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Direction-Dependent Responses To Traumatic Brain Injury In Pediatric Pigs

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Direction-Dependent Responses To Traumatic Brain Injury In Pediatric Pigs

Abstract
Traumatic brain injury (TBI) in children is a costly and alarmingly prevalent public health concern. Children (4-11 years of age) in the US have the highest rate of TBI-related emergency department visits. The plane of head rotation significantly affects neurocognitive deficits and pathophysiological responses such as axonal injury, but is largely ignored in TBI literature. In Chapter 1, an outline of existing research is provided, including the lack of attention to diagnosis, treatment, and prevention in children, who exhibit distinct biomechanical and neuropathological responses to TBI. Additionally, we hypothesize that the plane of head rotation in TBI induces a) region-specific changes in axonal injury, which lead to acute and chronic changes in electrophysiological responses; b) changes to event-related potentials and resting state electroencephalography (EEG) and c) tract-oriented strain and strain rate alterations in the white matter. All work in this dissertation is based on a well-established piglet model of TBI. In Chapter 2, we assess a novel rotational head kinematic metric, rotational work (RotWork), which incorporates head rotation rate, direction, and brain shape, as a predictor of acute axonal injury. This metric provides an improvement over existing metrics and could be useful in the development of effective child safety equipment used in recreation or transportation. In Chapter 3, we generate functional networks from auditory event-related potentials and use the patterns of change to distinguish injured brains from non-injured; the resulting algorithm showed an 82% predictive accuracy. In Chapter 4, we find elevations in network nodal strength, modularity and clustering coefficient after TBI across all frequency bands relative to baseline, whereas both metrics were reduced in shams. We report the first study using resting state EEG to create functional networks in relation to pediatric TBI, noting that this work may assist in the development of TBI biomarkers. In Chapter 5, we use a high-resolution finite element model to examine the effects of head rotation plane on the distribution of regional strains and strain rates. Sagittal rapid head rotations induced significantly larger volume fraction of damaged brainstem than axial and coronal rotations. We also found that local tissue deformation and histopathology were head direction- and region- dependent but poorly correlated at a local scale. Finally, in Chapter 6, we conclude that the work presented in this dissertation is novel and contributes valuable knowledge to the study of pediatric TBI, and that consideration of the plane of head rotation is critical to the understanding and accurate prediction of pediatric functional and region-dependent responses to TBI.

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This body of work is dedicated to my family, descendants of afro-Caribbean slaves, who have worked their way up the socio-economic ladder from the bottom. You have endowed me with the power to take on and complete this academic journey with strength and grace. I am especially grateful for your lessons of perseverance and sacrifice.

As the first person in my family to obtain a PhD and a racial minority, my dissertation work is a testament to the product of dreams, hard work and a little luck. For anyone who feels that the odds are stacked against them, I dedicate this work to you. Keep fighting, you can do it too!
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ABSTRACT

DIRECTION-DEPENDENT RESPONSES TO TRAUMATIC BRAIN INJURY IN PEDIATRIC PIGS

Lorre S. Atlan
Susan S. Margulies

Traumatic brain injury (TBI) in children is a costly and alarmingly prevalent public health concern. Children (4-11 years of age) in the US have the highest rate of TBI-related emergency department visits. The plane of head rotation significantly affects neurocognitive deficits and pathophysiological responses such as axonal injury, but is largely ignored in TBI literature. In Chapter 1, an outline of existing research is provided, including the lack of attention to diagnosis, treatment, and prevention in children, who exhibit distinct biomechanical and neuropathological responses to TBI. Additionally, we hypothesize that the plane of head rotation in TBI induces a) region-specific changes in axonal injury, which lead to acute and chronic changes in electrophysiological responses; b) changes to event-related potentials and resting state electroencephalography (EEG) and c) tract-oriented strain and strain rate alterations in the white matter. All work in this dissertation is based on a well-established piglet model of TBI. In Chapter 2, we assess a novel rotational head kinematic metric, rotational work (RotWork), which incorporates head rotation rate, direction, and brain shape, as a predictor of acute axonal injury. This metric provides an improvement over existing metrics and could be useful in the development of effective child safety equipment used in recreation or transportation. In Chapter 3, we generate functional networks from auditory event-related potentials and use the patterns of change to distinguish injured brains from non-injured; the resulting algorithm showed an 82% predictive accuracy. In
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CHAPTER 1:

ABSTRACT

To guide development of safety equipment that reduces sports-related head injuries, we sought to enhance predictive relationships between head movement and acute axonal injury severity. The severity of traumatic brain injury (TBI) is influenced by the magnitude and direction of head kinematics. Previous studies have demonstrated correlation between rotational head kinematics and symptom severity in the adult. More recent studies have demonstrated brain injury age- and direction- dependence, relating head kinematics to white matter tract-oriented strains (TOS). Now, we have developed and assessed novel rotational head kinematic parameters as predictors of white matter damage in the female, immature piglet. We show that many previously published rotational kinematic injury predictor metrics poorly predict acute axonal pathology induced by rapid, non-impact head rotations; and that inclusion of cerebral moments of inertia (MOI) in rotational head injury metrics refines prediction of diffuse axonal injury following rapid head rotations for two immature age groups. Rotational Work (RotWork) was the best significant predictor of traumatic axonal injury in both newborn and pre-adolescent piglets following head rotations in the axial, coronal and sagittal planes. An improvement over current metrics, we find that RotWork, which incorporates head rotation rate, direction and brain shape, significantly enhanced acute traumatic axonal injury prediction. For similar injury extent, the RotWork threshold is lower for the newborn piglet than the pre-adolescent.

