HHS Public Access

Author manuscript

Neurobiol Dis. Author manuscript; available in PMC 2021 July 01.

Published in final edited form as:

Neurobiol Dis. 2021 July ; 154: 105334. doi:10.1016/j.nbd.2021.105334.

A perfect storm: The distribution of tissue damage depends on seizure duration, hemorrhage, and developmental stage in a gyrencephalic, multi-factorial, severe traumatic brain injury model

Beth Costine-Bartell[a,b,*], **George Price**[a], **John Shen**[a], **Declan McGuone**[c], **Kevin Staley**[d,e], **Ann-Christine Duhaime**[a,b]

[a]Department of Neurosurgery, Massachusetts General Hospital, Charlestown, MA, United States

[b]Department of Neurosurgery, Harvard Medical School, Boston, MA, United States

[c]Department of Pathology, Yale School of Medicine, New Haven, CT, United States

[d]Department of Neurology, Massachusetts General Hospital, Boston, MA, United States

[e]Department of Neurology, Harvard Medical School, Boston, MA, United States

Abstract

The pathophysiology of extensive cortical tissue destruction observed in hemispheric hypodensity, a severe type of brain injury observed in young children, is unknown. Here, we utilize our unique, large animal model of hemispheric hypodensity with multifactorial injuries and insults to understand the pathophysiology of this severe type of traumatic brain injury, testing the effect of different stages of development. Piglets developmentally similar to human infants (1 week old, “infants”) and toddlers (1 month old, “toddlers”) underwent injuries and insults scaled to brain volume: cortical impact, creation of mass effect, placement of a subdural hematoma, seizure induction, apnea, and hypoventilation or a sham injury while anesthetized with a seizure-permissive regimen. Piglets receiving model injuries required overnight intensive care. Hemispheres were evaluated for damage via histopathology. The pattern of damage was related to seizure duration and hemorrhage pattern in “toddlers” resulting in a unilateral hemispheric pattern of damage ipsilateral to the injuries with sparing of the deep brain regions and the contralateral hemisphere. While “infants” had the equivalent duration of seizures as “toddlers”, damage was less than “toddlers”, not correlated to seizure duration, and was bilateral and patchy as is often observed in human infants. Subdural hemorrhage was associate with adjacent focal subarachnoid hemorrhage. The percentage of the hemisphere covered with subarachnoid hemorrhage was positively correlated with damage in both developmental stages. In “infants”, hemorrhage over the cortex was associated with damage to the cortex with sparing of the deep gray matter regions; without hemorrhage, damage was directed to the hippocampus and the cortex was spared. “Infants” had lower neurologic scores than “toddlers”. This multifactorial model of severe brain injury caused unilateral, wide-spread destruction of the cortex in piglets developmentally similar

*Corresponding author at: Building 149 13th Street, Room 3.405, Charlestown, MA 02129, United States. bcostinebartell@mgh.harvard.edu (B. Costine-Bartell).

to toddlers where both seizure duration and hemorrhage covering the brain were positively correlated to tissue destruction. Inherent developmental differences may affect how the brain responds to seizure, and thus, affects the extent and pattern of damage. Study into specifically how the “infant” brain is resistant to the effects of seizure is currently underway and may identify potential therapeutic targets that may reduce evolution of tissue damage after severe traumatic brain injury.

Keywords

Severe traumatic brain injury; Hemispheric hypodensity; Gyrencephalic; Abusive head trauma; Traumatic seizures; Subdural hemorrhage; Subarachnoid hemorrhage; Hypoxic ischemia

1. Introduction

Brain injury due to abusive head trauma (AHT) is the leading cause of injury-related death in children under the age of 4. Out of the 1.1 million documented cases of child abuse and neglect in the United States annually, 1200 cases result in death and 50% of these deaths are the result of AHT (Giardano et al., 1997). AHT is a silent epidemic stimulating little translational research to date. It results in long-term morbidity that may be as mild as learning disabilities and behavioral disorders, but is often severe with post-traumatic epilepsy, blindness, inability to speak, cognitive impairment, motor dysfunction, persistent vegetative state, or death (Duhaime et al., 1996). No interventions have been discovered to prevent the development of parenchymal damage after AHT (Foster et al., 2014).

Acute subdural hemorrhage (SDH) is the most common intracranial abnormality resulting from AHT and is observed in 80–90% of victims entering the pediatric intensive care unit; for reasons yet unknown, hemispheric hypodensity (HH) develops in 25–50% of cases, doubling the mortality rate (Foster et al., 2014; Dias et al., 1998; Gilles and Nelson Jr, 1998; Khan et al., 2017). HH is an injury pattern observed that is unique to infants and toddlers. Radiologically, SDH is associated with uniform swelling and loss of gray-white differentiation of the entire underlying hemisphere. These changes span multiple vascular territories, later followed by severe cortical atrophy. Subarachnoid hemorrhage (SAH) is often observed with the SDH (Foster et al., 2014; Duhaime and Durham, 2007). *Unilateral* HH is a striking radiological pattern where the SDH is restricted to one hemisphere and only the hemisphere underlying the SDH is hypodense (on CT). In the acute period, children often have seizures (overt or subclinical) with episodes of apnea and hypoventilation (Foster et al., 2014; Duhaime et al., 1998). The pathophysiology of unilateral HH remains largely unknown. There is no therapy.

Though mechanisms of pathophysiology are heavily studied in single-injury models of moderate TBI, there remains an urgent need to study the pathophysiology of multi-factorial, severe TBI in children. To date, modeling AHT has been studied by shaking gyrencephalic species, but the resulting damage does not include a substantial SDH, seizures, nor large areas of tissue damage (Finnie et al., 2012; Coats et al., 2017). Models that place an SDH alone do not result in the extensive damage pattern that occurs after AHT, including HH (Timaru-Kast et al., 2008; Shaver et al., 1996; Duhaime et al., 1994). We have created a

model of unilateral HH in piglets induced with cortical impact, placement of a SDH, local induction of seizures by kainic acid, and brief apnea and hypoventilation while subjects are anesthetized with a seizure-permissive protocol (Costine-Bartell et al., 2019a). In one-month old piglets (similar in development to human toddlers; “toddlers”), the HH model injuries result in cerebral edema, depressed neurologic status and metabolic acidosis requiring prolonged intensive care and results in a unilateral HH injury developing by 24 h in which the tissue damage encompasses the majority of the cortical ribbon, spanning multiple vascular territories (i.e., not hypoxic ischemic due to vessel occlusion), with relative sparing of the deep brain regions and the contralateral hemisphere. Though the resulting pattern of tissue damage is similar to hypoxic-ischemic damage and displays vasogenic edema, it is distinct from a classic asphyxial pattern as evidenced by with the sparing of one entire hemisphere (Costine-Bartell et al., 2019a; Bjorkman et al., 2010). The pathophysiological mechanisms by which damage affects the hemisphere underlying the SDH with relative sparing of the contralateral hemisphere, in the context of localized insults with global apnea, remain unknown.

In children, AHT is superimposed upon the individual’s stage of brain development in which the brain’s response to injury is influenced by inherent age differences. For reasons yet unknown, in infants, the damage is more typically bilateral while the injury is more commonly unilateral in toddlers (Gilles and Nelson Jr, 1998; Duhaime and Durham, 2007). The infant brain may be more resilient to focal injuries (Missios et al., 2009; Durham et al., 2000; Durham and Duhaime, 2007), but more susceptible to diffuse injuries compared to toddlers (Ibrahim et al., 2010; Tremblay et al., 1984). In infants, the majority of seizures are subclinical due to an immature neocortex (Clancy et al., 1988). The infant brain is less susceptible to experimentally induced seizures (Tremblay et al., 1984) but has less oxygen reserve during apnea. Infants have the lowest cerebral blood flow of all ages while toddlers are entering the development stage with a peak of cerebral blood flow (Takahashi et al., 1999). In our model of focal contusion, piglets of similar development to toddlers have greater swelling than piglets of similar development to infants (Duhaime et al., 2003).Therefore, the infant brain may be more susceptible to apnea but resistant to seizures while the toddler brain may be more prone to edema, but the specific factors that result in unilateral vs. bilateral HH are not known.

Here, we use our unique model of HH that replicates the potentially synergistic, multifactorial injury cascades within comparable developmental stages and brain morphology of human infants (one week old piglets; “infants”) and toddlers (1 month old piglets; “toddlers”) using a gyrencephalic species, physiological monitoring, and clinically relevant outcomes in our multi-injury, multi-insult model requiring overnight critical care management that mirrors the pediatric intensive care unit (Costine-Bartell et al., 2019a). The objective of this experiment is to utilize innate developmental differences to determine the role of seizures and edema in development of tissue damage after AHT. We demonstrate that combined insults lead to a bilateral “patchy” distribution of injury in “infants” but created a unilateral wide-spread distribution of injury in “toddlers” similar to the pattern observed in human children (Gilles and Nelson Jr, 1998; Duhaime et al., 1993). Age-specific therapies that abort the cascades of the pathophysiology and prevent injury progression after AHT in

children might reduce the severity of neural damage and reduce morbidity and mortality from these severe injuries.

2. Materials and methods

2.1. Surgery to create HH model injuries or sham surgery

Male, Yorkshire piglets were housed and fed as previously described (Costine-Bartell et al., 2019a; Missios et al., 2009). All protocols and procedures were in accordance with the guidelines of the American Veterinary Association and the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee at Massachusetts General Hospital.

A series of 31, 1-week ("infants") or 1-month old ("toddlers") piglets were alternatively assigned to receive model injuries or sham surgery. Shams served as controls. Surgery and anesthesia protocols were employed as previously described and adjusted to the age of the piglets (Table 1) (Costine et al., 2015). Briefly, general anesthesia was induced with isoflurane and oxygen then switched to room air. Prior to seizure induction, piglets were switched to a seizure-permissive anesthetic protocol with infusion of morphine and dexmedetomidine (Table 1), and then isoflurane was withdrawn. A combination of injuries scaled to brain volume and insults were induced to reflect the physiologic events in children with severe AHT as previously described: cortical impact, mass effect, placement of SDH, administration of kainic acid, apnea, and hypoventilation (Table 1) (Costine-Bartell et al., 2019a; Durham et al., 2000; Durham and Duhaime, 2007).

