

Maturation-dependent response of the piglet brain to scaled cortical impact

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Object. The goal of this study was to investigate the relationship between maturational stage and the brain's response to mechanical trauma in a gyrencephalic model of focal brain injury. Age-dependent differences in injury response might explain certain unique clinical syndromes seen in infants and young children and would determine whether specific therapies might be particularly effective or even counterproductive at different ages.

Methods. To deliver proportionally identical injury inputs to animals of different ages, the authors have developed a piglet model of focal contusion injury by using specific volumes of rapid cortical displacement that are precisely scaled to changes in size and dimensions of the growing brain. Using this model, the histological response to a scaled focal cortical impact was compared at 7 days after injury in piglets that were 5 days, 1 month, and 4 months of age at the time of trauma. Despite comparable injury inputs and stable physiological parameters, the percentage of hemisphere injured differed significantly among ages, with the youngest animals sustaining the smallest lesions (0.8%, 8.4%, and 21.5%, for 5-day-, 1-month-, and 4-month-old animals, respectively, $p = 0.0018$).

Conclusions. These results demonstrate that, for this particular focal injury type and severity, vulnerability to mechanical trauma increases progressively during maturation. Because of its developmental and morphological similarity to the human brain, the piglet brain provides distinct advantages in modeling age-specific responses to mechanical trauma. Differences in pathways leading to cell death or repair may be relevant to designing therapies appropriate for patients of different ages.

KEY WORDS • head injury • brain • development • contusion • swine

ALTHOUGH head injury is the most common cause of death and disability in childhood, the response of the immature brain to mechanical trauma has been incompletely studied. It is well recognized in the clinical realm that infants and young children exhibit brain injury syndromes that are unique to this age group. Interestingly, in some instances such as in nonaccidental injuries in infancy, damage may be profound, beyond that seen with similar-appearing injuries in adults, although after some types of injuries recovery is superior to that seen in adults, possibly due to increased brain plasticity early in life.^{2,18,29,31} The heterogeneity of clinical pediatric head injury makes an analysis of the effect of maturation on injury response difficult;³⁰ thus, whether specific therapies would be particularly effective or even counterproductive after brain injury in infants and children remains unclear.

Animal models of injury to the immature brain often do not account for changes in mass and morphological composition of the brain as it develops, making comparisons between injuries sustained at different ages difficult. It has been well established in isolated tissue and in vivo preparations that neural and vascular primary injury severity is

proportional to the magnitude and rate of tissue deformation.^{34,38,45,47} To create a consistent topographic deformation pattern in animals of different ages and with different brain sizes, we have developed a model of focal cortical impact in the piglet that has been scaled to deliver a volume of rapid tissue displacement proportional to the growing brain's changes in size and shape, thus enabling the specific response to injury to be compared between piglets of different ages. Because the development of the piglet brain closely parallels that of the human, the response of the brain to focal injury can be studied at different levels of maturation comparable to human infants, toddlers, and adolescents. We report our initial results using the scaled focal injury model in piglets of different ages.

Materials and Methods

Model Design

Cortical indentation was effected by a portable, stainless-steel spring-loaded device into which interchangeable blunted cylindrical stainless-steel indenter tips can be secured. When fired, the tips rapidly depress the exposed brain surface. The indenter device is attached directly to the skull with three-point screw fixation, there-

Abbreviation used in this paper: ANOVA = analysis of variance.

