

CENTRAL NERVOUS SYSTEMS INJURY CRITERIA FROM HUMAN SURROGATE RESEARCH: A PROSPECTIVE VIEW

David F. Meaney, Susan S. Margulies, Phil Whitley, Lawrence E. Thibault
Department of Bioengineering and Division of Neurosurgery
University of Pennsylvania, Philadelphia, PA
Naval Air Warfare Center - Aircraft Division Warminster, Warminster, PA

Paper was presented at the 22nd Annual Workshop on Human Subjects for Biomechanical Research. This paper has not been screened for accuracy nor refereed by any body of scientific peers and should not be referenced in the open literature.

The complex loading conditions that occur during an automotive crash present several challenges when relating the applied loads to occupant injury risk and developing effective injury prevention strategies. In this report, we detail previous efforts to describe the tolerance of the central nervous system to blunt mechanical trauma, and discuss how data from these in vivo and in vitro studies can complement human surrogate studies. We discuss the unique information generated from experiments using animal surrogates, and discuss their impact on developing estimates of injury thresholds. Following this review, we propose several new broad initiatives to form research links between animal and human surrogate research, and describe some preliminary efforts. It is expected that the combination of present and future efforts will provide the experimental information necessary to develop new estimates of injury tolerance that, in turn, will mitigate the incidence and morbidity of central nervous system injury.

INTRODUCTION

Central nervous system injury continues to represent a major economic and social burden in society, accounting for over 23 billion in annual costs and affecting over two million people each year in the United States. Although the mortality and morbidity of CNS injury has been well characterized, effective prevention and treatment strategies are under continuous development.

In our laboratory, we have focused on characterizing the lesion mechanisms and tolerance thresholds for both focal and diffuse brain injuries. Clinically, nearly three quarters of the brain injuries that result in death in severely head injured patients are due to two forms of brain injury - diffuse axonal injury and acute subdural hematoma. In this report, we discuss our previous modeling work that focused on defining the threshold for these two forms of brain injury. In turn, we review the findings from in vitro studies of CNS trauma that have focused on understanding the mechanisms of axonal injury. Finally, we review recent animal models of traumatic brain injury that have been developed, and discuss their implications in developing new tolerance threshold information.

MODELING OF FOCAL AND DIFFUSE BRAIN INJURIES

Scientists and engineers have proposed a number of injury criteria to provide a standard for protective equipment testing. Even in its latest form, however, the injury criteria has been criticized for its inability to distinguish between specific forms of brain injury and different head sizes, as well as the inconsistencies observed between injury measures and

injuries observed in human surrogate experiments. To address some of the shortcomings of head injury criteria (HIC), a recent effort has been made to separate and evaluate the mechanisms associated with specific forms of brain injury. To this end, two commonly observed brain injuries, acute subdural hematoma and diffuse axonal injury, have been reproduced in subhuman primates using either a coronal or sagittal plane noncentroidal head rotation. Physical models, or surrogates, of the nonhuman primate skull-brain structure subjected to kinematic and kinetic conditions identical to those used in the animal experiments produced an estimate of the intracranial deformations occurring during these experiments. By combining the structural and functional failure criteria for vascular and neural tissue, an estimate of the tolerance level for acute subdural hematoma and diffuse axonal injury in the nonhuman primate and man has been proposed.

Although these physical modeling experiments can offer an estimate of the contribution of multiple axis inertial loading parameters on the tolerance to specific forms of brain injury, they offer no direct means to assess the validity of these proposed thresholds. Alternatively, prudent use of human surrogates presents an opportunity to evaluate the effectiveness of proposed tolerance levels specific to brain injuries, and perhaps represents, when combined with physical modeling efforts, an efficient and complete means to directly identify more refined head injury tolerance criteria for the structural failure of neural and neurovascular tissues.

Formulation of DAI Tolerance Criteria

In the same manner as for other brain injuries, an animal model of diffuse axonal injury (DAI) was developed at the University of Pennsylvania and used to evaluate the injury mechanisms associated with prolonged coma and to develop a tolerance level for DAI in the nonhuman primate and man. Figure 1 depicts the effects of an impulsive rotation of the head, and demonstrates the graded forms of injury (concussion, DAI) produced using this device. 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 (Gennarelli, Thibault et al. 1987).

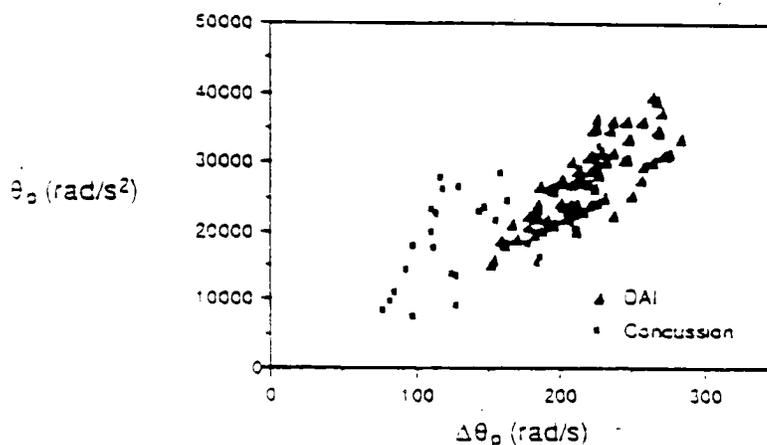


Figure 1

In order to assess the magnitude of the field variables associated with the loading conditions responsible for DAI, 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 in previous papers.

