The Pattern of Cerebral Injury in a Primate Model of Preterm Birth and Neonatal Intensive Care

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Abstract. Survivors of very premature birth face an increased risk of adverse motor, cognitive, and behavior sequelae. In order to understand the pathogenesis of these adverse outcomes, an animal model of premature birth and neonatal care in a species with a close similarity to the human infant is sought. In this histological and immunohistochemical study we have defined the pattern of cerebral injury in a premature baboon model undergoing similar neonatal intensive care to that of the human premature infant. Sixteen baboons were delivered at 125 days gestation (dg; term –184 dg) with 14 days neonatal intensive care and were compared with gestational control brains at 125, 140, and 160 dg. The premature baboons undergoing neonatal intensive care sustained a spectrum of neuropathologies including white matter injury, hemorrhage, and ventriculomegaly, which resemble lesions frequently observed in the human premature infant. These data suggest that the premature baboon is a model with similarities in maturation and pattern of cerebral injury to the human infant that may provide useful insights of relevance to the human preterm infant.

Key Words: Cerebral hemorrhage; Gyral development; Myelination; Periventricular leukomalacia; Ventriculomegaly.

INTRODUCTION

Advances in perinatal care have led to a significant improvement in the survival of very premature (<30 weeks gestational age) infants. However, up to 10% of these infants will later develop cerebral palsy (1) and another 25% to 50% will suffer developmental or behavioral disabilities with considerable educational, economic, and social implications (2–6). The most common cerebral neuropathology observed in premature infants, periventricular leukomalacia (PVL) (6), comprises focal cystic infarction in the periventricular region and diffuse injury characterized by reactive gliosis in the surrounding cerebral white matter (6, 7). Recent studies of premature infants with magnetic resonance imaging (MRI) have demonstrated that the diffuse component of white matter injury is very common (8, 9) with focal necrosis occurring rarely in only about 4% of infants (10). In addition to white matter injury, there is an increasing recognition that premature infants exhibit injury to cerebral grey matter, including the cerebral cortex (11), hippocampus (12), and cerebellum (13).

There are many potential contributors to the disabilities observed following preterm birth, including severity of the illness, malnutrition, and the nature of therapeutic regimens. Despite promising advances in the field of neuroprotection, the development of specific neuroprotective strategies for very premature infants remains limited by the current lack of an understanding of the cerebral pathologies and their causative pathways. Important insights into the nature of cerebral injury in the premature infant have been made with human autopsy studies (7, 14, 15). However, with the markedly improved survival of the premature infant over the last decade, autopsy material has become increasingly difficult to obtain and is limited by reflecting the neuropathologies associated only with the most severely ill infants. Thus, numerous animal models have been developed in an effort to replicate the major neuropathologies found in the human premature infant. These models have required an insult to be administered to the developing fetus or infant such as exposure to bacterial endotoxin (lipopolysaccharide, LPS) in sheep (16, 17), kittens (18), and E. coli in rabbits (19); by hypoxic protocols in the rat (20), sheep (17, 21, 22), and monkey (23); or by administration of excitatory amino acid receptor agonists in mouse (24) and rat (25, 26). In general within these models, the period prior to generalized myelogenesis has represented the developmental stage of increased white matter vulnerability (27).

In contrast to these previous animal models, this study focuses on the first non-human primate study of cerebral injury associated with premature birth. There is an enhanced likelihood that this primate model will more closely resemble the sequence of human cerebral development, although this has not been studied to date. These prematurely born primates differ from the human preterm infant in that delivery is elective in nature without infection or prenatal compromise, such as growth restriction.

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However, the model is also uniquely similar to that of the human infant in that the mothers received antenatal steroids and the infants are cared for in a neonatal intensive care unit with very similar interventions to that received by the human premature infant. Of particular note in comparison to other animal models, there were no specific experimental interventions aimed at producing a cerebral insult administered either before or after delivery. Our first aim was to establish a background for studies of the effects of premature delivery on brain structure by defining the normal ontogeny of gyral formation, cortical, and white matter development, including myelination, and of astrocytes, using appropriate histological techniques and immunohistochemistry. Animals were delivered at 125, 140, and 160 days gestation (dg) and immediately killed. This data was used to establish the comparative neurobiology to the human infant and allowed us to define the equivalent human gestational age matching each of the time points. Our second aim was to determine the nature and severity of the cerebral injury resulting from premature delivery. The respiratory and cardiovascular development of premature baboons (28, 29) suggests that 125 dg corresponds to 26 to 28 weeks in humans. Thus, the experimental cohort of baboons was delivered at 125 dg, nursed for 2 weeks in an intensive care facility, and killed at 140 dg. The neuropathological examination included assessment of any white matter damage, neuronal necrosis, presence of reactive astrogliosis or activated microglia, evidence of ventriculomegaly, and the presence of subarachnoid, germinal matrix, or intraventricular hemorrhage. This damage was compared to the profile of injury observed in the human premature infant. We hypothesized that prenatal brain growth and the pattern of cerebral injury will correlate with that seen in the preterm human infant and will validate the premature-born baboon as a highly appropriate model for the study of preterm human birth.

