JOURNAL OF NEUROTRAUMA
Volume 19, Number 7, 2002

Traumatic Axonal Injury after Closed Head Injury in the Neonatal Pig

RAMESH RAGHUPATHI[1] and SUSAN S. MARGULIES[2]

ABSTRACT

Closed head injury is the leading cause of morbidity and mortality in infants and children, and results in pathologies such as diffuse axonal injury (DAI) and subarachnoid hematoma (SAH). To better understand the mechanical environment associated with closed head injury in the pediatric population, animal models that include salient features of human infant brain must be utilized. Based on detailed information regarding the parallels between brain development in the pig and the human, the 3–5-day-old piglet was used to represent the infant at less than 3 months of age. Anesthetized piglets ($n = 7$) were subjected to rapid, inertial (nonimpact) rotation of the head about its axial plane and sacrificed at 6 h postinjury. Immediately following injury, five of seven piglets were apneic, with an absence of pupillary and pain reflexes. All piglets exhibited severe coma immediately postinjury, but recovered by sacrifice time. Blood was present on the surface of the frontal lobes, cerebellum, and brainstem, and subarachnoid hemorrhage was evident in the frontal cortex. In six of seven brain-injured piglets, accumulation of the 68-kDa neurofilament protein was evident in contiguous axons (swollen) and occasionally in disconnected axons (axonal bulbs), suggestive of traumatic axonal injury (TAI). Mapping of the regional pattern of TAI revealed injured axons predominantly in central and peripheral white matter tracts in the frontal and temporal lobes and in the midbrain. The number of injured axons was equivalent in both hemispheres, and did not correlate to the load applied to the head. Together, these data demonstrate that rapid rotation of the piglet head without impact results in SAH and TAI, similar to that observed in children following severe brain trauma.

Key words: axonal injury; head injury; infants; subarachnoid hematoma; white matter

INTRODUCTION

TRAUMATIC BRAIN INJURY is the most common cause of death in childhood in the United States, with at least a rate of over 200 children per 100,000 children requiring hospitalization annually (Fisher, 1997). In addition, closed head injury in infancy results in higher morbidity and mortality than that seen in older children, probably as a result of the significant incidence of inflicted injury in the youngest patients (Luerssen et al., 1991; Duhaime et al., 1992). The most common pathologies observed in severe head injuries in infants are subdural hematoma (SDH) and diffuse axonal injury (DAI). The underlying pathology of DAI is the widespread damage to axons in the white matter of the brain, while SDH is often caused by the rupture of the blood vessels in the subdural compartments. Data from biomechanical analyses of *in vitro* and *in vivo* adult models of brain trauma

Departments of [1]Neurosurgery and [2]Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania.

indicate a link between tissue strain and white matter and vascular injury (Lowenhielm, 1975; Margulies et al., 1990; Meaney et al., 1994, 1995).

Pediatric animal models for head injury used to evaluate the effect of contusional and/or diffuse brain injury have used immature (7-day-old to 17-day-old) rats (Adelson et al., 1996; Ikonomidou et al., 1996; Prins et al., 1996). Using a weight-drop model of diffuse injury in 17-day-old male rats, Adelson and colleagues (1996) observed the presence of diffuse subarachnoid hemorrhage and neuronal death in the hippocampus, while in younger animals (at postnatal day 7), weight-drop injury on to the fixed head resulted in focal cortical contusions (Ikonomidou et al., 1996). Lateral fluid-percussion brain injury, which in adult animals has been reported to cause traumatic axonal injury in white matter tracts (Bramlett et al., 1997; Saatman et al., 1998), was modified for use in immature rats (17-day-old), but was not associated with any microscopic abnormalities (Prins et al., 1996). These observations demonstrate that, while head injury mechanisms may be age-dependent, rodent models are of limited utility in providing data relevant to understanding head injury in human infants and young children. We sought to investigate age-dependent mechanisms of nonimpact brain injury in the piglet, a higher species with closer application to human head injury.

Analyses of the mechanical environment associated with pediatric brain injury must employ animal models that include salient features of human infant brains, such as the overall shape, gyral pattern, distribution of gray and white matter, the degree of myelination, regional cerebral metabolism, and blood flow. Investigators of cerebral insult have described the first weeks of life in the piglet as corresponding to the infant developmental stage (Armstead and Kurth, 1994; Hoehner et al., 1994; Brodhun et al., 2001). Contusional and diffuse injuries have been modeled in 3–6-week-old juvenile pigs (Madsen and Reske-Nielsen, 1987; Madsen, 1990; Armstead and Kurth, 1994; Brodhun et al., 2001). Contusional injuries in juvenile pigs were not associated with diffuse bleeding or white matter injury (Madsen and Reske-Nielsen, 1987; Duhaime et al., 2000), but lateral fluid percussion brain trauma in the juvenile pig resulted in mild traumatic axonal injury in white matter tracts, limited subdural and subarachnoid bleeding, and no evidence of contusions (Brodhun et al., 2001). While these studies attest to the utility of porcine models of brain trauma, little to no information exists characterizing the distribution of SDH and traumatic axonal injury following nonimpact, inertial head injury in neonatal (3–5-day-old) piglets.

The HYGE apparatus of University of Pennsylvania's Head Injury Center provides controlled, reproducible inertial rotational loads without impact (Meaney et al., 1995; Smith et al., 1997, 2000). Rapid rotation of heads of adult minipigs about the axial plane resulted in prolonged coma, which was correlated with axonal damage observed in the brainstem (Smith et al., 2000). In addition, subdural and subarachnoid bleeding was observed in 66% of the injured animals. Based on detailed information regarding parallels between pig and human development, we used piglets at 3–5 days of age to represent the human infant (<3 months of age) in a porcine head injury model using the HYGE apparatus. We demonstrate that nonimpact, inertial brain trauma resulted in SDH and traumatic axonal injury in the immature pig brain, and have determined the anatomic distributions of macroscopic (tissue tears, subdural hematoma, intracranial hemorrhage) and microscopic (axonal injury) damage following inertial (nonimpact) rotational loads applied to the head.

MATERIALS AND METHODS

Brain Injury and Physiologic Measurements

Neonatal (3–5 day, $n = 7$) farm piglets were sedated with an intramuscular injection of midazolam (0.5 mg/kg), and thereafter animals received inhalational anesthesia (2% isofluorane) via an endotracheal tube. Mean arterial pressure and arterial O_2 were measured using a pressure cuff on the hind limb (Vet/OX™ model 4402; San Diego Instruments, San Diego, CA) and oxymeter cuff on the tail (DINAMAP™ model 8300; Critikon, Tampa, FL), respectively. All measurements were periodically evaluated (≤30 min intervals) prior to injury and following injury. All protocols were approved by Institutional Animal Care and Use Committee of the University of Pennsylvania.

