The Strain Dependent Pathophysiological Consequences of Inertial Loading on Central Nervous System Tissue

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ABSTRACT

The complex problem of developing a predictive transfer function between the forces and/or motions experienced by vehicle occupants in the hostile mechanical environment of a collision and indicies relating to extent and severity of the various forms of central nervous system trauma can be made more tractable by understanding neural injury as a function of mechanical deformation at the cellular level.

A comparison of results obtained from animal, physical, and isolated tissue models indicates that an injury threshold for neural tissue can be expressed in terms of a maximum strain under conditions of dynamic loading.

Nonhuman primates were subjected to controlled inertial loading of the head which produced a spectrum of pathophysiological consequences, ranging from mild cerebral concussion to severe diffuse axonal injury with prolonged coma. Physical models of the skull/brain structural complex were loaded under identical kinematical conditions over the same range of accelerations and with precisely the same acceleration wave shapes. Nodal displacements of a printed grid contained within the surrogate brain material were measured from high speed film frames and the associated strains were computed. In parallel studies isolated single axons were subjected to high strain rate uniaxial elongation with extension ratios comparable to the levels suggested by the physical model experiments. The physiological response of the axon was examined by measuring membrane potential, voltage-clamp current and cytosolic free calcium concentrations pre and post loading.

Correlations are made between the maximum extension ratio and the physiologic variables which suggest an exponential dependence of injury severity upon the level of mechanical insult. At the isolated axon level the injury spectrum varies from mild and spontaneously reversible depolarization, to irreversible changes in membrane potential and accumulation of intracellular free calcium. The magnitude of the extension ratios which describes this spectrum of pathophysiological response is then correlated with the computed strain field in the physical model studies. A transfer function is developed which permits one to relate the inertial loading conditions under which the deformation response of the tissue will yield a specific level of neural tissue injury severity.

INTRODUCTION

Approximately 60,000 fatalities per year in the United States can be directly linked to traumatic brain injury, and 40,000 additional people are left severely disabled from such injuries. An estimated 400,000 citizens suffer mild head injuries which can produce some degree of functional disability for a period of months after the injury occurred, with no apparent physiological explanation. In all, nearly two million Americans each year suffer some type of head injury (1)

The relationships between the energies associated with the vehicle crash environment and the forces applied to the occupants which result in various levels of injury severity are difficult to determine. With respect to the central nervous system (CNS), impact to the head and the associated inertial loads produce deformation of the neural and neurovascular components which constitute the more macroscopic structures of the brain. The complex pathophysiological process which accompanies the mechanical distortion to the CNS can be studied by investigating the response of isolated tissue elements and single cells to mechanical stimuli that are designed to simulate the in-situ conditions asociated with trauma. By developing"failure" criteria for the components which constitute the brain we can begin to simplify this otherwise arduous task, and in the process provide a scientific basis for the development of improved head injury tolerance criteria.

In the past we have used a primate model to replicate specific forms of brain injury which are commonly seen in man. We were successful in being able to reproduce cerebral concussion and diffuse axonal injury to varying degrees of severity(2-4). Physical models, or surrogates of the skull-brain structure, were then employed to estimate the magnitude and temporal nature of the deformations which were experienced by the tissue under loading conditions which were identical to those used in the primate experiments(5,6). The data obtained from the physical model studies allowed us to prescribe the conditions under which we would test isolated tissue in order to examine injury at the cellular level.

This report will focus upon the effects of mechanical deformation on the axons of the central nervous system. Specifically, the effects of high strain rate uniaxial extension (to various levels of stretch ratio) is studied in concert with the changes in neurophysiology of the single axon. Dysfunction and/or structural failure at the cell level is then related to the spectrum of clinically relevant forms of central nervous system trauma. These relationships are based upon the electrophysiological and biochemical events which occur in response to controlled levels of cell membrane deformation. In order to apply these data to the problem of developing predictive indicies for CNS trauma , in its many forms, it remains to relate the macroscopic loading conditions to the field variables. In particular, we have found that the local stretch ratio for the isolated axon correlates with the pathophysiological changes observed over a broad range of cellular response. Physical and analytical models have been used to investigate the levels of rotational acceleration and changes in angular velocity which can produce deformations in excess of the threshold values which are estimated from the islated tissue data.

