JOURNAL OF NEUROTRAUMA
Volume 12, Number 4, 1995
Mary Ann Liebert, Inc.

Biomechanical Analysis of Experimental Diffuse Axonal Injury

DAVID F. MEANEY, DOUGLAS H. SMITH, DAVID I. SHREIBER, ALLISON C. BAIN, REID T. MILLER, DOUG T. ROSS, and THOMAS A. GENNARELLI

ABSTRACT

The purpose of this paper is to present results from methodologies used in our laboratory that are targeted toward identifying specific brain injury thresholds. Results from studying one form of brain injury, diffuse axonal injury, are presented in this report. Physical models, or surrogates, of the skull–brain complex are used to estimate the relationship between inertial loading and brain deformation. A porcine model of diffuse axonal injury, developed with information from these physical models and earlier *in vitro* tissue modeling studies, is used to correlate histologic and radiologic evidence of axonal injury to predicted regions of injury from the experimental and theoretical analysis. These results form the basis for developing improved diffuse brain injury tolerance levels, as well as identifying new means of diagnostic and treatment techniques for diffuse axonal injury.

Key words: diffuse axonal injury; physical models; animal models; diffuse brain injury tolerance levels

INTRODUCTION

DIFFUSE AXONAL INJURY (DAI) is a commonly encountered form of brain injury in mild, moderate, and severely head injured patients (Adams et al., 1982; Graham et al., 1993). In its mildest form, DAI is manifest as a reversible, concussive insult. In its most severe form, diffuse axonal injury often appears as immediate prolonged coma, is associated with nearly a third of the deaths in severly head injured patients, and is responsible for most cases of poor neurological outcome in survivors of head injury (Gennarelli et al., 1982, 1985).

Although clinically significant, available information detailing either the human tolerance or, in turn, preventive measures for reducing the morbidity of DAI in our population remains to be formulated. Currently, experimental models of traumatic brain injury have been designed to document the mechanisms and, to a much lesser extent, the pathomechanical features of various grades of diffuse brain injuries. Ultimately, it is expected that these models will yield information for new pharmacotherapies for brain injury, as well as assigning tolerable levels of forces or head motions experienced by the human before pathophysiological and pathobiologiocal changes occur.

This report is intended to present findings of a biomechanical analysis of an experimental diffuse axonal injury model in the miniature swine, and will discuss the features of this model in relation to previous experimental DAI models. Moreover, we discuss the biomechanical characteristics of this DAI model in relation to other models of traumatic brain injury. It is expected that as more tools become available to the biomechanician in the future, the role of the experimental traumatic brain injury model (see Lighthall et al., 1989; Gennarelli and Thibault, 1984 for review) will become an increasingly important means for identifying tolerance levels and predicting the

Department of Bioengineering and Division of Neurosurgery, University of Pennsylvania, Philadelphia, Pennsylvania.

long-term consequences of mechanical loading applied to the head.

MATERIALS AND METHODS

The tools used in this report to analyze experimental diffuse axonal injury include both inanimate simulations and a selected group of animal experiments. The animal model is developed using surrogate models of the skull–brain structure to identify the inertial loading conditions necessary to create intracerebral strains sufficient to elicit axonal injury *in vivo.* As a means of testing these thresholds, we use immunohistochemical techniques to determine the distribution and extent of axonal injury patterns in the miniature pig brain.

In the development stage, physical models of the miniature pig skull–brain structure were fabricated to measure the mechanical response of a surrogate brain to an impulsive head rotation delivered by a HYGE pneumatic impactor. The HYGE apparatus has been used in previous animal experiments to deliver sagittal, horizontal, oblique, and coronal plane rotations to the animal head (Gennarelli et al., 1982; Abel et al., 1978). Both duration and magnitude of the biphasic rotational acceleration can be modified through adjustments of the linkage and HYGE column. In its current form, the device is configured to deliver a purely impulsive coronal plane rotation, where the amount of rotation is adjustable through either the thrust column stroke or the linkage radius of rotation. For this testing series, models were subjected to either a full 65° or a 105° movement. Models were mounted with the approximate brain center of mass aligned with the linkage center of rotation, although the brain center of mass in the animal tests is slightly offset from the linkage center of rotation. The effects of this mounting procedure on the mechanical response are negligible, since the role of translational acceleration in the strains measured in physical modeling studies is not significant (Meaney et al., 1993b; Margulies, 1987).

