A MODEL OF THE INTRACELLULAR CALCIUM DISTRIBUTION THROUGHOUT THE BRAIN AS A FUNCTION OF INERTIAL LOADING IN VARIOUS PLANES

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ABSTRACT

Experiments that employ physical models of the skull and surrogate brain have permitted an approximation of the deformations that occur within the brain during dynamic loading. The kinematical conditions under which these experiments have been conducted thus far include sagittal and coronal plane noncentroidal rotations, while the kinetic conditions used have been guided by the matrix of inertial loading parameters used in subhuman primate experiments. Macroscopic load parameters, such as the acceleration, velocity and displacement, used in these tests are compared to the deformation computed in selected anatomic regions of interest.

Intracellular calcium measurements have been made on isolated single axons and neural-like cells in culture that have been mechanically stimulated. In these experiments, the magnitude and time course of the calcium transients have been analyzed with regard to the strains and strain rates that the individual cells experienced.

These data are combined in a model that presents the topographic distribution of the intracellular calcium changes throughout the brain following the inertial loading conditions imposed upon the model. These maps of the calcium events are then compared to pathology obtained from subhuman primate studies that were conducted under similar loading conditions. This approach is intended to provide insight into the relationships between the macroscopic loading parameters and the associated tissue injury, with implications for improved injury tolerance criteria and novel methods of therapeutic intervention.

INTRODUCTION

The complex nature of the head motion and impact phenomena occurring in the automotive crash environment in relation to the range of brain injuries observed clinically has presented challenges in proposing and evaluating head injury tolerance standards. In our laboratory, clinically relevant forms of brain injuries such as concussion, contusion, acute subdural hematoma, and diffuse axonal injury have been produced in subhuman primates using a controlled, inertial loading sufficiently distributed to eliminate skull fracture [1-3]. A companion series of experiments using surrogate models of the subhuman skull-brain subjected to identical kinematic and kinetic loading conditions have produced an estimate of the topographic and temporal nature of the intracranial deformation patterns occurring dynamically during these animal experiments [4-6]. These mechanical field parameter data have been compared to the injury lesion distribution determined from neuropathology examinations, and together have provided a means to formulate injury specific tolerance criteria for diffuse axonal injury and acute subdural hematoma [5, 7].

By focusing on anatomic regions most commonly associated with neuropathological lesions, the data derived from physical model studies also provide rational design criteria for

developing isolated tissue and cell culture models to study central nervous system trauma in vitro. Previous investigations have studied the response of neural and vascular preparations to a dynamic mechanical stimulus, and have led to the observation of a range of mechanically induced pathophysiologic events in isolated blood vessels, cellular components of the vessel wall, myelinated and unmyelinated axons, and neural-like cells [8-11].

The aim of this report was to combine these measured strain induced changes for in vitro neural components with the topographic estimates of strain occurring during an inertial loading to present a model for intracellular calcium changes immediately post impact. The relation of these strain induced calcium changes to the structural and functional changes in the axon, as well as future directions for the modeling effort, will be discussed.

MATERIALS AND METHODS

The methodologies for the study presented will be divided into a description of the physical model studies, used to relate to the macroscopic inertial loading conditions to approximations of the regional tissue deformation, and isolated axon studies, which provide an index of the pathophysiological responses elicited by a controlled mechanical stimulus. More complete descriptions of the methods for these studies can be found in previous reports.

Physical Model Studies

Human and subhuman primate skulls (Carolina Biological Supply, Burlington, NC, USA) were obtained and sectioned either to produce a coronal plane or sagittal plane view of the intracranial contents. Skull sections were fixed in position within aluminum cans using an epoxy resin, and an optically transparent silicone gel (Dow Corning, Midland, MI, USA) was poured into the intracranial cavity to a level 1-2 cm below the cut plane surface. An orthogonal enamel grid (3-5 mm spacing) was applied to the cured gel surface, and the remainder of the cranial cavity was filled with a second layer of silicone gel. An illustration of the planar physical models constructed to date is shown in Figure 1.

