Published in final edited form as:

J Mech Behav Biomed Mater. 2018 March ; 79: 30–37. doi:10.1016/j.jmbbm.2017.11.045.

Measurement of anisotropic mechanical properties in porcine brain white matter *ex vivo* using magnetic resonance elastography

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Abstract

The mechanical properties of brain tissue, particularly those of white matter (WM), need to be characterized accurately for use in finite element (FE) models of brain biomechanics and traumatic brain injury (TBI). Magnetic resonance elastography (MRE) is a powerful tool for non-invasive estimation of the mechanical properties of soft tissues. While several studies involving direct mechanical tests of brain tissue have shown mechanical anisotropy, most MRE studies of brain tissue assume an isotropic model. In this study, an incompressible transversely isotropic (TI) material model parameterized by minimum shear modulus (μ_2), shear anisotropy parameter (ϕ), and tensile anisotropy parameter (ζ) is applied to analyze MRE measurements of *ex vivo* porcine white matter (WM) brain tissue. To characterize shear anisotropy, “slow” (pure transverse) shear waves were propagated at 100, 200 and 300Hz through sections of *ex vivo* brain tissue including both WM and grey matter (GM). Shear waves were found to propagate with elliptical fronts, consistent with TI material behavior. Shear wave fields were also analyzed within regions of interest (ROI) to find local shear wavelengths parallel and perpendicular to fiber orientation. FE simulations of a TI material with a range of plausible shear modulus (μ_2) and shear anisotropy parameters (ϕ) were run and the results were analyzed in the same fashion as the experimental case. Parameters of the FE simulations which most closely matched each experiment were taken to represent the mechanical properties of that particular sample. Using this approach, WM in the *ex vivo* porcine brain was found to be mildly anisotropic in shear with estimates of minimum shear modulus (actuation frequencies listed in parenthesis): $\mu_2 = 1.04 \pm 0.12$ kPa (at 100 Hz), $\mu_2 = 1.94 \pm 0.29$ kPa (at 200 Hz), and $\mu_2 = 2.88 \pm 0.34$ kPa (at 300 Hz) and corresponding shear anisotropy factors of $\phi = 0.27 \pm 0.09$ (at 100 Hz), $\phi = 0.29 \pm 0.14$ (at 200 Hz) and $\phi = 0.34 \pm 0.13$ (at 300 Hz). Future MRE studies will focus on tensile anisotropy, which will require both slow and fast shear waves for accurate estimation.

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Graphical Abstract

Keywords

MR elastography; shear waves; anisotropy; transversely isotropic material; white matter brain tissue

Introduction

Traumatic brain injury (TBI) is prevalent in the United States (Coronado et al., 2011) and worldwide. During TBI, impacts to the head lead to large skull accelerations, and brain tissue is deformed in tension and shear (Bayly et al., 2005; Margulies and Thibault, 1992). Accurate models are needed to fully understand the mechanism of tissue damage from impacts. Finite element (FE) models are often proposed as a method to predict injurious conditions (Ueno et al., 1995; Zhang et al., 2004). These methods require accurate knowledge of brain tissue properties in shear and tension, including their directional properties.

Several recent studies suggest that white matter (WM) brain tissue is mechanically anisotropic. The response of brain tissue in shear was observed to be anisotropic under both small (Feng et al., 2013) and large deformations (Feng et al., 2017); these authors applied a transversely isotropic model to interpret their data. Velardi et al. (2006) studied the experimental behavior of *ex vivo* porcine brain tissue in extension and proposed an anisotropic, hyperelastic constitutive model to explain their data. Prange and Margulies (2002) studied *ex vivo* porcine and human brain tissue and found a directional dependence in WM. Ning et al. (2006) characterized brainstem experimental data as a transversely isotropic, viscoelastic material and compared observed behavior to the predictions of a corresponding numerical model.

Magnetic resonance elastography (MRE) allows the estimation of mechanical properties in soft tissue from images of shear waves (Muthupillai et al., 1995). MRE was originally developed using isotropic, elastic material models, which have evolved to include viscoelastic effects. Such isotropic models, either elastic or viscoelastic, have been applied in MRE studies involving liver (Asbach et al., 2008; Klatt et al., 2010; Mariappan et al., 2009), breast (Sinkus et al., 2007) and brain (Atay et al., 2008; Clayton et al., 2011; Green et al., 2008; Johnson et al., 2013). While most MRE studies assume “local homogeneity” of material properties, tissue heterogeneity has also been explored by Van Houten et al. (2001), using an inversion technique known as non-linear inversion.