EEG was recorded with an Olimex device (Plovdiv, Bulgaria; recorded in BrainBay, version 1.9; open source BioSignal Software; Open EEG project) with scalp electrodes or with an XL Tech EEG machine (recorded in NeuroWorks, Natus Neurology) using epidural strips containing 4 electrodes on each hemisphere (8 total, AdTech). EEG's were analyzed in a 4 bipolar montage. The duration of EEG recording was limited when using the Olimex device due to the difficulty of maintaining prolonged contact with electrodes, which required suturing to the scalp and the limited lifespan of the electrodes where signal: noise ratio was sufficient. Once the XL Tech EEG machine was obtained, pigs were recorded while sedated and instrumented. Additional convulsions and apneic episodes were observed in extubated, de-instrumented pigs and were included in the "total estimated seizure duration". Due to the limited EEG at the beginning of the experiment, "estimated seizure duration" was calculated by seizure-induced tachycardia that was a 40% increase of the baseline heart rate in each individual piglet prior to kainic acid administration. A 40–50% of heart rate above baseline has been previously shown to provide 35–99% sensitivity for electrographic seizures in human patients (Boon et al., 2015; Hampel et al., 2015). As expected, "infant" piglets had a greater heart rate baseline than "toddler" piglets (170 ± 6.4 vs. 128 ± 7.5 bpm, $P = 0.003$), and therefore, the heart rate threshold for seizures was 225 ± 6.2 bpm for "infant" piglets and 180 ± 9.0 bpm "toddler" piglets ($P = 0.001$). Seizure duration, seizure amplitude, and seizure type (focal vs. general) were determined using NeuroScore™ (Data Sciences International, St. Paul, MN). The efficacy of using elevated heart rate as a biomarker for seizures was tested in the subset of piglets that had EEG and the seizure duration as

measured via EEG or heart rate did not differ within “infants” (EEG: 80.4 ± 26.0 vs. HR: 160 ± 61.8, $P = 0.26$) or “toddlers” (EEG: 74.8 ± 23.5 vs. HR: 60 ± 17.3, $P = 0.64$).

Arterial blood was collected prior to injury, at regular intervals within the first 8 h post-injury, at 12–14 h post-injury and 24 h post-injury and a portion was analyzed with an i-STAT Handheld device (Abbott, Abbott Park, Illinois) for blood gases, which informed ventilation status in extubated piglets in the overnight ICU, glucose, and metabolites. In subjects that had a missing pre-injury sample the mean of the other pre-injury values within the age group was used. The post-injury peak or nadir was used for means comparisons.

As previously described, piglets were recovered from anesthesia six hours after cortical impact or sham surgery and were survived 24 h from injury to allow evolution of the pathophysiological cascades that might induce HH (Costine-Bartell et al., 2019a). All piglets receiving model injuries were constantly monitored and received critical care overnight either intubated or extubated as neurologic function allowed (Costine-Bartell et al., 2019b). If piglets did not have neurologic function sufficient for extubation, they were ventilated overnight and infused with an analgesic dose of morphine. Piglets that were able to be extubated received buprenorphine every 4 h and blood gases were analyzed every 2 h to determine if mechanical ventilation was required. After anesthetic recovery or a similar timepoint if not able to be extubated, neurologic function was tested at 8 and 20 h post-injury using an assessment previously described (Costine-Bartell et al., 2019a).

2.2. Brain collection and processing

Twenty-four hours after the injury, piglets were deeply anesthetized and euthanized via exsanguination by transcardial perfusion with saline followed by phosphate buffered formalin. The brain was removed and post-fixed for 5–7 days. Brains were weighed, photographed with and without dura. SDH was removed, cerebral hemispheres divided and weighed separately to detect any hemispheric swelling. Cerebral hemispheres were coronally sliced, photographed to determine SAH area, and processed into 8–9 large paraffin embedded tissue blocks spanning the entire hemisphere. The percentage of hemisphere covered by SDH or SAH was determined as previously described (Costine-Bartell et al., 2019a).

The percent of hemisphere damaged was calculated by adding the areas of damage among sections per hemisphere and dividing by the sum of the total areas of the sections (Costine-Bartell et al., 2019b). For our purposes here, unilateral HH was defined as >100% increase ipsilateral vs. contralateral with damage in the ipsilateral hemisphere >25%. To determine the interaction of hemorrhage and seizures on the location of damage, damage from piglets with >30-min seizure was determined in the cortex, deep gray matter, and hippocampus on two sections that included the hippocampus. The percentage of damage in each brain region was determined by taking the area of damage and dividing by the area of the brain region and averaging the two sections. Because the resulting SDH from injected blood was variable, piglets were binned into the groups: no SDH (< 15% of the hemisphere covered by SDH) or SDH (> 15% of the hemisphere covered by SDH). Because SDH resulting from blood injected into the dura on the ipsilateral hemisphere would sometimes result in a bilateral SDH (particularly in “infants”), unilateral subdural hematoma was defined when

the percentage of the ipsilateral hemisphere covered by SDH was 40% greater than the contralateral hemisphere.

2.3. Histology

Sections (10 μm) were mounted on poly-L-lysine-coated glass slides dried and stored in slide boxes. Sections were stained with hematoxylin and eosin (H&E) and the area of damage per section was determined utilizing a previously described mapping protocol (Costine-Bartell et al., 2019a). Briefly, an area was considered damaged if the tissue had 1.) "red neurons" 2.) acute tissue hemorrhage, or any of the following changes in combination with or adjacent to areas of "red neurons": 3.) vacuolization around blood vessels or 4.) vacuolization of interstitial neuropil, or 5.) vacuolization around neurons. "Red neurons" were classified by the presence of all three of the following features: 1.) cell shrinkage with perineuronal vacuolization, 2.) cytoplasmic hyper-eosinophilia, and 3.) nuclear pyknosis.

Immunohistochemistry was used to detect the distribution of amyloid precursor protein (APP) in sham and injured piglets 24 h after initiation of injuries and the distribution of APP in tissue was quantified in the same manner as previously described (Costine-Bartell et al., 2019a). APP was present either within axons or in neuron cell bodies. The area of antibody expression per hemisphere was determined by calculating the area of antibody positivity as a percentage of the entire hemisphere.

2.4. Exclusions

Six of the 31 piglets were excluded. Exclusion criteria included early death, breached dura such that placing a SDH was not possible, and intraparenchymal hemorrhage, which occurred exclusively in piglets in which kainic acid was injected into the cortex (Table 2). Intraparenchymal hemorrhage is not a feature in unilateral HH nor AHT. Because of the incidence of intraparenchymal hemorrhage in pigs in which kainic acid was injected in the cortex especially in "infants", kainic acid was administered by mixing with the blood that was injected under the dura. Additionally, an "infant" sham piglet failed to emerge from anesthesia and was euthanized early. As a result, the dose of seizure permissive anesthetics was reduced in subsequent "infant" piglets to allow for quicker recovery from anesthesia.

2.5. Statistics

Data are presented as means ± SEM and P values <0.05 were considered significant. All data were analyzed using GraphPad Prism® 8 (GraphPad Software, San Diego, CA). The proportion of focal or generalized seizures, unilateral vs bilateral SDH, unilateral vs. bilateral HH, and extubated vs. intubated overnight among "infants" vs. "toddlers" was tested with a Fisher's Exact Tests. The effect of hemisphere on EEG amplitude within each hemisphere pre-seizure, seizure, and post-seizure was tested via a one-way ANOVA repeated measures followed by Tukey's post-hoc tests. The difference in seizure length via EEG and via heart rate ("total estimated seizure") was tested in infants vs. toddlers with a t-test. The main effects of age and time on glucose, lactate, pH, base excess of the extracellular fluid, and neurological scores were tested with a repeated measures two-way ANOVA followed by Tukey's and Sidak post-hoc tests. Within treatments, the effect of age and hemisphere on % of the hemisphere damaged or positive APP was tested via two-way ANOVA followed by

Tukey's post hoc tests. The effect of SDH on damage per brain region was tested with a two-way ANOVA followed by Tukey's post hoc tests. There was no effect of age or hemisphere, therefore ages and hemispheres were combined. The difference in hemisphere weight ipsilateral vs. contralateral was determined with a paired *t*-test in "infants" and "toddlers" within each treatment. The correlation between the percent of hemispheric damage or hippocampal damage and seizure length, subdural hematoma area, and subarachnoid hemorrhage area were tested using Pearson Correlations.

3. Results

We used our unilateral HH model developed in "toddler" piglets and determined the damage distribution "infant" vs. "toddler" piglets at 24 h after initiation of injuries to investigate the age-dependent pathophysiology of this severe injury. The injuries included focal injuries on the right hemisphere: cortical impact, mass effect, a placed SDH, seizure induced by kainic acid, 1 min of apnea, and 10 min of hypoventilation. Focal injuries were scaled to piglet brain size (Table 1).

3.1. Seizures

Kainic acid-induced seizures with the seizure-permissive anesthesia protocol lasted an average of 3.0 ± 0.7 h among piglets and was not age-dependent. Most piglets had multiple seizures that were focal (40%) or started focally and then generalized (40%); a small proportion had exclusively generalized seizures (20%; ages combined; no effect of age; Fig. 1A). There was no effect of age on focal vs. generalized seizures. EEG amplitude was greater in the ipsilateral vs. contralateral hemisphere (ages combined; Fig. 1B).