Models were fabricated using previous model construction techniques (see Fig. 1 for illustration). Clean, dried miniature pig skulls were cut coronally and the interiors were painted white for photographic enhancement. A silicone gel was poured into the cranial cavity and alowed to cure before an orthogonal grid was applied to the cured gel surface. A second layer of silicone gel was used to fill the remainder of the intracranial cavity, and the skull–brain surrogate was encased within an aluminum can using epoxy resin to facilitate mounting on the HYGE apparatus. The position of the physical model on the HYGE device was nearly identical to that used in animal experiments.

High-speed film recorded the response of the model to a broad range of biphasic rotational accelerations. For each test, analysis of the high-speed film produced temporal measures of strain throughout the surrogate brain. These measures were used in an analytical model to determine the inertial loading conditions needed to exceed a critical strain level for axonal injury to occur. Proposed levels for mild and moderate diffuse axonal injury were formulated. Critical strain levels were derived from isolated tissue studies of axonal injury, and details of these tests can be found elsewhere (Galbraith et al., 1993; Thibault et al., 1990).

Using inertial loading conditions predicted from this analysis, a set of animals was injured both above and below the threshold proposed for mild DAI. Details of the experiments can be found in previous communications (Ross et al., 1994; Meaney et al., 1993a).

To document injury patterns under these loading conditions, injured animal brains were perfusion fixed in 3.7% paraformaldehyde and postfixed in 10% sucrose. Cornal cuts (1 cm thickness) were used to identify any gross hemorrhagic lesions or tissue tear, while thinner sections (40 μm) were used to analyze neuronal changes (cresyl violet) and mark areas where axonal swelling or retraction balls were found (SMI-31, SMI-32). The techniques used to identify axonal damage have been compared to silver labeling techniques, and have been found to be excellent indicators of axonal pathology (Ross et al., 1994). Maps of axonal injury patterns were produced

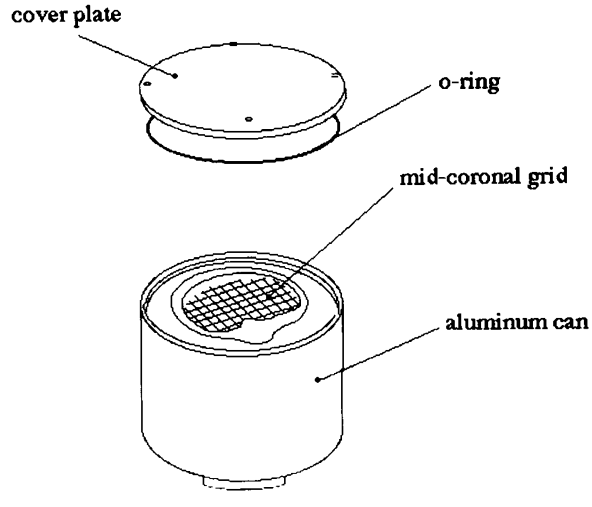

FIG. 1. Illustration of the physical model used in this study. A clean, dried porcine skull was embedded within an anluminum can and filled with a silicone gel that acted as a surrogate for brain tissue. An orthogonal grid placed between two gel layers was used to estimate the mechanical response of the brain surrogate to a coronal plane, rotational acceleration.