Studies were performed with head rotational acceleration in the axial plane with the center of rotation in the cervical spine (Fig. 1). Animals were subjected to rapid, purely impulsive, nonimpact rotations. Brain injury was induced using a well-characterized head rotational acceleration device to impart a rapid, single 110° axial rotation with its center in the cervical spine. To achieve this motion, the animals' heads were secured to a padded snout clamp, which, in turn, was mounted to the linkage assembly of a HYGE pneumatic actuator (Bendix Corp) that converts the impulsive linear motion to an angular (rotational) motion (Fig. 1). Axial angular velocities of 214–286 rad/sec have been used previously in the adult pig producing unconscious periods from 2 to over 8 h (Smith et al., 2000). To produce moderate injury in the piglets we determined the target rotational load (velocity) parameters by scaling the lowest load used previously

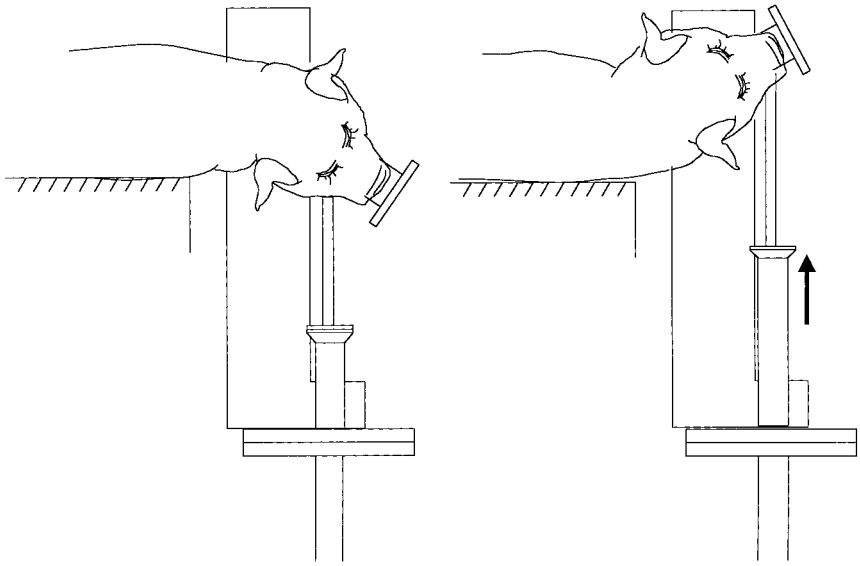

FIG. 1. Schematic depicting the linkage set-up of the HYGE device and the position of the piglet on the device. After securing the snout to the bite plate, the bite plate was rotated in the axial plane at an angle of 110°.

(214 rad/sec) that produced unconsciousness (2.5 h) in the adult minipig. Applying a relationship proposed by Ommaya and colleagues (1967) with a scaling factor defined as the inverse ratio of the brain masses raised to the one-third power, and using average brain mass of 35 and 72 g in the piglet and adult, respectively, results in a target angular velocity of 272 rad/sec for the piglet to provide a scaled mechanical load associated with moderate injury in the smaller brain. Immediately prior to inducing brain injury the animals were taken off anesthesia.

Activation of the HYGE device rotated the linkage assembly over the full desired angular excursion within 10–12 msec. The rotational velocity was measured by an angular rate sensor (ATA, Inc.) attached to the linkage sidearm. Signals were captured using a PC-based data acquisition (f = 8kHz), and accelarations were computed by differentiation of the rotational velocity signal, which was depicted as a half-sine curve with an average duration of 12 sec. Following the rapid rotation, the linkage was slowly moved back into its original position, the animal's snout removed from the bite plate, and respiratory, cardiac, and neurologic status was assessed. If apneic, mechanical ventilation was provided (tidal volume 10 mL/kg body weight). Neurologic status was monitored using corneal and pupillary reflexes and pinch tests to assess the response to physical stimuli. Once animals responded to the pinch reflex, anesthesia was resumed. Because animals were maintained on anesthesia until the time of euthanasia, body temperature was monitored using a rectal thermometer (Cole Parmer model 70002H) and maintained at 36–38°C using heating blankets (Table 2). One additional pig served as an uninjured control, and all gross and histological analyses are reported relative to this uninjured control animal.

Histology

The animals were euthanized 6–8 h after injury in order to evaluate and compare the regional patterns of traumatic axonal injury to finite element modeling of areas of tissue deformation, and to previously published patterns of axonal damage at 6–8 h following axial rotation of the adult porcine head. Piglet brains were fixed by transcardial perfusion with 1L heparinized saline (10,000 units/L), followed by 3.5 L of 10% formalin (Sigma Chemical Co., St. Louis, MO). Brains were removed from the cranial cavity and postfixed overnight at 4°C and blocked into 1-cm coronal sections for gross examinations and photography. Blocks were cryoprotected in sucrose. Brains were examined for microscopic neural and vascular injury. Sections (40-μm thick) from the cryoprotected blocks were stained with cresyl violet (Nissl) and evaluated using a light microscope for regional tissue tears in white matter, presence of intracerebral hemorrhage in white and gray matter, and overt neurodegeneration (pyknosis) in the gray matter. Microscopic evidence of axonal injury was investigated in the white matter of the brain using neurofilament (NF) immunohistochemistry. Reactive axonal changes, indicative of both primary and secondary axotomy, were determined by identifying the presence of swollen axons and terminal axonal bulbs containing neurofilament protein (see

