# Immediate coma following inertial brain injury dependent on axonal damage in the brainstem

DOUGLAS H. SMITH, M.D., MASAHIRO NONAKA, M.D., REID MILLER, PH.D.,
MATTHEW LEONI, B.S., XIAO-HAN CHEN, M.D., DAVID ALSOP, PH.D.,
AND DAVID F. MEANEY, PH.D.

*Departments of Neurosurgery, Bioengineering, and Radiology, University of Pennsylvania, Philadelphia, Pennsylvania*

*Object.* Immediate and prolonged coma following brain trauma has been shown to result from diffuse axonal injury (DAI). However, the relationship between the distribution of axonal damage and posttraumatic coma has not been examined. In the present study, the authors examine that relationship.

*Methods.* To explore potential anatomical origins of posttraumatic coma, the authors used a model of inertial brain injury in the pig. Anesthetized miniature swine were subjected to a nonimpact-induced head rotational acceleration along either the coronal or axial plane (six pigs in each group). Immediate prolonged coma was consistently produced by head axial plane rotation, but not by head coronal plane rotation. Immunohistochemical examination of the injured brains revealed that DAI was produced by head rotation along both planes in all animals. However, extensive axonal damage in the brainstem was found in the pigs injured via head axial plane rotation. In these animals, the severity of coma was found to correlate with both the extent of axonal damage in the brainstem ($p < 0.01$) and the applied kinetic loading conditions ($p < 0.001$). No relationship was found between coma and the extent of axonal damage in other brain regions.

*Conclusions.* These results suggest that injury to axons in the brainstem plays a major role in induction of immediate posttraumatic coma and that DAI can occur without coma.

KEY WORDS • coma • diffuse axonal injury • diffuse brain injury • brainstem • inertial brain injury • pig

TRAUMATIC brain injury affects more than 2,000,000 people each year in the United States and is the leading cause of death in children and young adults.[27,28] In the broad spectrum of brain injuries, the terms "focal" and "diffuse" are used to describe general pathological features that reflect distinct mechanisms of brain trauma. Focal brain injury is typically associated with blows to the head that may produce cerebral contusions and hematomas.[3,11,15] In contrast, DBI may occur in the absence of impact forces, but is dependent on inertial forces that are commonly produced by motor vehicle crashes and, in some cases, falls and assaults.[1,2,12,14] The principal mechanism inducing DBIs is the shearing of tissue caused by rapid head rotational motions leading to DAI.[11,12] In severe DAI, tissue tears in the white matter and intraparenchymal hemorrhage may occur. However, mild and moderate DAIs are commonly associated with only microscopic axonal lesions.[1,22]

Another important difference between focal brain injury and DBI is the source and character of posttraumatic coma resulting from these two general forms of injury.

Focal brain injury may produce mass effects from hemorrhage, contusion, or hematoma, which can induce herniation and brainstem compression.[3] Accordingly, resultant coma may not be immediate, but may develop later. In the case of DBI, Gennarelli and colleagues[12] demonstrated that nonimpact-induced rotational acceleration applied to the heads of nonhuman primates could induce an immediate and prolonged posttraumatic unconsciousness in the absence of mass lesions. This model also produced a DAI that appeared identical to that found in humans, the extent of which was found to be directly proportional to the duration of coma. Thus, it was concluded from these landmark studies that DAI is a major cause of immediate traumatic coma.

We have recently adapted the same apparatus used for the nonhuman primate DAI studies to induce DAI in pigs following head rotational acceleration in the coronal plane.[26] However, coma was not produced in the pigs, even though the relative amount of axonal injury appeared to be similar to that found in nonhuman primates in which coma was present. We also found that the distribution of axonal injury between the two species was substantially different. Most notably, we detected far less axonal damage in the brainstem and corpus callosum of brain-injured swine than that described of nonhuman primates. Thus,
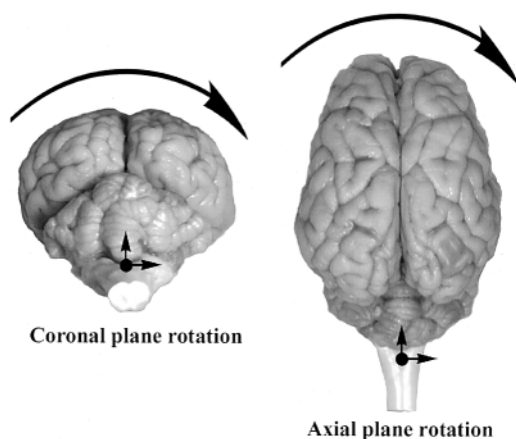
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*Abbreviations used in this paper:* βAPP = β amyloid precursor protein; DAB = 3,3′-diaminobenzidine; DAI = diffuse axonal injury; DBI = diffuse brain injury; ICP = intracranial pressure; MR = magnetic resonance.

