Characterization of Diffuse Axonal Pathology and Selective Hippocampal Damage following Inertial Brain Trauma in the Pig

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Abstract. Dynamic deformation applied to white matter tracts is a common feature of human brain trauma, and may result in diffuse axonal injury (DAI). To produce DAI in an experimental model, we have utilized nonimpact inertial loading to induce brain trauma in miniature swine. This species was chosen due to its large gyrencephalic brain with substantial white matter domains. Twenty anesthetized (2% isoflurane) miniature swine were subjected to pure impulsive centrotal rotation 110° in the coronal plane in 4 to 6 ms, peak accelerations ranged from 0.6 to 1.7 x 10⁷ rad/s². Seven days following injury, the brains were fixed (4% paraformaldehyde). Histopathologic examination was performed on 40 μm sections stained with cresyl violet (Nissl), antibodies targeting neurofilament (axonal damage), GFAP (astrocyte), and pig IgG (protein extravasation). Widespread multifocal axonal injury was observed in combination with gliosis throughout the brain, most commonly in the roof of the gray and white matter. Very little vascular disruption was noted in regions of axonal injury. Neuronal damage was primarily found in the CA1 and CA3 subfields of the hippocampus. These results suggest that this model is clinically relevant and useful for evaluating mechanisms of inertial brain trauma.

Key Words: DAI, Diffuse brain injury; Hippocampal damage; Inertial brain injury.

INTRODUCTION

Affecting over 2 million victims each year in the United States, traumatic brain injury (TBI) is the leading cause of death in children and young adults (1–3). Of the broad spectrum of pathologies resulting from TBI, the terms “focal” and “diffuse” brain injury have emerged as 2 major categories. Focal brain injury is typically associated with blows to the head leading to cerebral contusions and hematomas (4). In contrast, diffuse brain injury may occur in the absence of impact forces in some cases, but is dependent on inertial forces that rapidly rotate and deform the brain (4, 5). These inertial forces result in a nonuniform pattern of tissue stress and strain in the white matter, leading to a characteristic pattern of diffuse axonal injury (DAI) (4, 6–8). Accounting for a high percentage of mortality due to brain trauma, DAI is commonly observed following motor vehicle crashes and has been reported following fatal falls and assaults (9, 39, 40). While DAI may occur in isolation, it is important to note that some brain trauma patients suffer a combination of diffuse and focal damage (41).

Although many experimental animal models of focal brain injury have been developed, only one clinically relevant model of DAI has been previously characterized (10). The disparity in the experimental efforts dedicated to studying focal brain injury compared to diffuse brain injury reflects the difficulty in developing a model system that replicates the dynamics of diffuse injury, in particular the inertial loading conditions produced in automotive crashes. Thus, while DAI is widely considered the most important pathology in the majority of severely brain injured patients (7, 11), many characteristics of DAI remain incompletely explored.

It has been previously demonstrated that the principal mechanical loading associated with the induction of DAI is rotational acceleration of the brain resulting from unrestricted head movement in the instant after injury (8, 10, 12). This inertial loading to the brain induces shear, tensile, and compressive strains leading to dynamic tissue deformation. For the development of DAI, the size of the human brain plays an important role due to the substantial mass effects during injury that result in high strains between regions of tissue. The amount and location of strain distributed throughout the brain are related not only to the extent of rotational acceleration/deceleration, but also to the presence of intracranial dural compartments (e.g. falx, tentorium cerebelli) that may act as partial barriers in a given direction of motion contributing to the DAI pattern. Moreover, to produce characteristic DAI, both the magnitude and the rate of strain must fall above critical thresholds. It has been shown that the mechanical loading conditions at the time of injury must occur dynamically, usually in less than 50 msec, to produce widespread axonal pathology (8, 13).

Since the majority of lesions associated with DAI are microscopic, the clinical diagnosis is typically based on the appearance of prolonged unconsciousness accompanied by an intracranial mass lesion (4). In humans suffering from DAI at the highest severity (Grade 3M), microscopic lesions are typically located centrally and usually involve hemorrhage in the basal ganglia and dorsal lateral midbrain as well as tearing of the corpus callosum. In all grades of DAI, histopathologic evaluation...
reveals widely distributed multifocal appearance of swollen axons and terminal clubbing of axons, also referred to as “retraction balls.” This damage is found throughout the deep and subcortical white matter and is particularly common in the splenium of the corpus callosum. However, following mild to moderate “pure” diffuse brain trauma (Grades 1 and 2 mm), there may be a remarkable absence of macroscopic pathology and conventional imaging techniques may not reveal any abnormalities (14).