MATERIALS AND METHODS

Animal Model and Tissue Collection

Brain tissue was obtained from the Southwest Foundation for Biomedical Research, San Antonio, Texas. All animal husbandry, animal handling and procedures were reviewed and approved to conform to American Association for Accreditation of Laboratory Animal Care (AAALAC) guidelines.

Gestational Controls: For the ontogeny study, baboons (Papio sp) were delivered by hysterotomy under general anesthesia at 125 (n = 3), 140 (n = 5), and 160 dg (n = 2), killed immediately with sodium pentobarbitone (130 mg/kg i.v.), and the brains removed and prepared for histology as described below. These animals will be referred to as gestational controls (GC).

Preterm Models: Infants are delivered by elective hysterotomy under general anesthesia at 125 ± 2 dg. The experimental dams received antenatal steroid therapy (6 mg of betamethasone or dexamethasone at 24 and 48 hours prior to delivery). The infants were weighed, anesthetized with intramuscular ketamine (5 to 10 mg/kg) and intravenous valium (0.1 to 0.5 mg/kg), and intubated with a 2.0- to 2.5-mm-diameter endotracheal tube. All 125 dg animals received surfactant replacement therapy (Survanta, Abbot Laboratories, Abbott Park, IL; or Curosurf, Trinity Pharmaceuticals Ltd., Huddersfield, UK) immediately following the collection of lung fluid. An arterial line was placed via the umbilical cord into the descending aorta for blood pressure monitoring and blood gas sampling. A deep venous line (Per-Q-Cath, Bard Access Systems, Salt Lake City, UT) was placed percutaneously via the saphenous vein into the inferior vena cava for administration of fluids and drugs. All premature animals were maintained in servo-controlled, infrared-warmed body plethysmographs capable of continuous or intermittent pulmonary function monitoring (VitalTrends Body Plethysmograph, New York, NY) and ventilated on a standard, time-cycled, pressure-regulated infant ventilator (Infant Star, Soma Technology Inc., Cheshire, CT) with humidifier maintained at 36° to 37°C. Initial settings on the ventilator were FiO2 0.40, rate 40/min, inspiratory time 0.50 seconds, positive end expiratory pressure (PEEP) 4 to 5 cm H2O, and peak inspiratory pressure (PIP) as required for adequate chest excursion. FiO2 was regulated to keep PaO2 at 50 to 70 torr. PIP and PEEP were regulated to maintain measured tidal volumes between 4 and 6 ml/kg, and rate adjusted to maintain PaCO2 at 45 to 55 Torr. High frequency “rescue” was briefly occasionally necessary to support gas exchange. The need for high frequency ventilation was defined as a mean airway pressure requirement of ≥16 cm H2O to maintain PaO2 ≥60, or PIP ≥40 cm H2O to maintain tidal volume ≥4 ml/kg and/or PaCO2 < 60. For cardiorespiratory protocols, surgical ligation of the ductus arteriosis was undertaken routinely on day 5 of life. Intravenous administration of amino acids was initiated at 24 hours of age (1.5 gm/kg/d) and advanced at 48 hours of age (3.0 gm/kg/d). Hypotension was managed as previously described, including volume replacement, dobutamine, dopamine and epinephrine infusions as needed (29). Arterial blood pressure, heart rate, oxygen saturation, and EKG were monitored continuously. Blood gases, hematocrit, electrolytes, and blood counts were monitored at regular intervals. At 139 dg, prematurely delivered (PD) neonates were killed with sodium pentobarbital.

For all animals, brains were removed at postmortem, placed immediately into 10% buffered-formalin and shipped to the University of Melbourne. Brains were weighed, the cerebellum and brainstem detached from the cerebral hemispheres, and then the right or left hemisphere was randomly selected and blocked for paraffin processing as follows. Using the midline margin of the central sulcus on the dorsal surface of the cerebrum as a reference point, 5-mm coronal slices of the entire forebrain were taken relative to this point. Depending on the size of the brain, this ranged from 9 to 12 blocks (Fig. 1B). Blocks were then embedded in paraffin and 10 serial sections (8 μm) were cut from the rostral face of each slice. From each cerebellum, a 5-mm parasagittal block of vermis was collected, embedded in paraffin and cut as described above. For GC brains, serial frozen sections were cut (100 μm) from the remaining cerebrum at the level of the central sulcus.

