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A Revised Model for Estimating g-ratio from MRI

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Abstract

A key measure of white matter health is the g-ratio, which is defined as the ratio between the inner axon radius and the outer, myelinated, axon radius. Recent methods have been proposed to measure the g-ratio non-invasively using the relationship between two magnetic resonance imaging (MRI) measures. While this relationship is intuitive, it predicates on the simplifying assumption that g-ratio is constant across axons. Here, we extend the model to account for a distribution of g-ratio values within an imaging voxel, and evaluate this model with quantitative histology from normal and hypomyelinated mouse brains.

Keywords

g-ratio; myelin; magnetic resonance imaging; histology

1. INTRODUCTION

Myelin is a critical component of white matter, increasing speed of action potential conduction along axons and improving neurological function. It has been shown that there is a range of values between axon size and myelin thickness for optimal efficiency in healthy tissue (Chomiak and Hu, 2009; Rushton, 1951). The g-ratio describes the relationship between axon size and myelin thickness, and deviations in the g-ratio are thought to be involved in abnormal development and disease (Albert et al., 2007; Fields, 2008; Mason and Langaman, 2001; Paus and Toro, 2009). However, currently, the only way to assess properties of tissue microstructure such as axon diameter, myelin thickness, and g-ratio is through quantitative histology, such as electron microscopy. Such methods are time consuming, expensive, and destructive to the tissue. Magnetic resonance imaging (MRI)

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methods to measure these microstructural characteristics would be useful to more efficiently study white matter disease processes and treatments and, further, provide the potential for *in vivo* assessment.

Recently, it has been proposed that two quantitative MRI measures can be combined and interpreted with a geometric model of white matter to provide quantitative estimates of the g-ratio (Stikov et al., 2015, 2011). Specifically, Stikov and colleagues have suggested that using MRI estimates of 1) myelin volume fraction (from, for example, quantitative magnetization transfer measurements), and 2) axon or fiber volume fraction (from, for example, suitable analysis of diffusion-weighted imaging) can be used to estimate the g-ratio. These estimates were termed an “aggregate g-ratio” because the method is predicated on the assumption that the g-ratio is constant for all axons within a voxel, which is known not to be the case in both peripheral nerve (Friede and Beuche, 1985; Rushton, 1951) and central white matter (Berthold et al., 1983; Little and Heath, 1994). Here we extend their model to a more general one that makes no assumption about the distribution of g-ratio values within an imaging voxel, and we demonstrate the model in principal using quantitative evaluations of electron microscopy of the corpus callosum of control and hypomyelinated mice.

2. THEORY

Consider an ensemble of N myelinated fibers with the radius and g-ratio of the i^{th} fiber being R_i and g_i , respectively, and axon radius being r_i (hence, $g_i = r_i/R_i$, see Fig 1e). The total cross-sectional areas of fibers, axons, and myelin, are, respectively,

$$A_F = \pi \sum_{i=1}^N R_i^2$$

$$A_A = \pi \sum_{i=1}^N g_i^2 R_i^2 = \pi \sum_{i=1}^N r_i^2. \quad [1]$$

$$A_M = \pi \sum_{i=1}^N R_i^2 (1 - g_i^2)$$

From here, the ratio of myelin to fiber areas is

$$\frac{A_M}{A_F} = \frac{\sum_{i=1}^N R_i^2 (1 - g_i^2)}{\sum_{i=1}^N R_i^2}. \quad [2]$$

In the case that $g_i = g$ for all $i = 1$ to N , as assumed in the model presented by Stikov et al. (Stikov et al., 2015, 2011), this ratio reduces to

$$\frac{A_M}{A_F} = 1 - g^2. \quad [3]$$

Assuming one can measure myelin volume fraction (*MVF*) and fiber volume fraction (*FVF*) with MRI, the ratio A_M/A_F can be replaced with MVF/FVF , and the resulting MRI-measured g-ratio is then

$$g_{\text{MRI}} \equiv \sqrt{1 - MVF/FVF}, \quad [4]$$

as previously presented (Stikov et al., 2015, 2011).