INTRODUCTION

Traumatic Brain Injury (TBI) is a significant problem in the US that affects 1.7 million people each year and costs an estimated $221 billion annually in acute and chronic care
(Coronado et al., 2012). Children aged 0-4 years have the highest rate of TBI-related emergency department visits (1,256 per 100,000 population) (Faul et al., 2010, Cheng et al., 2016). Non-accidental and accidental events, such as falls and motor vehicle crashes, cause high childhood mortality, morbidity and disability. Bryan and colleagues estimate that 1.1 million to 1.9 million U.S. children and adolescents (under the age of 18 years) sustain sports and recreation-related concussions every year (Bryan, et al., 2016). TBIs may be sustained during falls or collisions while engaging in sports, recreational activities, such as bicycling, or on the playground. The majority of these children were not seen in health care settings, indicating that the number of pediatric TBIs may be greatly underestimated. A recent study of pediatric (0-17 years old) concussions diagnoses from electronic health records acquired over the 4 years reported that four out of five children were diagnosed at a primary care site and that one-third of the children were under the age of 12 years (Arbogast et al., 2016). This underscores the need for better screening tools outside of primary care practice sites (such as in emergency departments and schools) for children under 12 years. Although TBI is an active area of research, insufficient focus has been allocated towards the improvement of diagnosis, treatment and preventative measures specific to children, who exhibit distinct biomechanical and neuropathological responses to TBI compared to adults (Prange et al., 2002; Giza et al., 2007; Prins et al., 2003; Kochanek et al., 2010; Ibrahim et al., 2011). Increasingly, age-focused research is required in order to develop more effective diagnostic tools and therapies.

The use of pigs in neuroscience research has grown recently because the pig brain is similar to that of the human in anatomy and growth. Piglet aged 3-5 days, 4 weeks and 2 month old correspond to human infants, toddlers and pre-adolescents respectively in
brain development (Armstead, 2005). In addition to the similar rate of myelination and cerebral electrical activity development, piglets share similar cerebral hemodynamics and metabolism with humans. Furthermore, the pathophysiology of TBI in piglets compares well with that in human children (Armstead, 2000, 2005) due to similar gyral pattern, overall brain shape and distribution of gray and white matter (Buckley, 1986; Dickerson & Dobbing, 1967; Duhaime, 1998)

Head rotation direction significantly affects severity and outcome of axonal injury following head trauma. Our lab and other researchers have reported that head rotation direction significantly affects physiological and neurocognitive outcomes (Sullivan et al., 2013; Eucker et al., 2011; Ono et al., 1980; Gennarelli et al., 1982; Smith et al., 2000). Directional dependence of axonal injury has been reported in humans (Weaver et al., 2012), non-human primates (Gennarelli, 1982; Ommaya & Hirsch, 1971; Ommaya, Corrao, & Letcher, 1973), pigs (Browne, et al., 2011; Cullen et al., 2016) and piglets (Eucker et al., 2011; Ibrahim et al., 2010; Maltese, 2012).

In our neonatal piglet TBI model, we found that sagittal (SAG) rotations led to increased behavioral deficits, axonal injury (AI) and cerebral blood flow deficits in comparison to axial (AXI) rotations (Sullivan et al., 2013; Eucker et al., 2011). The time course of recovery from AI also varies with head rotation plane: AI at 6 hours post-injury by coronal (COR) head rotation was modest, but significantly elevated AI was noted at 6 hours post-SAG rotations (Eucker et al., 2011). In 3-5 day, 4 week and 2 month old piglets, coronal (COR) head rotations induce lower axonal injury volumes than axial (AXI) and sagittal (SAG) head rotations. SAG rotations led to increased behavioral deficits and axonal injury (AI) in comparison to AXI rotations (Sullivan et al., 2013). In the
3-5 day old and 2 month old pig, head rotations in the SAG plane induced longer durations of return to pinch reflex than sham, which was absent in the AXI and COR directions. Despite several reports of directional dependence in different species, the biomechanical mechanisms and neuropathological characterization of directional dependence are poorly understood, especially in the pediatric population which is distinctly vulnerable to TBI.

This dissertation seeks to address the critical issue of pediatric TBI by examining the effect of head rotation direction on the acute and chronic neuropathological and electrophysiological responses to injury in a pediatric animal model. Our central hypothesis is that head rotation direction significantly influences the regional deformation of brain tissue that in turn induces local axonal injury (Al) and neuronal synchrony dysfunction. This dissertation will examine the biomechanical and neuropathological responses to direction-dependent TBI in the pediatric population through use of a piglet TBI model via four specific aims:

Aim 1: To assess the novel rotational head kinematic metric, rotational work (RotWork), as a predictor of acute axonal injury.

Aim 2: To characterize the influence of injury via rapid head rotations about different planes on the cognitive processing of auditory stimuli at acute and long-term recovery time points in awake, immature piglets (4 weeks old).

Aim 3: To examine the effect of injury on resting state EEG at acute and chronic recovery time points in awake 4 week-old piglets.
Aim 4: To determine the effects of head rotation plane on the distribution of regional strains and strain rates using a high-resolution FEM of the immature pig brain.

Aim 1

The CDC reports that motor vehicle traffic-related crashes in the U.S. from 2006-2010 accounted for 29% of TBI-related deaths in 0-4 year olds and 55% (majority injury mechanism) in 5-14 years old children (CDC, 2017). An appropriately restrained 8-12 year old child has almost twice the head injury risk as a 1-3 year old child during frontal and side impact vehicular crashes (Kallan 2006). There is limited child-specific TBI tolerance data available for improved development of protective equipment, such as seat belts, airbags and helmets, for use during sports and transportation. This lack of child-specific data has led to the design of pediatric equipment based on the assumption that adults and children have equivalent injury tolerances (Kleinberger et al. 1998; Eppinger et al. 1999; Eppinger 2000; Ivarsson et al. 2004). There is a need to improve TBI prevention and protection measures for children. More extensive research into child-specific head injury risk criteria must be conducted in order to mitigate the harm and cost of brain injury in children.

