\theta$ terms yielded adjusted R^2 values that were not significantly different ($p>0.05$ based on the F-statistic in voxels with FA >0.6), indicating that incorporating the $\sin^2\theta$ term did not significantly alter the goodness of fit when accounting for the number of fitting parameters (results of fits shown in Sup. Fig. S1). Regions exhibiting a higher degree of orientation-dependent modulation of R_2^* appear hyperintense in the $A_{R_2^*}$ maps (Fig. 4b). A close correspondence is apparent between contrast in the voxel-wise fitted $A_{R_2^*}$ map and the parametric FA map from DTI (Fig. 4a, b). The fitted $A_{R_2^*}$ values were strongly correlated with FA values ($r=0.65$, $p<0.001$) in voxels with FA >0.2 . Notably, the CST exhibited a significantly ($p<0.005$) higher peak-to-peak amplitude of R_2^* modulation with B_0 -orientation compared to TPF ($A_{R_2^*}$ map in Fig. 4b), which was consistent across both brainstem specimens. The corresponding orientation map estimated from Eq. [1] (Fig. 4b) shows interleaved fiber orientations of CST and TPF that could be sensitively resolved and exhibit close agreement with the DEC map derived from DTI (Fig. 4a-b).

Results from fitting of frequency difference measurements to Eq. [2] based on $\sin^2\theta$ dependency are shown in Fig. 5, which shows $A_{\Delta f}$ and scaled orientation vector maps along with corresponding DTI-derived FA and DEC maps. The fitted orientation map (Fig. 5b) reveals interdigitating orientations of CST and TPF in the pons sensitively delineated based on Δf modulation with B_0 -orientation (Fig. 5, white arrows), which are in close agreement with the respective orientations resolved in the DEC map from DTI (Fig. 5a). Pronounced differences are also apparent, particularly in regions surrounding dense blood vessels (Fig. 5, white asterisks). Fitted $A_{\Delta f}$ values showed a substantial correlation with FA in the mid-pons ($r=-0.41$, $p<0.001$) in voxels with FA >0.2 . The correlation between $A_{\Delta f}$ values and FA was relatively poor ($r=-0.16$) in regions surrounding blood vessels, although these regions exhibited moderately high FA values (0.46 ± 0.13).

Quantitative A_{R2^*} and A_ϕ values for the two specimens are summarized in Table 1, along with FA and MSA values for comparison. ROIs for quantitative measurements in the CST and TPF in Table 1 were confined to voxels with angles of $0^\circ \pm 4^\circ$ and $90^\circ \pm 4^\circ$ between the B_0 -axis and primary eigenvector from co-registered DTI data in the 0° space, respectively, to ensure maximal angular sampling from 0° to 90° for fibers in each ROI at the acquired specimen orientations. This was done to reduce possible bias introduced by sampling orientations in the comparison of A_{R2^*} and A_ϕ measurements. The CST exhibited a significantly ($p < 0.005$) higher peak-to-peak amplitude of R_2^* modulation with B_0 -orientation (A_{R2^*}) compared to TPF for both specimens. In comparison, no significant differences were observed in FA and MSA values between the two white matter groups (Table 1). Fitted A_ϕ values were also slightly higher for CST compared to TPF (Table 1), but this difference was not statistically significant (at the $p < 0.01$ level).

Fig. 6 compares representative maps of FA, A_{R2^*} , A_ϕ and MSA for the same axial slice, and corresponding estimates of 3D vector maps scaled by the respective scalar indices. The STI data shown are from tensor fitting of GRE phase data at $TE = 26.8$ ms. To measure quantitative differences in estimated vector orientations across the maps, the angles made by the respective voxel-wise fitted 3D vectors with the corresponding DTI primary eigenvector were calculated. Mean angular differences were found to be $8.7^\circ \pm 4.8^\circ$ for R_2^* , $14.2^\circ \pm 8.1^\circ$ for A_ϕ , and $18.7^\circ \pm 11.5^\circ$ for STI fitting with respect to the DTI primary eigenvector (for white matter voxels with $FA > 0.6$). Comparison of zoomed-in sections through the mid-pons in an area relatively free of dense blood vessels in Fig. 6i-k demonstrates very fine crossing myelinated fibers resolved in the fitted R_2^* and A_ϕ -based maps, which exhibit close agreement with DTI data (Fig. 6i) and underlying fiber orientations seen with silver-impregnation at the same axial level (Fig. 6l).