The development of a cellular basis for central nervous system tolerance criteria is designed to provide a rational and comprehensive approach which utilizes patient data, animal studies, physical models,

analytical models, and single cell experiments. The patient studies provide the direction of the research program and define the clinical basis for the specific forms of injury to be investigated. The animal model experiments are used to determine the loading conditions which are related to the equivalent clinical pathophysiology. The analytical and physical model simulations enable one to estimate the deformations within the brain as a result of these specific loading conditions. Lastly, the isolated tissue studies enable one to investigate the direct physiological and biochemical effects of controlled mechanical stimulation at the level of the single cell. At the present time there does not appear to be a cellular basis for mechanical trauma. Presented in this report is a relationship between the stretch ratio, or the elongating strain, and the continuum of the cellular responses which are elicited under the conditions of high strain rate uniaxial extension. When comparing this functional relationship with the magnitude of the estimated strains which are obtained from the physical and analytical model simulations it becomes possible to establish thresholds for neural injury with varying degrees of severity. With the advent of improved finite element codes it is hoped that the topographic distribution of the tissue deformation can be related to the pathology in the clinical setting.

RESEARCH METHODS

The data bank of the Head Injury Center of the University of Pennsylvania was used to determine the nature of the brain injuries sustained by the patients. It has been shown that cerebral concussion.was the most frequent form of head injury.and that diffuse axonal injury (DAI), was one of the most common types of severe head injury.(7). Therefore,a study was undertaken to reproduce the clinical entities of cerebral concussion and diffuse axonal injury with graded levels of severity in the nonhuman primate and to determine the macroscopic loading conditions which relate to these phenomena. Earlier work in this area demonstrated that angular acceleration of the head was an important consideration with regard to the kinematics of the loading conditions (8-14). A system was therefore developed in collaboration with the National Highway Traffic Safety Administration which was capable of providing controlled accelerations to the head with the capability of independantly varying the kinematical constraints of the head motion. The details of the system design are described elsewhere (15,16). As a result of this effort we were able to reproducibly generate animal models of acute subdural hematoma, cerebral concussion, and diffuse axonal injury (DAI) with graded severity. This report, however, will focus upon the injuries associated with axonal damage only. Figure (1) depicts the effects of an impulsive rotation of the head on the pathophysiological outcome. The animals received cerebral concussion or DAI depending upon the loading conditions.

The data presented here are scaled to a 1200 gram brain based upon the scaling relationship proposed by Holbourn (16).One unique observation with regard to this study was that a substantial lateral (coronal plane) component of the rotation was required in order to produce DAI (17). The data sugested that cerebral concussion and DAI may represent a continuum of injury severity to the axons of the brain and that the anisotropic nature of the brain (axonal orientation) dictates to some degree the clinical portrait which results from mechanical trauma.

In order to assess the magnitude of the field variables associated with these loading conditions and to empirically evaluate the scaling function a series of physical model experiments were conducted. Skulls filled with an optically transparent surrogate brain material were subjected to identical loading conditions as the animals. The deformations of orthogonal grids painted on a plane within the gel were recorded with high speed photography and subsequently digitized for analysis. Details of this methodology have been presented elsewhere (18).

