Published in final edited form as:

Ultrasound Med Biol. 2015 March ; 41(3): 713–723. doi:10.1016/j.ultrasmedbio.2014.10.019.

Material Characterization of *In Vivo* and *In Vitro* Porcine Brain using Shear Wave Elasticity

Caryn A. Urbanczyk, Mark L. Palmeri, and Cameron R. Bass

Department of Biomedical Engineering, Duke University, Durham, NC 27708, United States

Abstract

Realistic computer simulation of closed head trauma requires accurate mechanical properties of brain tissue, ideally *in vivo*. A substantive deficiency of most existing experimental brain data is that properties were identified through *in vitro* mechanical testing. This study develops a novel application of shear wave elasticity imaging (SWEI) to assess porcine brain tissue shear modulus *in vivo*. SWEI is a quantitative ultrasound technique that has been used here to examine changes in brain tissue shear modulus as a function of several experimental and physiological parameters. Animal studies were performed using two different ultrasound transducers to explore the differences between physical response with closed skull and open skull arrangements. *In vivo* intracranial pressure (ICP) in four animal subjects was varied over a relevant physiological range (2-40 mmHg), and was correlated with shear wave speed and stiffness estimates in brain tissue. We found that stiffness does not vary with modulation of ICP. Additional *in vitro* porcine specimens (n=14) were used to investigate variation in brain tissue stiffness with temperature, confinement, spatial location, and transducer orientation. We found a statistically significant decrease in stiffness with increased temperature (23%) and an increase in stiffness with decreasing external confinement (22 - 37%).

This study demonstrated the feasibility of using SWEI to characterize porcine brain tissue both *in vitro* and *in vivo*. Our results underline the importance of temperature and skull derived boundary conditions on brain stiffness and suggests that physiological ranges of ICP do not significantly affect *in situ* brain tissue properties. SWEI allowed for brain material properties to be experimentally-characterized in a physiological setting and provides a stronger basis for assessing brain injury in computational models.

Keywords

Ultrasound; Shear wave elasticity; Acoustic radiation force; Brain stiffness; Intracranial pressure; Confinement

INTRODUCTION

Traumatic brain injury (TBI) has a high incidence of morbidity and mortality in the United States, contributing an estimated 1.7 million hospitalizations annually (Faul et al., 2010).

The highest injury rate is among children and young adults but the highest death rate is among the elderly (Langlois et al., 2006; Xiong et al., 2013). Falls and motor vehicle related accidents are the largest contributors to TBI and several modes of insult have been associated with delayed injury processes that can contribute to irreversible tissue damage (Vink et al., 1988). Tissue level symptoms may present in primary and secondary pathologies including cerebral swelling, diffuse axonal injury, or mild-to-moderate traumatic brain injury (Gennarelli, 1992). We rely on diagnostic, experimental and computational tools to appropriately classify and predict these injuries. Diagnostic imaging modalities, such as computed tomography and magnetic resonance, vividly show us demonstrable changes in brain anatomy and function with severe injuries (Munkeby and Lyng, 2004; Pickett et al., 2001). However, in milder cases, current clinical imaging often shows little or no pathology and is only weakly correlated with clinical outcome (Hammoud and Wasserman., 2002).

To understand these milder injuries, experimental models have been developed to explore relevant pathological conditions and to attempt to reproduce biomechanical response seen in human closed head injury (Anderson et al., 2003; Marmarou et al., 1994). Computational simulations provide insight into the mechanism of the physical response. Modeling of brain tissue may help predict brain injury severity and elucidate the effects of external forces on the brain during and after physical trauma (e.g. Bass et al., 2012; Miller et al., 2000; Shen et al., 2006). Experimental validation of these modeling studies provides the internal properties needed to modulate the behavioral response to that trauma. Taken in tandem, the physiological properties within the computational model may inform brain injury mechanisms that can be used to develop potential modes of therapy (Morrison et al., 2011).

Computational simulations depend on accurate descriptions of the material properties of the brain, but to date, most of the data from published literature has been collected *in vitro*, leaving *in vivo* properties largely unexplored (e.g. Galford and McElhaney, 1970; Miller and Chinzei, 2002; Ommaya, 1968; Prange and Margulies, 2002; Shuck and Advani, 1972). Previously reported *in vitro* complex shear stiffness and relaxation values have been highly variable, spanning roughly three orders of magnitude (Chatelin et al., 2010; Cheng and Bilston, 2007). This variation may partly be attributed to experimental technique and partly attributed to the brain's substantial geometric complexity, which encompasses the presence of structures including meningeal layers, fluid filled ventricles, white and gray matter tracts, sulci, and gyri, all of which present unique challenges to ascertaining accurate mechanical response.

Significant structural and functional diversity has motivated finding more accurate experimental approaches to measure physiological parameters and reduce the variability seen when characterizing brain material properties. Recently, successful preliminary studies with magnetic resonance elastography (MRE) have been used *in vivo* to estimate brain tissue elasticity. However, this technique has met with limitations related to low spatial resolution, long acquisition time, the need for external mechanical coupling, transmission through multiple tissue layers and establishment of standing wave patterns for imaging. (Green et al., 2008; Kruse et al., 2008; Sack et al., 2008). Shear wave elasticity imaging (SWEI) provides an alternate, viable solution to establishing accurate and biofidelic material properties for

brain. SWEI is an acoustic radiation force impulse (ARFI)-based imaging technique that provides the ability to non-invasively and non-destructively assess qualitative and quantitative stiffness (Doherty et al., 2013; Sarvazyan et al., 1998). ARFI-based methods have advantages over MRE in higher spatial and temporal resolution, the ability to focus the shear wave source directly into the tissue of interest, without need of coupling external vibration. Ultrasound is also lower cost and higher accessibility. Clinical studies have yielded consistent information in relatively homogenous tissues including breast, prostate, heart, and cervix (Carlson et al., 2014; Hsu et al., 2007; Li et al., 2009; Svensson and Amiras, 2006; Zhai et al., 2012), and SWEI has gained clinical acceptance for non-invasive staging of fibrosis in the liver (Palmeri et al., 2011, 2008) and experiments have demonstrated hepatic stiffening as a function of portal pressure (Rotemberg et al., 2013, 2012). Shear wave imaging has also recently seen implementation in the brain to determine material properties (Macé et al., 2011). SWEI implementation is novel in a system as structurally complex as the brain and with a catheter array, can be performed with minimal invasion, allowing for direct ultrasound coupling to the brain.