FIG. 1. Caudal *(left)* and dorsal *(right)* views of the pig brain, demonstrating coronal and axial plane rotations. *Black circles* mark the centers of rotation.

these observations do not support the "diffuse" component of DAI as the source of coma. Rather, these data suggest that posttraumatic coma may be dependent on the specific distribution of axonal damage in DBI. This distinction has important implications for the development of new diagnostic techniques and therapeutic strategies for DBI.

In the present study, we sought to elucidate potential anatomical origins of immediate posttraumatic coma following DBI. To accomplish this, we further modified the inertial injury device to compare the effects of head rotational acceleration in the axial plane with those in the coronal plane. With these modifications, we could evaluate the influence of the plane of head motion on the resulting distribution of injury and degree of neurological impairment. Results from previous computer-model simulations of inertial brain injury in the pig suggested a distinctly different distribution of tissue-shearing strain along midline structures for these two planes of head rotation.[20]

## Materials and Methods

In these studies, we carefully adhered to the animal welfare guidelines set forth in the U.S. Department of Health and Human Services publication, *Guide for the Care and Use of Laboratory Animals*. All animal procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

### Brain Injury Apparatus

Diffuse brain trauma in the pig was induced via head rotational acceleration (inertial) brain trauma, which was produced using a pneumatic actuator that delivers controlled acceleration without impact. The lack of impact allows us to examine DBIs separate from injuries caused by contact phenomena during impact, such as skull fracture and large cortical contusions. Triggering the pneumatic actuator generates linear motion, which is converted to angular motion by the use of a linkage assembly attached to the top of the injury device. An apparatus connected to the linkage transfers this rotational motion to the head of the animal. In the present study we used two separate linkage assemblies to produce pure impulsive head rotation in either the coronal or axial plane. Control of the head rotational acceleration profile was accomplished by adjusting both the hydraulic fluid level within the actuator and the pneumatic pressure delivered to the actuator. The inertial loading conditions produced by activation of this device have been shown to approximate close-

ly the conditions of inertial brain injury in humans, such as those encountered during automobile crashes.[20]

### Animal Preparation

Fourteen miniature female adult swine (6–7 months of age, Hormel strain), each weighing 17 to 22 kg, were used for this study (12 brain-injured animals and two uninjured controls). Female swine were used because of their high brain mass/head mass ratio compared with that of male swine. Food was withheld from the animals for 12 hours, after which anesthesia was induced by an initial injection of midazolam (400–600 mg/kg). Once sedated, the animals received 2 to 4% isoflurane via a snout mask until they reached a plane of surgical anesthesia. A venous catheter was inserted in the ear and the animals were endotracheally intubated and maintained in an atmosphere containing 1.5 to 2% isoflurane. The physiological monitoring apparatus included noninvasive electrocardiographic electrode leads affixed to the chest and extremities, a pulse oximeter placed on the skin of the tail, a rectal thermometer, and sampling tubes for end-tidal $CO_2$ measurement attached to the endotracheal tube. Arterial pressure was measured using an inflated cuff placed on a hindlimb. Arterial blood gas levels were periodically evaluated before and after injury. The pigs were continuously monitored and the physiological data were collected in a computer-driven storage system.

### Induced Brain Injury

The heads of the anesthetized animals were secured to one of the two linkage assemblies by a snout clamp composed of a padded bite plate and spring steel bands encircling the snout. The anesthetic agent was discontinued 10 seconds before the injury device was activated. The linkage assemblies were adjusted to produce pure impulsive head rotation, with the center of rotation located close to the skull base for coronal plane rotation or at C1–2 for axial plane rotation (Fig. 1). Activation of the pneumatic actuator rotated the linkage assembly over the full desired angular excursion of 110º in 20 msec, with peak acceleration at approximately 6 msec. The loading conditions were measured using a piezoresistive accelerometer and an angular rate sensor attached to the linkage sidearm. Signals were captured using a personal computer–based data acquisition system (sampling at a frequency of 8000 samples/second) and were post-processed using appropriate filtering algorithms (analog filtering with a cutoff frequency of 1000 Hz). Following acceleration, the linkage was slowly moved back into its original position, the animal's snout was removed from the biteplate and the animal's respiratory, cardiac, and neurological statuses were assessed. Mechanical ventilation was provided for animals unable to breathe on their own.

Three different transducers were used to determine the transient rotational acceleration, rotational velocity, and rotational displacement profiles. Rotational acceleration was computed for the duration of the experiment by using the recorded linear acceleration measured at a fixed point on the linkage. In comparison, rotational velocity and displacement were recorded directly from the transducers. Each of these three measurements was downloaded onto a computer oscilloscope emulation program for storage. Brain mass was determined when the brain was removed for histological analysis and was used to normalize the loading parameters to a 80-g brain mass according to Holbourn's scaling relationship. Once we confirmed that the measurements of rotational acceleration and velocity were equal, we used peak angular velocity as the numerical representative of the kinetic loading conditions for each animal.