examples in Fig. 3B,C). Adjacent coronal brain sections were immunostained for the 68-kDa (light) and 200-kDa NF (heavy) protein subunits, respectively. Sections were incubated with anti-NF68 antibody (1:400, clone NR4; Sigma Chemical Co.) or anti-NF200 antibody (1:400, clone N52; Sigma Chemical Co.) overnight at 4°C, followed by biotinylated donkey anti-mouse IgG (1:2,000; Jackson Immunoresearch Labs, West Grove, PA). Antibody complexes were detected using avidin-biotin-peroxidase (ABC) histochemistry (Vector Labs, Burlingame, CA) and 3,3′-diaminobenzidine as chromogen. Omission of primary antibody on selected sections of uninjured and injured pig tissue provided a negative control. White matter tracts in the frontal, parietal, and temporal lobes were evaluated throughout each of four coronal sections between plates 1 and 12 (Yoshikawa 1968), and identified as central and peripheral white matter tracts, external capsule, midbrain, hippocampus, and basal ganglia. The entire area of each white matter tract was evaluated in both the left (leading) and the right (lagging) hemispheres using a Leica microscope at 20× magnification (area of viewing field = 0.71 mm^2), and counts and location of injured axons were mapped onto schematics of the four coronal sections. Data from a viewing field was included if at least three swollen axons/axonal bulbs were observed. The numbers of injured axons in each region were summed in each animal, and then averaged across animals. In addition, the number of microscopic viewing fields in each region containing injured axons were summed. The density of injured axons (injured axons/mm^2) was calculated by dividing the total number of axons in both hemispheres by the total field area, where total field area is the number of fields included times the viewing field area of 0.71 mm^2. In order to compare our data to that in the adult mini-pig following axial rotation (Smith et al., 2000), we calculated the density of injured axons in each adult minipig by dividing the total number of axons in the hemisphere (values in Table 1 of Smith et al., 2000) by the total field area, where total field area is the number of fields included times the field area of 1.2 mm^2 (number of fields obtained from Dr. D.H. Smith, University of Pennsylvania, personal communication). The values from individual adult animals were averaged across all animals and are included in the Discussion.

Data Analysis and Statistics

Physiological data in injured animals were compared to baseline values using a repeated measures one-way ANOVA, followed by a Student-Neuman Keuls post-hoc *t* test. Because uninjured animals did not contain any injured axons, counts of injured axons were compared in only the injured animals using a two-way ANOVA to determine the propensity for axonal injury in either the leading or the lagging hemisphere, and in a specific white matter tract (hemisphere × region). A p value of less than 0.05 was considered significant.

RESULTS

Physiology

Rapid rotation of the piglet head in the axial plane with an average peak angular velocity of 250 ± 10 rad/sec (average total load duration, Δt, = 11.4 ± 0.8 sec; Table 1). This load is equivalent to 196 ± 9 rad/sec when scaled for brain weight of 72 g, the average weight of an adult minipig. Immediately following the load, five of seven piglets were apneic with an absence of pupillary, corneal, and pain reflexes, and received mechanical ventilator support until spontaneous breathing was restored. Pain reflex returned within 5 min postinjury in three piglets,

Table 1. Brain Injury Parameters in 3–5-day-old Piglets

Animal no.	*Body weight (kg)*	*Brain weight (g)*	*Peak angular velocity (radian/sec)*	*Δt*	*Peak angular decelaration (radian/sec²)*
2	n.d.	35	236.6	11.6	138,000
3	n.d.	33	256.78	12.2	155,249
4	n.d.	37	264.17	12.0	102,267
8	2.0	34	244.46	10.6	104,731
11	1.5	34	246.43	10.6	105,964
13	3.0	35	244.46	12.2	104,731
14	3.0	38	259.24	10.4	105,964
n = 7	2.0 ± 1.0	35 ± 2	250 ± 10	11.4 ± 0.8	116,701 ± 21,076

n.d., not determined.

Table 2. Physiologic Variables in Brain-Injured Infant Piglets

Time (min postinjury)	*MAP (mm Hg)*	*HR (beats/min)*	*Temperature (°C)*
0	50.2 ± 10.1	159 ± 11	36.4 ± 1.1
10	46.8 ± 11.4	161 ± 19	35.7 ± 1.0
20	80.3 ± 34.3	181 ± 28	n.d.
45	46.8 ± 14.9	163 ± 46	36.2 ± 1.7
90	43.8 ± 13.5	142 ± 10	36.3 ± 2.1
180	39.8 ± 11.2	136 ± 20	36.3 ± 2.6
360	49.6 ± 12.6	143 ± 41	37.5 ± 2.9

The mean arterial pressure (MAP), heart rate (HR), and core body temperature (Temp) were measured in anesthetized piglets for 30 min prior to and up to 6 h following application of load using noninvasive techniques. Values presented as means ± standard deviation of $n = 7$ piglets.

n.d., not determined.

between 15 and 25 min in three piglets and at 80 min postinjury in one piglet. Using the coma scale developed by Smith et al. (2000), seven of seven brain-injured piglets had a score of 0–1 (severe coma) immediately postinjury but recovered to a score of 8 (no coma) by sacrifice time at 6 h postinjury. By approximately 20–35 min posttrauma, all injured piglets were breathing spontaneously. No overt changes in mean arterial pressure and PO_2 were observed in any injured animal (Table 2).

