Material characterization of the brainstem from oscillatory shear tests

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Abstract

Traumatic damage to the brainstem occurs frequently when the brain-skull complex experiences injurious loading especially during those traumatic situations that produce diffuse axonal injury (DAI). DAI has been shown to be dependent on load direction and correlated with regional tissue deformation in response to rotational inertial loads. Possible mechanisms for the selective vulnerability of the brainstem are (1) the geometry of the central nervous system is responsible for producing high tissue strains in these regions, (2) regional differences in overall material stiffness result in larger deformations at these sites, and (3) the anisotropic mechanical properties of these regions lead to a sensitivity to the rotational load direction and magnitude. This paper investigates the latter two hypotheses by performing oscillatory shear tests on adult porcine brainstem in three mutually perpendicular directions.

The complex shear moduli were calculated over a range of frequencies (20–200 Hz), for three levels of peak engineering strain (2.5%, 5.0%, and 7.5%). The directional data demonstrated that the brainstem exhibits significant transversely isotropic behavior. Both components of the complex modulus in which the axonal fibers are oriented parallel to the plane of shear but transverse to the shear direction were significantly higher than those of the other two, mutually indistinguishable test cases across the range of strains tested. By comparison with similar tests on cerebral tissue, these data demonstrated that the brainstem displays a stiffer biomechanical response. These differences were present for both components of the complex shear modulus and were greater as the magnitude of the applied strain increased. The regional stiffness and anisotropic response of the brainstem coupled with its location as a narrow bridge between CNS regions interact to result in the selective vulnerability of this region in rotational loading.

Keywords: Constitutive properties; Material properties; Biomechanics; Viscoelastic

1. Introduction

Trauma to the brainstem is a hallmark of severe head injury, occurring in 53% of all head injuries and in 70% of those with survival times of less than 48 h (Jellinger, 1983). Often, brainstem injury occurs as a part of diffuse axonal injury (DAI). DAI is characterized by widespread injury to the cerebral white matter with a propensity for lesions in the brainstem and the corpus callosum. Damage to these regions are associated with high mortality because these sites serve as neural relay stations and as centers for vital functions (Gentry et al., 1989; Rosenblum et al., 1981). Previous studies have demonstrated that DAI is correlated with regional tissue deformation in response to rotational inertial loads (Margulies et al., 1990), and is dependent on the load direction (Gennarelli et al., 1987). These clinical and experimental findings lead to three possible mechanisms for the selective vulnerability of the brainstem and the corpus callosum to rotational accelerations of the head: (1) the geometry of the central nervous system is responsible for producing high tissue strains in these regions, (2) regional differences in overall material stiffness result in larger deformations at these sites, and (3) the anisotropic mechanical properties of these regions lead to a sensitivity to rotational load direction and magnitude.

The isolated contribution of the first mechanism, that the particular location of the brainstem and corpus callosum within the central nervous system (CNS) makes these sites vulnerable to high strains and therefore injurious loading (Gennarelli, 1986), was investigated using physical models of the brain. The models were composed of skull sections filled with homogeneous tissue-like...
viscoelastic material, subjected to rotational loads shown to produce DAI (Margulies et al., 1990). The spatial distribution of deformation was captured on high speed movie film, and the regional strains showed that the brainstem and corpus callosum regions experience significantly higher strains during rotational loading than other regions of the cerebrum that are less likely to be injured in DAI (Arbogast et al., 1994). Thus, CNS geometry has been confirmed as contributing to brainstem and corpus callosum injury.

The contribution of regional material properties to regional injury was investigated using three-dimensional human finite element (FE) models by Zhou and colleagues. They simulated frontal impact and sagittal rotation, and compared the responses of models composed of homogenous material properties and those containing regional material distinctions (Zhou et al., 1995). Correspondence between regions of high stress in the FE simulations with topographical lesion data from an experimental animal model was obtained only when the regional variations in mechanical properties were incorporated. Based on their previous simulations, they selected material properties such that the brainstem was isotropic and less stiff than the isotropic cerebral hemispheres. Thus, their simulations appear to confirm the importance of regional material properties in the corpus callosum and brainstem.