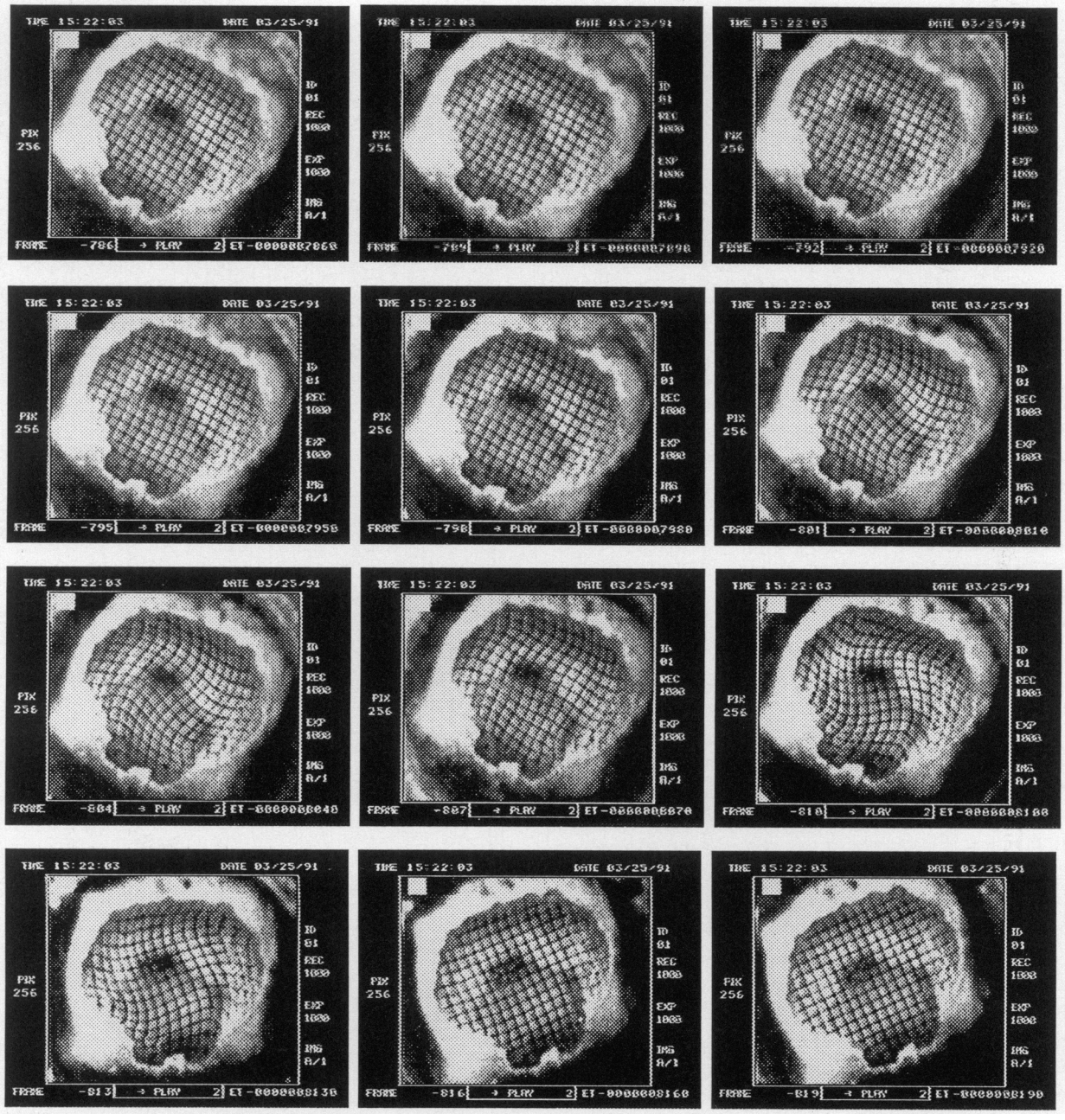

FIG. 2. High-speed video (1000 frames/s) captured the distortion of the grid within the physical model in response to a biphasic rotational acceleration/deceleration. Analysis of a consecutive series of grid frames allowed for the calculation of strain throughout the grid plane of the physical model.

for selected coronal planes of the brain, and regions where axonal injury was found were marked on the anatomic drawings.