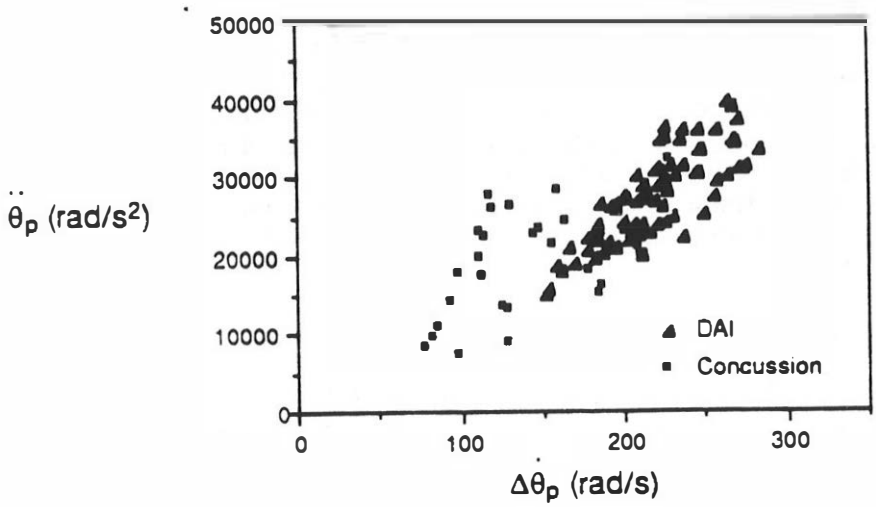

FIGURE 1

Figure 2. shows examples of computer-reconstructed digitized images of both the Baboon Model and the Human Adult Model. The undeformed images are on the left and the figures to the right represent the peak deformation which correlates closely in time with the peak deceleration.

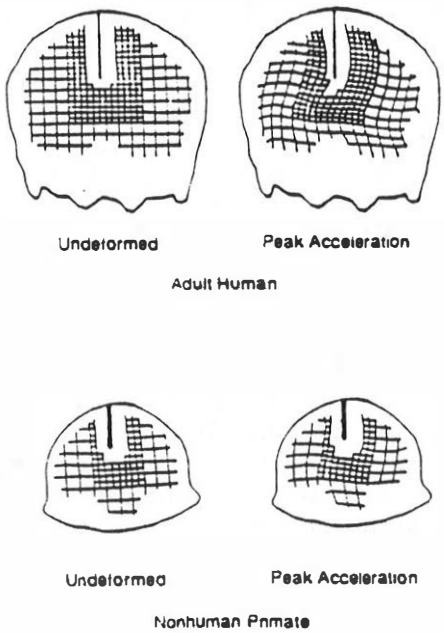

FIGURE 2

From these experiments the strains associated with the loads were estimated and related to those used to produce specific forms of injury. These data can also be used to validate various analytical or numerical simulations of the event.

In order to perform a simplified parametric analysis of this problem we constructed an analytical model of a right circular cylinder of the surrogate material undergoing centroidal rotation (19). The model was exercised for variations in the constitutive properties of the surrogate material, the mass of the model, and a variety of loading conditions which span those values which were measured in the animal model experiments.

Figure 3. presents some results of the model for illustrative purposes. In the top figure the model approximates a 145 gram brain which is equivalent to the mass of the baboon brain. The lower figure represents a mass of 1067 grams and is designed to model the surrogate of the adult human. The strains are calculated at a nondimensional radius of 0.3 in each case. This equivalent anatomic location was selected to represent the deep white matter of the brain where significant morphologic damage is observed in association with DAI in both the nonhuman primate and in man. The dashed lines are drawn at the values of 5, 10, 15, and 20% strain for reasons which will be discussed in the results section.

