# Frequency Dependence of Mouse Brain Tissue Stiffness Measured in vivo with MR Elastography

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**Introduction** Magnetic resonance elastography (MRE) is a non-invasive imaging technique for quantitative measurement of the mechanical properties of biologic tissue *in vivo* [1]. The clinical interest in MRE has largely been driven by the direct relationship between tissue health and stiffness. As a result, MRE may provide significant clinical value for the non-invasive diagnosis of pathology and response to therapy by tracking tumor development and monitoring therapeutic response. MRE may also have considerable value in the development of treatment protocols in pre-clinical, rodent models of cancer. Because of cost and versatility, the mouse, in particular, is widely employed in oncologic studies. To resolve its small anatomic features, MRE experiments in mice must be performed with high driving frequencies (>600 Hz). However, high-frequency waves exhibit increased attenuation, reducing wave penetration depth and making it more difficult to impart motion deep into tissue with sufficient amplitude to overcome background noise. Also, biologic tissue is viscoelastic; hence, its response to load depends on the driving frequency. Recent MRE studies in mouse brain have been performed in high-field scanners (7 - 11.7T) at single driving frequencies of 1000 and 1200 Hz [2,3]. Here, we perform elastography in mouse brain tissue at 4.7T and report viscoelastic material properties over a range of driving frequencies (600 - 1800 Hz).

**Methods** Acquisition: All experiments were conducted at 4.7T using a Varian DirectDrive™ small-animal MR scanner. The scanner is built around an Oxford horizontal-bore magnet, and is equipped with Magnex high-performance gradients and IEC gradient power amplifiers. MRE data were collected using an actively decoupled surface (receive)/volume (transmit) coil pair. Six female nude mice, 7 to 8 weeks-old, (Hsd:Athymic Nude-Foxn1$^{nu}$, Harlan) were studied. All procedures were approved by the institutional Animal Studies Committee in accordance to the NIH Guide on the Care and Use of Animals. A custom-built stereotaxic head holder incorporated a piezoceramic-actuated (APA100M-NM, Cedrat Technologies) bite bar to introduce propagating shear waves into the brain (Fig 1, top). A TTL-equipped function generator and low-current voltage amplifier were used to drive the actuated bite bar harmonically at 600, 800, 1200, and 1800 Hz. Motion-encoding gradients were synchronized with actuator vibration to encode an isochromatic phase shift proportional to tissue displacement, at four time points, using a custom phase-locked spin-echo pulse sequence [2]. Two sets of motion-encoded data were acquired; one each with positive and negative polarity motion encoding gradients. The entire mouse brain was imaged with 29 contiguous trans-axial slices. The acquired in-plane resolution was 250 x 250 μm$^2$; the slice thickness was 500 μm. TR/TE: 1000/27.5 ms. In order to hold TR/TE constant for all data sets, the number of MR motion encoding cycles ($N_{MS}$) varied with driving frequency. $N_{MS}$ = 4, 5, 8, and 10 for 600 Hz, 800 Hz, 1200 Hz, and 1800 Hz, respectively. Sinusoidal, motion encoding gradients with amplitudes of 18 G/cm were used at all driving frequencies. In this study, only through-plane $u_z(x,y)$ displacements were sensitized. Data processing: Data from a central region of the brain were interpolated in all three dimensions to yield isotropic voxels that were 125 μm on a side. Motion-sensitized, phase-contrast images were obtained by complex division of positive and negative polarity phase images. Parasitic phase wrapping was removed via commercial software (Phase Vision Ltd, Loughborough, UK). Phase- contrast data were converted into displacements, $u_z(x,y,t)$, by standard methods and the fundamental harmonic coefficient $U_z(x,y,\omega)$ was extracted by Fourier transform along the time dimension. Both real and imaginary parts of $U_z(x,y,\omega)$ were retained. $U_z(x,y,\omega)$ was smoothed with a circular, 2$^{nd}$-order Butterworth bandpass filter (in: 0.158 mm$^{-1}$, out: 0.630 mm$^{-1}$) in the spatial frequency domain. A central difference scheme was used to approximate the Laplacian; $\Delta^2U_z(x,y,\omega)$. Inversion of the linear isotropic homogeneous material equation of motion, $(G' + iG'')\Delta^2U_z(x,y,\omega) = -\rho\omega^2U_z(x,y,\omega)$, was performed by local least-squares fit. For each voxel, the complex modulus was found that minimized the squared error between this equation and data from a 3-D kernel surrounding that voxel. The residual error of each fit, normalized by the variance in that kernel, was calculated to assess the "goodness-of-fit" of the linear isotropic homogeneous material model at that location. A normalized residual error (NRE) of zero indicates a perfect local fit; a residual of 1.0, a poor fit. All modulus approximations were thresholded; if the local model fit produced an NRE > 0.5, the estimated modulus was discarded.