RESULTS

A range of kinetic loading conditions was used in the inanimate modeling studies to define a relationship between inertial loading parameters and surrogate material strains. Figures 2 and 3 clearly show the surrogate brain response to a biphasic loading pulse. In this case, the rotation was fixed at 65°. Grid pattern response follows the biphasic loading used in the experiment, while analysis of the grid pattern indicates that the strain along the cortical margins is larger than the strain within the deep white matter structures (Fig. 3). It is interesting to note the substantial difference in the spatial response of the model com-

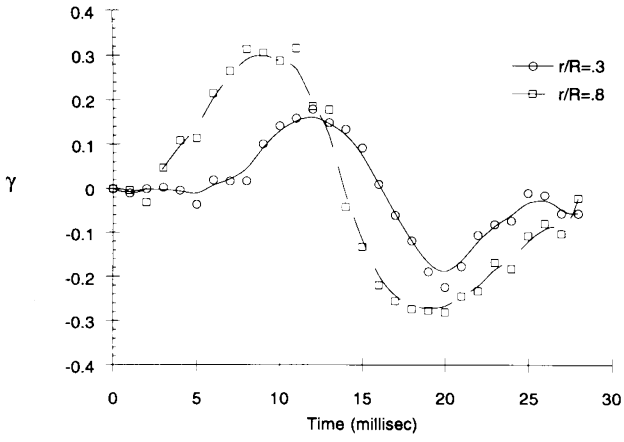

FIG. 3. Strains measured in the surrogate modeling tests patterned the biphasic acceleration, and were larger along the periphery (r/R = 0.8) of the model when compared to the inner core (r/R = 0.3).

pared to previous nonhuman primate studies, where the falx cerebri membrane redistributed the strain pattern so that appreciable strains appeared within the deep white regions, even at low loading levels (Margulies et al., 1990).

Physical models were tested under a broad range of loading conditions to yield estimates of the relationship between surrogate material strains and kinetic loading parameters (Fig. 4). Regression analysis allowed the calculation of a critical loading level necessary to exceed the tissue tolerance for primary axonal injury. From these studies, it was proposed that a peak rotational acceleration of 105,000 rad/s^2 and peak change in rotational velocity of 290 rad/s was necessary for mild DAI to appear in the animal experiments.

These conditions were used as a guide for the injury parameters employed in a subsequent set of animal experiments. Animals were injured using loading parameters both below and above the proposed mild DAI threshold. In all experiments, animals were subjected to a 105° coronal plane rotation. Analysis of the injured brains used both immunohistochemical markers for axonal injury (SMI-31, SMI-32) as well as standard Nissl staining for morphological cellular changes. Axonal injury was absent in animals injured below the proposed threshold, although the time before sacrifice may not have been sufficient for axonal changes to surface in this set of animals. In the remainder of the animals studied, immunohistochemistry revealed that axonal injury appeared first along the subcortical margin of the white matter and propagated inward as the level of acceleration and velocity increased (Fig. 5). These injury patterns, described in detail in another report (Ross et al., 1994), follow the change in strain patterns determined from the surrogate modeling studies.

DISCUSSION

This paper describes a systematic approach to first develop and then test an experimental model of diffuse axonal injury in miniature swine. Physical models were tested to determine the response of brain surrogates to impulsive head rotation. The mechanical response data were compared to results from isolated tissue studies to form a basis for predicting loading conditions that would create axonal injury *in situ*, and provided guidelines for a limited series of animal experiments. Animal experiments revealed that axonal injury was created using the proposed loading conditions, and that the extent of damage was related to the magnitude of the loading parame-