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. From these experiments the strains associated with the loads were estimated and related to those used to produce specific forms of injury.

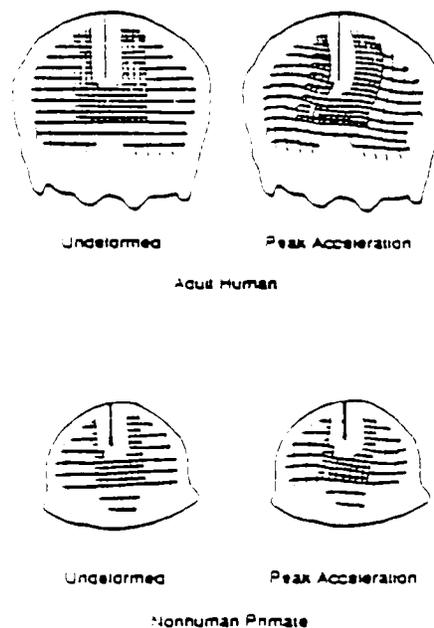


Figure 2

The data derived from physical model experiments 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 (Margulies and Thibault 1989). 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 results of the model for an idealized baboon brain (145 grams) and adult human brain (1067 grams). 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 based on results from isolated tissue experiments (see below).

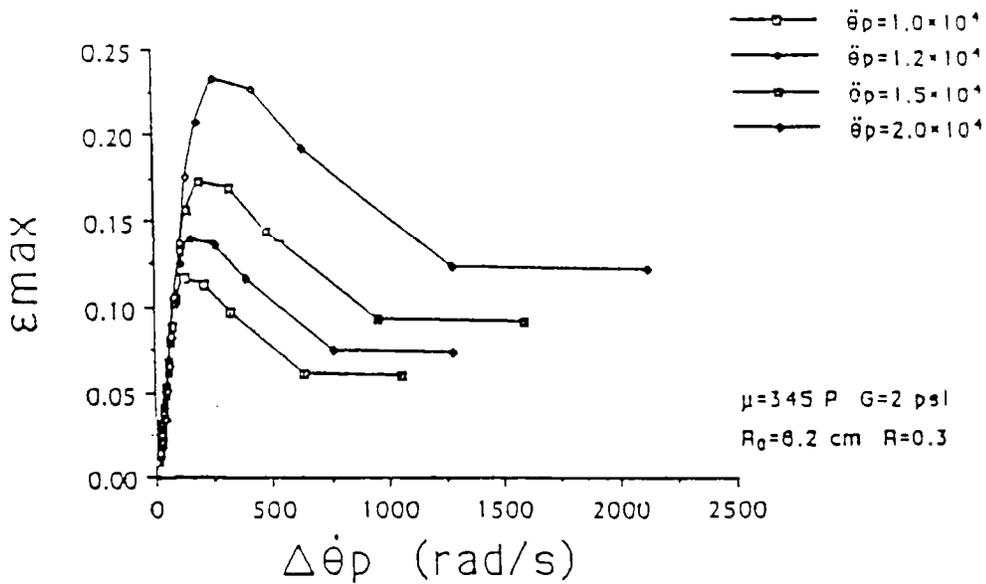
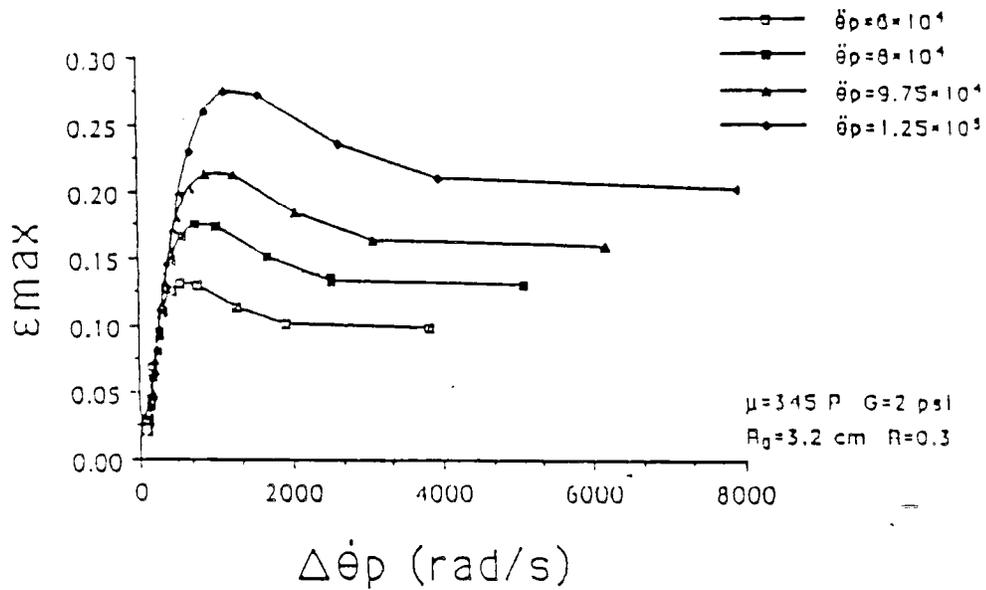