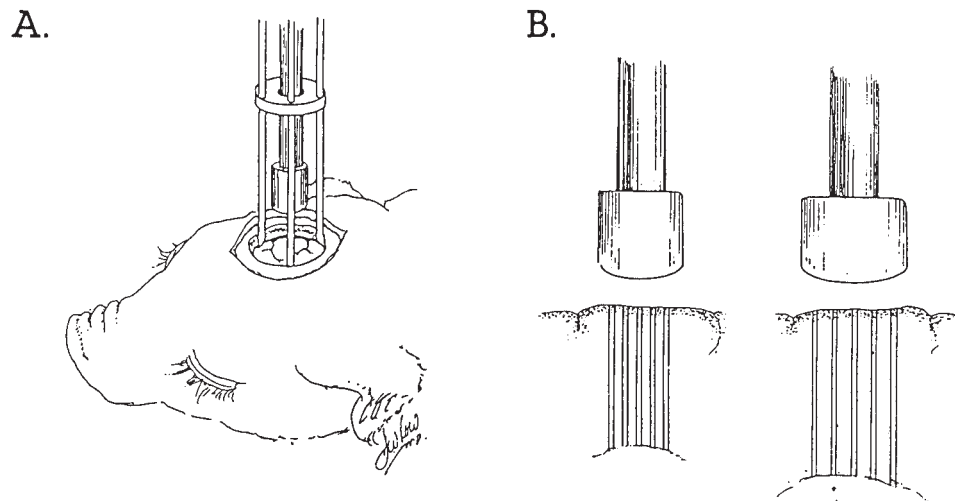


FIG. 1. A: Schematic representation of the cortical injury device. B: Illustration of the scaled indenter tips and their use in histological sampling. The tip diameter and indentation depth increase in proportion to increases in brain dimension with age, from youngest (left) to oldest (right), as described in the text. Samples are taken at the 0%, 25%, 50%, 75%, and 100% regions of tissue under the indenter tip for each age.

by allowing visible confirmation of the initial position of the indenter tip, ensuring a directly perpendicular indentation, and eliminating relative motion of the head and indenter (Fig. 1). The device was tested using a laser displacement transducer and was shown to deliver highly reproducible firing traces with a time course of 4 msec and an indentation velocity of 1.7 m/second.

To scale indentation volumes, the maximum diameter of the brain in three dimensions (superior-inferior, transverse, and anterior-posterior) and its total mass were measured in nine normal animals (three at each age of interest), and mean measurements were obtained for each age. Using these data, the indentation volumes used to create the injury in piglets in each age group were chosen such that the total volume and specific dimensions of indentation would be proportional to these changes in physical dimensions with maturation. By varying and matching both diameter and depth of indentation to be proportional to growth, displacement of specific structures is held as consistent as possible despite changes in brain size (such as similar span of the indenter top across comparable proportions of gyri and vessels and similar proportionate downward displacement of gray and white matter structures).

For this initial study, we chose to test a single injury severity across all three ages of interest. Because other forms of brain injury in infants, particularly hypoxia/ischemia, demonstrate vulnerability of the white matter, the specific injury used in this study was chosen such that it would ideally affect both gray and white matter. Thus, indenter dimensions were chosen so that the indented volume would extend well into the subcortical white matter for comparable distances in all three age groups. A 1-week survival time was chosen to minimize the possible effect of differences in timing of injury evolution that might be seen with shorter survival times. To avoid pathological changes in the brain that might reflect complications of neurological impairment during the survival interval, an injury severity level that did not produce clinical symptoms was sought. During development of the current model, preliminary studies in which varying indentation volumes were used on 26 piglets at these three ages demonstrated that indentation volumes of approximately 1% of total brain volume produced reproducible, histologically visible lesions with an uncomplicated long-term (7-day) survival period. Based on the brain dimensions of the nine control animals, this percentage of deformation was accomplished in the current study by using proportionally scaled indenter tips measuring 1.04 cm in diameter and indenting to a depth of 4.8 mm for the 5-day-old piglets; 1.07 cm in diameter with 5.9-mm indentation for the 1-month-old piglets; and 1.267 cm in diameter with 7-mm indentation for the 4-month-old piglets.

Surgical Procedure

This study was approved by the Children's Hospital of Philadelphia/Joseph Stokes, Jr. Research Institute, an animal care facility accredited by the American Association for the Accreditation of Laboratory Animal Care, in compliance with the Guide for Care and Use of Animals and all applicable laws and regulations. Twelve domestic Yorkshire piglets of random sex (four each of age 5 days, 1 month, and 4 months) underwent the experimental protocol. All animals were restricted from consuming solid food overnight prior to surgery but were allowed free access to water and/or milk. Animals received medication before surgery, consisting of midazolam (0.5 mg/kg). General anesthesia was maintained using 1 to 2% isoflurane/21% O₂, which was administered by bag and mask ventilation throughout the course of the experiment. Surgical procedures and recovery took place on a feedback-controlled temperature blanket. Continuous physiological monitoring, including heart rate, end-tidal CO₂, respiratory rate, O₂ saturation, and body (rectal) temperature, was performed, and experimental conditions were modified as necessary to maintain all physiological variables within the normal range prior to the start of the experiment. Physiological parameters, including heart rate, end-tidal CO₂, O₂ saturation, respiratory rate, and body temperature, were recorded at baseline, immediately post-injury, and at 5, 10, and 15 minutes postinjury.

