INFANT RAT BRAIN TISSUE IS SIGNIFICANTLY STIFFER THAN ADULT

Amit Gefen,[1] Nurit Gefen,[1] Ramesh Raghupathi,[2] and Susan S. Margulies[1]

Departments of [1]Bioengineering and [2]Neurosurgery
University of Pennsylvania, Philadelphia PA, E-mail. margulies@seas.upenn.edu

INTRODUCTION

Biomechanical computer models are increasingly being used to provide insight into the mechanisms of traumatic brain injury. The accuracy of these simulations in predicting the severity and distribution of injury is strongly dependent on adequate mechanical description of material characteristics of the brain tissue. Until recently, biomechanical models of a pediatric head injury considered the infant and young children's brains as having the same mechanical properties of adults, with only the size scaled to fit the simulation. However, the brain is developing rapidly over the first four years of life. Dendritic and axonal branching are accelerated and accompanied by rise in lipid content as axonal segments are being myelinated. Concurrently, the mechanical properties of the infant/child brain (such as stiffness under compression and shear) may change during development. The experimental evidence of age-dependent mechanical properties was provided by Thibault and Margulies (1998) and Prange and Margulies (2002) who measured the shear moduli of fully-developed one-year-old and 2-5 day-old porcine cerebrum undergoing shear strains. For strains larger than 5%, shear modulus of adult cerebral tissue was significantly lower than for newborn piglets. However, there is no quantitative description of the time course for these changes during development, nor is there a report of such age-dependency in other species.

Rat models predominant for studying brain injury due to ease of behavioral, histopathological and molecular analyses. In terms of brain development, 10 postnatal rat days of age are equivalent to human birth and at 20 postnatal days the rat is equivalent to 1-year-old human child (Porterfield and Hendrich, 1993). The goal of this study is, therefore, to measure the elastic properties of brain in the rat during development from neonatal (13-day and 17-day-old rats) to adulthood (43 and 90-day-old). These mechanical property data provide important information for scaling of coefficients to account for brain properties in computer simulations as well as for development of pediatric animal models of head injury in which parameters such as loads and accelerations can be scaled with development.

METHODS

The protocol was approved by the Institutional Animal Care and Use Committee at the Univ. of Pennsylvania. An electromechanical calibrated PC-controlled indentor with a hemispherical tip comprising a miniature linear stepper motor, force transducer and a linear variable displacement transducer was used to indent the exposed brain at a rate of 1 mm/s (Fig. 1). For an indentor with a hemispherical tip, shear moduli G of brain tissue were calculated using Lee and Radok's (1960) solution, $G(t) = 3RP/16(R\delta)^{1.5}$, for an elastic half-space (representing brain) that is indented to depth δ by an indentor of radius R subjected to load P. The resulted relaxation curves $G(t)$ were used to calculate the long-term (G_∞) and instantaneous (G_i) shear moduli of the brain *in situ* and *in vitro*. A total of 44 animals were grouped from 9 different litters and divided into four sub-groups according to their age: 13-days-old (N=11), 17-days-old (N=12), 43-days-old (N=10), and 90-days-old (N=11). For each age sub-group, 6-7 animals were randomly selected for testing the brain *in situ,* and the remaining 4-5 animals were assigned to *in vitro* (excised) testing to verify that tissue properties measured for the brain were not affected by the confining nature of the skull.

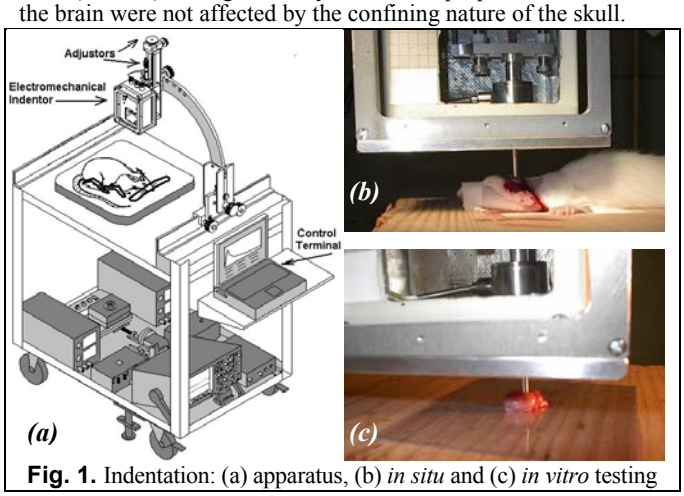

Fig. 1. Indentation: (a) apparatus, (b) *in situ* and (c) *in vitro* testing

Prior to measurements, the tip of the indentor was lubricated with surgical gel to prevent tissue adhesion and allow free slip of tissue during deformation. A lethal sodium pentobarbital dose (200 mg/Kg) was intraperitoneally injected and death was verified by cessation of heartbeat. Post-mortem, the scalp was reflected, a cranial window was opened above the left or right (randomized) brain hemisphere and the dura was removed. The indentor was subsequently positioned using its fine system of adjusters (Fig. 1a,b) until delicate contact was made with the skull surface as determined by monitoring the force transducer signal on an oscilloscope. Force data were continuously measured at a sampling rate of 25 Hz using a PC laptop equipped with A/D board and LabView 6i software package (National Instruments). The brain was then tested 5 times to measure the tissue's stable mechanical response: indented 1 mm (1 mm/s) and held for 90 seconds, followed by a rest period of 60 seconds before the next indentation.