SWEI quantifies transverse wave propagation speed via time-of-flight (TOF) based reconstruction of ultrasonically-tracked data (Palmeri et al., 2008). A feature, such as the maximum displacement or leading edge of a secondary mechanical wave, is identified in displacement-versus-time curves at known lateral positions spatially-offset from the focused region of excitation. The shear wave speed is then estimated assuming a linear relation between the feature arrival time and the lateral positions. After shear wave speed estimation, the relationship between shear wave speed and underlying tissue properties can be inferred by fitting data to a specific mechanical model. Often, isotropy, incompressibility, and linear elasticity are assumed. Under these conditions, the shear modulus (μ) is related to the density (ρ) times the square of the shear wave speed (c_T).

$$c_T = \sqrt{\frac{\mu}{\rho}} \quad (\text{Eqn. 1})$$

SWEI has the substantial benefit over previous methods to characterize brain material properties in that it is able to determine material properties *in vivo*, at physiological temperatures, with minimal disruption to living physiology for large local strain levels. This is in contrast to many of the techniques used to attain the results seen in the literature. Although there have been a few *in vivo* classical mechanics experimental studies (Gefen and Margulies, 2004; Miller et al., 2000), much of the historical work was completed on cored samples at ambient temperature, and sheared under large strains (0.25 – 25.0 %) while synthetically fixed between two platens (Bilston et al., 2001; Fallenstein et al., 1969; Shuck and Advani, 1972). There are also implications to material properties associated with time, temperature, and sample preparation. Fixatives, formaldehyde in particular, stiffens nervous tissue measurably and Dommelen et al., (2009) discusses the viability of fresh brain samples as limited to a 6 hour post-mortem testing window. Cellular breakdown and synthetic molecular cross-linking may comparison of *in vitro* to *in vivo* material properties imprecise.

In the following experimental studies, we used SWEI in the brain to study differences in tissue stiffness with several environmental variables (temperature, transducer orientation, confinement) and physiological variables (intracranial pressure, spatial location). While there is clearly much to examine, we limited our focus to the cerebral and cerebellar lobes. These brain regions represent a large spatial extent, have pathophysiological and clinical significance, abundant data from existing literature allows for comparison of our work to published results (Chatelin et al., 2010).

MATERIALS AND METHODS

Ultrasonic parameters

Shear waves were generated with focused acoustic radiation force (ARF) and radiofrequency (RF) data was collected using a customized Siemens ACUSON™ S2000 scanner (Siemens Medical Systems, Ultrasound group, Issaquah, WA, USA). The system has been modified to allow for user control of acoustic beam sequences and intensities. Two different transducers were used over the course of the experimental studies: For *in situ* and *in vitro* imaging, an ACUSON 14L5 handheld, linear array (footprint: 50 mm x 10 mm) was used and, for the *in vivo* portion of the experiments as well as additional *in situ* and *in vitro* testing, an ACUSON AcuNav 10F catheter-based, phased array (footprint: 10 mm x 3 mm) was used. This smaller array allowed for minimal disruption of the natural *in vivo* setting. Relevant ultrasonic system parameters and transducer specifications are presented in Table 1. Transmit power and the number of transmitted cycles was increased above standard diagnostic ultrasound settings for all ARF excitations to achieve adequate displacement SNR in the brain tissue. Beam sequences specified, with reference to cadaveric and MRI stereotaxic brain data, to acquire images deep to the exterior gray matter layer of the brain (Félix et al., 1999; Saikali et al., 2010). Beam sequences were designed with a single acoustic “push” on the left hand side of the ROI and unidirectional propagation. Shear wave displacements, propagating in the lateral direction, were tracked over an 8 mm x 4 mm region of interest (ROI) with 2 mm axial averaging for 6ms in both array configurations (Figure 3).

Experimental procedures

All experimental animals were obtained from the Duke University vivarium and all procedures were approved by the Duke Institutional Animal Care and Use Committee (IACUC). Three different imaging procedures were used: (1) *in vivo* imaging of anesthetized porcine animal subjects with the AcuNav 10F, (2) *in situ* imaging of cadaveric porcine specimens with the AcuNav 10F and 14L5, (3) *in vitro* imaging of cadaveric porcine specimens using the AcuNav 10F and 14L5. All *in-vitro* and *in-situ* samples were fresh tissue acquisitions and were neither fixed, nor heparinized. Specimens were tested within 4-5 hours post-mortem.

In Vivo

A pig was anesthetized and secured to a spine board in prone position (n=4). Surgery was minimally invasive, with bilateral 5 mm holes drilled through the temporal skull, allowing access to the brain. A catheter pressure transducer (SP-524, Millar Instruments, Houston,

TX, USA) was placed in the first hole. The AcuNav 10F probe was inserted into the epidural space through the second hole. Each hole was then back filled with saline and sealed from atmosphere. (Figure 1). Intracranial pressure (ICP) was modulated above normal by caudal elevation of the animal via the spine board (0° – 34°). Physiological ICP range was initially binned into three ranges: normal baseline ICP (2 mmHg – 8 mmHg), mild ICP increase (15 mmHg – 25 mmHg), and moderate ICP increase (25 mmHg – 40 mmHg). To compare biological response across subjects, normalization to individual baseline was used. Because of orientation of the access holes, cortex B-mode and SWEI images were acquired in sagittal plane.

In Situ

After euthanasia, *in-situ* SWEI images were captured with the entire skull intact, using the AcuNav 10F inserted through each of the access holes and in the same horizontal configuration as used during the *in-vivo* experiment (n=4). For imaging with the 14L5 transducer, a custom experimental setup was constructed. Additional porcine specimens (heads) were acquired from the Duke University vivarium specifically for non-surgical testing *in situ* and *in vitro* (n=9). A craniotomy was performed, creating a 5 cm x 4 cm imaging window. Specimens were tested while submerged in a phosphate buffered saline bath at 37°C to mimic native fluid environment and keep neural tissue from drying out. Images were acquired in coronal and sagittal planes for the cerebral lobe.