After routine skin sterilization and application of a local anesthetic (1% lidocaine), a T-shaped skin incision was made centered over the sagittal and right coronal suture. A craniectomy was performed beginning with a 1.1-cm burr hole at the posterior junction of the right coronal and sagittal sutures. This was enlarged to 2 to 3 cm in diameter by using bone rongeurs in the posterior and lateral direction, proportional to the size of the parietal bone; care was taken to avoid trauma to the cortical surface. The dura was incised in a cruciate fashion. The sterilized cortical indentation device was secured to the bone opening, and the appropriately sized indenter tip for each age group was threaded securely into the indenter collar. Scaled cortical displacement was performed to the appropriate indentation depth by release of the spring-loaded indenter tip, as described earlier.

After injury, the lesion site was gently irrigated with sterile saline to ensure hemostasis. The dura was reapproximated over the lesion and the skin closed in the usual fashion. The animal was allowed to recover from general anesthesia and was monitored until it could ambulate. The piglets were observed for a period of 7 days postinjury and any abnormal neurological function was noted.

After 7 days, the animals were medicated with sodium pentobar-

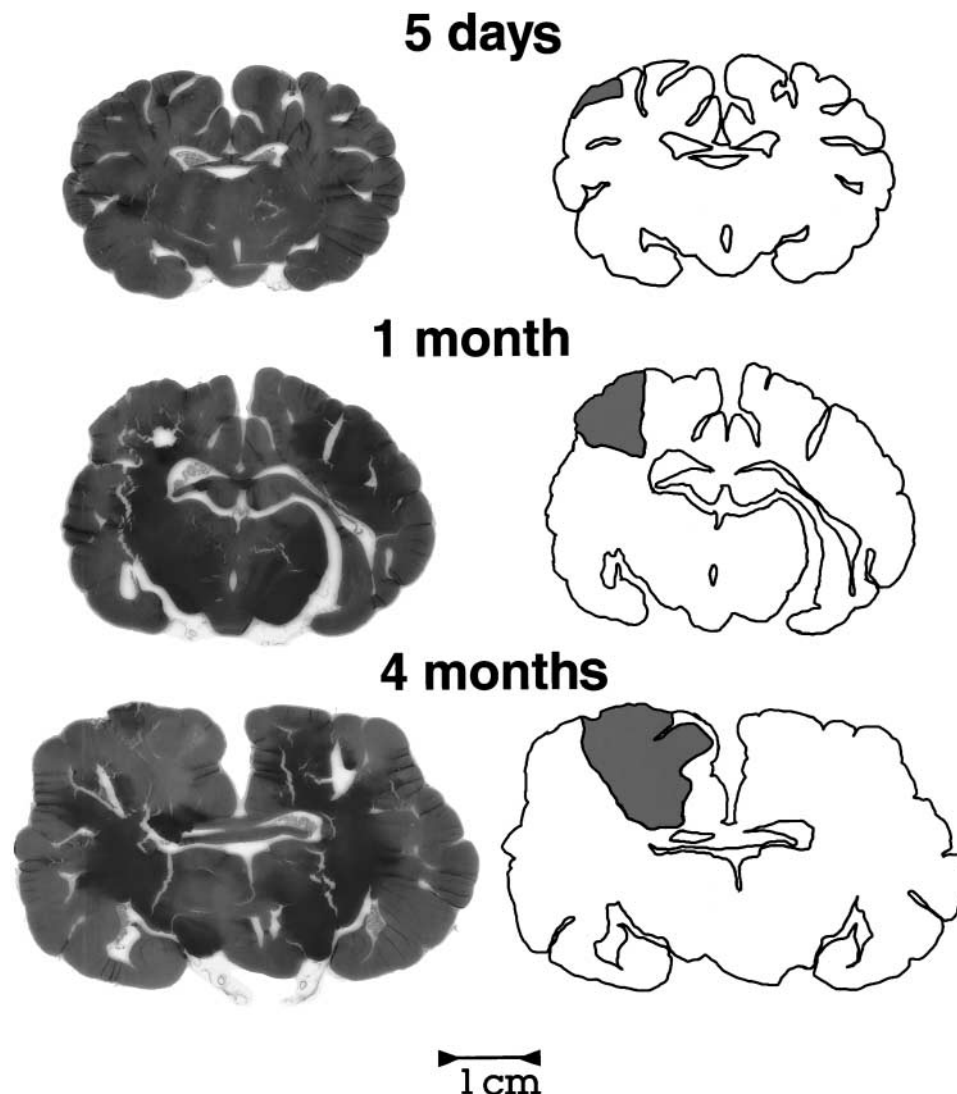


FIG. 2. Representative piglet brain slices from the three age groups stained with hematoxylin and eosin. Corresponding areas of cell loss and necrosis seen by light microscopy have been outlined.

bital (approximately 50 mg/kg) to achieve anesthesia. A right thoracotomy was performed and intracardiac perfusion was initiated with 0.9% normal saline solution followed by 10% buffered formalin. The brain was removed, weighed, and further fixed in 10% buffered formalin for a period of 3 to 5 days. A 1.5-cm coronal section centered over the lesion site was blocked, processed, and embedded in paraffin for histological analysis. Sections of 10- μ m thickness were taken every 0.25 mm and mounted on poly-L-lysine-coated slides and stained with hematoxylin and eosin. To compare relative lesion size, five serial brain slices were analyzed per animal, corresponding to the 0%, 25%, 50%, 75%, and 100% regions of tissue under the indenter tip (Fig. 1). Adjacent sections were also stained with antibody to glial fibrillary acidic protein to look for evidence of glial reaction.