The device used to accelerate these physical models was based on a HYGE shock testing apparatus (Consolidated Vacuum Corp. , Rochester, NY, USA) custom modified to produce a noncentroidal rotation of the model. Modifications to the deceleration metering pin, as well as the loading pressures supplied to the HYGE cylinders, provided control of the biphasic waveform to produce inertial loading with deceleration pulse durations of 3-11 milliseconds, and peak rotational accelerations up to 1.5×10^5 rad/s^2. Tangential acceleration at a point on the linkage assembly was measured using an uniaxial accelerometer, and was recorded on an Endevco Smart IIA recorder system (Endevco, Inc., San Juan Capistrano, CA, USA).

High speed cinematography (Redlake Inc., Redlake, CA, USA) captured the grid deformation during the inertial loading period, and susequent analysis of a select series of high speed film frames allowed for the calculation various mechanical field parameters. From this digitized information, a spatial representation of the shear strain at selected time points was calculated and plotted (Golden Software, Golden, CO, USA).

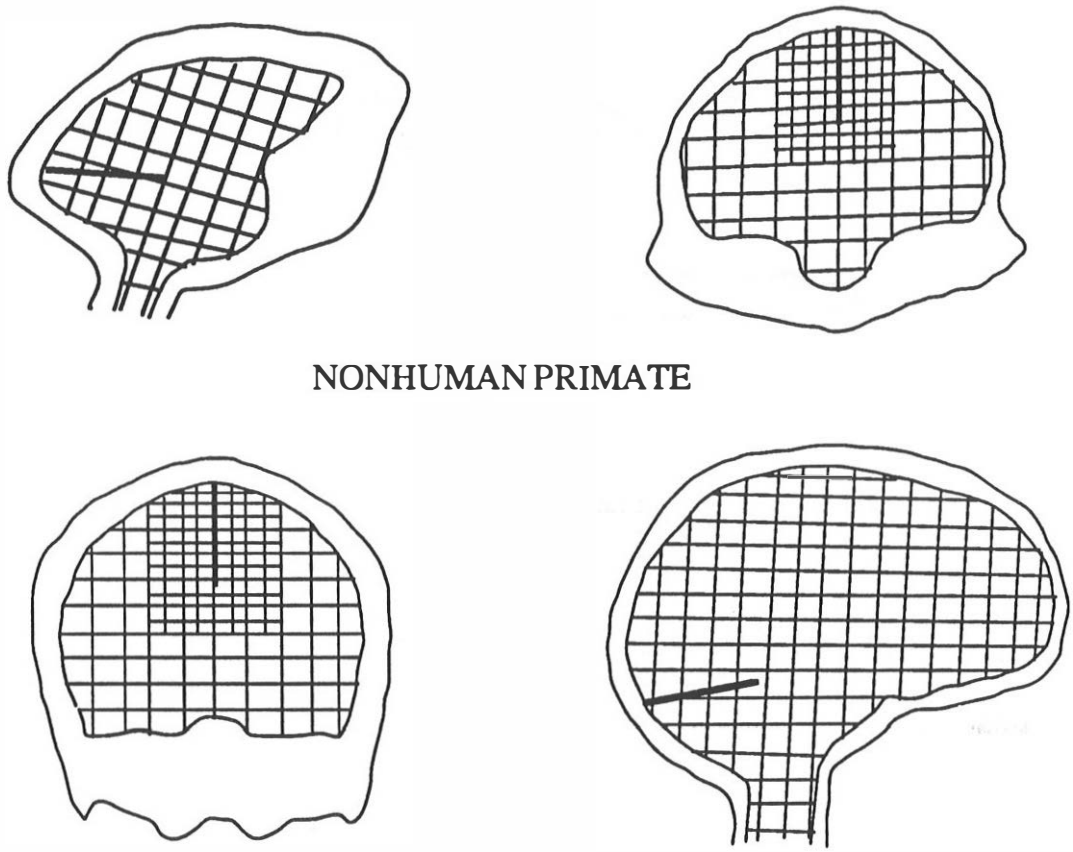


Figure 1

Isolated Axon Studies

A squid giant axon specimen (Loligo Pealei) was dissected and mounted on a custom designed material testing system designed to uniaxially elongate the isolated axon over a range of strain rates. A pulley/speaker coil system was used to dynamically elongate the axon, while a Statham force transducer was utilized to preload the axon specimen and measure the dynamic force. Electrodes could be incorporated into the system to measure changes in the membrane potential and intracellular calcium concentration elicited by the dynamic stimulus. For these studies, a calcium selective microelectrode was placed intraxonally and used to measure axoplasmic calcium concentration. A schematic of the apparatus used in these studies appears in Figure 2.