The possibility of mechanical anisotropy in biological tissues should also be addressed in studies utilizing MRE. A transversely isotropic (TI) model is the simplest anisotropic model, with a single fiber orientation defining a plane of isotropy. Five parameters are required to completely define a general elastic TI material, while three parameters are sufficient to define an incompressible TI material. Recently, several MRE studies based on anisotropic material models have been performed. Most of this work has focused on estimation of two different shear moduli in planes parallel and perpendicular to the fiber orientation (2-parameter models). Sinkus et al. (2005) has published studies of shear anisotropy in breast tissue; Green et al. (2013), Wuerfel et al. (2010), Papazoglou et al. (2006), Qin et al. (2013) have studied muscle tissue. Shear anisotropy has also been estimated in anisotropic biomaterials: Qin et al. (2013) studied anisotropic phantoms of a composite material and Namani et al. (2009) studied aligned fibrin gels.

More complete transversely isotropic models account for both shear and tensile anisotropy. Romano et al. (2012) estimated five stiffness parameters in brain white matter corticospinal tracts utilizing spatial-spectral filters, Helmholtz decomposition, and waveguides. Three-parameter models have recently emerged as more compact, but still accurate, models of soft tissue, which is nearly incompressible. Guo et al. (2015) estimated three parameters in skeletal muscle using inversions of the curl of the displacement field. Tweten et al. (2017, 2015) used finite element (FE) simulations to establish basic requirements for estimation of three material parameters. Schmidt et al. (2016) measured both “slow” and “fast” shear waves in muscle tissue *ex vivo* and aligned fibrin gels, and used complementary information in these fields to estimate all three material parameters.

In the human brain, Romano et al. (2012) obtained estimates of five anisotropic stiffness parameters in corticospinal tracts in human subjects *in vivo*, but such estimates remain speculative since parameter values have not been confirmed by direct mechanical test or comparison to simulation. Qualitatively, however, anisotropy of WM has been detected by MRE *in vivo* in the human brain by Anderson et al. (2016), who used multiple excitation methods and showed that estimates of isotropic material parameters depended on the directional properties of the wave field. Still missing are clear estimates of a minimal set of intrinsic, anisotropic material parameters for WM brain tissue, in which confidence has been established by comparison to direct test and simulations.

The goal of the current study is to quantify the anisotropic shear properties of *ex vivo* WM using MRE, by estimating parameters of simulations to fit experimental data. Shear waves

were visualized and measured in *ex vivo* porcine WM embedded in gelatin (experiment) and in numerical (FE) simulations of shear waves in a TI material embedded in an isotropic material. Results from simulations were compared to experiment to estimate anisotropic shear moduli over multiple frequencies. By comparing experimental wave fields to numerical simulations that incorporate material anisotropy in a sub-domain of a finite-size sample, typical assumptions underlying estimates of material properties (i.e., an unbounded domain with uniform, isotropic properties) are not necessary. The results indicate mild, but non-negligible, mechanical anisotropy of WM brain tissue in shear.

Methods

Theory

In an incompressible TI, linear, elastic material model, valid for small deformations, the three independent material parameters can be defined by shear modulus, μ_2 , shear anisotropy, ($\phi = \mu_1/\mu_2 - 1$), and tensile anisotropy ($\zeta = E_1/E_2 - 1$), where μ_1 is the shear modulus that governs shear in planes containing the dominant fiber axis (the normal to the plane of isotropy, $\mathbf{a}$), μ_2 is the minimum shear modulus governing shear in the plane of isotropy (normal to the fiber axis), E_1 is the tensile modulus in the fiber direction and E_2 is the tensile modulus in directions perpendicular to fiber axis (Jones, 1998; Schmidt et al., 2016; Spencer, 1984; Tweten et al., 2015). Two types of shear waves, dependent on these parameters, are possible in incompressible TI materials: (i) “slow” (pure transverse) waves, where the polarization of the shear wave, $\mathbf{m}_s$, is perpendicular to both the wave propagation direction, $\mathbf{n}$, and the fiber direction, $\mathbf{a}$; and (ii) “fast” (quasi-transverse) waves, where the polarization of the shear wave, $\mathbf{m}_f$, is perpendicular to the polarization of the slow shear wave, and lies in the plane defined by $\mathbf{n}$ and $\mathbf{a}$. Wave speeds of slow and fast shear waves can be defined, respectively, by the following expressions (Schmidt et al., 2016; Tweten et al., 2015):

$$c_s^2 = (\mu_2 / \rho) (1 + \phi \cos^2 \theta), \quad (1)$$

$$c_f^2 = (\mu_2 / \rho) (1 + \phi \cos^2 2\theta + \zeta \sin^2 2\theta). \quad (2)$$