Kinematic tolerances for diffuse axonal injury may be used to predict TBI risk and severity and determine biomechanical mechanisms behind TBI, where head kinematic measures (such as velocity and acceleration) are correlated with pathology. The ability to predict pediatric TBI from head kinematic parameters is necessary for the effective design of child safety equipment and for the accurate assessment of risk during recreation and transportation. Rotational kinematic metrics capture the relationship between the magnitude and/or rate of head rotational motion with risk of TBI. However,
the majority of previously published rotational kinematic metrics do not account for rotational direction–dependence of acute AI, which may lead to poor predictions of TBI risk. To mitigate brain injury in children, we will evaluate current and novel head rotational kinematic metrics for two pediatric populations of pigs (equivalent to the infant and pre-adolescent child). We hypothesize that RotWork will be larger in SAG rotations than AXI or COR for both age groups. Brain injury predictors with rotational moment of inertia will correlate more strongly with Axonal Injury Volume (AIV)

Aim 2

There are currently no child-specific assessments of TBI that are both non-invasive to the subject as well as independent of the administration environment, subject baseline, subject participation, and administrator bias. Clinicians may use balance, eye-tracking or neuropsychological tests, such as the Immediate Post-Concussion Assessment and Cognitive Test (ImPACT); however, there is little data to support the use of these tests to assess TBI in children aged 5-12 years (Davis et al., 2017). There are currently few biomarkers available for the diagnosis and prognosis of TBI in children (Davis et al., 2017), despite active research on the diagnostic capabilities of CSF or serum-based biomarkers for mild to severe TBI in adults (Berger et al., 2005; Nakhjavan-Shahraki, Yousefifard, Oraii, Sarveazad, & Hosseini, 2017; Papa et al., 2016; Paziana & Korley, 2015). CSF or serum-based biomarkers such as glial fibrillary acidic protein has been reported to detect head lesions in children 10 years and younger (Papa et al., 2016), but require invasive procedures to acquire. Subsequently, there is an urgent need for objective, non-invasive diagnostic tools to identify TBI in the young brain. Our second goal is to determine the effect of head rotation on neuropathological responses.
We hypothesize that SAG rotations will cause distinct changes in the synchrony across various brain regions compared to sham as well as COR and that head rotation about different planes will lead to acute (1 day) and chronic (7 days) changes in electrophysiological responses. We also hypothesize that rotational direction will influence both event-related potentials (ERPs) and resting state electroencephalography (EEG) in the piglet brain. Event-related potentials (ERPs) are cortical electrical responses to repeated sensory stimuli (as measured by scalp EEG electrodes). ERPs capture neural dynamics generated by cortical and sub-cortical neurons that occur during cognitive processing. The primary advantages of ERPs for the assessment of TBI in children are that ERPs do not require prior training of the subject, are involuntary (the subject cannot feign responses), are independent of subject verbal capability (and therefore will be equally effective in non-verbal subjects as in those who can communicate) and that they can be acquired quickly (under 30 minutes per subject). ERPs may provide the multimodal framework needed for age-appropriate diagnosis and prognosis of TBI.

Aim 3

EEG measure synaptic activity across the brain via scalp or depth electrodes. The temporal dynamics of EEG activity control information retention and integration across brain regions (Buzsáki, Anastassiou, & Koch, 2012). When the brain engages in certain functions, certain oscillations are dominant e.g. processing sensory stimuli, directing attention or orientating in space. There is also preservation of cortical oscillations across mammals (Buzsáki, Logothetis, & Singer, 2013). Functional Networks may be clinically relevant to the diagnosis of neurological diseases.
Hyperconnectivity has been observed in MEG, EEG, fMRI functional networks following TBI in adults (Hillary et al., 2011; 2014; 2015, Nunez, et al., 2015; Porter et al., 2017; Sharp et al., 2011). Up to the time of publication, there were no resting state EEG functional connectivity studies in children after TBI. There is also a paucity of EEG studies in pigs & piglets after TBI.

Resting state EEG measurements (using scalp electrodes) will elucidate the neural mechanisms and time course of recovery from diffuse brain injury. We hypothesize that hyperconnectivity of the resting state network will occur in the immature pig brain after TBI compared to sham.

Aim 4

Chronic (6 day) AI persistence was reported after SAG rotations in neonatal piglets, but was absent following AXI rotations despite SAG and AXI rotations presenting similar magnitudes of AI at 6 hours when angular velocity was similar (Sullivan et al., 2013). This discrepancy in AI may be explained by a higher density of AI after SAG rotations compared to AXI. Our current quantification methods of axonal histopathology may not be sensitive enough to detect region-specific differences in the numbers of injured axons per unit volume of brain tissue.

Finite element modelling (FEM) is a numerical technique that provides estimates of deformation at various regions throughout the brain. We will develop an anatomically accurate FEM of the 3-5 day old piglet with separately meshed gray and white matter regions. We will also assess the dependence of axonal injury density (AID) following rapid head rotation (at 6 hrs) in immature (3-5 day old) piglets on regional tract-oriented strains (TOS) and strain rates (TOSR). We hypothesize that regions of high local TOS
and TOSR will cause greater structural dysfunction among all three planes of head rotations, where SAG rotations result in the highest AID across all regions.

Furthermore, we hypothesize that tract-oriented strain (TOS) and strain rate (TOSR) profiles in the white matter will be altered by changing the plane and the velocity of head rotation.

The biomechanical causes of axonal injury (AI) across different brain regions following rapid head rotation about different planes are not well understood. The ability to predict pediatric TBI from head movement metrics during recreation and transportation is critical to the effective design of child safety equipment and the accurate assessment of brain injury risk. The electrophysiological response to head rotation direction in the immature piglet has not been previously characterized. This dissertation seeks to advance the field by presenting the kinematic, biomechanical and electrophysiological causes of the direction-dependence of AI following diffuse, mild TBI in the immature piglet. We will illustrate the necessity for considering head rotation direction when designing preventative TBI measures. We will develop and assess novel rotational head kinematic and electrophysiological metrics as predictors of acute axonal pathology in the immature brain that show validated TBI predictive capability in the pediatric population.


Ono et al. (1980). Human Head Tolerance to Sagittal Reliable Estimation deduced from experimental head injury using subhuman primates and human head cadaver skulls. 24th Stapp Car Crash Conference


Abstract

To guide development of safety equipment that reduces sports-related head injuries, we sought to enhance predictive relationships between head movement and acute axonal injury severity. The severity of traumatic brain injury (TBI) is influenced by the magnitude and direction of head kinematics. Previous studies have demonstrated correlation between rotational head kinematics and symptom severity in the adult. More recent studies have demonstrated brain injury age- and direction- dependence, relating head kinematics to white matter tract-oriented strains (TOS). Now, we have developed and assessed novel rotational head kinematic parameters as predictors of white matter damage in the female, immature piglet. We show that many previously published rotational kinematic injury predictor metrics poorly predict acute axonal pathology induced by rapid, non-impact head rotations; and that inclusion of cerebral moments of inertia (MOI) in rotational head injury metrics refines prediction of diffuse axonal injury following rapid head rotations for two immature age groups. Rotational Work (RotWork) was the best significant predictor of traumatic axonal injury in both newborn and pre-adolescent piglets following head rotations in the axial, coronal and sagittal planes. An improvement over current metrics, we find that RotWork, which incorporates head rotation rate, direction and brain shape, significantly enhanced acute traumatic axonal injury prediction. For similar injury extent, the RotWork threshold is lower for the newborn piglet than the pre-adolescent.
Introduction