Hematoxylin and Eosin-Stained Paraffin Sections

Sections from each block were stained with hematoxylin and eosin (H&E) in both GC and PD brains to provide an overview.
of the general organization of the grey and white matter. In the GC brains, the surface folding index (SFI) (21), which gives an estimate of the expansion of the surface area relative to volume, was calculated for the cerebral hemispheres. In each GC brain, 3 sections were taken at each of 3 matched levels (Fig. 1B); rostral (slice 2); mid (slice 6); caudal (slice 10). Using an image analysis system (Image Pro; version 4.1, Media Cybernetics, San Diego, CA), the area (A) of the hemisphere (excluding the basal ganglia and diencephalon) and the length (L) of the pial boundary of the cerebral cortex were measured and used to calculate the SFI (SFI = L²/A). To estimate cortical growth, sections were taken from slices 2 to 8 and the depth of the cortex measured at 6 sites on the cingulate and parasagittal cortices. The area of the combined basal ganglia and thalamus was also measured on sections from slices 5 to 8.

Black-Gold Staining

Myelinated fiber tracts in GC animals were detected using the Black-Gold technique (30). Frozen sections were washed in distilled water and then immersed in 0.2% Black-Gold (a gift from Dr. Larry Schmued, National Center for Toxicological Research, Jefferson, AR) in 0.9% NaCl solution for 20 to 30 min at 60°C. Myelin staining was enhanced by treating sections with 0.2% potassium tetrachloroaurate (Sigma Chemical Company, St. Louis, MO) in 0.9% NaCl for 5 min at 60°C. Sections were washed in distilled water, and the stain was fixed by washing the sections in 0.5% sodium thiosulfate solution for 3 min. Sections were then mounted, dehydrated, and coverslipped.

Immunohistochemistry

Gliafibrillary acidic protein (GFAP) immunohistochemistry was used to examine the ontogeny of astrocytes and radial glia and the effects of premature delivery on the distribution and morphology of these cells. Calretinin immunohistochemistry was used to examine the ontogeny of Cajal-Retzius cells. De-waxed sections were washed in 0.1 M phosphate buffer (PB) and treated with 0.3% H₂O₂ in methanol for 20 min to block endogenous peroxidase activity. Sections were then treated with 4% bovine serum albumin (BSA; Sigma Chemical Company) in 0.1 M PB for 30 min before incubation at 4°C overnight with polyclonal anti-GFAP antisera (1:500; DakoCytomation, Glostrup, Denmark) or for 3 days with polyclonal anti-calretinin antisera (1:2,000; Swant, Bellinzona, Switzerland). GFAP-immunoreactive (IR) sections were incubated with secondary antibodies for 1 hour at room temperature (biotinylated goat antirabbit antibody, 1:200, Vector Laboratories, Burlingame, CA) and reacted with an avidin-biotin-peroxidase solution (1:100, Vectastain ABC Elite Kit, Vector Laboratories). For Calretinin-IR sections, sections were reacted with the streptavidin-biotin-peroxidase method (Amersham, Piscataway, NJ). For both antibodies, the reaction was visualized with diaminobenzidine tetrahydrochloride (DAB) solution (Sigma Chemical Company) containing 0.01% H₂O₂ in PB. Sections were counterstained with hematoxylin to visualize nuclei. When the primary antibody was replaced with PB, staining failed to occur. The cohorts of GC or PD brains were stained simultaneously to minimize procedural differences.

Lectin Histochemistry

Lectin histochemistry was used to label brain macrophages, including reactive microglia, in PD brains. Sections were processed as described for the GFAP immunohistochemistry except that they were incubated in biotinylated-Lycopersicon esculentum (tomato) lectin (1:100, Sigma Chemical Company) for 2 nights in place of the primary antibody and the secondary antibody was omitted.

Analysis of Gestational Control and Prematurely Delivered Brains

In gestational control brains at each age, the distribution of myelin as evidenced by Black-Gold staining was assessed qualitatively. The density of GFAP-IR cells in the white matter was quantified using an image analysis system (ImagePro Plus v4.1, Media Cybernetics). Two regions were examined: adjacent to the ventricle and in the subcortical region. Six to 7 sections (extending through the parietal and temporal and occipital lobes) were analyzed per animal, with 2 fields from these regions being randomly selected for sampling. The density of GFAP-IR cells within each region was pooled for each animal, and a mean value for each gestational age calculated. GFAP-IR sections were also examined to determine the distribution of radial glial processes in the grey and white matter. Calretinin-IR sections were examined to determine the distribution and morphology of Cajal-Retzius cells. Gestational controls at 140 dg (n = 5) and all of the PD brains were coded to avoid experimenter bias and sections were analyzed for the following: general microscopic appearance, including the presence of hemorrhages, hippocampal pyramidal cell loss, ventriculomegaly, and reactive gliosis in the grey or white matter. To assess white matter injury the proportion of white matter damage in each section was quantified using a point counting technique (21). Combining all sections for each brain, this value was then expressed as a proportion of the total white matter damage. The types and distribution of injury were plotted on line diagrams of sections from each block of tissue to determine whether there was a predilection for damage in a particular brain region.

