Journal of Magnetic Resonance 277 (2017) 117–130

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

Mapping axonal density and average diameter using non-monotonic time-dependent gradient-echo MRI

Daniel Nunes [a], Tomás L. Cruz [a], Sune N. Jespersen [b,c], Noam Shemesh [a,*]

[a] Champalimaud Neuroscience Programme, Champalimaud Centre for the Unknown, Av. Brasilia 1400-038, Lisbon, Portugal
[b] Center of Functionally Integrative Neuroscience (CFIN) and MINDLab, Clinical Institute, Aarhus University, Aarhus, Denmark
[c] Department of Physics and Astronomy, Aarhus University, Aarhus, Denmark

ARTICLE INFO

Article history:
Received 3 January 2017
Revised 23 February 2017
Accepted 25 February 2017
Available online 28 February 2017

Keywords:
Axon density
Axon diameter
Susceptibility
Gradient echo
Multi-gradient echo
T2*
Magnetic resonance imaging (MRI)
Ultrahigh field MRI

ABSTRACT

White Matter (WM) microstructures, such as axonal density and average diameter, are crucial to the normal function of the Central Nervous System (CNS) as they are closely related with axonal conduction velocities. Conversely, disruptions of these microstructural features may result in severe neurological deficits, suggesting that their noninvasive mapping could be an important step towards diagnosing and following pathophysiology. Whereas diffusion based MRI methods have been proposed to map these features, they typically entail the application of powerful gradients, which are rarely available in the clinic, or extremely long acquisition schemes to extract information from parameter-intensive models. In this study, we suggest that simple and time-efficient multi-gradient-echo (MGE) MRI can be used to extract the axon density from susceptibility-driven non-monotonic decay in the time-dependent signal. We show, both theoretically and with simulations, that a non-monotonic signal decay will occur for multi-compartmental microstructures – such as axons and extra-axonal spaces, which were here used as a simple model for the microstructure – and that, for axons parallel to the main magnetic field, the axonal density can be extracted. We then experimentally demonstrate in *ex-vivo* rat spinal cords that its different tracts – characterized by different microstructures – can be clearly contrasted using the MGE-derived maps. When the quantitative results are compared against ground-truth histology, they reflect the axonal fraction (though with a bias, as evident from Bland-Altman analysis). As well, the extra-axonal fraction can be estimated. The results suggest that our model is oversimplified, yet at the same time evidencing a potential and usefulness of the approach to map underlying microstructures using a simple and time-efficient MRI sequence. We further show that a simple general-linear-model can predict the average axonal diameters from the four model parameters, and map these average axonal diameters in the spinal cords. While clearly further modelling and theoretical developments are necessary, we conclude that salient WM microstructural features can be extracted from simple, SNR-efficient multi-gradient echo MRI, and that this paves the way towards easier estimation of WM microstructure *in vivo*.

1. Introduction

Axon density, degree of myelination, and the regional size distribution, all play a paramount role in both healthy and diseased Central Nervous System (CNS) function [1]. In normal white matter (WM), axonal conduction velocities are determined by these properties, with larger and more myelinated axons producing higher conduction velocities [2–6], which in turn facilitate rapid information transduction between remote CNS regions [2,4,7–9]. Severe deficits arise from even slight aberrations to the axonal microstructure: changes in the axonal size distribution and myelin structure in optic nerve lead to severe and progressive vision loss [10,11];

Abbreviations: CNS, Central Nervous System; dCST, dorsal corticospinal tract; DTI, Diffusion Tensor Imaging; EPI, Echo Planar Imaging; FG, Fasiculus Gracilis; FC, Fasiculus Cuneatis; FOV, Field of View; GLM, General Linear Model; GLTA, Generalized Lorenzian Tensor Approach; MGE, Multi-Gradient-Echo; MRI, Magnetic Resonance Imaging; NMR, Nuclear Magnetic Resonance; OGSE, Oscillating Gradient Spin-Echo; PBS, Phosphate Buffer Saline; QSI, q-space Imaging; ReST, Reticulospinal tract; RST, Rubrospinal tract; SNR, signal to noise ratio; SAR, Specific Absorption Rate; STT, Spinothalamic tract; TE, echo time; TR, repetition time; VST, Vestibulospinal tract; WM, White Matter.

* Corresponding author.
E-mail address: noam.shemesh@neuro.fchampalimaud.org (N. Shemesh).

http://dx.doi.org/10.1016/j.jmr.2017.02.017
1090-7807/

decreases in axon density (axonal loss) is observed in Multiple-Sclerosis (MS) histology [12], and areas characterized by a specific range of axonal diameters may be more prone to axonal loss [12,13]. Axonal losses, as well as changes in average size and myelination, can be found in the vicinity of MS plaques but also in normal appearing white matter [14], suggesting their involvement in slow and potentially chronic damage to the tissue. Upon trauma, dramatic changes in average axon size, density, and myelination are observed with time [15,16]. Other microstructural abnormalities, such as axonal beading, are thought to be involved in, e.g., stroke [17]. Such changes to the neuronal morphology can be followed by pronounced cognitive impairments [18].