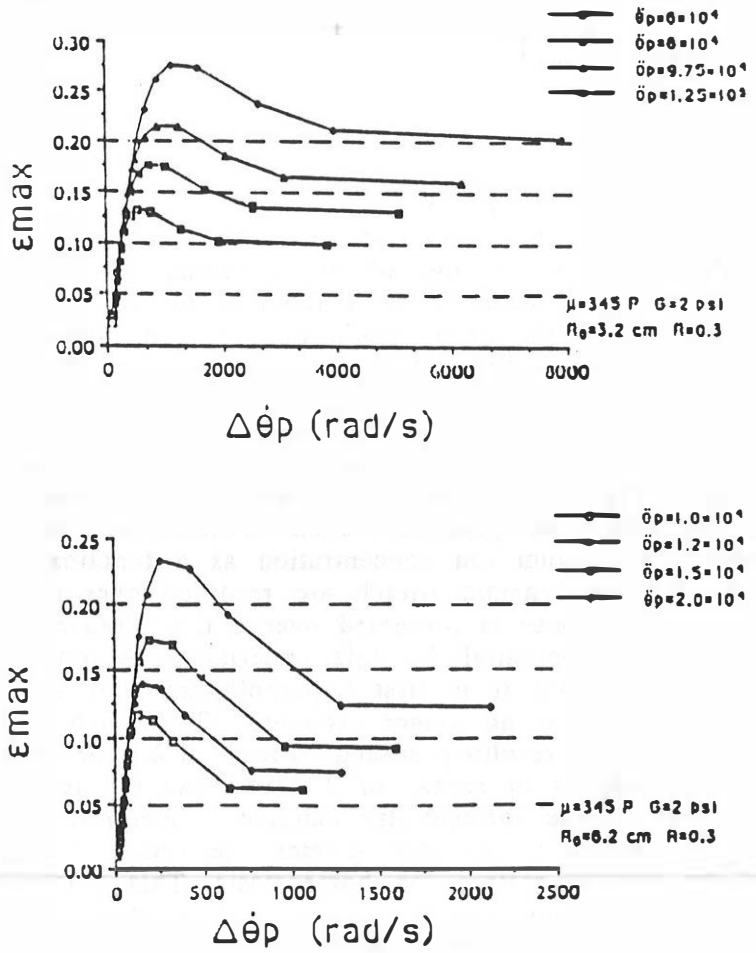

FIGURE 3

Having produced the clinically relavent injuries of cerebral concussion and DAI in the animal model, and having estimated the strains associated with the concommitant loading parameters, we conducted a series of experiments to determine the cellular response to dynamic deformation of comparable magnitude.

The giant axon of the squid, Loligo Pealei, was selected as the isolated tissue model and a system was designed to apply uniaxial extension at high strain rates to the preparation. The system consists of an electromagnetic actuator, displacement transducer, isometric force transducer, membrane potential electrodes, and custom designed calcium ion selective electrodes; all of which are mounted on the stage of a microscope. The details of the design of this system are presented elsewhere (20). A simplified schematic representation of the system is shown in figure 4.

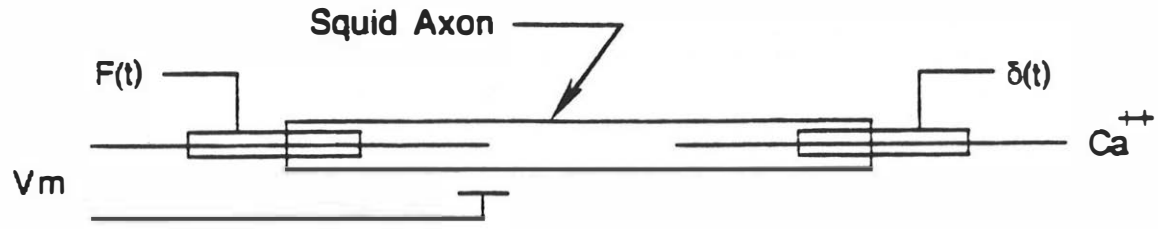

FIGURE 4

The actuator was programed to deform the axons to various stretch ratios at specific strain rates. Recordings of the membrane potential and the cytosolic free calcium ion concentrations as a function of the strain and the tensile forces developed within the axon enable one to study the response of the isolated tissue to mechanical stimulation. The intention of these experiments was to attempt to elucidate the thresholds for the tissue response to a well controlled mechanical insult. Our ultimate aim was to be able to relate the field variables from the physical and analytical model studies to this isolated tissue response. Figure 5. demonstrates the typical experiment which displays, in order, the membrane potential, axon deformation, developed tension, and cytosolic free calcium ion concentration as a function of time. As can be seen the data for the dynamic stretch are recorded over the interval of 100 ms while the calcium response is presented over a time course of 30 sec.

The resting membrane potential for this experiment is modified by the rapid stretch in such a way that it is first hyperpolarized and subsequently depolarized to an extent that it is no longer excitable. The stretch of the axon is approximately .32 mm and the resulting stretch ratio is 1.2. The developed tension under these conditions is in excess of 2 grams and the result of this insult is a dramatic rise in the intracellular calcium concentration. Note that this rise in calcium is followed by an even greater rise which is indicative of a complete failure of the cell to restore ion homeostasis. This particular experiment was selected to demonstrate the severe end of the pathophysiological spectrum. It has been shown previously that the effects of elevated cytocolic free calcium above 50 micro molar will result in calcium activated neutral protease which can damage the protein structures of the cell.

