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Inelastic Behavior in Repeated Shearing of Bovine White Matter

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Abstract

Understanding the brain's response to multiple loadings requires knowledge of how straining changes the mechanical response of brain tissue. We studied the inelastic behavior of bovine white matter and found that when this tissue is stretched beyond a critical strain threshold its reloading stiffness drops. An upper bound for this strain threshold was characterized, and was found to be strain-rate dependent at low strain rates, and strain-rate independent at higher strain rates. Results suggest that permanent changes to tissue mechanics can occur at strains below those believed to cause physiological disruption or rupture of axons. Such behavior is characteristic of disentanglement in fibrous networked solids, in which strain-induced mechanical changes may result from fiber realignment rather than fiber breakage.

Keywords

Brain tissue mechanics; inelastic straining

1. Introduction

Rapid skull motion may injure cells when the resulting intracranial strains locally exceed a critical threshold (e.g. Bain and Meaney (2000), Ommaya et al. (1968)). Studies of cultured axons suggest that axial strains exceeding 5 to 10 percent may physiologically disrupt axons (e.g. Bain et al. (2001), Geddes and Cargill (2001), Morrisson et al. (2000,2003), Smith et al. (1999)).

Since only limited measurements exist of the brain's mechanical response *in vivo* to low levels of acceleration (Bayly et al. (2004,5)), predictions of the strain levels that result from a particular head insult rely largely on computer models of the brain (e.g. Zhang et al. (2001)). These computer models rely on the many excellent data and constitutive models of the mechanical response of brain tissue to monotonic straining (Meaney (2003), Margulies and Meaney (1998), Miller (2002)). However, data and models of the brain's response to repeated loading are still needed in order to begin computer simulation of clinical pathologies related

to multiple head insults, such as shaken baby syndrome (Duhaime et al. 1987) and second concussion syndrome (Cantu, 2003).

This brief communication presents some first steps towards qualitatively characterizing how repeated deformation alters the mechanical properties of brain tissue. To study the effect of repeated deformation on the mechanical properties of white matter, simple torsion tests were performed on freshly isolated cylindrical cores of bovine white matter. Basic observations of strain induced mechanical property changes and an upper bound on strain and strain-rate thresholds for these changes are presented.

2. Methods and Materials

2.1 Sample acquisition

Bovine brains were delivered in 4°C 2% saline solution within 20 minutes of slaughter following standard FDA procedures. With the brain at 4°C, 6.70 mm-radius ($\pm 150\mu\text{m}$) cylindrical cores were removed from the frontal lobes using a circular stainless steel knife. These were placed into a plastic cylindrical mold and sliced into 1.3 mm-thick ($\pm 50\mu\text{m}$) discs using a razor blade. Discs containing gray matter or obvious signs of pre-slaughter injury were discarded. Each brain yielded 3–6 samples, which were tested within 90 minutes of slaughter.

2.2 Experimental set-up

Samples were tested in 2% saline solution, maintained at 22°C or 37°C, on a Rheometrics RSA-II hydraulic rheometer (TA Instruments, New Castle, Delaware). The rheometer's opposing circular platens were covered with 80-grit sandpaper. Samples were centered on the lower platen, and the upper platen was lowered to compress the sample by 10%.

2.3 Experimental protocols

Tests were designed to determine: (1) the response of specimens to repeated loading; and (2) upper-bound threshold-strain estimates for permanent mechanical changes. In each test, linear strain amplitude changes were applied at a constant strain rate. No preconditioning stretches were applied.

2.3.1 Repeated loading protocol—Eight samples from 4 brains were repeatedly twisted and untwisted at prescribed rates to prescribed angles while torque was monitored. Six specimens, three tested at a rate of 0.0015 rad/s and three at 0.003 rad/s, were twisted at least four times to each of the following angles, with all tests at lower angles being completed before proceeding to larger angles of twist: 0.0045 rad, 0.022 rad, 0.22 rad, and 0.34 rad. Each twist was followed by an immediate untwisting back to an angle of zero at the same prescribed rate. Specimens were held at an angle of zero after each cycle of twisting and untwisting until viscoelastic effects had relaxed. To illustrate the trends observed in all of these tests, the final two specimens were tested at a rate of 0.0015 rad/s to an angle of 0.34 rad; data from one of these tests were used for Figure 4.

2.3.2 Plasticity threshold protocol—Eighteen samples from 4 additional brains were twisted to a prescribed peak strains at prescribed rates, then untwisted at the same prescribed rate. Each specimen was twisted so that the nominal engineering shear strain at its outermost radial extremity reached $\gamma = \{0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.125, 0.15\}$. Six different strain rates of the outermost radial boundary were tested: two specimens at 0.25 s^{-1} , four at 0.50 s^{-1} , three at 0.75 s^{-1} , four at 1.0 s^{-1} , two at 1.3 s^{-1} , and three at 1.5 s^{-1} .

Data were analyzed to identify the degree of straining required for the secant modulus at $\gamma=0.015$ strain to drop to 85% of the value from the virgin curve (Figure 1) (e.g. Hill, 1950). The maximum strain applied to the sample prior to this reduction in secant modulus was recorded as a plasticity threshold. In most cases this threshold was found using the linear interpolation scheme described in Figure 1. The strain level of $\gamma=0.015$ was chosen because this was the largest level to which specimens could be repeatedly strained without affecting the secant modulus.