By starting with the ratio A_M/A_A , a similar relationship is found,

$g_{\text{MRI}} \equiv \sqrt{1/(1 + MVF/AVF)}$, where *AVF* is the axon volume fraction as measured by MRI.

However, without the simplifying assumption that *g* is constant for all axons, Eq [2] can be simply reduced to

$$\frac{A_M}{A_F} = \frac{\sum_{i=1}^N R_i^2 - \sum_{i=1}^N g_i^2 R_i^2}{\sum_{i=1}^N R_i^2} = 1 - \frac{\sum_{i=1}^N g_i^2 R_i^2}{\sum_{i=1}^N R_i^2}. \quad [5]$$

Again, replacing the A_M/A_F with MVF/FVF and using Eq [4], we get

$$g_{\text{MRI}}^2 = \frac{\sum_{i=1}^N g_i^2 R_i^2}{\sum_{i=1}^N R_i^2}, \quad [6]$$

which shows that the squared value of the previously proposed MRI measure of g-ratio is equal to the area-weighted mean of g^2 values across the *N* fibers.

3. MATERIALS AND METHODS

3.1 Tissue Preparation

Animal studies were approved by the Vanderbilt University Institutional Animal Care and Use Committee. Histology was acquired from control and *Rictor* conditional knockout (CKO) mice, similar to a previously described mouse model of tuberous sclerosis complex (Carson et al., 2013). Six adult mice were anesthetized with isoflurane and sacrificed via transcardial perfusion of 1× phosphate-buffered saline (PBS) wash followed by 2.5% glutaraldehyde + 2% paraformaldehyde in PBS (modified Karnovsky solution). Following perfusion, brains were quickly removed from the skull and immersed in the fixative solution for 1 week. For MRI studies not presented here, the perfusion and immersion solutions included a paramagnetic MRI contrast agent and the fixative was washed out of brains prior

to imaging and subsequent histology. For histologic preparation, a 1–2 mm sagittal slice of tissue was cut from the left hemisphere beginning at the mid-brain from each of 6 brains (n=4 control and n=2 CKO). Subsequently, 2 regions of white matter from the corpus callosum (genu- GCC and midbody - MidCC) were cut from each slice. Two regions were analyzed to account for potentially different axon populations between regions of the corpus callosum (Barazany et al., 2009). Tissue samples were then processed for transmission electron microscopy in the Vanderbilt Cell Imaging Shared Resource-Research Electron Microscopy facility. Thick sections (0.5 – 1 μm) were collected using a Leica Ultracut microtome (UC-7), then stained with 1% toluidine blue. Ultra-thin sections (70–80 nm) were then cut and collected on 300-mesh copper grids. Copper grids were post-section stained at room temperature with 2% uranyl acetate (aqueous) for 15 minutes and then with lead citrate for 10 minutes. Ultra-thin sections were imaged on the Philips/FEI Tecnai T12 electron microscope at 15,000 $\times$ magnification. From each section, six images were acquired using a side-mounted AMT CCD camera, resulting in a total of 6 mice $\times$ 2 regions $\times$ 6 images/region/mouse = 72 images.

3.2 Data Analysis

The pipeline of histology analysis is summarized in Fig 1. Images were segmented using the histogram of pixel gray scale values, defining the threshold between myelin and non-myelin pixels at the nadir. This provided a binary image where myelin = 1 and non-myelin = 0 (Fig 1c) and an estimate of *MVF*. From the binary image, each myelinated axon was manually identified and its area (A_{Ai} , for the i^{th} axon) was computed using a region growing

algorithm. This value provided an estimate of axon radius $r_i = \sqrt{A_{Ai}/\pi}$, and the sum of all axon areas provided an estimate of *AVF*. For each axon, the thickness of the surrounding myelin (δ_i) was calculated as the average of manual measurements made in two locations, and the g-ratio was estimated as $g_i = r_i/(r_i + \delta_i)$.

