Instantaneous and Equilibrium Responses of the Brain Tissue by Stress Relaxation and Quasi-Linear Viscoelasticity Theory

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Human brain and brainstem tissues have viscoelastic characteristics and their behaviours are functions of strains, as well as strain rates. Determination of the equilibrium and instantaneous stresses happening at low and high strain rates provide insights into a better understanding of the behaviour of such tissues. In this manuscript we present the results of a series of stress relaxation tests, at six different values of strains conducted on porcine brainstem tissue samples to indirectly measure the equilibrium and instantaneous stresses. The equilibrium stresses at low strain rates are measured from long-term responses of the stress relaxation test. The instantaneous stresses at high strain rates are determined using Quasi-Linear Viscoelasticity (QLV) theory at six strains. The results show that the instantaneous stresses are much larger (almost 11 times) than the equilibrium stresses and across all the strains. It can be concluded that the instantaneous response can be reasonably estimated from the long-term response which can be easily measured experimentally. The experimental results also show that the reduced relaxation moduli, estimated from the QLV theory, vary for the six strains tested.

**Keyword:** Quasi-Linear Viscoelasticity Theory, brain response, brainstem tissue, stress relaxation test, instantaneous response, long term stress.

1. **INTRODUCTION**

   Under an impact load on the head, the brain tissue experiences diverse mechanical stresses. A traumatic brain injury (TBI) may occur when induced stresses exceed the tolerance limits. In most biomechanical studies on human concussions, the attempts are focused on relating the size of the mechanical assault to the localized tissue deformation, pressure, and stress-strain response in order to diagnose the brain injury [1-6]. In such efforts, accurate measurement of generated stresses in the brain tissue are necessary for predicting the size of the injuries. It is known that brain tissue has both elastic and viscous behavior [7-10]. A great deal of effort has been devoted, therefore, to describing the brain tissue material properties in terms of linear viscoelastic behavior [11-13]. Quasi-linear and nonlinear viscoelastic theories have also been
developed for large deformations [14-16]. The majority of these studies often characterize the tissue under simple compression, tension, or shear tests [17-21]. In some studies, the brain is characterized by simultaneously measuring the elastic and viscous responses [22, 23]. In some others, only the elastic characteristics are addressed to identify the tissue response [24-26].

Similar to soft biological tissues and other viscoelastic materials, brain tissue has a strong strain rate dependency [27, 28]. It is also known that brain response stiffens with an increase in strain rates [29, 30]. As shown in Fig. 1, the stress-strain curve of a viscoelastic material, at infinitely slow rate loadings, takes the A-B path known as the equilibrium response. At infinitely high rate loadings, such as an impact or blast, the response follows the A-C path known as the instantaneous response [30, 31]. Both equilibrium and instantaneous responses are purely elastic responses and obtainable only at extremely low or high strain rates. Such responses cannot be achieved using commercial testing machines for simple monotonic ramp experiments [32]. The viscosity domain is bounded by these two elastic responses and directly related to the material viscosity and strain rate. The maximum and minimum stress levels that can possibly be applied under a specific compression level to brain and brainstem tissues, has not yet been clearly addressed.

The main aim of the current study is to estimate the instantaneous and equilibrium stresses for brainstem tissue. To this end, six sets of stress relaxation experiments were conducted at 6 different strain amplitudes. For these series of tests, long-term responses were measured directly from the experiments as the equilibrium stresses of the tissue. Instantaneous stresses, defined as the highest induced stresses in the tissue, were estimated by employing the QLV constitutive model [14, 33].
2. MATERIALS AND METHODS

2.1. Sample preparation

Twelve fresh porcine brains were acquired from the Animal Science Department facilities at North Dakota State University (NDSU). The animals were six to eight months old, and all were healthy. The brains were carefully removed from the heads and cut along their mid-sagittal planes. They were then placed immediately in a physiological saline solution to prevent dehydration and kept cold while they were transported to the lab. The brains were kept at 3 to 5 °C, and all tests were conducted within 3 to 10 hours as were the post mortem times proposed by Garo et al. [34]. Samples were prepared from both the brain and brainstem. Samples from brain tissue were tested only to validate the experimental method. Samples from brainstem tissue were then tested for the purpose of finding the instantaneous and long-term elastic responses of the tissue.