Figure 3

Formulation of ASDH Tolerance Criteria

A combination of animal studies, physical model tests, numerical simulations, and tissue testing were integrated to derive tolerance levels for ASDH in subhuman primates (macaca mulatta) and humans (Meaney 1991) for sagittal plane head motions. The results of 43 animal experiments performed by Abel et al were reviewed to establish an injury threshold (Abel, Gennarelli et al. 1978). The animals were subjected to sagittal plane angular acceleration of short duration and relatively constant angular excursion (approximately 60 degrees). The injuries produced were ranked in terms of an experimental trauma scale (ETS), where higher ETS values indicated more serious injuries. ETS values of 4 or less were classified as varying levels of concussion, while scores of 5 or 6 indicated injuries which were fatal within hours. Large subdural hematomas (>2% of the intracranial volume) located in the frontal region of the brain occurred often in animals with these high ETS scores (5-6), but were absent in animals classified with lower ETS score (≤ 4). Nineteen of the 43 animals were diagnosed with ETS scores of 4 or lower.

To estimate the deformations experienced by the parasagittal bridging veins in these experiments, a subhuman primate skull/brain model was constructed and subjected to the loading conditions within the range used in animal experiments (Meaney 1991). Since results from these animal studies indicated that the rupture of bridging veins in the frontal region of the brain was the most common cause of acute subdural hematoma, a nodal grid element in the frontal region of the rhesus skull/brain model was selected for analysis. The measured cortical stretch ratio in the primate surrogate model ($\theta'' = 7 \times 10^5 \text{ rad/s}^2$, $t_d = 5.8 \text{ msec}$), along with the measured acceleration trace, is shown in Figure 4. Using the results from a series of model tests over a broad range of loading conditions, a relationship between the peak stretch ratio and select kinematic parameters (peak angular acceleration, peak change in angular velocity) was formulated.

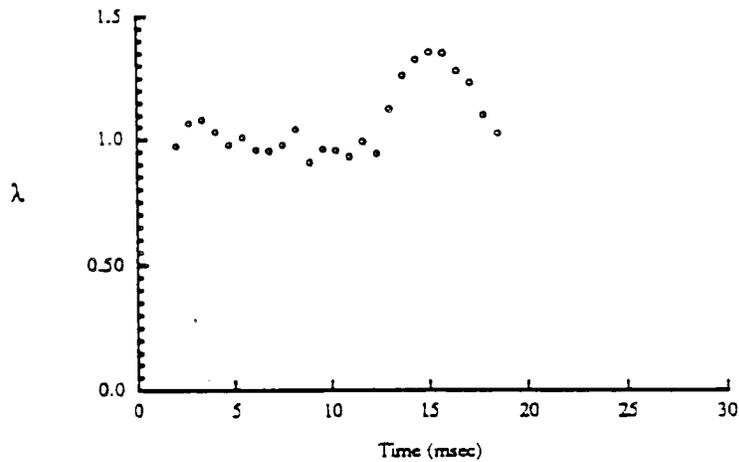
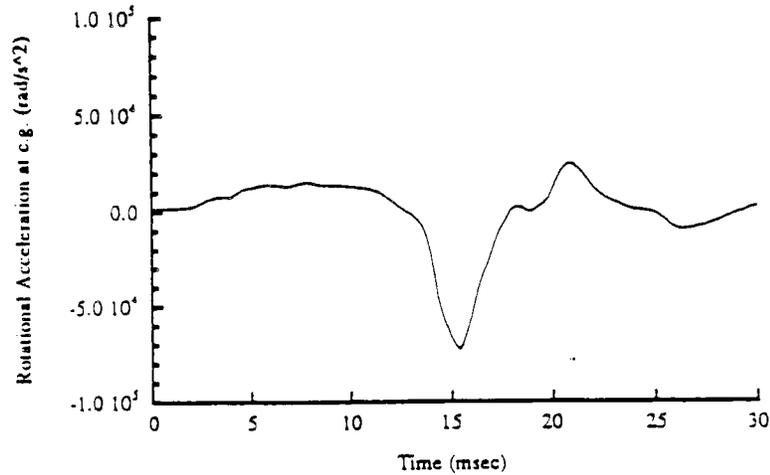


Figure 4

To develop a relationship for the structural failure of parasagittal bridging veins, a total of 129 bridging veins from 10 unembalmed human cadavers (3-62 yrs. old) were elongated over a range of strain rates, until failure (Meaney and Thibault 1990). Vessels were mounted on the material testing section, perfused with isotonic saline, and elongated at constant velocity until structural failure occurred. The velocity was controlled to apply strain rates within the range of strain rates witnessed in earlier physical modeling studies (approximately 25 s⁻¹ to 170 s⁻¹). Displacement and force signals were sampled and recorded. Bridging veins from three age groups (3-9 yrs. old, 27-47 yrs. old, and >62 yrs. old) were perfused (10 cm H₂O) and tested over a range of strain rates (1-220 s⁻¹). Kruskal-Wallis analysis ($\alpha=0.05$) showed no difference in the measured parameters for the age groups studied. Thus, an average stretch value ($\lambda_{ult} = 1.55$) was used as the failure limit for parasagittal bridging veins.