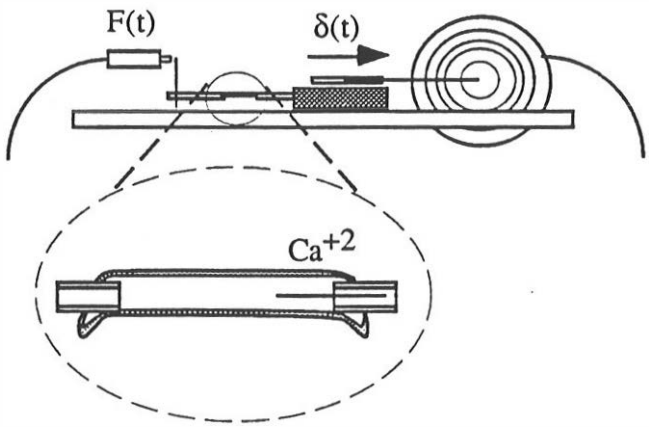
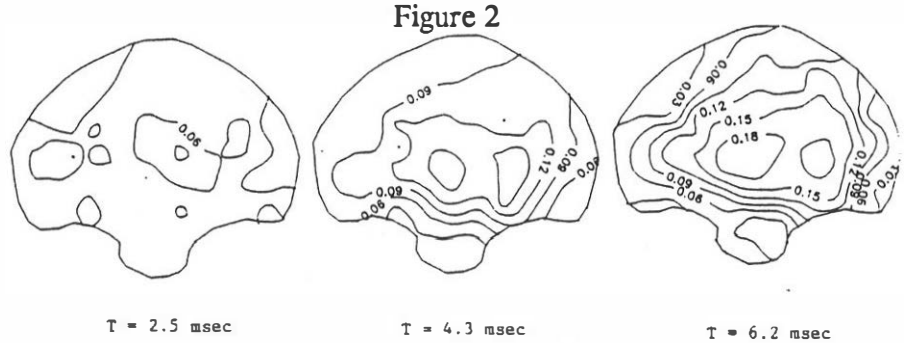
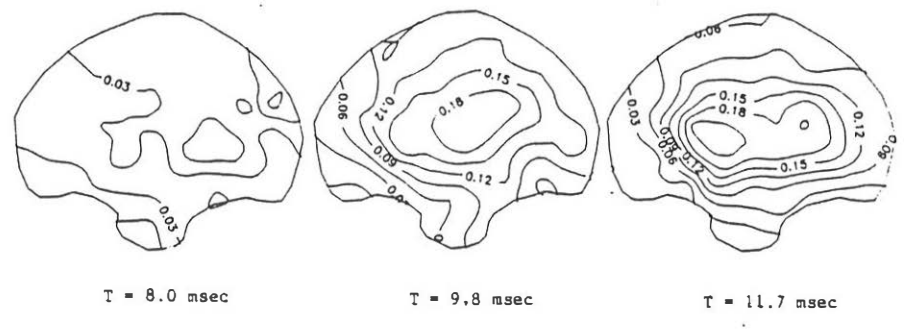


