MATERIAL PROPERTIES OF THE DEVELOPING PORCINE BRAIN

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ABSTRACT

The objective of this project is to quantify the age-dependent material properties of porcine brain tissue and to correlate them with structural alterations associated with growth and development. Samples of frontal cortex from neonatal (2-3 days) and adult pigs were harvested and tested within 3 hours post-mortem. The complex shear modulus of the samples was measured in a custom-designed oscillatory shear testing device. Samples were tested at a shear strain amplitude of 2.5% from 20-200 Hz at 25°C and 100% humidity. The elastic and viscous components of the complex shear modulus change significantly with the development of the cortical region of the brain. These changes in material properties correlate with increases in myelin, brain mass, total cell number, and a decrease in water content. This project is the first step in developing head injury tolerance criteria for the infant and young child.

SEVERE HEAD INJURY is the most frequent cause of death in the pediatric population (Weston 1968, Kraus et al. 1987), comprising 50% to 80% of all trauma-related deaths each year. Annual pediatric head injury statistics describe an enormous emotional and financial burden of 300,000 to 400,000 hospitalizations, 6,000 to 7,000 deaths, and an estimated $10 billion in costs for children under 14 years of age (Waller et al. 1989). Importantly, the majority of traumatic head injuries to children less than 6 years old result from motor vehicle accidents and falls (Pascucci 1988, Bruce 1990).

Trauma in the under four years-of-age group is particularly important because it is a period of accelerated growth and development (Calder et al. 1984) and it is a time associated with a relatively high frequency of non-accidental injury caused by custodial abuse (Bruce 1990). Approximately 10 % of all traumatic injuries in children will have a non-accidental etiology (McClelland et al. 1980), and in urban populations, 25 children per 100,000 people will be victims of abuse annually (Helfer and Pollock 1968). The frequent incidence of morbidity and mortality associated with abuse emphasizes the clinical and socio-economic importance for a greater

understanding of the biomechanics and clinical outcome of pediatric head injury.

Although investigations studying the biomechanics of pediatric head injury have been performed (Dejeammes et al 1984, Duhaime et al 1987, Stürtz 1980, Mohan 1979), their results and their interpretation have relied on scaling applied loads based on brain mass to infer the effect of external loads on the neural and vascular elements of the pediatric brain (Ommaya et al 1967). The differences between adult head injury and pediatric head injury are intrinsically linked to both the anatomy and mechanical properties of the developing skull/brain structure. Currently, brain mass is the only physical characteristic used to distinguish the mechanical response of the pediatric head from the adult head when developing separate head injury criteria, because there are no data available that characterize the properties of brain and skull in children.

The focus of this communication is on the mechanical properties of the developing brain. The first four to five years of life is a period of rapid growth and development in the human. This growth spurt is characterized by increases in neural and glial cell number, rapid myelination, decreases in water content, increases in dendritic branching and synaptic connections. In this study we report mechanical properties of tissue obtained from the pig. Dobbings and colleagues (Dickerson and Dobbing 1966, Dobbing) compared human central nervous system (CNS) development with several other species to develop a means for interspecies scaling of CNS development. Using the guideline proposed by Dobbings, one can extrapolate the stage of CNS development between mammalian species if one matches the timing of component growth, and assumes that interspecies differences in the fundamental units, like myelin composition, are minor. Using this approach Dobbings and coworkers found that during the first decade of human life, months of life in a human were roughly comparable to weeks in a pig. Thus, a 1 year-old pig would correspond to a >4 year-old human child with a fully developed CNS, and a 2-3 day old pig would correlate roughly with a <1-month old human newborn. In this study, to examine the mechanical properties in the developing brain, we compare properties measured in tissue from 1 year-old pigs with those from young (2-3 day old) pigs.

This study reports, for the first time, the properties of developing brain tissue. Samples of cortical tissue were tested sinusoidally in simple shear over a broad frequency range (20-200 Hz) and the complex shear modulus was determined. This information is essential for predicting the unique kinematics associated with brain injury in the pediatric population and, thus, is a first-step in identifying brain injury thresholds for children.