The one previous animal model shown to replicate all of the clinical features of DAI was initially characterized in nonhuman primates, utilizing nonimpact head rotational acceleration to produce coma in association with diffuse axonal damage (10). This model, sometimes referred to as “Penn II,” produced DAI throughout the white matter of both hemispheres, corpus callosum, brainstem, and cerebellum. Nonhuman primates were originally chosen for this experimental model due to their relatively large brain mass. As the brain size decreases, the rotational acceleration necessary to induce similar strains as seen in humans increases exponentially. To exemplify this point, the Penn II device is capable of producing 20,000 kg of thrust, just enough to generate sufficient impulsive loading (nonimpact rotational acceleration) that result in DAI in a 50 to 75 g nonhuman primate brain.

To explore diffuse brain injury in another animal model, we have recently adapted the same injury apparatus as in the Penn II studies to induce rotational acceleration brain injury in miniature swine. Miniature swine were chosen due to their relatively low body weight (17 to 20 kg) and large brain mass (70 to 80 g). This new animal brain injury model was established through physical model experiments using miniature swine skulls filled with surrogate brain material that were subjected to scaled rotational acceleration (5). Analysis of point by point deformations of the surrogate brains at differing accelerations established experimental parameters predicted to produce DAI in miniature swine. In preliminary experiments, we employed these parameters in miniature swine and observed widely distributed axonal pathology (15). In the present study, we sought to extensively characterize the histopathologic consequences in this new injury model, including severity and distribution of axonal injury, subtypes of axonal pathology, blood brain barrier disruption, gliosis, and selective neuronal injury at one week following trauma.

MATERIALS AND METHODS

Twenty-two miniature young adult (4 months of age) swine (Hanford strain), both male and female, weighing 17 to 20 kg, were used for this study. Twenty animals received rotational acceleration brain injury, and 2 served as unjured controls. In preparation for injury, the animals were made fast for 12 h, and following this, anesthesia was induced with an initial injection of midazolam (400 to 600 mg/kg). Once sedated, animals received 2 to 4% isoflurane via a snout mask until they reached a plane of surgical anesthesia, at which time a venous catheter was inserted in the ear, and the animals were endotracheally intubated and maintained on 1.5 to 2% isoflurane. The initial 10 animals received sterile placement of an intracranial pressure (ICP) probe housed in a subarachnoid bolt, which was screwed into the parietal bone. In addition, sterile placement of a femoral arterial catheter was also performed. Additional monitoring apparatus included noninvasive EKG electrode leads affixed to the chest and extremities, 8 channel EEG with leads placed over the top of the head, a pulse oximeter placed on the skin of the tail to measure heart rate and PO2, a rectal thermometer to measure core body temperature, and sampling tubes for end tidal CO2 measurement attached to the endotracheal tube. Arterial blood gases (pO2, pCO2, pH) were also periodically evaluated prior to injury and following injury. The pigs were continually monitored and all data from physiologic monitoring were collected on a computer-driven storage system.

All incisions were closed and dressed. No substantial physiologic changes were noted in any of the initial 10 animals prior to or following injury, leading to a simplification of the monitoring protocol in the remaining animals. The remaining 12 animals did not receive placement of the ICP probe or arterial cannulation, but were monitored for heart rate, respiration rate, end tidal CO2, rectal temperature and oximetry.

To induce brain injury, the heads of the animals were secured to the rotational acceleration injury apparatus, the HYGE pneumatic impactor, via a padded snout clamp. The HYGE apparatus has previously been described in detail (5). The snout clamp is directly mounted to the linkage assembly of the HYGE device that converts the linear motion to an angular motion. For these experiments, the linkage was adjusted to produce a pure impulsive head rotation in the coronal plane, with the center of rotation close to the brain center of mass. Head rotation was fixed at 110°, and rotational acceleration was biaxial with a predominant deceleration phase. Once the animals were securely fastened to the HYGE device, inhalation anesthesia was withdrawn 10 seconds prior to activating the HYGE device.