Brain slices were reviewed by a single neuropathologist (J.A.G.) without knowledge of the age of the animal or the experimental conditions. Areas of brain injury, defined by the presence of necrosis with or without hemorrhage, neuronal dropout or injury, and/or reactive gliosis, were then identified by light microscopy and outlined on the slides (Figs. 2 and 3). To compare relative lesion dimensions among the age groups, the area of the lesion and the area of the cor-

responding contralateral hemisphere were computed using an image analysis system (MCID; Imaging Research, Inc., St. Catharines, ON, Canada).

The following data were obtained for each animal. 1) The average lesion area over the five serial sections (mm^2) was computed to allow for a comparison of lesion size between piglets of the same age. 2) The average ratio of lesion area to the area of the corresponding contralateral hemisphere was calculated to allow for a comparison of lesion sizes in piglets of different ages by providing an estimate of the percentage of hemisphere injured.

One-way ANOVA was used to identify differences in percentage of hemisphere injured as a function of age; post hoc comparisons between groups were made using the Neuman-Keuls t-test. Pearson correlation coefficients were used to determine the relationship between body weight and brain weight and the percentage of injured hemisphere. Repeated-measures ANOVA was used to determine the effect of age and time from injury on physiological parameters over the course of the experiment. One-way ANOVA was used to assess differences in physiological variables between age groups at each time point. All results were considered statistically significant if the probability value was less than 0.05.

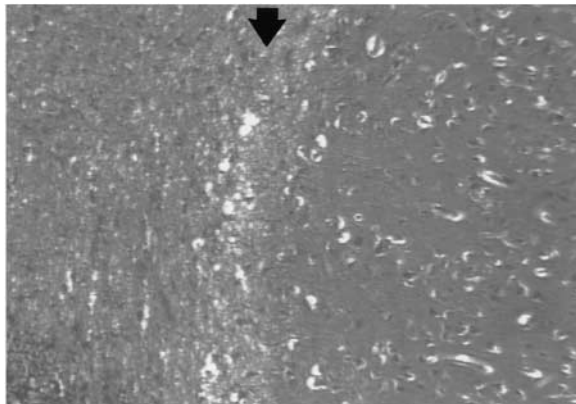


FIG. 3. High-power photomicrograph showing the boundary between the area of lesion (left side of figure) and normal brain (right side of figure). The boundary is marked by an arrow. H & E, original magnification $\times 100$.