In the simplest viscoelastic model of TI material behavior, dissipation can be modeled by an isotropic loss factor η , so that each real-valued, elastic modulus, for example μ_2 , is replaced by a complex-valued, viscoelastic modulus $\mu_2^* = \mu_2' + i\mu_2''$, where $\eta = \mu_2''/\mu_2'$.

Experiments

Sample preparation—Disk-shaped samples (~42mm diameter, ~14 mm thick), consisting of both WM and GM, were dissected from the corpus callosum and associated superior cortical GM in female domestic pigs (N=8, age 3 months, 40–45 kg) immediately after euthanasia (Fig. 1, a–d). Samples were embedded in gelatin mixed with 50% water and 50%

glycerol (Okamoto et al., 2011) in a cylindrical container (48 mm inner diameter) The embedded samples were punctured axially by a 3-mm diameter plastic rod (Fig. 1,e). A piezoelectric actuator (APA150M, Cedrat Technologies, Meylan, France) powered by a low-current, high-voltage amplifier (LA75C, Cedrat Technologies, Meylan, France) was used to provide harmonic vibrations of the plastic rod in the central axis (z) at 100, 200, and 300 Hz, generating radially-propagating shear waves ($\mathbf{n} \approx \mathbf{e}_R$). This preparation does not probe tensile anisotropy (no “fast” shear waves are produced). This is because shear wave propagation is normal to fiber orientation, producing no stretch along the fiber direction.

Imaging—Images of shear-wave propagation in the disk-shaped WM/GM samples were acquired using previously-described spin-echo MRE sequences (Clayton et al., 2011; Schmidt et al., 2016). Imaging was performed at 4.7 Tesla at room temperature ($\sim 21^\circ\text{C}$) with an Agilent/Varian DirectDrive imaging system. MRE imaging parameters were: voxel size = 1.0 mm isotropic, TR = 1100 – 1300 ms, TE = 30–47 ms. Multiple (1–3) sinusoidal motion encoding cycles of gradient strength 10–12 G/cm were synchronized with motion to induce phase contrast proportional to displacement. Eight temporal samples were acquired per sinusoidal excitation period, by incrementing the phase delay between the imposed vibration and acquisition. Anatomical (spin-echo, T1-weighted, TE = ~ 10 ms, TR = 1000 ms, 2 averages) MRI was performed (Fig. 1, c) to identify the boundaries of the brain tissue sample and to distinguish white matter and gray matter. Diffusion weighted images (30 directions, $b=3000 \text{ s/mm}^2$) were acquired over the same volume to confirm the myelinated axon orientation. Time from euthanasia to the start of the experiments was ~ 1 –2 h.

Computational modeling and simulations—A 3D, finite-element (FE) model of the MRE experiment was created using COMSOLTM Multiphysics 5.1. The model consists of a cylindrical slab (42 mm diameter, 14 mm thick) representing brain tissue embedded in a cylinder of gelatin (48 mm diameter, 48 mm long). The inclusion was assigned TI material properties with a single fiber direction and the gelatin was assigned isotropic properties (Fig. 2). Axial harmonic excitation of $25 \mu\text{m}$ was provided at 100, 200, and 300 Hz on the inner radius ($1.5 \mu\text{m}$) of the model. Assigned properties of the inclusion were: baseline storage modulus, μ_2 , varied from 0.5 – 1.5 kPa (at actuation frequency of 100 Hz), 1.2 – 2.5 kPa (at 200 Hz), 2 – 5 kPa (at 300 Hz), a loss factor, $\eta = 0.5$, typical of brain tissue (Feng et al., 2013), density, $\rho = 1000 \text{ kg/m}^3$. Shear anisotropy, ϕ , was varied from 0 – 0.6. Tensile anisotropy (nonzero ζ) was not included as ζ does not affect pure transverse (“slow”) shear waves induced by excitation normal to fiber direction. The material properties assigned to gelatin were: shear storage modulus, $\mu = 1.0, 1.1$, and 1.2 kPa (Okamoto et al., 2011) at 100, 200, and 300 Hz actuation frequencies respectively; loss factor, $\eta = 0.1$; and density, $\rho = 1100 \text{ kg/m}^3$. Loss factors taken from prior studies were varied over a limited range to check that attenuation of wave amplitudes in simulations was similar to experiment. The parameters μ_2 and ϕ of the FE simulations with shear wave propagation most similar to the MRE experiment were taken as the estimates of μ_2 and ϕ within the selected slices of the *ex vivo* tissue sample.