3.2. Clinical characteristics

Injuries and insults caused metabolic acidosis (Fig. 1 D–F) and depressed neurologic scores (Fig. 2) in both ages but "infants" were worse. A subset of subjects displayed acute hypotension and cardiac dysfunction with both ages requiring epinephrine for hypotension and chest compressions for resuscitation (Table 3). Early in the series, an "infant" piglet had prolonged status epilepticus with hypotension and died before the end of the experiment. Thereafter, if persistent hypotension accompanied status epilepticus lasting 6–7 h, then seizures were stopped with isoflurane (Table 3). More "toddler" piglets were able to be extubated for the duration of the overnight period than "infants" while some "toddlers" had emergency re-intubations (Table 3).

Model injuries reduced neurological scores in both ages and to a greater degree in "infant" piglets (Fig. 2A). At 20 h post-injury, the main effect of model injuries persisted (P = 0.01) but was not different within age-groups (Fig. 2B). Neurological score was inversely correlated with the total brain damage in "infants", but not in "toddlers" ("infants": − 0.736, $P = 0.037$; "toddlers": −0.36, $P = 0.25$), perhaps reflective of the bilateral distribution of damage in "infants". "Toddlers" may be afforded some compensation in neurologic function with damage that is largely restricted to one hemisphere.

3.3. Distribution of damage and hemorrhage

In response to HH model injuries, the amount and pattern of damage were age-specific. “Toddlers” typically had unilateral wide-spread hypoxic-ischemic-type damage encompassing the majority of the cortex with sparing of deep brain regions, the hippocampus, and the contralateral hemisphere while “infant” piglets typically had bilateral diffuse, patchy, areas of damage (Fig. 3). “Toddlers” had a greater amount of injury, which was restricted to the ipsilateral hemisphere whereas damage was less and equivalent between hemispheres in “infants” (Fig. 3G). The proportion of piglets that had unilateral HH was greater in “toddlers” vs. “infants” (Fig. 3H).

The placed SDH often resulted in a concomitant, focal subarachnoid hemorrhage (Fig. 4). The SDH itself may not contribute to damage directly, but instead, may provide a focal SAH. Consistent with this, the SDH was not thick nor space filling, but thin similar to children with SDH from AHT (Fig. 4). “Toddlers” had a greater amount of the hemisphere covered by subdural blood and a greater SDH on the ipsilateral hemisphere, but for yet unknown reasons that may reflect anatomic differences, “infants” had equivalent subdural blood ipsilateral vs. contralateral. Subarachnoid hemorrhage covered a greater percentage of the hemisphere ipsilateral vs. contralateral (main effect, $P = 0.03$) and tended to be different within “toddlers” ($P = 0.08$) but was not different ipsilateral vs. contralateral within “infants” ($P = 0.8$, Fig. 5B). The percentage of the cortex covered by SAH but not SDH was positively correlated to the percent hemisphere damaged in both ages (Table 4).

3.4. The effect of seizure and hemorrhage on the distribution of damage

Seizure duration affected damage distribution in an age-specific manner. Seizure duration, which was not different between “infants” and “toddlers” was positively correlated with the percentage of damage in the ipsilateral hemisphere in “toddlers” but not “infants” (Table 4). Prolonged seizures may drive damage in “toddlers”, but “infants” may be resistant to seizure-induced damage in combination with the other injuries and insults. Seizures at least one hour long appear to be required for the hemispheric pattern in toddlers (Fig. 5C).

In rodent models of seizure or seizure with anoxia injury, the hippocampus is often selectively damaged with minimal damage to the cortex (Wirrell et al., 2001). In contrast, in this multi-insult TBI model with hemorrhage, the cortex was predominately damaged with relative sparing of deep brain areas and hippocampus (Fig. 5E). The effect of hemorrhage on the location of the seizure-driven damage was explored after it was observed that an “infant” piglet without a SDH, with a prolonged seizure, had minimal damage in the cortex, but extensive damage in the hippocampus (Fig. 5F) - opposite to the typical pattern. In “infants” without a SDH, damage was equivocal among brain regions, but with a SDH, damage was greater in the cortex than in the hippocampus and deep gray areas (Fig. 5D). The SDH or SAH overlying the cortex may incite damage to the cortex while sparing the hippocampus and deep gray areas. In “toddlers” with unilateral HH, 5 of 6 had seizure lasting over 60 min and 5 of 6 had a unilateral SDH indicating that status epilepticus and a unilateral SDH are the “perfect storm” typically required for unilateral HH to occur.

The model injuries resulted in slight cerebral swelling and was specific to age as the hemisphere ipsilateral to the injuries was heavier than the contralateral hemisphere (23.4 ± 1.19 vs. 19.5 ± 1.14; $P = 0.005$) in toddlers, but weight was not different between hemispheres in infants (16.5 ± 0.4 vs. 16.0 ± 0.32; $P = 0.146$). Ipsilateral hemisphere weight was positively correlated with seizure duration in "toddlers"($r = 0.91$, $P = 0.001$) but not in "infants" ($r = 0.58$, $P = 0.13$).

"Toddlers" had a greater propensity for spread of damage that was primarily restricted to the ipsilateral hemisphere. Although the SDH was bilateral in "infants", even when an individual "infant" piglet had a unilateral SDH (Fig. 4C), damage was still bilateral (Fig. 3F). In the few animals where kainic acid was administered intracortically (Table 2), damage spread was restricted to the ipsilateral hemisphere in "toddlers" but still spread bilaterally in "infants". This may indicate that there are factors that promote unilateral spread and confinement of damage in "toddlers" that are unique from "infants".

3.5. Distribution of APP

The overall area of APP-positive neurons per hemisphere (Fig. 6D–F) paralleled that of the hypoxic-ischemic-type damage via H&E staining (Fig. 3G) with a greater amount of APP-positive neurons ipsilateral vs. contralateral in "toddlers" but equivalent among hemispheres in injured "infant" piglets. In addition, the type of APP staining pattern was noted to correspond to previously described patterns of expression in human infants and toddlers: "traumatic axonal", "geographic", "metabolic", or "diffuse axonal injury" (Geddes et al., 2001a) (Fig. 6 A–C). In piglets with the greatest amount of damage via H&E or via metabolic APP, APP distribution was wide-spread similar to the "geographic" or global as described by Geddes et al. (Geddes et al., 2001a). Diffuse axonal injury was not observed and therefore, does not appear to be required for this injury pattern.

4. Discussion

This gyrencephalic, large animal model of severe TBI is multifactorial modeling contusion, hemorrhage, mass effect, apnea, hypoventilation, and traumatic seizures and was associated with reduced neurologic scores, cardiac dysfunction, and sometimes, death. This line of experiments is unique in its requirement to employ a critical care unit to allow prolonged survival in a non-accidental pediatric traumatic brain-injury model where subjects had depressed neurologic function. Here, we use this model to understand the age differences in the pathogenesis and resulting damage patterns in order to unravel the potentially relevant pathophysiological cascades. Identifying the drivers of the spreading damage may lead to therapeutic targets. Modeling unilateral instead of bilateral HH is a key feature to ensure that the unilateral hypoxic-ischemic-type damage observed is not merely due to an anoxic effect. Here, we seek to understand the multiple insults, which when sustained alone would not lead to destruction of the cortex, and we are testing the hypothesis that various insults work synergistically together causing the extensive tissue destruction. Here, we have determined that seizure drives the damage and hemorrhage directs the location of the damage.

The damage pattern described here was similar to what has been observed in humans with HH and is distinct from other animal models of severe brain injury including neonatal

asphyxia and rotational injury. In “toddlers”, the damage extended from the site of the cortical impact and spread rostral and caudal along the cortical ribbon in the hemisphere ipsilateral to the focal injuries while the deep gray matter, hippocampus, and contralateral hemisphere were spared. The unilateral pattern in “toddlers” vs. bilateral pattern in “infants” is similar to the observed frequency of patterns in human children.

The “infant” pattern was patchy in both hemispheres and if there was not a significant SDH, then the hippocampus was damaged instead of the cortex. The few animal models that evaluate multi-factorial injuries and insults result in bilateral, diffuse damage. In large animal models of rotational injury, high angular accelerations can cause severely depressed neurologic function and apnea, but the resulting hemorrhage is bilateral/diffuse, the damage is exclusively diffuse axonal injury, and does not mimic the unilateral pattern of HH observed in humans (Gennarelli et al., 1981; Gennarelli et al., 1982). In a piglet model of birth asphyxia, where seizures develop spontaneously after hypoxia and ischemia, albumin extravasation is global without a distinct pattern of damage (Goasdoue et al., 2019). In a model of seizures induced with kainic acid after asphyxia in PND9 rat pups, the damage induced occurs in both hemispheres mainly in the hippocampi with minimal damage in the cortex (Wirrell et al., 2001). In the present model, hemorrhage on the cortex appears to direct the location of the damage to the cortex. Sparing of the contralateral hemisphere ensures that it is not purely a hypoxia mechanism of injury. Though the biilateral pattern in “infants” could be confounded by a lack of unilateral SDH pattern, even in “infants” with a unilateral SDH or “infants” where kainic acid was administered directly into the cortex, damage still spread to both hemispheres. The pattern of SDH distribution was similar in our study to human infants and toddlers where infants often have bilateral SDH and HH and toddlers more often have unilateral SDH and HH. Maturational differences of the dura and subarachnoid space may enable travel of blood across hemispheres in both humans and swine. Similar to autopsy description of infants with abusive head trauma (Geddes et al., 2001b), we observed a lack of diffuse axonal injury but instead observe a wide-spread metabolic pattern of APP along with hypoxic-ischemic type of damage.