Results

Clinical Findings

All piglets made a rapid and uneventful clinical recovery, returning to baseline feeding and ambulation levels during the early postoperative period. No motor deficits or changes in activity level were noted.

Physiological Findings

All physiological variables remained relatively stable compared with baseline; there was no evidence of an age-dependent physiological response to injury over time. Significant differences were found in baseline heart rate between the oldest animals and the two younger groups, with the 4-month-old animals having lower heart rates, and this difference persisted throughout the experiment ($p < 0.02$). At baseline, the rectal temperatures of the youngest animals were on average approximately 1°C cooler than those of both older groups, which was a significant difference ($p < 0.05$), but the two older groups did not differ from each other. At the time of injury and thereafter, there were no significant differences in temperature between any of the three age groups.

Histological Findings

The lesions were readily visualized on hematoxylin and eosin-stained sections as discrete areas of cell loss or necrosis, with or without hemorrhage, possessing relatively sharp boundaries (Fig. 3). Adjacent tissue was generally well preserved and exhibited essentially normal architecture. Glial fibrillary acidic protein-stained sections showed reactivity to be confined to regions immediately adjacent to the lesion, without widespread reactive gliosis.

Quantification of Variables

The values are given as the means \pm the standard deviations.

Five-Day-Old Group. Four animals were studied, three males and one female. Body weight ranged from 1.8 to 2.4 kg (mean 2 ± 0.3 kg). Brain weight ranged from 43.3 to 51.8 g (mean 47.6 ± 3.5 g). The average lesion area was

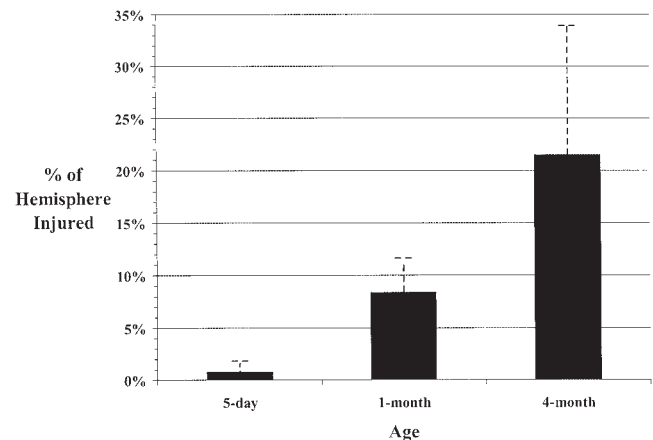


FIG. 4. Bar graph showing the percentage of hemisphere injured in the sampled region as a function of age (mean and standard deviation) in the three age groups. There is a significant increase in lesion size with increase age of the piglets.

$2.8 \pm 2.1 \text{ mm}^2$ (range $0.4\text{--}6.4 \text{ mm}^2$). The percentage of hemisphere injured was $0.8 \pm 1\%$ (range $0.1\text{--}1.9\%$) (Fig. 4).

One-Month-Old Group. Four animals were studied, one male and three females. Body weight ranged from 4.7 to 10.4 kg (mean 7.7 ± 3.1 kg). Brain weight ranged from 47 to 64.56 g (mean 56.1 ± 7.3 g). The average lesion area was $34.9 \pm 16.8 \text{ mm}^2$ (range $22.8\text{--}59.1 \text{ mm}^2$). The percentage of hemisphere injured was $8.4 \pm 3\%$ (range $6\text{--}12.6\%$).