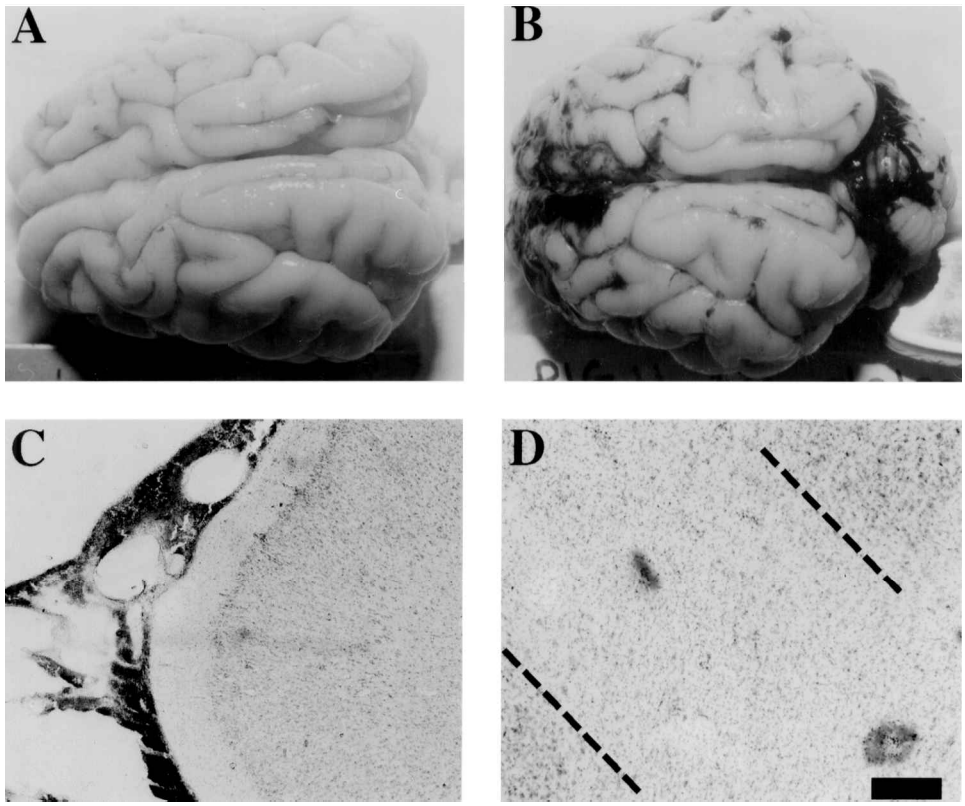

FIG. 2. Macroscopic and microscopic evaluation of histological damage to the brain of the piglet following axial head rotation. (**A**) Brain of an uninjured 3–5-day-old piglet. (**B**) Brain of an injured piglet at 6 h postinjury removed from the head following perfusion-fixation with neutral-buffered formalin. Note the presence of blood over the frontal lobes of both hemispheres and over the cerebellum. (**C**) Representative micrograph illustrating the presence of hemorrhage in the subpial and subarachnoid layers above the frontal cortex. (**D**) Representative Nissl-stained section illustrating the presence of petechial hemorrhage in the central white matter tract within the frontal lobe. Dotted lines in panel D delineates white from gray matter. Bar represents 375 μm (C), 100 μm (D).

Histology

Compared to a control piglet brain (Fig. 2A), macroscopic examination of injured brains revealed the presence of blood on the surface of the cerebellum, brainstem, and left (leading edge of the brain) frontal and temporal lobes, indicative of subdural bleeding, but was not restricted to the leading edge of the brain (Fig. 2B). On gross evaluation of brain tissue blocks, blood was observed in the lateral ventricles but macroscopic cerebral tissue tears were absent. In coronal brain sections stained with cresyl violet (Nissl), no evidence of neuronal loss (pyknosis) was observed in any region of the injured brain. Subpial and subarachnoid blood was visible over the frontal cortex (Fig. 2C), and petechial hemorrhage was evident in both the gray and white matter (Fig. 2D). No overt evidence of blood–brain barrier disruption (using anti-swine IgG antibody) was observed in the piglet (data not shown).

Axonal Injury

Control piglet brains exhibited normal staining for the 68-kDa neurofilament protein in all regions of the white matter (Fig. 3A). In all but one of the seven brain-injured piglets examined, axonal swellings (Fig. 3B), and occasional axonal bulbs (Fig. 3C) were detected. Evidence of axonal injury was further substantiated using an antibody that recognizes the full-length amyloid precursor protein (APP, data not shown). Injured axons were located both at roots of the gyri and in the deep hemispheric white matter (Fig. 4). Mapping of the distribution of the injured axons revealed that almost all brain-injured piglets exhibited axonal injury in the central and peripheral white matter tracts, and midbrain, but injured axons in other regions such as the basal ganglia, external capsule and hippocampus occurred in only 16–50% of the brain-injured animals (Fig. 4 and Table 3). Interestingly, the damaged axons in the piglet brain were interspersed with axons that appeared morphologically normal, and did not correlate with the regional presence of petechial hemorrhage. In fact, the injured piglet which had no evidence of axonal injury, exhibited subdural bleeding and intraparenchymal hemorrhage. Little to no axonal injury was detected in the piglet brain when an antibody that detects the heavy (200-kDa) NF protein subunit was used (data not shown).

Injured axons exhibiting characteristics illustrated in Figure 3B,C were counted, and quantitative analysis of NF-68 positive injured axons revealed that the density of injured axons in the different white matter tracts (severity) were equivalent in both hemispheres (Fig. 5). In addition, there was no apparent correlation between loading conditions (angular peak velocity) and the density of

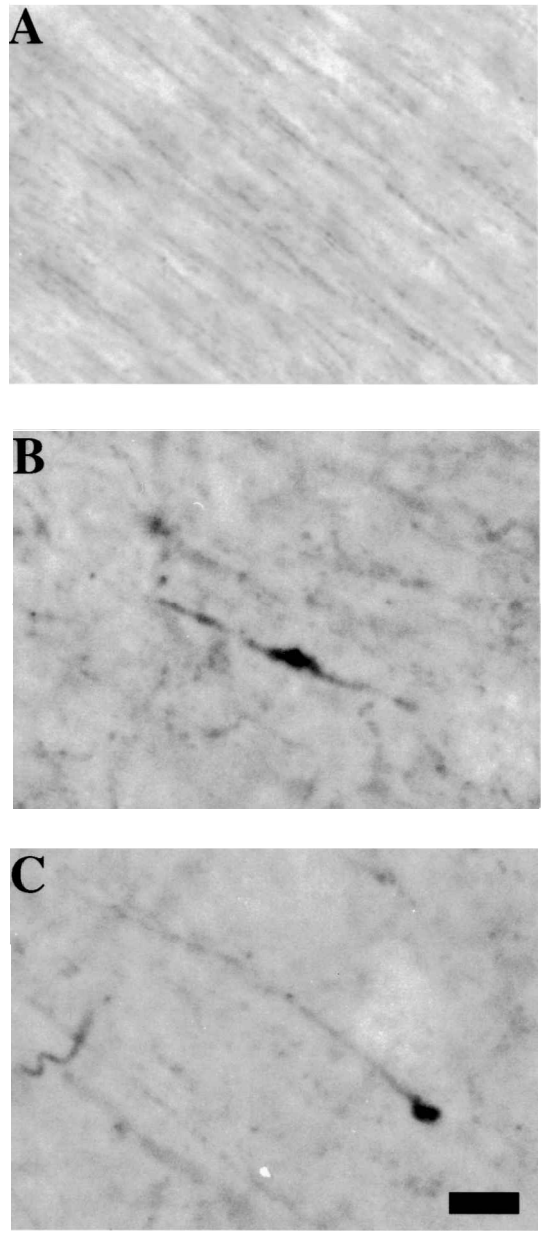

FIG. 3. Evidence of traumatic axonal injury in the white matter of piglet brains following axial head rotation. (**A**) Immunoreactivity for the 68-kDa subunit of neurofilament protein within the peripheral white matter tract in the temporal lobe of an uninjured piglet brain. Note the organized and regular staining pattern of the axons. (**B**) Representative photomicrograph of a traumatically injured axon obtained from the peripheral white matter within the frontal lobe (slice A in Fig. 4). Note the presence of accumulated 68-kDa neurofilament protein in an otherwise contiguous axon. (**C**) Representative photomicrograph of an axonal bulb present in the white matter tract within the midbrain (slice B in Fig. 4). Note the presence of accumulated 68-kDa neurofilament protein at the end of the disconnected axon. Bar represents 25 μm for all panels.