METHODS

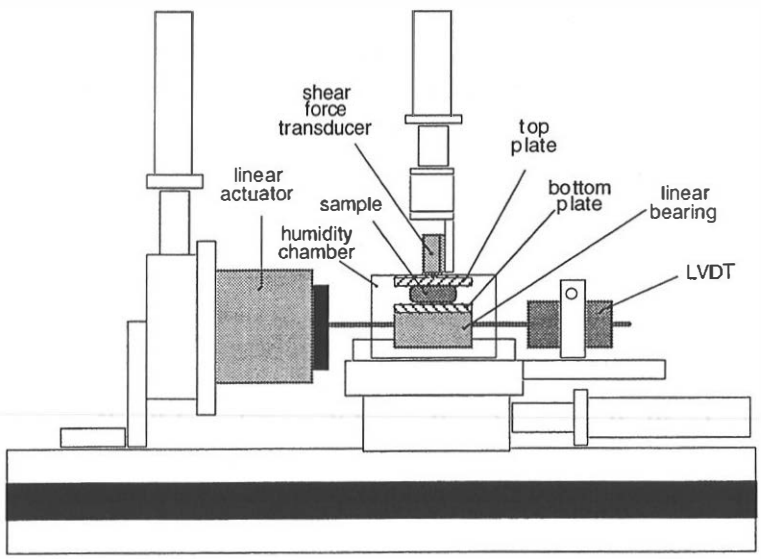
Fresh porcine brain tissue was obtained from 2-3 day old (N=6) and 1 year old (N=6) domestic pigs in compliance with the Animal Care and Use Committee of the University of Pennsylvania's and the United States Department of Agriculture's regulations regarding remnant tissue

procurement (IACUC protocol #100). Time of death and brain weight were recorded for each animal immediately post-mortem. The cerebrum and thalamus of each pig was removed from the skull en bloc, stored in separate airtight containers filled with chilled mock cerebrospinal fluid, and refrigerated until preparation for testing. Samples were tested within 1-5 hours post mortem.

The focus of our study was to determine the age-related behavior of brain tissue, independent of its inhomogeneous and anisotropic properties. Therefore, regardless of age, all specimens were removed from the same location in the frontal cortex and tested in the same orientation. Each brain tissue specimen was prepared by first hemisecting the cerebrum along the longitudinal fissure. A cylindrical sample of cortical tissue was removed from the cerebrum by plunging a cylindrical cork borer laterally into the medial surface of the cingulate gyrus, just superior to the genu of the corpus callosum. The cylindrical sample was then sliced perpendicular to its long axis to remove a disc-shaped tissue specimen approximately 1mm thick and 10-12 mm in diameter. Care was taken to ensure that the disc-shaped specimen was composed entirely of cortical tissue and was free of any penetrating sulci. One sample per animal was tested. Samples were randomized with respect to right or left hemisphere but the location and orientation of each sample remained constant across all studies. The stress-free sample diameter and thickness (h) were measured while the sample was floating in mock CSF solution. Cross-sectional area (A_o) was computed.

Mechanical properties of the porcine brainstem were determined in shear because brain tissue has a high bulk modulus but a very low shear modulus, and is therefore most likely to fail in shear (McElhaney et al (1976), Holburn 1943). Shear properties were determined using a custom-designed device capable of performing both oscillatory (20-200Hz) and stress relaxation tests (Figure 1).

Figure 1 - Brain Tissue Testing Device



Frequency and strain amplitude were independently controlled in the oscillatory tests reported in this communication. The device used a horizontal parallel plate configuration with a variable gap distance, delivered a displacement to the bottom face of the test sample, and measured the shear force transmitted through the sample at its top face. The bottom plate was mounted on a linear bearing for support and control, and was connected to a LVDT (Trans-Tek Inc., Ellington, CT) to measure the amplitude of the input displacement. An isometric force transducer (Kulite Semiconductor Products Inc., Leonia, NJ) was connected to the top plate (a glass cover slip) to measure the shear force transmitted through the specimen. Force and displacement transducers were calibrated before every experiment. Tissue samples were mounted in the testing apparatus and the shear sandwich assembly was enclosed within a humidity chamber to prevent dehydration during testing. Once in place, the sample was allowed to equilibrate to the temperature and humidity conditions within the chamber (approximately 25°C and 100% humidity) for five minutes. Micrometers supported the top plate/force transducer assembly and were used to adjust the gap between the plates to the thickness (h) of the test specimen, and eliminated any pre-stress on the sample.