Discussion

The effect of white matter microstructure on GRE signal phase and magnitude evolution has been investigated recently [16,31,38]. Here, we found distinct contrast between CST and TPF fibers based on R_2^* modulation with B_0 orientation - with significantly higher A_{R2^*} evident in the CST versus TPF, while no significant difference in FA was observed between these (Fig. 4, Table 1). Interestingly, the fitted θ -independent offset (B_{R2^*}) maps (Sup. Fig. S1) showed negligible contrast between the CST and TPF, although they exhibited clear contrast between gray and white matter regions. Theoretically, the B_{R2^*} maps can be interpreted to represent the rate of signal decay when all fibers are aligned parallel to B_0 (although not anatomically feasible). This finding suggests that the observed contrast between the CST and TPF is entirely encoded in their respective dependencies on B_0 -orientation - which has important implications for probing fiber-specific white matter microstructure, as discussed below.

In our study, the dependence of R_2^* on B_0 orientation could be approximated closely by a $\sin^4 \theta$ modulation. This suggests that for the TE range (0-30.8 ms) used here, signal dephasing in the extra-axonal compartment is in the quadratic regime described in refs. [31,36,39]. Based on simulations, Wharton and Bowtell [31] showed that a pure $\sin^4 \theta$ fit yields a good approximation for R_2^* dependence on B_0 , when contributions to signal decay

from the myelin compartment can be ignored. Based on our observations (Fig. 3) and published estimates of short- T_2 of myelin water component (~ 6 ms at 7T) for *in vivo* human brain [40], this is a reasonable assumption for fixed tissue at 11.7T, where T_2^* is even shorter. However, a precise form of relationship underlying R_2^* dependence on B_0 orientation is likely to depend significantly on sequence parameters, particularly on TE. For instance, a recent theoretical study suggested that signal contributions specific to the myelin sheath are expected to exhibit oscillatory and decaying components [16]. In addition, the transition time from quadratic to linear dephasing regimes for cylindrically-shaped perturbers in white matter is itself dependent on orientation (θ) [36].

As seen from f -maps (Fig. 2), the temporal phase evolution in brainstem white matter was not linear, particularly for fibers at larger angles to the B_0 -field. This observation is consistent with previous findings reported in freshly excised [31] and *in vivo* [28] brain tissues. The specificity of relatively strong frequency differences to white matter fibers aligned perpendicular to B_0 is clearly demonstrated in our data by comparison with histological staining (Fig. 2). Moreover, as shown in Figs. 3 and 5, f -contrast between CST and TPF fibers was found to modulate significantly with B_0 orientation, which could be used to sensitively reconstruct their interdigitating orientations that showed close agreement with primary eigenvector maps from DTI. A significant θ -dependency was not observed in tissue regions surrounding blood vessels in f -maps as compared to R_2^* data (Fig. 6). This effect could possibly stem from time-dependent dephasing associated with magnetic fields induced by the dense network of microscopic blood vessels traversing this region [41].

A possible source of contribution to the relatively higher angular differences (with respect to DTI) observed in f -fitted maps compared to R_2^* data in our study (Fig. 6) can also arise from errors propagated during phase preprocessing, and residual RF transmit phase effects [19] that could not be effectively eliminated owing to substantially non-linear behavior of signal phase and hardware constraints on receiver bandwidth which limited the minimum achievable inter-echo spacing at the acquired resolution. Our results in Fig. 6 also demonstrate marginally higher angular differences observed with STI fitting ($18.7^\circ \pm 11.5^\circ$) as compared to f -fitted maps ($14.2^\circ \pm 8.1^\circ$) when using DTI data as the reference. However, note that instead of using f -maps, STI fits the rank-two susceptibility tensor based on frequency-shift (f) maps, which includes the susceptibility-induced nonlocal frequency shift without consideration of microstructure in its theoretical framework, therefore larger errors in the estimation of fiber orientations are not unexpected [30].