Four-Month-Old Group. Four animals were studied, two males and two females. Body weight ranged from 34.4 to 55.2 kg (mean 46.7 ± 10.2 kg). Brain weight ranged from 87.3 to 107.1 g (mean 99 ± 9.5 g). The average lesion area was $965.2 \pm 53.8 \text{ mm}^2$ (range $26.8\text{--}125.6 \text{ mm}^2$). The percentage of hemisphere injured was $21.5 \pm 12\%$ (range $5.8\text{--}35.5\%$).

Scaled Indentation Volumes

Because the brain dimensions used to calculate an approximately 1% scaled indentation volume were derived from normal control animals prior to these experiments, we wished to confirm that scaling was comparable across ages in the specific piglets used in this series. Therefore, the percentage of total brain volume displaced was calculated by comparing the indentation volumes used in these experiments to the mean brain volumes for each piglet after it was killed. Actual percentages were 0.89%, 0.98%, and 0.92% for 5-day-, 1-month-, and 4-month-old piglets, respectively, thus confirming comparable displacements among age groups. Pressure, force, and energy expended when using this model were also calculated using the spring constant $K = 5 \times 10^4 \text{ N/m}$ and were found to be comparable between groups, with the exception that less force was expended per cubic millimeter of brain in the oldest animals (Table 1).

Results of Statistical Analysis

There was a significant difference in the ratio of lesion/

Maturation-dependent response of piglet brain to cortical impact

TABLE 1

Characteristics of scaled cortical impact model in the piglet*

Age Group	Dtip (m)	DI (m)	Atip (m ² × 10 ⁻⁵)	% V Brain Indented	Pressure (N/m ² × 10 ⁶)	Force (N/m ³ × 10 ⁶)	Energy (J/m ³ × 10 ⁴)
5 day	0.0104	0.0048	8.49	0.89	1.41	2.65	1.27
1 mo	0.0107	0.0059	8.99	0.98	1.64	2.76	1.63
4 mo	0.0127	0.0070	12.61	0.92	1.39	1.86	1.30

* Atip = area of indenter tip; DI = depth of indentation; Dtip = diameter of indenter tip; V = volume.

contralateral hemisphere between age groups, with percentage of hemisphere injured increasing with age (one-way ANOVA, $p = 0.0018$, $F = 13.79$; Fig. 4). Post hoc comparisons between groups made using the Neuman-Keuls t-test showed the following differences in percentage of hemisphere injured between age groups: 5-day compared with 1-month, $p = 0.026$; 5-day compared with 4-month, $p = 0.0015$; 1-month compared with 4-month, $p = 0.029$.

Body weight and the percentage of hemisphere injured were highly correlated ($r = 0.8853$, $p = 0.0001$; Fig. 5). Brain weight and the percentage of hemisphere injured were also highly correlated ($r = 0.7894$, $p = 0.0023$).

Although body weight in the oldest animals was greater in males compared with females, brain weights were similar between sexes at all ages studied.

To explore further the relationship between body temperature and lesion size, a Pearson's correlation coefficient was calculated for these two variables; this relationship did not reach statistical significance ($r = 2.08$).

Discussion

In this model of focal brain injury, precise scaling of injury input to changes in physical dimensions of the gyrencephalic brain reveals age to be significant in the histological response to injury, with the degree of damage increasing progressively with maturation. These results are somewhat surprising in light of the known tendency for poor outcome after head injury in the youngest age groups.^{29,31} As in other aspects of brain injury research, characterization of injury response during immaturity depends on the particular species, developmental stage, injury type, and outcome measure studied. In the present study, scaling was performed by keeping volume and specific dimensions of tissue displacement proportionate to changes in the brain at each age. This method of creating contusion injury enables strain distribution to be held constant, which has been shown to be an extremely reliable determinant of exact injury extent.^{34,38,45} Force correlates less precisely with injury extent, because a given force can be produced by varying both mass and area; such differences can significantly affect the pattern of resulting injury. Direct displacement of the cortical surface was chosen rather than fluid-percussion injury to minimize differences experienced by the brain parenchyma due to developmental changes in the subarachnoid space and sutures.⁴¹ Thus, this model was developed so that differences seen among ages would likely reflect differences in the brain's specif-