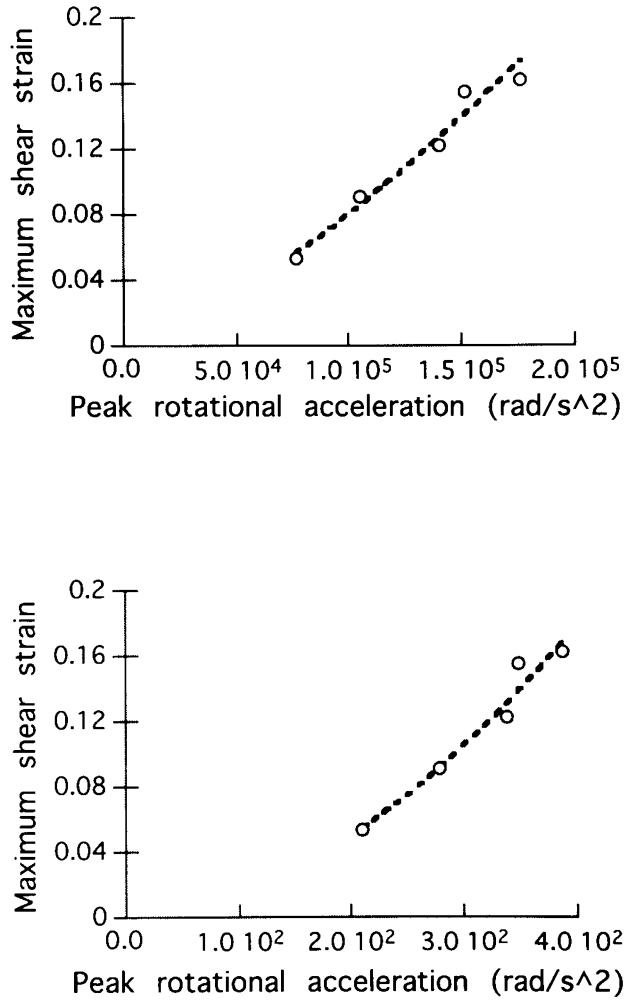

FIG. 4. Several physical model tests were conducted to characterize the response of a region approximating the subcortical white matter to changes in the peak rotational acceleration and peak change in rotational velocity. A critical strain value for axonal injury (0.1) yielded estimates for the loading conditions required for mild DAI.

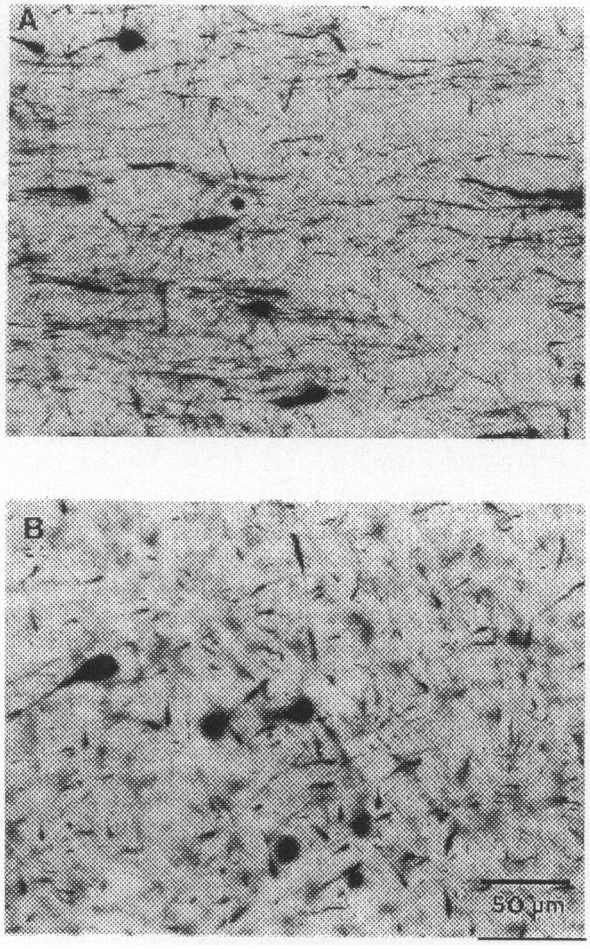

FIG. 5. Animals injured above the proposed threshold for mild DAI exhibited regions of axonal damage along the subcortical margin of the brain (5a; SMI-31 immunohistochemistry, frontal gyrus) and along the white matter/gray matter interface (5b; SMI-31 immunohistochemistry). As the inertial loading increased, the distribution of injury was more widespread, extending into the periventricular regions.

ters. These results form the basis for a more detailed analysis of the experimental model using alternative modeling tools, as well as analyzing possible treatment and diagnostic strategies for diffuse axonal injury.