The relatively higher degree of θ -dependent R_2^* modulation for CST compared to TPF suggests that the contrast mechanism can exhibit potential sensitivity to specific features of underlying white matter microstructure. Based on the hollow cylinder model in ref. [7], signal contribution to B_{R2^*} maps is dominated by the T_2 -relaxation constant of axonal and extra-axonal water. Results in Fig. S1 reveal a near absence of contrast between CST and TPF in the fitted B_{R2^*} maps. In comparison, the CST is more prominently highlighted in fitted A_{R2^*} maps compared to the TPF (Figs. 4, S1), and shows relatively higher amplitude of θ -dependent R_2^* modulation (Fig. 3, Table 1). No significant differences in FA between the two fiber-groups are seen (Table 1), suggesting that the orientation-dependent amplitude maps can probe specific aspects of white matter microstructure that are distinct from those

probed using both T_2 -relaxation and diffusion MRI. Possible features that can influence the observed A_{R2^*} and A_f contrasts include fiber volume fraction, myelin g-ratio, and ratio of water to lipid layer thicknesses in the myelin sheath, as suggested by recent studies modeling GRE signal evolution [7,16]. Although details on these geometric parameters for CST and TPF fibers are not available and require more detailed electron microscopy analysis, our data indicate that the A_{R2^*} and A_f measures are potentially sensitive to tract-specific differences in underlying white matter microstructure.

In conclusion, our findings show significant modulation of R_2^* and A_f contrasts in the human brainstem with B_0 -field orientation, and demonstrate 3D estimation of white matter fiber orientations based on the observed dependencies. The relative amplitudes of R_2^* and A_f modulation observed in CST and TPF further suggest that the anisotropic evolution of GRE contrasts can be potentially used to probe local tract-specific white matter microstructure in the human brain. The orientation-dependence and phase non-linearity observed in our study can also have useful implications for interpretation of QSM data in the human brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Barbara Crain in the Department of Pathology for help in obtaining the brainstem specimens. This work was funded by the National Institutes of Health (NIH) grant P41EB015909 and NIH R03 EB017806 (to M.A.).

References

1. Fukunaga M, Li TQ, van Gelderen P, de Zwart JA, Shmueli K, Yao B, Lee J, Maric D, Aronova MA, Zhang G, Leapman RD, Schenck JF, Merkle H, Duyn JH. Layer-specific variation of iron content in cerebral cortex as a source of MRI contrast. *Proc Natl Acad Sci U S A*. 2010; 107(8):3834–3839. [PubMed: 20133720]
2. Lodygensky GA, Marques JP, Maddage R, Perroud E, Sizonenko SV, Huppi PS, Gruetter R. In vivo assessment of myelination by phase imaging at high magnetic field. *Neuroimage*. 2012; 59(3): 1979–1987. [PubMed: 21985911]
3. Shmueli K, de Zwart JA, van Gelderen P, Li TQ, Dodd SJ, Duyn JH. Magnetic susceptibility mapping of brain tissue in vivo using MRI phase data. *Magn Reson Med*. 2009; 62(6):1510–1522. [PubMed: 19859937]
4. Marques JP, Maddage R, Mlynarik V, Gruetter R. On the origin of the MR image phase contrast: an in vivo MR microscopy study of the rat brain at 14.1 T. *Neuroimage*. 2009; 46(2):345–352. [PubMed: 19254768]
5. Denk C, Hernandez Torres E, MacKay A, Rauscher A. The influence of white matter fibre orientation on MR signal phase and decay. *NMR Biomed*. 2011; 24(3):246–252. [PubMed: 21404336]
6. Lee J, van Gelderen P, Kuo LW, Merkle H, Silva AC, Duyn JH. T_2^* -based fiber orientation mapping. *Neuroimage*. 2011; 57(1):225–234. [PubMed: 21549203]
7. Wharton S, Bowtell R. Fiber orientation-dependent white matter contrast in gradient echo MRI. *Proc Natl Acad Sci U S A*. 2012; 109(45):18559–18564. [PubMed: 23091011]