Analysis of Physiological Measurements

The primates underwent extensive physiological monitoring with continuous measurement of cardiovascular and respiratory parameters. These measures, including PaO₂, PaCO₂, FiO₂, pH, diastolic, systolic and mean arterial blood pressure, and the calculated oxygenation index, were summarized for the 14 day post-delivery period with minimal and maximal measures every 12 hours for the first 48 hours and then daily for the remaining period (i.e. measures of the most extreme values recorded for each physiological variable were recorded from each period).

Statistical Analysis

Results are expressed as mean ± SEM unless otherwise stated. Differences in SFI and cortical thickness at 125, 140, and 160 dg were analyzed with a one-way analysis of variance (ANOVA). Primates with moderate-severe cerebral injury were compared with those with mild or no cerebral injury for each physiological measure by a t-test for normally distributed data.
### TABLE 1
Summary of Histopathology in the Brains of Prematurely Delivered Baboons

<table>
<thead>
<tr>
<th>Infant #</th>
<th>Hemorrhage</th>
<th>White matter injury</th>
<th>Grey matter injury</th>
<th>Ventriculomegaly</th>
<th>Hc cell loss</th>
<th>Gliosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+ subarachnoid</td>
<td>+ medial temporal/prefrontal/parieto-occipital regions</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>+ subarachnoid + WM bleeding (Fig. 2H)</td>
<td>+ occipital lobe</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+ subcortical astrogliosis</td>
</tr>
<tr>
<td>3</td>
<td>+ intraventricular (Fig. 2F) + germinal matrix (Fig. 2G) + subarachnoid (Fig. 2E)</td>
<td>+ parietal cortex (Fig. 2A–D)</td>
<td>+ (Fig. 2O)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>+ frontal and occipital regions</td>
<td>+</td>
<td>+ (Fig. 2E) med/sup-temporal gyri</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>+ germinal matrix</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>+ WM bleeding (Fig. 2H)</td>
<td>+ frontal WM, corpus callosum</td>
<td>+ (Fig. 2L)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>+ subarachnoid WM bleeding</td>
<td>+ frontal cortex</td>
<td>+</td>
<td>+</td>
<td>+ (Fig. 2L)</td>
<td>astrogliosis in hippocampus</td>
</tr>
<tr>
<td>9</td>
<td>+ subarachnoid</td>
<td>+ occipital cortex</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>+ subarachnoid + cerebellar (Fig. 2I)</td>
<td>+ medial temporal gyrus</td>
<td>–</td>
<td>–</td>
<td>+ (Fig. 2J)</td>
<td>astrogliosis in hippocampus</td>
</tr>
<tr>
<td>11</td>
<td>+ subarachnoid</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+ astrogliosis in hippocampus microglia in septal area</td>
</tr>
<tr>
<td>12</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>–</td>
<td>+ parietal cortex</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>+ subarachnoid + cerebellar</td>
<td>+ IC, parieto-occipital WM + reduced gyral formation (Fig. 2M)</td>
<td>+ head of caudate, putamen</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>15</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>16</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviations: cx, cortex; Hc, hippocampus; IC, internal capsule; p-f, prefrontal; WM, white matter; +, injury; –, no injury.
or Kruskall-Wallis for non-parametric analysis. A probability of p < 0.05 was considered to be significant.

RESULTS

Ontogeny of the Forebrain

Weight Increase and Gyral Formation: The fetal baboon brain undergoes a period of considerable growth from 125 to 160 dg. Between 125 and 140 dg there is a 42% increase in whole brain weight (125 dg: 38.8 ± 1.3 g; 140 dg: 55.3 ± 2.4 g), followed by a further 16% increase in weight between 140 and 160 days (160 dg: 64.1 ± 3.5 g). At 125 dg, the main sulci are present, including the lateral, central, lunatus, calcareine, superior, temporal, and frontal (Fig. 1A); the inferior temporal and inferior frontal are present but rudimentary. Between 125 and 140 dg (Fig. 1B) these primary indentations deepen, gyri become more prominent, and secondary gyri are seen in the orbital, frontal, insular, and occipital regions. Gyral formation further increases by 160 dg with tertiary gyri now evident (Fig. 1C). There was no obvious asymmetry in gyral development.

Ontogeny of Cortical Development: Examination of H&E-stained sections from brains at 125 dg (Fig. 1D) revealed that all 6 cortical layers and the subplate were present. Cells in layer 2 exhibited an elongated morphology, suggesting that they had only recently completed their migration. Large polymorphic calretinin-immunoreactive Cajal-Retzius cells were located at the pial surface in layer 1 with their processes extending in a dense meshwork throughout the deeper regions of the layer. Fewer cells and a less dense fiber layer were present at 140 and 160 dg. Cortical thickness did not increase significantly (p = 0.43) between 125 dg (1.49 ± 0.03 mm) and 140 dg (1.54 ± 0.03 mm; Fig. 1E), but did increase by 20% (p < 0.001) between 140 and 160 dg (1.84 ± 0.04 mm; Fig. 1F). Analysis of the SFI showed that there was a dramatic increase of 73% (p < 0.001) in gyral formation between 125 dg (28.4 ± 2.0) and 140 dg (49.2 ± 3.8), followed by a slower increase of gyral formation to 30% (p < 0.05) from 140 to 160 dg (63.6 ± 5.0).