The HYGE device rapidly rotated the miniature swine heads in the coronal plane over 20 to 30 msec. To measure the mechanical injury parameters, an uniaxial accelerometer (Endevco Instruments, San Juan Capistrano, CA) mounted to the linkage sidearm was used to determine tangential deceleration along the linkage motion. From the recorded linear acceleration, rotational acceleration was determined for the duration of the experiment. Injury severity parameters were based on preliminary studies (5, 15). Brain mass was determined when the brain was removed for histological analysis, and was used to normalize the loading parameters to a 70 g brain mass according to Holbourn’s scaling relationship (Holbourn, 1956).

Following injury, the animals’ heads were released from the clamps. Upon stabilization of vital signs (less than 15 minutes [min] following injury), arterial and venous lines and the ICP bolt were removed, all incisions sutured, and a topical antibiotic and dressing applied to the wounds. Following these procedures, the animals were extubated and continuously monitored for vital signs (respiration and pulse) until awake and ambulatory, which always occurred within 8 hours (h) of injury. In addition, all animals received buprenorphine (0.1 mg/kg, i.m., q 12 h, p.r.n) for postoperative analgesia.
At 7 days following brain injury, the animals received an overdose of pentobarbital (150 mg/kg I.V.), and the descending aorta was clamped and transectedally perfused with 4 liters of saline following by 10 liters of 4% paraformaldehyde. The brains were carefully removed, postfixed in 4% paraformaldehyde for 2 h, and stored in phosphate buffer solution. The brains were blocked into 0.5 cm coronal sections for gross examinations and photography. All blocks were cryoprotected in sucrose and a series of 40-μm frozen sections were cut from the front face of each block and mounted on microscope slides. One set of adjacent sections was mounted and stained with Nissl stain (eves blue), hematoxylin and eosin, and acid fuchsin. Additional sets were evaluated with immunohistochemical techniques using the following primary antibodies: monoclonal antibodies, including the NR4 antibody, targeting the 68 KD neurofilament subunit (Boehringer Mannheim; 1:4); the NN18 antibody, targeting the 160 KD neurofilament subunit (Sigma; 1:40); N52 antibody, targeting the 200 KD neurofilament subunit (Sigma; 1:400); SMI-31 antibody, targeting selected epitopes located primarily on the 170 to 200 KD neurofilament subunits and reacting with extensively phosphorylated NF-H and to a lesser extent with NF-M (Sternberger and Meyer monoclonals 1: 5000); GA-5 antibody, targeting the glial fibrillary acidic protein (Sigma; 1:400); Biotin-sp-conjugated affinipure goat anti-swine IgG (Jackson Immunoresearch Lab, Inc. 1:10000), targeting endogenous pig IgG. The sections were incubated with primary antibody overnight at 4°C and then incubated at room temperature for 1 h, each with the appropriate secondary antibodies and ABC solution (1:1000). Peroxidase activity was revealed with 0.025% 3,3′-diaminobenzidine, 300 mg Imidazole, and 0.25% H.O. for 10 min. Initial examination of all anti-neurofilament antibodies was performed on alternate brain sections of 5 animals to determine the best antibodies for revealing axonal pathology in this model. In these studies, we did not extensively evaluate axonal injury with anti-amyloid precursor protein (APP) antibodies. Anti-APP immunohistochemistry is often used for evaluation of human AD for acute postinjury fatalities (42). The utility of this approach is based on the fast transport of APP, which accumulates more rapidly in damaged axons than in slow transport neurofilament. However, by 7 days postinjury, slow transport proteins have had ample time to accumulate. In initial studies using our pig model, we found that immunohistochemical analysis with anti-APP antibodies was slightly less sensitive than the anti-neurofilament antibodies for detecting axonal pathology.

All sections were examined under light microscopy and a semi-quantitative analysis was performed to determine the extent and distribution of axonal damage throughout the brain. Two sections from each block of tissue were examined under light microscopy and the following brain regions were evaluated: frontal, parietal, temporal, and occipital lobes, lateral ventricle region, external capsule, cerebral peduncles, thalamus and brainstem. The entire area of each region was evaluated for axonal pathology. Anatomic regions were based on the Atlas of the Brains of Domestic Animals (43).

Scoring of axonal pathology (terminal clubbing or substantial swelling of axons) demonstrated by immunohistochemical staining was performed by rating 1 to 5 damaged axons/anatomic region/section as mild, 6 to 15 damaged axons as moderate, and over 15 damaged axons as severe. The total number of injured axons and the relative severity of injury per brain region were computed for comparison.