The experimental model reported here is distinct from previous models of DAI in some aspects. The species difference from previous models in the nonhuman primate not only brings changes in brain shape and orientation, but also in the intracranial partitioning of the cerebral hemispheres. The lack of a pronounced falx cerebri membrane in the miniature swine affects the mechanical response of the brain by lowering the magnitude of strain experienced in both the deep white matter structures and corpus callosum. As a result, the corpus callosal damage in even mild diffuse axonal injury will not appear as prominent as in the nonhuman primate. Second, damage in the dorsolateral quadrant of the rostral brain stem noted in severe grades of DAI may appear under different circumstances in the miniature swine, due to the different alignment of the brain stem in the minipig versus the primate. It is expected that the influence of multiplanar loading conditions in this experimental model will be investigated in the future, and the information from injury patterns will prove invaluable in reproducing severe grades of DAI in this experimental model.

Nevertheless, the similarities of the neuropathological features of this model to previous models of DAI, in concert with the ability to predict specific threshold levels for DAI in the miniature swine, now form the possibility of bringing additional tools to conduct more detailed studies to identify DAI tolerance levels in the human. These coordinated studies could include advanced experimental and computational mechanics tools that further elucidate the structural response of the brain to complex mechanical loads. Isolated tissue and cell injury models (see Lucas, 1992, for review) provide a means for transferring the structural response derived from these efforts to a functional and/or pathobiological response of the brain, and form an integrative framework for identifying hazardous loads and designing products to mitigate the incidence of diffuse brain injuries.

These biomechanical modeling efforts, both experimental and computational, should not be viewed as applicable to only selected experimental models but rather to include both the large and small animal models of brain injury that are currently in use. Due to limited resources and equipment, the use of large animal models will continue to represent only a small fraction of the experimental surrogates used in studying brain injury mechanisms and treatment. Other well-developed rodent models, including both central and lateral fluid percussion, cortical impact, weight drop, and cortical evacuation (Dixon et al., 1988, 1991; Feeney et al., 1981; McIntosh et al., 1987; Shreiber et al., 1995), can be viewed as providing not only a platform for studying mechanistic and intervention aspects of brain injury, but also as a means for further clarifying the biomechanical aspects of functional and structural neural damage. While some preliminary studies have been accomplished that describe the pathomechanical features of these models (Meaney et al., 1994; Thibault et al., 1992), little is still known on the biomechanical relationship between these models and the human brain injury. Further, the independent control of the biomechanical parameters in these experimental models to selectively control tissue damage remains elusive. These items are not only crucial in developing effective injury prevention strategies, but may also offer advanced experimental models to identify new pharmacotherapies and diagnostic strategies for particular forms of brain injury.

ACKNOWLEDGMENTS

Funds for this work were provided by the National Institutes of Health Grant NS-08803 and the Centers for Disease Control Grant R49.CCR-304684. Control

REFERENCES

ABEL, J.M., GENNARELLI, T.A., and SEGAWA, H. (1978). Incidence and severity of cerebral concussion in the rhesus monkey following sagittal plane angular acceleration. Proc. of the 22nd Stapp Car Crash Conf. pp. 33–53.

ADAMS, J.H., GRAHAM, D.I., MURRAY, L., and SCOTT, G. (1982). Diffuse axonal injury due to non-missile head injury in humans: An analysis of 45 cases. Ann. Neurol. **12,** 557–563.

BLUM, R., THIBAULT, L., and GENNARELLI, T. (1985). In-vivo indentation of the cerebral cortex. *Proc. of 35th ACEMB.* Chicago, IL.

DIXON, C., LIGHTHALL, J.W., and ANDERSON, T.E. (1988). Physiologic, histopathologic, and cineradiographic characterization of a new fluid percussion model of experimental brain injury in the rat. J. Neurotrauma **5**(2), 91–104.

DIXON, C.E., CLIFTON, G., LIGHTHALL, J., YAGHAMI, A., and HAYES, R. (1991). A controlled cortical impact model of traumatic brain injury in the rat. J. Neurosci. Methods **39**(3), 253–262.