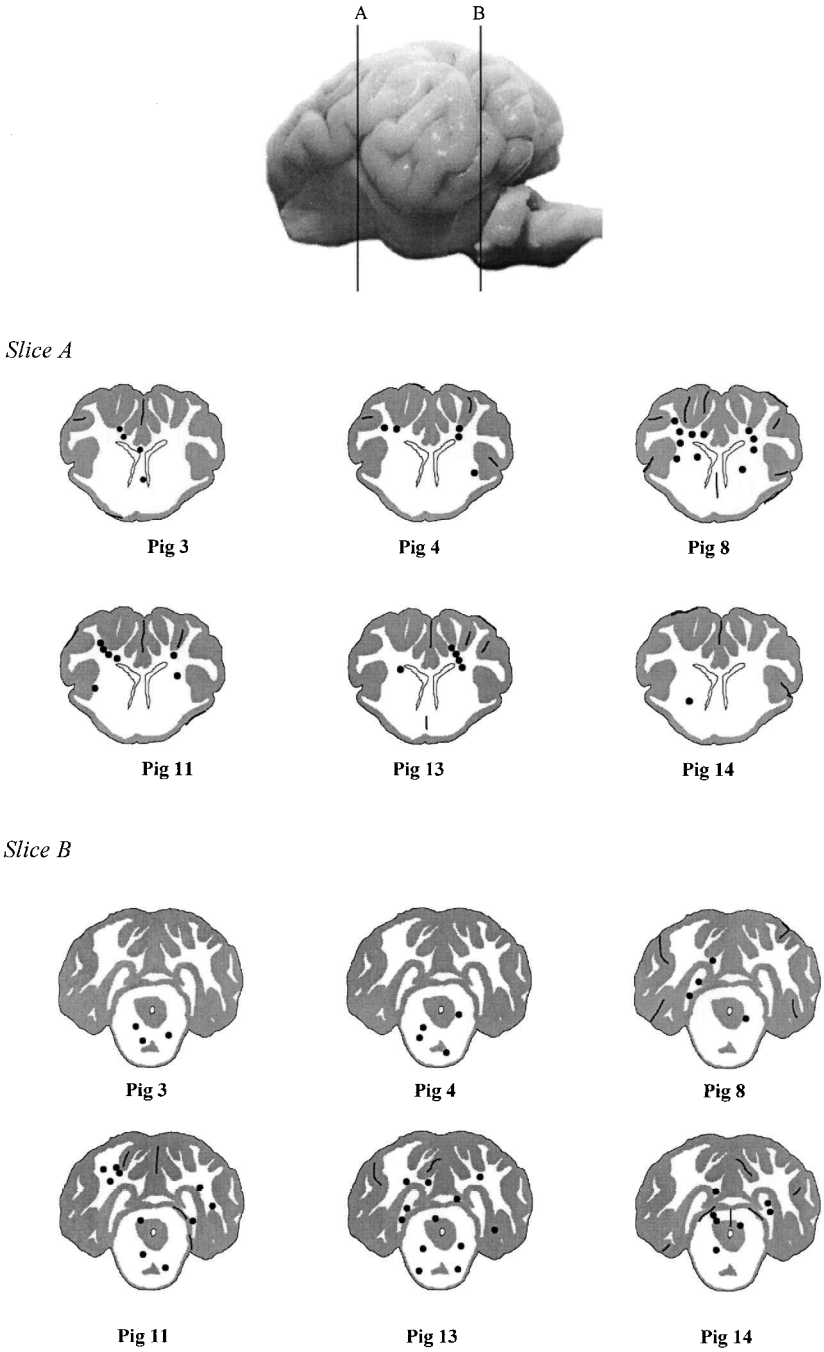

FIG. 4. Schematic depicting the regional distribution of injured axons within white matter tracts in brains of six of seven injured piglets. All fields shown contained at least three swollen axons and/or axonal bulbs. Injured axons were defined based on the presence of accumulated 68-kDa neurofilament protein leading to a swollen appearance of the axon, or the presence of axonal bulbs. White matter tracts were examined at ×20 magnification encompassing a field of view of 0.71 mm^2, which is represented in the schematic by a filled dot. Slice A represents plate 3, while slice B represents plate 12 from the *Atlas of Brains of Domestic Animals* (Yoshikawa, 1968). Although four coronal sections between plates 1 and 12 were evaluated in each animal, plates 3 and 12 were chosen for illustration purposes because they were common to all six brain-injured piglets.

TABLE 3. REGIONAL QUANTIFICATION OF INJURED AXONS AFTER ROTATIONAL-ACCELERATION BRAIN TRAUMA IN THE INFANT PIGLET

	Number of injured axons (number of fields)							
Piglet	*WM-c*	*WM-p*	*EC*	*MB*	*HC*	*BG*	*Total*	*Injured axons/mm²*
3	17 (5)	9 (2)	0	10 (3)	0	7 (1)	43 (11)	5.4
4	15 (5)	10 (3)	0	12 (4)	0	9 (2)	46 (14)	4.5
8	30 (7)	6 (2)	0	22 (3)	11 (2)	18 (3)	87 (17)	7.2
11	39 (7)	63 (9)	9 (1)	13 (3)	4 (1)	9 (1)	137 (22)	8.7
13	46 (12)	58 (10)	0	19 (5)	11 (3)	0	134 (30)	6.2
14	3 (1)	29 (6)	0	24 (4)	12 (2)	3 (1)	71 (14)	7.0

Injured axons were identified in four coronal planes using immunohistochemistry for the 68-kDa neurofilament (NF68) protein, and defined based on the appearance of accumulated NF-68 protein in contiguous axons (swellings) or in disconnected axons (axonal bulbs). Numbers in parentheses denote the total number of microscopic fields (0.71 mm^2) in each region containing the injured axons. A microscopic field was considered counted if it contained at least three injured axons. Because there did not appear to be a hemispheric difference in the occurrence and distribution of injured axons, the numbers from both hemispheres were combined. The density of injured axons (denoted by injured axons/mm^2) was calculated by dividing the total number of injured axons by the total field area, where field area is the number of included fields times the field area of 0.71 mm^2.

