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Magnetic Susceptibility Induced White Matter MR Signal Frequency Shifts - Experimental Comparison between Lorentzian Sphere and Generalized Lorentzian Approaches

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Abstract

Purpose—The nature of the remarkable phase contrast in high field gradient echo MRI studies of human brain is a subject of intense debates. The Generalized Lorentzian Approach (GLA) (He & Yablonskiy, PNAS 2009;106:13558) provides an explanation for the anisotropy of phase contrast, the near absence of phase contrast between WM and CSF, and changes of phase contrast in multiple sclerosis. In this study we experimentally validate the GLA.

Theory and Methods—The GLA suggests that the local contribution to frequency shifts in WM does not depend on the average tissue magnetic susceptibility (as suggested by Lorentzian sphere approximation), but on the distribution and symmetry of magnetic susceptibility inclusions at the cellular level. We use *ex vivo* rat optic nerve as a model system of highly organized cellular structure containing longitudinally arranged myelin and neurofilaments. The nerve's cylindrical shape allowed accurate measurement of its magnetic susceptibility and local frequency shifts.

Results—We found that the volume magnetic susceptibility difference between nerve and water is -0.116ppm , and the magnetic susceptibilities of longitudinal components are -0.043ppm in fresh nerve, and -0.020ppm in fixed nerve.

Conclusion—The frequency shift observed in the optic nerve as a representative of WM is consistent with GLA but inconsistent with Lorentzian sphere approximation.

Keywords

Phase contrast; Generalized Lorentzian Approach; optic nerve; magnetic susceptibility; quantitative susceptibility mapping

Introduction

Phase MR images obtained by gradient-recalled echo protocols provide greatly enhanced contrast in the brain at high magnetic fields (1–5). This contrast, which is distinct from that obtained with conventional T1-weighted and T2-weighted images, allows visualization of biological structures within gray matter (GM) and white matter (WM). However, the

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biophysical origin(s) of the phase (frequency) contrast is not well understood and have been investigated from different angles: (i) susceptibility effects induced by differing tissue chemical composition, specifically including differences in iron (6–8), deoxyhemoglobin (9, 10), protein (11, 12), and myelin (13, 14) content; (ii) magnetization exchange effects between “free” water and macromolecules (2, 15, 16); (iii) anisotropy of tissue microstructure (17, 18) and (iv) possible anisotropy of tissue magnetic susceptibility (19, 20).

A role for myelin was suggested by a report demonstrating that demyelination leads to a loss of phase contrast between WM, a myelin rich structure, and GM (13, 14). Indeed, it has also been shown that the phase contrast between WM and cortex can be principally attributed to variations in myelin content (21). However, in contradistinction, phase contrast is very small between WM and cerebrospinal fluid (CSF), where myelin is essentially absent (1, 17).

To explain these curious phenomena, He and Yablonskiy (17) introduced a new theoretical concept called the Generalized Lorentzian Approach (GLA). An important insight from this conceptual framework is that the local contribution to the MRI signal phase does not directly depend on the bulk magnetic susceptibility of the tissue, but on the “magnetic micro-architecture” of the tissue – i.e., the distribution of magnetic susceptibility inclusions (lipids, proteins, iron, etc.) at the cellular and sub-cellular levels. This theory provides an explanation why phase contrast is essentially absent between WM and CSF and provided a conceptual platform for quantitative interpretation of data from MR phase imaging of white matter diseases (22).

In this paper we compare two theoretical approaches that are used for analyzing phase data - a Lorentzian Sphere approach used in most publications and a generalized Lorentzian approach. The goal of this manuscript is to validate the concept of GLA by using *ex vivo* rat optic nerve as a model system of pure white matter. The simple geometry of isolated optic nerve provides a well-defined shape (circular cylinder) that minimizes distortions from global magnetic field inhomogeneity and contamination from neighboring tissues. Preliminary results of this paper were reported in (23).

Theory of Susceptibility Induced MR Frequency Shifts

In liquid samples, it is usually assumed that the magnetic susceptibility induced frequency shift Δf can be expressed as a sum of two terms (24). The first term describes the effects of the sample's global shape by means of a shape factor (SF):

$$\left. \frac{\Delta f_e}{f_0} \right|_{shape} = SF \cdot \chi, \quad [1]$$

and the second term describes the effects of Lorentzian sphere factor:

$$\frac{\Delta f}{f_0} = \frac{1}{3} \chi. \quad [2]$$

Here f_0 is the base Larmor frequency ($f_0 = \gamma B_0 / 2\pi$) for a nuclide with gyromagnetic ratio γ , and χ is the volume magnetic susceptibility of the sample (sometimes called bulk or average magnetic susceptibility). Since biological tissues are mostly water, it is convenient to reference the frequency of a MR signal in biological tissue to that of pure water. From here on all magnetic susceptibilities used in this paper are volume magnetic susceptibilities referenced to water which is the difference between the volume magnetic susceptibilities of the tissue (water+inclusions) and that of pure water.

The concept of a Lorentzian sphere factor has played an important role in the evaluation of magnetic susceptibility effects on the water MR signal frequency shift δf in biological systems. It originated from the method proposed by Lorentz (25) for calculating a local electric field in a solid system composed of electrically polarized particles, but can also be applied for calculation of local magnetic fields created by a system of magnetically polarized particles – magnetic susceptibility inclusions – in liquid samples. In this approach, the susceptibility inclusions creating magnetic field at the position of the nucleus of interest are considered as point dipoles. Spatially they can be separated into nearby and distant groups by an imaginary *Lorentzian Boundary*. For randomly distributed dipoles, a spherically shaped boundary, surrounding nucleus of interest, is a natural choice reflecting the system spherical symmetry. The radius of Lorentzian sphere should be much bigger than the average distance between dipoles. In this case dipoles outside the Lorentzian boundary can be considered as continuous media while the contribution of dipoles inside the Lorentzian boundary to the MR signal frequency shift cancels out. This is happening because an average magnetic field around point dipole is zero, thus resulting in zero accumulated phases by fast diffusing water molecules (17, 22). Hence, the magnetic susceptibility-induced Larmor frequency shift can be evaluated *as if* the nucleus was moving inside an imaginary hollow sphere in a magnetized media with the volume magnetic susceptibility χ - Eq. [2]. This concept can also be applied to systems where local uniformity and isotropy, i.e., spherical symmetry, is achieved by averaging over random microscopic configurations (26), e.g. red blood cells (27, 28) (see also detail discussion of blood magnetic properties in a recent review paper (29)).