The third possible mechanism that may explain the selective regional vulnerability of the brainstem is its regional anisotropy. The brainstem and the corpus callosum are distinct from the other CNS regions, in that they have a primarily longitudinal arrangement of axonal fibers, rather than a more random distribution. Previous investigations of cerebral tissue, a region of random fiber orientation, have showed its nearly isotropic response (Shuck and Advani, 1972). However, there is no data available regarding regional anisotropy of the brainstem or corpus callosum. The distinct alignment of these regions may result in high strains when loaded in a particular direction as evidenced by the directional dependence of neural injury of the corpus callosum and the brainstem (Gennarelli et al., 1987).

It is the central hypothesis of this paper that due to its microscopic architecture, the brainstem shows a transversely isotropic response to mechanical deformation. We propose to characterize the anisotropic mechanical response of the brainstem by performing high frequency, oscillatory shear tests in a variety of tissue orientations. Due to the large bulk modulus to shear modulus ratio of CNS tissue (McElhaney et al., 1976), these materials are most likely to deform in shear when loaded. Oscillatory shear testing techniques have been used previously to provide a measurement of the complex modulus of cerebral tissue (Fallenstein et al., 1969; Shuck and Advani, 1972); however, no similar tests have investigated the mechanical response of the brainstem. If our hypothesis is confirmed, then clinical and experimental findings support the rationale that the vulnerability of the brainstem and the corpus callosum during rotational loading is caused by a combination of mechanisms: CNS geometry, regional differences in overall stiffness, and anisotropic properties.

2. Materials and methods

Brains were obtained from adult pigs (age 1 yr) immediately after the animals were killed at a local slaughterhouse (n = 63). The tissue was immersed in a mock cerebrospinal fluid to preserve the ionic balance during transport. The two hemispheres of the cerebrum were separated down the longitudinal fissure and freed from the brainstem by a cut through the cerebral peduncles. Incisions through the middle cerebellar peduncles removed the cerebellum from the brainstem.

Cylindrical core samples were taken in two orientations from the middle to the upper brainstem using a right circular cylindrical trephine (diameter = 11 mm). Core A consisted of an inferior–superior cut along the axis of the upper brainstem. In this section the main fibers of the brainstem are coincident with the long axis of the cylinder. Core B was taken in a ventral–dorsal direction perpendicular to the long axis of the brainstem. In this section, the majority of the brainstem fibers are oriented transverse to the long axis of the cylinder (Fig. 1).

From core A, a 1 mm thick disk-shaped test sample was cut. We hypothesized that this plane was the plane of isotropy and that properties in this plane will be independent of shear direction. For this reason, these samples were randomized with respect to shear direction. This orientation was denoted direction ‘transverse–transverse’ (TT). The convention used for describing the orientations specifies the shearing plane and shearing direction relative to the main vertically oriented fibers of the brainstem, i.e. TT corresponds to a shear plane transverse to the fibers and a shear direction transverse to the fibers. From core B, two orientations were selected, ‘parallel–parallel’ (PP) and ‘parallel–transverse’ (PT) (Fig. 2). Care was taken to cut these two orientations from the portion of core B that does not include the horizontally oriented basis pontis fibers.

We hypothesize that the horizontal anatomic plane represents the brainstem’s plane of isotropy and that properties in this plane will be directionally independent. In a transversely isotropic material, the shear modulus measured in this plane (axonal fibers oriented transverse to the shear plane and transverse to the shear direction) will be equivalent to the modulus of specimens with the fibers of the brainstem oriented parallel to the shear plane and parallel to the shear direction. Furthermore, the shear modulus of specimens in the third orientation
Mechanical properties of the porcine brainstem in shear were determined using a custom designed shear testing apparatus (STA) (Arbogast et al., 1997). The apparatus is based on the work of Fallenstein and colleagues (Fallenstein et al., 1969) with augmented capabilities of oscillatory testing over a frequency range. Briefly, a specimen is sandwiched between two parallel plates constructed of roughened glass coverslips. The bottom plate is solidly attached to a linear bearing for support and control, and the top plate is fixed. The displacement of the bottom plate is measured by a LVDT connected to the linear bearing, whereas the force transmitted through the sample is measured by an isometric force transducer attached to the top plate. The test specimen is placed between the two plates and the top plate is lowered until it comes into contact with the top face of the sample. A function generator in series with a power operational amplifier is used to excite a linear voice coil actuator to drive the bottom plate sinusoidally with a specified amplitude and frequency. To maintain high humidity, saline heated to 100°C is injected into the compartments within a clear Plexiglas™ chamber surrounding the specimen and the plates taking care that no liquid comes in contact with the test sample. Periodically during an experiment, the saline is replaced with fresh hot saline. The hot saline raises the temperature surrounding the sample <5°C above the ambient room temperature.