*Head Coronal Plane Rotation.* For these injuries (six animals), ICP monitoring was not used because extensive previous studies had demonstrated that there would be almost no ICP changes. On stabilization of the animal's vital signs, the arterial and venous lines were removed, all incisions were sutured closed, and topical antibiotic and dressing were applied to the wounds. All animals received intramuscular buprenorphine hydrocholoride (0.1 mg/kg) every 12 hours as needed for analgesia. Animals injured by coronal plane rotation were killed 7 days postinjury.

*Head Axial Plane Rotation.* Following the head coronal plane rotation studies, we changed the linkage assembly to prepare for head rotational acceleration in the axial plane (6 animals). Sterile

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placement of an ICP probe housed in a subarachnoid bolt was performed immediately following injury. All other physiological monitoring was continued. Corneal and pupillary reflexes and pain responses were monitored every 5 to 15 minutes. Animals that did not breathe on their own received mechanical ventilation with compressed air. For animals emerging from coma, 1.5 to 2% isoflurane anesthesia was reinstated. Because of the comatose state and extensive support needs of these animals, it was determined that the animals could not be maintained for a prolonged postinjury survival time. Therefore, all animals in this group were killed 8 hours posttrauma. We have previously observed that this postinjury interval is of sufficient length to allow determination of the extent and distribution of axonal injury.[9,26] Furthermore, the extent and distribution of axonal damage observed within hours after brain injury was found to be similar to that observed 3 to 10 days posttrauma.

### Assessment of Coma

We developed a numerical coma scale scoring system to characterize the depth of unconsciousness in brain-injured pigs by using the following categories: corneal reflex (0 = absent, 1 = unilateral, 2 = bilateral); response to pain (0 = absent, 1 = movement without any sign of intention, 2 = movement with intention); spontaneous eye opening (0 = negative, 2 = positive); and righting reflex (2 = positive). The severity of coma was determined by the sum of the scores: 0 to 1 represented severe coma, 2 or 3 moderate coma, 4 or 5 mild coma, and 6 to 8 emergence from coma. A coma scale score was determined for each animal at 30-minute intervals beginning immediately following the injury.

### Tissue Preparation

The animals were killed by an intravenous overdose of pentobarbital (150 mg/kg) and were transcardially perfused with 4% paraformaldehyde. The brains were removed, weighed, postfixed in paraformaldehyde, and blocked into 0.5-cm coronal sections for gross examination and photography. Tissue blocks from some animals were cryoprotected in sucrose, and a series of 40-$\mu$m frozen sections were cut from the front face of each block and mounted on microscope slides. Some blocks were cut into coronal blocks 3 to 5 mm thick and processed for paraffin embedding in an automated tissue processor. Serial paraffinized sections 6 $\mu$m thick were cut on a rotary microtome, mounted on poly-L-lysine–coated slides, and stained with cresyl violet, hematoxylin and eosin, or immunostain.

### Immunohistochemical Analysis

Immunostaining was performed on free floating sections and paraffin-embedded sections by using the avidin–biotin immunoperoxidase complex method. The brain sections were incubated with a primary antibody overnight at 4ºC and then incubated at room temperature for 1 hour each with the appropriate secondary and tertiary antibodies. They were visualized using the avidin–biotin complex method. Peroxidase activity was revealed using 0.025% DAB, 300 mg imidazole, and 0.25% hydrogen peroxide or a DAB kit for 10 minutes. Omission of primary antibody or application of control serum instead of primary antibody on selected sections of pig tissue provided a negative control. Primary antibodies used to detect axonal damage included the monoclonal NR4 antibody, targeting the 68-kD neurofilament protein subunits (dilution 1:400); the N52 antibody, targeting the 200-kD neurofilament subunits (dilution 1:400); and the 22c11 antibody, $\beta$APP (dilution 1:40).

### Histopathological Analysis

All sections were examined using light microscopy and a semiquantitative analysis was performed to determine the extent and distribution of axonal damage throughout the brain. We have previously developed sector scoring techniques to determine the number of profiles representing axonal injury in the pig.[26] For each immunostain, three sections from each block of tissue were examined using 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. Although the anatomical regions under investigation were generally based on those listed in the *Atlas of the Brains of Domestic Animals*,[29] we recently developed our own atlas schematics of the pig brain to normalize (register) the specific anatomical regions to be evaluated.[9,20,26] Scoring of axonal pathological characteristics (axonal bulbs or axonal swelling) was performed by rating one to five damaged axons per 1.2 mm$^2$ of a specific anatomical region as mild, six to 15 damaged axons as moderate, and more than 15 damaged axons as severe. After all sections were examined, two-dimensional injury patterns were created and assigned to representative schematic brain sections to semiquantitatively map the severity and extent of axonal injury.

### Magnetic Resonance Imaging

In one animal injured by head axial plane rotation, we performed routine MR imaging at 90 minutes postinjury using a 1.5-tesla imaging system. The animal was comatose during the examination, but did not require mechanical ventilation. The pig was placed in the prone position on the bed of the MR imager and a 13-cm surface coil was positioned on the surface of the head immediately above the brain. Following acquisition of a quick $T_1$-weighted sagittal image for localization, proton-density and $T_2$-weighted axial images through the brain were obtained using a two-echo fast spin–echo sequence. Axial orientation in the human MR imaging unit corresponds to approximately coronal slices through the brain of the pig. We selected the following parameters: field of view 20 cm, slice thickness 5 mm, matrix $192 \times 256$, echo train length of 8, repetition time of 4 seconds, and effective echo times of 17 and 85 msec. Images through 15 contiguous slices through the brain were acquired. These images were then compared with macro- and microscopic analyses of corresponding tissue sections from the same animal.