First, the samples from the brain were cut from frontal and parietal lobes of each hemisphere. The procedure followed the experimental protocol by Miller and Chinzei [15] designed to prepare samples for uniaxial unconfined compression tests. Similar to that study, a steel pipe, with sharp edges and surgical scalpels, were used to cut cylindrical brain samples into pieces with a diameter of 25.4 mm and a height of 9 to 10 mm.

Next, the brainstem samples were cut perpendicular to the axons from the medulla oblongata and from the transitional area between the medulla and the spinal cord. Considering brainstem size limitations, the samples were prepared with a height of 5±0.7 mm (mean ± SD). The cross-sectional areas of the brainstems varied between samples and had complex shapes that were difficult to measure. Before each individual test, photographs of the cross-sectional areas were taken, and ImageJ software version 1.48 (Wayne Rasband, National Institutes of Health,
USA) was used to calculate the size of the areas of interest. The cross-sectional area for these samples was in the range of 93±30 mm².

2.2. Test method

Tests were performed at room temperature (~22 °C) using a BOSE 3200 Electroforce machine (BOSE Corporation, Bloomington MN, USA) designed for testing soft tissue materials. Two low-force load cells, rated at 2.5 N and 20 N (BOSE Corporation, Bloomington MN, USA), were utilized for the experiments on the brainstem and brain tissue samples, respectively. Fig. 2 shows the brain and brainstem samples as well as the experimental setup of the experiments.

The surface of the platens was treated with a surgical lubricant (Surgilube, Fougera Pharmaceuticals Inc.) to approximate unconfined compression tests. Samples were then carefully located between the two platens, and the upper platen was cautiously moved downward until it touched the sample. The zero force and displacement were carefully found. Based on the force and displacement signals, obtained directly from the testing machine, nominal stresses and strains were calculated for the analyses.

For experimental validation, the brain tissue samples were tested in stress relaxation experiments at 50% initial strain (n=2 samples), similar to the experiments performed by Prevost et al. [23]. Simple monotonic compression ramp tests, at strain rates of 0.64/s (n=2) and 0.1/s (n=3), were also done, resembling the studies by Miller [15] and Prevost et al. [23]. For brainstem tissue samples, using the same protocol used for brain tissue, the stress relaxation tests were performed at 6 nominal strain amplitudes of 5% (n=4), 10% (n=3), 15% (n=4), 20% (n=4), 25% (n=4), and 30% (n=3). The ramp portion was set to 20 mm/s for all of the tests, and at the end of each experiment no signs of dehydration was observed. No notable differences were
observed in mechanical properties for brainstem between different animals of the same species, even between samples of the same animal at different locations.

2.3. Numerical Formulations

2.3.1. Elastic responses of the tissue

When using monotonic ramp experiments, it is very difficult to find the equilibrium stress, or the lowest strain rate, at which viscosity has no impact on the tissue response [35]. Stress relaxation tests can, however, simply explain this elastic property of the tissue by recording the tissue behavior over a time period until the viscosity effect has disappeared and stress has remained constant [36]. The equilibrium stress was, therefore, recorded directly from the long-term responses of each stress relaxation test. In this regard, the tissue stress values were estimated from 30 to 50 seconds after applied initial displacement, when the measured force remained constant and the estimated stress levels did not change significantly with time. This procedure was used for all of the initial strain values to find the lower bound response of the material. Unlike the equilibrium response, for the instantaneous stress level, it is impossible to be directly measured by the stress relaxation test, as well as it is impracticable to experimentally implement a very sudden ramp. Thus, for instantaneous stresses, the QLV theory was employed to capture this elastic behavior of the tissue. The QLV mathematical model was fitted to the results of stress relaxation experiments. The *fmincon* solver in Matlab (Version R20102a, MathWork, MA, USA) was then used to find the optimal constants of the QLV model. The method provided the global minimum solution by starting from several initial values in the search domain. JMP software (Version 10.0.0, SAS Institute Inc., NC, USA) was used for statistical analyses and the significance level was set to 0.05.
2.3.2. Quasi-linear Viscoelasticity (QLV) Theory