Analysis of experimental and simulated image data—The Fourier transform of the displacement data from all of the 8 acquisition phases was found using the FFT, and the

coefficients at the fundamental harmonic were extracted, producing a three-dimensional field of Fourier coefficients. Data were then smoothed (Gaussian smoothing with a 3x3x3 voxel convolution kernel and a standard deviation of 1) in 3D. For estimation of apparent shear modulus and shear wavelength, the curl of the smoothed 3D displacement field was expected to eliminate effects of longitudinal waves.

As a simple measure of anisotropy, radially-propagating shear waves in WM were fitted to ellipses (see Fig. 3). Peaks of elliptical wave fronts were manually picked on a 2D image of axial (w) displacement in the xy plane (interpolated in the xy plane from 1 mm resolution to 0.5 mm resolution), in 3 contiguous slices within slices containing WM from each sample. The various FE simulations (across a plausible range of baseline shear moduli and shear anisotropy) were also subjected to the same analysis, except that peaks of elliptical wave fronts were picked from 3 contiguous, central slices by an automated 2D method. Points picked either manually or by automated method were fitted using an algorithm that minimized the squared-error between the ellipses and the picked points (Fitzgibbon et al., 1999). The ratio, R , between ellipse semi-axis lengths was recorded for each experimental sample or FE simulation, along with the angle of the longer semi-axis.

The apparent complex shear modulus $\mu^* = \mu' + i\mu''$ was estimated in both MRE experiments and FE simulations, using local direct inversion (LDI) of the three components of the curl of the displacement field (Okamoto et al., 2011). To express these parameters in terms of kinematic features of the wave field, analogous to elliptical shape, storage modulus

estimates were converted to local shear wavelength, defined as $\lambda^+ = \frac{1}{f} \sqrt{\frac{\mu'}{\rho}}$. To quantify directional variations in wavelength maps from LDI, regions of interest (ROIs, see Fig. 4) were defined by squares centered at equal distances from the cylinder axis, in directions parallel and perpendicular to the longer semi-axis.

Dynamic shear testing (DST)—For comparison with MRE, estimates of viscoelastic shear modulus (μ_1^* and μ_2^*) were also obtained by dynamic shear testing (DST) (Feng et al., 2013; Namani et al., 2012). Samples consisting of predominantly WM brain tissue ($N = 10$) acquired from the corpus callosum and samples consisting of predominantly GM brain tissue ($N=10$) acquired from the region superior to the corpus callosum within the frontal and parietal lobes of the brain were acquired from 3 different female domestic pigs (age 3 months, 40–45 kg). The mean $\pm$ std. deviation sample thickness was 2.75 ± 0.63 mm and the sample diameter was 13.39 ± 3.42 mm in WM samples, and in GM the average sample thickness was 3.37 ± 0.84 mm and a sample diameter of 14.93 ± 1.31 mm. Samples were tested in shear both parallel and perpendicular to the WM fiber axis (determined visually), and in two arbitrary perpendicular directions in GM. Shear modulus estimates were averaged over the frequency range from 20 to 30 Hz. This range was chosen to avoid inertial effects (shear waves) within the sample (Feng et al., 2013).

Results

MRE experiments and FE simulations exhibit elliptical shear wave propagation

MRE experiments were performed on cylindrical brain samples from eight pigs. Example shear wave images from a representative MRE experiment, and from the corresponding FE simulation found to best approximate this particular MRE experiment are shown in Fig. 3. Shear wave patterns are consistent with theoretical predictions based on wave speeds in a uniform, unbounded, TI material (Tweten et al., 2015). Elliptical waves were observed in regions with known white matter (corpus callosum). Circular waves were observed in slices containing only isotropic gelatin (Fig. 3,d).