Ontogeny of Myelination: Myelination in the baboon forebrain was examined at the level of the central sulcus and 5 mm rostral and caudal to this point. Black-Gold staining revealed a moderate degree of myelination in the thalamus and internal capsule at 125 dg (Fig. 1G, J). At 140 dg, myelination had increased in the thalamus and there was some staining in the alveus of the hippocampus, posterior limb of the internal capsule, and the white matter. At 160 dg, myelination was extensive and apparent throughout the brain. Myelination was also apparent in the lateral ventricles (arrow) and choroid plexus at 125 dg (Fig. 1H). At 140 dg, myelination had increased in the thalamus and internal capsule at 125 dg (Fig. 1G, J). At 140 dg, myelination had increased in the thalamus and there was some staining in the alveus of the hippocampus, posterior limb of the internal capsule, and the white matter.

![Fig. 2.](http://jnen.oxfordjournals.org/) Various forms of injury in the brains of prematurely delivered baboons (animals exhibiting these major pathologies are indicated within Table 1). A, B, E, F–J, M–O: H&E-stained sections. A: Coagulative necrosis in the periventricular and subcortical white matter (dotted outline). B: High-power view of coagulative necrosis from (A, arrows). C: GFAP-IR illustrating reactive astrocytes in the white matter (arrowheads). D: Tomato lectin immunohistochemistry illustrating activated macrophages (arrowheads) in white matter adjacent to a cortical infarct. E: Subarachnoid hemorrhage in the occipital lobe (arrows) and cortical infarct (dotted outline). F: Intraventricular hemorrhage (not associated with the choroid plexus) in posterior horn of lateral ventricle (arrow). Boxed area shown at high magnification in (G). Germinal matrix hemorrhage (arrows) at tip of inferior horn. H: Small hemorrhage in the subcortical white matter (I). Hemorrhage in the cerebellar cortex (arrows). J: Hippocampus; box outlines area shown in the underlying photomicrograph, showing cell loss in the stratum pyramidale (SP). Arrows delineate border of SP. K, L: Dark-field images of GFAP showing gliosis in the hippocampus of a PD neonate (L) compared to a gestational control (K). M: Retarded gyral formation. N: Gestational control brain section through the level of the posterior-inferior horns of the lateral ventricles (arrow). O: Ventriculomegaly in posterior-inferior horns of a PD neonate (arrow). Abbreviations: DG, dentate gyrus; GFAP, glial fibrillary acidic protein; PF, primary fissure; SP, stratum pyramidale; SR, stratum radiatum. Scale bars: A, E, F, J, M, N, O = 5 mm; H = 50 μm; B, = 140 μm; C, D = 25 μm; G = 100 μm; I = 1 mm; K, L = 200 μm.

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Premature baboons with nil-mild cerebral injury (n = 10)</th>
<th>Premature baboons with moderate-severe cerebral injury (n = 6)</th>
<th>p value (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FiO₂ (%)</td>
<td>66 ± 21</td>
<td>62 ± 12</td>
<td>0.9</td>
</tr>
<tr>
<td>Oxygenation index</td>
<td>8.4 ± 1.4</td>
<td>8.8 ± 1.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Minimum mean arterial blood pressure (mmHg)</td>
<td>25.2 ± 3.8</td>
<td>22.8 ± 4.0</td>
<td>0.4</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>29.8 ± 3.4</td>
<td>25.2 ± 3.2</td>
<td>0.057</td>
</tr>
<tr>
<td>pH</td>
<td>7.2 ± 0.1</td>
<td>7.0 ± 0.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* Data represent the mean of the most extreme values measured over the post-delivery period. Abbreviations: FiO₂, fraction of inspired oxygen; PaCO₂, partial pressure of arterial carbon dioxide.
TABLE 3
Comparative Ages for Development of Key Parameters in the Human Infant and Baboon