The QLV theory has been employed to determine the relaxation modulus and instantaneous response of biological tissues, ranging from ligaments and tendons [37, 38] to the brain [14, 39, 40]. The general QLV theory function, which incorporates both nonlinearity and time dependence of the tissue, can be expressed as:

\[ \sigma(t) = \int_{-\infty}^{t} G(t-\tau) \frac{\partial \sigma^c(\varepsilon)}{\partial \varepsilon} d\varepsilon(\tau) \]  \hspace{1cm} (1)

where \( \sigma^c(\varepsilon) \) is the elastic response of the material to a suddenly applied strain, \( \varepsilon \); \( G(t) \) is the reduced relaxation function; and \( \sigma^c(\varepsilon) \) is assumed to be an exponential function to capture the ramp of the stress relaxation tests as follows [33]:

\[ \sigma^c(\varepsilon) = A(e^{B\varepsilon} - 1) \]  \hspace{1cm} (2)

where \( A \) and \( B \) are elastic related tissue constants. Although for \( G(t) \), a robust formula introduced in references [33, 41-43], and called box-shaped spectrum can be implemented, the researchers for the study presented here employed a simpler expression [14, 39] as the reduced relaxation function,

\[ G(t) = ae^{-bt} + ce^{-dt} + ge^{-ht} + k \]  \hspace{1cm} (3)

In this equation, \( a, b, c, d, g, h, \) and \( k \) are material constants (\( a+c+g+k=1 \)), that are estimated from the test data. According to the “strain history approach” [38], the stresses have resulted from a ramp phase with a constant strain rate, \( \dot{\varepsilon} \), over time. Thus the subsequent stress relaxation of the eq. (1) (from \( t_0 \) to \( t_\infty \)) is turned into in the following equation,

\[ \sigma(t) = AB\dot{\varepsilon} \left[ \frac{ae^{-bt}e^{(b+B\dot{\varepsilon})\tau}}{b+B\dot{\varepsilon}} + \frac{cdt e^{(d+B\dot{\varepsilon})\tau}}{d+B\dot{\varepsilon}} + \frac{ge^{-ht}e^{(h+B\dot{\varepsilon})\tau}}{h+B\dot{\varepsilon}} + \frac{ke^{(B\dot{\varepsilon})\tau}}{B\dot{\varepsilon}} \right] t_0 \]  \hspace{1cm} (4)
3. RESULTS

3.1. Experimental tests and validations

Fig. 3 shows the results from stress relaxation and simple ramp experiments on brain tissue samples along with similar results from two other previous studies by Miller and Chinzei [15] and Prevost et al. [23]. The results are very similar among studies with the root-mean-square deviation (RMSD) of 224 and 72 (Pa) at ramp test (Fig. 3a, b) and 349 (Pa) in stress relaxation (Fig. 3c). The results of nominal (Lagrange) stresses on the brainstem samples were averaged at each strain level as shown in Fig. 4. The initial maximum stresses from stress relaxation tests varied from about 400 kPa at 5% strain value to 3600 kPa at 30% strain value.

The long-term stress values for all strain amplitudes were determined from the experiment presented here and shown in Fig. 5. The constants of the QLV model in Eq. (4) are shown in Table 1. A significant and negative association between constant B and all strain values (P=0.005) was found. The association for constant A was insignificant (P=0.25). The remaining constants did not show any associations with strain. The QLV model was then independently used to estimate the corresponding instantaneous stress value. In this regard, the instantaneous stress of the brainstem was calculated at each strain level from Eq. (4) when $t = 0^+$ [i.e., $G(0^+)$ =1, such that $\sigma(0^+)=\sigma^\varepsilon(\varepsilon)$] and are shown in Fig. 5. As the strain increased, the instant response also increased, as illustrated in Fig. 5.

Based on a linear regression analysis, a strong linear correlation was found between the instantaneous and equilibrium stresses ($P<0.001$). The relationship was captured by the linear equation $y=ax–b$ where $y$ and $x$ were instantaneous and long-term stresses, respectively. The constants were estimated along with 95% confidence interval (CI) as follows: $a$ (as the slope of the regression line) = 11.0 (10.2, 11.9) and $b = 666.36$ (365.1, 967.6). Both elastic stress values
for bovine brain tissue were also derived from the data published in the study of Laksari et al. [39] and performed a similar regression analysis. Similar to our porcine brainstem tissue, the resulting relationship between the two elastic responses was linear with instantaneous stresses. The corresponding determined constants were, however, different at $a = 6.4$ and $b = 274.27$ (Fig. 6).