“Infants” were clinically worse than “toddlers”. Both ages required overnight critical care, treatment for hypotension, and chest compressions. Resuscitation failed in one “infant”. While lactate increased and pH fell similarly in “infants” and “toddlers”, “infants” continued to display metabolic acidosis at 24 h. While mean arterial pressure in this model (Costine-Bartell et al., 2019b) is similar to models of neonatal asphyxiation (Bjorkman et al., 2010) with a low around 20 mmHg after the end of the insults, pH was lower in the neonatal asphyxiation model vs. our model (7.0 vs. 7.3) highlighting the difference in the length and severity of the insults with our model relying on multi-factorial insults to cause the brain damage vs. pure anoxia. Though metabolic status and seizure duration was similar in “infants” and “toddlers”, “infants” were clinically worse with lower neurologic scores 8 h after injury and were unable to be extubated but reliant on the ventilator. In contrast, some “toddlers” were able to be extubated overnight. Though “toddlers” had more extensive damage, the damage was unilateral in “toddlers” but “patchy” and bilateral damage in “infants”, which may be more debilitating. Both stages of development made improvement at 24 h post-injury though the main effect of injury was still significant.

Though “infants” were clinically worse than “toddlers”, they had less damage than “toddlers”. Seizure duration was not different among developmental stages indicating that it is *how* the brain responds to seizures that may the key difference among developmental ages. Certainly, early in postnatal life, high intracellular [Cl^-] results in GABA that is depolarizing and a decreased threshold for seizures (Glykys et al., 2017). High intracellular [Cl^-] is due in part to an abundance of $Na^+K^+Cl^-$co-transporter 1 and low K^+Cl^-transporter 2 as well as Gibbs-Donnan effects with a lower amount of impermeant anions in the extracellular matrix early in development affecting the direction of chloride current as well as cell swelling (Glykys et al., 2017; Kahle et al., 2008). We do not know when the GABA switch happens during development in swine. “Infants” may be resistant to scaled injuries and resistant to the effects of seizures. Our previous work demonstrates that the areas of hypoxic-ischemic injury displays vasogenic edema via extravasation of albumin (Costine-Bartell et al., 2019b). Here, the hemisphere ipsilateral to injuries was heavier than the contralateral hemisphere and positively correlated with seizure duration while hemisphere weight was not different between hemispheres in infants. Prolonged seizures cause opening of the blood brain barrier via matrix metalloproteinases (Goasdoue et al., 2019; Dubey et al., 2017; Cudna et al., 2017). Future studies will aim to determine if development affects the baseline or increase in mediators of blood brain barrier opening. Matrix metalloproteiniase-9 is a biomarker for intracranial hemorrhage, which is common in abusive head trauma (Berger et al., 2017). Inherent age-dependent differences in the composition of the extracellular matrix, baseline abundance of enzymes that degrade it, or age-dependent upregulation of extracellular matrix enzymes after hemorrhage and seizure may be key to the extensive spreading of damage in “toddlers” but not in “infants”.

In patients with severe brain injury, including children with abusive head trauma, severely affected patients have more seizures, but it is not known if seizures are an effect of severe injury or if seizures directly contribute to damage. Though induced seizures do not cause damage in immature rodents (prior to PND18) (Nitecka et al., 1984), seizures have been shown to cause damage when the brain is already compromised (Wirrell et al., 2001). Kainic acid after a mild arterial occlusion creating ischemia exacerbated damage in a rodent pup model (PND9) (Wirrell et al., 2001). In a piglet model of birth asphyxiation, piglets with spontaneous seizures induced by hypoxia and ischemia had greater damage, which was greatest in the cortex, compared to piglets without spontaneous seizures (Bjorkman et al., 2010). In our model, seizures are induced and the generation of seizures is not dependent upon the severity of the other injuries allowing us to directly measure the effect of seizure. “Infants” and “toddlers” had the same duration of seizures, but to different effect. “Infants” had less damage that was not correlated to seizures while “toddlers” had extensive damage positively correlated to seizure duration. In “toddlers” the unilateral pattern was only observed in those with seizures lasting over one hour. Prolonged seizures appear to drive the damage in the tissue that already compromised with hemorrhage, focal trauma, and mass effect. A limitation of the present study is the variation in EEG acquisition among subjections. Early in the study, only scalp EEG was available and was recorded for a short duration of time, and therefore, heart rate above baseline was used as a proxy for seizure duration and was validated in subjects with epidural EEG. Future work will characterize ictal and interictal EEG signatures to determine if interictal electrographic activity drives

damage as ictal events are observed to do. Future studies can aim to determine if seizures are preferentially focal in “toddlers” vs. “infants”. Additionally, investigation of whether mediators of seizure-induced blood brain barrier opening are age-dependent is ongoing.

As detected via amyloid precursor protein (APP), children that died from AHT displayed traumatic axonal injury as well as a geographic pattern that is hypothesized to indicate metabolic stress/hypoxic ischemia but did not have diffuse axonal injury (Geddes et al., 2001a; Shannon et al., 1998; Hefter and Draguhn, 2017). Here, we demonstrate a similar pattern of overlap with hypoxic-ischemic injury. Diffuse axonal injury, which is created by large angular acceleration forces, is not required for extensive hypoxic-ischemic type damage. APP may serve as a proxy for hypoxic-ischemic type damage as the percentage of the hemisphere damaged (via visualization of H&E staining), though in some subjects, APP was more prominent in the white matter and did not overlap with damaged gray matter.

Work is currently underway to identify clinically relevant biomarkers that may serve as correlates to damage and indicate therapeutic efficacy both in this model and in human patients. Studies of permeability, perfusion and diffusion changes using MRI may identify early indicators of evolving tissue damage. Quantification of electrographic seizures, interictal spikes, periodic lateralized epileptiform discharges, and delta waves and their potential correlation to cortical tissue damage is underway. A substantial advantage of this severe TBI model is the ability to directly test if induced electrographic seizures drive tissue damage. Patients with severe TBI often have seizures, but if it not known if seizures drive the tissue damage or if they are a symptom of the tissue damage. The efficacy of peripheral serum biomarkers of neuronal damage (neuron specific enolase) and hemorrhage (matrix metalloproteinases) to indicate hemispheric damage at 24 h post-injury is also being tested (Berger et al., 2017; Costine et al., 2012; Price et al., 2018; Price et al., 2019).

5. Conclusion

In this model of severe TBI scaled to brain size, the clinical status and pattern of damage was age-dependent. “Infants” were clinically more severely impaired than “toddlers”. While both ages had equivalent durations of induced seizure, “infants” had less damage than “toddlers”. In this multi-factorial, severe injury model of severe brain injury the extensive damage required the “perfect storm” of hemorrhage pattern and prolonged seizures. “Infants” appear to have endogenous mechanisms that calm the storm, reducing damage. “Toddlers”, however, have pathophysiologic mechanisms that tend to limit the damage, though profound, to one hemisphere. Future work will evaluate age-dependent differences in the mediators of seizure and hemorrhage-induced opening of the blood brain barrier and determine if interictal electrographic signatures are correlated with tissue damage. Understanding the downstream mediators of blood brain barrier opening may serve as therapeutic target in subjects at a stage of development when status epilepticus is difficult to treat. Current anti-epileptic drug guidelines are not specific to TBI but are applied to status epilepticus induced by any cause for children of any age (Sauro et al., 2016). Future research may inform broader critical care management guidelines. This model can be used as a platform for testing of both therapeutics and clinical management of severe TBI in the pediatric intensive care unit.

Acknowledgments

This work is supported by NICHD grant K01HD083759 and R01HD099397 to BCB. The authors express their gratitude to Eleanor Crawford, Andrew Bourque, Kyle Vynorious, Jacqueline Andrews, Madeline Perlewitz, Zoe Silsby, Scott Henderson, Natalie Escobar, Carla Fortes-Monteiro, Madeline Karsten, Luis Fernando Martinez Ramirez, and Anna Akkara for their assistance with animal surgeries, tissue sectioning, and map analysis.

Abbreviations:

AHT	abusive head trauma
APP	amyloid precursor protein
CT	computed tomography
EEG	electroencephalogram
H&E	hematoxylin and eosin
HR	heart rate
SAH	subarachnoid hemorrhage
SDH	subdural hematoma

References

Berger RP, Pak BJ, Kolesnikova MD, Fromkin J, Saladino R, Herman BE, et al., 2017. Derivation and validation of a serum biomarker panel to identify infants with acute intracranial hemorrhage. JAMA Pediatr. 171 (6), e170429. [PubMed: 28395008]

Bjorkman ST, Miller SM, Rose SE, Burke C, Colditz PB, 2010. Seizures are associated with brain injury severity in a neonatal model of hypoxia-ischemia. Neuroscience. 166 (1), 157–167. [PubMed: 20006975]

Boon P, Vonck K, van Rijckevorsel K, El Tahry R, Elger CE, Mullatti N, et al., 2015. A prospective, multicenter study of cardiac-based seizure detection to activate vagus nerve stimulation. Seizure. 32, 52–61. [PubMed: 26552564]

Clancy RR, Legido A, Lewis D, 1988. Occult neonatal seizures. Epilepsia. 29 (3), 256–261. [PubMed: 3371282]

Coats B, Binenbaum G, Smith C, Peiffer RL, Christian CW, Duhaime AC, et al., 2017. Cyclic head rotations produce modest brain injury in infant piglets. J. Neurotrauma 34 (1), 235–247. [PubMed: 26953505]

Costine B, Quebeda-Clerkin P, Dodge CP, Harris BT, Hillier SC, Duhaime AC, 2012. Neuronal specific enolase, but not S100B nor myelin basic protein, increases in peripheral blood corresponding to lesion volume after cortical impact in piglets. J. Neurotrauma 29 (17), 2689–2695. [PubMed: 22867012]

Costine B, Missios S, Taylor SR, McGuone D, Smith CM, Dodge CP, et al., 2015. The subventricular zone in the immature piglet brain: anatomy and exodus of neuroblasts into white matter after traumatic brain injury. Dev. Neurosci 37, 115–130. [PubMed: 25678047]