### Statistical Analyses

Linear regression analysis was performed to determine potential statistical relationships between coma scores and the applied kinetic loading conditions for each animal. In addition, we evaluated potential relationships between the total or regional number of immunoreactive axonal profiles and the coma score for each animal. Only animals demonstrating immediate and prolonged posttraumatic coma were included in these analyses. The coma scores determined at 8 hours posttrauma were chosen for these analyses because the greatest range in scores between individual animals was found at this time point.

### Sources of Supplies and Equipment

We procured the pneumatic actuator from Bendix Corp. (Rochester, NY), the piezoaccelerator and angular rate sensor from Applied Technology Associates, Inc. (Albuquerque, NM), and the ICP pressure probe from Camino Laboratories (San Diego, CA). The Hypercenter XP automated tissue processor was obtained from Shandon Scientific Instruments (Cheshire, UK) and the Leitz rotary microtome from Leica (Malvern, PA). The DAB kit was purchased from Vector Laboratories (Burlingame, CA), the NR4 and N52 antibodies from Sigma Chemical Co. (St. Louis, MO), and the $\beta$-APP from Boehringer–Mannheim Corp. (Indianapolis, IN). The GE Horizon MR imager was obtained from General Electric Medical Systems (Milwaukee, WI).

## Results

### Injury-Loading Conditions

The peak angular velocity for coronal-plane rotations ranged between 310 and 352 rad/second; the peak angular velocity for axial-plane rotations ranged between 214 and 286 rad/second.

### Physiological Parameters and Behavior Following Injury

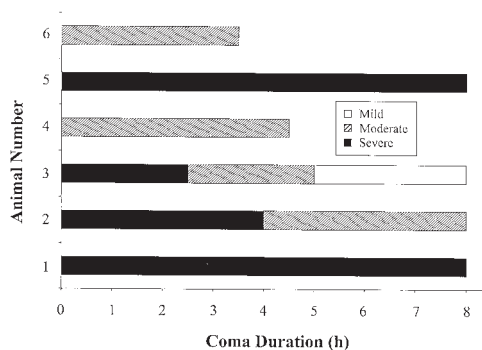*Head Coronal Plane Rotation.* Following trauma, no co-

FIG. 2. Bar graph showing the duration and severity of coma in six animals following head axial plane rotation during an 8-hour period. Coma severity was determined by a numerical coma scale scoring system; see *Materials and Methods* for details.
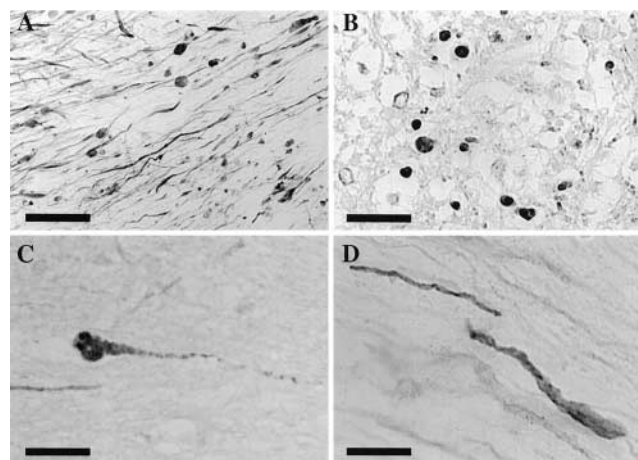


FIG. 3. Representative photomicrographs revealing axonal damage following inertial brain injury. A: High density of neurofilament immunoreactive profiles demonstrating swollen axons in the subcortical white matter after head coronal plane rotation. Bar = 100 μm. B–D: Following head axial plane rotation, damaged axons were identified in the brainstem by βAPP immunoreactivity (B, bar = 50 μm) and by neurofilament immunoreactivity (C and D, bar = 25 μm).

ma was noted in any animal. In addition, no substantial changes in mean arterial blood pressure, pulse oximetry, or end tidal $CO_2$ were observed after the injury. All animals began to awaken within 5 to 15 minutes after injury and demonstrated a righting reflex. Inhalation of anesthetic agent was reinstated for these animals and they were transported back to their cages where the agent was discontinued. Although these animals were able to ambulate typically within 1 hour after injury, they displayed slightly sluggish responses to sensory stimuli (startle reflex and tactile response) for up to 8 hours posttrauma. These findings are consistent with those of our previous reports.[9,25,26]

*Head Axial Plane Rotation.* Immediately following injury, all animals demonstrated either moderate or severe coma based on our pig coma scale score. Four animals were unconscious for the duration of the study (8 hours), whereas the remaining two animals remained in mild to moderate coma for 3.5 to 4.5 hours (Fig. 2). By 5 minutes postinjury, three of the animals had an ICP approximately 15 to 30 mm Hg above baseline (average baseline was previously determined in uninjured animals). This elevation of ICP was transient in two of the animals, lasting less than 15 minutes. In one animal, ICP remained moderately elevated for the duration of the study. This animal was later found to have a large subdural hematoma.