The Center for Disease Control has designated traumatic brain injury (TBI) as a silent epidemic that affects as many as 1.9 million children aged ≤18 years annually in the United States (Bryan, et al., 2016; Faul, et al., 2010). The need for effective TBI prevention is underscored by the 1 billion USD cost of hospitalization and care (Schneier, et al., 2006). The ability to predict pediatric TBI from head kinematic parameters is necessary for the effective design of child safety equipment and for accurate assessment of brain injury risk during recreation and transportation. Motor vehicle traffic-related crashes account for the majority of TBI-related deaths in youth 0-14 years old in the United States (CDC Injury Center, 2016) and across the world (Diamond et al., 2009; Gawryszeski, 2007; Kypri et al., 2000; Wang et al., 2003; Wu et al., 2008).

Previous research has increased our understanding of the mechanisms behind TBI through computational simulations, analysis of helmet kinematics during sports games and animal models of TBI. Rotational head motion, as opposed to linear head motion, is widely believed to cause TBI by excessive shearing of brain tissue, which is a consequence of its low shear modulus and high bulk modulus (Gennarelli, 1994; Kimpara & Iwamoto, 2012; Ommaya, et al., 2002). Most published brain injury criteria use either rotational or linear head motion for brain injury prediction. Even fewer criteria, such as Head Impact Power (HIP) (Newman, et. al, 2000), utilize both linear and rotational components of motion.

Most TBI prediction metrics were formulated from unspecific, non-functional brain trauma severity classification systems. Previously published rotational injury predictors, such as Brain Injury Rotational Criteria (BRIC), were correlated to the head component...
of the Abbreviated Injury Scale (AIS), which is a general measure of the threat of injury to a subject’s life, rather than a direct and objective measure of the severity of brain injury. Several studies show that axonal damage strongly correlates to head injury severity (Benson et al., 2007; Johnson, et al., 2013; Sullivan et al., 2013)), whereas AIS and other injury severity assessments, such as Glasgow Coma Scale and Injury Severity Score, have been shown to poorly predict 12 month outcomes after TBI (Foreman et al., 2007). In order to better inform new injury predictors through increased accuracy of TBI severity measurement, we utilized immuno-histological assessment of axonal damage ≤ 6 hours TBI.

To relate head movements to axonal damage, sophisticated computational finite element models have been used to determine mechanistic underlying associations that relate head movements, tissue distortions and axonal injury. Finite element simulations of rapid head rotations in animals (Coats, et al., 2012; Maltese & Margulies, 2016; Sullivan et al., 2015) and humans (Post et al., 2015; Sahoo, et al., 2014; Weaver, et al., 2012; Zhao & Ji, 2015) demonstrate that high magnitude axonal tract strains strongly correlate with the direction of head rotation. Age, brain size and direction of head rotation (Eucker, et al., 2011; Ommaya, et al., 2002; Prange & Margulies, 2002; Sullivan et al., 2013) directly modulate brain injury risk. Of the rotational brain injury kinematic metrics, BRIC uses resultant rotational velocity and acceleration, which is independent of head rotation direction. The Revised Brain Injury Rotational Criteria (Revised BrIC) addresses directional rotational velocity sensitivity by including direction-specific critical thresholds of injury. In comparison to previously mentioned metrics, Power Rotational Head Injury Criterion (PRHIC36)(Kimpara & Iwamoto, 2012) and HIP include the head rotation direction by including moment of inertia (MOI) to capture directional and brain size
sensitivity of axonal injury (Eucker et al., 2011; S. Sullivan et al., 2015). MOI is the resistance to rotational motion about an axis (x, y or z).

In this study, we hypothesized that kinematic injury predictors that incorporate MOI, namely, Rotational Kinetic Energy (RotKE), Rotational Work (RotWork) and rate of RotKE (rKE), would provide improved correlation of acute axonal injury volume (AIV) in our animal model of diffuse TBI compared to previously published metrics. We evaluated these three novel rotational kinematic brain injury predictors and 8 published metrics for newborn and pre-adolescent pigs to identify those strongly correlated with volume of acute (6 hours post-injury) axonal injury assessed via histology. Histopathology from our most acute time point of 6 hours post injury limits the effects of secondary injury cascades that may not be directly caused by biomechanical forces that deform the brain during a rapid head rotation. We assumed that the histopathology detected at 6 hours would be due to the biomechanically damaged (stretched) axons instead of ischemic or hypoxic axons due to later (1 day post-injury) pathophysiological cascades. Using data obtained in two populations of piglets, representative of human infants and pre-adolescents, we examined the relationships between age, axonal injury and rotational kinematic metrics.

Methods
All studies were approved by the University of Pennsylvania Institutional Animal Care and Use Committees (IACUC). Previously published axonal injury and angular velocity data (Eucker et al., 2011; Sullivan et al., 2015) from 3-5 day old (n=32) and new data from 2 month old (n=17) female piglets sacrificed at 6 hours after TBI were analyzed in a novel manner for this publication. These piglet ages correspond to human infants (<2 years) and pre-adolescents (10-13 years) respectively in brain development (Armstead,
The use of pigs in neuroscience research has grown recently because the pig brain is similar to that of the human in anatomy (Lind et al., 2007), and piglets share similar cerebral hemodynamics and metabolism with humans (Buckley, 1986; Dickerson & Dobbing, 1967; Duhaime, 1998). The rate of myelination and cerebral electrical activity during development has been correlated with the human (Lind et al., 2007). Furthermore, the pathophysiology of TBI in piglets compares well with that in human children due to similar gyral pattern, overall brain shape and distribution of gray and white matter (Armstead, 2000, 2005)