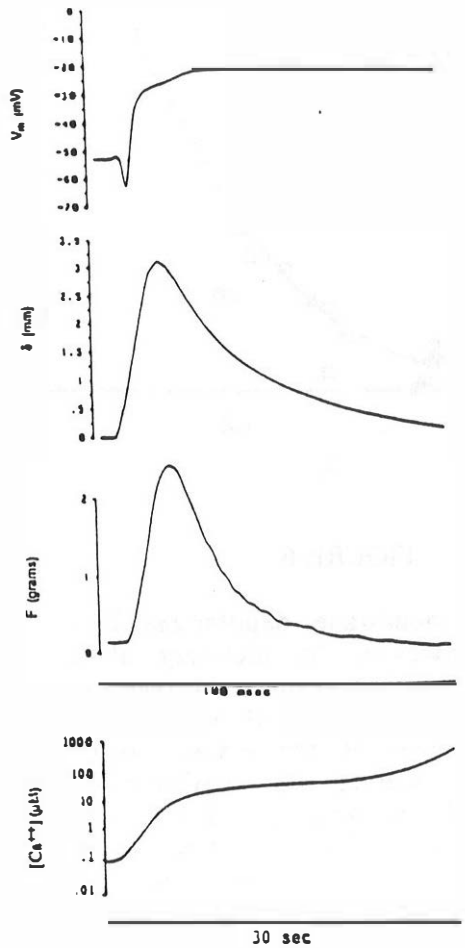

FIGURE 5

RESULTS

The isolated tissue studies afford the opportunity to investigate the issue of whether there is a direct correlation between the mechanical stimulation and the resulting pathophysiological manifestations at the level of the axon. If such a correlation exists then an injury tolerance criterion may be assigned on the basis of this correlation provided we have the ability to relate the field variables within the brain to the loads which are applied to the head.

The first measure of the results of this report is presented in Figure 6. Alterations in the membrane potential of the axon is a sensitive measure of the injury to this living structure. Depicted in this figure is the change in membrane potential (expressed as a percent change from the resting state in order to normalize the data) as a function of the mechanical stimulus (which is expressed as the stretch ratio). As can be seen there is an exponential dependance of the depolarization upon the magnitude of the mechanical stimulus. It should be noted that the membrane fails in a structural sense when the stretch ratio exceeds 1.25 at these levels of strain rate. These experiments were conducted under a variety of chemical conditions which present the membrane as normal or modified with sodium and potassium channel blockers present. There is no difference in the outcome with respect to the depolarization, the interpretation of which will be discussed latter.

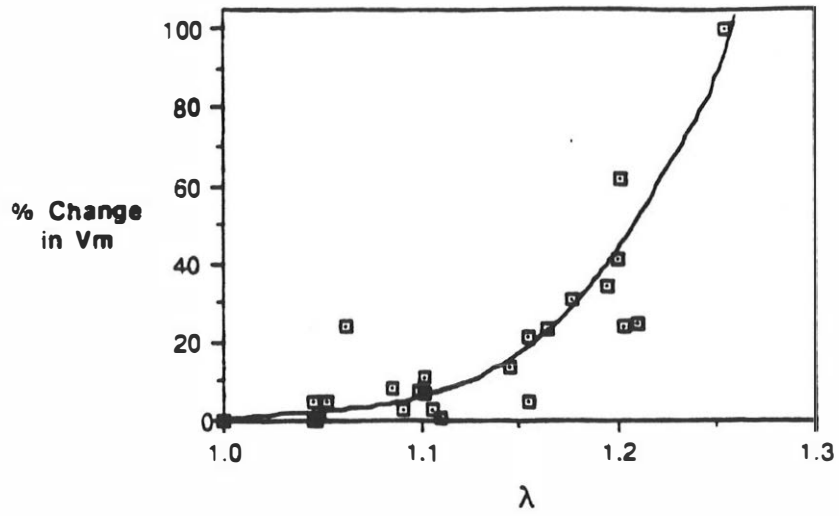

FIGURE 6

The degree to which the membrane depolarizes is a reasonable measure of the severity of the injury, however, the recovery of the resting membrane potential to a point where it is once again excitable is a important consideration from a purely functional point of view. Figure 7. shows three different experiments where the level of the insult spans the range of stretch ratios from 1.12 to 1.20. In these studies the membrane potential is recorded over a period of ten minutes and the recovery of the membrane is examined during this interval. Shown on the ordinate is the membrane potential immediately post injury. As can be seen the three levels of mechanical stimulation which were selected demonstrate the following; spontaneous recovery at a stretch ratio of 1.12, a residual deficite at a stretch ratio of 1.15, and an irreversible injury at a stretch ratio of 1.20.