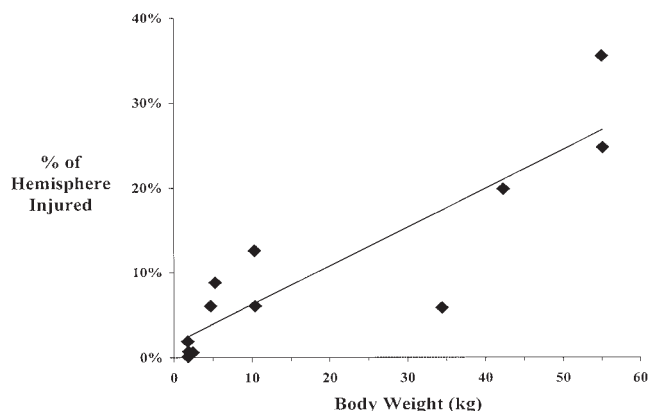


FIG. 5. Scatterplot showing the correlation between body weight and percentage of hemisphere injured. This correlation is significant ($r = 0.8853$, $p = 0.0001$).

ic response to focal mechanical injury as a function of maturation, rather than reflecting different injury inputs.

In previous investigations in which response to mechanical trauma has been compared at different stages of maturation, a variety of injury models, subject ages, and outcome measures have been used. Armstead and Kurth⁴ compared the cerebrovascular response of infant and juvenile piglets subjected to the same severity of fluid-percussion injury and noted greater physiological instability in the younger animals. Grundl, et al.,²¹ compared cerebral edema and lesion size in immature rats (26–32 days of age) with mature rats (60–84 days of age) after the rats had been subjected to a weight-drop injury in which force was scaled to brain weight by varying the drop height. Edema was more prolonged in the immature animals, but the percentage of the hemisphere injured did not differ at 24 hours after trauma. Biagas, et al.,⁹ used a similarly scaled weight-drop model to compare posttraumatic hyperemia in immature, mature, and aged rats and found that hyperemia was more extensive in the younger animals. Bittigau and colleagues¹⁰ studied the degree of apoptotic damage seen 24 hours after weight-drop injury in rodents of various ages in which pressure was scaled to brain weight. They found that apoptotic cell death was most pronounced in 7-day-old rat pups, being more apparent at this age than in either younger or older animals. Prins and Hovda⁴¹ measured Morris water maze acquisition after administering the same severity of fluid-percussion injury to rats of different ages and found that immaturity was associated with preserved function. Investigators in other studies of head injury in immature animals have used rodents of a single age.^{1,24}

The rodent brain is lissencephalic, has little white matter, and follows a very different maturational sequence from that of humans. The brain growth spurt of rodents occurs entirely in the postnatal period, whereas pigs and humans peak in brain growth at the time of birth.¹⁶ Because of its similarity in overall shape, gyral pattern, distribution of gray and white matter, and growth pattern to human infants, the piglet brain has been used extensively to model human development.^{6,16,17,19,49} The response of the piglet to hypoxia and ischemia appears to parallel that in human infants; cerebral blood flow and metabolism are

similar, and the piglet brain matures in a similar manner with respect to myelination and electrical activity.^{11,16,40,50} The size of the pig brain is ideally suited for clinically relevant biomechanical modeling of human head trauma, including both focal and inertial injuries involving gray and white matter.^{26,37,46} Contusion injury, diffuse axonal injury, acute subdural hematoma, and epidural hematoma have been characterized in pigs or piglets.^{7,32,33,44,46}

The piglet ages selected for this study were based on parallels between pig and human development. These three time points were chosen to help answer the question of when maturational differences occur that might be relevant to injury response; it is not clear from clinical experience whether only infants show different injury patterns or whether vulnerability changes in a continuum or in a step-wise manner. There may be peaks and valleys of vulnerability, similar to those shown for neuronal plasticity, related to cycles of cell growth, apoptosis, synaptic connectivity, and myelination.^{10,12,27,28} For this reason, the three age groups were chosen to correspond to specific human developmental stages. During the first few weeks of life in piglets (infant stage), myelination and electroencephalographic patterns remain similar to those of the human newborn.⁴⁰ Neonatal patterns of cerebral metabolism are present at 3 and 5 days of life,¹⁴ and cerebral circulation and blood flow characteristics are established during this time.^{11,50} Visual evoked responses remain immature until 2 weeks after birth.³⁵ Investigators of cerebral insult in the piglet have described the 1st weeks of life as corresponding to the infant developmental stage.^{5,20,22,36}