Figure 2





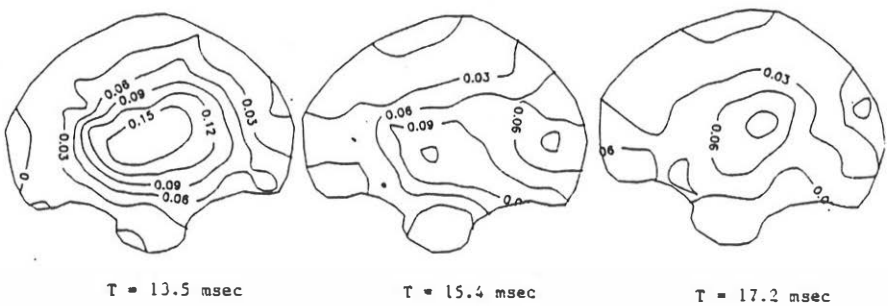


Figure 3

RESULTS

Physical model studies outlined in the methods section were conducted using both human and subhuman primate skull sections. Figure 3 shows a time series of shear strain contour plots for a coronal plane physical model of the baboon skull-brain structure subjected to a prescribed inertial loading condition ($\ddot{\theta}_p$= 1.04 x 10^5 rad/s^2, τ_d = 5.3 ms). The characteristics of this loading waveform, as described by the magnitude and time course of the acceleration, are identical to those used to produce severe diffuse axonal injury in the subhuman primate. While shear strain patterns depicted in Figure 3 achieve maximum values during both the acceleration (t=6.2 milliseconds) and deceleration (t=11.7 milliseconds) periods, the state of maximum strain for the acceleration loading period consistently appears during the deceleration phase.

Additionally, a series of isolated axon experiments were conducted in which the ultimate uniaxial strain (ε) applied to the axon ranged from .03 to .20. The measured axoplasmic calcium concentration (Figure 4) displayed a transient change at low levels of stretch, a more pronounced residual deficit at higher levels of strain, and an irreversible accumulation at the highest level of insult. Structural failure of the axon occurred at an ultimate strain of .25.

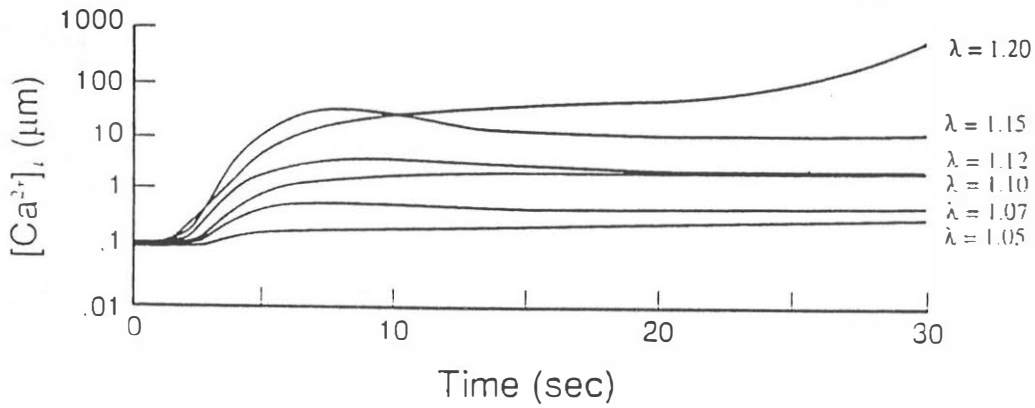


Figure 4

The strain information shown in Figure 4 can be integrated with the isolated axon studies to predict the resulting distribution of intracellular calcium occurring within the axonal segments brain tissue in response to a prescribed inertial loading. For these correlations, the shear strain (γ) was related to the stretch ratio by the following formula:

$$\lambda = [\ 1 + \sin(2\gamma)\ ]^{.5} - 1$$

Figure 5 presents the predicted temporal intracellular calcium distribution for the inertial loading shown to produce severe DAI in the nonhuman primate. We have compared this distribution of intracellular calcium with the lesions observed in the brains of injured subhuman primates, where the mechanical parameters used to injure these animals are similar to those observed in this modeling study, and have found a reasonable correlation between these calcium changes (Figure 5) and the topographic distribution of the lesions.