However, straight application of the Lorentzian Sphere approximation in non-liquid samples (brain tissue in particular) might not be correct under all circumstances. As was suggested in (17), due to the anisotropic and inhomogeneous nature of the brain's cellular structure (especially considering elongated cells, such as axons and dendrites), Eq. [2], should be modified since the susceptibility inclusions cannot be modeled as randomly distributed point magnetic dipoles anymore thus violating system spherical symmetry. Indeed, at the sub-cellular level, protein-rich cytoskeleton fibers, lipid-rich endoplasmic reticulum and cell membranes, as well as iron-rich oligodendrocytes related to myelin, are primarily arranged in a highly anisotropic manner - mainly longitudinally along the axonal direction.

To incorporate the presence of these longitudinal structures into the theory of MRI signal phase contrast, the concept of GLA was introduced (17). Similar to the application of the concept of Lorentzian sphere, a cylindrical shaped Lorentzian boundary consistent with the cylindrical symmetry of longitudinal structures was used to separate the magnetic field felt by the nucleus of interest into nearby and distant components. Since the accumulative phase

of the nucleus of interest moving/diffusing around longitudinal structures within the Lorentzian cylinder is zero (recall that an average magnetic field created by a cylinder outside itself is zero), the contribution of longitudinal structures inside the Lorentzian cylinder to the signal phase cancels out and MR signal can be calculated *as if* the nuclei of interest were moving inside a hollow cylinder embedded in the homogeneous media. For such structures the relationship in Eq. [2] describing the effect of Lorentzian sphere is no longer valid and should be substituted by the expression followed from Generalized Lorentzian Approach (GLA) (17):

$$\frac{\Delta f}{f_0} = \frac{1}{2} \chi_L \cdot \sin^2 \alpha. \quad [3]$$

Here χ_L is a contribution to tissue magnetic susceptibility from longitudinally arranged components and α is the angle between B_0 and the principal axis of the collection of longitudinal structures. This theory was also confirmed by our computer Monte-Carlo simulations in Ref. (22).

Note that both the Lorentzian sphere approach and GLA describe frequency shifts, induced by magnetic susceptibility inclusions in water, assuming that magnetic susceptibility inclusions themselves do not give MR signal (or their weighted contribution to MR signal is negligible). Also note that Eq. [3] does not take into account possible anisotropy of magnetic susceptibility of WM. This will be introduced and discussed later in the paper (see Eq. [12] and discussion thereabouts).

In our experimental setup we position an optic nerve in a liquid-filled NMR tube and conduct MRI experiments with different orientations of NMR tube with respect to the external magnetic field B_0 (see details in the Methods section). The optic nerve is suspended parallel to the NMR tube and the imaging plane is always placed perpendicular to the optic nerve. Given the simple structure of an optic nerve, consisting of a bundle of myelinated axonal fibers, it is straightforward to apply the Generalized Lorentzian concept to our system. Both the NMR tube and the optic nerve can be considered as infinite cylinders because their diameters are much smaller than their lengths. The optic nerve's *total magnetic susceptibility* is the sum $\chi_L + \chi_{iso}$ where χ_{iso} represents a contribution to tissue magnetic susceptibility from isotropic components/structures.

Frequency Shift of MR Signal in Homogeneous Medium Surrounding the Optic Nerve

In the imaging plane, which presents a cross-sectional view of the nerve in the glass tube, the nerve-induced MR signal frequency shift f_e/f_0 in the homogeneous medium outside the nerve can be obtained based on the well-known expression for magnetic field created by a circular cylinder. The result is:

$$\left. \frac{\Delta f_e}{f_0} \right|_{r > r_0} = \frac{1}{2} (\chi_{iso} + \chi_L - \chi_e) \cdot \left(\frac{r_0}{r} \right)^2 \cdot \cos(2\theta) \cdot \sin^2 \alpha. \quad [4]$$

This frequency shift is proportional to the magnetic susceptibility difference between the nerve ($\chi_{iso} + \chi_L$) and the surrounding solution (χ_e , which is also referenced to water).

Moving radially away from the nerve, the frequency shift decays as $1/r^2$, where r is the length of the radial vector $\mathbf{r}$ extending from the center of the nerve to the in-plane coordinate of interest; r_0 is the radius of the nerve. The angular dependence of the pattern of the frequency shift in the plane orthogonal to the principal axis of the nerve (the MRI slice plane) is described by the angle θ between $\mathbf{r}$ and the projection of B_0 onto the slice plane. When the nerve is parallel to B_0 ($\alpha = 0^\circ$), there should be no nerve-induced frequency shifts in the homogeneous medium outside the nerve; whereas, when the nerve is perpendicular to B_0 ($\alpha = 90^\circ$), the nerve-induced frequency shift effects are maximal. For each given r

integrating over all angles θ , the *average nerve-induced frequency shift*, $\left\langle \frac{\Delta f_e}{f_0} \right\rangle$, within the external medium is zero, independent of α .