Combining the relationships from the physical modeling studies with a mean ultimate failure limit of $\lambda_{ult} = 1.55$, critical values of $\theta'' > 1.4 \times 10^5 \text{ rad/s}^2$ and $\Delta\theta' > 390 \text{ rad/s}$ to produce tearing of parasagittal bridging veins in the rhesus monkey were calculated.

MODELING OF CENTRAL NERVOUS SYSTEM INJURY IN VITRO

The high incidence and mortality associated with traumatic brain injury has prompted the development of several experimental models to identify the mechanisms of specific brain injuries in a controlled laboratory environment. Advantages for utilizing these experimental models are numerous, and include the ability to vary model input parameters to produce controlled and graded levels of injury, and the capability for producing and investigating isolated forms of brain injury. Experimental models also allow monitoring of macroscopic, cellular, and molecular physiological variables immediately following the injury, filling an important void in the clinical data base. The important endpoints or applications of these experimental models are twofold. Mechanically induced traumatic brain injury models present an opportunity for testing and comparing therapeutic intervention techniques in a more controlled, reproducible manner than is possible clinically. In addition, the models aid in the development of improved CNS injury tolerance criteria, providing an essential first step in the design of protective devices and in the more general goal of injury prevention.

A number of cell culture and isolated tissue models have been developed to investigate the sequence of events following mechanical and/or ischemic injury to nervous system tissue. The primary advantage of cell culture models is that the environment of the cells is greatly simplified, removing confounding factors that exist in whole animal traumatic brain injury models. The simplicity of cell culture models also presents an opportunity to focus on identifying the primary pathophysiological consequences of traumatic mechanical injury to isolated cells in culture, and to evaluate the synergistic cellular response to mechanical and ischemic insult.

In an initial series of in-vitro studies, the response of an isolated axon to dynamic elongation was evaluated, with the goal of establishing criteria for both the non-structural, i.e. functional, failure of the axon and the ultimate elongation limit before failure of the axon occurred. The giant axon of the squid, *Loligo Pealei*, was selected as the isolated tissue model since this structurally uniform unmyelinated axon allows a direct correlation between a mechanical input and the electrophysiological response to be established. A system was designed to apply a uniaxial extension at high strain rates to the axon and consisted 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 (Galbraith 1988). A simplified schematic representation of the system is shown in figure 5.

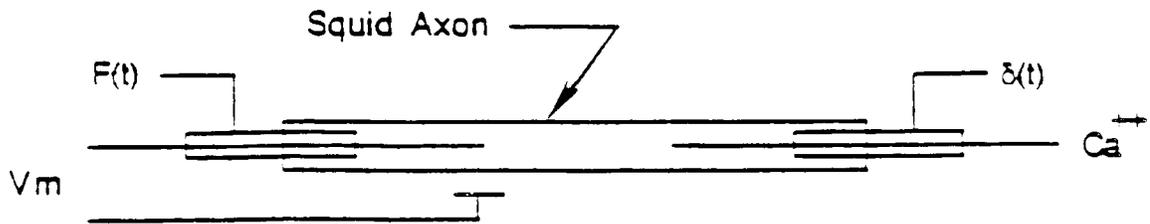


Figure 5

The actuator was programmed to deform the axons to various stretch ratios at strain rates of $5\text{-}25\text{ s}^{-1}$. 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 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 6 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.

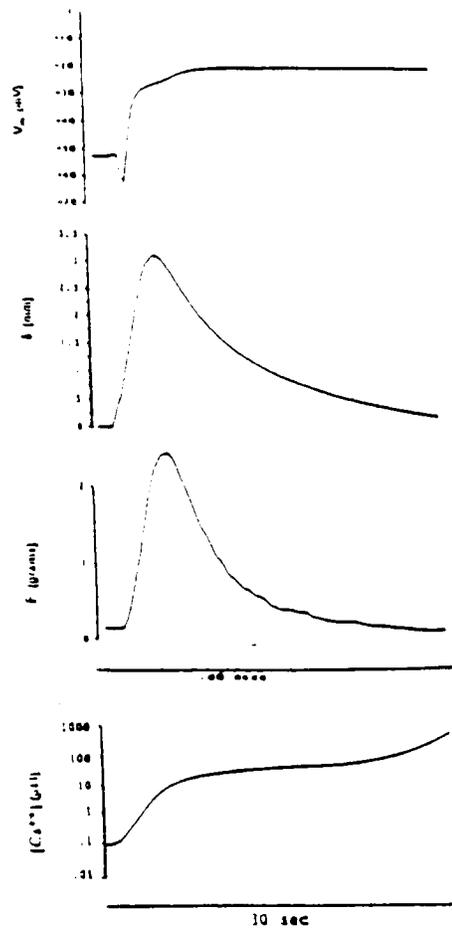


Figure 6

The resting membrane potential for this experiment ($\lambda_{ult}=1.2$) 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 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 cytosolic free calcium above 50 micromolar will result in calcium activated neutral protease which can damage the protein structures of the cell.