8. Lee J, Shmueli K, Fukunaga M, van Gelderen P, Merkle H, Silva AC, Duyn JH. Sensitivity of MRI resonance frequency to the orientation of brain tissue microstructure. *Proc Natl Acad Sci U S A*. 2010; 107(11):5130–5135. [PubMed: 20202922]
9. Bender B, Klose U. The in vivo influence of white matter fiber orientation towards B(0) on T2* in the human brain. *NMR Biomed*. 2010; 23(9):1071–1076. [PubMed: 20665897]
10. Rudko DA, Klassen LM, de Chickera SN, Gati JS, Dekaban GA, Menon RS. Origins of R2* orientation dependence in gray and white matter. *Proc Natl Acad Sci U S A*. 2014; 111(1):E159–167. [PubMed: 24374633]
11. Liu C. Susceptibility tensor imaging. *Magn Reson Med*. 2010; 63(6):1471–1477. [PubMed: 20512849]
12. He X, Yablonskiy DA. Biophysical mechanisms of phase contrast in gradient echo MRI. *Proc Natl Acad Sci U S A*. 2009; 106(32):13558–13563. [PubMed: 19628691]
13. Luo J, He X, Yablonskiy DA. Magnetic susceptibility induced white matter MR signal frequency shifts—experimental comparison between Lorentzian sphere and generalized Lorentzian approaches. *Magn Reson Med*. 2014; 71(3):1251–1263. [PubMed: 23637001]
14. Yablonskiy DA, Sukstanskii AL. Generalized Lorentzian Tensor Approach (GLTA) as a biophysical background for quantitative susceptibility mapping. *Magn Reson Med*. 2015; 73(2):757–764. [PubMed: 25426775]
15. Lee J, Shmueli K, Kang BT, Yao B, Fukunaga M, van Gelderen P, Palumbo S, Bosetti F, Silva AC, Duyn JH. The contribution of myelin to magnetic susceptibility-weighted contrasts in high-field MRI of the brain. *Neuroimage*. 2012; 59(4):3967–3975. [PubMed: 22056461]
16. Sukstanskii AL, Yablonskiy DA. On the role of neuronal magnetic susceptibility and structure symmetry on gradient echo MR signal formation. *Magn Reson Med*. 2014; 71(1):345–353. [PubMed: 23382087]
17. Liu C, Li W, Johnson GA, Wu B. High-field (9.4 T) MRI of brain dysmyelination by quantitative mapping of magnetic susceptibility. *Neuroimage*. 2011; 56(3):930–938. [PubMed: 21320606]
18. Langkammer C, Schweser F, Krebs N, Deistung A, Goessler W, Scheurer E, Sommer K, Reishofer G, Yen K, Fazekas F, Ropele S, Reichenbach JR. Quantitative susceptibility mapping (QSM) as a means to measure brain iron? A post mortem validation study. *Neuroimage*. 2012; 62(3):1593–1599. [PubMed: 22634862]
19. Schweser F, Deistung A, Lehr BW, Reichenbach JR. Quantitative imaging of intrinsic magnetic tissue properties using MRI signal phase: an approach to in vivo brain iron metabolism? *Neuroimage*. 2011; 54(4):2789–2807. [PubMed: 21040794]
20. Wang Y, Liu T. Quantitative susceptibility mapping (QSM): Decoding MRI data for a tissue magnetic biomarker. *Magn Reson Med*. 2014
21. Lim IA, Faria AV, Li X, Hsu JT, Airan RD, Mori S, van Zijl PC. Human brain atlas for automated region of interest selection in quantitative susceptibility mapping: application to determine iron content in deep gray matter structures. *Neuroimage*. 2013; 82:449–469. [PubMed: 23769915]
22. Liu C, Li W, Tong KA, Yeom KW, Kuzminski S. Susceptibility-weighted imaging and quantitative susceptibility mapping in the brain. *J Magn Reson Imaging*. 2015; 42(1):23–41. [PubMed: 25270052]
23. Bilgic B, Pfefferbaum A, Rohlfing T, Sullivan EV, Adalsteinsson E. MRI estimates of brain iron concentration in normal aging using quantitative susceptibility mapping. *Neuroimage*. 2012; 59(3):2625–2635. [PubMed: 21925274]
24. Li W, Wu B, Liu C. Quantitative susceptibility mapping of human brain reflects spatial variation in tissue composition. *Neuroimage*. 2011; 55(4):1645–1656. [PubMed: 21224002]
25. Wisnieff C, Liu T, Spincemaille P, Wang S, Zhou D, Wang Y. Magnetic susceptibility anisotropy: cylindrical symmetry from macroscopically ordered anisotropic molecules and accuracy of MRI measurements using few orientations. *Neuroimage*. 2013; 70:363–376. [PubMed: 23296181]
26. Li W, Wu B, Avram AV, Liu C. Magnetic susceptibility anisotropy of human brain in vivo and its molecular underpinnings. *Neuroimage*. 2012; 59(3):2088–2097. [PubMed: 22036681]
27. Li X, Vikram DS, Lim IA, Jones CK, Farrell JA, van Zijl PC. Mapping magnetic susceptibility anisotropies of white matter in vivo in the human brain at 7 T. *Neuroimage*. 2012; 62(1):314–330. [PubMed: 22561358]