Frequency Shift of MR Signal Inside the Optic Nerve

When the B_0 field is parallel to the nerve, the global shape factor $SF = 0$ (long cylinder approximation), the frequency shift induced by the longitudinal structure is also equal to

zero, Eq. [3], and only the frequency shift induced by the isotropic component, $\frac{1}{3}(\chi_{iso} - \chi_e)$ per Eq. [2], would contribute to the frequency shift between nerve and surrounding liquid. In the case when B_0 forms an angle with the principal axis of the nerve, the frequency shift

$\left\langle \frac{\Delta f_{i-e}}{f_0} \right\rangle$ between inside the nerve and the homogeneous medium (recall that integral of f_e over θ at each r is zero regardless of α per Eq. [4]) has a contribution from the global

circular cylindrical shape per Eq. [1] with $SF = -\frac{1}{2}\sin^2\alpha$, a Lorentzian contribution from

intra-nerve longitudinal structures which is $\frac{1}{2}\chi_L \cdot \sin^2\alpha$ (Lorentzian cylinder, Eq. [3]), and

from isotropic susceptibility structures $\frac{1}{3}(\chi_{iso} - \chi_e)$ (Lorentzian sphere). The first two terms have opposite signs because the shape factor (SF) reflects magnetic field induced by a “real” magnetized cylinder, and the Lorentzian term represents the magnetic field created by the near environment outside the Lorentzian cavity (17). Including a possible macromolecule-

water exchange (MWE) effect (2, 15, 16), which would not depend on α , the $\left\langle \frac{\Delta f_{i-e}}{f_0} \right\rangle$ between inside the nerve and the homogeneous external medium is:

$$\left\langle \frac{\Delta f_{i-e}}{f_0} \right\rangle = -\frac{1}{2}(\chi_{iso} - \chi_e) \cdot \sin^2\alpha + \frac{1}{3}(\chi_{iso} - \chi_e) + MWE. \quad [5]$$

A very important feature of Eqs. [4] and [5] is the difference in their dependence on magnetic susceptibility: while Eq. [4] depends on the total magnetic susceptibility of the optical nerve ($\chi_{iso} + \chi_L$), Eq. [5] depends only on the isotropic component χ_{iso} (longitudinal structures do not contribute to the frequency shift in circular cylindrical structures) (17). That would not be the case in a traditional assumption of the Lorentzian sphere, where both Eqs. [4] and [5] would depend on the total magnetic susceptibility ($\chi_{iso} + \chi_L$).

Materials and Methods

All procedures were in compliance with the Washington University Institutional Animal Care and Use Committee. Pairs of optic nerves were harvested from three euthanized healthy adult Sprague-Dawley rats. For each subject, one optic nerve was soaked in 1% phosphate buffered saline (PBS), and examined 2~3 hours after death. The other nerve was subsequently fixed with 10% formalin and examined 1~2 days later.

Two pieces of thin, coated copper wires (0.1 mm diameter) were tied the optic nerve at both ends. The total length of the optic nerve is about 1.1 cm, while the distance between the copper wires is 2mm shorter. The wire-nerve-wire structure was threaded through a glass tube (2.97 mm ID, 4.2 mm OD, and 15 cm in length) with two open ends. The free ends of the copper wires were wrapped around both ends of the tube, with the nerve suspended in the middle of the tube (Fig. 1). The tube was filled with either 1% PBS or 10% formalin fixative, and sealed with Parafilm.

MRI Procedures

Experiments were performed on an Agilent/Varian DirectDrive™ 4.7-T MR scanner using a 1.5 cm diameter surface transmit/receive RF coil. Localized shimming employed a STEAM sequence on a $5 \times 5 \times 5 \text{ mm}^3$ voxel, selected at the mid-point of the optic nerve's longitudinal length. Typical linewidth was ~ 8 Hz (range 5 to 12 Hz). Data were acquired using a multi-echo gradient echo sequence, on a 1-mm thick slice with $75 \times 75 \mu\text{m}^2$ in plane resolution. Other pulse sequence parameters were: TR 170 ms, first TE 7.4 ms, echo spacing 13.2 ms, 4 echoes, flip angle 30° , imaging matrix 128×128 , bandwidth 30 kHz, total acquisition time 17 min (50 averages). The tube was first oriented parallel to B_0 and then rotated 6 times in ~15 degree increments until a perpendicular orientation was achieved. Following each rotation: (i) the orientation angle was determined/confirmed from scout images (with resolution of $0.23 \times 0.16 \text{ mm}^2$ in plane, 2-mm slice thickness); (ii) the imaging plane of the multi-echo gradient echo sequence was oriented perpendicular to the optic nerve; and (iii) localized shimming was performed to minimize field distortions. Experiments were repeated for each pair of optic nerves.

Separate NMR experiments of susceptibility and exchange effects were performed to exclude the contribution from external media since the fresh nerve was measured while suspended in 1% PBS solution and fixed nerve was fixed and suspended in formalin. This experiment applied the method for simultaneously determining the susceptibility effect and exchange effect employed in a previous publication (15) and details of experiments can be found there. A scheme employing coaxial tubes was used; the inner tube (2mm outer diameter) was filled with water and 0.5% dioxane; the outer tube (5mm outer diameter) was filled with aqueous solutions containing 0.5% Dioxane and either 1% PBS or 10% formalin (exact same solutions that were used in optic nerve experiments). MR experiments were conducted on a Varian INOVA 500-MHz (11.74-T) vertical bore analytical spectrometer. A separate coaxial tube containing a $\text{D}_2\text{O}/\text{H}_2\text{O}$ mixture was used for shimming (maximizing B_0 homogeneity). Radiation damping was minimized by detuning the receiver coil and employing a reduced filling factor (5 mm sample tube outer diameter placed within an RF coil with greater than 10 mm diameter).

Data processing

Data were processed with MATLAB® software (The MathWorks, Inc., Natick, MA). Eight fold zero-filling was applied to k-space data to increase digital resolution for more accurate estimation of r_0 . The data were then Fourier transformed into the image domain and a Hanning filter applied to reduce Gibbs-ringing and signal leakage.

Frequency maps f were determined from phase maps ϕ corresponding to different gradient echo times TE according to equation $\phi = \phi_0 + 2\pi \cdot f \cdot TE$. Phase unwrapping was performed as described in (30).