**RESULTS**

**Physiologic/Neurologic Response**

Prolonged coma was not produced in this model and only transient loss of consciousness (less than 15 min) was observed. This length of unconsciousness may have even been prolonged due to the effects of residual anesthesia. The animals typically were able to ambulate within a few hours following injury, but appeared slightly disoriented, often not responding or having delayed responses to sensory stimuli for up to 6 h following injury. By 24 h following injury, all the animals appeared completely normal based on gross neurosensory examination.

Immediately following injury, the ICP, MAP, and pulse became elevated. By 5 min following injury, the ICP typically rose to 20 to 30 mmHg above baseline and in a few animals as high as 50 mmHg above baseline. However, by 5 to 10 min following injury, the ICP was found to have returned to relatively normal levels. MAP was elevated immediately postinjury, but only to 30 to 40 mmHg above baseline, and this also rapidly resolved within 10 min. Relatively modest increases in pulse rate were observed following the same timecourse as changes in MAP. No significant changes were noted in EKG waveform, arterial blood gases, end tidal CO2, or rectal temperature.

Although no tonic clonic seizure activity or tremors were noted following injury, acute changes in postinjury EEG waveforms were observed in all animals. EEG was performed only as long as the animal remained unconscious following injury. Therefore, EEG was recorded for a maximum of 15 min following injury. Post-trauma EEG changes included slowing of the alpha rhythm in the frontal and parietal regions and intermittent rhythmic high amplitude theta and delta activity on all channels. In addition, approximately half of the animals showed asymmetries in focal intermittent slow wave discharges with substantial differences in the amplitude of the waves between hemispheres. In a few cases, we observed synchronized sharp waves and slow-spike and wave complexes.

**Axonal Pathology**

We observed diffuse axonal damage in all 20 animals injured by rotational acceleration brain injury, while no axonal pathology was noted in the sham animals. The axonal pathology included terminal clubbing (retraction balls) of axons and swelling of axons. These pathologic characteristics of traumatically damaged axons are very similar to previous reports in humans and...
nonhuman primates. The terminal clubs appeared to be discrete spherical profiles, up to 40 μm in diameter, connected to an almost normal diameter proximal portion of the axon. In contrast, the swollen axons often included multiple varicosities and were observed involving up to several hundred μm of a single axon (Fig. 1). All primary anti-neurofilament antibodies evaluated (NR 4, NN 18, N52, SMI-31 and SMI-32) demonstrated these axonal pathologies. However, the antibodies that revealed the most axonal pathology were NR 4 (vs NF 68) and N52 (vs NF 200). The most common regions demonstrating extensive axonal injury included the deep white matter at the root of the gyri and at the junction of white and gray matter in the frontal, parietal, temporal and occipital lobes. In addition, axonal pathology was also commonly observed at the margin of lateral ventricles, external capsule, cerebral peduncle, and basal ganglia. Variable densities of terminal clubbing and swollen axons were seen in 19 of 20 cases in the dorsal piriform region and insular cortical white matter, dorsal hippocampal commissure, anterior commissure, internal capsule, thalamus, lateral margin of the third ventricle, cerebellum, midbrain and pons (Figs. 2–4). The total amount of axonal pathology in all injured animals appeared consistent with Grades 1 and 2 DAI seen in humans. Axonal pathology was also noted in the cerebral cortex of injured animals in approximately half of the cases (Fig. 5).

In 14 animals, we also observed variable amounts of pyramidal neuron perikarya stained with antibodies targeting phosphorylated neurofilaments (NN 18, N52, and SMI-31). These perikarya were in association with damaged axons in the layers 3 to 5 of cerebral cortex in the frontal, parietal and temporal lobes as well as in the hippocampus (Fig. 5).

**Neuronal Damage**

Cresyl violet, HE and acid-fuchsin staining revealed neuronal changes at 1 week postinjury characterized by pyknosis and loss of neurons in the cerebral cortex. Although this damage was found in the pyramidal cells in layer III of the frontal, parietal, temporal or occipital lobes of most animals, relatively few neurons were affected. Modest neuronal injury was also observed in the cerebellum of 5 animals, including pyknosis and apparent loss of Purkinje cells. The hippocampus was the only subcortical region demonstrating neuronal injury. Although the extent of damage varied between animals, we consistently observed bilateral damage to the CA1, CA3 and dentate hilar subfields of the hippocampus. This damage was observed as substantial thinning of the pyramidal cell layer of the CA1 and CA3 subfields, suggesting loss of neurons, and the pyknotic or swollen appearance of many remaining neurons in CA1, CA3 and the dentate hilus (Figs. 5, 7, 8).