In Vitro

Whole brain specimens were removed from the skull intact and were placed in a small dish with saline (unconstrained). To examine temperature effects, specimens were tested at either 22°C or 37°C , by submergence in a phosphate buffered saline bath (n=8). Bath temperature was regulated with a heating coil and continuously recorded with a digital thermometer. After examining temperature effects, later specimens were tested exclusively at 37°C (n=14). All samples were maintained in a warm and humidified environment until necropsy and given approximately 10 minutes to equilibrate to the appropriate temperature (Figure 2). Images were acquired in coronal and sagittal planes for both cerebral and cerebellar lobes (Figure 3).

SWS Estimation and Processing

We used focused acoustic radiation force to remotely insonate our region of excitation and then, with the same transducer, we tracked off-axis tissue motion. All datasets were processed offline using MATLAB (R2012b, Math Works, Natick, MA, USA). Local displacements were calculated by comparing pre-push references and post-push tracks using a Loupas phase-shift estimator (Loupas et al., 1995). A complex correlation threshold value of 0.95 was used to remove inaccurate displacement estimates. A quadratic motion filter was used to minimize the effects of physiological motion (Fahey et al., 2008). Transducer lateral beam-width was calculated from the center operating frequency and excitation focal configurations, as 0.35 mm for AcuNav and 0.21 mm for 14L5. 3-dimensional data sets were averaged over the excitation depth-of-field to yield 2-dimensional displacement versus time curves (Figure 4). Shear wave speeds were estimated with a Radon sum (LATSUM)

projection scheme (Rouze et al., 2010) using an axial kernel of 2.0 mm. Shear wave speeds are calculated as the inverse slope along the vector with the largest sum of local displacements. A 0.5 mm offset is used before applying the Radon summation to insure that the analysis region is outside the shear wave diffraction zone immediately adjacent to the region of excitation (Figure 4). Under isotropic, incompressible, linear elastic assumptions, shear wave speeds were converted to shear modulus by Eqn. 1. Frequency content of shear wave profiles was assessed by Fourier transform of shear wave velocities. For the fundamental frequency range, spectral density curves show -6dB crossings of 300 Hz and 350 Hz for the AcuNav and 14L5 transducers, respectively (Figure 5).

Statistical Analysis

Shear modulus estimates were averaged across repetitions, within each specimen, after determining acquisition order did not affect shear wave speed. Grand means were calculated for each parameter and were compared across all subjects and experimental conditions. Statistical tests were implemented in SYSTAT13 (SysStat, Chicago, IL, USA). Condition for statistical significance was set at $p < 0.05$. Analysis of variance (ANOVA) and co-variance (ANCOVA) were used to compare shear modulus estimates to intracranial pressure levels and confinement conditions. Tukey-Kramer corrected T-tests were used for direct comparisons between individual parameters: (1) coronal vs. sagittal planes, (2) cerebral vs. cerebellar spatial locations, (3) ambient vs. physiological temperature.

RESULTS

Shear modulus is significantly decreased in cortex at physiological temperature, compared to ambient temperature

Experiments on *in vitro* brains yielded a statistically significant decrease in stiffness values with variation in temperature ($p < 0.001$). Testing at 22° C produced a shear modulus of 3.36 ± 0.28 kPa while brains maintained at 37° C yielded a shear modulus of 2.42 ± 0.06 kPa (Figure 6a), suggesting that samples must be tested at physiological temperature to obtain material properties in a lab setting with a tight confidence interval.

Shear modulus does not vary between white matter tracts in the cerebral and cerebellar brain lobes, nor between sagittal and coronal imaging planes within brain regions

To study variation by brain region and directionality, we examined stiffness for the cerebral lobe and the cerebellar lobe independently *in vitro*. Average elasticity values along the anterior-posterior axis (sagittal) were compared to those along the left-right axis (coronal) (Figure 6b). We found no statistical effect on tissue stiffness related to imaging plane for either cerebral or cerebellar brain regions ($p = 0.71$, $p = 0.35$). Mean values and standard deviations are presented in Table 2. Since the differences between coronal and sagittal values within brain regions were not significant, we pooled displacement data from both directions to compare tissue stiffness between spatial locations. Shear modulus values were 2.73 ± 0.63 kPa and 2.76 ± 1.11 kPa for the cerebrum and cerebellum, respectively and were not statistically significantly different ($p = 0.94$).

Removing the brain from confinement within the skull increases tissue stiffness

We pooled data by spatial location to examine the effects of external confinement by the skull. Using the 14L5 transducer, the average shear modulus for cerebral specimens partially constrained within the skull was 3.33 ± 0.22 kPa and for unconstrained specimens was 2.69 ± 0.17 kPa. We also examined confinement effects using the AcuNav 10F transducer in three conditions: *in vivo*, *in situ* (partially constrained), and *in vitro* (unconstrained). The mean shear moduli estimated from the AcuNav 10F are similar to those obtained with the 14L5 transducer for its respective constraining conditions. Average values were 2.44 ± 0.41 kPa, 3.36 ± 1.22 kPa, 2.94 ± 0.93 kPa for *in vivo*, *in situ*, and *in vitro* constraining conditions (Figure 7). *In situ* and *in vitro* conditions measured with the AcuNav showed a 37% and 22% increase in stiffness, respectively, over the *in vivo* condition. From the *in situ* to *in vitro* setting, there was a stiffness decrease of 14% seen in the AcuNav data and a 19% drop seen in the 14L5 data. Fourier transforms of shear wave velocity profiles yielded similar spectral profiles for both transducers. Power spectral density plots of the shear wave velocity profiles yielded major contributions from 200 – 500 Hz. Average center frequency and -12 dB bandwidth for the AcuNav was of 408.8 ± 161.5 Hz and 1065.9 ± 379.4 Hz. Center frequency and -12 dB values for the 14L5 were 302.5 ± 127.7 Hz and 773.1 ± 189.5 Hz (Figure 5). Based on the average center frequency for each of the transducers, average shear wavelengths were calculated as 7.11 ± 1.41 mm for the AcuNav and 5.49 ± 3.06 mm for the 14L5.