Determination of r_0 —Knowledge of r_0 is required to determine the magnetic susceptibility of the optic nerve as per Eq. [4]. To find the center of the optic nerve and subsequently measure r_0 , a map of the image intensity gradient was generated based on the magnitude image $I(x,y)$, Eq. [6]:

$$G(x, y) = \sqrt{[I(x+1, y) - I(x-1, y)]^2 + [I(x, y+1) - I(x, y-1)]^2}, \quad [6]$$

where x and y are voxel coordinates in the imaging plane. Starting from an initial estimate of the nerve's center coordinate (origin), radial rays were traced across and covering the nerve's full circumference at 2° intervals (i.e., 180 rays in total). The coordinates of maximal ray intensity (maximal image gradient) at each 2° increment outline the first estimate of the edge of the optic nerve. An updated estimate of the nerve's center was found by averaging coordinates of the edge points and the entire process was repeated until convergence was achieved. Finally, r_0 was determined by calculating the average distance from the center to all the edge points.

B_0 inhomogeneity correction—Before determining the susceptibility of the optic nerve based on the frequency maps, B_0 inhomogeneities resulting from imperfect shimming must be accounted for. This was done by expanding the angular dependence of the frequency map in terms of a Fourier series:

$$f(r, \theta) = F_0(r) + \sum_{n=1,2,\dots} \cos(n \cdot \theta) \cdot F_{nc}(r) + \sum_{n=1,2,\dots} \sin(n \cdot \theta) \cdot F_{ns}(r), \quad [7]$$

where θ is the azimuthal angle, and r is the distance from the nerve center. The coefficients in this series can be expressed in a standard manner:

$$F_{ns}(r) = \frac{1}{\pi} \int_0^{2\pi} f(r, \theta) \cdot \sin(n\theta) d\theta, F_{nc}(r) = \frac{1}{\pi} \int_0^{2\pi} f(r, \theta) \cdot \cos(n\theta) d\theta, F_0(r) = \frac{1}{2\pi} \int_0^{2\pi} f(r, \theta) d\theta \quad [8]$$

All coefficients in front of the harmonics can be further expressed as Taylor series:

$$F_n(r) = \sum_{m=0,1,2,\dots} a_{nm} \cdot r^m. \quad [9]$$

The only exception to this expression is the $F_{2c}(r)$ term that is associated with $\cos(2\theta)$ because the field generated by the optic nerve on the surrounding homogeneous medium also contains the term $\cos(2\theta)$, Eq. [4], hence this term should be written as

$$F_{2c}(r)|_{r>r_0} = \sum_{m=0,1,2,\dots} a_{2m} \cdot r^m + \Delta f_c \cdot (r_0/r)^2. \quad [10]$$

f_c here represents the frequency shifts induced by the inner cylinder,

$\frac{\Delta f_c}{f_0} = \frac{1}{2} (\chi_{iso} + \chi_L - \chi_e) \cdot \sin^2 \alpha$. As the dipolar (susceptibility) term is proportional to $1/r^2$, when one is close to the inner cylinder (the minimum point) the local field is mainly affected by this term, while distant from the inner cylinder at larger r the B_0 inhomogeneity plays greater role. The $F(r)$ curves were first calculated by numerical integration of frequency maps for the distant regions ($2r_0 < r < r^*$, where $r^* \sim 3.5r_0$ to make sure that the fitting area is always inside the phantom) according to Eq. [8]. Then coefficients a_{nm} were calculated by fitting Eqs. [9] and [10] to these curves. Finally, these fitting results were used to calculate all terms in Eq. [7] (usually up to second order) for all r and θ . The frequency maps were subsequently corrected by subtracting these terms except for the term $\cos(2\theta) \cdot F_{2c}(r)$.

Evaluation of imaging errors—Computer simulations of the actual MRI experiment were conducted to evaluate errors in parameter estimates possibly arising from imaging protocol itself. All programs for *in silico* data generation and analysis were written in MATLAB®. Imaging protocol parameters in the simulations were set to the experimentally employed parameters. 2D *in silico* imaging data mimicked the cross-sectional slices of two infinitely long co-axial cylinders filled with different homogenous media.

Simulations were performed using Eq.[11] below, both with the nerve parallel to and perpendicular to B_0 . Effects of different data processing procedures, such as zero filling and Hanning filtering were evaluated. Different combinations of spin densities for the media assumed to fill the inner and outer cylinders (inner : outer) were selected as 1 : 2, 2 : 1, 3 : 2 and 2 : 3, bracketing those observed in actual experiments. Finally, the specific TE, image intensity, and b field corresponding to experimental data presented in Results were used to estimate r_0 from simulated data with B_0 parallel to the principal axis of the concentric cylinders. To calculate the “true r_0 ” of the optic nerve, the experimentally measured optic nerve radius was multiplied by the ratio of r_0 used in simulations (the input value) to r_0 estimated from simulated data.

The inner cylinder in the simulations had radius $r_0 = 0.32$ mm, corresponding to the optic nerve, and the outer cylinder had radius $R = 1.87$ mm. The field of view was 9.6×9.6 mm² and the matrix size was 128×128 . Noise was added to obtain SNR of 30 typical to experimental data. The k-space sampling of the object was calculated according to the following equation:

$$S(k_x, k_y) = \iint \rho(x, y) \cdot \exp(-i \cdot 2\pi \cdot (k_x \cdot x + k_y \cdot y) - i \cdot \gamma \cdot b(x, y) \cdot (TE + t)) \cdot dx dy, \quad [11]$$

where TE is the gradient echo time, t is time during readout ($2\pi k_x = -\gamma G_x t$), γ is the gyromagnetic ratio and $b(x,y)$ is the secondary field generated by the susceptibility difference between the different homogeneous medium assumed to fill the inner and outer cylinders (χ_{i-e}). In simulations of a nerve perpendicular to the B_0 , this susceptibility difference was set according to a preliminary measurement: $\chi_{i-e} = -0.117$ ppm. $b(x,y)$ inside the inner cylinder was described by the Lorentzian sphere approach, in this case requiring shape factors for sphere and cylinder, hence $b(x,y) = -(1/6) \cdot \chi_{i-e} \cdot B_0$. The field surrounding the inner cylinder (present in the annulus between the two concentric tubes) is

$b(x,y) = \frac{1}{2} \cdot \Delta\chi_{i-e} \cdot \left(\frac{r_0}{r}\right)^2 \cdot \cos(2\theta) \cdot B_0$. To focus on magnetic susceptibility effects, the global field inhomogeneity was neglected (i.e., set to zero) and the T2 relaxation time constant was assumed to be infinite for both compartments. The integral in Eq. [11] was calculated as a discrete sum over 2048×2048 points, which is 16×16 times greater than the simulated imaging resolution (128×128). The simulated data was processed by exactly the same protocol as experimental data.