It is important to note that these changes do not appear to have been preparation artifact, since none of the uninjured animals demonstrated this pathology.

**Astrocytosis**

GFAP immunohistochemistry revealed reactive astrocytosis in the molecular layer of the cerebral cortex and throughout the white matter in regions that also demonstrated axonal injury. Astrocytosis was also consistently observed in the corpus callosum, where little axonal pathology was detected. A much greater extent of reactive astrocytosis was found in the CA1 and CA3 subfields of hippocampus, consistent with regions of neuronal loss and damage (Figs. 6, 7).

**Vascular Disruption**

Petechial hemorrhages were seen along the root of gyri of the frontal, parietal and temporal lobes, as well as in insula and piriform regions. Small subarachnoid hemorrhages were also found in 4 animals. Congestion of small vessels with sludging red blood cells was observed in the subarachnoid space, cerebral cortex, subcortex and periventricular regions in half of the injured animals. Slightly increased immunostaining against endogenous IgG was restricted to the cortex, and subcortical white matter was observed in 4 cases, suggesting that no overt breakdown of the blood brain barrier was present 1 week following injury (not shown due to modest change). However, acute changes in extravasation of endogenous IgG cannot be determined by this examination. There were no large tissue tears or contusions observed in any animals.

**DISCUSSION**

Nonimpact head rotational acceleration/deceleration in miniature swine reproducibly induced diffuse damage to axons in the white matter and selective damage to neurons in the hippocampus 1 week following injury. The distribution of the damage to these sites in the injured animals appeared to be remarkably similar. The regions where axonal injury was most consistently observed and most severe included the subcortical white matter at the roots of the gyri in the parietal, frontal and temporal lobes. This damage most likely reflects the effects of high strains in these regions due to increased centripetal forces in the periphery of the plane of rotation. However, the gray matter situated even more distal to the center of rotation was not commensurately damaged. Therefore, this model appears to replicate the vulnerability of the white matter to injury following inertial loading conditions as is seen in humans.

Injury in the coronal plane in these animals did not induce more than a modest and transient change in ICP and MAP and no hematomas were observed. Although
lesions were detected in the deep (parasagittal) white matter. Axonal pathology in the pig was typically found at the interface of anatomic borders. These regions included white matter/gray matter junctions and the area along the margins of the internal capsule and ventricles. This distribution of injury may suggest that there is a general vulnerability of axons spanning the borders of distinct anatomic structures to rotational acceleration. Despite the generalized tissue injury, no major vascular disruptions were detected. In addition, only minimal extravasation of endogenous pig IgG was found 1 week postinjury, suggesting little blood brain barrier disruption at this timepoint. However, it is possible that greater IgG extravasation occurred at earlier timepoints and is no longer detectable by this examination. Nonetheless, gliosis was observed in all regions of axonal pathology 1 week following injury.

The spectrum of morphologic differences in axonal pathology following inertial brain injury in the pigs was quite striking. It has not yet been established if the variability in appearance of axonal damage represents a continuum of the same injury process at various stages of degeneration, or if different morphologic characteristics are progressing in parallel (16–18). In the present study, we observed what appeared to be 2 distinct pathologic mechanisms of axonal injury—generalized swelling of axons and terminal clubbing of axons (retraction balls). When sectioned longitudinally, axonal swellings were visualized extending up to several hundred microns, often with multiple varicosities along their length. This morphology likely reflects accumulation of transported organelles at multiple regions within a single axon. In contrast, terminal clubs sectioned longitudinally appeared relatively spherical, sharply tapering down to an almost normally sized axonal diameter proximally and ligated distally. In addition, we never observed more than one ball-like formation in a single axon, or a string of disconnected balls in longitudinal sections of white matter tracts.
A possible explanation for a potential spectrum of axonal pathologies may be found in the varying loading conditions assumed by axons traversing different planes. A uniform strain distributed throughout an axon, such as tensile elongation, may cause a generalized deformation and injury. Expansive regions may then differentially swell according to the relative extent and distribution of pathology, resulting in varicosities or continuously swollen stretches. On the other hand, dynamic nonuniform shear strain, essentially focusing the deformation of axons at one point, may result in more localized axonal pathology. This localized shear strain may cause primary or secondary axotomy with concomitant accumulation of transported organelles in the distal portion of the axon. In contrast to elongated swellings, this accumulation appears to be tightly contained at the proximal portion of the characteristic ball-like formation. The nature of the barrier preventing substantial "backswelling" from these ball-like terminal clubs is as of yet unknown. In addition to varying loading conditions, the different axonal pathologies may also represent a variety of degenerative responses to trauma.