28. Schweser F, Deistung A, Gullmar D, Atterbury M, Lehr BW, Sommer K, Reichenbach JR. Non-linear evolution of GRE phase as a means to investigate tissue microstructure. *Proceedings of the International Society for Magnetic Resonance in Medicine*. 4527; 19:2011.
29. Sati P, van Gelderen P, Silva AC, Reich DS, Merkle H, de Zwart JA, Duyn JH. Micro-compartment specific T2* relaxation in the brain. *Neuroimage*. 2013; 77:268–278. [PubMed: 23528924]
30. Wharton S, Bowtell R. Effects of white matter microstructure on phase and susceptibility maps. *Magn Reson Med*. 2015; 73(3):1258–1269. [PubMed: 24619643]
31. Wharton S, Bowtell R. Gradient echo based fiber orientation mapping using R2* and frequency difference measurements. *Neuroimage*. 2013; 83:1011–1023. [PubMed: 23906549]
32. Aggarwal M, Zhang J, Pletnikova O, Crain B, Troncoso J, Mori S. Feasibility of creating a high-resolution 3D diffusion tensor imaging based atlas of the human brainstem: a case study at 11.7 T. *Neuroimage*. 2013; 74:117–127. [PubMed: 23384518]
33. Aggarwal M, Mori S, Shimogori T, Blackshaw S, Zhang J. Three-dimensional diffusion tensor microimaging for anatomical characterization of the mouse brain. *Magn Reson Med*. 2010; 64(1): 249–261. [PubMed: 20577980]
34. Wu B, Li W, Guidon A, Liu C. Whole brain susceptibility mapping using compressed sensing. *Magn Reson Med*. 2012; 67(1):137–147. [PubMed: 21671269]
35. Woods RP, Grafton ST, Watson JD, Sicotte NL, Mazziotta JC. Automated image registration: II. Intersubject validation of linear and nonlinear models. *J Comput Assist Tomogr*. 1998; 22(1):153–165. [PubMed: 9448780]
36. Yablonskiy DA, Haacke EM. Theory of NMR signal behavior in magnetically inhomogeneous tissues: the static dephasing regime. *Magn Reson Med*. 1994; 32(6):749–763. [PubMed: 7869897]
37. Aggarwal M, Nauen DW, Troncoso JC, Mori S. Probing region-specific microstructure of human cortical areas using high angular and spatial resolution diffusion MRI. *Neuroimage*. 2015; 105:198–207. [PubMed: 25449747]
38. Chen WC, Foxley S, Miller KL. Detecting microstructural properties of white matter based on compartmentalization of magnetic susceptibility. *Neuroimage*. 2013; 70:1–9. [PubMed: 23268785]
39. Yablonskiy DA. Quantitation of intrinsic magnetic susceptibility-related effects in a tissue matrix. Phantom study. *Magn Reson Med*. 1998; 39(3):417–428. [PubMed: 9498598]
40. van Gelderen P, de Zwart JA, Lee J, Sati P, Reich DS, Duyn JH. Nonexponential T(2) decay in white matter. *Magn Reson Med*. 2012; 67(1):110–117. [PubMed: 21630352]
41. Sukstanskii AL, Yablonskiy DA. Theory of FID NMR signal dephasing induced by mesoscopic magnetic field inhomogeneities in biological systems. *J Magn Reson*. 2001; 151(1):107–117. [PubMed: 11444944]