BG, basal ganglia; EC, external capsule; HC, hippocampus; MB, midbrain; WM-c, white matter tracts in deep subcortical regions; WM-p, white matter tracts immediately underlying the cortical gray matter.

injured axons in the whole brain (Fig. 5). Two-way ANOVA (hemisphere × region) of either the total number of sites containing injured axons or the total number of injured axons in each region (severity) revealed that region, rather than hemisphere, was the main effect ($p < 0.001$ for sites, $p < 0.029$ for severity). Post-hoc analyses revealed that, of all regions in which axonal injury was observed, the peripheral and central white matter tracts contained both the greatest number of sites exhibiting injured axons and the greatest number of injured axons ($p < 0.05$ compared to all other regions; Table 3).

DISCUSSION

Inertial rotation of the piglet's head in the axial plane resulted in traumatic axonal injury in white matter tracts in multiple regions of the brain. Subdural and subarachnoid hemorrhage was observed over the frontal lobes, and intraparenchymal hemorrhaging was observed to a limited extent. Despite the presence of injured axons in multiple white matter tracts, little to no evidence of cellular necrosis and tissue tears was present in any region of the injured brain. Brain-injured piglets did not suffer overt and extensive loss of consciousness, and were able to spontaneously respire in the acute posttraumatic period. There was no hemispheric preponderance for axonal injury with equivalent numbers of injured axons in the lead-

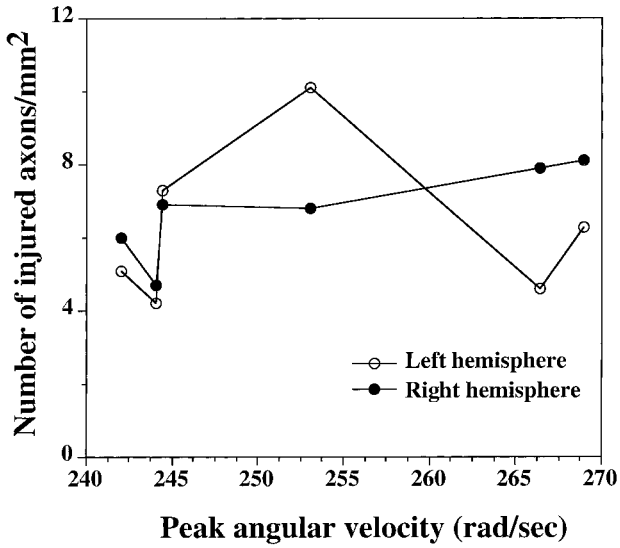

FIG. 5. Density of axonal injury depicted as a function of injury load. The density of injured axons (swollen, contiguous axons and axonal bulbs) present in all white matter tracts within each hemisphere of each injured brain was calculated as described in Methods and plotted as a function of the angular velocity (in radians/sec) to which that brain was subjected. Note that there does not appear to be a strong correlation between the velocity of the angular rotation and the density of injured axons. Also note the equivalent density of injured axons between the leading (left) and lagging (right) hemispheres.

ing (left) and lagging hemispheres. Similarly, there was no statistical correlation between the severity of axonal injury and the peak angular velocity. Together these data are indicative of the establishment of a model of inertial brain trauma in the neonatal piglet that exhibits histopathologic features relevant to those observed in human neonates—traumatic axonal injury *and* subarachanoid hemorrhage.

Traumatic axonal damage in the injured piglet brain was characterized by axonal swelling and the presence of axonal bulbs, pathologies that have been observed in both porcine and nonhuman primate models of diffuse brain trauma, and in humans (Smith et al., 1997, 2000; Maxwell et al., 1993, 1997; Adams et al., 1982; Gennarelli et al., 1982; McKenzie et al., 1996). In the nonhuman primate, axonal shearing accompanied by dissolution of the cytoskeleton and alterations in organelle morphology provided evidence for primary axotomy, and was demonstrated at 1 h postinjury (Maxwell et al., 1993). Traumatic axonal injury observed in humans as early as 2 h postinjury (McKenzie et al., 1996), and in animals at 4–6 h postinjury (Maxwell et al., 1997; Smith et al., 2000), may be indicative of secondary axotomy. Together, these observations suggest that the axonal swellings observed in the brain-injured piglet may likely be suggestive of secondary axotomy.

It must be noted that the peak angular velocity (250 $\pm$ 10 rad/sec) was not significantly different between the present study and that used for adult minipigs (249 $\pm$ 31 rad/sec; Smith et al., 2000). However, scaling these inertial loads to the same brain mass, the equivalent peak angular velocity experienced by the piglet was 22% lower than that experienced by the adult minipigs ($p < 0.001$). Whether considering scaled or unscaled loads, the immature piglet brain appears to be more vulnerable to traumatic axonal injury than the adult animal: axial rotation of the neonatal porcine head resulted in 6.5 $\pm$ 1.5 (mean $\pm$ standard deviation) injured axons/mm^2 of the cerebrum in the injured piglet brain, compared to our calculations of 1.9 $\pm$ 0.7 injured axons/mm^2 in the cerebrum of the brain-injured adult minipig rotated in the same direction. However, as reported for either coronal or axial head rotation of the adult minipig, our data suggests that the extent of axonal damage in the cerebral hemispheres does not correlate with the length of coma.

Our data suggesting that piglets may exhibit traumatic axonal damage to a greater extent that adults support observations in both the clinical and experimental literature. Thus, infants and preadolescent children that have suffered a closed head injury appear to exhibit suffer a greater mortality rate and more behavioral damage than older children and adults (Luerssen et al., 1991; Duhaime et al., 1992). Similarly, brain-injured neonatal rats (postnatal day 7–17) exhibited greater mortality rates and physiologic instability (Grundl et al., 1994; Prins et al., 1996) and significantly more apoptotic cell death (Pohl et al., 1999) compared to adult rats. Furthermore, diffuse brain trauma in neonatal (3–5-day-old) and juvenile (3–4-week-old) pigs resulted in a greater alteration in cerebrovascular parameters in the neonatal animals than in the older pigs (Armstead and Kurth, 1994; Armstead, 1999). In contrast to these observations of increased vulnerability to brain trauma, a vast body of literature (reviewed in Kolb et al., 2000; Villablanca and Hovda, 2000) suggest that neonatal animals exhibit an enhanced ability to recover from penetrating brain injuries compared to adult animals, perhaps as a result of increased plasticity in the immature brain. In support of these findings, we have recently reported that experimental contusive brain injury resulted in a lower degree of cerebrovascular alterations (at 2–4 h postinjury) and a smaller lesion (at 7 days postinjury) in 3–5-day-old piglets than in 1-month-old pigs (Durham et al., 2000; Duhaime et al., 2000). It is tempting to speculate that these apparent differences in the sensitivity of the immature brain to traumatic injury may reflect the type of injury, that is, penetrating (focal) versus nonpenetrating (diffuse).