Estimated local wavelength comparisons between experiment and FE models

Each MRE experiment was matched individually to the FE simulation with shear wave propagation that most resembled that of the experiment, in terms of both (1) local shear wavelength in parallel and perpendicular ROIs and (2) the ratio of elliptical semi-axis fit to shear wave fronts. Normalized root mean squared error (NRMSE) was found between local wavelength (λ^+) estimates in experiment and simulation, in both parallel ($NRMSE_{\lambda_1}$) and perpendicular ($NRMSE_{\lambda_2}$) ROIs, in the 8 image slices of the experimental sample that contained the most WM (closest to the center of the corpus callosum), and in the middle 8 slices of the TI inclusion of the FE models. Normalized RMS error ($NRMSE_R$) was also found between the ellipse axis ratio (R) of the MRE experiment and FE simulations for the 3 slices in which ellipses were fitted. These three NRMSE estimates were combined to identify the FE simulation that most closely represented the specific experiment. The NRMSEs of each wavelength and the ellipse ratio were weighted and summed to provide an overall objective function that reflected both metrics (local wavelength and ellipse shape) equally. The following weighting scheme was used for this study:

$$NRMSE_{weighted} = \frac{1}{4}NRMSE_{\lambda_1} + \frac{1}{4}NRMSE_{\lambda_2} + \frac{1}{2}NRMSE_R.$$

Fig. 5 shows surfaces of NRMSE from the comparison between all FE simulations and a single representative MRE experiment. These surfaces show (i) the wavelength error between simulations and experiment in the perpendicular ROI ($NRMSE_{\lambda_2}$, Fig. 5, a), (ii) wavelength error in the parallel ROI ($NRMSE_{\lambda_1}$, Fig. 5, b), (iii) the error between ellipse axis ratios ($NRMSE_R$, Fig. 5, c), and (iv) a plot showing the weighed NRMSE combining all estimates into one weighted estimate. Parameter estimates for all samples (N=6 for 100 Hz, and N=8 for 200 and 300 Hz) were analyzed statistically and results are shown in Fig. 6.

WM in the *ex vivo* porcine brain was found to be anisotropic in shear with estimates of minimum shear modulus shown in Table 1. The shear modulus in planes parallel to fibers, μ_1 , was significantly larger than shear modulus in the plane of isotropy, μ_2 (Wilcoxon signed rank test) at 100, 200, and 300 Hz ($P < 0.05$, $P < 0.05$, $P < 0.05$, respectively). Both shear moduli showed significant increases with actuation frequency (μ_2 : $P < 0.005$; μ_1 : $P < 0.005$; Friedman test) as expected for viscoelastic materials. Notably the increase in the anisotropy factor ϕ was also statistically significant ($P < 0.05$).

Dynamic shear testing (DST) results

Shear moduli in planes parallel and perpendicular to the fiber axis for WM brain tissue (N=10) were estimated in a frequency range from 20 to 30 Hz using DST. This frequency range is as high as possible, while avoiding instrument resonances and inertial effects in the sample. The storage modulus was estimated to be $\mu'_{\parallel} = 0.61 \pm 0.06$ kPa when fibers were aligned parallel to the direction of imposed shear displacement and $\mu'_{\perp} = 0.54 \pm 0.08$ kPa when fibers were aligned perpendicular to shear displacement. The loss modulus was $\mu''_{\parallel} = 0.34 \pm 0.05$ kPa for the parallel orientation and $\mu''_{\perp} = 0.30 \pm 0.05$ kPa for the perpendicular orientation. The mean ($\pm$ std. dev.) ratio between paired parallel and

perpendicular moduli was $\mu'_{\parallel} / \mu'_{\perp} = 1.14 \pm 0.08$ for storage modulus, and

$\mu''_{\parallel} / \mu''_{\perp} = 1.11 \pm 0.06$ for loss modulus. Shear moduli from two perpendicular arbitrary directions (denoted as *a* and *b*, since there is no reference fiber direction) in GM brain tissue (N=12) were averaged over frequencies from 20 to 30 Hz using DST. The storage modulus was found to be $\mu'_a = 0.49 \pm 0.09$ kPa in one direction of imposed shear displacement and $\mu'_b = 0.50 \pm 0.11$ kPa when the shear displacement was imposed in the perpendicular direction. The loss modulus was $\mu''_a = 0.20 \pm 0.05$ kPa for the first orientation and $\mu''_b = 0.23 \pm 0.06$ kPa for the second orientation. In GM, the ratio between moduli in these

two arbitrary, perpendicular directions, was $\mu'_a / \mu'_b = 0.99 \pm 0.12$ for storage modulus and