Intracranial pressure variation under external constraint does not affect the shear modulus of the cerebral brain

Under closed-skull, physiological conditions, *in vivo* imaging of sagittal plane cortex produced average shear moduli of 2.49 ± 0.18 kPa at normal, baseline ICP levels. Linear regression of tissue stiffness values versus normalized ICP for mild increase (1-3 times) and moderate increase (3-5 times) yielded correlation coefficients that were neither statistically different from each other nor statistically different from a horizontal line. Absolute shear modulus values and p-values for linear regressions are presented in Table 3. Changes in intracranial pressure did not modulate *in vivo* shear modulus (Figure 8) and there is little predictive power of ICP for shear modulus within a physiologically relevant range.

DISCUSSION

This experimental study is the first to use ultrasound imaging to examine the effects of several environmental and physiological parameters on shear modulus in both the laboratory (*in vitro*) and surgical (*in vivo*) setting. Our SWEI technique was able to successfully discern propagating shear waves inside the brain. We achieved realistic tissue stiffness values *in vitro*, *in situ*, and *in vivo* with both the handheld linear array (14L5) and the catheter-based probe (AcuNav 10F). Confinement provided to the brain, by the skull, affected the largest change on tissue stiffness, suggesting that skull confinement affects nonlinear material behavior. We noted a large increase in tissue stiffness after removing the calvarium, but a slight decrease in estimated shear modulus between *in situ* and *in vitro* testing at constant atmospheric pressure. Shear modulus *in situ* and *in vitro* were 37% and 22% higher than *in vivo* moduli (Figure 7). The linear elastic shear modulus values calculated here match the

short-term complex shear modulus terms calculated by Gefen & Margulies (2004). The authors also noted an increase in shear modulus in non-preconditioned tissue from *in vivo* to *in situ* test modes and a subsequent decrease in modulus from *in situ* to *in vitro* test modes. In that study, the authors used a mechanical indentation technique, thus it is encouraging that such disparate experimental designs display similar trends with external constraint. The effect of constraining condition on tissue stiffness shown here in brain may be related to tissue stiffness results published in excised, constrained-versus-unconstrained canine livers (Rotemberg et al., 2012). The authors found that increasing portal pressure in an excised liver while the organ was externally confined, did not lead to increased hepatic stiffening, but under increased portal pressure without confinement, stiffness increased. To elucidate biological similarities, a complimentary study of unconstrained brain under increasing pressure would be necessary.

White matter brain properties show strong changes with temperature (Figure 6a). This result is particularly important because it allowed us to control for a potential confound. Although the effect of temperature is important for biological tissues (e.g. Bass et al., 2007), few studies have been published on its effects. Several studies instead take advantage of the time-temperature superposition principle (Brands et al., 2000; Shen et al., 2006), but this has not been found to be tenable for rheologically complex material such as the brain (Brands et al., 2000). The purpose of this study was to explicitly determine the effect of temperature on mechanical properties measured for brain with particular emphasis on the difference between room temperature and body temperature. Statistically significant stiffening of the sample response was obtained with decreasing temperature. Continuing work will allow us to discriminate detailed temperature dependence of soft tissues with respect to other environmental changes.

Modulation of intracranial pressure under fully constrained conditions did not show significant differences in brain stiffness. It has been suspected that increased brain tissue stiffness following severe traumatic brain injury is an important factor in the chronic elevation of intracranial pressure (Bouma et al., 1992). We hypothesized that brain stiffness would increase with increased ICP, potentially due to compensatory vasodilation; Similar to the changes seen in pressure volume index as a function of cerebral perfusion pressure (CPP) and ICP (Marmarou et al., 1991). However, we observed that the brain did not display significant changes under increased ICP over a physiologically relevant range (Table 3, Figure 8). Our results are similar to Gefen & Margulies (2004) who tracked cerebral perfusion pressure and modulated arterial blood brain pressure/volume flow. They also did not find any difference in shear modulus with CPP change. It is likely that the mechanisms governing CPP, ICP and stiffness change are related but these findings indicate that perfusion pressure does not affect living brain tissue directly. Since brain, ICP and blood are essentially incompressible, in a largely rigid container, the potential for vasodilation to change material properties over short terms may be limited. There remains a paucity of data of this physiological response on brain mechanical properties. Temporal patterns of ICP increase after TBI are not precisely known. An improved understanding of time course of post-traumatic ICP changes would offer an advantage for clinical and research purposes. Secondary pathophysiological effects, including swelling and edema may also cause

changes in material properties. However these aspects are not addressed in this experimental study and continued study is needed to elucidate the relationship among ICP, brain tissue stiffness, and traumatic head injury.

To examine potential anisotropy within and between brain regions, the long axis of the transducer was matched to the long axis of the medium, so that direct comparisons could be made. Qualitative inspection of traditional B-mode images taken with the 14L5 transducer show that each of the four spatially separate sectors, appear structurally distinct (Figure 3); however, average stiffness in each ROI was independent of B-mode appearance and we may not draw conclusions about the stiffness of the substructures contributing to the B-mode image architecture. We could not establish a significant difference in shear modulus between cortical and cerebellar lobes ($p=0.95$). Within each brain region, tissue stiffness was slightly lower in sagittal plane than in coronal plane, but this finding was not statistically significant ($p=0.71$, $p=0.35$). Increasing the sample size ($n=8$) may elucidate the relevance of this finding. We were unable to image the cerebellar lobe while the brain was fully- or partially-constrained because of ultrasonic shadowing from intact occipital skull bone. It is important to consider the frequency range when comparing results to literature and our findings on elastic properties of various brain regions is consistent with results from other groups. Nicolle et al., (2004, 2005) investigated variation within the corona radiata resulting in complex shear moduli of 2.1 – 18.7 kPa for a range of 0.1 – 6310 Hz, but could not find conclusive evidence of anisotropy at small strain or strain rates, comparable to those applied in the current work. Sack et al., (2008) measured average shear moduli of 1.17 – 1.56 kPa over a 25-50 Hz frequency range in humans using MRE. Our conclusions are similar to the ultrasound shear wave imaging paper, Mace et al 2011, who did not find a significant difference comparing sagittal plane (12.9 ± 1.3 kPa) and coronal plane (13.3 ± 2.9 kPa) shear wave imaging in comparable cortical regions and also found similar overall shear modulus values for several difference brain regions including cortex, hippocampus, thalamus. While experimental methodology was similar, our work expanded to include closed skull *in-vivo* mode and unconstrained *in-vitro* mode. We also selected a large animal model comparable to human brain in its gyrencephalic structure. We plan to investigate for property differences between the white matter interior and the gray matter exterior layers of the brain. Neural cell bodies and axons have distinct functions and structures but there is no consensus in the literature on whether one is mechanically different from the other (Kruse et al., 2008). Brain elasticity maps generated from diffusion tensor images indicate that imaging plane is not a direct surrogate for neural tract directionality, and may merit additional study into shear anisotropy associated with myelinated axons (Hrapko et al., 2008; Prange and Margulies, 2002).