Diffuse white matter injury was induced in juvenile female piglets using a well-characterized rotational acceleration device (Raghupathi & Margulies, 2002). A single non-impact, closed-head, rapid head rotation (12-20 ms) was applied in the purely coronal (COR), axial (AXI) or sagittal (SAG) plane, centered at the mid-cervical spine (Eucker et al., 2011). The angular velocity of head rotation was recorded at 10 kHz using an angular rate sensor (ATA Engineering, Model#: ars--06, Inc., Herndon, VA) attached to the linkage sidearm of HYGE pneumatic actuator (via data acquisition system in LabView, National Instruments, Austin, TX). A 10-point binomial smoothing filter was applied to all triangular-shaped velocity-time histories. The mean and standard deviation of the peak angular velocities in each direction measured in the 3-5 day and 2 month old are summarized in Table 2.1. During AXI and COR rotations, the piglet head rotated 90°, while SAG spanned 60° because of limited cervical spine flexibility in this direction and we excluded hyperflexion and hyperextension. Our SAG standard deviations are smaller in our previous study because we limited the range of infant piglet head velocity due to increased mortality in this direction at lower velocities than those used in COR or AXI. The means and standard deviations of peak absolute angular acceleration were for
2.96e+8 ± 1.48e+8 and 1.58e+6 ± 3.55e+5 rad/s² in the 5 day and 2 month old piglets respectively.

Our rapid head rotation injury model captures the short, high deceleration aspects of impact events, without a contact event. To confirm, we scaled our piglet angular velocities and accelerations (using Ommaya’s brain mass scaling laws) to that of three monkey species (rhesus, chimpanzee, and squirrel) presented in Ommaya’s studies comparing head kinematics from separate head acceleration and impact injuries (Ommaya et al., 1968, 1970; Ommaya & Hirsch, 1971; Ommaya et al., 1973). We observed that loading conditions for impact and whiplash associated with concussion overlap for all three nonhuman primate species. Furthermore, we note that when we scaled pig kinematics to each species’ brain mass, the pig kinematics overlap with both impact and whiplash loading conditions for all three primate species. Our HYGE injury kinematics encompass both impact and whiplash. We conclude that our porcine rapid head rotation injury model captures whiplash kinematics as well as the short, high deceleration aspects of impact events, without a contact event.

<table>
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<th>Coronal ( \text{VEL}_{\text{avg}} ) (rad/s)</th>
<th>Axial ( \text{VEL}_{\text{avg}} ) (rad/s)</th>
<th>Sagittal ( \text{VEL}_{\text{avg}} ) (rad/s)</th>
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<tr>
<td><strong>Newborn Pig</strong></td>
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<td>(average brain mass = 27.8 g)</td>
<td>199.81 ± 17.76 (n=6)</td>
<td>171.65 ± 29.03 (n=21)</td>
<td>158.66 ± 2.21 (n=5)</td>
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<tr>
<td><strong>Pre-adolescent Pig</strong></td>
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<tr>
<td>(average brain mass = 58.8 g)</td>
<td>170.72 ± 12.12 (n=5)</td>
<td>142.27 ± 18.47 (n=9)</td>
<td>126.08 ± 21.25 (n=3)</td>
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**Table 2.1:** Sample size, mean and standard deviations of average peak angular velocities for porcine subjects.

The staining, imaging and quantification procedures for immunohistochemical processing of piglets brains are detailed elsewhere (Eucker et al., 2011) and are summarized here. Six hours after injury, brains were perfusion-fixed with 10% neutral
buffered formalin for 24 hours. Fixed brains were weighed before being cut into serial coronal 3mm sections; from each section a thin slice (6 μm thickness) was examined for histopathology. Histopathological assessment of axonal damage was conducted by staining coronal slices for beta-amyloid precursor protein (βAPP). Positive βAPP immuno-staining indicates damaged axons with disrupted fast axonal transport. A neuropathologist blinded to the animal injury group then identified regions with > 2 positive βAPP-stained axonal profiles per field (scanning power 5-40x) in each slice. For every animal, the cumulative sum of positively stained regional area throughout all examined brain slices (6 μm thickness) yielded an AIV, expressed as a percent of total cerebral volume. AIV is individual-specific response.

In our new analysis, we sought to identify a biomechanical “biomarker” that related to acute axonal injury. We computed the MOI ($I_i$), or the resistance to rotational motion, for each age group using our previously published (Sullivan et al., 2015) finite element models of the piglet head in Abaqus Explicit v6.9-EF1 (Dassault Systèmes, Vélizy-Villacoublay, France). We did not use individual MOI measurements because brain mass within an age group was very consistent (coefficient of variation <10%); we did not obtain individual MRI images from each pig. Instead, we obtained one representative MRI for each age, and calculated the MOI from that image. For this new analysis, we determined the MOI (about the x-, y- and z- axes) with respect to the center of rotation for our typical 3-5 day old and 2 month old piglet. For the 3-5 day old piglet mesh, the MOI with respect to the mid-cervical spine about the x-axis (SAG rotations), y-axis (AXI rotations) and z-axis (COR rotations) were defined from Abaqus to be 81.57 gmm$^{-2}$, 61.68 gmm$^{-2}$ and 25.05 gmm$^{-2}$ respectively. The MOI (about the x-, y- and z- axes) for the 2 month old piglet brain mesh were determined to be 254.63 gmm$^{-2}$, 172.83 gmm$^{-2}$.
and 102.11 gmm\(^2\) respectively. Due to brain geometry, in both ages, piglet MOI was largest for SAG rotations, because the caudal-rostral (x) dimension is the largest in the pig brain, and smallest for COR rotations.

For each animal (n=32 newborn piglets, n=17 pre-adolescent piglets), the filtered angular velocity-time profiles were used to calculate eight previously published rotational head injury prediction metrics: peak angular velocity (VEL\(_i\)), peak angular acceleration (ACCEL\(_i\)), Rotational Injury Criterion (RIC36)\(^1\),(Kimpara & Iwamoto, 2012), Head Impact Power (HIP)\(^2\),(Newman et al., 2000), Brain Rotational Injury Criteria (BRIC)\(^3\),(Takhounts, et al., 2011), Revised Brain Rotational Injury Criteria (Revised BrIC)\(^3\),(Takhounts, et. al, 2013), Power Rotational Head Injury Criterion (PRHIC36)\(^4\),(Kimpara & Iwamoto, 2012) and Rotational Velocity Change Index (RVCI)\(^5\),(Yanaoka & Takahashi, 2015).