$\mu''_a / \mu''_b = 0.94 \pm 0.21$ for loss modulus. Wilcoxon signed rank test statistical analysis was performed to show WM μ_1 and μ_2 shear moduli to be significantly different than each other (storage modulus: $P < 0.005$, loss modulus: $P < 0.005$). Similar statistical analysis showed GM μ_a and μ_b shear moduli to be not significantly different (storage modulus: $P = 0.38$, loss modulus: $P = 0.084$).

Discussion

In this study, slow shear waves were imaged using MR elastography techniques in WM *ex vivo* brain tissue. In centrally-excited, cylindrical samples, outwardly propagating slow shear waves exhibited elliptical wave fronts and local wavelengths that depended on the direction of propagation, consistent with TI behavior (Schmidt et al., 2016). Baseline shear moduli and shear anisotropy of *ex vivo* porcine WM brain tissue were estimated by comparing experimental shear wave data to simulations of shear waves in TI materials.

MRE and direct mechanical tests (DST) in the current study both indicated the presence of mild shear anisotropy in porcine WM brain tissue. Shear modulus estimates obtained by fitting the current MRE results with FE simulations (shear modulus magnitudes 1.0 kPa at 100 Hz, 1.9 kPa at 200 Hz, and 2.9 kPa at 300 Hz) were larger than estimates obtained by DST (average shear modulus magnitude ~ 0.65 kPa). These trends are consistent with the expected increase in modulus with increase in actuation frequency in viscoelastic biological tissue. Shear anisotropy factors (ϕ) of 0.25–0.35, estimated by fitting the current MRE

studies with FE simulations, were roughly double those observed by DST in the current study, but closer to the shear anisotropy factors observed by DST (Feng et al., 2013) in ovine corpus callosum WM. Differences may reveal limitations of each method, but we particularly note limitations of DST. DST estimates can be affected by sample flatness, normal force, order of testing (which direction is tested first), slip, nonlinearity, or non-affine deformation. MRE estimates of parameters are limited by the practical challenges of image resolution (discretization of shear waves) and domain size (which limits the number of wavelengths). Both methods potentially obscure local variations on a scale smaller than the wavelength of a shear wave (for our MRE experiments this was ~5–10 mm), or the size of the DST sample (~13–15 mm). Shear anisotropy may also depend on frequency. Differences between shear anisotropy estimates from MRE and DST are similar to those found in previous studies involving muscle tissue (Schmidt et al., 2016). Given the limitations of each method, we believe that the shear anisotropy estimates from MRE, which are also consistent with simulation and visual observations of wavefronts, are more accurate. It should be noted that these results do not validate or prove the ability of this model to predict behavior in another situation, but illustrate the ability of this model, with these parameters, to explain the current observations.

While the focus of this study is on MRE estimation of material properties, we note that the current estimates of material properties from DST in the porcine brain between 20 – 30 Hz are comparable to those from previous DST studies on *ex vivo* ovine brain (Feng et al., 2013). Shear storage modulus in planes parallel to the fiber orientation in WM were very similar between the two studies, while shear storage modulus perpendicular to the fiber axis is ~20% larger in the current study, leading to the lower shear anisotropy ratio observed by DST in the current study. Both studies found GM to be isotropic by DST, while Feng et al. (2013) found a lower modulus in GM. Both studies found similar, approximately isotropic, loss factors, in both GM and WM by DST.