All three tissue orientations were tested dynamically in shear over a range of frequencies (20–200 Hz in 10 Hz increments) at each of the three levels of maximum engineering shear strain (2.5, 5.0, 7.5%). To minimize
post mortem tissue degeneration, specimens for tests performed on each of the three orientations at each of the three strain levels were taken from different animals. A total of seven samples were tested in each orientation at each strain amplitude (7 animals × 3 strain levels × 3 orientations = 63 samples). All tests were approved by the Institutional Animal Care and Use Committee and were completed within 4 h post mortem.

The complex shear modulus, $G^*$, was calculated at steady state at each frequency from the following formulas:

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G' = \frac{\tau_0}{\gamma_0} \cos \delta, \quad G'' = \frac{\tau_0}{\gamma_0} \sin \delta,
\]

where $\tau_0$ and $\gamma_0$ are the amplitudes of the sinusoidally varying shear stress and shear strain, respectively, and $\delta$ is the phase difference between the two signals.

A two-way analysis of variance (ANOVA) (factors: orientation and frequency) with repeated measures on one factor (frequency) was performed to determine any differences in the complex moduli among the three tissue orientations. To investigate the hypothesis of transverse isotropy, orthogonal contrasts were performed to test the hypothesis that (1) $G_{TT}$ and $G_{PP}$ were indistinguishable from one another and (2) $G_{PT}$ was significantly different from the other two orientations. ($G_{xy}$ indicates any of the three moduli, $G'$, $G''$, and $G^*$, in the $xy$ orientation.) A least-squares fit linear regression line was fit through modulus-frequency data for each orientation. Both regression parameters, slope and $y$-intercept, were compared among orientations to assess the nature of the differences.

3. Results

The complex modulus ($G^*$), and its two components, storage ($G'$) and loss modulus ($G''$) (averaged over the seven samples tested at each strain-orientation combination) varied with testing frequency (Figs. 3–5) indicating a viscoelastic response. The standard deviation associated with $G^*$ (averaged over all frequencies and strains) was 37.0, 43.0, and 85.0 Pa for $G_{TT}$, $G_{PP}$, and $G_{PT}$, respectively.

For each component of the complex shear modulus of the brainstem, the data indicated a transversely isotropic relationship. Specifically, for $G'$, $G_{TT}$ and $G_{PP}$, the two moduli hypothesized to be equivalent, were statistically indistinguishable ($p > 0.3$) from one another at two out of three strain levels. The modulus, $G_{PT}$, in which the axonal fibers are oriented parallel to the plane of shear but transverse to the shear direction, was significantly higher ($p < 0.05$) than the other two orientations at all three strain levels. Similarly, for $G''$, $G_{TT}$ and $G_{PP}$ were equivalent ($p > 0.3$) and $G_{PT}$ was significantly higher ($p < 0.05$) at two out of three strain levels. For $G^*$, these relationships were evident at all levels of strain. These differences in orientation were characterized by parallel vertical shifts in the modulus versus frequency curve,
rather than changes in the slope of this curve. A comparison of regression slopes detected no differences among orientations ($p > 0.10$). However, comparison of $y$-intercepts using analysis of covariance (ANCOVA) showed that $G_{PT}$ was significantly higher than $G_{PP}$ ($p < 0.001$) and $G_{TT}$ ($p < 0.001$) for $G'$, $G''$, and $G^*$. These results confirm our hypothesis of the transversely isotropic behavior of the brainstem.