Costine-Bartell BA, McGuone D, Price G, Crawford E, Keeley KL, Pareja JM, et al., 2019a. Development of a model of unilateral hemispheric hypodensity (“Big Black Brain”). J. Neurotrauma 36 (5), 815–833. [PubMed: 30039743]

Costine-Bartell BA, McGuone Declan, Price George, Crawford Eleanor, Keeley Kristen L., Munoz-Pareja Jennifer, Dodge Carter P., Staley Kevin, Duhaime Ann-Christine, 2019b. Development of a model of unilateral hemispheric hypodensity. J. Neurotrauma 36, 815–833. [PubMed: 30039743]

Cudna A, Jopowicz A, Mierzejewski P, Kurkowska-Jastrzebska I, 2017. Serum metalloproteinase 9 levels increase after generalized tonic-clonic seizures. Epilepsy Res. 129, 33–36. [PubMed: 27886560]

Dias MS, Backstrom J, Falk M, Li V, 1998. Serial radiography in the infant shaken impact syndrome. Pediatr. Neurosurg 29 (2), 77–85. [PubMed: 9792961]

Dubey D, McRae PA, Rankin-Gee EK, Baranov E, Wandrey L, Rogers S, et al., 2017. Increased metalloproteinase activity in the hippocampus following status epilepticus. Epilepsy Res. 132, 50–58. [PubMed: 28292736]

Duhaime AC, Durham SR, 2007. Traumatic brain injury in infants: the phenomenon of subdural hemorrhage with hemispheric hypodensity ("big black brain"). Prog. Brain Res 161, 293–302. [PubMed: 17618985]

Duhaime AC, Bilaniuk L, Zimmerman R, 1993. The "big black brain": radiographic changes after severe inflicted head injury in infancy. J. Neurotrauma 10 (Suppl. 1), S59.

Duhaime AC, Gennarelli LM, Yachnis A, 1994. Acute subdural hematoma: is the blood itself toxic? J. Neurotrauma 11 (6), 669–678. [PubMed: 7723066]

Duhaime AC, Christian C, Moss E, Seidl T, 1996. Long-term outcome in children with the shaking-impact syndrome. Pediatr. Neurosurg 24, 292–298. [PubMed: 8988494]

Duhaime AC, Christian CW, Rorke LB, Zimmerman RA, 1998. Nonaccidental head injury in infants - the "shaken baby syndrome". New Engl. J. Med 338, 1822–1829. [PubMed: 9632450]

Duhaime AC, Margulies SS, Durham SR, O'Rourke MM, Golden JA, Marwaha S, et al., 2000. Maturation-dependent response of the piglet brain to scaled cortical impact. J. Neurosurg 93 (3), 455–462. [PubMed: 10969944]

Duhaime A-C, Hunter JV, Grate LL, Kim A, Golden J, Demidenko E, et al., 2003. Magnetic resonance imaging studies of age-dependent responses to scaled focal brain injury in the piglet. J. Neurosurg 99, 542–548. [PubMed: 12959443]

Durham SR, Duhaime AC, 2007. Maturation-dependent response of the immature brain to experimental subdural hematoma. J. Neurotrauma 24 (1), 5–14. [PubMed: 17263666]

Durham SR, Raghupathi R, Helfaer MA, Marwaha S, Duhaime AC, 2000. Age-related differences in acute physiologic response to focal traumatic brain injury in piglets. Pediatr. Neurosurg 33 (2), 76–82. [PubMed: 11070433]

Finnie JW, Blumbergs PC, Manavis J, Turner RJ, Helps S, Vink R, et al., 2012. Neuropathological changes in a lamb model of non-accidental head injury (the shaken baby syndrome). J. Clin. Neurosci 19 (8), 1159–1164. [PubMed: 22705132]

Foster KA, Recker M, Lee P, Bell MJ, Tyler-Kabara E, 2014. Factors associated with hemispheric hypodensity after subdural hematoma following abusive head trauma in children. J. Neurotrauma 31 (19), 1625–1631. [PubMed: 24693985]

Geddes JF, Hackshaw AK, Vowles GH, Nickols CD, Whitwell HL, 2001a. Neuropathology of inflicted head injury in children. II. Microscopic brain injury in infants. Brain 124, 1299–1306. [PubMed: 11408325]

Geddes JF, Vowles GH, Hackshaw AK, Nickols CD, Scott IS, Whitwell HL, 2001b. Neuropathology of inflicted head injury in children. II. Microscopic brain injury in infants. Brain. 124 (Pt 7), 1299–1306. [PubMed: 11408325]

Gennarelli TA, Adams JH, Graham DI, 1981. Acceleration induced head injury in the monkey.I. The model, its mechanical and physiological correlates. Acta Neuropathol. Suppl 7, 23–25. [PubMed: 6939241]

Gennarelli TA, Thibault LE, Adams JH, Graham DI, Thompson CJ, Marcincin RP, 1982. Diffuse axonal injury and traumatic coma in the primate. Ann. Neurol 12 (6), 564–574. [PubMed: 7159060]

Giardano A, Christian C, Giardano E, 1997. A Practical Guide to the Evaluation of Child Physical Abuse and Neglect. Sage, Thousand Oaks, CA.

Gilles EE, Nelson MD Jr., 1998. Cerebral complications of nonaccidental head injury in childhood. Pediatr. Neurol 19 (2), 119–128. [PubMed: 9744631]

Glykys J, Dzhala V, Egawa K, Kahle KT, Delpire E, Staley K, 2017. Chloride dysregulation, seizures, and cerebral edema: a relationship with therapeutic potential. Trends Neurosci. 40 (5), 276–294. [PubMed: 28431741]

Goasdoue K, Chand KK, Miller SM, Lee KM, Colditz PB, Wixey JA, et al., 2019. Seizures are associated with blood-brain barrier disruption in a piglet model of neonatal hypoxic-Ischaemic encephalopathy. Dev. Neurosci 1–16.

Hampel KG, Vatter H, Elger CE, Surges R, 2015. Cardiac-based vagus nerve stimulation reduced seizure duration in a patient with refractory epilepsy. Seizure. 26, 81–85. [PubMed: 25799907]

Hefter D, Draguhn A, 2017. APP as a protective factor in acute neuronal insults. Front. Mol. Neurosci 10, 22. [PubMed: 28210211]

Ibrahim NG, Ralston J, Smith C, Margulies SS, 2010. Physiological and pathological responses to head rotations in toddler piglets. J. Neurotrauma 27 (6), 1021–1035. [PubMed: 20560753]

Kahle KT, Staley KJ, Nahed BV, Gamba G, Hebert SC, Lifton RP, et al., 2008. Roles of the cation-chloride cotransporters in neurological disease. Nat. Clin. Pract. Neurol 4 (9), 490–503. [PubMed: 18769373]

Khan NR, Fraser BD, Nguyen V, Moore K, Boop S, Vaughn BN, et al., 2017. Pediatric abusive head trauma and stroke. J. Neurosurg. Pediatr 20 (2), 183–190. [PubMed: 28574318]

Missios S, Harris BT, Dodge CP, Simoni MK, Costine BA, Lee YL, et al., 2009. Scaled cortical impact in immature swine: effect of age and gender on lesion volume. J. Neurotrauma 26 (11), 1943–1951. [PubMed: 19469691]

Nitecka L, Tremblay E, Charton G, Bouillot JP, Berger ML, Ben-Ari Y, 1984. Maturation of kainic acid seizure-brain damage syndrome in the rat. II. Histopathological sequelae. Neuroscience. 13 (4), 1073–1094. [PubMed: 6527790]

Price G, Li M, McGuone D, Shen J, Escobar N, Bourque A, et al., 2018. Amyloid precursor protein, matrix metalloproteinase-9, and albumin in a multi-injury, unilateral model of SDH-associated TBI. National Neurotrauma Annual Meeting. 35 (16), A91.

Price G, Li M, McGuone D, Shen J, Lok J, Staley K, et al., 2019. Developmental regulation of matrix metalloproteinase-9 may blunt the spread of damage in a severe, multi-injury, unilateral model of SDH-associated TBI. J. Neurotr. 35th Ann. Natl Neurotr. Symp 36 (13), A97.

Sauro KM, Wiebe S, Dunkley C, Janszky J, Kumlien E, Moshe S, et al., 2016. The current state of epilepsy guidelines: a systematic review. Epilepsiax. 57 (1), 13–23.

Shannon P, Smith CR, Deck J, Ang LC, Ho M, Becker L, 1998. Axonal injury and the neuropathology of shaken baby syndrome. Acta Neuropathol. 95 (6), 625–631. [PubMed: 9650755]

Shaver EG, Duhaime AC, Curtis M, Gennarelli LM, Barrett R, 1996. Experimental acute subdural hematoma in infant piglets. Pediatr. Neurosurg 25 (3), 123–129. [PubMed: 9144710]

Takahashi T, Shirane R, Sato S, Yoshimoto T, 1999. Developmental changes of cerebral blood flow and oxygen metabolism in children. Am. J. Neuroradiol 20 (5), 917–922. [PubMed: 10369366]

Timaru-Kast R, Meissner A, Heimann A, Hoelper B, Kempski O, Alessandri B, 2008. Acute subdural hematoma in pigs: role of volume on multiparametric neuromonitoring and histology. J. Neurotrauma 25 (9), 1107–1119. [PubMed: 18771396]

Tremblay E, Nitecka L, Berger ML, Ben-Ari Y, 1984. Maturation of kainic acid seizure-brain damage syndrome in the rat. I. Clinical, electrographic and metabolic observations. Neuroscience. 13 (4), 1051–1072. [PubMed: 6527789]

Wirrell EC, Armstrong EA, Osman LD, Yager JY, 2001. Prolonged seizures exacerbate perinatal hypoxic-ischemic brain damage. Pediatr. Res 50 (4), 445–454. [PubMed: 11568286]