It must be emphasized 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 7 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 millimolar. Under this circumstance the membrane would be considered incompetent.

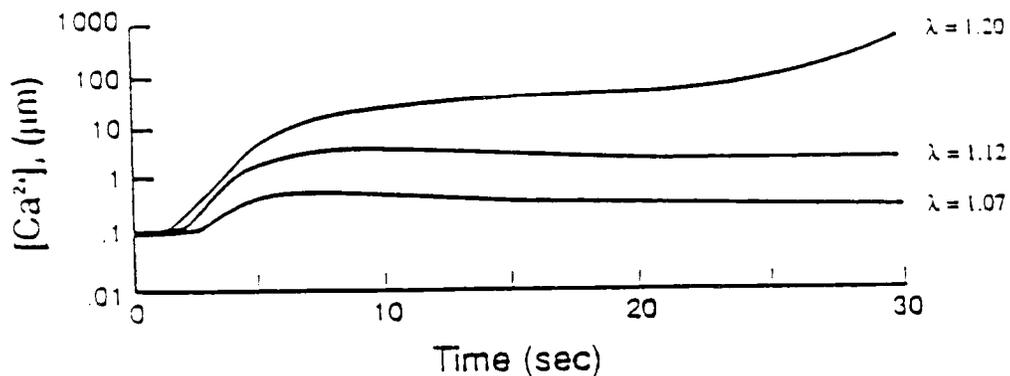


Figure 7

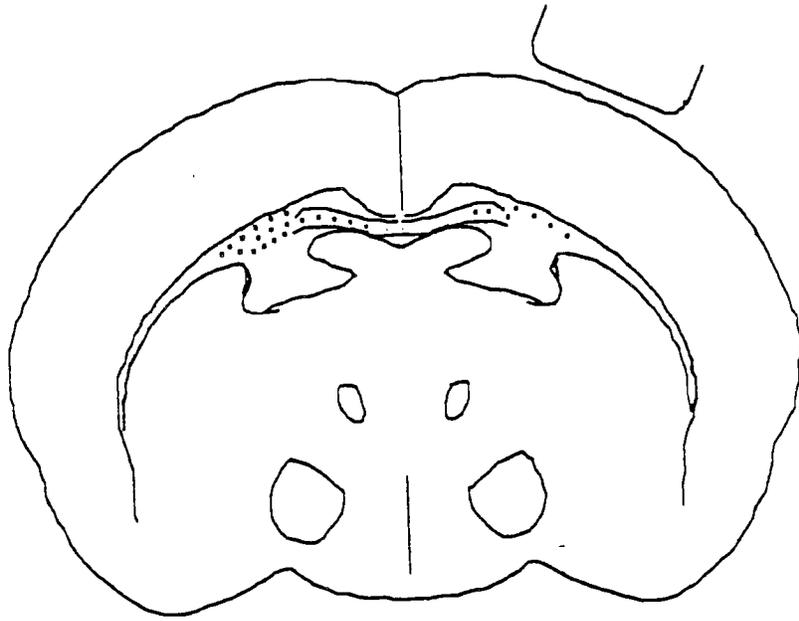
ANIMAL MODELS OF BRAIN INJURY

While the cell and tissue culture studies provide an indication of the immediate pathophysiologic changes occurring in neural cells and isolated axons as a consequence of stretch, methodological constraints prevent these techniques from monitoring long term changes. Moreover, these cell and tissue culture models do not provide an assessment of behavioral, physiological, and cognitive deficits caused by mechanical trauma.