The researchers of the study presented here also determined the reduced relaxation moduli, $G(t)$, of the brainstem, at different strains. Fig. 7 shows how the time dependency effect of the tissue behaved at the first 10 seconds of the test time interval, during which the responses varied considerably. As illustrated, the relaxation modulus varied from one strain level to another. The relaxation moduli derived, however, at 15%, 20%, and 25% strain values were, to some extent, similar to each other.

4. DISCUSSION

Using stress relaxation experiments, the lowest stress bound at the infinitely slow strain rates and the highest stress extent at the maximum possible strain rates on brainstem tissue were determined. Theoretically, these two boundaries should have had completely elastic responses and confined the viscoelastic behavior of a very soft biological tissue. In this study, the effect of tissue viscosity was minimized to indirectly estimate these two elastic responses. Based on a strong linear correlation, it was found that the highest level of stress that could possibly be applied to the brainstem was 11-fold greater than its lowest possible stress value at any tested strain amplitude. It was also found that characterization of the brain tissue, using the QLV theory at only one strain amplitude, as performed in the literature [13, 14], may not well describe the applicability of the theory at different strain levels.
It is known that the instantaneous stress cannot be directly obtained from experiments due to technical issues such as overshoot, vibrations, and testing machine limitations [38]. A strong correlation was found, however, between the instantaneous and equilibrium responses ($R^2=0.99$) suggesting that the instantaneous response could be estimated from the long-term response of the tissue. This result is noteworthy because the long-term response can be easily measured directly from experiments. A similar strong linear correlation between the two elastic responses for the bovine brain tissue was also found in the literature [39]. This similarity in the linear relationships between the two elastic responses for both porcine and bovine suggests that a linear correlation might also hold between the two elastic responses for other types of brain tissues, including possibly human brain tissue. The slope of the regression line for the bovine brain tissue was, however, found to be smaller than that of the porcine brainstem tissue which underscores the need to perform experiments with each particular brain tissue. This discrepancy in slopes may be due to the use of different tissues from different animals.

Reduced relaxation moduli for all strain amplitudes using the QLV theory were also measured in this study. Surprisingly, while the moduli should be independent of strain values, the experiments showed that they varied considerably with strain. This strain dependency of the relaxation modulus may have been due to model truncation, as the viscous part of the tissue is considered to be linear. In reality, the viscous part of the tissue might be nonlinear [13, 44], thus the application of the QLV theory may not be capable of representing the complexity of tissue behavior in the nonlinear viscoelastic regions. This finding suggests that the characterization of brain tissue using the QLV theory at only one strain amplitude may not be applicable for the whole range of tissue deformations, and multiple strain amplitude experiments must be performed to calculate a reasonable set of constants.
The two elastic responses have been addressed differently to propose brain tissue constitutive equations in prior studies [24]. Mendis et al. [45] assumed a strain rate of 0.08/s as quasi-static and neglected the effect of viscosity. The respective strain rate in the study by Miller and Chinzei [15] was performed at a very small value of $0.64 \times 10^{-5}$/s. In addition, Miller and Chinzei [15] assumed that the strain rate of 0.64/s was high enough to ignore the effects of the time-dependent relaxation parameters in their proposed model. Pervin and Chen [46], however, were able to increase the strain rates on brain tissue up to 3000/s using a modified split Hopkinson pressure bar and observed an increase in stress values, showing the effect of viscosity even at such high strain rates. In fact, the presence of viscosity makes it difficult to identify the long term and instantaneous responses of the tissue at very low and very high strain rates by applying monotonic ramps. In contrast, several individual stress relaxation experiments were performed, in the study presented here, at different strain amplitudes on tissue samples to address both elastic responses. The results showed that under initial strain levels of larger than 20%, the rate of change in both stresses increased. This may hint to the nature of tissue nonlinearity which, unfortunately, cannot be well addressed with simple monotonic ramps on the tissue. The application of the QLV theory gives a better estimation of the instantaneous response as the highest possible stress experienced by the tissue.