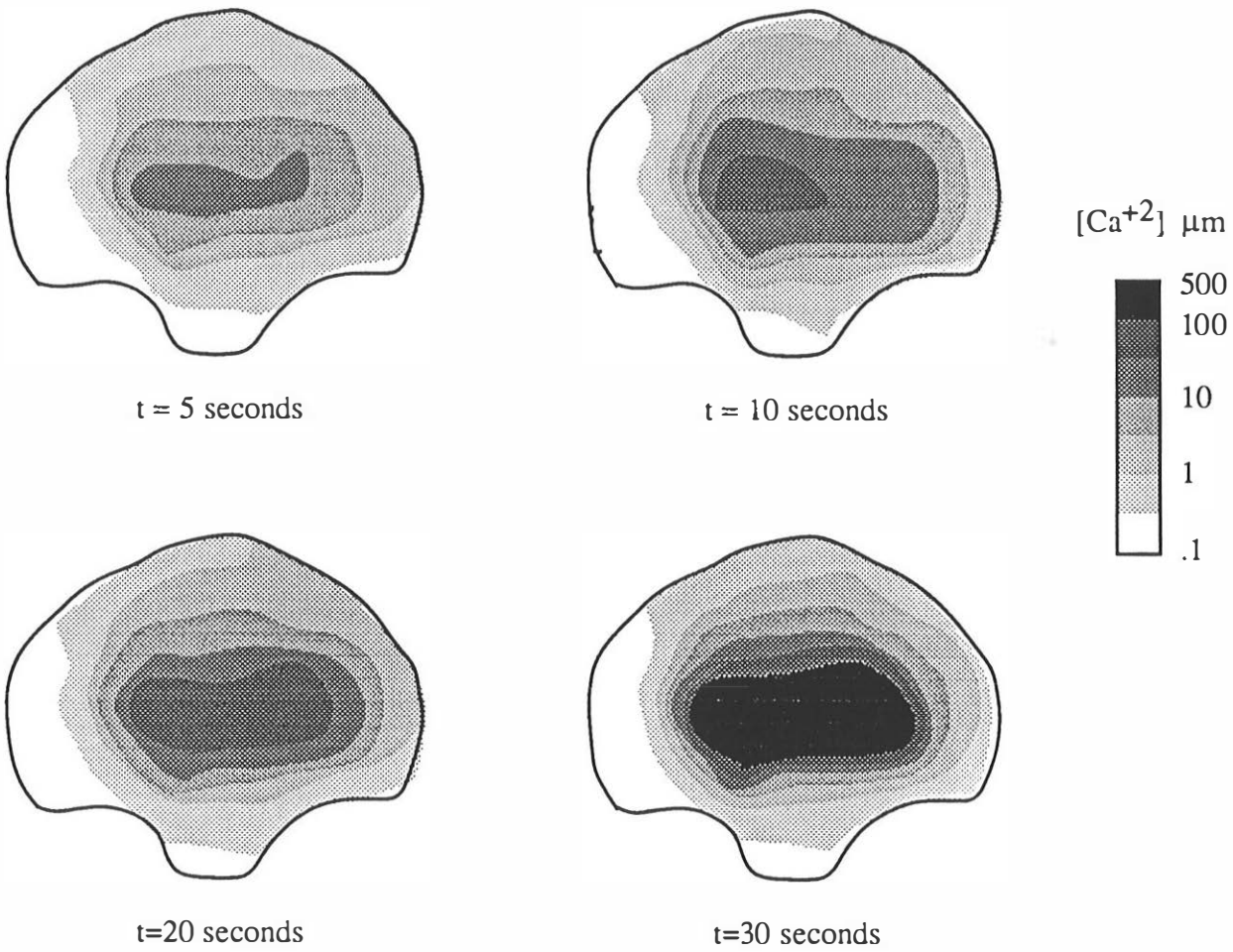


Figure 5

For a lower magnitude of inertial loading ($\ddot{\theta}_p$ = 4.7 x 10^4 rad/s^2, τ_d = 8.1 ms), the magnitude of peak deformation is reduced and the distribution of intracellular calcium displays no accumulative elevation. (Figure 6)