Mechanical ventilation after the injury was necessary for four animals; two of these animals required continuous respiratory support, whereas the other two needed 1 to 2 hours of respiratory support. The two animals that began to emerge from coma received the anesthetic agent isoflurane for the remaining portion of the study. Only modest changes in blood gas levels, end-tidal $CO_2$, mean arterial blood pressure, electrocardiographic readings, pulse, respiration (for nonventilated animals), and core body temperature were found in these injured animals.

### Gross Pathological Findings

The average brain weight was 80 g. Brains from the animals injured via head coronal plane rotation appeared completely normal, with no visible signs of contusions or hematomas. Macroscopic examination of the brains from animals injured via head axial plane rotation revealed blood on the surface of four of the brains, which was located over the frontal, parietal, and temporal lobes, cere-

bellum, and brainstem. This blood had the classic appearance of subdural and subarachnoid space bleeding. In addition, we found that two of these animals also had small hemorrhagic contusions at the base of the frontal lobes. Blood was also found in the ventricles of three of the animals. In all animals, a small number of tissue tears (typically not more than 15 per animal) identified by small hemorrhages (0.5–2 mm in diameter) were visible in the brainstem, in structures at the base of the brain, and along the ventricles.

### Microscopic Pathological Findings

Axonal bulbs and varicose axonal swellings were observed following trauma in all animals and were not found in uninjured animals (Fig. 3). However, the distribution of axonal damage was markedly different between the two planes of head rotation.

*Head Coronal Plane Rotation.* In these animals, neurofilament and βAPP immunoreactivity demonstrated a distribution and amount of axonal injury that was virtually identical with previous observations.[26] Axonal damage was observed in the roots of gyri, at the interface of the gray and white matter, adjacent to ventricles, and in the deep hemispheric white matter. Regionally, axonal damage was most predominantly found in the frontal, parietal, and temporal lobes. To a lesser extent, axonal damage was also found in the basal ganglia and brainstem (pons and midbrain) (Figs. 3–5). No tissue tears and almost no vascular disruption were noted in regions of axonal injury. However, small petechial hemorrhages, only identified using microscopy, were seen along the roots of gyri in the frontal, parietal, and temporal lobes and periventricular regions, as well as in the brainstem.

*Head Axial Plane Rotation.* In these animals neurofilament and βAPP immunoreactivity demonstrated an asym-
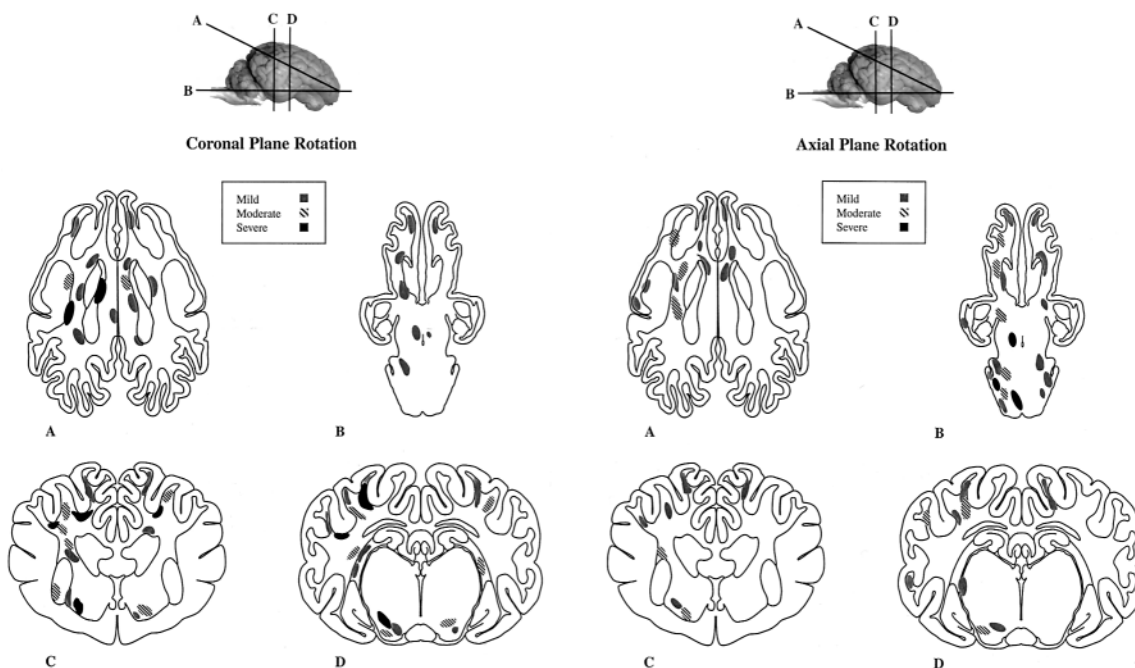
FIG. 4. Schematic representation of the distribution and severity of axonal injury following head coronal plane *(left)* and axial plane *(right)* rotation. Lines through the brain shown at the top of the figure demarcate anatomical regions of interest: frontal lobe, basal ganglia, and occipital lobe (A), brainstem through brain base (B), rostral–thalamic level (C), and dorsal–hippocampal level (D). Regions of axonal injury are shaded according to severity (mild, moderate, or severe).