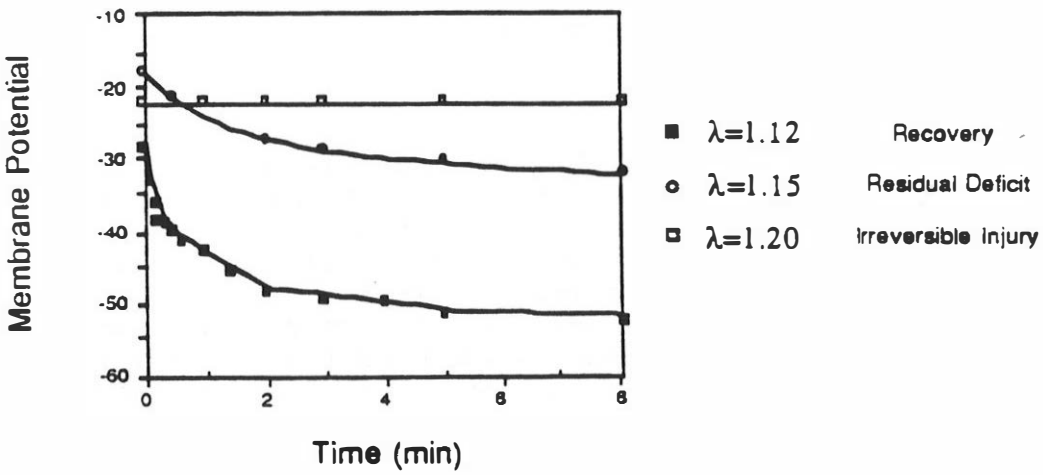

FIGURE 7

It must be emphasised that these studies are conducted in-vitro and therefore the interpretation of the data should reflect the relative aspects of the results, as opposed to the absolute values of the numerical values. However, this study demonstrates that the degree of mechanical injury to the axon influences both the magnitude of the depolarization as well as the time course of the recovery phase.

This observation is not unlike the clinical aspects of brain injury with regard to the duration of the neurological changes which accompany a head injury. In order to investigate the mechanisms of injury to the isolated axon and to further explore the functional relationships between mechanical deformation and neuropathophysiology we measured the changes in intracellular calcium following injury. Figure 8. demonstrates the temporal response of the cytosolic calcium ion concentration following three levels of mechanical stimulation. Within a period of 30 seconds the calcium concentrations of those axons which were subjected to stretches of 7 and 12% respectively were exhibiting recovery toward the control levels of intracellular calcium. In the case of the experiment which produced a stretch of 20% the free calcium ion concentration continued to rise to equilibrium with the external medium which was sea water with a calcium concentration of one milli molar. Under this circumstance the membrane would be considered incompetent.

We believe that the effects of mechanical deformation of the axon membrane lead to an alteration in membrane permeability as a result of the development of non-specific defects in the membrane. This phenomena which we will refer to as mechano-poration is dependent upon the strain in the membrane and the strain rate with which the loads are applied. This mechanism of injury can produce a broad spectrum of cellular responses and will be discussed latter.