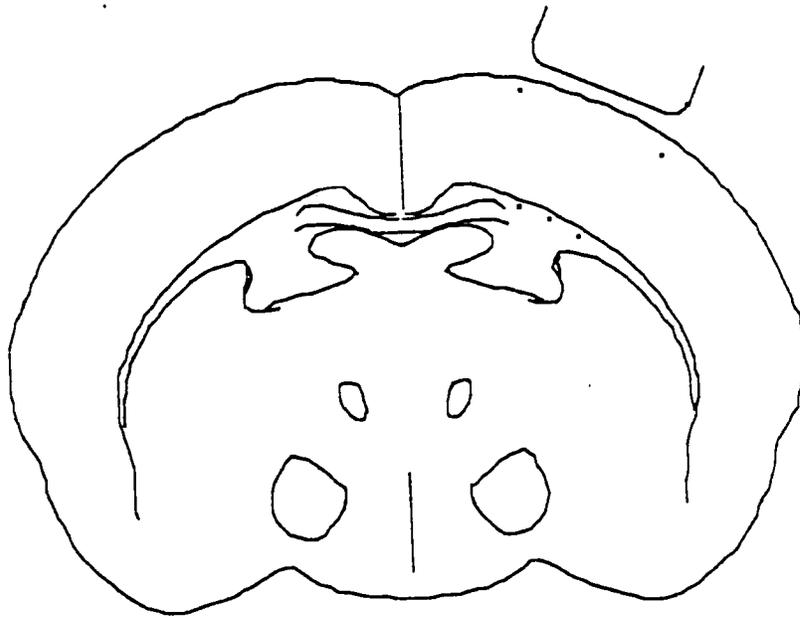
In response to these needs, we have developed an experimental model of traumatic brain injury that uses a controllable mechanical input to produce axonal injury in the rat. Details of this technique can be found elsewhere (Meaney and Ross 1993). In short, we have produced forebrain axonal injury in rats using a cortical impact injury model by directing the translation of tensile strain across the midline by opening the dura mater over the contralateral hemisphere. This is a modification of the original cortical impact technique (Lighthall, Dixon et al. 1989) and was designed to maximize forebrain axonal injury while minimizing injuries to other regions of the brain. The left cortex is indented 1.5 mm at a 23° angle with a velocity of 2.5 m/sec and a 22 msec dwell time. We have performed several series of experiments and found no evidence of forebrain axonal injury in 18 cases where the dura mater over the contralateral cortex was left intact (Ross and Ebner 1990). In another set of experiments (Gennarelli, Thibault et al. 1991) we performed bilateral craniotomies over the parietal cortex and opened the dura mater on the contralateral side prior to impact. Forty-two animals were sacrificed 1-28 days after injury, their brains sectioned and processed for cresyl violet, H&E, GFAP, GABA, or neurofilament (SMI-31 and SMI-32) immunohistochemistry.

Neurofilament immunohistochemistry revealed perikaryal and dendritic hypertrophy in the region beneath the craniotomy in the non-impacted cortex as well as numerous large axonal retraction balls in the underlying cortical white matter. Similar immunohistochemical techniques indicated the presence of axonal damage in the brainstem for cases with no contralateral craniotomy, and the significant reduction of these brainstem lesions caused by incorporating a contralateral opening. Numerous layer III pyramidal neurons in the adjacent cortex had initial axonal segments characterized by multiple large (20-30 μ m) dilatations and terminal clubs, indicative of axonal injury. Neurons with this appearance were never seen in the impacted cortex or in the non-impacted cortex of cases in which the contralateral dura was not opened.

A diagram depicting the distribution of axonal retraction balls for the cases with contralateral opening, shown in Figure 8 (a), indicate the increase in axonal injury caused by this modification. In comparison, this contralateral axonal injury was not observed for quasistatic indentations; see Figure 8 (b).



(a)



(b)

Figure 8

REFERENCES

- Abel, J., T. Gennarelli, et al. (1978). *Incidence and severity of cerebral concussion in the rhesus monkey following sagittal plane acceleration*. Proc. 22nd Stapp Car Crash Conf. SAE: 33-53.
- Galbraith, J. A. (1988). *The Effects of Mechanical Loading on the Electrophysiology of the Giant Squid Axon*. University of Pennsylvania.
- Gennarelli, T., L. Thibault, et al. (1987). *Directional Dependence of Axonal Brain Injury Due to Centroidal and Non-Centroidal Acceleration*. Proc. of 31st Stapp Car Crash Conf. SAE: 49-53.
- Gennarelli, T. A., L. E. Thibault, et al. (1991). *Axonal injury in the rat cerebral cortex in a modified rigid indenter cortical impact model*. Neurotrauma Society Annual Meeting, New Orleans.
- Lighthall, J. W., C. E. Dixon, et al. (1989). *Experimental models of brain injury*. J Neurotrauma 6(2): 83-97.
- Margulies, S. and L. Thibault (1989). *An analytical model of traumatic diffuse brain injury*. J. Biomech. Eng. 11: 241-249.
- Meaney, D. F. and L. E. Thibault (1990). *Tissue Failure Criteria For Isolated Parasagittal Bridging Veins (abstract)*. Proceedings of the First World Congress in Biomechanics.
- Meaney, D. F. (1991). *The Biomechanics of Acute Subdural Hematoma in the Subhuman Primate and Man*. University of Pennsylvania.
- Meaney, D. F. and D. Ross (1993). *Modification of a cortical impact brain injury model to produce axonal damage in the rat cerebral cortex*. : (submitted).
- Ross, D. T. and F. F. Ebner (1990). *Thalamic retrograde degeneration following cortical injury: an excitotoxic process?* Neurosci. In Press.

DISCUSSION

PAPER: Central Nervous Systems Injury Criteria From Human Surrogate Research: A Prospective View

PRESENTER: David F. Meaney, University of Pennsylvania

Q: Narayan Yoganandan, Medical College of Wisconsin

This MR is exciting stuff. Are they somewhat similar to what we call the GSE meridian spinnacle? You just take some snapshots at a certain interval.