A surprising finding in this model was the absence of axonal pathology in midline structures, particularly in the corpus callosum. This difference compared with human and nonhuman primate DAI may suggest an important role of extracerebral anatomy in the development of regional axonal damage. The pig has a smaller falx compared to humans and nonhuman primates. During coronal plane rotation in humans, the falx may impinge on the rotational movement of the trailing brain hemisphere while the leading hemisphere pulls away, resulting in high strain along the sagittal midline. This may potentially explain the large extent of midline damage that has been historically noted in human diffuse brain injury (19). The paucity of midline injury in pig DAI may suggest that both hemispheres more freely rotate in the coronal plane compared with the human brain. Nonetheless, we did observe marked gliosis in the corpus callosum, suggesting that this region did receive enough strain to induce an astrogial response without overt damage to the axons.
Fig. 4. Representative photomicrographs of SMI-31- and NF-200-immunostained sections demonstrating axonal pathology in multiple structures throughout the injured brain of the pig. (A) basal gangli, (B) thalamus, (C) cerebellum, (D) pons, (E) cerebral peduncle, and (F) external capsule. Bar = 50 μm.

Despite the production of DAI in the pig model, we did not observe prolonged loss of consciousness. The relative extent of damage produced in the pig appeared to be easily within the range that is associated with coma in humans and nonhuman primates (10). Nonetheless, only transient loss of consciousness was observed following injury in pigs, usually less than 15 min, and even this finding may have been exaggerated.
Fig. 5. Representative photomicrographs of the cerebral cortex in swine. HE staining demonstrates normal neurons in the temporal lobe (A) compared with pyknotic neurons found in brain-injured swine (B). NF-200 immunostaining demonstrates normal gray matter in the parietal lobe (C) in contrast to phosphorylation of perikaryal neurons (D) and terminal clubbing of axons (E) in brain-injured swine. GFAP immunostaining demonstrates normal molecular layer of the cortex (F) compared with astrocytosis in brain-injured swine (G). Bar = 50 µm.
due to residual anesthesia. This observation of DAI without coma compels us to revisit the debate of potential anatomic substrates responsible for the production of coma in human victims of diffuse brain injury (4). Based on our present data, DAI alone may not explain the morbidity associated with diffuse brain injury. In humans, one of the most distinctive features of severe diffuse brain injury is substantial injury to the brainstem, including contusion, hemorrhage, and axonal injury (6, 9, 20), which may in itself result in coma. In the pig, the brainstem projects caudally, positioning it in a less vulnerable position to coronal plane rotation as reflected by the relatively paucity of axonal pathology or vascular lesions in the brainstem. Future studies will explore the effect of brain rotation in the axial plane in pigs that will produce a transverse strain on the brainstem to evaluate the presence of coma.