The researchers of the study presented here could not find any studies on similar regions of the porcine brainstem to compare with their results. Good agreement was found, however, between results of the study presented here and the results from previous studies (Fig. 3) on porcine brain tissue comparing the results as a validation of the results of monotonic ramps and stress relaxation experiments. The largest difference was found between the results of the stress relaxation study presented here and those of Prevost et al. [23]. This difference was likely due to
the fact that the samples in [23] were preloaded by about 30 Pa which possibly affected the estimated stress values.

The main limitation of the study presented here was due to the fact that it could not be proven that the instantaneous stresses, measured indirectly using the QLV theory, were purely elastic. In addition, stress relaxation experiments were conducted at only one strain rate (2 s\(^{-1}\)). To find the limits of the QLV theory for a wide range of strain values, similar stress relaxation experiments needed to be performed at different test speeds. An understanding of the test speed effect may contribute to a better understanding of the theory. The rate-independent hyperelastic constitutive models, such as Mooney-Rivlin and Ogden, also have this limitation. They represent considerable error with other loading speeds than with those for which they were determined [8]. However, this deserves to be further investigated and it is suggested that other strain energy functions such the Money-Rivlin or Neo-Hookean relations to be examined. It is strongly suggested that the instantaneous behavior be determined by using different strain rates for acquiring the mean and standard deviation of that response. It is also possible that a unified model to determine the relaxation constants, using all six strain amplitudes simultaneously, may lead to a more robust estimation of relaxation constants over a large range of strains. On the other hand, the irregular geometries of the brainstem samples, varying from sample to sample, may have affected the estimated constants of the QLV model.

5. CONCLUSION

In this paper a method how to measure the instantaneous stress (at extremely high-speed loading) and the equilibrium stress (at remarkably slow loading) for the brainstem tissue at different strains has been introduced. A series of stress relaxation test data was examined and
analyzed. For equilibrium stress, the long-term responses of tissue were considered while the QLV model was employed to independently estimate the corresponding instantaneous stress at each strain. A strong linear correlation was found between the instantaneous and equilibrium behavior of porcine brainstem tissue. Such linear trend was also matched with the data from previous studies on bovine brain tissue. The instantaneous stress for the brainstem tissue was found to be eleven times larger than the long-term stress, almost similar at all strain levels. Such findings could be used to improve evaluation of the stresses generated in brain tissue under a mechanical assault as well as to predict the injury. The reduced relaxation modulus was found to vary with strain, suggesting multiple strain experiments are still needed to investigate the range of applicability of the QLV theory.

ACKNOWLEDGMENTS
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DEDICATION
G. Karami dedicates this manuscript to Professor Abolhasan Vafai, the long lasting Editor-in-Chief of the Journal of Scientia Iranica for his excellent editorial job.

REFERENCES


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Table 1. Results of QLV theory for stress relaxation
Fig. 1. A schematic of possible range of stress-strain curve for viscoelastic material (brain).

- \( \dot{\varepsilon} \to \infty, \text{Elastic instantaneous response} \)
- \( \dot{\varepsilon} \to 0, \text{Elastic equilibrium response} \)
- Minimum and maximum possible stress range
Fig. 2. Sample preparation and experimental setup: (a) a porcine brainstem, (b) medulla part of the brainstem, (c) Electroforce machine used for testing, and (d) a brain tissue sample.
Fig. 3. Results of the experimental data compared with previous studies; simple monotonic compression tests (a) at the rate of 0.64/s; (b) at the rate of 0.1/s; and (c) stress relaxation at 50% strain level.
Fig. 4. Six stress relaxation results of brainstem samples at strains from 5% to 30% with SDs
Fig. 5. Instantaneous and equilibrium (Long-term) stress states limiting the possible range of stress that the brainstem may experience at a specific strain value.
Fig. 6. Correlation between Instantaneous and equilibrium stresses of the brainstem, starting at 5% strain level and show the CI for our porcine brainstem; similar regression lines for bovine data derived from previous studies are also presented.
Fig. 7. Reduced relaxation moduli at different strain levels for brainstem

Biographies

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