At 1 month of age the piglet brain has characteristics of early but incomplete maturation. The brain weight has increased by approximately 1.3 times, and doubles by 4 months of age. This increase at 1 month of age is proportionally similar to that which occurs during the infant and toddler stage in humans (approximately ages 1–3 or 4 years) compared with adult brain weight, whereas after this peak the rate of increase in brain mass declines.^{13,16,49} At 1 month of age the animals are not yet weaned, which begins to occur during the 2nd month of life.⁴⁹ Myelination is significantly increased compared with that of the infant, but is not yet complete, also similar to humans in the toddler stage.^{19,40,43} The electroencephalographic pattern is similarly close to the mature pattern, like that of young humans at approximately 3 years of age.^{3,40} Thus although exact comparisons in all modalities are not possible, both changes in brain mass and changes in neurological function and functional independence in piglets at 1 month of age appear to parallel that in humans during the toddler stage, which is the early postinfancy prepubertal developmental stage.

The term adolescent has been used by many investigators when studying porcine models to apply to a variety of ages beyond infancy and weaning but before full growth.^{22,23,48} With respect to neurological function, the brain is nearly but not quite full size (approximately 90% of adult mass) in both 4-month-old piglets and human adolescents.^{16,25} Myelination has progressed through the rapid growth phase, but continues at a slowed rate.¹⁹ Skeletal and sexual maturation characteristics in the piglet at this age similarly best correspond to early adolescence in humans.^{8,15,39,42}

Several possible explanations for the age-related differ-

ences seen in this study can be considered. Changes in gene expression might confer relative resistance to injury at earlier ages by influencing such processes as apoptosis and injury repair. Changes in tissue mechanical properties with age also might influence pathological response. Additionally, differences in systemic or cerebrovascular physiological variables might play a role in age-dependent vulnerability. In this study, no differences among ages were seen with respect to changes in physiological variables after injury, although, as expected, some differences in baseline values (such as heart rate) were found. Temperature, in particular, may affect lesion size. In this study, although the youngest animals were on average 1°C cooler than the older groups at baseline, likely reflecting more rapid cooling under anesthesia similar to that seen in human infants, this difference did not persist at the time of injury or thereafter. No differences in temperature were found between the two older groups at any time point, despite differences in lesion size; no significant correlation between temperature and lesion size was found across ages. Thus, it appears unlikely that temperature differences alone can explain the differences in lesion size by age seen in this study. More detailed studies in this model of brain temperature, cerebral blood flow, cortical excitability, and other physiological variables are currently underway.

Finally, although immaturity appears to confer relative resistance to damage in this focal injury model, this protection cannot be generalized to different injury conditions. The immature brain might vary in its response to mechanical trauma of greater severity, to the additional physiological stress conferred by hypoxia or ischemia, or to different types of injury such as subdural hematoma or diffuse inertial injury. The piglet model is advantageous for the study of these varied conditions. The present study demonstrates that for moderate focal mechanical trauma, the immature brain is particularly well suited to resisting injury, which, in this setting, contradicts the notion of infant fragility. This would suggest that the poor outcome seen in clinical series of head-injured infants does not reflect intrinsic increased vulnerability to specific focal deformational mechanical trauma in this age group, but likely represents different magnitudes of injury, different injury types (such as inertial injury and/or subdural hematoma seen in nonaccidental injury), or other factors such as superimposed hypoxia/ischemia.

Conclusions

Using this precisely scaled model of focal contusion injury in piglets, progressively larger lesions were seen as the piglets' age increased from infancy to adolescent stages. Understanding the specific cellular and molecular events subserving cell death and repair processes that vary with age will require further study. Because of its developmental and morphological similarity to the human brain, the piglet brain provides distinct advantages to modeling various types of mechanical trauma and may provide insight into age-specific changes in injury response.

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