Figure 1.

B_0 -orientation dependent modulation of R_2^* and frequency shift (f) contrasts in the human brainstem. Axial sections at the level of the pons show comparison of fitted R_2^* (b) and f maps at TE=16.8 ms (c) for two representative orientations of the brainstem axis (0°, 89°) relative to the external B_0 field. The maps reveal a distinct inversion of R_2^* and frequency shift contrasts between the corticospinal tract (CST) and transverse pontine fibers (TPF) at the two orientations, with no apparent change evident in gray matter (GM) areas. The respective fiber orientations of the two white matter groups can be seen in the corresponding direction-encoded colormap derived from DTI on the left (red: anterior-posterior, green: medial-lateral, blue: superior-inferior).

Figure 2.

Phase processing and generation of frequency difference (Δf) maps. From left to right, axial sections through the brainstem illustrate: **a)** the raw phase data, **b)** unwrapped phase after Laplacian unwrapping, **c)** frequency shift (f) map at TE=26.8 ms after SHARP-filtering, **d)** corresponding Δf map calculated from the 1st and 7th echoes ($f_{TE=26.8\text{ ms}} - f_{TE=2.8\text{ ms}}$), **e)** Luxol fast blue (LFB)-stained section at approximately the same axial level as the MRI slice shown in a-d. The B_0 field is oriented perpendicular to the plane of the sections for the sampling orientation shown. Note the relatively large frequency difference apparent in fibers oriented perpendicular to B_0 , as evidenced by comparison with staining of in-plane myelinated transverse pontine fibers (TPF) in the LFB section.

Figure 3.

a-b) R_2^* and frequency difference (Δf) measurements in the CST and TPF white matter groups plotted as a function of rotation of the brainstem axis with respect to the static B_0 -field. Data points are mean measurements over regions-of-interest in the CST (filled circles) and TPF (hollow circles), plotted for 12 sampling orientations with specimen rotation about the x -axis (angular variation about y -axis = 6°). Error bars represent standard deviations over voxels in each ROI. Representative regions for the two fiber groups are indicated in the axial T_2 -weighted section on the left. **c-d)** Scatter plots of voxel-wise R_2^* and Δf measurements from multiple specimen orientations plotted against angle measured between the B_0 -axis and the corresponding primary diffusion eigenvector from co-registered DTI data. Data points plotted are measured R_2^* and Δf values at single voxels in the CST (blue) and TPF (orange), from 3D GRE data at 12 specimen orientations with respect to the B_0 -axis.

Figure 4.