Heterogeneous propagation paths can be associated with phase aberration, dispersion and frequency dependence, which act to complicate simple relations derived in the case of lossless, elastic media. This is a potential challenge for SWEI in quantifying elastic moduli of viscoelastic soft tissues and as such, assessing the spectral content of the shear wave profiles is important in the context of phase vs. group velocity calculations. Phase velocity is determined by both elasticity and viscosity, where group velocity is associated with superposition of a group of waves of similar frequency. In non-dispersive materials, the

group and phase velocities are equal but in a viscous tissue, which can be considered anomalous dispersive, the phase velocity differs from the group velocity (Rose, 2004). Examining the spectral content of the shear waves generated by both transducers (Figure 5) and confirming a consistent, narrow band frequency distribution was important because a change in shear wave bandwidth can lead to slight changes in shear wave group velocity. For the associated shear wave frequency range of 200-500 Hz, shear modulus values from our SWEI data match several subsets of published *in vivo* and *in vitro* results (Arbogast and Margulies, 1997; Thibault and Margulies, 1998; Vappou et al., 2008).

Limitations of this study may include the image reconstruction method and choice of mathematical fit. The experimental task of finding a peak becomes more difficult for dispersive media, especially in the presence of noise. Shear wave SNR may be improved by implementing a Bayesian adaptor model or a random sample consensus (RANSAC) algorithm. This technique has shown previous success *in vivo* (Wang et al., 2010). We chose an isotropic, incompressible, linear elastic solid, such that the shear modulus would be proportional to the square of the wave speed. This first study is a hyper-elastic approximation across a small frequency range. While nonlinear models better describe complex behaviors, they are experimentally and computationally costly. Anatomical and mechanical variation at the micron scale of ARF excitation is small. Reduction in complexity can capture simple behavioral response and the isotropic, elastic model has been used successfully to describe several other viscoelastic organs (Rotemberg et al., 2013; Vappou et al., 2006). We appreciate the limitations of applying linear elastic assumptions for organs such as the brain and we have strong incentives to characterize distortion and diminishing shear wave profiles in lossy tissues as well as examine viscoelasticity with higher order mathematical models.

The results of this study indicate that temperature and boundary conditions are important factors and necessary to include in design by both modelers and experimentalists. Since much of the published experimental results are derived from room temperature testing, it should prompt us to reassess standing numerical models, the established values used to derive them, and refocus onto more biofidelic ones. We found a dependency of tissue stiffness on neither spatial location nor transducer orientation. We also found that ICP increase does not result in stiffness change and infer that perfusion has little to no effect on the mechanical properties of *in vivo* brain tissue.

Brain injury is an important public health issue because of the high frequency of incidence, potential for long term functional and behavioral deficits, and possible links to conditions like Alzheimer's (Thurman and Alverson, 1999). It is not currently known how much, if at all, the stiffness of brain tissue changes in disease processes, particularly in degenerative disorders, but there is a substantial need for tools to recognize brain injuries, and to understand and predict associated biological response. Classical mechanics testing of brain has provided an abundance of low rate, *in vitro*, compression or shear loading experimental data. Elastic displacement and velocity estimation techniques based on real-time ultrasound imaging have substantial advantages over classical mechanics testing. SWEI can provide robust and repeatable measurements of tissue strain, useful in generating relative stiffness profiles and absolute mechanical property values. Unlike shear, indentation, or tension/

compression testing, SWEI allows for the maintenance of the organization and continuity all the organs structural elements. The prospect of utilizing a diagnostic ultrasound imaging technique as a means of acquiring accurate *in vivo* material properties in a non-invasive or minimally invasive scenario is novel and important. Shear wave elasticity imaging, with continuing improvements in data acquisition and processing techniques, will find use *in vivo* for the evaluation of local or diffuse pathological conditions in brain tissue.

CONCLUSIONS

This experimental study is the first to use ultrasound imaging to examine the effects of several environmental and physiological parameters on shear modulus in both the laboratory (*in vitro*) and surgical (*in vivo*) setting. Results suggest that skull confinement affects nonlinear material brain behavior: with a 22% increase in shear modulus from *in-vivo* to *in-situ* and 37% from *in-vivo* to *in-vitro*. Decreased testing temperature showed 23% increased stiffening of brain tissue response, but modulation of intracranial pressure under fully constrained conditions did not significantly affect brain tissue stiffness. Our results underline the importance of temperature and skull derived boundary conditions on brain stiffness and suggests that physiological ranges of ICP do not significantly affect *in situ* brain tissue properties. SWEI allowed for brain material properties to be characterized in a physiological setting, which provides a stronger basis for assessing brain injury in computational models.