From each image, the *MVF* and *AVF* estimates were used to compute g_{MRI} , as shown above. Also from each image, the N (~50) measures of g were fitted to a gamma distribution as done and observed previously for axon diameter distributions (Assaf et al. 2008, Barazany et al., 2009; Olivares et al., 2001), from which three descriptive measures were calculated: arithmetic mean (g_{mean}), the area-weighted mean (g_{awm}), the square-root of the area-weighted g^2 (g_{awmg} , following the right hand side of Eq [6]). Calculating these descriptive measures of g from the fitted gamma distribution parameters rather than directly from the samples g_i , $i = 1$ to N , reduced the influence of one or two large axons amongst a relatively small sampling of the population.

4. RESULTS AND DISCUSSION

Figure 1f displays a representative histogram of the g-ratios obtained from one histology image. Each characterization of the g-ratio (g_{mean} , g_{awm} , g_{awmg} , and g_{MRI}) is displayed on the histogram. It is apparent that g_{mean} (~0.8) is significantly lower than the area-weighted means and g_{MRI} . This characteristic will be true in general for distributions of finite width, but will also depend on the skewness of the distribution. For further information on the histology results, including statistical evaluations of fitting axon diameters and g-ratios to

gamma distributions, refer to our Data in Brief article, Quantitative Analysis of Mouse Corpus Callosum from Electron Microscopy Images.

Figure 2 displays comparisons of g_{MRI} with g_{mean} , g_{awm} , and g_{awmgs} , respectively, from left to right. Each point represents the measurements from one image with both MidCC and GCC measures displayed (control = black, CKO = red). The hypomyelination present in the CKO mice is apparent from the generally greater g-ratios. Qualitatively, g_{MRI} shows a reasonable correspondence with all three measures, but the comparisons between g_{MRI} and the area-weighted measures are noticeably closer to the line of identity (dashed line). Quantitatively, these differences are reflected in the root mean squared difference (RMSD), between the two measures for each plot.

Similar relationships are found between g_{MRI} and the two area-weighted measures, g_{awm} and g_{awmgs} . While theory shows that $g_{MRI} = g_{awmgs}$ for circular geometries, the two experimental measures will deviate for elliptical or other non-circular axonal shapes. Nonetheless, the close correspondence of g_{MRI} and g_{awmgs} in Fig 2 indicates that this is not a substantial issue. Also, while g_{awmgs} will deviate most from g_{awm} for broad axon diameter distributions with a small mean, the difference between measures of g_{awm} and g_{awmgs} in this study is small (1.33%). Thus, these three measures are effectively the same and g_{MRI} can be reasonably interpreted as an axon-area-weighted measure of g , which is somewhat easier to intuit than the square root of the axon-area-weighted g^2 .

Both area-weighted measures show a slight trend toward being underestimated by g_{MRI} at lower values of g , which may reflect the limitations of the histology analysis. Axons with lower g-ratios tended to be smaller in diameter and more densely packed, which may have resulted in a tendency to overestimate local *MVF* due to limitations of the segmentation. This overestimation of *MVF* would reduce estimates of g_{MRI} but not affect the other characterizations of g which were derived from direct measures of myelin thickness.

The histology data are also summarized in Table 1, which displays the mean $\pm$ standard error of the mean of the four g-ratio measures across the images from each region (MidCC, GCC) for control and CKO mice. The g_{mean} values from control mice (~0.81) are somewhat higher than the previously predicted optimal value of 0.77 (Chommiak and Hu, 2009) but agree with some previous histological evaluations of the mouse corpus callosum (Arnett et al., 2001; Mason and Langaman, 2001). Because the measures of myelin thickness from histology depended on the segmentation level, it is possible that a small systematic underestimation of myelin thickness has resulted in overall elevated values of g_{mean} , but that would not change the conclusions of this study.