Peak Angular Velocity (VEL\(_i\)):

\[
VEL_i = \max(\omega_i(t)) \quad \text{(Equation 1)}
\]

where \(\omega_i(t)\) is the absolute value of the head’s angular velocity-time history from a rotation about axis i (e.g. x, y, or z).

Peak Angular Acceleration (ACCEL\(_i\)):

\[
ACCEL_i = \max(\alpha_i(t)) \quad \text{(Equation 2)}
\]

where \(\alpha_i(t)\) is the absolute value of the head’s rotational acceleration-time history from a rotation about axis i (e.g. x, y, or z). Angular acceleration was calculated by differentiating the angular velocity time history.

Rotational Injury Criterion (RIC36):

\[
RIC36 = \left[ (t_2 - t_1) \left\{ \frac{1}{(t_2-t_1)} \int_{t_1}^{t_2} \alpha_i(t) dt \right\}^{2.5} \right]_{\max} \quad \text{(Equation 3)}
\]

where \(t_2 - t_1 = 36\) ms and \(\alpha_i(t)\) is defined above \(^6\)(Kimpara & Iwamoto, 2012).

Head Impact Power (HIP):

\[
HIP = (\sum m \cdot a_i \cdot \int a_i(t)dt) + (\sum I_{ii} \cdot ACCEL_i \int \alpha_i dt) \quad \text{(Equation 4)}
\]
where \( m \) is mass of head (kg), \( a_i \) is linear acceleration (ms\(^2\)) and \( I_{ii} \) is MOI about axis \( i \) (kgm\(^2\)), where the skull is assumed to be rigid. Linear (tangential) accelerations from the center of mass to the center of rotation for each animal were used in the HIP calculation.

Brain Injury Rotational Criterion (BRIC):

\[
BRIC = \frac{\text{VEL}_i}{\omega_{cr}} + \frac{\text{ACCEL}_i}{\alpha_{cr}} \quad \text{(Equation 5)}
\]

where \( \omega_{cr} \) and \( \alpha_{cr} \) are newborn pig’s mass scaled critical angular velocity (415 rad/s) and acceleration (2.55e+5 rad/s\(^2\)) from diffuse axonal injury (DAI) threshold load values in the baboon as published in (Margulies & Thibault, 1992).

Revised Brain Injury Rotational Criterion (Revised BrIC):

\[
\text{Revised BrIC} = \sqrt{(\frac{\text{VEL}_x}{\omega_{xc}})^2 + (\frac{\text{VEL}_y}{\omega_{yc}})^2 + (\frac{\text{VEL}_z}{\omega_{zc}})^2} \quad \text{(Equation 6)}
\]

where \( \omega_{xc} = 330 \) rad/s, \( \omega_{yc} = 281 \) rad/s and \( \omega_{zc} = 213 \) rad/s for newborn pig’s mass scaled critical angular velocities in their respective directions based on the average of cumulative strain damage measure and max principal strain in simulated injury monitor from (Takhounts, et al., 2013).

Power Rotational Head Injury Criterion (PRHIC36):

\[
PRHIC36 = \left[ (t_2 - t_1) \left\{ \frac{1}{(t_2 - t_1)} \int_{t_1}^{t_2} (\sum l_{ii} \cdot a_i \int \alpha_i dt) \right\}^{2.5} \right]_{max} \quad \text{(Equation 7)}
\]

where the maximum integral time duration was set to 36 ms (Kimpara & Iwamoto, 2012). The contents under the initial integral correspond to rotational component of HIP.

Rotational Velocity Change Index (RVCI):

\[
RVCI = \left[ R_x \left( \int_{t_2}^{t_1} \alpha_x dt \right)^2 + R_y \left( \int_{t_2}^{t_1} \alpha_y dt \right)^2 + R_z \left( \int_{t_2}^{t_1} \alpha_z dt \right)^2 \right]_{max} \quad \text{(Equation 8)}
\]

where \( R_x, R_y \) and \( R_z \) are weighting factors about the x, y and z axes respectively and \( t_1 \) and \( t_2 \) are the initial and final times over a 10ms duration (Yanaoka & Takahashi, 2015). The Cumulative Strain Damage Measure weighting factors, \( R_x = 1 \), \( R_y = 2.29 \) and \( R_z = 1.98 \), were used because they were more appropriate for predicting diffuse injuries than weighting factors determined from correlation with Max Principal Strain (Yanaoka & Takahashi, 2015).
In addition to the eight previously published metrics, three novel metrics were calculated using the rotational MOI we identified. The novel head injury metrics calculated for each animal includes: rotational kinetic energy (RotKE), rotational work (RotWork) and rate of change of rotational kinetic energy (rKE), shown in Equations 9–11 respectively. Each piglet within an age group was assumed to have the same direction-specific MOI ($I_{ii}$).

RotKE, RotWork and rKE of the head during rotation may describe the global forces on the brain responsible for axonal injury through deformation of tissue.

$$RotKE = \frac{1}{2} I_{ii} VEL_i^2 \quad \text{(Equation 9)}$$

$$RotWork = \tau \theta = ACCEL_i I_{ii} \theta \quad \text{(Equation 10)}$$

$$rKE = \frac{1}{2} I_{ii} VEL_i^2 \frac{1}{t_{max}} \quad \text{(Equation 11)}$$

where $\theta$ is maximum change in angular displacement; $t$ is number of seconds to reach max angular velocity, $VEL_i$.