To drive the system for oscillatory tests, a function generator in series with a power operational amplifier was used to excite a linear voice coil actuator (BEI Motion Systems, San Marco, CA). By varying the generator signal, the bottom plate of the oscillating assembly was displaced sinusoidally with a specified amplitude and frequency. Each sample was subjected to a sequence of sinusoidal shear strains with an amplitude of 2.5% over the frequency range of 20 to 200 Hz in 10 Hz increments, randomized to an increasing or decreasing frequency protocol. The displacement of the bottom plate and the resulting force at the top plate were measured. Shear prestress was eliminated at each frequency by adjusting the plate alignment until the DC offset of the force signal was nulled. The voltages corresponding to displacement and force amplitudes and the phase difference between the two signals were measured at each frequency with a digitizing oscilloscope (Hewlett Packard, Loveland, CO) at 100 ksamples/sec.

Several precautions were take to ensure that these measurements represent the true response of the material. Electronically, the frequency response of both the displacement and force transducer are adequate over the frequency range, and the two transducers were conditioned identically to remove alterations in the data due to a phase shift from the filtering components. Mechanically, the inertial effects of the system were minimized by reducing the mass of moving portion of the device. Likewise, the inertial effects associated with the acceleration of the material itself were reduced by limiting the size and therefore, the mass of the sample. The measurements of the device were validated for a viscoelastic silicone gel with mechanical properties similar to brain (Arbogast et al, in review). The assumption of a non-slip surface at the tissue-plate interface was confirmed previously in oscillatory shear tests between 20 and 200 Hz.

DATA ANALYSIS

Shear stress, τ_o, and Lagrangian shear strain, γ_o, were computed from the amplitude of the sinusoidal force (F) and displacement (D) signals at each test frequency using the following relationships:

$$\tau_o = \frac{F}{A_o} \qquad \text{and} \qquad \gamma_o = \frac{D}{h}$$

where A_O is the shear area of the sample and h is the thickness of the sample.

A viscoelastic material subjected to a periodic shear strain, $\gamma(t) = \gamma_o cos(\omega t)$, will respond with a periodic shear stress that leads the strain by a phase angle, δ. The complex shear modulus, $G^* = G_1 + iG_2$, of a viscoelastic material may be determined from periodic stress and strain measurements using the following equations:

$$G_1 = \left(\frac{\tau_o}{\gamma_o}\right)\cos(\delta)$$

$$G_2 = \left(\frac{\tau_o}{\gamma_o}\right)\sin(\delta)$$

where G_1 and G_2 represent the elastic and viscous components of the complex shear modulus, respectively, τ_o is the amplitude of the shear stress, γ_o is the amplitude of the shear strain, and δ is the phase angle between the signals. This formulation assumes linearity of the material and neglects inertial effects of accelerating the mass of the sample. The measured force and displacement signals were sinusoidal, permitting the use of these equations to calculate the complex modulus (G^*, G_1 *and* G_2) at each frequency.

The significance of the material properties' (G^*, G_1 *and* G_2) dependence on age and frequency were evaluated using analysis of variance (ANOVA $p<0.01$).

RESULTS

The frequency response of the complex modulus, G^*, and the elastic and viscous components G_1 and G_2 are shown in Figures 2 and 3a and 3b, respectively (mean±SE). Each of the material property parameters were larger in the adult tissue than the pediatric tissue, and values increased with increasing frequency. Analysis of variance revealed that G^*, G_1 and G_2 each depend significantly ($p<0.01$) on age and frequency. In addition, analysis of the cross-correlation (age x frequency) indicated that the slopes of G^* and G_2