metrical distribution of axonal injury in the white matter, which was more predominantly found in the leading hemisphere relative to the direction of rotation (Figs. 3–5). Axonal injury was primarily found in the brainstem (pons and midbrain), the only region in which severe axonal damage (high concentrations of swollen axons) was demonstrated. However, a mild-to-moderate extent of axonal damage was found in the frontal, parietal, and temporal lobes; along the anterior, medial, and posterior periventricular regions; and in the basal ganglia. Petechial hemorrhages were found in the brainstem, along the base of the brain, and throughout the hemispheres. Tissue tears, also observed during macroscopic examination, were found in association with intraparenchymal hemorrhage (Figs. 6 and 7).

### Findings on MR Imaging

Prior to histopathological analysis, routine visual inspection of the MR images obtained in one pig injured via head axial plane rotation revealed no overt focal hyperintensities suggestive of pathological changes. However, subsequent histopathological analysis demonstrated DAI, multiple petechial hemorrhages, and several hemorrhagic tissue tears throughout the brain. No contusions were found in the brain of this animal. Direct matching of brain sections with corresponding MR images only elucidated questionable changes in signal intensity on the MR images, even in regions that included tissue tears up to 2 mm in length (Fig. 7).

### Correlation With Coma Severity

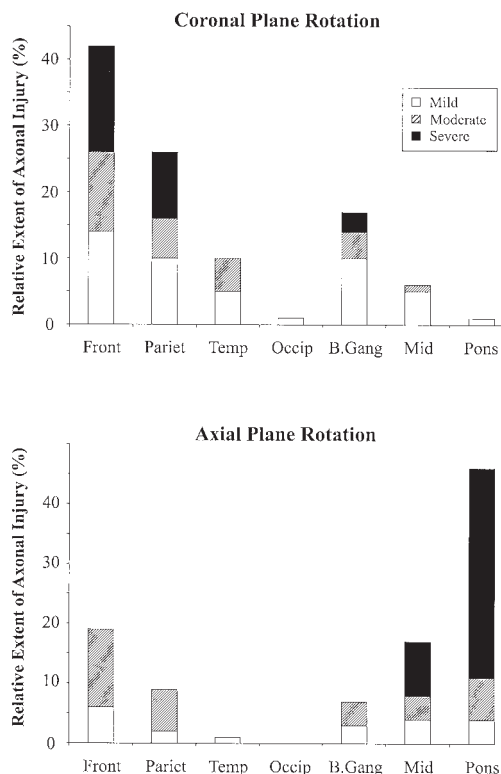Only the animals injured via head axial plane rotation



FIG. 5. Bar graphs showing the distribution and severity of axonal injury in several brain regions following head coronal and axial plane rotations. B.Gang = basal ganglia; Front = frontal lobe; Mid = midbrain; Occip = occipital lobe; Pariet = parietal lobe; Temp = temporal lobe.
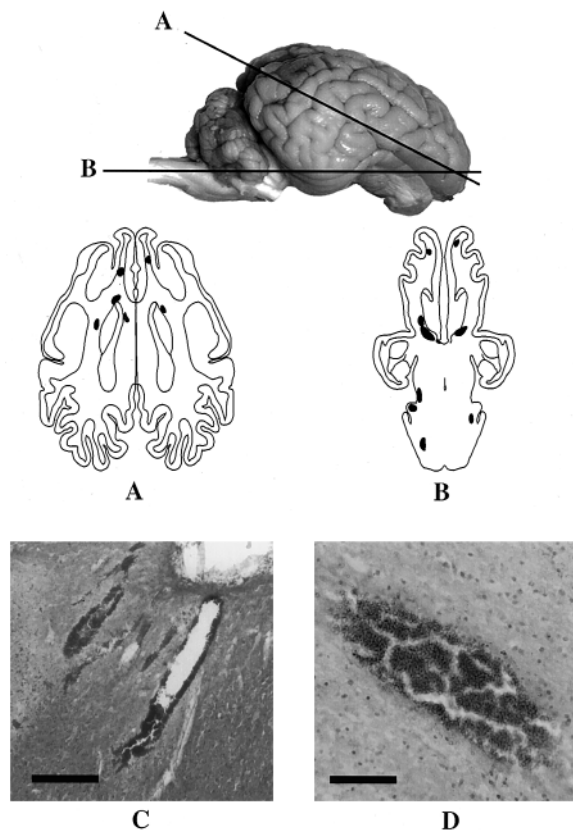
FIG. 6. A and B: Schematic representations showing the distribution of tissue tears (0.5–2.0 mm) following head axial plane rotation. Lines through brain at the top of the figure demarcate anatomical regions of interest: frontal lobe, basal ganglia, and occipital lobe (A), and brainstem through brain base (B). C and D: Representative photomicrographs demonstrating the appearance of the tissue tears. Bar = 250 μm for C and 100 μm for D.