REFERENCES

- Anderson RWG, Brown CJ, Blumbergs PC, McLean AJ, Jones NR. Impact mechanics and axonal injury in a sheep model. *J. Neurotrauma*. 2003; 20:961–974. [PubMed: 14588113]
- Arbogast K, Margulies S. Regional differences in mechanical properties of the porcine central nervous system. *Proceedings of the 41st Stapp Car Crash Conf*. 1997:293–300.
- Bass CR, Planchak CJ, Salzar RS, Lucas SR, Rafaels KA, Shender BS, Paskoff G. The temperature-dependent viscoelasticity of porcine lumbar spine ligaments. *Spine*. 2007; 32:436–442.
- Bass CR, Panzer MB, Rafaels KA, Wood G, Shridharani J, Capehart B. Brain injuries from blast. *Ann. Biomed. Eng*. 2012; 40:185–202. [PubMed: 22012085]
- Bilston L, Liu Z, Phan-Thien N. Large strain behaviour of brain tissue in shear: some experimental data and differential constitutive model. *Biorheology*. 2001; 38:335–345. [PubMed: 11673648]
- Bouma GJ, Muizelaar JP, Bando K, Marmarou A. Blood pressure and intracranial pressure-volume dynamics in severe head injury: relationship with cerebral blood flow. *J. Neurosurg*. 1992; 77:15–19. [PubMed: 1607958]
- Brands D, Bovendeerd P, Peters GWM, Wismans JS. Large Shear strain dynamic behavior of in vitro porcine brain tissue and a silicone gel model material. *Stapp Car Crash J*. 2000; 44:249–260. [PubMed: 17458730]
- Carlson LC, Feltovich H, Palmeri ML, Dahl JJ, Munoz Del Rio A, Hall TJ. Estimation of shear wave speed in the human uterine cervix. *Ultrasound Obstet. Gynecol*. 2014; 43:452–458.
- Chatelin S, Constantinesco A, Willinger R. Fifty years of brain tissue mechanical testing: from in vitro to in vivo investigations. *Biorheology*. 2010; 47:255–276. [PubMed: 21403381]
- Cheng S, Bilston LE. Unconfined compression of white matter. *J. Biomech*. 2007; 40:117–124. [PubMed: 16376349]
- Doherty J, Trahey G, Nightingale K, Palmeri M. Acoustic Radiation Force Elasticity Imaging in Diagnostic Ultrasound. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control*. 2013; 60:685–701. [PubMed: 23549529]

- Dommelen J, Van Hrapko, M. Peters GWM. Mechanical properties of brain tissue: characterisation and constitutive modelling. *Mechanosensitivity of the Nervous System*. 2009:249–279.
- Fahey BJ, Hsu SJ, Trahey GE. A novel motion compensation algorithm for acoustic radiation force elastography. 2008; 55:1095–1111.
- Fallenstein G, Hulce V, Melvin J. Dynamic mechanical properties of human brain tissue. *J. Biomech.* 1969; 2:217–226. [PubMed: 16335085]
- Faul M, Xu L, Wald M, Coronado V. Traumatic brain injury in the United States: emergency department visits, hospitalizations and deaths 2002–2006. *Centers Dis. Control Prev. Natl. Cent. for Inj. Prev. Control*. 2010
- Félix B, Léger ME, Albe-Fessard D, Marcilloux JC, Rampin O, Laplace JP. Stereotaxic atlas of the pig brain. *Brain Res. Bull.* 1999; 49:1–137. [PubMed: 10466025]
- Galford J, McElhaney J. A viscoelastic study of scalp, brain, and dura. *J. Biomech.* 1970; 3:211–221. [PubMed: 5521539]
- Gefen A, Margulies SS. Are in vivo and in situ brain tissues mechanically similar? *J. Biomech.* 2004; 37:1339–1352. [PubMed: 15275841]
- Gennarelli TA. Mechanisms of brain injury. *J. Emerg. Med.* 1992; 11:5–11. [PubMed: 8445204]
- Green MA, Bilston LE, Sinkus R. In vivo brain viscoelastic properties measured by magnetic resonance elastography. *NMR Biomed.* 2008; 21:755–764. [PubMed: 18457350]
- Hammoud DA, Wasserman BA. Diffuse axonal injuries: pathophysiology and imaging. *Neuroimaging Clin. N. Am.* 2002; 12:205–216. [PubMed: 12391632]
- Hrapko M, Dommelen JAW, Van Peters, G.W.M. Wismans JSHM. Characterisation of the mechanical behaviour of brain tissue in compression and shear. *Biorheology*. 2008; 45:663–676. [PubMed: 19065013]
- Hsu SJ, Bouchard RR, Dumont DM, Wolf PD, Trahey GE. In vivo assessment of myocardial stiffness with acoustic radiation force impulse imaging. *Ultrasound Med. Biol.* 2007; 33:1706–1719. [PubMed: 17698282]
- Kruse S, Rose GH, Glaser KJ, Manduca A, Felmlee JP, Ehman RL. Magnetic resonance elastography of the brain. *Neuroimage*. 2008; 39:231–237. [PubMed: 17913514]
- Langlois JA, Rutland-brown W, Wald MM. The Epidemiology and Impact of Traumatic Brain Injury A Brief Overview. *J Head Trauma Rehabil.* 2006; 21:11–14.
- Li C, Duric N, Littrup P, Huang L. In vivo breast sound-speed imaging with ultrasound tomography. *Ultrasound Med. Biol.* 2009; 35:1615–1628. [PubMed: 19647920]
- Loupas T, Peterson RB, Gill RW. Experimental evaluation of velocity and power estimation for ultrasound blood flow imaging, by means of a two-dimensional autocorrelation approach. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control*. 1995; 42:689–699.
- Macé E, Cohen I, Montaldo G, Miles R, Fink M, Tanter M. In vivo mapping of brain elasticity in small animals using shear wave imaging. *IEEE Trans. Med. Imaging*. 2011; 30:550–558. [PubMed: 20876009]
- Marmarou A, Anderson RL, Ward JD, Choi SC, Young HF, Eisenberg HM, Foulkes MA, Marshall LF, Jane JA. Impact of ICP instability and hypotension on outcome in patients with severe head trauma. *J Neurosurg.* 1991; 75:559–566. [PubMed: 1653309]
- Marmarou A, Foda M, Brink W. A new model of diffuse brain injury in rats: Part I: Pathophysiology and biomechanics. *J. Neurosurg.* 1994; 80:291–300. [PubMed: 8283269]
- Miller K, Chinzei K, Orsengo G, Bednarz P. Mechanical properties of brain tissue in-vivo: experiment and computer simulation. *J. Biomech.* 2000; 33:1369–1376. [PubMed: 10940395]
- Miller K, Chinzei K. Mechanical properties of brain tissue in tension. *J. Biomech.* 2002; 35:483–490. [PubMed: 11934417]
- Morrison B, Elkin BS, Dollé J-P, Yarmush ML. In vitro models of traumatic brain injury. *Annu. Rev. Biomed. Eng.* 2011; 13:91–126. [PubMed: 21529164]
- Munkeby B, Lyng K. Morphological and hemodynamic magnetic resonance assessment of early neonatal brain injury in a piglet model. *J. Magn. Reson. Imaging*. 2004; 20:8–15. [PubMed: 15221803]