Figure 2 - G* vs. Frequency for Porcine Brain Tissue

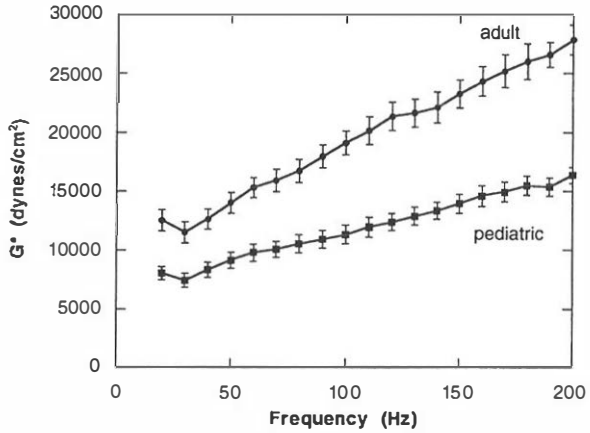


Figure 3a - G_1 vs. Frequency for Porcine Brain Tissue

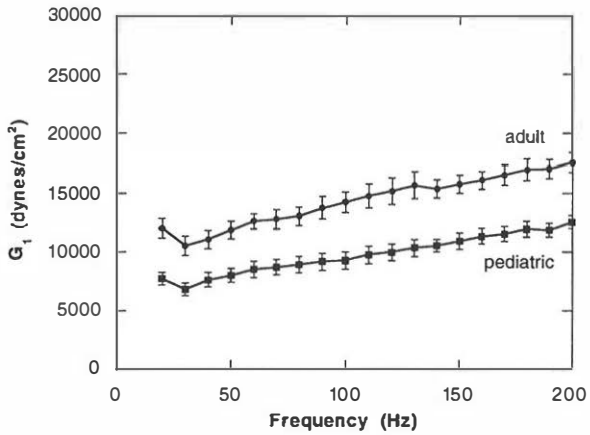
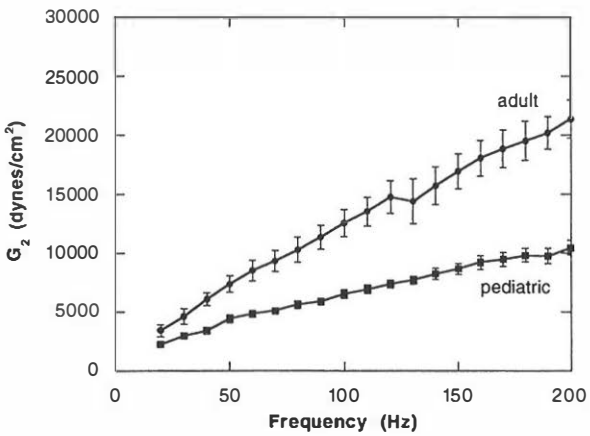


Figure 3b - G_2 vs. Frequency for Porcine Brain Tissue



are affected significantly by age, whereas age resulted in only a parallel shift in G_1 with no significant change in slope.

The composition (percent total sample area that was white or gray matter) of each sample was determined using a microscope-based video image analysis system. The average (mean±SD) white matter surface area fraction was 40±10 percent in the pediatric brain samples and 81±12 percent in the adult tissue samples. This difference is attributable to keeping the diameter of the cylindrical cork borer tool constant, and using the same test site and orientation for both age groups. Because the test site was centered about a white matter core, identical-sized samples from adult cortex contained less gray matter than pediatric animals because the white matter nearly filled the entire diameter in that site. Therefore, the adult samples contained a proportionally larger fraction of white matter than the pediatric samples. Importantly, the data within each group was quite consistent, and we could find no consistent relationship between variation of gray/white matter content and properties within each group.

DISCUSSION

Our study has two major limitations: that porcine tissue was tested rather than human tissue, and that properties were determined only in one location and one orientation. There are two aspects we will address regarding porcine tissue. First, that 2-3 day old pig brain tissues are similar to those of human infants, and similarly that 1-year old pigs brain tissue is representative of adult tissue. Second, that pig brain tissue at the same phase of CNS development resembles human tissue.

The first four to five years of life is a period of rapid growth and development in the human. This growth spurt is characterized by increases in neural and glial cell number, rapid myelination, decreases in water content, increases in dendritic branching and synaptic connections. Dobbings and colleagues (Dickerson and Dobbing 1966, Dobbing) measured the magnitude and rate of change of brain mass, cholesterol content (indicating lipid content, which is a major component in myelin), water content, and DNA-P content (an indicator of total cellularity) in the cerebrum . For the purposes of direct comparison between pig and man, we normalized each parameter by the maximum value and replotted the results in Figure 4, 5, 6, 7, respectively. The x-axis in all plots is time, in units of weeks for pigs, and months for humans, with zero indicating birth, and the y- axis is percent maximum value. While not a perfect correlation for all four aspects of development, when comparing the Dobbings et al. (Dickerson and Dobbing 1966, Dobbing) CNS developmental data for humans and pigs, we concur with Dobbings et al. proposal that during the first decade of human life, months of life in a human are roughly comparable to weeks in a pig. Thus, assuming that cellularity, brain mass, water content and myelin content are important contributors to material properties, we believe that measurements of brain tissue from 1 year-old pigs correspond to those in a >4 year-old human child with a fully developed CNS, and that our measurements of tissue from 2-3 day old pigs correlate roughly with those from a <1-month old human newborn.