It is apparent from Table 1 that the two area-weighted measures (g_{awm} , g_{awmgs}) are nearly identical in all cases and similar to g_{MRI} values. All measures show statistically significant differences (using a two-tailed student's t-test, $\alpha=0.05$) between control and CKO mice, demonstrating that for this example, g_{MRI} , which reports a significantly different value than g_{mean} , is sufficient to detect differences in microstructure between the control and CKO mice. However, we note the time course of demyelination and remyelination may not always be well captured by a scalar value, and because of the area-weighting effect, g_{MRI} in

particular will be less sensitive to microstructural changes in smaller axons. For example, a previous study of microstructure in the mouse corpus callosum during and following exposure to cuprizone in the diet (Mason and Langaman, 2001) observed periods with changes in myelin thickness and axon diameter that were not captured by the mean g-ratio, and found that recovery periods involved preferential remyelination of smaller axons. Perhaps these limitations can be overcome with more sophisticated models that incorporate axon diameter distributions (such as is done with the AxCaliber method, Assaf et al. 2008, Barazany et al. 2009) and established relationships between axon diameter and g-ratio (Berthold et al., 1983; Chomiak and Hu, 2009); however, the practical limits on MRI measures of g-ratio may come from the ability to make robust estimates of *MVF* and *FVF*, which remains an area of active study.

4.2 Conclusions

As quantitative MRI methods strive to provide more detailed information about underlying tissue properties, such as the g-ratio, histologic comparisons are vital to understand microstructural meaning of derived imaging measures. We have shown here that the recently proposed approach to estimate the aggregate g-ratio index with MRI will, in principal, provide a measure that is close to the axon-area-weighted measures of g across all axons in a voxel. This measure will naturally be more sensitive to changes or differences in larger diameter axons and should be interpreted with this knowledge.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

MRI	Magnetic resonance imaging
MVF	myelin volume fraction
FVF	fiber volume fraction
CKO	conditional knockout
PBS	phosphate-buffered saline
MidCC	midbody of corpus callosum
GCC	genu of corpus callosum

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Figure 1.

Histology analysis methods. a) Demonstration of the middle corpus callosum region chosen for electron microscopy tissue preparation. b) A transmission electron microscope image is acquired from the middle region of the mouse corpus callosum. c) A threshold is applied to separate myelin and non-myelin pixels and obtain a binary myelin mask, providing a myelin volume fraction (*MVF*). d) A region growing algorithm is used to fill all axon areas, and the sum of all axon areas provides an axon volume fraction (*AVF*). Myelin thickness is measured manually in two locations per axon. e) Axon radius (r) is derived from the area of each axon, assuming circular geometry. f) The g-ratio is calculated per axon and the histogram of values is fitted to a gamma distribution, from which the following measures are computed: mean (g_{mean}), area-weighted mean (g_{awm}), and the square root of the area-weighted g^2 (g_{awmgs}). The proposed MRI measure, g_{MRI} , was computed from measures of *MVF* and *AVF*.

Figure 2.

Scatter plots of (left) g_{MRI} versus g_{mean} , (middle) g_{MRI} versus g_{awm} , and (right) g_{MRI} versus g_{awmgs} where black and red points signify control and CKO image measures, respectively. The root mean squared difference (RMSD) between each pair of measures is shown above each plot.

Table 1

g_{mean} , g_{awm} , g_{awmgs} , and g_{MRI} mean $\pm$ SEM from the middle and genu regions of the corpus callosum across all images for control and Rictor CKO mice.

Region	g_{mean}	g_{awm}	g_{awmgs}	g_{MRI}
<i>Control</i>				
MidCC	0.815 $\pm$ 0.003	0.848 $\pm$ 0.003	0.850 $\pm$ 0.003	0.844 $\pm$ 0.004
GCC	0.803 $\pm$ 0.005	0.847 $\pm$ 0.003	0.849 $\pm$ 0.003	0.834 $\pm$ 0.005
<i>CKO</i>				
MidCC	0.845 $\pm$ 0.006	0.873 $\pm$ 0.007	0.875 $\pm$ 0.007	0.870 $\pm$ 0.010
GCC	0.854 $\pm$ 0.006	0.880 $\pm$ 0.009	0.883 $\pm$ 0.009	0.889 $\pm$ 0.008