- Nicolle S, Lounis M, Willinger R. Shear Properties of Brain Tissue over a Frequency Range Relevant for Automotive Impact Situations: New Experimental Results. *Stapp Car Crash J.* 2004; 48:239–258. [PubMed: 17230269]
- Nicolle S, Lounis M, Willinger R, Palierne J-F. Shear linear behavior of brain tissue over a large frequency range. *Biorheology.* 2005; 42:209–223. [PubMed: 15894820]
- Ommaya AK. Mechanical properties of tissues of the nervous system. *J. Biomech.* 1968; 1:127–138. [PubMed: 16329300]
- Palmeri ML, Wang MH, Dahl JJ, Frinkley KD, Nightingale KR. Quantifying hepatic shear modulus in vivo using acoustic radiation force. *Ultrasound Med. Biol.* 2008; 34:546–558. [PubMed: 18222031]
- Palmeri ML, Wang MH, Rouze NC, Abdelmalek MF, Guy CD, Moser B, Diehl AM, Nightingale KR. Noninvasive evaluation of hepatic fibrosis using acoustic radiation force-based shear stiffness in patients with nonalcoholic fatty liver disease. *J. Hepatol.* 2011; 55:666–672. [PubMed: 21256907]
- Pickett W, Ardern C, Brison RJ. A population-based study of potential brain injuries requiring emergency care. *C. Can. Med. Assoc. J.* 2001; 165:288–292.
- Prange M, Margulies S. Regional, directional, and age-dependent properties of the brain undergoing large deformation. *J Biomech Eng.* 2002; 124:244–252. [PubMed: 12002135]
- Rose, JL. Ultrasonic waves in solid media. Cambridge University Press; 2004.
- Rotemberg V, Palmeri M, Nightingale R, Rouze N, Nightingale K. The impact of hepatic pressurization on liver shear wave speed estimates in constrained versus unconstrained conditions. *Phys. Med. Biol.* 2012; 57:329–341. [PubMed: 22170769]
- Rotemberg V, Byram B, Palmeri M, Wang M, Nightingale K. Ultrasonic characterization of the nonlinear properties of canine livers by measuring shear wave speed and axial strain with increasing portal venous pressure. *J. Biomech.* 2013; 46:1875–1881. [PubMed: 23726184]
- Rouze NC, Wang MH, Palmeri ML, Nightingale KR. Robust estimation of time-of-flight shear wave speed using a radon sum transformation. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control.* 2010; 57:2662–2670. [PubMed: 21156362]
- Sack I, Beierbach B, Hamhaber U, Klatt D, Braun J. Noninvasive measurement of brain viscoelasticity using magnetic resonance elastography. *NMR Biomed.* 2008; 21:265–271. [PubMed: 17614101]
- Saikali S, Meurice P, Sauleau P, Eliat P-A, Bellaud P, Randuineau G, Vérin M, Malbert CH. A three-dimensional digital segmented and deformable brain atlas of the domestic pig. *J. Neurosci. Methods.* 2010; 192:102–109.
- Sarvazyan A, Rudenko O, Swanson S, Fowlkes J, Emelianov S. Shear Wave Elasticity Imaging: A New Ultrasonic Technology of Medical Diagnostics. *Ultrasound Med. Biol.* 1998; 24:1419–1435. [PubMed: 10385964]
- Shen F, Tay TE, Li JZ, Nigen S, Lee PVS, Chan HK. Modified Bilston nonlinear viscoelastic model for finite element head injury studies. *J. Biomech. Eng.* 2006; 128:797–801. [PubMed: 16995770]
- Shuck L, Advani S. Rheological response of human brain tissue in shear. *J. Basic Eng.* 1972; 94:905.
- Svensson WE, Amirás D. Ultrasound elasticity imaging. *Breast Cancer Online.* 2006; 9:1–7.
- Thibault KL, Margulies SS. Age-dependent material properties of the porcine cerebrum: effect on pediatric inertial head injury criteria. *J. Biomech.* 1998; 31:1119–1126. [PubMed: 9882044]
- Thurman D, Alverson C. Traumatic brain injury in the United States: a public health perspective. *J. Head Trauma Rehabil.* 1999; 14:602–615. [PubMed: 10671706]
- Vappou J, Willinger R, Breton E, Choquet P, Goetz C, Constantinesco A. Dynamic viscoelastic shear properties of soft matter by magnetic resonance elastography using a low-field dedicated system. *J. Rheol.* 2006; 50:531–541.
- Vappou J, Breton E, Choquet P, Willinger R, Constantinesco A. Assessment of in vivo and postmortem mechanical behavior of brain tissue using magnetic resonance elastography. *J. Biomech.* 2008; 41:2954–2959. [PubMed: 18805534]
- Vink R, McIntosh TK, Demediuk P, Weiner MW, Faden AI. Decline in intracellular free Mg²⁺ is associated with irreversible tissue injury after brain trauma. *J. Biol. Chem.* 1988; 263:757–761. [PubMed: 3335524]

- Wang MH, Palmeri ML, Rotemberg VM, Rouze NC, Nightingale KR. Improving the robustness of time-of-flight based shear wave speed reconstruction methods using RANSAC in human liver in vivo. *Ultrasound Med. Biol.* 2010; 36:802–813. [PubMed: 20381950]
- Xiong Y, Mahmood A, Chopp M. Animal models of traumatic brain injury. *Nat. Rev. Neurosci.* 2013; 14:128–142. [PubMed: 23329160]
- Zhai L, Polascik T, Foo W, Rosenzweig S, Palmeri M, Madden J, Nightingale K. Acoustic Radiation Force Impulse Imaging of Human Prostates: Initial in vivo demonstration. *Ultrasound Med. Biol.* 2012; 38:50–61. [PubMed: 22104533]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1. Fluoroscopic image of *in vivo* instrumentation within porcine animal subject skull. Red arrow indicates placement of Millar pressure sensor and blue arrow indicates location of AcuNav 10F catheter probe.