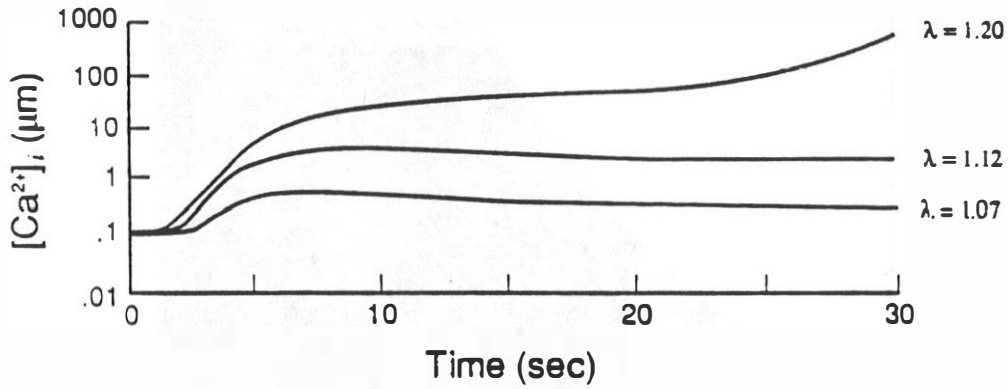

FIGURE 8

Figure 9. demonstrates the functional relationship between the magnitude of the mechanical strain, expressed as the stretch ratio, and the peak values of the intracellular calcium changes following the injury. This curve is devided into five regions which we believe represent the approximate ranges of stretch ratio that delineate the physiological changes associated with the ultimate outcome of the experiment. These ranges are labled A-E and are defined as follows:

A- The axon will spontaneously recover quickly with no residual deficite;

B- The axon will recover, but the time course of recovery is prolonged, and there will be no residual deficite;

C- The axon will attempt to recover but there will be a residual deficite, and the ultimate outcome of the recovery will depend strongly upon the ability of the cell to pump calcium (i.e., metabolic factcrs will strongly influence this outcome);

D- The axon is irreversibly injured by the initial calcium insult, and will eventually die;

E- The axon will fail structurally as a result of the mechanical deformation.

The response of the isolated axon to mechanical stimulation appears to exhibit an injury pattern which is continuous in the sense that the severity level is graded and dependent upon the level of insult in an exponential manner. The thresholds for specific forms of injury are reasonably well defined at the single cell level when one measures the membrane potential and the intracellular free calcium ion concentration. A plateau region of the previous curve indicates that the calcium ion concentration will rise to external levels once one reaches region D. This region must therefore be considered as fatal for the single cell.

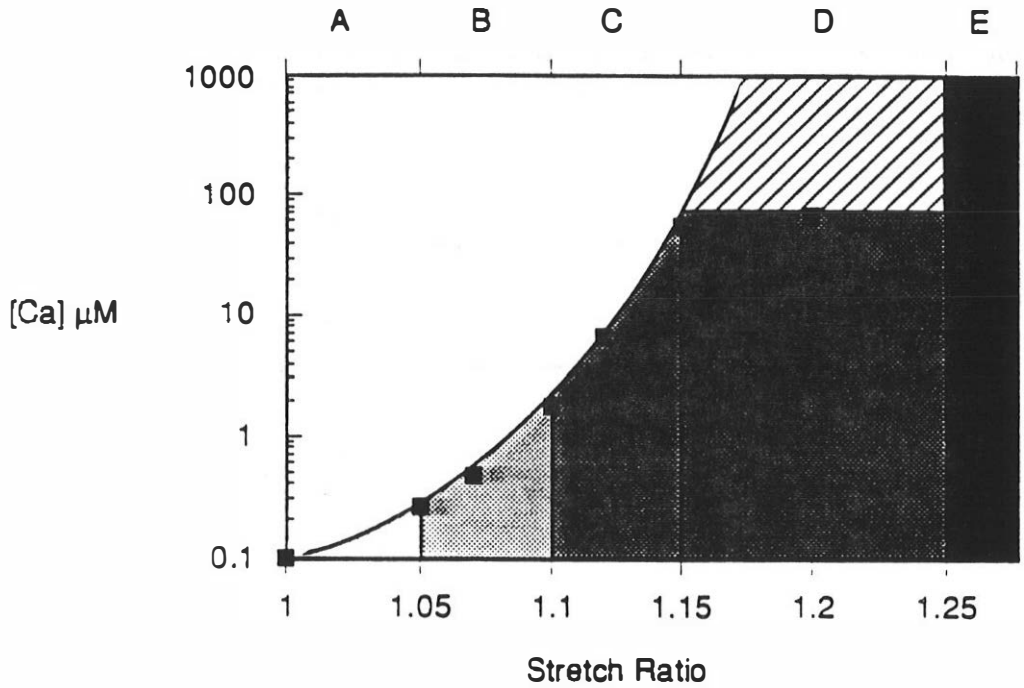

FIGURE 9

DISCUSSION

Based upon the experimental and analytical studies presented in this report we submit that neural tissue injury can be directly related to the magnitude of the imposed mechanical strain under conditions of dynamic loading. It may therefore be possible to generate INJURY-SPECIFIC TOLERANCE CRITERIA in the near future.