<table>
<thead>
<tr>
<th>Anatomical region of interest</th>
<th>Human infant</th>
<th>Baboon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development of primary sulci</td>
<td>Primary sulci and gyri established by 26 to 28 weeks (35)</td>
<td>Primary sulci and gyri all present at 125 dg, although inferior temporal and inferior frontal rudimentary</td>
</tr>
<tr>
<td>Prominent Growth in cortical grey matter</td>
<td>Brain growth spurt about 26 weeks (35)</td>
<td>Rapid increase in weight between 125 dg and 140 dg</td>
</tr>
<tr>
<td>Complex gyral development including secondary and tertiary gyri</td>
<td>Secondary gyri evident at 32 to 34 weeks and tertiary sulci at 40 to 44 weeks (35)</td>
<td>Secondary gyri seen in orbital, frontal, insular and occipital regions at 140 dg and some tertiary sulci by 160 dg</td>
</tr>
<tr>
<td>Cortical cytoarchitecture</td>
<td>Formation of 6 cortical layers by at least 28 weeks (34); volumetric MR techniques indicate prominent cortical growth from 29 to 40 weeks (37); Reduction in subplate zone from 26 to 40 weeks (31)</td>
<td>Six cortical layers and subplate zone identified at 125 dg</td>
</tr>
<tr>
<td>Myelination of the posterior limb of internal capsule (PLIC), pre and post central gyri and corona radiata</td>
<td>Mature myelin at 44 weeks in PLIC and 52 weeks in the optic radiation (38, 39)</td>
<td>Myelin present at 125 dg in IC and diencephalon</td>
</tr>
<tr>
<td>Myelination of the superior temporal gyrus</td>
<td>Mature myelinization by 48 weeks</td>
<td>Early myelination in the dorsal aspect of parietal and frontal gyrus at 140 dg Mature myelin by 160 dg Moderate myelination by 160 dg</td>
</tr>
</tbody>
</table>

mature of the pre-central and post-central gyri (Fig. 1H, K). At 160 dg, myelination had increased in the internal capsule, alveus, and lateral geniculate nucleus (Fig. 11); subcortical myelination (Fig. 1L) extended dorsally, ventrally, and laterally, with staining now apparent in the gyri flanking the lateral fissure, including the superior temporal gyrus.

Ontogeny of Astrocytes: At 125 dg (Fig. 1M), GFAP-IR astrocytes were abundant in the deep white matter (346 ± 32 cells/mm²) across the rostrocaudal extent of the brain, but less dense in the subcortical white matter (263 ± 74 cells/mm²; Fig. 1P). Astrocytes were evident in the internal capsule, the corpus callosum, the septal area, and the basal ganglia. At 140 dg, the gradient of GFAP-IR cells from deep to subcortical white matter was still evident (Fig. 1N); cell density throughout the deep white matter was similar to 125 dg (374 ± 30 cells/mm²) but had increased in the subcortical regions (405 ± 26 cells/mm², Fig. 1Q). This trend continued at 160 dg (Fig. 1O, R). At 125 dg, the pial surface was covered with glial end-feet forming the glia limitans. GFAP-IR radial glial processes were evident in the intermediate, subventricular, and ventricular zones but appeared varicose and in the process of dismantling; this process was more pronounced at 140 and 160 dg. At these ages it was not possible to identify any stained fibers traversing from one cerebral surface to the other.

Histopathological Analysis of Premature Baboon Brains

Histological examination of PD neonatal baboon brains at 139 dg revealed the presence of various neuropathologies; none of these neuropathologies were evident in gestational controls. The 2 most common forms of injury in the PD brain were white matter injury and hemorrhage. The frequency of occurrence of each of these pathologies in the cerebrum of individual animals is summarized in Table 1.

White Matter Injury: Evidence of white matter injury was found in 8/16 PD neonates; the injury ranged from small patches of reactive astrocytosis (Fig. 2C) to more extensive damage, including small cystic lesions with dense cellular borders (Fig. 2A, B), endothelial hypertrophy, and activated microglia (Fig. 2D). The extent of white matter injury for each brain ranged from 0.4% to 2.6% of total white matter (mean 1.24%). The animal with the greatest extent of injury (2.6%) also had the

Fig. 3. Representative coronal sections of the brains from infants #3 and #8, showing both the distribution and type of injury across the rostral-caudal (R-C) extent. Key to the neuropathology: Hatched area = subarachnoid hemorrhage; Stars = gliosis; Graded shading = white matter injury; Black shading = ventriculomegaly; Shaded spheres = grey matter injury; Small circles with bolded outline = intraventricular hemorrhage; Dotted area = germinal matrix hemorrhage; Checkered area = cortical infarct. Scale bar = 1 cm.
most extensive distribution of damage across the rostral-caudal extent of the brain, with damage observed in 4/10 blocks (infant #8, Table 1). In another animal (infant #1, Table 1), white matter damage was confined to 1/10 blocks (occipital lobe), however, this damage occurred 17% of the white matter and was the most extensive damage observed within any 1 brain region. There was no evidence of specific disruption of radial glial fibers within the white matter of PD infants; as noted for gestational controls radial glia appear to be in the process of dismantling at this stage of development.