Figure 4 - Brain Growth Spurt

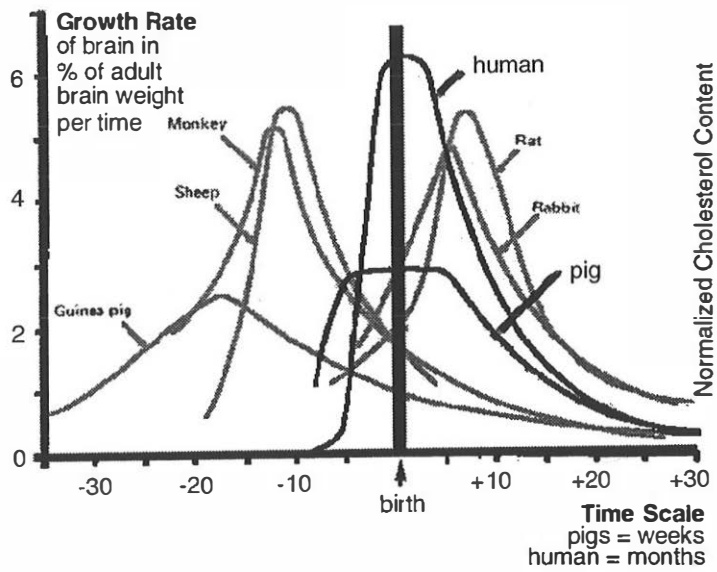


Figure 5 - Normalized Cholesterol

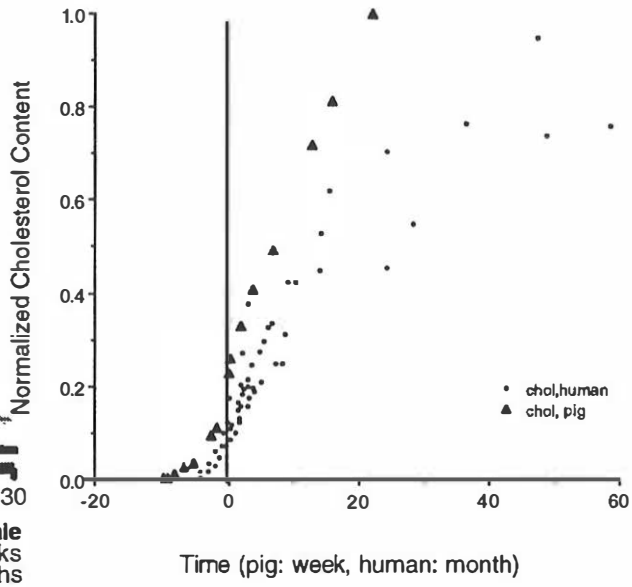


Figure 6 - Normalized Water Content

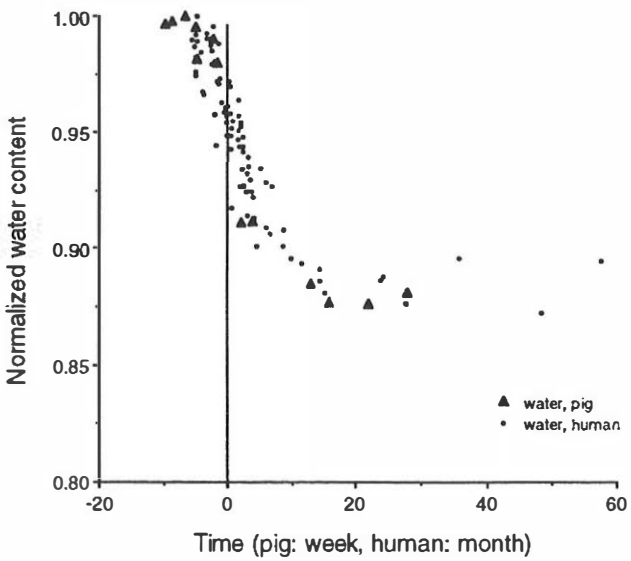
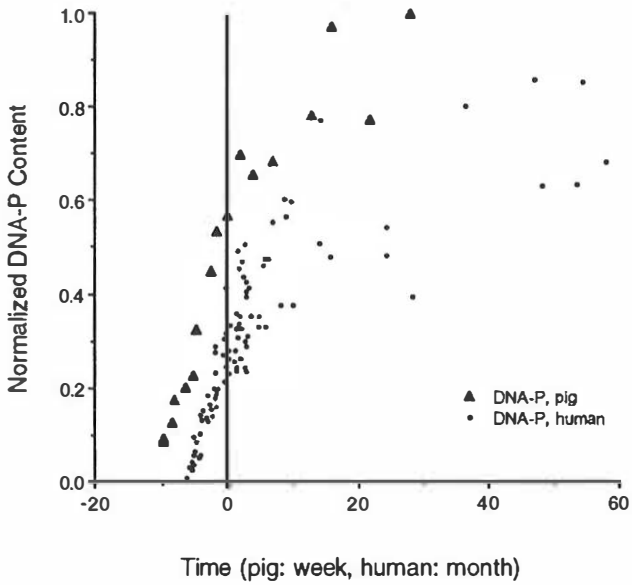