The finding of hippocampal pathology in the pig model of diffuse brain injury appears very much in concert with findings following inertial brain injury in humans and nonhuman primates (21, 22). All subfields of the hippocampus appeared to be involved, demonstrating neuronal damage and death as well as marked gliosis. Moreover, the damage to the hippocampus appeared to be selective, as no other subcortical regions demonstrated neuronal pathology. While damage was detected in the cortex, including pyknosis of neurons, phosphorylation of neuronal perikaryal neurofilament and gliosis, this damage was not extensive nor consistently observed. At present, it is not clear why the hippocampus is selectively vulnerable to inertial injury, but we do not believe it is due to superimposed ischemia. While superimposed hypoxia/ischemia does play an important role in some cases of human diffuse brain injury (7), it cannot be confirmed simply by the presence of pyknotic hippocampal
Fig. 7. Representative photomicrographs of HE-stained CA3 region of the hippocampus in uninjured swine (A) compared with pyknosis and loss of neurons in the CA3 of brain injured swine (B). (C) HE-stained hilar neurons of the dentate gyrus in uninjured swine compared with pyknosis and loss of neurons in the dentate hilar region of brain-injured swine (D). (E) GFAP-immunostained sections demonstrating the CA3 region of the hippocampus in uninjured swine compared with marked gliosis in the CA3 region in brain injured swine (F). Bar = 50 μm.
neurons. Nonhumans primates subjected to head rotational acceleration in the coronal plane demonstrated hippocampal pathology despite normal ICP and physiologic control (21). In addition, in previous studies of diffuse brain injury in 4 pigs utilizing $^{31}$P and $^1$H magnetic resonance spectroscopy (MRS) during the acute period following injury (20 min to 4 h), $^{31}$P MRS demonstrated no changes in the intracellular concentrations of ATP, pH, or changes in PCR/Pi, while localized $^1$H MRS showed no changes in lactate concentration (23). Nonetheless, in all of these animals DAI and hippocampal injury was confirmed with histopathologic analysis. These results suggest that there was no substantial metabolic stress to the brains of the pigs following injury. We did observe acute EEG changes in the present study that may suggest some seizure-like activity; however, it cannot presently be determined if these EEG abnormalities contributed to the damage in the hippocampus.

The hippocampus also appears particularly sensitive to focal brain injury, demonstrated by a series of studies characterizing selective hippocampal damage in rodent contusion models of brain trauma (24–29). Hippocampal damage in these models has been shown to be produced even though blood flow measures in the hippocampus were well above the ischemic threshold (30, 31). Moreover, in contrast to ischemic injury alone, the distribution of hippocampal neuronal injury following brain trauma includes substantial disruption of the CA1 subfield as well as the CA4 and the dentate hilar regions. Taken together, these data suggest that selective hippocampal damage may be universally produced in all forms of brain trauma, appears to have a characteristic distribution, and may occur in the absence of ischemic influences.

In consideration of the importance of axonal pathology following all types of brain trauma, we and others have identified widely distributed axonal injury in rodent models of impact brain injury (32–35). However, these findings have led to some confusion of terminology. It has been debated whether the term “DAI” is appropriate to describe the axonal damage in rodents. It should be noted...
that “DAI” originated as a clinical classification reflecting the response of the human gyrencephalic brain to high strain resulting in characteristic axonal pathology due to tissue against tissue tearing (9). Nonimpact inertial brain injury may only be possible in models using animals with relatively large gyrencephalic brains, such as the nonhuman primate and the pig. The lissencephalic brain of rodents are very small and contain few purely white matter domains. Therefore, inertial loading forces necessary to produce nonimpact brain injury in rodents would be astronomical. As such, only impact injury has been shown to produce axonal damage in these models. The distribution of the axonal injury in rodent models is predominantly found in gray matter structures, the strip of subcortical white matter, and the brainstem. The brainstem injury in these models is most likely due to extrusion during injury.

A strength of our new model of inertial brain injury in the pig is that it may be used to develop and evaluate new noninvasive diagnostic techniques for human diffuse brain injury. Conventional imaging techniques, including magnetic resonance imaging (MRI) and computed tomography (CT), often fail to identify the minute lesions of DAI when little blood or edema is associated with the injury (14). Therefore, new techniques have been proposed that identify changes in the macromolecular arrangement of tissue (37). These techniques are thought to be ideal to study changes in white matter due to its highly organized, anisotropic nature. While much preliminary evaluation of several techniques is currently being performed in humans, the obvious advantage of the pig DAI model is the availability of histopathologic correlation. We have recently evaluated one of these techniques, magnetization transfer imaging (MTI), to evaluate DAI following inertial brain injury in the pig. We found that MTI appears to have enhanced sensitivity over conventional MRI for detection of DAI based on comparisons between histopathologic samples and corresponding images (37, 38). These studies were performed concomitantly with MTI evaluation of brain-injured patients, thus providing corroboration for the clinical data.

The new pig model of DAI currently stands as the only animal model in use that appears to replicate the inertial loading conditions produced in many instances of human brain injury. Although the pig DAI model does not reproduce all of the salient features of human DAI, the mechanisms of injury and morphologic character of axonal damage are conserved. The differences in coma production and distribution of axonal injury in the pig compared with the human may actually have advantages. With this animal model, we may be able to determine anatomic substrates responsible for coma and identify mechanisms responsible for the distinctive pattern of brain damage in humans, including axonal injury and selective hippocampal damage.

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REFERENCES


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