Taking into account differences in frequency and anatomical region, the current parameter estimates from MRE may be compared to results in the literature obtained by direct mechanical testing. Arbogast and Margulies (1998) studied porcine WM brain tissue (brainstem) using DST from frequencies ~20 – 200 Hz, spanning much of the range of the MRE experiment. They found shear modulus values at 100 and 200 Hz similar to ours (~1.7 – 2.25 kPa) and shear anisotropy ratio similar to the current estimate ($\phi \sim 0.3$). It should be noted that Arbogast and Margulies (1998) used a much higher strain (2.5 – 7.5 %) than was applied in either the current DST or MRE tests. Hrapko et al. (2008) studied porcine brain (corona radiata, acquired within three different directional planes) using rotational rheometry within the frequency range of 1 – 10 Hz. The shear anisotropy ratio in the corpus callosum region, estimated in the current MRE-based study is similar to the corresponding estimate from Hrapko et al. (2008) (~0.3 vs ~0.2 – 0.4, respectively), despite the significant difference in actuation frequency (100 – 300 Hz vs 1 – 10 Hz, respectively). Average shear moduli from Hrapko et al. (2008) are consistent with our DST estimates (~0.4 – 0.6 kPa at 1–10 Hz vs ~0.65 kPa at 20 – 30 Hz, respectively).

The anisotropy estimated in the current study is mild (i.e., not an order of magnitude) and not as large as what might be expected given the diffusion anisotropy of brain WM. The degree

of anisotropy is also smaller than the level assumed in recent modeling studies (Giordano et al., 2014). The level of anisotropy is consistent with the magnitude of the directional dependence of shear moduli observed by Anderson et al. (2016).

The current study is limited by the assumption that the tissue is linearly viscoelastic with loss factors typical of brain ($\eta \approx 0.5$) and gelatin ($\eta \approx 0.1$). Loss factors were not optimized by fitting, but provided attenuation of shear waves similar to that observed in experiments. The assumption of incompressibility may introduce some errors in the current study and *in vivo* studies, due to the actual slight compressibility of tissue and the consequent existence of longitudinal waves (although the curl operation is intended to eliminate longitudinal waves from the MRE analysis). The assumption of an unbounded, uniform domain is not necessary because the FE model includes bounded sub-domains with different properties. It should be noted, however, that shear waves traveling through WM tissue may be influenced by nearby GM, as perfect segmentation between tissue types was not possible. Structural anisotropy of the experiment as a whole does affect wave motion, and thus may influence estimates of material anisotropy (intrinsic) based on global features of wave propagation (elliptical axis ratio). Local estimates of wavelength should not be strongly affected. Manual identification of points on ellipses may also introduce imprecision. Importantly, we have made no attempt to quantify the tensile anisotropy, which is likely to exist and to be important in brain behavior, but which is not manifested under these experimental conditions. Future studies will focus on: (i) complementary estimates of tensile anisotropy of WM using MRE with “fast” or quasi-transverse/QT shear waves which introduce fiber stretch; and (ii) estimation of shear and tensile anisotropy in the human brain *in vivo*.

Conclusion

White matter in the porcine brain *ex vivo* was found to be mildly anisotropic in shear using MRE. Anisotropic material parameters were estimated from the parameters of FE simulations that most closely matched data from the MRE experiments. These material parameter estimates enhance our understanding of the mechanical properties of WM in brain tissue *ex vivo*, and provide confidence in our future ability to estimate anisotropic mechanical properties of WM in the intact, living brain. Ultimately, MRE-derived estimates of anisotropic properties of WM *in vivo* will lead to improved computational models of brain biomechanics and deeper understanding of TBI.

Acknowledgments

This work was supported by National Science Foundation grant CMMI-1332433 (Bayly) and National Institutes of Health grant R01 NS-055951 (Bayly).

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Highlights

- Shear waves in white matter ex vivo were compared to corresponding simulations.
- Baseline shear modulus and shear anisotropy were found by fitting models to data.
- Wave fronts were elliptical and exhibited fastest propagation parallel to fibers.
- Shear waves in white matter reveal transversely isotropic material behavior.

Figure 1.

(a–b) T1-weighted (T1W) *in vivo* anatomical images from prior work (Bayly et al., unpublished) showing tissue volume used for current study (red dashed outlines). (c) T1W images of *ex vivo* sample used in the current study. Fiber orientation is left-to-right. (d) Photo of *ex vivo* sample of brain tissue used for this study. (e) Apparatus for MRE experiments: tissue embedded in gel inside cylindrical container, excited by harmonic motion of a central axial stinger.

Figure 2.