Figure 2.

In order to minimize the potential for temperature gradient across the tissue from the surface to the center of the brain, all of the samples were acquired fresh and kept in a warm / humidified enclosed space until necropsy. This enabled us to keep the specimen internal temperature relatively high and decrease the necessary equilibration time.

Figure 3.

B-mode images taken with the 14L5 displays the geometric complexity of the brain, including presence of gyri, sulci, and fluid filled ventricles. We examined stiffness for the cortical and cerebellar lobes independently *in vitro* in both the coronal and sagittal directions. The entire imaging window for the 14L5 transducer is visible here. The overlaid red box outlines the 4 mm x 4 mm ROI over which we tracked shear wave propagation.

(A)

(B)

Figure 4.

We used Acoustic Radiation Force (ARF) to remotely insonate the region of excitation and generate shear waves. The same transducer tracked shear wave propagation. shown are axial displacement-versus-time curves for five select lateral locations, with 2 mm axial averaging extending 2 –4 mm to the right of the push location for 4 - 5 ms. Shear wave speeds were estimated by lateral summation algorithm that uses a Radon projection, averaging axially 2mm. A 0.5 mm offset (Lat_{start}) is used before applying the Radon summation to insure that the analysis region is outside the shear wave diffraction zone immediately adjacent to the region of excitation. The maximum sum identifies the optimal trajectory between time and lateral extents. (A) *In vivo* case from the AcuNav 10F array, (B) *In vitro* case from the 14L5 array.

Figure 5. Spectral density curves for the frequency content of shear wave velocity (A) for the AcuNav transducer and (B) for the 14L5 transducer after filtering with a quadratic motion filter. Frequency ranges for the fundamental and its harmonics are visible.

(A)

(B)

Figure 6.

(A) Shear moduli reconstructed from sagittal scans from the cortical midbrain region of interest in the *in vitro* setting with the 14L5 reveal substantial decrease in stiffness as well as stiffness variance with increasing temperature. ($p < 0.001$). (B) For unconstrained specimens, *in vitro*, no difference in shear modulus was noted between cortical and cerebellar lobes ($p = 0.95$). Within each region, tissue stiffness tended to decrease from coronal to sagittal planes; however, this finding was not statistically significant ($p = 0.71$, $p = 0.35$).

Figure 7. Decreasing external brain constraint saw 37% and 22% increases in stiffness over *in vivo* condition. For specimens examined with the Acunav ($p < 0.0001$). For data collected with the 14L5 ($p < 0.02$). No statistical difference was detected between the two transducers used in the experiments.

Figure 8.

Across all subjects we did not see any visible effect in shear modulus with increased intracranial pressure. For the physiologically relevant range (2-40 mmHg) all linear regression lines were statistically indistinguishable from horizontal, indicating shear modulus is not affected by ICP.

Table 1

Important ultrasonic scanner (Siemens S2000) and transducer (Siemens 14L5, AcuNav 10F) parameters.

Probe	Linear (14L5)	AcuNav 10F
Ultrasound Scanner	S-2000	S-2000
Push Frequency	7.27 MHz	6.15 MHz
Track Frequency	6.15 MHz	6.15 MHz
Push F#	1.0	1.4
Push Focal Depth (axial)	5.5 mm	10 mm
Push Cycles	500	400
Push Duration	100 μ s	80 μ s
Lateral Positions	34	41
Beam Spacing	0.15 mm	0.23 mm
Pulse Repetition Frequency	13,298 Hz	16,234 Hz

Table 2

Shear Modulus values (kPa) plus/minus standard deviations calculated from shear wave velocities in the 14L5 for porcine samples *in situ* and *in vitro* in both the coronal and sagittal planes. Shear modulus values decreased significantly between *in situ* and *in vitro* experiments ($p < .05$), but shear modulus between imaging plane and brain region were not statistically different. We were unable to image the cerebellum *in situ* due to ultrasonic shadowing from the skull.

	<u>Cerebrum</u>		<u>Cerebellum</u>	
	Coronal	Sagittal	Coronal	Sagittal
In Situ (Partially Constrained)	3.59±0.41	3.07±1.08	--	--
In Vitro (Unconstrained)	2.81±0.59	2.66±0.31	3.07±1.99	2.36±0.28

Table 3

In vivo tissue stiffness for mild and moderate ICP increase. Values are presented as mean $\pm$ standard deviation. P-values indicate poor predictive power of ICP for shear modulus within the physiologically relevant range.

Animal #	Baseline ICP (mmHg)	Shear Modulus Values at Each ICP Level (kPa)			ICP dependence (p-value)
		Baseline ICP	Mild ICP Increase	Moderate ICP Increase	
1	6.33 $\pm$ 0.74	2.59 $\pm$ 0.05	2.61 $\pm$ 0.11	2.60 $\pm$ 0.09	0.679
2	5.08 $\pm$ 2.08	2.29 $\pm$ 0.23	2.23 $\pm$ 0.13	2.30 $\pm$ 0.12	0.327
3	20.64 $\pm$ 0.34	2.71 $\pm$ 0.26	2.67 $\pm$ 0.44	2.68 $\pm$ 0.18	0.811
4	6.80 $\pm$ 1.53	2.39 $\pm$ 0.59	2.64 $\pm$ 1.06	1.80 $\pm$ 0.35	0.237