A: That's exactly it. It's an offshoot of that technique. We've had the radiologist tell us that a lot of this stuff is limited to the software that they have in the MR facilities nowadays, and it's a somewhat general purpose technique in that many centers can do it, but you just have to invest the time to get it to a certain point. Our eventual hope is to do, not the quasi static motions that you see there, but, of course, some dynamic motions.

Q: How much of stretch did you observe when you went all the way from flexion to extension in any given subject?

A: Well, we don't know if we took extension as our zero point. We don't know how much of the full stretch would be in full flexion. What we did is we had a normal subject sit in there as if they were just standing upright. We took that as our zero point, so to speak, so they went forward about 65 degrees in flexion and they went back 60 degrees in extension, and we had strains up to 10-12 percent, if I'm not mistaken, so it was all quasi static.

Q: Because the studies that were done at the Medical School in Wisconsin about 10-15 years ago on the monkeys observed changes in the potentials with flexion extension, indicating that the actual tension in a small car should be the cause for injuries and this would be a nice technique if you can measure the SSEP's, then it will be very nice. One other quick question I have, "How did you track your earlier models in the 1980's?" On the inner shelling, used motions about 60 degrees with the monkeys, is that right?

A: Yes, that's correct.

Q: Now you are trying to do it with the miniature pig. Can you comment on how did you translate that data and plan to use the monkey data with the pig, because the pig has a real thick skull and a real small brain.

A: Yes. It turns out the size of the mini pig brain is somewhere between 65 and 70 grams, which is about the size of Rhesus monkey's brain. It's on the low end for a Rhesus monkey. We have about sixty experiments with the Rhesus monkey on hand. I think, more importantly, and what you were talking a little bit about, is the distinctly different neuroanatomy that's present in the miniature pig. For example, they don't have a significant Faulk's membrane that penetrates between the two hemispheres. What we found out to date is that we don't necessarily give the

extensive damage, to say the corpus colossal areas, that we saw on the non-human primate and which we always thought was a function of the Faulk's membrane, and how it was this stiff fin that extended into the hemispheres. So it's actually interesting in a sense that we can study an animal model that has a different neuroanatomy. We get accidental injury in both models and we try to understand the biomechanics of it, but it is an issue that we need to look into a bit further and get a bit more sophisticated with our models and as our loading conditions become not only single plane but multi-planar. I think those issues will surface once again.

Q: Thank you.

Q: Tyler Kress, University of Tennessee

Good work on your talk. I was wondering, on the curve you put up there for the calcium concentration, you had the two plots: one of them for recovery and one of them for residual deficit. Did that correspond to the ten percent and the fifteen percent for the squid axon for the two respectively?

A: I think I know the slide that you're talking about. Yes. It did.

Q: And you discussed how on the irreversible, around the 20 percent stretch, how the calcium just continues to build up. Does that behave linearly or is it the concentration of that calcium? I was kind of curious about what kind of duration you measured that for.

A: Yes. The experiments were done at Woods Hole and we did periodic sampling of calcium, once we found that it was going up unchecked, so to speak. In that one, we actually came back two hours later and we found that the concentration had now become the same as the external bath. So we have a series of points from T equals zero, when the insult was given, to about 30-35 minutes after that. We found an initial rise that came up and had some kind of linear portion to it, but then eventually started to kind of creep up.

Q: OK. Thank you.

Q: Guy Nusholtz, Chrysler Corporation

A physical model that you had, how did you take into account the blood vessels running through the brain and the anisotropic process in the brains?

A: Excellent question. We didn't take that into account per se. What we tried to do is to match the properties of our silicone with those that have been measured for brain, so that it fell within the range of those measured for brain. I think all of us realize that the data that we have on mechanical properties for brain need some additions made to it to account for say, age, as well as the anisotropic nature of brain and, at this point, when that data becomes available, we're going to attempt to put in some anistrophe in our models, but we'd like to see some of that data.

Q: You had a slide which appeared to be strain-rate percentage of cells that responded and there was a strain rate and non-high strain. Could you explain a little bit more when you say, "percentage of cell." You're not talking about the degree that responded, but you are talking about the number of cells.

A: Yes.

Q: But some cells, even at the high strain rate didn't respond and other cells did.

A: Right. One of the things that we saw, as you can probably infer from those strain contour plots that I had for a cell, is that from cell to cell the level of strain differs, not only within the cell but also the absolute magnitude of strain, even though you are trying to be as uniform as possible to this population. It does change and we think that is a primary factor in giving us what you saw and you mentioned; that is, you didn't have a 100 percent of the cells responding under high strain rate deformation and we have a feeling that is due to the attachment between the cell and the monolayer itself.

Q: OK. So, that is due to the experimental process.

A: Yes.

Q: Did you try different rates outside of, I interpret that as 500 percent strain per second, right?

A: No, we actually tried to look at the two ends of the spectrum, so to speak, to see if there was any distinct change and what we're trying to do right now is to fill in between, so to speak, to see what that injury curve, if you will, looks like and how it changes over strain-rate.