In addition to traumatic axonal injury, subarachanoid hemorrhage is a significant feature of the neuropathology in infants and children that suffered a closed head injury (Luerssen et al., 1991; Duhaime et al., 1992). Despite reports of multiple models of closed head injury in neonatal animals, little to no information exists regarding the neuropathologic sequelae of brain trauma in neonates. Adelson and colleagues (1996) observed ventriculomegaly, SAH, and cerebral swelling in postnatal day17 rats that received a severe weight-drop injury. In juvenile piglets, SAH and axonal injury was observed only in animals that received both the fluid-percussion trauma and hypovolemic hypotension that resulted in increased intracranial pressure (Brodhun et al., 2001). Although we observed SAH and subpial blood over the frontal cortex and an accumulation of blood in the lateral ventricles, intracerebral tissue tears and petechial hemorrhages were not observed to any great extent, perhaps indicative of mild brain trauma. Both petechial hemorrhages and primary tissue tears were present in the brains of the adult minipig exposed to higher loads (Smith et al., 2000) and following lateral head accelaration in nonhuman primates (Adams et al., 1982; Gennarelli et al., 1982). Closed head injury in neonatal rats using a pneumatic piston resulted in skull fractures and the development of a cortical contusion that contained an increased number of apoptotic cells (Pohl et al., 1999). Consistent with the features of a nonimpact trauma, we did not observe skull fractures or any microscopic evidence of neu-

ronal necrosis in gray matter regions of the injured piglet brain. However, in part, this may reflect the short postinjury survival time (6 h); cell death following lateral fluid-percussion injury in adult rats begins to manfest at 12–24 h posttrauma (Dietrich et al., 1994; Hicks et al., 1996; Conti et al., 1998).

Although the neuropathology of head injury in adult humans has been extensively described (Gennarelli et al., 1998), similar information in the pediatric population is limited. Shannon and colleagues (1998) reported that, in a cohort of 14 cases of inflicted head injury in infants under age 2, all exhibited evidence of traumatic axonal injury in the cerebral white matter and subdural hematoma. Here, we present a model of nonimpact brain trauma in the infant piglet that only resulted in short periods of apnea but was accompanied by subdural and subarachanoid hemorrhage, and traumatic axonal injury. However, more recently, Geddes and colleagues (2001a,b) conducted a meticulous analysis of the clinicopathology of nonaccidental pediatric head injury and reported that the predominant histologic abnormality in inflicted brain damage is that of brain swelling and hypoxic damage, accompanied by focal areas of axonal damage (axonal bulb formation) in the craniocervical junction (Geddes et al., 2001b). Further investigations into the role of hypoxia on the distribution of traumatic axonal injury are warranted.

ACKNOWLEDGMENTS

We gratefully acknowledge Drs. Douglas H. Smith and David F. Meaney for the insightful discussions and technical expertise. We also thank Matthew Leoni and Florence Bareyre for their excellent technical assistance. These studies were supported by NIH grants R01-NS39679, NS41561, and CDC grant R49-CCR312712.

REFERENCES

ADAMS, J.H., GENNARELLI, T.A., and GRAHAM, D.I. (1982). Brain damage in non-missile head injury: observations in man and subhuman primates, in: *Recent Advances in Neuropathology* W.T. Smith and J.B. Cavanagh, (eds), Churchill Livingston: New York, pps. 165–190.

ADELSON, P.D., ROBICHAUD, P., HAMILTON, R.L., et al. (1996). A model of diffuse traumatic brain injury in the immature rat. J. Neurosurg. **85,** 877–884.

ADELSON, P.D., DIXON, C.E., and KOCHANEK, P.M. (2000). Long-term dysfunction following diffuse traumatic brain injury in the immature rat. J. Neurotrauma **17,** 273–282.

ARMSTEAD, W.M. (1999). Age-dependent impairment of K_{ATP} channel function following brain injury. J. Neurotrauma **16,** 391–402.

ARMSTEAD, W.M., and KURTH, C.D. (1994). Different cerebral hemodynamic responses following fluid-percussion brain injury in the newborn and juvenile pig. J. Neurotrauma **11,** 487–497.

BRAMLETT, H.M., KRAYDIEH, S., GREEN, E.J., et al. (1997). Temporal and regional patterns of axonal damage following traumatic brain injury: a beta-amyloid precursor protein immunocytochemical study in rats. J. Neuropathol. Exp. Neurol. **56,** 1132–1141.

BRODHUN, M., FRITZ, H., WALTER, B., et al. (2001). Immunomorphological sequelae of severe brain injury induced by fluid-percussion in juvenile pigs—effects of mild hypothermia. Acta Neuropathol. **101,** 424–434.

CONTI, A.C., RAGHUPATHI, R., TROJANOWSKI, J.Q., et al. (1998). Experimental brain injury induces regionally distinct apoptosis during the acute and delayed post-traumatic period. J. Neurosci. **18,** 5663–5672.

DIETRICH, W.D., ALONSO, O., and HALLEY, M. (1994). Early microvascular and neuronal consequences of traumatic brain injury: a light and electron microscopic study in rats. J. Neurotrauma **11,** 289–301.

DUHAIME, A.C., ALARIO, A.J., LEWANDER, W.J., et al. (1992). Head injury in very young children: mechanisms, injury types, and opthalmologic findings in 100 hospitalized patients younger than 2 years of age. Pediatrics **90,** 179–185.

DUHAIME, A.C., MARGULIES, S.S., DURHAM, S.R., et al. (2000). Maturation-dependent response of the piglet brain to scaled cortical impact. J. Neurosurg. **93,** 455–462.

DURHAM, S.R., RAGHUPATHI, R., HELFAER, M.A., et al. (2000). Age-related differences in acute physiologic response to focal traumatic brain injury in piglets. Pediatr. Neurosurg. **33,** 76–82.