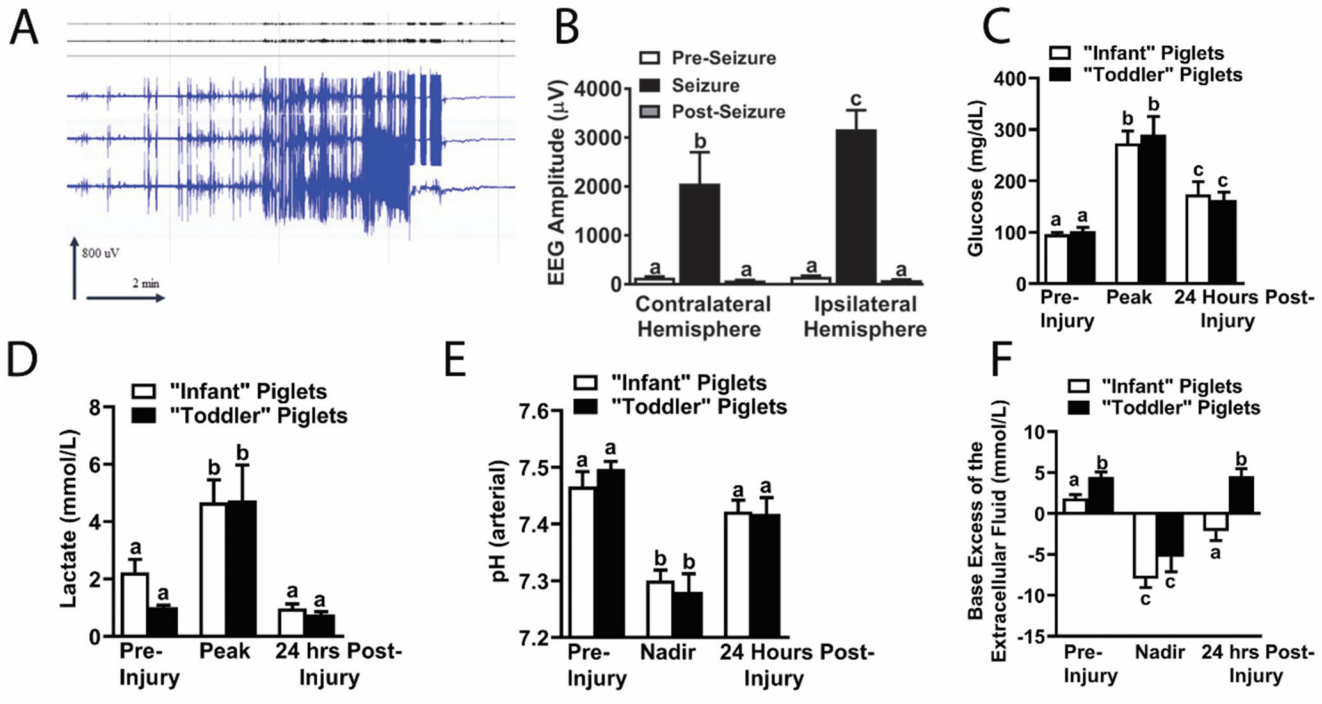

Fig. 1.

The model injuries caused focal seizures and metabolic acidosis. **A**. A kainic acid-induced seizure that was focal to the ipsilateral hemisphere (blue; contralateral hemisphere: black). **B**. EEG amplitude was greater in the ipsilateral vs. contralateral hemisphere (ages combined) during seizures. Model injuries increased peripheral blood glucose concentrations (**C**) and caused metabolic acidosis (**D**–**F**) in both ages. By 24 h post-injury, metabolic acidosis resolved in “toddlers” but not “infants” (**F**). [a,b,c]Means ± SEM with different letters differed, $P < 0.05$.

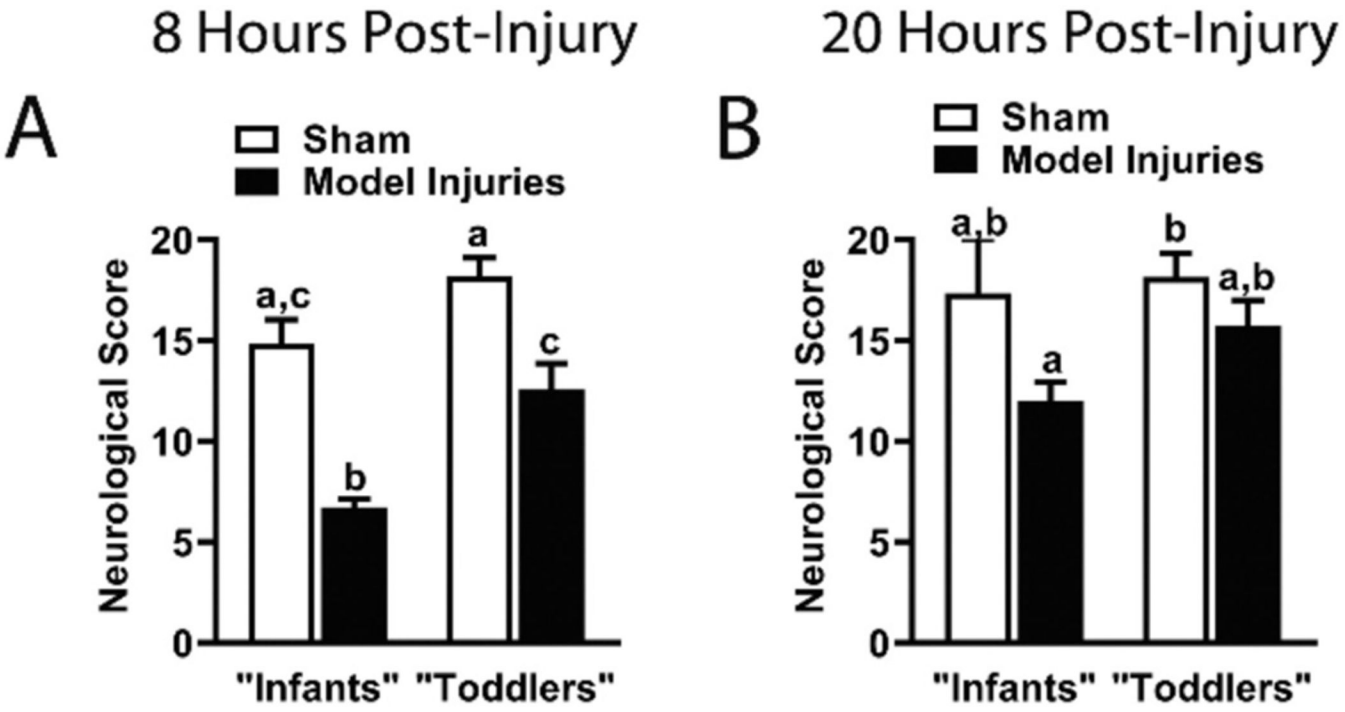

Fig. 2.

Model injuries reduced neurologic scores that were worse in “infants” than “toddlers”. **A**. At 8 h post-injury, piglets with model injuries had lower neurological scores than their sham counterparts (subjected to anesthetics, burr hole, and femoral artery line) in both ages and was lower in “infants” vs. “toddlers”. **B**. At 20 h post-injury, there was still a main effect of injury ($P = 0.01$) on neurological score, but neurological score was no longer different within ages, model injuries vs. sham. [a,b,c]Means ± SEM with different letters differed, $P < 0.05$.

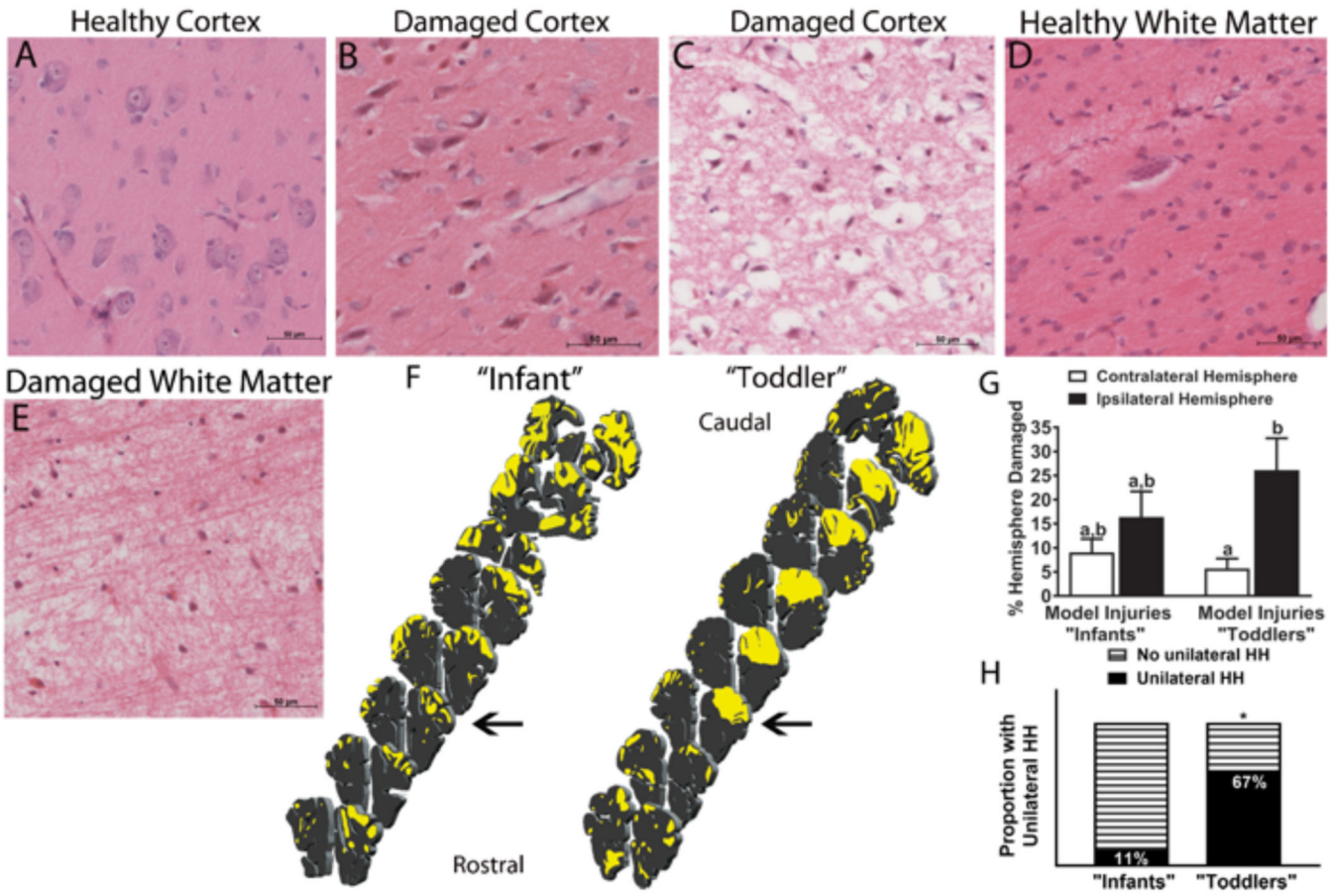

Fig. 3.