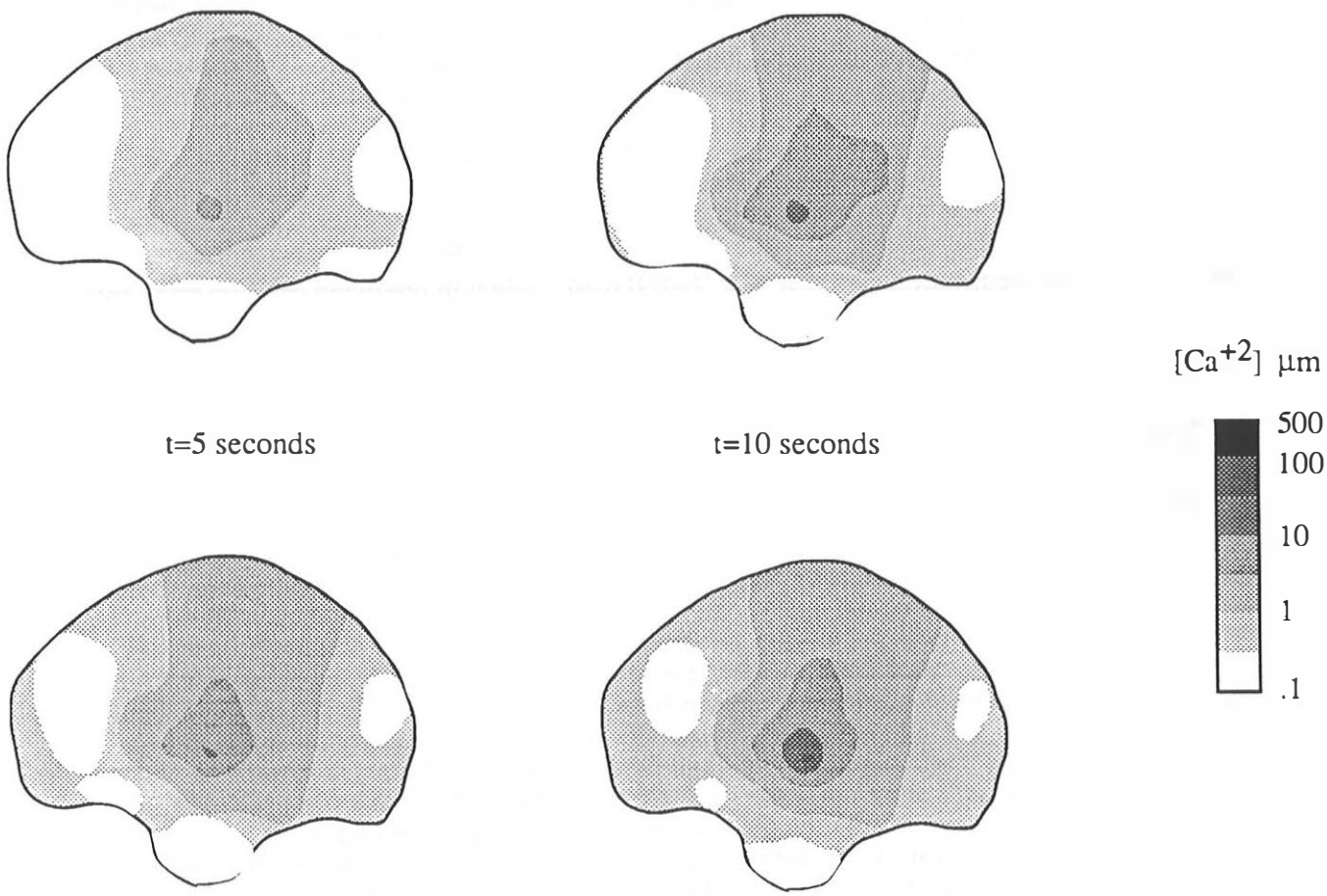


Figure 6

DISCUSSION

This report is intended to bring together the results of animal studies, physical model experiments, and isolated tissue investigations to derive a better understanding of the mechanisms and etiology for diffuse brain injury. Neuropathological evidence of axonal degeneration has led us to focus our investigations thus far on mechanically induced axonal injury to both unmyelinated and myelinated axons, and has led to the establishment of

functional and structural failure criteria for these neural components expressed in terms of measurable mechanical parameters. In parallel, physical models of the skull-brain have provided the opportunity to obtain an estimate of the brain deformation caused by a blunt impact loading, thereby creating a means to relate macroscopic load parameters to the damage of isolated components of the brain.