Q: Have you speculated on a potential mechanism for it?

A: Yes. We think there might be something happening to the membrane itself, the cell membrane, and there might be some distinct tears or pores that appear in the membrane that are transient for the reversible types of injuries, so you have a transient increase in cytosolic calcium. That the cell is, in fact, able to deal and can actually bring the concentration of calcium down and the membrane repairs itself, and on the other end of the spectrum, the tears are so big and pervasive that the cell really can't do much and it almost throws up its hands and goes on to die, so to speak.

Q: Have you measured or attempted to measure any sort of change in the physical response to the cell; like, does it become stiffer or softer?

A: No. We haven't and you would think that there are some distinct changes especially with that sort of skeleton plot that's shown there. There are some people that talk about the role of the cytosolic skeleton in mechanical properties. There's some people at Georgia Tech, Bob Nearma, in particular, that have done some good work in there, but we haven't looked at that particular aspect.

Q: Thank you.

Q: Marc Weiss, Navy, New Orleans

First a question, and then a comment. On your magnetic imaging technique, which was really nice to see, does that provide the possibility for doing the same sort of analysis you're doing on

the cord, taking a closer look at the vertebrae and the soft tissue, particularly the ligaments between them? In terms of modifying how you take and analyze the image, does it have that potential?

A: I think long term, yes, if we invest enough energy into it. There is this spatial resolution problem right now, at this point in time, but certainly, a potential is there to do that.

Q: A comment on the stretch issue in terms of static versus dynamic. We have done some preliminary analysis of our volunteer data, looking at neck stretch. Historically, we haven't looked at displacement much and under high levels of frontal impact, two-points. First of all, we do get changes in length. Now this is based on external measurements, obviously, in terms of defining the neck, but of the range 15 to 20 percent, we can't with no sequela. That would be called injurious. That's number one.

Number two, when you comment in terms of voluntary motion, the two types where somebody rotates and then flexes, or someone who translates and comes down in the dynamic situation, it's the latter case that obtains. You get the linear motion. That's clear and unambiguous. You get the linear translation before the rotation, and that's one of the real problems with the anthropomorphic necks we have. It doesn't demonstrate that kind of behavior. We also have some data which, if we had't looked at, no one else is, and if anyone is interested. We have some instrumented voluntary head/neck data, so we've done what you've done, but not made the tissue measurements but mechanical measurements. We do have that in our database if somebody's interested in looking at those numbers. So one could get some idea of some direct mechanical measurements.

A: I think you and I will be talking later.

Q: Shashi Kuppa, Conrad Technologies

I'm a little confused. Two weeks ago, I heard Dr. Pavlishok say that there was no correlation between the calcium concentration and strain, and you use it as a measure for strain. Could you comment on that?

A: Yes. John and I have some differences in opinion, I guess, but John actually talks about really the molecular response and epigenesis of axonal injury. He doesn't get as involved, in fact, he doesn't get involved in correlating that with some mechanical stimulus. We try to get some measure of the cell viability or the health of the cell as a consequence of this mechanical load that we are applying to it. We do it from two standpoints. We measure a change in an ion which many people think is responsible for maintaining the health of the cell, mainly calcium, and we try to measure some functional response, some electrophysiological measure of injury and the two appear to coincide with each other when you look at the squid giant axon.

One of the things that John and I have talked about is to perhaps extend some of these models that we have in isolation to some more sophisticated models where we can look at axons inside too. We can programmably deform these axons, and we can try to understand how the function of this group or bundle of axons changes and how that can ultimately come to some criteria for injury to the bundle of axons of the composite tissue nature. So actually, John and I are kind of on the same page. It's probably a little bit of language that we have to resolve.

Q: Thank you.

Q: David Porter, University of Louisville

I certainly enjoyed your talk. I had a question about the stretch issue. In your study, you started off with a giant squid axon, which presumably is a peripheral nerve, and from there, as I understand it, you determined that, at a 20 percent stretch, there was basically permanent impairment of the nerve due to equilibrium of the calcium inside and outside of the cell, right?

A: Um, hum.

Q: My question is, would you consider it a logical conclusion to state that a peripheral nerve stretched 20 percent would be impaired? A peripheral nerve in a human body.

A: I'm a bit cautious in taking the squid data and just saying it applies to humans. What we are doing is we're developing more sophisticated models that go, for example, to the frog myelinated fiber in the sciatic nerve to take a look at myelination and how that may affect the course of injury criteria. More recently, we're looking at the optic nerve in a guinea pig because that's a nice arrangement of axially oriented axons. These approximate central nervous system axons and you can get some measure of functional deficit, via the visual evoked potential caused by stretch. In the future, we can feel more comfortable putting this package together and saying in a human it's going to be "X." And, I'm cautious and I wouldn't want to say that at this point.

Q: Along the same lines, do you think MRI technique could be used for a peripheral nerve?

A: No.

Q: It would be more difficult to find the nerve?

A: Yes. I think it goes back to the comment before about spatial resolution. If there is enough energy invested, you could probably do that.

Q: Thank you very much.