FE model (COMSOLTM Multiphysics v5.1) showing axial (w) displacement. A disk-shaped inclusion representing transversely isotropic (TI) WM is enclosed in an isotropic soft material representing gelatin. Inclusion: $\mu_2 = 2.0$ kPa, $\mu_1 = 2.6$ kPa ($\phi=0.30$), $\eta = 0.5$, $\rho = 1000$ kg/m³. Surrounding gelatin: $\mu = 1.1$ kPa, $\eta = 0.1$, $\rho = 1100$ kg/m³. Actuation frequency: 200 Hz. The black arrow points in the direction normal to the TI material's plane of isotropy (i.e., the fiber axis, $\mathbf{a}$).

Figure 3.

Shear-wave propagation in WM brain tissue (experiment, top row) and transversely isotropic FE models (simulation, bottom row) with fitted ellipses outlined. (a–c) MRE images of shear wave propagation in WM at (a) 100 Hz, (b) 200 Hz, and (c) 300 Hz. Shear-wave fronts are fitted by ellipses (black or white). The boundary of the tissue sample is outlined by a thin dotted white line. (d) Shear-wave propagation in a slice containing only gelatin at 300 Hz. (e–g) Shear wave propagation in FE simulations with similar mechanical properties to the experiment: (e) 100 Hz, $\mu_2 = 1100$ Pa, $\phi = 0.30$; (f) 200 Hz, $\mu_2 = 1600$ Pa, $\phi = 0.45$; (g) 300 Hz, $\mu_2 = 2300$ Pa, $\phi = 0.35$. (h) Shear-wave propagation in the isotropic/gelatin portion of the FE model at 300 Hz.

Figure 4.

Apparent shear modulus in WM brain tissue and transversely isotropic (TI) FE models. Regions of interest (ROIs) parallel ($\parallel$) and perpendicular ($\perp$) to fitted ellipse semi-major axis are highlighted. (a–c) Maps of apparent shear modulus at (a) 100 Hz, (b) 200 Hz, (c) 300 Hz, estimated from MRE data in slices containing brain tissue. (d) Maps of apparent shear modulus at 300 Hz, estimated from MRE data in slices containing only gelatin. (e–g) Maps of apparent shear modulus estimated using data from FE simulations with mechanical properties matched to the experiment: (e) 100 Hz: $\mu_2 = 1100$ Pa, $\phi = 0.30$; (f) 200 Hz: $\mu_2 = 1600$ Pa, $\phi = 0.45$; (g) 300 Hz: $\mu_2 = 2300$ Pa, $\phi = 0.35$. (h) Maps of apparent shear modulus at 300 Hz, estimated using data from the isotropic/gelatin portion of the FE model.

Figure 5.

Parameter estimation by comparison of experiment to simulation. Results from inversion of data from MRE experiments are matched to results of inversion of data from a library of FE simulations performed with a range of plausible transversely isotropic (TI) material parameters. Results from $\perp$ and $\parallel$ ROIs are compared separately to identify effects of anisotropy. (a) Normalized RMS error (NRMSE) between wavelength (λ_2) estimates from experimental data and TI FE simulations in the $\perp$ ROI. (b) NRMSE between wavelength (λ_1) estimates from experimental data and FE simulations in the $\parallel$ ROI. (c) NRMSE between the ratio of semi-axes of ellipses fitted to shear-wave fronts in the experimental data and FE simulations. (d) Weighted total NRMSE between experiment and FE models (weighted sum of wavelength NRMSEs and axis ratio NRMSE).

Figure 6.

Summary of shear modulus and shear anisotropy estimates in WM brain tissue. (a) Shear moduli in planes parallel ($\mu_{\parallel}$) and perpendicular ($\mu_{\perp}$) to fiber orientation. (b) Shear anisotropy ($\phi = \mu_{\parallel}/\mu_{\perp} - 1$). Solid bars (100 Hz, 200 Hz, 300 Hz) represent results from MRE/FE analysis. Cross-hatched bars (20–30 Hz) show results obtained by direct mechanical testing (DST). Lines across plots with stars indicate statistical significance (* $P < 0.05$, ** $P < 0.005$). Curly bracketed lines signify Friedman tests across 100 Hz, 200 Hz, and 300 Hz. Straight lines signify Wilcoxon signed rank tests between two sets of data.

Table 1

Estimated parameters of the TI material model

Frequency [Hz]	μ_2 [kPa]	ϕ
100	1.04 ± 0.12	0.27 ± 0.09
200	1.94 ± 0.29	0.29 ± 0.14
300	2.88 ± 0.34	0.34 ± 0.13