Hempsheric hypodensity model injuries cause wide-spread bilateral damage in “infants” and unilateral hemispheric damage in “toddlers”. **A**. Healthy cortex. **B**. Hypoxic-ischemic-type damage with “red neurons”. **C**. Severehypoxic-ischemic-type damage displaying vacuolization of the neuropil and blood vessels **D**. Healthy white matter. **E**. Damaged white matter demonstrating pallor and rarefaction. **F**. Schematics of each section rostral to caudal in an “infant” and “toddler”. Damaged tissue is highlighted in yellow. Both piglets had unilateral SDH on the ipsilateral hemisphere (arrow). Damage expanded beyond the cortical impact site (arrows indicate the section where the rostral gyrus was impacted) and was diffuse in the “infant” while mainly restricted the ipsilateral hemisphere with spreading in the cortex rostral to caudal in the “toddler”. **G**. There was a main effect of hemisphere ($P = 0.005$) with “toddlers” having greater amount of damage ipsilateral to the injuries vs. contralateral ($P = 0.02$); in contrast, the percent of the hemisphere damaged was not different ipsilateral vs. contralateral in “infant” piglets ($P = 0.67$) **F**. “Toddlers” had a greater proportion of piglets displaying unilateral hemispheric hypodensity-type injury (HH) than “infants”. *Proportion is different, $P = 0.049$. ^{ab}Means ± SEM with different letters differ, $P < 0.05$.

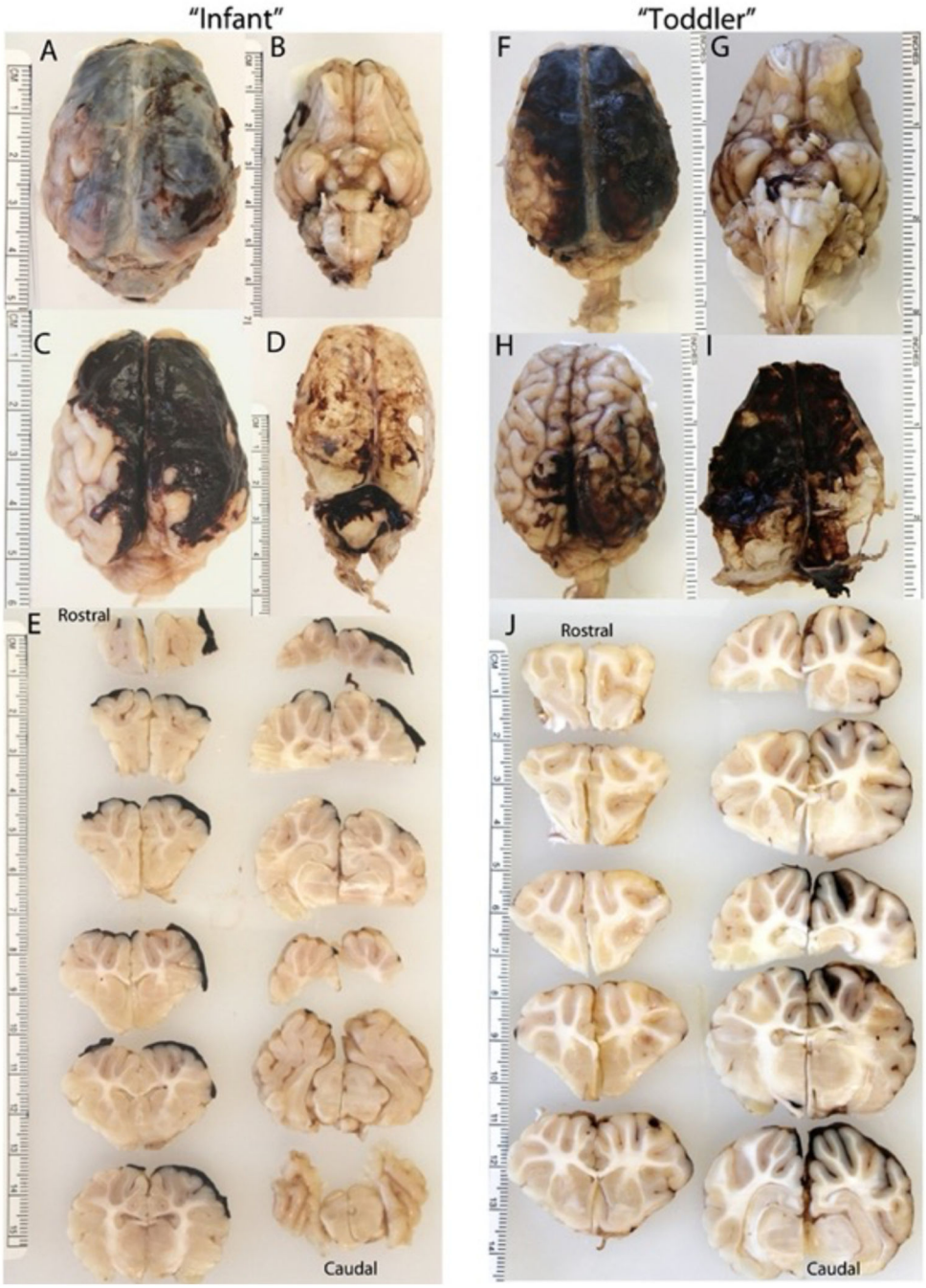

Fig. 4.

Distribution of SDH and SAH in "toddlers" and "infants". **"Infant brain": A**. Brain with dura. **B**. Ventral view of brain. **C**. Thick SDH on the brain. **D**. Ventra Ventral view of dura with subdural blood. **E**. Coronal sections demonstrate SDH with minimal SAH. **"Toddler brain": F**. Brain with dura. **G**. Ventral view of brain. **H**. Brain with dura and SDH removed demonstrating diffuse SAH on the right hemisphere. **I**. dura Ventral view of dura with the SDH. **J**. Coronal sections demonstrate SDH with extensive SAH and "dusky" cortex.

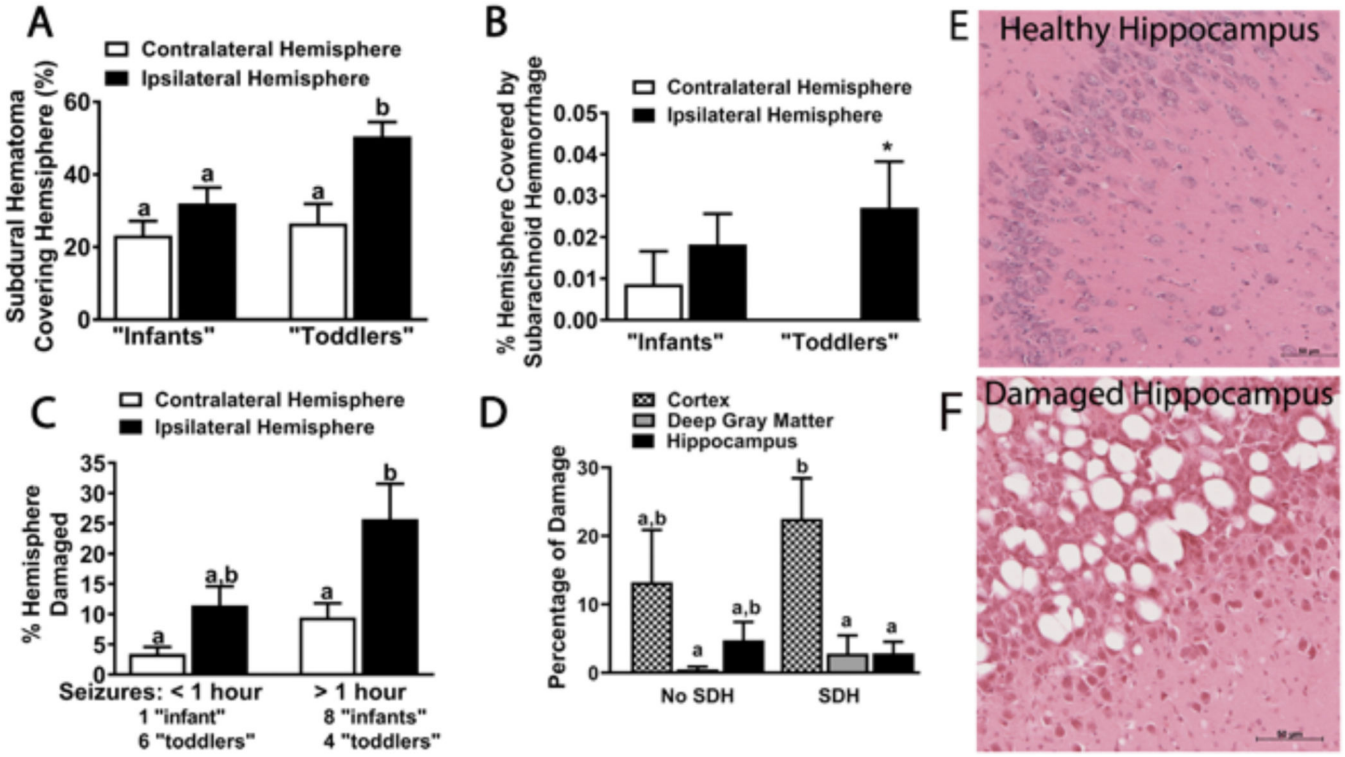

Fig. 5.