Determination of Magnetic Susceptibility—The magnetic susceptibility difference between the optic nerve and the surrounding medium was determined following field inhomogeneity correction using Eq. [10]. Since r_0 is known from measurements described above, fitting $F_{2c}(r)$ against $(r_0/r)^2$ yields the coefficient f_c . Then a fit of f_c to a linear function of $\sin^2\alpha$ yields $(\chi_{iso} + \chi_L - \chi_e)$.

The frequency shift experienced in the interior of the optic nerve vs. average frequency of the surrounding homogeneous medium was also determined after background field inhomogeneity correction. The frequency shift experienced in the interior of the optic nerve was calculated by averaging over the area inside the nerve (an area around the center point covering less than half of the nerve diameter to avoid partial volume effects). A fit to a linear function of $\sin^2\alpha$ gives us $(\chi_{iso} - \chi_e)$.

Results

Computer Simulations

Two examples of the results of the computer simulations are shown in Fig. 2. Limited imaging resolution, partial volume effects, and Gibbs ringing artifacts create uncertainties in determining the inner cylinder radius (see Appendix II Table A1). Moreover, susceptibility-induced distortion of the cylinder is demonstrated clearly in the plot of radius as measured at different angles about the inner cylinder. This results from $b(x,y)$, which adds an additional gradient (although small) to the imaging gradient (31). Since the simulations employed a susceptibility difference similar to that between actual nerve tissue and surrounding medium, it was possible to use results of the simulation to correct (scale) the experimentally determined r_0 (e.g., see Appendix II Table A2).

Figure 3 shows the deviation between the actual field distribution and the field obtained from the profile of phase images. This deviation arises from the finite sampling of the MR signal, as revealed by computer simulations. Major deviations from the input model parameters are seen at the edges of the inner cylinder. However, the central portions and the

“tails” of frequency maps around the inner cylinder more closely follow the ideal phase. In fitting *in silico* data to Eqs. [9] and [10], the data points to be modeled were chosen to be sufficiently far from the edge of the nerve so as to avoid the distortions shown in Fig. 4. For the case of the cylinders oriented perpendicular to B_0 , the frequency shift of the inner

cylinder is: $\frac{\Delta f_{i-e}}{f_0} = -\frac{1}{6}\Delta\chi_{i-e}$. Considering only the central area of the inner cylinder (avoiding partial volume effects) yields $\chi_{i-e} = -0.1192$ ppm, which differs from the input model parameter ($\chi_{i-e} = -0.1167$ ppm) by 2.1 %. Modeling the frequency shifts outside of the inner cylinder yields, Eq. [4], $\chi_{i-e} = -0.1160$ ppm, which differs from the input model parameter by only 0.6%.

Excised Optic Nerve Experiments

Figure 4 demonstrates dependence of the signal phase inside the optic nerve on gradient echo time TE . The data show that the linearity of phase change as function of TE is very good ($R^2 > 0.99$). Thus, the set of echo times used in these experiments is not sensitive to the previously observed non-linear phase behavior (32–36).

Figure 5 shows examples of magnitude and phase images obtained from the optic nerve as well as frequency maps after field inhomogeneity correction. Figure A2 in Appendix shows the effects of zero-filling and Hanning filtering of raw data. Typical SNR for the first echo is around 30 both inside and outside the nerve. (Specifically for the data in Figure A2, SNR in the magnitude image is 32 for the nerve at the first echo and reduces to 12 at the fourth echo due to the signal $T2^*$ decay. SNR for the area outside of the nerve (formalin or PBS solution) is about 32 for all echoes.

B_0 inhomogeneity corrected profiles of the phase images for parallel and perpendicular cases are displayed in Fig. 6, taken through the center of the nerve in both horizontal and vertical directions. The frequency shifts described in Eq. [4] are clearly demonstrated here. When the nerve is parallel to B_0 , it does not induce frequency shift in the surrounding media, whereas when nerve is perpendicular to B_0 , it induces maximal frequency shift to the surrounding that decays as $1/r^2$. In agreement with theoretical prediction, the readout (parallel to B_0) and phase encode (perpendicular to B_0) directions show frequency shift with opposite signs, as $\cos(2\theta)$ changes from 1 to -1 .

Magnetic Susceptibility Measurements

Figure 7 shows the dependence of the frequency shifts on $\sin^2 \alpha$ after accounting for (correcting/removing) B_0 inhomogeneities. The predicted linearity of the data when plotted vs. $\sin^2 \alpha$ is clearly and definitively observed. Table 1 provides parameter estimates for each individual optic nerve. The Lorentzian sphere approach predicts equal slope magnitudes in plots of f_{i-e}/f_0 vs. $\sin^2 \alpha$ and f_c/f_0 vs. $\sin^2 \alpha$. The results presented herein are clearly not in agreement with this prediction. Hence, alternate approaches such as GLA should be applied to explain our observations. The model parameters estimated from the fresh nerves – where

$\frac{1}{2}(\chi_{iso} + \chi_L - \chi_e) = -0.55 (\pm 0.06)$ ppm and $\frac{1}{2}(\chi_{iso} - \chi_e) = -0.29 (\pm 0.02)$ ppm – are $(\chi_{iso} - \chi_e) = -0.044 (\pm 0.004)$ ppm and $\chi_L = -0.043 (\pm 0.009)$ ppm. Mean values of the

estimated model parameters from three data sets representing both fresh and fixed nerves are given in Table 2. Interestingly, the magnetic susceptibility of longitudinal structures χ_L observed in fixed nerves is substantially smaller compared to fresh nerves. Also note that the bulk susceptibility difference between nerve and surrounding media is consistently different ($\sim 20\%$) comparing fresh nerve and fixed nerve. This difference can be attributed to the difference in experimental conditions – fixed nerves were studied in 10% formalin solution while fresh nerves were studied in 1% PBS solution. To account for these differences, the magnetic properties of formalin and PBS were determined (see Appendix). While there is no measurable susceptibility difference between formalin solution and water, the susceptibility difference between PBS and water is -2.2 ppb.