When compared with similar work done in this laboratory on cerebral tissue (Thibault, 1997), the complex modulus of the brainstem (averaged over all orientations and frequencies) was greater than that of the cerebrum. In particular, the storage modulus of samples from the porcine brainstem was significantly higher ($\sim 20\%$ at 2.5% strain ($p < 0.05$), $\sim 100\%$ at 5.0% strain ($p < 0.001$)) than the storage modulus from the porcine cerebrum. This relationship was evident at the two levels of strain tested. The loss modulus showed no regional variation at the lower strain level ($p > 0.4$), however at the higher strain, the brainstem was significantly greater ($\sim 80\%$) than the cerebrum ($p < 0.001$). The two regions showed a parallel storage modulus-frequency relationship that was evident at both strain levels tested; however, a region-dependent variation in the loss modulus-frequency relationship was present at the higher strain level. In general, these regional relationships can be summarized by examining the complex modulus, $G^*$. For the 2.5% strain tests, the regions were indistinguishable ($p > 0.1$), while at the higher strain the brainstem was statistically greater ($p < 0.001$).

4. Discussion

The data in this paper provide essential information about the mechanical response of the brainstem in order to understand its selective vulnerability seen clinically in rotational head injury. Previous work has shown that this region may be vulnerable simply due to its location within the central nervous system as a narrow bridge from the cerebral hemispheres to the spinal cord and cerebellum (Arbogast et al., 1994; Margulies et al., 1990). Two other possible reasons for the high injury rate of the brainstem center around the mechanical properties of this region. This region may be overall more or less stiff than the surrounding regions or due to its axonal architecture, the brainstem may show an anisotropic mechanical response. This paper addresses these two latter hypotheses by presenting region-specific anisotropic material properties for the brainstem obtained under carefully controlled experimental conditions. Specifically, the complex shear modulus of the porcine brainstem was measured \textit{in vitro} over a broad frequency range including those loading rates seen in traumatic situations.

By comparing these results with similar work done in this laboratory on cerebral tissue (Thibault, 1997), the complex modulus of the brainstem (averaged over all orientations and frequencies) is $20-80\%$ greater than that of the cerebrum. Specifically, these differences were present for both components of the complex shear modulus and were greater as the magnitude of the applied strain increased. Recall that isotropic regional differences were incorporated into a previous geometrically detailed model (Zhou et al., 1995). Two points from this previous work are of specific interest to our analysis. First, the shear modulus of the brainstem was selected to be 168 kPa; a two order of magnitude increase from the experimentally determined values presented here. Second, the cerebrum was selected to be stiffer than the brainstem, whereas in our study we find that this property ratio is reversed. Implementation of the less stiff material description for the brainstem into Zhou’s model should result in greater intracranial strains for the brainstem. Furthermore, the inverse relationship between the cerebrum and the brainstem properties should alter the distribution of strain at the interface between the two regions, an area characterized by axonal pathology.

Our anisotropic analyses showed that the brainstem exhibits statistically significant transversely isotropic behavior. Specifically, both components of the complex modulus for orientation PT, in which the fibers are oriented parallel to the shear plane, but transverse to the shear direction, were significantly higher than the other two test cases across the range of strains tested. The moduli of the remaining two orientations, PP and TT, were equivalent. The standard deviation of the $G^*$ measurements of TT, the orientation in which tests were
randomized with respect to shear direction, was comparable to those of the other orientations, consistent with our hypothesis that this horizontal plane is a plane of isotropy. The data presented in this study suggest it may be the transverse isotropy of the brainstem that produces the pathological pattern seen in diffuse axonal injury rather than simply a variation in the magnitude of the isotropic description of various CNS regions.

Interestingly, this transverse isotropy was evident in the oscillating shear tests while concurrent stress relaxation tests of porcine brainstem showed an isotropic material behavior (Arbogast, 1997, in review). This dichotomy lends support to the claim that the material response may be loading rate dependent. Further inspection of the oscillatory data shows that although over the entire frequency range the material response was dependent on direction, at the lowest frequencies (20 and 30 Hz) the responses of the three orientations were indistinguishable ($p > 0.10$) at each of the three strain magnitudes. Although these two frequencies are much higher than the infinitely low frequency associated with equilibrium modulus of the stress relaxation tests, this information indicates a trend toward isotropy at lower loading rates. These data suggest that the structural components responsible for directional differences — the neural fibers, the matrix of glial cells, or the interaction between the two — are highly dependent on the loading rate.