Figure 10. presents the values of stretch ratio which are predicted to occur as a function of a rotational acceleration of the head. The stretch ratio was calculated using the analytical model of a right circular cylinder undergoing centroidal rotation (as described in 19). ·

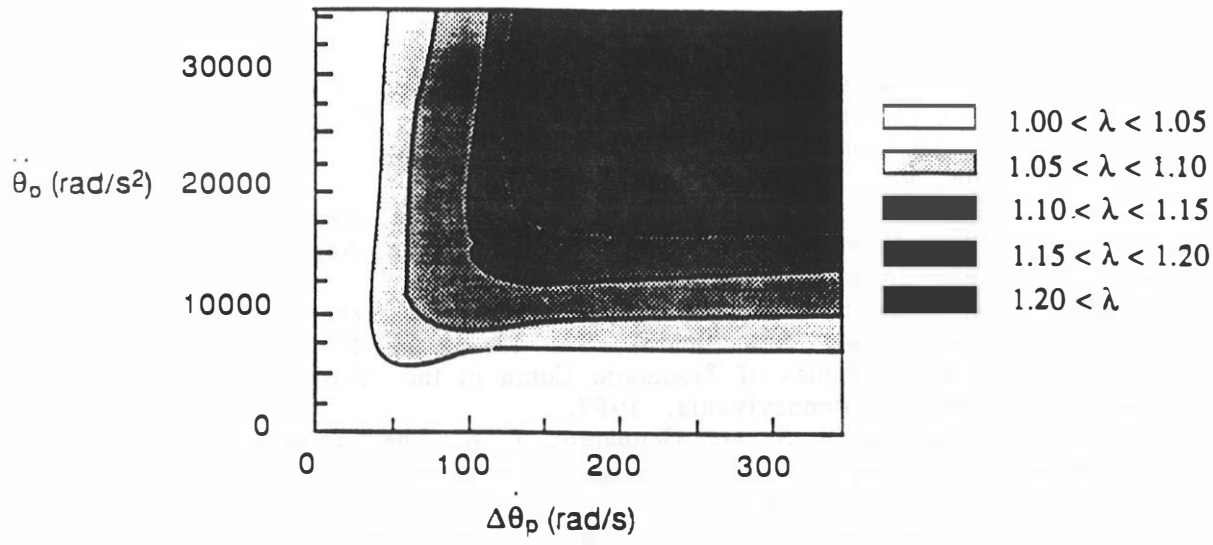

FIGURE 10

The regions of this curve are drawn for strains of 5, 10, 15, and 20%. At this time there is no direct way to relate the magnitude of the strain at the level of the single cell to the complex portrait of brain injury and the various clinical manifestations; we must also know the anatomic distribution of the strain pattern in order to assess the global effects of injury to the whole brain. For example the injuries which are described as cerebral concussion, mild DAI, moderate DAI, and severe DAI with prolonged coma may represent the continuous spectrum of injury to the axons of the brain. To relate the data in this study to those phenomena requires additional investigation. However, the data presented in this report strongly suggest that the injury tolerance threshold for the individual components of the central nervous system are reflected in this continuum response..With the improved finite element codes,which are becoming increasingly available, it is hoped that correlations will be made between the macroscopic loading conditions and the topographic distribution of the strains experienced by the neural elements. Issues such as brain anisotrophy and detailed anatomic distributions of the axonal elements may be simulated in future numerical model simulations.

The isolated tissue model has provided the opportunity to study the detailed mechanisms of injury to the axon under these loading conditions and we hope that this model will enable us to further investigate improved methods of injury prevention and to develop strategies for theraputic intervention.

ACKOWLEDGEMENTS

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