FIG. 7. Proton-density *(upper)* and $T_2$-weighted *(center)* MR images obtained 2 hours after head axial plane rotation in a pig compared with schematic representations *(lower)* showing regions of tissue tears and hemorrhage *(black markings)* up to 2 mm in length at the level of the brainstem *(left)* and hippocampus *(right)*. This animal also had substantial axonal damage in the brainstem. Note the lack of obvious signal changes in the MR images, despite the presence of tissue tears and axonal injury.

were included in the statistical analyses because coma was only produced by head rotation in this plane. Using the coma scale score determined at 8 hours postinjury, linear regression analysis revealed a strong correlation between the severity of coma and the applied kinetic loading conditions (peak angular velocity) for each animal ($p < 0.001$). We also found a relationship between the severity of coma and the extent of axonal damage in the brainstem ($p < 0.01$) by matching our coma scale score with the number of immunoreactive axonal profiles in this region. However, no statistical relationship was found between the severity of coma and the extent of axonal damage in other individual brain regions or all other hemispheric regions combined ($p > 0.05$; Table 1).

## Discussion

In this study, we found that inertial loading applied to the heads of pigs along either the coronal or axial plane induced DAI throughout the white matter. However, immediate and persistent coma was only observed in animals injured by head axial plane rotation, even though the severity of the loading conditions was less than that experienced by animals injured via coronal rotation. We also
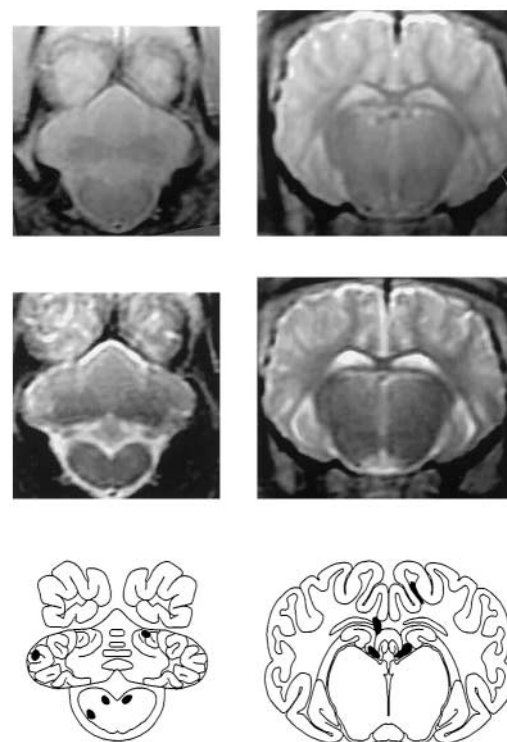
found a differential distribution of axonal injury between the two planes of rotation. Substantially more axonal damage was observed in the dorsolateral region of the rostral brainstem of animals injured with head axial plane rotation. Furthermore, the extent of axonal damage in the brainstems of these animals directly correlated with the severity of resultant coma. In contrast, no statistical relationship was found between the severity of coma and the total extent of axonal damage throughout the brain or in separate brain regions, excluding the brainstem. These findings are the first to demonstrate the important relationship between the plane of head rotational acceleration, the distribution of axonal injury, and the generation of immediate and prolonged posttraumatic coma. Specifically, these results suggest that: 1) injury to axons in the brainstem plays a major role in induction of immediate posttraumatic coma; and 2) DAI can occur without coma.

Seminal observations made using the nonhuman primate model of inertial brain injury established that DAI alone could induce immediate coma in the absence of mass effects.[12] However, it has also been observed that coma may not be present in some patients with presumed DAI,[21] as observed following head coronal plane rotational acceleration in pigs.[9,26] Even though the relative extent of coma in brain-injured patients has been found to be generally predictive for outcome,[10,17] large disparities re-

main. It has not been resolved why some patients with DBI may have good recoveries despite a history of prolonged coma, whereas others may remain severely disabled. At issue may be the potentially misleading "diffuse" portion of the term "diffuse axonal injury." Most accounts of DAI describe a widely distributed, multifocal pattern of axonal damage rather than a diffuse distribution in the strict sense.[1,3,15] Although Gennarelli and colleagues[12] found that the severity of coma appeared to be related to the plane of rotational acceleration, the role of axonal injury distribution in the development of coma was not previously determined. Our present results suggest that coma may be more of a reflection of the severity of axonal damage in specific regions, most notably the brainstem, rather than the total sum of axonal injury distributed throughout the brain. Accordingly, comatose patients who achieve good recoveries may have axonal damage primarily limited to the brainstem.[23] Conversely, lack of coma may not necessarily suggest a good recovery if substantial axonal damage is present throughout the subcortical and deep white matter. Nonetheless, it is important to consider that for severe diffuse brain trauma in humans, the most common distribution of damage includes a high concentration of axonal damage and tissue tears in the brainstem and midline structures.