To date, only Shuck and Advani have characterized the directional dependence of the material properties of any CNS tissue (Shuck and Advani, 1972). At low frequencies ($2$–$10$ Hz) they tested both gray and white matter of the cerebral hemispheres in oscillatory torsion in three perpendicular directions and concluded that both the storage and the loss modulus of gray and white matter were essentially isotropic. Our study also indicated isotropic behavior at low frequencies. Most other experimentally determined CNS tissue material properties have measured the isotropic response of human cerebral hemispheres (Donnelly et al., 1997; Fallenstein et al., 1969; Galford and McElhaney, 1970; Shuck and Advani, 1972). To compare our findings with these studies, we averaged the moduli from our studies over all strains and orientations (Fig. 6). Differences may be attributed to variability in parameters such as post mortem testing time, and the rate and magnitude of the applied loading. Also, the tissue used in the current study was from porcine brainstem rather than human cerebrum, and can vary to some degree from previous studies. Despite these factors, our values are comparable in magnitude to those measured by Fallenstein et al. and Donnelly et al. and within a particular study, at a given frequency, the relative magnitudes of the storage and loss moduli are similar in our study and all previous investigations.

Certain experimental limitations are associated with this study: in vivo versus in vitro measurement, the use of pigs as an experimental model, and the use of a simple shear testing configuration. To measure the true response of any soft tissue, material testing should be performed in an in vitro environment where the vasculature, supported by an average blood pressure, provides additional stiffness. Few testing protocols allow this possibility, and as a result most mechanical testing is performed in vitro. One limitation of in vitro testing is that samples are obtained after a finite time post mortem, after pH and temperature sensitive proteases can initiate tissue degeneration and affect the mechanical properties. In our study, we minimized post mortem tissue deterioration by completing the experiments within $3$–$4$ h post mortem.

Strict control of post mortem time, and other variables such as age or cause of death is unachievable when testing cadaveric human tissue. For these reasons, adult pigs of the same age and free from any disease were chosen as an adult human surrogate in these experiments. In all quadrupeds, the brainstem, a primitive portion of the CNS in control of basic physiological functions, is continuous with the forebrain. These reasons suggest that the brainstem of a highly developed mammal, such as the pig, is an anatomically appropriate surrogate for the human brainstem.

The simple shear testing mode has been selected because CNS tissues show a high bulk modulus to shear modulus ratio, and thus, are most likely to deform in shear when loaded (McElhaney et al., 1976). The selected
frequency range (20–200 Hz), includes those rates associated with rapid tissue deformations (5–30 ms) (Margulies et al., 1990) that may accompany traumatic injury. The strain field was assumed to be uniform; each point throughout the thickness experiences the same shear strain. By using relatively small strains, the stresses in the plane normal to the direction of shear displacement, necessary to maintain a constant distance between the plates in simple shear, are minimized ($\sigma_{\text{simple}} > 0.999\sigma_{\text{pure}}$ for $\varepsilon = 2.5\%–7.5\%$) and a state of pure shear is approximated.

Our findings show first, that the brainstem is globally stiffer than the cerebral hemispheres, and second, that the brainstem responds anisotropically to shear loading. In particular, the brainstem was 80–100% stiffer than the cerebrum and the brainstem shear modulus in the transverse direction was significantly greater (10–20%) than the shear modulus in the plane of isotropy. Owing to these material differences, the next step would be to combine all three mechanisms for the selective vulnerability of the brainstem in rotational head injury (geometry, regional stiffness variation, and anisotropy) in a detailed FE model such as the one discussed earlier (Zhou et al., 1995). With this data, one can now determine conclusively the interplay of these characteristics on the distribution of intracranial strains during pathological loading conditions, and thus assess mechanisms of the clinically present primary brainstem damage. These simulations would provide an insight into the biological response of the brainstem to pathological loading conditions and why this region is injured in some traumatic circumstances and protected in others.

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