**Hemorrhage:** Subarachnoid hemorrhages were observed in 6/16 PD neonates. They most frequently occurred on the dorsolateral gyri of the parietal and occipital lobes but also occurred on the medial surface of the hemispheres (Fig. 2E). Hemorrhage sites were characterized by an organized center of damage, with surrounding fibroblast and macrophage activity and thickened capillary walls. Hemorrhages also occurred in the lateral ventricles (1/16 PD neonates; Fig. 2F), germinal matrix (2/16 PD neonates; Fig. 2G), white matter (3/16 PD neonates; Fig. 2H), and cerebellum (2/16 PD neonates; Fig. 2I).

**Hippocampal Damage:** Distinct regions of cell loss were seen in the CA2/3 region of the pyramidal cell layer in the hippocampus of 3/16 PD neonates (Fig. 2J); reactive astrocytosis was frequently observed in association with this cell loss (Fig. 2K, control; Fig. 2L, PD).

**Cerebral Cortical and Deep Grey Matter Injury:** Necrotic cortical neurons (layers 5 and 6) and small patches of cell loss were seen in 4/16 PD neonates; one of these animals sustained more extensive cortical damage associated with adjacent intraventricular hemorrhage (Fig. 2E). In another infant there was cell loss in the basal ganglia and in another, significant failure of normal gyral development (Fig. 2M); the hemispheres had the appearance of GC animals at 125 dg.

**Ventriculomegaly:** Four/sixteen PD neonates displayed enlarged lateral ventricles (Fig. 2O) in relation to the surrounding brain tissue when compared to GC brains (Fig. 2N). In particular, the posterior and inferior horns were markedly enlarged in these animals.

Representative series through 2 PD brains are presented in Figure 3. As seen in these diagrams and from Table 1, cerebral injury occurred at sites throughout the rostrum-caudal extent of the hemispheres.

**The Relationship of Cerebral Injury to Cardiovascular and Respiratory Measures:** The premature primates with moderate to severe cerebral injury displayed a trend toward the worst measures for physiology. For each animal the most extreme measure of physiology was analyzed against the extent of cerebral injury. The premature primates with moderate to severe cerebral injury displayed lower minimal PaCO$_2$ (p = 0.057), lower minimal pH (p = 0.2), and lower minimal mean arterial blood pressure (p = 0.4) in comparison to those with none or mild cerebral injury. These recordings suggest that the infants with cerebral injury were displaying more instability in their respiratory (lowered PaCO$_2$), cardiovascular (lower blood pressure), and metabolic states (lower pH despite lower PaCO$_2$; Table 2).

**DISCUSSION**

This study has shown that there are marked similarities in the sequence of cerebral development and in the pattern of cerebral injury between the prematurely delivered baboon and the premature human infant. Most notably, the premature baboon displays a marked predominance of cerebral white matter injury in the absence of an experimental interventional insult.

In establishing the gestational ages at which equivalent brain development occurs in the baboon and human, we examined parameters such as the extent of hemispheric gyral folding, cortical growth, astrocyte development,
and myelination. Although many aspects of cerebral development have been studied in rhesus and macaque monkeys (31–33), a normative pattern of development for the baboon with its longer gestation period (~184 days vs. ~165 days in the rhesus monkey) has not been as well documented. It was not the purpose of the current study to establish quantitative data for every domain of development of the cerebral hemispheres, but rather the major features against which to define cerebral injury in this model. At 125 dg in the baboon, all layers were present in the cerebral cortex, the main sulci and gyri had developed, and myelination had commenced in the internal capsule and diencephalon (Table 3). Mature Cajal-Retzius cell bodies and processes were prominent in layer 1 and radial glial processes were beginning to dismantle. In humans, the 6 cortical layers are established by at least 28 weeks (34), the primary gyri by 26 to 28 weeks (35), and mature Cajal-Retzius cells are present by 24 weeks (36). Taken together, it would appear that development of the baboon brain at 0.7 of gestation (125 dg) equates to human development at 26 to 28 weeks (182 to 196 dg). It is possible that with the examination of younger baboons this date might be revised, but it conforms to the age estimated from the cardiovascular and respiratory developmental milestones (28, 29).

In baboons there was a brain growth spurt from 125 to 140 dg followed by a slower rate of growth from 140 to 160 dg. However, cortical thickness increased more significantly in the latter time period. By 140 dg, mature myelin, detected with the high resolution Black-Gold technique (30), was present in the posterior limb of the internal capsule and extended into the dorsal gyri of the frontal and parietal lobes of the hemispheres in the region around the central sulcus. Fetal human brains display a growth spurt around 28 weeks (35) and there is prominent growth in cortical grey matter between 29 and 40 weeks (37). Myelinogenesis begins in the periventricular white matter at around 30 weeks in the human (27) although mature myelin is not evident in the forebrain until much later at about 44 weeks in the posterior limb of the internal capsule, 48 weeks in the temporal gyrus, and at 52 weeks in the optic radiation (38, 39). Luxol fast blue staining, which was used in these studies, is not optimal for labeling individual myelinated fibers (30) and therefore the earliest myelinating fibers might not have been detected. Taken together, we suggest that the baboon brain at 140 dg is equivalent to 32 to 34 weeks, and at 160 dg to term in humans. Thus, there is a marked similarity in the sequence of development, albeit with a somewhat more advanced pattern of myelination in the baboon.