For each age group and metric, we performed separate linear regressions between each metric and AIV(R, version 3.2.4, 2016-03-10). Our objective was to identify all metrics with promising injury predictive capability (defined as $R^2 > 0.5$ with slopes greater than zero) and then determine which of these metrics are strong in both age groups. While we recognize that metrics may have some co-dependency (e.g. on acceleration), collinearities between metrics were not assessed. We evaluated outliers, and excluded all data greater than three standard deviations from the metric mean for that age cohort. We excluded one outlier pre-adolescent animal from the ACCEL, HIP, RVCI and rKE metrics and removed a single infant animal from the PRHIC36, HIP and RotWork metrics.
Results

Rotational Head Injury Metrics and Axonal Injury

We calculated the mean tangential accelerations: 23,499 ± 836 m/s² (infants) and 26,426 ± 1,287 m/s² (pre-adolescents). The mean and standard errors of angular accelerations were: 43,071 ± 13,018 rad/s² (infants) and 34,594 ± 8,608 rad/s² (pre-adolescents). For both ages, animals injured in the COR plane (Fig. 2.1, triangles) had relatively low AIV, while rotations in the AXI and SAG planes resulted in markedly higher AIV (Fig. 2.1, circles, squares respectively). Eight rotational kinematic metrics had positive and significant linear relationships with AIV in one or both ages (Table 2.2). Ellipses in Fig. 2.1 indicate 95% confidence intervals for these relationships, where stronger relationships (R²<0.5) are designated by gray-filled ellipses and are bolded in Table 2.2. Although Revised BrIC accounted for the effect of head rotation direction on head injury by weighting peak head velocities by critical threshold velocities, Revised BrIC did not correlate significantly with axonal injury. For all of the strong metrics shown in Fig. 2.1, all slopes in the newborn age group exceeded that of the pre-adolescent.
Figure 2.1: Axonal injury volume by rotational plane and piglet age.
Table 2.2: Table of all rotational head injury predictors (and whether moment of inertia in their calculation), R² values (greater than 0.5 shown in bold), p-values (asterisk indicates p-value <0.05) and slopes from separate linear regressions with AIV in 3-5 day (n=32) and 2 month old (n=17) piglets.

<table>
<thead>
<tr>
<th>Rotational Head Injury Metric</th>
<th>Use moment of inertia</th>
<th>3-5 day old piglet</th>
<th>Pre-adolescent piglet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R²</td>
<td>p-value</td>
</tr>
<tr>
<td>VEL&lt;sub&gt;i&lt;/sub&gt;</td>
<td>No</td>
<td>0.132</td>
<td>0.04056*</td>
</tr>
<tr>
<td>ACCEL&lt;sub&gt;i&lt;/sub&gt;</td>
<td>No</td>
<td>0.199</td>
<td>0.0105*</td>
</tr>
<tr>
<td>RIC36</td>
<td>No</td>
<td>0.068</td>
<td>0.1490</td>
</tr>
<tr>
<td>HIP</td>
<td>Yes</td>
<td>0.677</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>BRIC</td>
<td>No</td>
<td>0.117</td>
<td>0.0558</td>
</tr>
<tr>
<td>Revised BrIC</td>
<td>No</td>
<td>0.004</td>
<td>0.7205</td>
</tr>
<tr>
<td>PRHIC36</td>
<td>Yes</td>
<td>0.280</td>
<td>2.203e-3*</td>
</tr>
<tr>
<td>RVCI</td>
<td>No</td>
<td>0.125</td>
<td>0.0474*</td>
</tr>
<tr>
<td>RotKE</td>
<td>Yes</td>
<td>0.717</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>RotWork</td>
<td>Yes</td>
<td>0.632</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>rKE</td>
<td>Yes</td>
<td>0.601</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

All rotational head injury metrics that used MOI and rotational kinematics had strong, significantly positive correlations with axonal injury in one or both age groups. RotKE had the strongest correlation for all 3-5 day old piglets, and PRHIC36 performed the best in pre-adolescent piglets (Table 2.2, Fig. 2.1). Both HIP and rKE are head power measures and are strong predictors of AIV for the infant, but not for the pre-adolescent pig. HIP quantifies the summation of both translational and rotational head power, while rKE only measures rotational power. RotWork was the only strong metric in both age
groups (Fig. 2.2). The RotWork-AIV slope for the 3-5 day old is more than six times larger than that of the 2 month old pig (Fig. 2.2), indicating that the newborn-aged pig sustained much more AIV per joule of RotWork than the pre-adolescent piglet.

Figure 2.2: Cerebral axonal injury volume vs. rotational work for 5 day and 2 month old piglets.

Translation to the Human Pediatric Population

Based on observations made in the neonatal pig, the largest MOI was in the SAG direction and correlated with the largest volume of axonal injury. To translate our findings to humans, we hypothesized that the principal axis with the highest MOI for the human head would exhibit the largest sensitivity to head injury. In a child, the skull is more spherical than that of an adult, due in part to increased skull bone curvature.
(Winkelstein, 2012). Up to the time of publication, we found one study that reported 14 children (aged 5 months to 16 years) head MOI values for all three principal axes (Loyd et al., 2010). We note that only measurements of head (not brain) MOI about all three principal axes in humans were previously published and that developmental changes in the geometry of the skull are non-linear (Loyd et al., 2010) and may differ from that of the brain over time. In this study, COR rotations corresponded to head movement about the x-axis, defined from the dorsum sellae to the nasion; SAG head rotations corresponded to movement about the y-axis, defined as left to right; while AXI rotations corresponded to rotations about the z-axis, defined as inferior to superior along the spine. We observed that across all ages, MOI values about the y-axis (SAG) were consistently the largest. To determine if brain development altered the shape (MOI) of the head, we performed a linear regression of MOI (for each axis) by age (Fig. 2.3). Comparison of each axis’ regressions by analysis of covariance revealed that the MOI in all directions increases significantly (p<0.0001) with development (y axis slope with age 12.82 ± 1.40; x axis 10.21±1.40; z axis 9.57 ± 1.40, all in units of kg-cm²/year), but ANOVA revealed no interaction between the effect of age and direction (p=0.23). The lack of significant interaction between age and direction on MOI indicates that the brain grows in an isotropic manner, without a significant change in shape. Future studies should collect more data from children to confirm how MOI varies with development.
Discussion

Our goal was to identify TBI mechanisms that inform motor vehicle and protective equipment design strategies. We believe that the novel predictors of rotational head injury presented in this study are scientifically valuable as well as potentially clinically relevant to injury prevention and public health. In this study, our objective was to compare previously published rotational head kinematic metrics with new kinematic metrics that included MOI and to correlate these metrics with acute, histopathological axonal injury in the newborn- and pre-adolescent-aged piglet populations.

