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Brain stiffens post mortem

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ABSTRACT

Alterations in brain rheology are increasingly recognized as a diagnostic marker for various neurological conditions. Magnetic resonance elastography now allows us to assess brain rheology repeatably, reproducibly, and non-invasively in vivo. Recent elastography studies suggest that brain stiffness decreases one percent per year during normal aging, and is significantly reduced in Alzheimer's disease and multiple sclerosis. While existing studies successfully compare brain stiffnesses across different populations, they fail to provide insight into changes within the same brain. Here we characterize rheological alterations in one and the same brain under extreme metabolic changes: alive and dead. Strikingly, the storage and loss moduli of the cerebrum increased by 26% and 60% within only three minutes post mortem and continued to increase by 40% and 103% within 45 minutes. Immediate post mortem stiffening displayed pronounced regional variations; it was largest in the corpus callosum and smallest in the brainstem. We postulate that post mortem stiffening is a manifestation of alterations in polarization, oxidation, perfusion, and metabolism immediately after death. Our results suggest that the stiffness of our brain–unlike any other organ–is a dynamic property that is highly sensitive to the metabolic environment. Our findings emphasize the importance of characterizing brain tissue in vivo and question the relevance of ex vivo brain tissue testing as a whole. Knowing the true stiffness of the living brain has important consequences in diagnosing neurological conditions, planning neurosurgical procedures, and modeling the brain's response to high impact loading.

1. Introduction

With less than 3% of our body weight, our brain consumes 15% of cardiac output, 20% of oxygen, and 25% of total body glucose (Mergenthaler et al., 2013). It seems intuitive that our brain, more than any other organ, is highly sensitive to alterations in its biochemical environment. Here we probe the brain's sensitivity to biochemical alterations and expose a single brain to the most extreme change in metabolic conditions, from alive to dead. We characterize changes in brain rheology within three minutes post mortem using magnetic resonance elastography (Muthupillai et al., 1995), a rapidly developing technology that allows us to quantify the viscoelasticity of the living brain, repeatably, reproducibly, and non-invasively in vivo and in situ (Muthupillai and Ehman, 1996). Magnetic resonance elastography generates shear waves in soft tissues, images wave propagation, and correlates wave propagation to tissue stiffness in the form of elastograms (Kruse et al., 2000). While the technology was initially developed to identify regional stiffness variations and detect tumors in breast, liver, kidney, and prostate cancer (Mariappan et al., 2010), it is now widely used to characterize stiffness profiles across various living tissues including the breast, the heart, kidneys, lung, skeletal muscle, and the brain (Glaser et al., 2012). With increasing confidence in the method itself, elastography is emerging as a diagnostic biomarker for various neurological conditions (Hiscox et al., 2016). Recent studies have reported an annual stiffness decay of 0.8% during normal aging (Sack et al., 2009), and a stiffness reduction of 20% in chronic progressive multiple sclerosis (Enzinger et al., 2015) and 8% in Alzheimer's disease (Murphy et al., 2011). Its inherent high regional specificity makes elastography uniquely suited to differentiate between specific subtypes of dementia including Alzheimer's disease, frontotemporal dementia, normal pressure hydrocephalus, and dementia with Lewy bodies (ElSheikh et al., 2017).

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Overwhelming evidence suggests that elastography can quantify significant differences between certain subject groups (Sack et al., 2011; Streitberger et al., 2012; Arvin et al., 2015; Murphy et al., 2016). However, to serve as a truly diagnostic tool, the technology seems to lack a thorough verification and validation. Elastography recordings depend on multiple factors including the method to generate shear waves (Badachhape et al., 2017), the selection of the actuation frequency (Hiscox et al., 2016), the inversion technique to extract the dynamic moduli (Johnson and Telzer, 2017), and, more recently, the analysis of physiological vibration (Zorgani et al., 2015). Few studies have calibrated their elastography measurements against phantom gels with known properties (Atay et al., 2008; Kruse et al., 2008) or against finite element simulations (Miller et al., 2000; Bayly et al., 2012). Yet, to date, there is no consistent comparison of in vivo and in situ elastography recordings with ex vivo measurements on one and the same sample. The only study that directly compared elastography to standard mechanical testing compared ex vivo elastography to ex vivo rheometry on explanted brains at frequencies that differed by two orders of magnitude (Vappou et al., 2008). The only study that compared same-sample measurements in vivo and in situ perturbed the natural environment by removing the skull to access the brain for indentation testing (Gefen and Margulies, 2004).

Comparing in vivo recordings to ex vivo measurements seems to be critical to correctly interpret the ex vivo stiffness values of brain tissue that have been recorded and reported for more than half a century (Chatelin et al., 2010). Despite comprehensive efforts to characterize the rheology of the brain ex vivo (Franze et al., 2013), the literature continues to provide contradictory results with respect to tissue anisotropy (Feng et al., 2013a; Budday et al., 2015), regional variations (Prange and Margulies, 2002; Forte et al., 2017), gray and white matter stiffness ratios (Christ et al., 2010; Weickenmeier et al., 2016), and stiffness alterations post mortem (Budday et al., 2015; Nicolle et al., 2004). These ongoing controversies point towards an urgent need to better understand the mechanistic origin of measurement discrepancies. Knowing the in vivo stiffness of the living brain has important consequences for understanding brain form, function, and failure (Goriely et al., 2015). The stiffness is the most critical input parameter for various computational brain models to predict safety level thresholds (Cloots et al., 2013), simulate surgical procedures (Goriely et al., 2016), model blast and impact situations (Cotton et al., 2016), and design protective devices (Nyein et al., 2010).