Our study of the pattern of cerebral injury in the prematurely born baboon indicates a selective vulnerability of injury to the cerebral white matter. It has also been previously demonstrated in the immature brain of both human infants and other animal species that the white matter is most vulnerable to injury in the presence of late oligodendrocyte precursors and immature oligodendrocytes. The risk declines with the onset of myelination (40). In this premature baboon model demonstrating significant white matter injury, a study of the distribution and timing of oligodendrocyte progenitors and their relationship to the onset of myelination would thus indicate the periods of regional vulnerability in the white matter and clearly allow relevant comparative time points with the human infant. This will be undertaken by our group in the next series of studies.

Astrocytosis in the cerebral white matter was observed in many of the prematurely delivered brains. It is important to note that GFAP-IR astrocytes were a prominent feature of the developing white matter in gestational control brains; they were abundant in the deep hemispheric white matter at 125 days, increasing in density in this region and into the subcortical white matter by 140 days. Astrocyte density increased in concert with myelination. Although GFAP-IR is not the earliest marker for astrocytes it is specific and provides a sensitive method for comparison with human material. In the premature human cerebral white matter, GFAP-positive cells are seen as early as 16 to 18 weeks in large fiber tracts (41), in the deep periventricular white matter by at least 28 weeks, extending into the more superficial subcortical white matter in the perinatal period (42). In the human, gliogenesis continues throughout the second half of gestation and continues for several years after birth (41). The dense distribution of astrocytes throughout the cerebral white matter in this baboon model and in the human infant is important to note as it may be otherwise mistaken as a pathological finding.

With an understanding of the normative ontogeny in the premature baboon model, we continued to be struck by the similarity in the distribution and frequency of cerebral injury to that seen in the prematurely born human infant (Table 4). Injury in the baboon brain occurred commonly in structures associated with the temporal lobe, including the medial and superior temporal gyri, the hippocampus, and the posterior and inferior horns of the lateral ventricles. Neonatal white matter injury, the most common form of damage in human infants, was also the most common form of injury in the baboon. This damage was usually the diffuse form with cystic infarction occurring in only 1 case. Diffuse white matter injury was associated with the attraction of infiltrating cells, although the injury was not as extensive as that reported in humans, which may be due to the absence of potentiating or priming factors such as perinatal infection or hypoxia. White matter injury occurred most frequently in the parietal and occipital lobes. The frequency of intraventricular and subarachnoid hemorrhage also closely
correlated with human data (11). Subarachnoid hemorrhages in the human newborn usually resolve without any apparent consequences, although it is possible that there could be subtle developmental problems as yet not identified. Hippocampal cell loss occurred in approximately 20% of the premature baboons and may be directly relevant to the recent observations that there is hippocampal atrophy in older prematurely born children (12). It has also been reported that the subiculum of the hippocampus is vulnerable in premature infants (43). The evidence of necrosis in the cerebral cortex and basal ganglia in this model is relevant to the loss of cortical grey matter observed in premature infants as detected by MRI and histological evidence of injury in the basal ganglia and thalamus (11).

We have shown that the baboon brain displays strong structural similarities to the human brain with regards to its sequence of white and grey matter maturation, including gyral formation, myelination, and cortical laminar development. Our initial histopathological data suggests that the prematurely born baboon sustains cerebral injury similar to that in the prematurely born human. Thus, we believe that this model has the potential to assist in shedding light on the nature of cerebral injury in the prematurely delivered human. We are now in a position to evaluate whether the pattern of cerebral injury varies in relation to specific ventilatory regimes employed during neonatal intensive care. Clearly there are important differences between this model and the human setting, which limit the generalization of these findings to all preterm infants. These differences include the absence in the baboon model of any perinatal potentiating or priming factors precipitating preterm labor including perinatal fetal infection (44, 45). There was a range in the severity of illness in the animals, with many animals displaying significant cardiorespiratory instability, which is more consistent with sicker preterm infants. Postnatal neonatal therapies, such as analgesic care and patent ductal arteriosus ligation vary between this protocol and within many neonatal intensive care settings. The protocol of routine surgical ligation of the ductus does not reflect common neonatal practice. Such therapeutic differences to that of the human infant have been reviewed for the next series of protocols, with enhancing similarities to the human preterm infant for the improved translation of the findings from this model. We also plan to undertake advanced and conventional MR imaging of the model to allow direct correlation of the neuropathology with MR images of the brain. This will further our understanding of the extent to which imaging reflects the true extent of cerebral damage.

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