FISHER, M.D. (1997). Pediatric traumatic brain injury. Crit. Care Nurs. Q. **20,** 36–51.

GEDDES, J.F., HACKSHAW, A.K., VOWLES, G.H., et al. (2001a). Neuropathology of inflicted head injury in children. I. Patterns of brain damage. Brain **124,** 1290–1298.

GEDDES, J.F., VOWLES, G.H., HACKSHAW, A.K., et al. (2001b). Neuropathology of inflicted head injury in children. II. Microscopic brain injury in infants. Brain **124,** 1299-1306.

GENNARELLI, T.A., THIBAULT, L.E., ADAMS, J.H., et al. (1982). Diffuse axonal injury and traumatic coma in the primate. Ann. Neurol. **12,** 564–574.

GENNARELLI, T.A., THIBAULT, L.E., and GRAHAM, D.I. (1998). Diffuse axonal injury: an important form of traumatic brain damage. Neuroscientist **4,** 202–215.

GRUNDL, P.D., BIAGAS, K.V., KOCHANEK, P.M., et al. (1994). Early cerebrovascular response to head injury in immature and mature rats. J. Neurotrauma **11,** 135–148.

HICKS, R.R., SOARES, H.D., SMITH, D.H., et al. (1996). Temporal and spatial characterization of neuronal injury following lateral fluid-percussion brain injury in the rat. Acta Neuropathol. **91,** 236–246.

HOEHNER, P.J., KIRSCH, J.R., HELFAER, M.A., et al. (1994). Dihydropyridine ligand binding decreases earlier in adolescent than in infant swine after global cerebral ischemia. Stroke **25,** 2060–2066.

IKONOMIDOU, C., QIN, Y., KIRBY, C., et al. (1996). Prevention of trauma-induced neurodegeneration in infant rat brain. Pediatr. Res. **39,** 1020–1027.

KOLB, B., GIBB, R., and GORNY, G. (2000). Cortical plasticity and the development of behavior after early frontal cortical injury. Dev. Neuropsychol. **18,** 423–444.

LOWENHIELM, P. (1975). Mathematical simulations of gliding contusions. J. Biomech. **8,** 351–356.

LUERSSEN, T.G., HUANG, J.C., McLONE, D.G., et al. (1991). Retinal hemorrhages, seizures, and intracranial hemorrhages: relationships and outcomes in children suffering traumatic brain injury, in: *Concepts in Pediatric Neurosurgery.* A.E. Marlin (ed), Karger: Basel, pps. 87–94.

MADSEN, F.F. (1990). Regional Cerebral blood flow after a localized cerebral contusion in pigs. Acta Neurochir. (Wien) **105,** 150–157.

MADSEN, F.F., and RESKE-NIELSEN, E. (1987). A simple mechanical model using a piston to produce localized cerebral contusions in pigs. Acta Neurochir. (Wien) **102,** 65–72.

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

MAXWELL, W.L., WATT, C., GRAHAM, D.I., et al. (1993). Ultrastructural evidence of axonal shearing as a result of lateral accelaration of the head in non-human primates. Acta Neuropathol. **86,** 136–144.

MAXWELL, W.L., POVLISHOCK, J.T., and GRAHAM, D.I. (1997). A mechanistic analysis of nondisruptive axonal injury: a review. J. Neurotrauma **14,** 419–440.

McKENZIE, K.J., McLELLAN, D.R., GENTLEMAN, S.M., et al. (1996). Is beta-APP a marker of axonal damage in short-surviving head injury? Acta Neuropathol. **92,** 608–613.

MEANEY, D.F., ROSS, D.T., WINKELSTEIN, B.A., et al. (1994). Modification of the cortical impact model to produce axonal injury in the rat cerebral cortex. J. Neurotrauma **11,** 599–609.

MEANEY, D.F., SMITH, D.H., SHREIBER, D.I., et al. (1995). Biomechanical analysis of experimental diffuse axonal injury. J. Neurotrauma **12,** 689–694.

OMMAYA, A.K., YARNALL, P., HIRSCH, A.E., et al. (1967). Scaling of experimental data on cerebral concussion in subhuman primates to concussive thresholds for man. 11th Stapp Car Crash Conf. **SAE670906,** 73–80.

POHL, D., BITTIGAU, P., ISHIMARU, M.J., et al. (1999). *N*-Methyl-D-aspartate antagonists and apoptotic cell death triggered by head trauma in developing rat brain. Proc. Natl. Acad. Sci. USA **96,** 2508–2513.

PRINS, M.L., LEE, S.M., CHENG, C.L.Y., et al. (1996). Fluid percussion brain injury in the developing and adult rat: a comparative study of mortality, morphology, intracranial pressure and mean arterial blood pressure. Dev. Brain Res. **95,** 272–282.

PRINS, M.L., and HOVDA, D.A. (1998). Traumatic brain injury in the develoiping rat: effects of maturation on Morris water maze acquisition. J. Neurotrauma **15,** 799–811.

SAATMAN, K.E., GRAHAM, D.I., and McINTOSH, T.K. (1998). The neuronal cytoskeleton is at risk after mild and moderate brain injury. J. Neurotrauma **15,** 1047–1058.

SHANNON, P., SMITH, C.R., DECK, J., et al. (1998). Axonal injury and the neuropathology of shaken baby syndrome. Acta Neuropathol. **95,** 625–631.

SMITH, D.H., CHEN, X.-H., XU, B.-N., et al. (1997). Characterization of diffuse axonal pathology and selective hippocampal damage following inertial brain trauma in the pig. J. Neuropathol. Exp. Neurol. **56,** 822–834.

SMITH, D.H., NONAKA, M., MILLER, R.T., et al. (2000). Immediate coma following inertial brain injury dependent on axonal damage in the brainstem. J. Neurosurg. **93,** 315–322.

VILLABLANCA, J.R., and HOVDA, D.A. (2000). Developmental neuroplasticity in a model of cerebral hemispherectomy and stroke. Neuroscience **95,** 625–637.

YOSHIKAWA, T. (1968). The brain of the pig, in: *The Atlas of the Brains of Domestic Animals, Vol. 5, Sect. 1.* Universivity of Tokyo Press: Tokyo, pps. 1–32.

Address reprint requests to:
Susan S. Margulies, Ph.D.
Department of Bioengineering
University of Pennsylvania
105D Hayden Hall, 3320 Smith Walk
Philadelphia, PA 19104

E-mail: margulies@seas.upenn.edu