Finally, the intercept in the plot of f_{i-e}/f_0 vs. $\sin^2 \alpha$ is equal to $\frac{1}{3}(\chi_{iso} - \chi_e) + MWE$, Eq. [5]. This allows estimation of the water-macromolecule exchange effect in the optic nerve. Results indicate that MWE in the fresh nerve is negligible, while MWE in the fixed nerve is 0.038 ± 0.007 ppm. See further comments in the Discussion section below.

Discussion

It is generally assumed that the MR signal frequency shift induced in biological objects due to magnetic susceptibility effects is dependent on the objects bulk/average magnetic susceptibility and shape. However, this is not adequate to explain frequency shifts observed in brain white matter such as a general “darkness” of white matter phase map (22) and the nearly absence of phase contrast between WM - a myelin rich structure and CSF. The GLA (17) proposed that the object's underlying microstructure at the cellular and sub-cellular levels should be included in the model describing the MR signal frequency shift. This is especially important for brain structures such as white matter, which is composed mainly of longitudinally arranged cells (i.e., neurons). Validating this phenomenon in the intact brain is highly challenging because of complicated underlying structure and generally insufficient resolution of MRI experiments. In this paper, the MR signal frequency shift induced by an excised optic nerve, which is a representative of white matter tract, was determined and compared to the predictions of two theoretical concepts – the Lorentzian sphere approach, Eq. [2], and the GLA, Eq. [3]. For the experiments with the optical nerve reported in this paper, the Lorentzian sphere approach predicts equal slope magnitudes in plots of f_{i-e}/f_0 vs. $\sin^2 \alpha$ and f_c/f_0 vs. $\sin^2 \alpha$. The results presented herein are clearly not in agreement with this prediction but are in agreement with the prediction of GLA. The circular cylindrical geometry of isolated optic nerve, a tract of axonal bundles running parallel to each other, provided a well-defined shape that minimized distortions in B_0 and avoided signal contamination from the surrounding medium. These attributes made it possible to accurately measure the magnetic susceptibility of all components of “magnetic microarchitecture” of the optic nerve. For a freshly harvested optic nerve we found that $\chi_{iso} + \chi_L = -0.116 \pm 0.010$ ppm and its longitudinal component $\chi_L = -0.043 \pm 0.009$ ppm. Recall that all these volume susceptibilities are referenced to the magnetic susceptibility of water (-9.035 ppm). We should emphasize that different WM tracts might have different biological structure but from the perspective of their “magnetic micro-architecture” they are not that different – they all are bundles of neurons covered by myelin sheath. Hence, their magnetic

properties should be similar, though quantitative numbers for magnetic susceptibility might be different.

Quantitative measurement of frequency shifts is crucial for this study. Computer simulations generating *in silico* image data were performed to test for bias that could be introduced by imaging protocol and post-processing procedures. As demonstrated in Fig. 3, the transition point from inner cylinder to outer cylinder is greatly affected by imaging resolution and also field inhomogeneities. Similar profiles are also observed in experimental data (Fig. 6). Moreover, image definition of the circular edge of the inner cylinder can be distorted when the cylinder is not parallel to B_0 (Fig. 2h'). Fitting image pixels outside the nerve substantially distant from the transition point ($r > 2r_0$) avoids this artifact and provides an accurate determination of f_c . In our analysis employing the input r_0 and α computer simulated image data, the deviation between the derived magnetic susceptibility and the input model parameter value(s) was only 0.17% confirming the robustness of the imaging protocol and analysis procedures.

With insight from the computer simulations, the r_0 measured from experimental data with cylinder parallel to B_0 (minimal distortions, Eq. [4]) was used as the “true r_0 ” – uncorrected or corrected/scaled – for all data (all α) when calculating susceptibility (Table 2). Finally, although $\sin^2 \alpha$ determined from each time we rotate the tube might not be perfectly accurate, fitting to multiple measurements at 5 to 7 different α s ensures the accuracy of the result. While the frequency shift inside of the nerve is, in principle, independent of r_0 , partial volume effects on the measurement were avoided by evaluating only the area for which $r < r_0 / 2$.

The effects of magnetization exchange might also be a significant factor in determining frequency shifts in biological tissue (2, 15, 16). In the experiments described herein, magnetization exchange would contribute to the frequency shifts separately from the susceptibility effect. As magnetization exchange will not be dependent on the angle formed between nerve tissue and B_0 , it enters Eq. [5] as a constant term. In principle, these experiments should allow determination of the exchange effect, as described in Results. These findings, however, cannot be directly attributed to water-macromolecule exchange inside the optic nerve since data reported in the Appendix show that both formalin and PBS shift the water MR frequency by non-susceptibility mechanisms and the volume fraction of either formalin or PBS that penetrates inside of the nerve is unknown.

A multiple compartment model has been proposed to explain the MR frequency shifts in the brain (32–36). The measurements obtained from voxels inside the optic nerve would need to be reexamined if multiple frequency compartments were found to originate within one voxel. This phenomenon would lead to a non-linear behavior of signal phase as a function of gradient echo time, which does not appear to be significant within the TE range in our data (Fig. 4).

GLA, by taking into account anisotropy of tissue geometry at the cellular level, predicted the anisotropic relationship between the MR signal phase/frequency and the direction of the external magnetic field B_0 (17). This anisotropic relationship exists even if the underlying

tissue magnetic susceptibility would be isotropic. It is in fact important to observe that the lipid bilayers as part of white matter fiber tracts are highly ordered which suggests that the magnetic susceptibility of white matter could be anisotropic (19, 20). In light of the longitudinal geometry of the axonal fibers, it is feasible to assign anisotropic magnetic susceptibility to the above considered longitudinal structures of optic nerve (myelin sheath and neurofilaments). Assuming their cylindrical symmetry we can introduce instead of χ_L two components – axial, $\chi_{L\parallel}$, along the fiber direction and radial, $\chi_{L\perp}$, perpendicular to the fiber direction. Note that χ_{iso} remains isotropic since it describes isotropic components/structures. Incorporating this consideration in the GLA, we arrive at a modification to Eq [4], which now becomes

$$\Delta f_e/f_0 = \frac{1}{2} \cdot (\chi_{iso} + \chi_{L\perp} - \chi_e) \cdot \left(\frac{r_0}{r}\right)^2 \cdot \cos(2\theta) \cdot \sin^2\alpha \quad [12]$$

Subsequently, results reported in Tables 1 and 2 represent radial component, $\chi_{L\perp}$, rather than “total” χ_L . Hence, we obtain $\chi_{L\perp} = -0.043 \pm 0.009$ ppm.