Estimation of white matter orientation maps based on B_0 -orientation-dependent R_2^* modulation in the brainstem. **a)** Representative axial slice showing DEC and FA maps[†] reconstructed from DTI, **b)** Corresponding orientation vector map and peak-to-peak amplitude of R_2^* modulation (A_{R2^*} map) from voxel-wise fits of the R_2^* data at multiple specimen orientations to Eq. [1]. 3D vector components estimated in Eq. [1] are scaled by the fitted A_{R2^*} parameter at each voxel to generate the orientation map. Red, green, and blue represent orientations along anterior-posterior, medial-lateral, and superior-inferior axes, respectively, in both (a) and (b). [†]Note that the relative smoothness of the DTI maps in (a) is due to the wider point-spread-function of 3D-GRASE acquisition along the phase encoding directions, as discussed in ref. [33].

Figure 5.

Estimation of fiber orientation maps of the brainstem based on frequency difference (Δf) mapping at multiple specimen orientations with respect to B_0 . DEC and FA maps from DTI are shown in a coronal view through the pons in the left panel (a-a'). Corresponding fitted orientation vector and Δf amplitude parameter ($A_{\Delta f}$) maps from fitting of the 3D-GRE phase data at sampled orientations to Eq. [2] are shown in the right panel (b-b'). White arrows indicate interdigitating fiber orientations of the CST and TPF resolved in the fitted Δf orientation and DEC maps. Asterisks indicate regions containing dense blood vessels, where apparent differences are discernible between the DTI and fitted Δf maps. Red, blue, and green represent anterior-posterior, superior-inferior, and medial-lateral orientations, respectively, in (a) and (b).

Figure 6.

Comparison of white matter orientation maps estimated from DTI, B_0 -orientation dependent modulations of R_2^* and Δf , and fits using the susceptibility tensor model (STI). Color-coded orientation maps (a-d) and corresponding FA, $A_{R_2^*}$, $A_{\Delta f}$ and MSA parametric maps (e-h) are shown in the same axial slice through the brainstem. Red, blue, and green represent vector orientations as indicated by the color index. White asterisks (in c, d) denote tissue regions containing dense blood vessels. i-k) Zoomed-in views of the region indicated by the white box in panel (a) demonstrate very fine crossing fiber structures in the mid-pons resolved in the DTI data (i) and in corresponding results from voxel-wise fits of the experimental R_2^* and Δf data to Eqs. [1] and [2], respectively (j, k). l) Corresponding silver-stained section at the same axial level as the panels in (i-k) is shown for comparison. Scale bar = 1.5 mm.

Table 1
FA, fitted AR2*, A_f and MSA values for the two brainstem specimens

ROI	Specimen A					Specimen B				
	FA	AR2* (s ⁻¹)	A _f (Hz)	MSA (ppm)	FA	AR2* (s ⁻¹)	A _f (Hz)	MSA (ppm)	FA	MSA (ppm)
CST	0.69 ± 0.05	23.3 ± 2.1 *	-4.42 ± 0.81	0.019 ± 0.007	0.71 ± 0.06	24.2 ± 1.5 *	-5.16 ± 0.83	0.027 ± 0.008		
TPF	0.67 ± 0.04	17.5 ± 1.8 *	-3.67 ± 0.52	0.017 ± 0.006	0.72 ± 0.04	16.6 ± 1.1 *	-4.48 ± 0.63	0.025 ± 0.006		
GM	0.06 ± 0.01	3.3 ± 0.8	-0.43 ± 0.18	0.003 ± 0.001	0.11 ± 0.02	2.5 ± 0.2	-0.98 ± 0.62	0.006 ± 0.001		

Values shown are mean ± standard deviation of measurements over ROIs in the CST, TPF, and gray matter nuclei (GM). ROIs in the CST (and TPF) were defined based on co-registered DEC maps, and confined to voxels with primary eigenvector orientations parallel (and perpendicular) to the B0-axis at the 0° specimen position, to ascertain maximal angular sampling of fiber orientations relative to B0 for each white matter group (angular variation within each ROI ±4°). GM ROIs were defined in pontine nuclei delineated in DTI trace maps. MSA values listed are from STI fitting of the phase data at TE=26.8 ms.

* denotes statistically (p<0.005) significant differences in the fitted AR2* values from Eq. [1] observed between the CST and TPF white matter groups.