3. Results

Typical torque vs. rotation angle curves from ramp tests are pictured in Figure 2 for a specimen repeatedly twisted at a rate of ± 0.0015 rad/s. Repeated reloadings involving small tissue deformations caused no mechanical changes to the specimens (Figures 2 and 3a). Unloading of the specimens produced a sharp drop in torque due to a change in sign of the rate-dependent component of the mechanical response. A negative torque was required to return the angle of rotation to zero due to viscous effects.

Specimens twisted beyond a critical threshold exhibited diminished secant moduli (Figures 2 and 3b). The reloading curve here showed a marked reduction in initial slope; however, the peak torque exceeded that of the previous (lower strain-amplitude) cycle. The increasing strain levels in subsequent cycles resulted in progressively reduced secant moduli. For repeated twisting to a single peak strain, little change in secant moduli were observed after the first twisting (Figure 4).

Plasticity thresholds measured on 18 different specimens were found to exhibit both rate-dependent and rate-independent modes, with the threshold strain increasing monotonically with strain rate at lower strain rates (Figure 5).

4. Discussion

After a single “preconditioning” twist of the tissue beyond a rate-dependent threshold, mechanical resistance upon reloading dropped an amount that increased with the degree of twist. Subsequent twisting to this same degree had little effect on mechanical response, meaning that the major reduction in reloading modulus occurred in response to the initial loading. Thresholds for the degree of twist required to permanently change tissue mechanical properties were characterized as a function of shear strain and strain rate under the following two assumptions. First, the observed changes in mechanical modulus were due predominantly to changes in the outermost annulus of tissue. This is reasonable in light of observations that brain tissue stiffens with straining (Miller, 2002), meaning that the regions of highest strain could be expected to dominate the secant modulus. Second, the variations in specimen dimensions were negligible when comparing torque-vs.-peak strain plots from different specimens. This caused errors in estimated thresholds on the order of $((\Delta R)/R + (\Delta h)/h)$ for a specimen of radius R , height h , and tolerances for radius and height measurements ΔR and Δh . For $\Delta R=150\mu\text{m}$ and $\Delta h=50\mu\text{m}$, this yields errors on the order of 6%.

The critical strain thresholds measured appeared to exhibit strain-rate dependence at lower strain-rates, and strain-rate independence at higher strain rates. The measured upper-bound strain levels were lower than the strain thresholds reported in the literature for causing physiological changes to cultured axons (e.g., Morrison III et al., 2003), implying that permanent changes to the mechanical properties of brain tissue can occur at loading levels smaller than those known to injure cells.

When strained repeatedly to the same level, samples exhibited little change in mechanical response after the first cycle (Figure 4). Such behavior is characteristic of entangled fibrous

solids, and can result from two sources. The first is fiber realignment, as occurs in some yarns and fabrics in tension (Boisse et al., 2005), and in fibrous mats in compression (Durville, 2005; Poquillon et al., 2005). Here, disentanglement and realignment of fibers leads to a reduction in secant moduli for reloading for strains up to the peak strain of the previous cycle. The second is stochastic fiber fracture (Curtin, 1999), in which this behavior is due to friction holding together fragmenting fibers (Godfrey and Rossettos, 2001). Since the modulus changes were observed at strain levels far too low to fracture cultured axons, evidence points to rearrangement of fibers that occurs with little fiber breakage.

In some such materials (e.g. collagenous tissues), “pre-conditioning” stretches are applied before mechanical testing to de-tangle fibrils into a configuration more representative of conditions *in vivo* (e.g. Pryse et al., 2003; Nekouzadeh et al., 2007). Whether “pre-conditioning” stretches are appropriate for brain tissue is a topic of debate (e.g. Darvish and Crandall, 2001). Pre-conditioning and details of cutting may underlie differences between the current results and those of Hrapko, et al. (2006) who observe no mechanical changes to white matter over a broad range of loading conditions.

Our results suggest insight into mechanisms of repeated-acceleration induced traumatic brain injury. At strain-concentrating features such as the boundaries of anatomical inhomogeneities, reductions in secant moduli can lead to local amplification of strains on subsequent loading cycles (e.g., Genin and Hutchinson, 1997): a second, identical loading may produce local strains much higher than those resulting from the first loading.

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Figure 1.

Example of analysis. The curves are normalized by the value of torque on the first (top) loading curve at 1.5% peak engineering shear strain. The first reloading results in a normalized torque at $\gamma_{\max}=1.5\%$ of about 0.9, and the second in a normalized torque of about 0.8. The threshold strain at this particular strain rate would be found by interpolating halfway between the peak strain of the virgin curve and that of the first reloading curve. The dotted line represents a 15% drop in normalized torque, or the threshold at which significant changes were determined to have occurred.

Figure 2.
Typical torque vs. angle data for reloading of specimens tested at 37°C.

Figure 3.

Typical torque vs. angle data for specimens tested at 37°C. (a) Reloading cycles at low strain levels caused no permanent mechanical changes: the curves for the ninth and tenth cycles (pictured) followed those of the eight previous cycles. (b) Higher strain levels in later reloading cycles led to reduced secant moduli upon reloading.

Figure 4. Bovine white matter (here tested at 22°C) exhibited a reduction in secant modulus upon reloading, with subsequent twisting of the same magnitude resulting in relatively minor reductions of the tissue's mechanical resistance.

Figure 5.

Estimated strains at which permanent mechanical changes occur in bovine white matter. Data is shown for 18 specimens. The data appear to exhibit a rate-dependent mechanism for mechanical changes at low strain rates, and a rate-independent mechanism at higher strain rates.