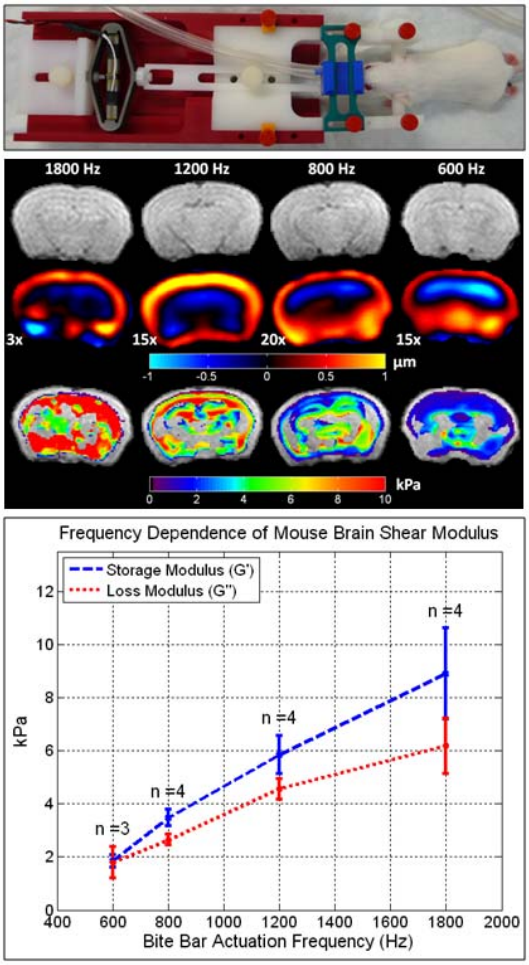


**Figure 1: (Top) Custom head holder with piezo-actuated bite bar introduces shear waves into brain; (Middle) Images of anatomy, wave displacement (Re{$U_z(x,y,\omega)$}) with scaling factor, and storage modulus elastogram at 4 different driving frequencies; (Bottom) Mean storage and loss moduli as a function of actuation frequency; bars indicate standard deviation.**

**Results** Standing wave fields shown in Figure 1 (middle) indicate motion initiating from the actuated bite bar penetrated with sufficient amplitude into the brain at all driving frequencies. Note that the wavelength decreases as the actuation frequency increases. However, subtle evidence of material stiffening with increasing frequency is evident in the wave images, since driving frequency and wavelength are inversely proportional for a material with constant density and stiffness. One would expect a 3:1 ratio in wavelength between driving frequencies of 600 and 1800 Hz. Representative storage modulus elastograms quantitatively depict global stiffening within brain tissue as frequency increases. Evidence of a strong frequency dependence in the average storage (G') and loss (G") moduli of brain tissue is shown in Figure 1 (bottom). Variability in the mean modulus increases with driving frequency; however, this does not invalidate the observed trend.

**Discussion** Results indicate mouse brain tissue exhibits pronounced frequency-dependent stiffening from 600-1800 Hz. This stiffening hinders efforts to increase the spatial resolution of MRE elastograms, since wavelength does not shorten in direct inverse proportion to frequency. However, the frequency dependency itself highlights dynamic properties of brain tissue subjected to high strain rates, and may provide an additional MRE-based diagnostic marker. Future work will assess the sensitivity of tissue viscoelasticy to disease or injury and therapeutic treatment over this actuation bandwidth.

**References** [1] Muthupillai et al., Science, 1995, 269:1854-57; [2] Atay et al., J Biomech Eng., 2008, 130(2):21013. [3] Diguet et al., Proc ISMRM, 2009, 714.