While the axial component $\chi_{L\parallel}$ of the magnetic susceptibility of the longitudinal structures remains “invisible” in our data due to the cylindrical symmetry of the fibers, it could have become “visible” in destroyed optic nerve with randomly distributed tissue debris. Such a process naturally occurs in multiple sclerosis (MS). Numerical simulations (22) demonstrated that in MS lesions where longitudinal structures get destructed, the frequency shifts induced by those inclusions would start to comply with the Lorentzian Sphere approximation. Since phase shifts in brain MS lesions are typically more positive than the surrounding tissue the average magnetic susceptibility $(\chi_{L\parallel} + 2\chi_{L\perp})/3$ should be more ‘paramagnetic’ compared to water. Given that $\chi_{L\perp}$ is negative, this can only happen if $\chi_{L\parallel}$ is positive (more ‘paramagnetic’ compared to water). Moreover, estimates of myelin susceptibility based purely on its chemical component also suggest that its average susceptibility is more ‘paramagnetic’ than water (17).

The above consideration of anisotropic magnetic susceptibility might need further improvement by incorporating the effects of the anisotropic structure of the lipid bilayers (34, 36). This effect was proposed after our paper was already submitted for publication, and is also under further experimental investigation in our laboratory.

An alternative to the GLA approach (19) suggesting that the anisotropy of MR signal phase can be accounted for solely by introducing a magnetic susceptibility tensor in the Lorentzian Sphere approximation described by Eq. [2], does not account for the longitudinal configuration of WM structure and symmetry.

Finally we note that understanding the influence of tissue microstructure on MRI signal formation is crucial for numerous aspects of different quantitative MRI methods including the qBOLD technique that relies on detail analysis of BOLD signal based on multi-compartment tissue structure (11, 32).

Conclusions

The GLA, by taking into account the anisotropy and symmetry of tissue geometry at the cellular level, provides adequate description of the anisotropic behavior of the MR frequency shifts in white matter. Freshly harvested and fixed nerves show quite different results for the longitudinal component of magnetic susceptibility, which suggests that care should be taken when analyzing phase data in fixed tissue and projecting findings to *in vivo* or freshly excised tissues.

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Appendix I: Magnetic Susceptibility and Exchange Effects in External Media

Results

Data are shown in Figure A1.

As the spectra in Fig. A1 show, the MR frequency near 3.6 ppm is identified as dioxane and the peaks near 4.7 ppm are identified as water. Regarding neighboring resonances originating from the same ^1H species, the resonance with greater intensity is from species in the outer tube of the co-axial set, and the lower intensity resonance is from species in the inner tube. Since the dioxane MR frequency is considered reflective only of a susceptibility effect, the frequency shifts between dioxane in the inner and outer tubes reflects the susceptibility deviation of PBS or formalin solutions (here compared to pure water). Formalin did not induce a discernible frequency shift of dioxane resonances, whereas PBS induced a -0.0093 ppm shift compared to pure water. However, the ^1H water MR resonance frequency is affected not only by the susceptibility effect, but also by other effects, e.g., pH, exchange with other labile protons, etc. Figure A1a shows that the principal formalin ^1H resonance has a frequency shift induced by non-susceptibility effects of $+0.050$ ppm. Figure A1b shows that the PBS solution has frequency shifts induced by non-susceptibility effects of -0.013 ppm. These results are important factors to consider when understanding the results of the current manuscript and other such experiments in fixed tissue.

Figure A1.
NMR spectra of formalin (a) and PBS (b) solutions obtained from a double tube experiment. Peaks around 4.7 ppm represent water resonances, peaks around 3.7 ppm (shown also in insets) represent dioxane resonances.

Appendix II: Bias in determination of the optic nerve radius: computer simulated data

Table A1

Computer-simulation-measured r_0 of inner cylinder obtained for different spin densities.

Spin density Ratio (Inner : Outer)	Estimated r_0 (pixels)	Deviation from Input (%)
2 : 1	33.16	-5.3%
3 : 2	32.43	-7.3%
1 : 2	36.15	3.3%
2 : 3	37.06	5.9%

Simulations were performed with $\chi_{i-e} = -0.1167$ ppm and an echo time TE of 20 ms; both Hanning filter and zero filling were applied during data processing. The input r_0 was 35 pixels. We can see that different combinations of spin densities in the inner and outer cylinders affect r_0 estimation differently.

Table A2

Examples of the bias in measured r_0 when coaxial cylinders (inner and outer) are parallel to B_0 and are modeled as having different magnetic susceptibilities.

Spin Density Ratio (Inner : Outer)	Fresh Optic Nerve Mimic (pixels)	Deviation from Input (%)	Fixed Optic Nerve Mimic (pixels)	Deviation from Input (%)
3 : 2	34.16	-2.40%	33.30	-4.86%
1 : 2	34.89	-0.31%	37.38	6.80%

Input r_0 was 35 pixels; $b(x,y)$ inside inner cylinder was assigned based on experimental results.

Top row shows the cases when cylinder spin density ratio inner : outer = 3 : 2, sampled at TE = 7 ms; Bottom row shows the cases when spin density ratio inner : outer = 1 : 2, at TE = 25 ms. The two coaxial cylinders were modeled as having essentially the same frequency shifts as encountered in the actual optic nerve experiments.

Numbers from the Table A2 are used for correction of r_0 measurement in experimental data as described in the Methods section.