Hemorrhage distribution and seizure duration effects damage distribution. **A**. The placed SDH covered a larger proportion of the brain in “toddlers” (main effect of age, $P = 0.02$) and was greater ipsilateral vs. contralateral hemisphere ($P = 0.004$) in “toddlers” but was equivocal among hemispheres in “infant” piglets ($P = 0.47$). **B**. SAH was greater in the ipsilateral vs. contralateral hemisphere (main effect, $P = 0.03$) and tended to be greater ipsilateral vs. contralateral within “toddlers” ($P = 0.08$) but not in “infants” ($P = 0.8$). **C**. Subjects were binned according to seizure duration. The percent of the hemisphere damaged was greater in the ipsilateral vs. contralateral hemisphere only in those with seizures longer than 1 h. **D**. Within “infants”, SDH may direct damage to the cortex as damage was equivocal among brain regions in those without a SDH, but greater in the cortex vs. deep gray matter and hippocampus in those with a SDH. In piglets with a SDH, the hippocampus was not damaged (**E**), but was extensively damaged in those without a SDH (**F**). ^{ab}Means ± SEM with different letters differ, $P < 0.05$. *Tended to be different from contralateral hemisphere in “toddlers”, $P = 0.08$.

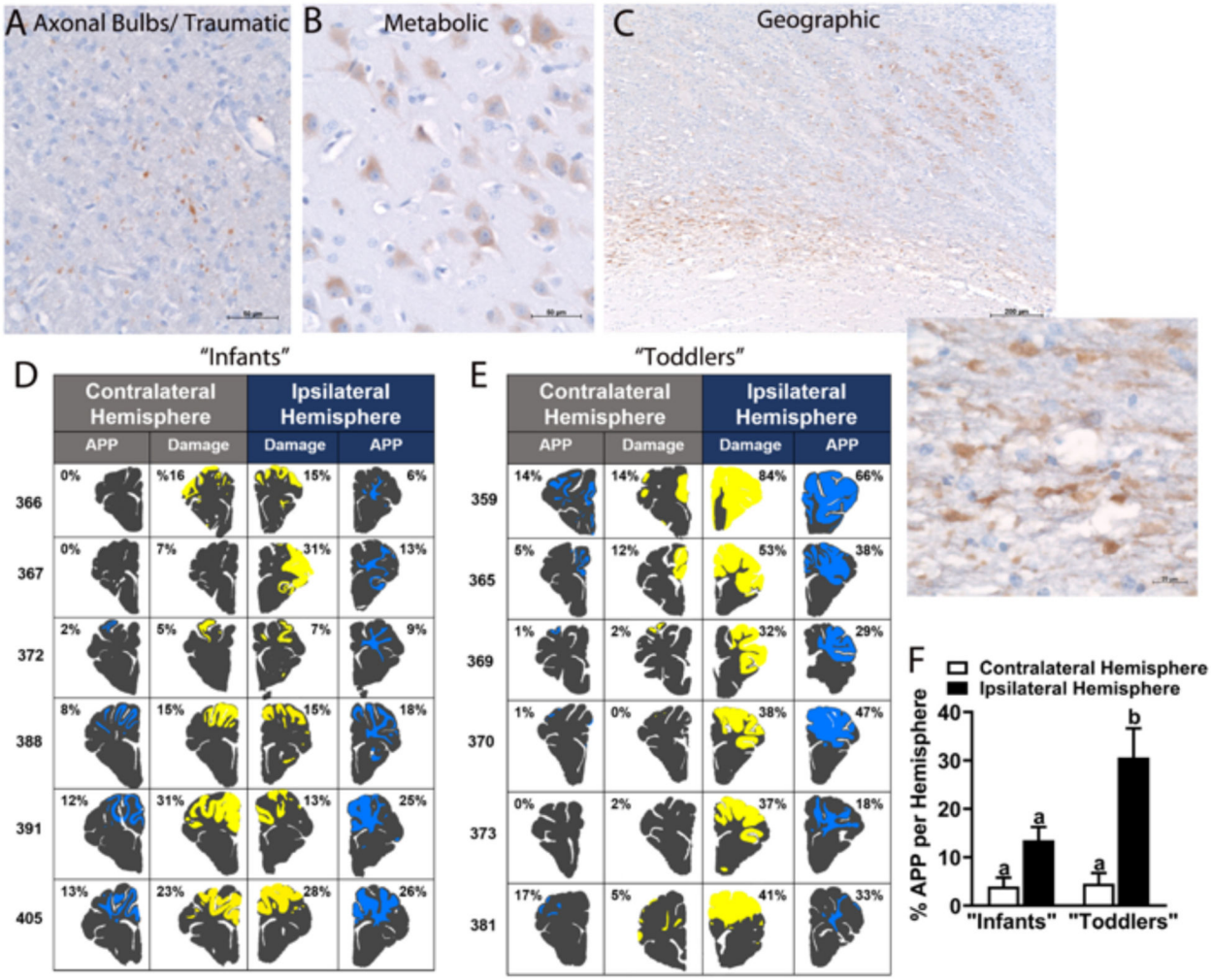

Fig. 6.

Distribution of amyloid precursor protein (APP) compared to damage. APP revealed axonal bulbs from traumatic injury (blue, **A**), overlapped with damage in a metabolic pattern (**B**), and in the severe cases, was geographic/vascular spanning large regions in deep gray matter (**C, C inset**; 359 ipsilateral). “Infants” (**D**) or “toddlers” (**E**) demonstrate different total APP pattern (blue) vs. damage patterns (yellow) in individual subjects. Table displays APP and damage via H&E in individual subjects contralateral or ipsilateral to the focal injuries. Some APP overlapped with damage patterns and some APP extended in white matter tracts beyond the area of damage visible with H&E. **F**. The percentage of the hemisphere positive for APP was a similar pattern as the damage pattern with ipsilateral APP being greater than contralateral in “toddlers” but not “infants”. ^{ab}Means ± SEM with different letters differ, $P < 0.05$.

Table 1

Details of anesthetic doses and experimental injuries and insults with age-specific difference in dose or scaling.

Parameter	"Infants" (1-week old)	'Toddlers" (1-month old)
Analgesic dose	5 μg/kg bolus buprenorphine	10 μg/kg bolus buprenorphine
Seizure permissive anesthesia	Dexmedetomidine (5–20 μg/kg/h), Morphine (1.75 mg/kg/h), Chlorpromazine (0.5–1 mg/kg bolus, to effect)	Dexmedetomidine (10–30 μg/kg/h), Morphine (3.5 mg/kg/h), Chlorpromazine (1–2 mg/kg bolus, to effect)
Mass effect: 5% of brain volume	2.25 mL balloon	2.7 mL balloon
Subdural Hematoma: 10% of brain volume	4.5 mL blood (Durham and Duhaime, 2007)	5.4 mL blood (Durham and Duhaime, 2007)
Cortical impact: displace 1% of brain volume	The indentor was 1.04 cm in diameter and indented to a depth of 4.8 mm (Durham et al., 2000).	The indentor was 1.07 cm in diameter and indented to a depth of 5.9 mm (Duhaime et al., 2000).
Epileptic agent	Kainic acid (42–84 μg/kg) injected into the cortex or mixed with subdural blood.	
Apnea	1 round for 1 min	
Hypoventilation	1 round for 10 min	

Table 2

Number of piglets assigned to the experiment, exclusions, and route of kainic acid administration.

Characteristic	"Infant" Sham	"Toddler" Sham	"Infant" Injured	"Toddler" Injured
Total	4	4	13	10
Excluded	1 (anesthetic complications)	0	4 (1 early death, 1 ripped dura, 2 intraparenchymal hemorrhage)	1 (Intraparenchymal hemorrhage)
Total included in the experiment	3	4	9	9
Intracortical kainic acid (included in experiment)	0	0	2	4
Kainic acid with SDH blood only (included in experiment)	0	0	7	5

Table 3

Clinical characteristics of piglets receiving model injuries.

Characteristic	Number “Infant” Injured (%)	Number “Toddler” Injured (%)
Epinephrine for hypotension	2 (14%)	2 (20%)
Chest compressions for cardiac arrest	1 (7%)	0 (0%)
Seizures stopped due to prolonged hypotension	3 (21%)	2 (20%)
Able to be extubated all night	0 (0%)	7 (70%) *
Early death (excluded from pathology)	1 (7%)	0 (0%)

* Percentage differs, $P = 0.002$. *Re*-intubations were due to spontaneous apnea, labored breathing or failure to remain oxygenated as determined by blood gas analysis.

Table 4

Correlation of subdural hemorrhage, subarachnoid hemorrhage and seizure duration to the percentage of damage in the ipsilateral hemisphere in “infants” vs. “toddlers”.

	“Infants”	**“Toddlers”**
Area of subdural hemorrhage	$P = 0.61$, $r = 0.13$	$P = 0.19$, $r = 0.34$
Area of subarachnoid hemorrhage	$P = 0.005$, $r = 0.63$ *	$P < 0.001$, $r = 0.87$ *
Seizure duration	$P = 0.178$, $r = 0.178$	$P = 0.012$, $r = 0.784$ *

* Correlation is significant $P < 0.05$.