FEENEY, D., BOYESON, M., LINN, R., MURRAY, H., and DAIL, W. (1981). Responses to cortical injury: Methodology and local effects of contusion in the rat. Brain Res. **211,** 67–77.

GALBRAITH, J.A., THIBAULT, L.E., and MATTESON, R.A. (1993). Mechanical and electrical responses of the squid giant axon to simple elongation. J. Biomech. Eng. **115,** 13–22.

GENNARELLI, T.A., and THIBAULT, L.E. (1984). Biological models of head injury. Central Nervous System Status Report 391–403.

GENNARELLI, T.A., THIBAULT, L.E., ADAMS, H., GRAHAM, D.I., THOMPSON, C.J., and MARCINCIN, R.P. (1982). Diffuse axonal injury and traumatic coma in the primate. Ann. Neurol. **12** (6), 564–574.

GRAHAM, D.I., ADAMS, J.H., DOYLE, D., FORD, I., GENNARELLI, T.A., LAWRENCE, A.E., MAXWELL, W.L., and McLELLAN, D.R. (1993). Quantification of primary and secondary lesions in severe head injury. Acta Neurochir. Suppl. **57,** 41–48.

LIGHTHALL, J.W., DIXON, C.E., and ANDERSEN, T.E. (1989). Experimental models of brain injury. J. Neurotrauma **6**(2), 83–97.

LUCAS, J.H. (1992). In vitro models of mechanical injury. J. Neurotrauma **9**(2),117–120.

MARGULIES, S.S. (1987). Biomechanics of traumatic coma in the primate. Ph.D. dissertation, University of Pennsylvania.

MARGULIES, S., THIBAULT, L., and GENNARELLI, T. (1990). Physical model simulations of brain injury in the primate. J. Biomech. **23,** 823–836.

MCINTOSH, T.K., R., VINK, L., NOBLE, et al. (1987). Traumatic brain injury in the rat: Characterization of a lateral fluid percussion model. Neuroscience **28**(1), 233–244.

MEANEY, D.F., SMITH, D.H., ROSS, D.T., and GENNARELLI, T.A. (1993a). Diffuse axonal injury in the miniature pig: Biomechanical development and injury threshold in: Crashworthiness and Occupant Protection in Transportation Systems. J.D. Reid, and K.H. Yang (eds.). AMD-vol. 169, pp. 169-175.

MEANEY, D.F., THIBAULT, K.L., GENNARELLI, T.A., and THIBAULT, L.E. (1993b). Experimental investigation of the relationship between head kinematics and intracranial tissue deformation. *ASME SAM* **24,** 8–11.

MEANEY, D.F., THIBAULT, L.E., WINKELSTEIN, B.A., BRASKO, J., ROSS, D.T., and GENNARELLI, T.A. (1994). Modification of the cortical impact brain injury model to produce axonal damage in the rat cerebral cortex. J. Neurotrauma **11**(5), 320–329.

ROSS, D.T., MEANEY, D.F., SABOL, M.K., SMITH, D.H., and GENNARELLI, T.A. (1994). Distribution of forebrain axonal injury following inertial closed head injury in miniature swine. Experimental Neurology **126,** 291–299.

SHREIBER, D.I., MEANEY, D., WON, J.Y., SUN, D.A., ARBOGAST, K., ROSS, D.T., McINTOSH, T.K., and GENNARELLI, T.A. (1995). Evaluation of a new model of cerebral contusion (abstract). J. Neurotrauma **12**(1), 142.

THIBAULT, L.E., MEANEY, D.F., ANDERSON, B., and MARMAROU, A. (1992). Biomechanical aspects of the fluid percussion model of brain injury. J. Neurotrauma **9**(2), 311–322.

THIBAULT, L.E., GENNARELLI, T.A., MARGULIES, S.S., MARCUS, J., and EPPINGER, R. (1990). The strain dependent pathophysiological consequences of inertial loading on central nervous system tissue. Proc. Int. Conf. Biomech. Impact, Lyon, France, 191–202.

Address reprint requests to:
D. Meaney
Department of Bioengineering and
Division of Neurosurgery
University of Pennsylvania
Philadelphia, PA 19104