Here we compare the in vivo, in situ, and ex vivo rheology of one and the same brain under similar dynamic conditions. We recorded the region-specific in vivo and in situ brain viscoelasticity using elastography at five activation frequencies, 40, 60, 80, 90, and 100 Hz, in five distinct regions of the brain, the cerebrum, cerebellum, corpus callosum, thalamus, and brainstem, and compared the results to the ex vivo viscoelasticity from nanoindentation in the same frequency regime. In addition to the mechanical characterization, we performed a histochemical analysis to reveal a mechanistic correlation between stiffness and myelin density. Finally, we converted our recordings to the region-specific parameters of four widely used viscoelastic models, the Maxwell, Voigt, spring-damper, and standard linear solid models.

2. Materials and methods

This study was approved by the Research Compliance Office at Stanford University. It complies with IRB and Animal Care and Use guidelines and was conducted according to Stanford University policy. To characterize regional mechanical and microstructural changes in the living and dead porcine brain–in vivo, in situ, and ex vivo–we combined magnetic resonance elastography, dynamic nanoindentation, and a histochemical analysis.

Animal preparation

Our study is based on one and the same brain of a 50.2 kg female Yorkshire pig. Prior to scanning, we sedated the animal with 6 mg/kg intramuscular tiletamine zolazepam (Telazol, Fort Dodge, IA), which we induced with 3% isoflurane (Henry Schein Animal Health Dublin, OH) in oxygen delivered by a face mask. We intubated the trachea with an endotracheal tube, 8 mm in diameter, and maintained anesthesia with 1% to 3% isoflurane in oxygen using mechanical ventilation. To administer drugs and monitor the systemic arterial blood pressure, we placed percutaneous catheters in the left femoral vein and artery. We monitored heart rate, electrocardiogram, pulse oximetry, and end-tidal CO2 throughout the imaging sequence. Throughout anesthesia, we intravenously administered lactated Ringer's solution (Abbott Laboratories, Deerfield, IL) at a rate of 10 to 15 mL/kg per hour to compensate for fluid loss. We performed magnetic resonance elastography in vivo, then euthanized the animal with Euthanasia Solution (Vedco, Inc., St. Joseph, MO), confirmed death by an electrocardiogram and auscultation, placed the animal back into the scanner, and performed post mortem magnetic resonance elastography in situ.

Magnetic resonance elastography

For magnetic resonance elastography, we used the 3 T research scanner (GE Healthcare, Waukesha, WI) at the Richard M. Lucas Center for Imaging at Stanford University following our established imaging protocols (Weickenmeier et al., 2018). To accommodate the full pig head and activation pillow, we used a single-channel split head coil and positioned the animal in the supine position for optimal transmission of the mechanical activation. We placed the acoustic passive actuator (Mayo Clinic, Rochester, MN) underneath the pig's head and connected the internal activation pillow to an external acoustic active actuator to generate continuous vibrations at a selected frequency (Kruse et al., 2008). These vibrations transmit through the skull and induce shear waves inside the brain. We used three-dimensional magnetic resonance elastography and imaged the wave field using three directions of motion encoding at multiple time points in the wave cycle. We acquired the three-dimensional data with a 48-slice two-dimensional spin echo-planar imaging pulse sequence in the axial plane, at a resolution of 96 × 96, two shots, at a repetition time of TR = 2000 ms, an echo time of TE = 60 ms, and a field of view of FOV = 24 cm, using an array spatial sensitivity encoding technique, and applied a full three-dimensional inversion algorithm (Murphy et al., 2017). To ensure a synchronized receiver signal, we set the frequency of the motion encoding gradient equal to the actuation frequency. We acquired phase images at eight offset points sampled over one period of motion, and determined the elastograms from these images using three-dimensional direct inversion (Murphy et al., 2013; Manduca et al., 2001). For each actuation frequency ω, we determine the isotropic storage and loss moduli $G'(\omega)$ and $G''(\omega)$ and map the frequency-dependent dynamic shear modulus $G(\omega)$,

$$G(\omega) = G'(\omega) + i\, G''(\omega),$$

and the effective shear stiffness $G^{\text{eff}}(\omega)$,

$$G^{\text{eff}}(\omega) = [\, G'(\omega)^2 + G''(\omega)^2 \,]^{1/2}.$$

In total, we performed a structural T1-weighted scan, in vivo elastography at five frequencies, 40, 60, 80, 90, and 100 Hz, and in situ elastography three and 45 min post mortem at a frequency of 80 Hz.

Region-specific moduli

To assign the elastography measurements to specific brain regions, we performed a semi-automatic segmentation from T1-weighted magnetic resonance images using Simpleware (Synopsys, Mountain View, USA). To identify individual brain regions, we combined initial grey-scale thresholding, manual segmentation, and volumetric Gaussian