For the purpose of *in vitro* testing, the brain was delicately excised from the skull after euthanasia using fine surgical scissors. Harvested brains were placed on a pre-lubricated smooth plate that allowed non-confined deformation (Fig. 1c). Tissues were maintained moist during measurements using saline spray. Long-term plateau portions of the relaxation curves were averaged over 10 seconds ($78 \le t \le 88$ sec after indentation) to calculate G_∞ values. Shear moduli G_∞, G_i calculated for brain *in situ* and *in vitro* for different animal ages and at preconditioning cycles 1-5 were evaluated using a 3-way ANOVA (Systat v10.2) to test dependence on the following factors: (i) *in situ* vs. *in vitro* results – to determine effects of the brain's confinement within the skull on shear moduli *in situ*, (ii) age of animals and (iii) preconditioning of brain tissue. Subsequent ANOVA tests were used to determine the preconditioning cycle for which G_∞, G_i measurements had been stabilized (i.e. statistical similarity between results of subsequent cycles). Finally, Tukey-Kramer tests for G_∞, G_i values across age groups were carried out for the non-preconditioned (1st loading cycle) and fully-preconditioned (5th cycle) measurements. A value of p lower than 0.05 was considered statistically significant.

RESULTS

Decrease in values of both G_∞, G_i shear moduli with age was well demonstrated (Fig. 2). 3-way ANOVA yielded that for 1mm indentation, G_∞, G_i are the same when the brain is tested *in situ* and *in vitro*, indicating no significant confining effect of the skull. Hence, *in situ* and *in vitro* measurements were pooled. In addition, G_i and G_∞ readings stabilized after 3 and 4 preconditioning cycles, respectively. Therefore, the 5th loading cycle was used to compare preconditioned shear moduli across ages by means of Tukey-Kramer tests for each modulus type. "Neonatal" (13-day-old) rat brains were significantly stiffer than "adult" (90-day-old), in both non-preconditioned and preconditioned G_i and G_∞ (Table 1). The younger animals representing the infancy and childhood stages of brain development (13, 17-day-old) were similar in all four mechanical characteristics and, likewise, the adult brains (43 and 90-day-old) were similar in all characteristics.

DISCUSSION

Material properties of the rat brain in development stages equivalent to human infancy are mechanically different from those equivalent to human adulthood, and hence, infant and adult brains cannot be treated as being the same material in biomechanical analyses. The younger brain tissue is stiffer, and becomes more compliant with growth, very likely due to myelination of axons.

The present results specify the relevant material scaling factors to account for the stiffer pediatric brain in biomechanical computer simulations. The infant to adult stiffness property ratio obtained in this study, ~1.9 in average, is in excellent agreement with the one recently reported for porcine brains (Prange and Margulies, 2002).

The rat is commonly used to study traumatic brain injury. The results of this study indicate that if the same injury parameters are applied to infant and adult rats, e.g. acceleration, shaking, blunt impacts to the skull, etc. they will produce different brain tissue stresses and strains, not only due to differences in brain size but also due to the different tissue material properties. Hence, additional scaling of the injury-causing accelerations and loads between ages is needed to account for age-dependent brain stiffness in experimental design of head trauma in the rat.

Table 1: Instantaneous G_i [Pa] and long-term G_∞ [Pa] shear moduli of the rat brain. NPC = non-preconditioned, PC = preconditioned.

	Neonatal (averaging 13,17 days)		Adult (averaging 43,90 days)	
	NPC	PC	NPC	PC
G_i	3335	1782	1723	1237
G_∞	783	626	512	399

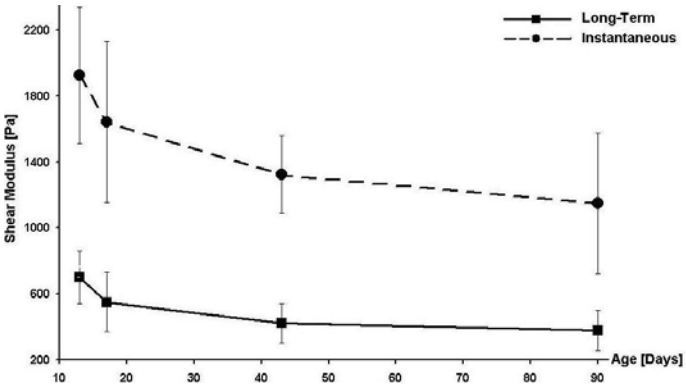

Fig. 2. Preconditioned shear moduli (mean ± standard deviation) of the rat brain versus age

ACKNOWLEDGEMENTS

Funding was provided by the NIH grants R01-NS-39679 and R01-NS-41561 and by the CDC grant R49-CCR-312712.

REFERENCES

Lee, E.H., and Radok, J.R.M., 1960, "The contact problem for viscoelastic bodies," *J. Appl. Mech.* Vol. 27, pp. 438-444.

Porterfield, S.P., and Hendrich, C.E., 1993, "The role of thyroid hormones in prenatal and neonatal neurological development - current perspectives," *Endocr. Rev.* Vol. 14, pp. 94-106.

Thibault, K.L, and Margulies S.S., 1998, "Age-dependent material properties of the porcine cerebrum: effect on pediatric inertial head injury criteria," *J. Biomech.* Vol. 31, pp. 1119-1926.

Prange, M.T., and Margulies, S.S. 2002, "Regional, directional and age-dependent properties of the brain undergoing large deformations," *J. Biomech. Eng.* Vol. 124, pp. 244-252.