Figure A2.

Results of zero-filling and Hanning filtering of raw data from a fixed rat optic nerve. Images shown are obtained while optic nerve is perpendicular to the B_0 direction. Profiles through the center of the optic nerve are displayed. a) Profile directly resulting from IFFT of k-space data. b) Profile after Hanning filter was applied to a) to eliminate Gibbs ringing artifact. c) Profile after eight fold zero filling was applied to b) to increase digital resolution. e) Profile after only eight fold zero filling was applied to a), i.e., no Hanning filter. Magnitude and phase images corresponding to cases a) and c) are displayed in d) and f), respectively.

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Figure 1.
Picture of the experimental setup.

Figure 2.

An illustration of computer simulation results and procedure for determining r_0 of the inner cylinder (optic nerve). Input value for r_0 was 0.328 mm which was equivalent to 35 pixels after 8 times zero filling. Left panel - results without including susceptibility effect in the simulation; Right panel - results with susceptibility effect. a,a' - objects created for simulation; b,b' - original magnitude images obtained from simulated data; c,c' - magnitude images obtained after zero filling and Hanning filter; d,d' - field maps used in simulations; e,e' - original phase images obtained from simulated data; f,f' - phase images obtained after zero filling and Hanning filter; g,g' - gradient maps of the magnitude of the processed image (c,c' respectively); h,h' - plots of the distance from edge points to estimated center of the inner cylinder vs. position of the edge points.

Figure 3.

Example of the profiles of phase images obtained from *in silico* data for B_0 oriented perpendicular to the cylinder axis and along the readout direction. The frequency distribution arising in the simulation resulted from an assumed susceptibility for homogeneous medium (Lorentzian sphere approach) in the inner cylinder of $\chi = -0.117$ ppm. Vertical scale is phase value in radians; horizontal scale is pixel number with origin at the center of inner cylinder. a) Profiles through the center of the inner cylinder along B_0 direction; b) Profiles through the center of the inner cylinder perpendicular to B_0 direction. Solid black lines represent the phase after simulation (processed with zero-filling and Hanning filter), blue dotted lines represent the “ideal” phase result, which is proportional to the input of susceptibility induced b field.

Figure 4.

Representative examples of signal phase evolution inside the optic nerve with echo time at different α . The phase at TE = 0 was set to zero. The solid lines are linear fits. All four data sets show excellent linearity.

Figure 5.

An example of experimental data obtained from an excised fixed rat optic nerve. Upper row - magnitude images, middle row - corresponding frequency maps, and bottom row - frequency maps after B_0 inhomogeneity correction. Maps from left to right show cross-sections of the optic nerve as the nerve forms angle $\alpha = 1, 30, 60, 90$ degrees with B_0 .

Figure 6.

Profiles through the center of the nerve of zero-filled and Hanning-filtered phase images after B_0 inhomogeneity correction. Data is obtained from excised fixed rat optic nerve. Vertical scale is value of phase in radians, horizontal scale is pixel number in the image matrix. a) Profile along readout direction when nerve is nearly parallel to B_0 ; b) profile along phase encode direction when nerve is parallel to B_0 ; c) profile along readout direction (parallel to B_0) when nerve is perpendicular to B_0 ; d) profile along phase encode direction (perpendicular to B_0) when nerve is perpendicular to B_0 .

Figure 7.

Example of experimental data for one fresh nerve and one fixed nerve. Filled circles - experimental data; lines - linear fits against $\sin^2 \alpha$. Panels a) and c) are frequency shifts of inside nerve vs. surrounding media; b) and d) are describing frequency shifts induced outside the nerve by magnetic susceptibility difference between nerve and surrounding media. Error bars on a) and c) are standard deviations of frequencies inside of the nerve region with $r < r_0 / 2$; error bars on b) and d) describe combined errors of r_0 estimation and fitting errors of coefficients in front of $1 / r^2$ in Eq. [10]. The Lorentzian sphere approach predicts equal slope magnitudes between upper and lower plots. The results presented herein are clearly not in agreement with this prediction.

Table 1

Summary of fitting results from three fresh and three fixed optic nerves without r_0 correction.

Fitting Results [ppm]		$\frac{1}{2}(\chi_{iso} + \chi_L - \chi_e)$	R^2	$\frac{1}{2}(\chi_{iso} - \chi_e)$	MWE	R^2
Fresh Optic Nerve	Rat 1	-0.043	0.978	-0.021	-0.003	0.99
	Rat 2	-0.050	0.999	-0.025	0.002	0.98
	Rat 3	-0.040	0.989	-0.023	-0.003	0.91
	Average +/- std	-0.044 ± 0.005		-0.023 ± 0.002	-0.001 ± 0.003	
Fixed Optic Nerve	Rat 1	-0.062	0.997	-0.052	-0.017	0.99
	Rat 2	-0.056	0.998	-0.049	-0.004	0.99
	Rat 3	-0.049	0.995	-0.035	-0.013	0.97
	Average +/- std	-0.056 ± 0.006		-0.045 ± 0.009	-0.011 ± 0.007	

Table 2

Mean $\pm$ standard deviation of estimated volume magnetic susceptibilities referenced to water. (Parameter estimates for individual optic nerves are given in Supplementary Material, Table S2.)

[ppb] r_0 Uncorrected	χ_{iso}	$\chi_L - \chi_e$	χ_L	χ_e	$\chi_{iso} + \chi_L$
Fresh Optic Nerve	-0.088 ± 0.010	-0.046 ± 0.004	-0.043 ± 0.009	-0.028	-0.116 ± 0.010
Fixed Optic Nerve	-0.112 ± 0.013	-0.090 ± 0.018	-0.020 ± 0.007	0	-0.112 ± 0.013
[ppb] r_0 Corrected					
Fresh Optic Nerve	-0.091 ± 0.010	-0.044 ± 0.004	-0.045 ± 0.009	-0.028	-0.119 ± 0.010
Fixed Optic Nerve	-0.106 ± 0.028	-0.090 ± 0.018	-0.015 ± 0.016	0	-0.106 ± 0.028