Several Magnetic-Resonance-Imaging (MRI) methods have been proposed for mapping axonal microstructures, and in particular, diffusion weighted imaging was shown to map regional average axon size changes in diseased tissues [19–24]. In normal CNS, Ong et al. have demonstrated that the histologically well-characterized spatial distribution of mean axon diameter in the spinal cord can be mapped noninvasively by q-space imaging (QSI) using a gradient system capable of producing 50 T/m [25,26]. Assaf et al. proposed the AxCaliber model for characterizing variations in the (histologically well-known [27]) axon diameter distributions in the corpus callosum [28] (though note recent concerns [29]), and Duval et al. used AxCaliber on the 300 mT/m gradients of the Connectome Scanner to map spinal cord axonal diameters *in vivo* in humans [30]. Very recently, Xu et al. demonstrated that axon microstructure in the rat spinal cord could be estimated [31] using oscillating gradients spin echo (OGSE) diffusion MRI, showing good correlations between the OGSE-derived maps and histology [31]. Non-uniform oscillating gradients MRI – a technique exhibiting greater sensitivity towards smaller dimensions [32] – was recently shown to contrast the corpus callosum's histologically known [27] five different tracts [33]. When powerful gradients are not available [34] the axon index can be mapped [35], which preserves some of the axon diameter contrast [36]. Other diffusion-based methods use more sophisticated modelling [35,37–43] or pulse sequences [44–49] to extract other features of white matter, such as its underlying orientation dispersion, neurite density, and microscopic anisotropy.

Susceptibility-driven contrasts [50,51] have been gaining increasing attention in recent years, both in terms of anatomical contrasts, and, more recently, in terms of microstructure. Lee et al. showed that phase images arising from gradient-echo data in white matter show a strong orientation dependence with respect to the main magnetic field [52]; Liu introduced susceptibility tensor imaging (STI), which provides information on the absolute orientation of anisotropic systems when samples are rotated with respect to the main magnetic field [53] or when a more elaborate multipole acqusition scheme is employed [54]. Several models have been put forward to describe the biophysical origins of the susceptibility anisotropy [55–66]. T_2^* anisotropy has likewise been emerging as a highly useful contrast for orientation-mapping in WM [67–71], and several models have again been put forward to explain its origins [72–74]. Non-mono-exponential signal decay has been recently observed in WM when gradient echo measurements reached relatively high TEs, suggesting the contributions of multiple microstructural compartments to the signal, and allowing their spatial mapping [73,75]. Chen et al. recently simulated how multiple compartments would impact the full signal decay, finding a pattern in orientation- and TE-dependences of the signal [76], which could potentially be used to map specific compartments within the white matter. Importantly, the new contrasts revealed in these studies were derived from one of MRI's simplest sequences: the MGE, which is time-efficient and typically high in SNR. However, to our knowledge, MGE's potential to actually extract – or even qualitatively contrast – crucial microstructural metrics such as axonal density and average sizes has not been heretofore studied, though an analysis of non-exponential relaxation in the context of microstructural disorder has been presented very recently [77].

In this study, we harness a simplistic model of white matter tissue and its full susceptibility-driven time-dependent signal response, to establish that the axon density can in fact be determined from simple MGE experiments. We then validate our theoretical findings in *ex-vivo* rat spinal cords, demonstrating experimentally that remarkable axon density contrasts can be obtained that highlight the major tracks within the SC, and which correlate with histological findings. We further show that other parameters of the model, such as the susceptibility-driven frequency shift, seem to qualitatively reflect the regional variation in average axon diameters. The potential of time-dependent MGE experiments for characterizing more specific features in WM is discussed.

2. Theory

An object placed in a homogeneous magnetic field $\mathbf{B_0}$, will impart a local shift in the Larmor frequency $\Delta\omega(\mathbf{r})$ of a magnitude proportional to the susceptibility difference $\Delta\chi$ between water and the object. In general, there is a complex relationship between $\Delta\omega(\mathbf{r})$ and $\Delta\chi$ [55–57,61–66] depending on the objects' shape and geometrical arrangement. Here we restrict ourselves to a very simple model of spinal cord white matter as a collection of parallel cylinders representing the axons oriented along the main magnetic field. In this case, the induced magnetic fields inside and outside axons are homogeneous but differ by an amount proportional to $\Delta\chi$. Furthermore, echo times are assumed to be sufficiently large to ignore the signal contribution from myelin water, as we hypothesized that at the ultrahigh field used for these experiments (16.4 T), myelin water is likely to have very short T_2^*. Hence, the magnitude signal can be computed from a sum of two terms: an intra-cylindrical compartment with a volume fraction f_i, arbitrarily selected to be on-resonance, and an extra-cylindrical compartment with a volume fraction $1-f_i$ and frequency shift $\Delta\omega$. Each compartment is assumed to exhibit monoexponential transverse relaxation with relaxation rates $R_{2i}=1/T_{2i}$ and $R_{2e}=1/T_{2e}$, corresponding to intra- and extra-axonal relaxation rates, respectively. Hence, the magnitude signal can be written as:

$$S(TE)=S_0\left|f_i e^{-\frac{TE}{T_{2i}}}+(1-f_i)e^{-TE\left(\frac{1}{T_{2e}}+i\Delta\omega\right)}\right| = S_0*\sqrt{f_i^2 e^{-2TE*R_{2i}^*}+2f_i(1-f_i)*e^{-TE\left(R_{2i}^*+R_{2e}^*\right)}*\cos(\Delta\omega*TE)+(1-f_i)^2 e^{-2TE*R_{2e}^*}} \quad (1)$$

Eq. (1), though extremely simple, is central in this study, because it predicts a non-monotonic and oscillatory signal decay, from which the four model parameters can be extracted. This obviates the need to extract the signal phases in every TE, which may involve quite elaborate signal processing, yet still obtain the information contained within the model.

In reality, we expect small deviations from axonal misalignment with the main magnetic field, which will induce inhomogeneous magnetic field in the compartments. Violations of axons as simple cylinders will have similar implications, and such effects will lead to a more complicated time-dependent phase shift between the compartments, as well as non-exponential T_2^* decay in each of the compartments. Nevertheless, here we assume these deviations to be sufficiently small and hence restrict ourselves to exploring