The information contained in this report is a first step toward integrating the information from physical model and isolated tissue studies to present a temporal picture of the events occurring at the isolated tissue level immediately ($t < 1$ minute) post-impact. The data presented predict a clear regional change in the intracellular calcium concentrations of axons residing within regions of the brain such as the corpus callosum, cerebral cortex, and parasagittal white matter. These changes are not only spatially selective, but are affected by the macroscopic head acceleration parameters. For example, a relatively mild acceleration level capable of producing concussion in the subhuman primate, produces only minor regional changes in intracellular calcium concentration. No areas are predicted to cause neurofilament degradation due to calcium activated neutroproteases ($Ca^{+2} > 50\text{-}100\ \mu m$), although predicted calcium transients in the medial third of the coronal brain section are sufficiently large to begin disassembly of microtubules ($Ca^{+2} > 1\mu m$) (Figure 6). In comparison, head acceleration levels capable of producing severe diffuse axonal injury in the subhuman primate create a more widespread elevation in calcium concentration (Figure 5). In particular, regions within the deep white matter display calcium levels indicative of neurofilament degradation, subsequent osmotic pressure imbalances, and possible axonal swelling.

Future refinements for the model presented herein include accounting for the anisotropic mechanical properties of the brain, the directionality of axonal fibers within selected anatomic regions of the brain, and including the effect of ischemia on intracellular calcium changes. Currently, physical models are constructed with a silicone gel material selected to match measured in vitro mechanical properties of brain tissue. However, no attempt is made to simulate the heterogeneous composition of the brain, such as the probable structural differences between the gray and white matter. As more detailed constitutive property information becomes available on the brain, these characteristics can be included in future physical models. In the same vein, both the direction of the nerve fiber projections and the orientation of the principal strain vectors contribute in estimating the tensile deformation experienced by an axon or group of axons residing within a select anatomic region. In the future, more sophisticated models are planned which will merge information on the mechanical field parameters with neuroanatomic data to present a more refined model for the intracellular calcium distribution within the brain. Finally, experiments are currently in progress to elucidate the effect of superimposed hypoxia on the accumulation of intracellular calcium.

ACKNOWLEDGMENTS

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REFERENCES

1. Abel, J., T. Gennarelli, and H. Segawa, *Incidence and Severity of Cerebral Concussion in the Rhesus Monkey Following Sagittal Plane Acceleration*. Proc. of the 22nd Stapp Car Crash Conf., 1978. SAE: p. 33-53.

2. Gennarelli, T., *et al., Diffuse Axonal Injury and Prolonged Coma in the Primate.* Ann. Neurol., 1982. **12**: p. 564-574.

3. Gennarelli, T.A., L.E. Thibault, and A.K.. Ommaya, *Comparison of Translational and Rotational Head Motions in Experimental Cerebral Concussion.* SAE Trans., 1971. p. 797-803.

4. Margulies, S., L. Thibault, and T. Gennarelli, *Physical Model Simulations of Brain Injury in the Primate.* J Biomechanics, 1990. **23**: p. 823-836.

5. Meaney, D., Biomechanics of acute subdural hematoma in the subhuman primate and man. 1991, Ph.D. dissertation, University of Pennsylvania:

6. Thibault, L., *et al. Analysis of the Strains Induced in Physical Models of the Baboon Brain Undergoing Inertial Loading.* in *Proc. of 35th ACEMB.* 1982.

7. Margulies, S., Biomechanics of Traumatic Coma in the Primate, Ph.D. dissertation, University of Pennsylvania, 1987

8. Winston, F.K., The Modulation of Intracellular Free Calcium Concentration by Biaxial Extensional Strains of Bovine Pulmonary Artery Endothelial Cells., Ph.D. dissertation, University of Pennsylvania, 1988

9. Barbee, K.A., Cellular Response of Vascular Smooth Muscle to Mechanical Stimuli. 1991, Ph.D. dissertation, University of Pennsylvania:

10. Galbraith, J.A., The Effects of Mechanical Loading on the Electrophysiology of the Giant Squid Axon. Ph.D. dissertation, University of Pennsylvania, 1988

11. Boock, R., Vascular Response to Mechanical Deformation. Ph.D. dissertation, University of Pennsylvania, 1991