Figure 7 - Normalized DNA-P



The focus of this communication is on the mechanical properties of the developing brain. A review of the mechanical properties of adult human and animal brain (Ommaya 1968, Fallenstien et al 1969, Shuck and Advani 1972, Metz et al 1970, Koeneman 1966, McElhaney et al. 1976) reveals that the reported properties of brain tissue vary by as much as an order of magnitude, as well as species of donor. There is no published data regarding porcine and human tissue mechanical tests with matched experimental conditions. Shuck and Advani (1972) measured complex shear modulus in human brain tissue over a range of frequencies that includes 20-200 Hz. They tested adult human tissue undergoing sinusoidal torsion at small amplitudes and computed complex shear moduli. The frequency-dependent behavior they report for "unyielded white and gray" adult human cortex bears remarkable resemblance to our findings, including a larger slope in G_2 than G_1 that results in a "cross-over" between 100 and 150 Hz. However, the magnitude they report for G_1 and G_2 are on the order of a magnitude higher than our values for adult porcine tissue. The reasons for this difference may be attributed to differences in experimental protocol or species. Their protocol included testing tissue a few hours after autopsy (which might have occurred hours or days after death) and it is unclear the effect post mortem time may have had on their results. Regardless, it is important to note that the frequency dependent behavior of adult porcine tissue was confirmed by previous findings in the literature for human tissue, and that the frequency dependence of the viscous component of pediatric porcine tissue differed considerably from adult tissue.

The range in reported tissue properties may also be related to the anisotropic and inhomogeneous nature of brain tissue. Only Shuck and Advani (1972) included brain's anisotropy and inhomogeneity in their study design. They reported that gray (thalamic nuclei) matter displayed some anisotropy in its storage modulus, and that multi-oriented white matter from the corona radiata was nearly isotropic. Their data also shows overall differences in the two types of brain tissue, indicating heterogeneity in the brain. On average, they report that the storage modulus for these two regions are similar, and that the loss modulus for gray matter is approximately half that for randomly-oriented white matter. However, Shuck and Advani's important and encouraging data on gray and white matter differences were obtained over a frequency range of 2-10 Hz, considerably lower than frequencies associated with traumatic head injury loads. In this communication we sought to measure the age-related properties of brain tissue independent of the anisotropic and inhomogeneous nature of brain tissue, and therefore tested samples oriented in the same direction and harvested from the same location. Future studies may be conducted to investigate the inhomogeneity and anisotropic properties of brain tissue in more detail, and their changes with development.

The response of the head to traumatic injury is influenced by the mass, shape, dimensions, and material properties of the skull and the brain. Previous investigations have determined the material properties of mature human skull and brain to predict the mechanical response of the adult head to traumatic loads and to determine thresholds for brain injury. However, the

brain, cranial bone, and sutures undergo rapid and dramatic developmental changes following birth. These changes affect the material and structural properties of the individual tissues, and substantially affect the response of the head to trauma during childhood. Presently, little is known about how the material and structural properties of the brain change with age. We measured the age-dependent material properties of young (2-3 day old) and adult (>1 year old) porcine brain tissue during sinusoidal tests (20-200 Hz) at a strain amplitude of 2.5 percent, using a unique shear test apparatus. Age significantly affected the magnitude of both the storage and loss components of the brain tissue properties, and the frequency-dependence of the viscous component. Characterizing the developmental changes of the brain's material properties is an essential component to determine the unique response of the pediatric brain to traumatic loading conditions. As such, this project is the first step in developing injury tolerance criteria for the infant and young child, and eventually to more effective preventive, therapeutic, and rehabilitative strategies for the pediatric community.

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