By virtue of the prone location of the brainstem in humans and nonhuman primates, almost any mechanical loading to the head will cause strain and stress in this region. However, few studies have investigated the specific mechanical loading conditions that are most injurious to the brainstem. Using computational models, we have recently found evidence that the extent of deformation of the brainstem in the swine is dependent on the specific plane of head rotational acceleration.[20] For head coronal plane rotation in pigs, the caudally projecting brainstem is near the center of rotation and experiences relatively low strains, whereas higher strains are produced at the periphery, such as in the subcortical white matter. For head axial plane motions in the pig, the brainstem is deformed transverse to its longitudinal axis, producing much greater focal tensile and shear forces compared with those in coronal plane rotation. In accordance with data from the computational models, our present results demonstrate that following head axial plane rotation, substantially more axonal damage was found in the brainstem, compared with that incurred in head coronal plane injuries. Furthermore, tissue tears in the brainstem were only found after head axial plane rotation. Therefore, our results suggest that the plane of head rotational acceleration plays an important role in determining both the distribution of axonal damage and the production of coma.

It is important to note that following either plane of head rotation in pigs, the splenium of the corpus callosum was not damaged to nearly the same extent as typically found in brain-injured humans and in the nonhuman primates in the inertial brain injury studies.[1,6,12] We believe this difference is due to the relatively small falx in pigs compared with that in primates. During head rotation in primates, the falx may impinge on the trailing brain hemisphere, while the leading hemisphere pulls away, resulting in high strain along the sagittal midline. This high strain through the midline structures may not be produced in pigs during head rotational acceleration because the brain

TABLE 1
*Characteristics of head axial plane rotation and resultant injuries in six pigs\**

| Animal No. | Peak Angular Velocity (rad/sec) | Coma Scale Score (8 hrs postinjury) | Damaged Axons/1.2 mm² | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Bstem | Front | Pariet | Temp | Bgang | Hemis |
| 1 | 277 | 0 | 55 | 20 | 9 | 0 | 9 | 38 |
| 2 | 234 | 2 | 20 | 2 | 1 | 2 | 1 | 6 |
| 3 | 232 | 5 | 8 | 2 | 3 | 1 | 2 | 8 |
| 4 | DNC | 8 | 4 | 19 | 4 | 1 | 11 | 25 |
| 5 | 286 | 0 | 61 | 27 | 16 | 0 | 0 | 43 |
| 6 | 214 | 6 | 14 | 11 | 5 | 0 | 1 | 17 |

\* Regression analysis demonstrated a correlation between the coma score and the peak angular velocity ($p < 0.001$) and between the coma score and the extent of axonal damage in the brainstem ($p < 0.01$). Abbreviations: Bgang = basal ganglion; Bstem = brainstem; DNC = data not counted; Front = frontal lobe; Hemis = Hemisphere; Pariet = parietal lobe; Temp = temporal lobe.

hemispheres are freer to rotate past the sagittal midline. For our present study, this difference was advantageous for deciphering anatomical origins of coma. Our results demonstrate that immediate coma can be induced, despite a paucity of corpus callosal and other midline damage.

The identification of mechanical loading conditions leading to DAI and coma is paramount for the development of preventive measures to reduce injury. An understanding of the most and least injurious angles of head rotation could be incorporated into new safety designs for automobiles and sports equipment. In addition, characterization of the distribution of axonal damage contributing to coma could facilitate the diagnosis and prognosis of DBI. Although conventional brain imaging techniques are useful for revealing macroscopic changes often seen in severe diffuse brain trauma, such as white matter tears and parenchymal hemorrhage, these techniqes cannot easily detect the predominant injury, microscopic axonal swellings. Accordingly, in patients with little macroscopic injury following DBI, images of the brain may appear normal,[7,21] leading many to believe that DAI is substantially underdiagnosed. This diagnostic shortfall was demonstrated in the present study, in which conventional MR imaging assessment of one animal injured by head axial-plane rotation failed to exhibit clearly extensive axonal damage or tissue tears. In an attempt to enhance diagnostic capabilities for DBI, studies from our laboratory and others have identified several new imaging and spectroscopic techniques that appear to illuminate regions of axonal injury more clearly.[4,5,8,13,16,18,19,24] Thus, in the near future, some of these techniques may be used as the standard to identify axonal damage more accurately and to improve prognostication.

Although the initial report of the relationship between DAI and immediate and prolonged coma following brain injury was published in 1982,[12] the present study is the first to corroborate this mechanistic link. In addition, the present results extend this doctrine to include the importance of specific planes of head rotation and the distribution of axonal damage in the generation of posttraumatic coma.

### References

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