Figure 2.3: Moment of Inertia versus Age.
Rotational Moment of Inertia Improves Prediction of Axonal Injury

Consistent with our hypothesis, kinematic metrics that included MOI and rotational direction improved prediction of axonal injury. The strongest metrics were RotKE, RotWork and rKE for newborn piglets and PRHIC36 and RotWork for the pre-adolescent pigs. RotKE was the strongest predictor of axonal injury severity for newborn animals, and PRHIC36 was the strongest predictor for pre-adolescent pigs. RotWork had strong predictive capability in both ages. Therefore, we recommend that future rotational injury metrics should consider including RotWork to enhance robustness of brain injury prediction.

Age Influences the Axonal Injury Threshold

For both pigs and children, the increase in head size with age significantly increased the magnitude of MOI about each axis. We hypothesized that acute axonal injury would increase with MOI and increasing rotational injury metric values. Thus, we expected older larger brains with larger MOI and RotWork to have more severe axonal injury than the newborn. However, with larger brains, pre-adolescent-aged piglets experienced less AIV for many rotational head injury metrics than newborn-aged piglets (Fig. 1). For example, comparison of AIV-RotWork relationships across age suggests that newborn-aged pigs have a lower RotWork threshold to axonal injury (Fig 2). Experimentally, even when accelerations were scaled by brain mass alone (Ommaya, et al., 1967), we expected larger, older brains exposed to similar accelerations to have more severe axonal injury. Instead, rapid head rotations caused newborn piglets to sustain worse outcomes (Maltese, 2012b; Sullivan et al., 2015; Sullivan et al., 2013) and larger AIV (Fig. 1) than that experienced by pre-adolescent pigs. Thus, scaling by brain mass and size is inappropriate when comparing across age because injury risk in the young brain
is not mitigated by its small size. Potential mechanisms responsible for these findings include differences in material properties, white matter tract orientation or deformations experienced in the older brain that are protective. Previously we incorporated tract and age-related constitutive property differences into a computational model to evaluate the contributions of these factors, and found smaller tract-oriented deformation thresholds for acute axonal injury in the newborn than pre-adolescent piglets (Sullivan et al., 2015). The risk of axonal injury in the 3-5 day old piglet was surprisingly high in spite of a relatively small MOI compared to the 2 month old. The biomechanics of injuries between 3-5 day old and 2 month old piglets does not explain the age-specific vulnerability to axonal injury; we believe that age-specific pathophysiology may explain the more severe axonal injury presented in the 3-5 day old piglet. We speculate that age-specific responses to injury such as pro-inflammatory cascades may cause increased hypoxic/ischemic microenvironment leading to more axonal injury. Acute white matter inflammation after TBI is altered by age where post-injury neuroinflammation was increased in old mice yet with fewer post-injury neurofilament-positive swellings compared to young mice (Cheng et al., 2018). Another mechanism for age-dependent axonal injury following TBI may be cerebral blood flow deficits, which promote larger axonal injury volumes. Larger decreases in cerebral blood flow for the 5 day old piglet were noted compared to 4 week old piglets at 3 hours following fluid-percussion TBI (Armstead and Kurth et al., 1994). Ibrahim reported significant decreases in cerebral blood flow in 3-5 day old and 4 week old piglets at 6 hour after unscaled non-impact, rapid head rotation in the axial plane (Ibrahim et al., 2010). Future studies should focus on the biological and physiological mechanism responsible for the vulnerability in the newborn.
Axonal Injury Vulnerability to Sagittal Head Rotations

MOI may be considered a weighting factor for each axis when predicting injury from head rotations about combinations of the x, y and z axes. In both ages, RotWork, MOI and AIV were both highest when the piglet brain was rotated in the SAG plane and lowest in the COR plane. For newborn-aged pigs, SAG head rotations were previously shown to cause global reductions in cerebral blood flow, persistent axonal injury and significant functional deficits, which were absent following COR and AXI rotations (Eucker et al., 2011; Sullivan et al., 2013). Similarly, newborn-aged pigs rotated in the SAG direction had significantly longer unconscious durations than the sham group, as did AXI head rotations at similar angular velocities (Eucker, 2011). For the 2 month old pigs, SAG had comparable mean AIV (at 6 hours post-injury) to AXI over a range of angular velocities, but SAG was the only direction with significantly longer time to return of pinch reflex (return to consciousness) compared to sham (Maltese, 2012b). Finite element simulations of rapid head rotations in the pig reveal that white matter tract orientation relative to the deformation field may be a key driver of head rotation direction-dependent axonal damage (Sullivan et al., 2015), whereby larger stretching along white matter tracts produces higher AIV. We propose that MOI and white matter tract orientation both contribute to axonal vulnerability to sagittal head rotations in the pig.

The translational applications of our results from pig to human must incorporate differences in brain size, architecture and tissue properties. These differences also contribute to limitations of mass-scaling across species. In the nonhuman primate, more severe brainstem injury was observed following coronal head rotations, compared to horizontal and sagittal rotations (Gennarelli et al., 1982, Gennarelli et al., 1987). The orientation of white matter tracts and brainstem may account for discrepancy between
bipedal non-human primates and quadrupedal pigs. The presence of the cerebrospinal fluid-filled pontine cistern that separates the primate brain stem from the skull in the sagittal plane may dampen stresses caused by sagittal rotations in the primate relative to the piglet. Inter-species differences in skull thickness and composition and neck tissue properties may also contribute discrepancies in axonal injury across species. These cross-species direction-dependent responses are important avenues of future study.

The real-world relevance of the rotational head kinematics becomes more apparent after mass-scaling the angular velocities from the 3-5 day old and 2 month old piglet to the human adult and infant, which allows comparison with previously reported injury thresholds. A 3-5 day old piglet with mean angular velocity of 175 rad/s (brain mass of 27.8 g) scales to 72 rad/s for human newborn (brain mass of 400 g) and 47 rad/s for a human adult (brain mass of 1400 g). The 2 month old pig with mean angular velocity of 142 rad/s scales to 75 rad/s and 49 rad/s for human infants and adults respectively. Because Rowson reports 50% risk of concussion as 28.3 rad/s using impact data from American football games, our piglet studies are likely associated with a larger than 50% risk of concussion (Rowson et al., 2012). Sullivan and colleagues reported the range of sagittal head rotational angular velocity as 44-49 rad/s from parietal and occipital head impacts during low height falls in infants (Sullivan, Coats, & Margulies, 2015), which is smaller than the 72-75 rad/s infant brain mass-scaled load used in our 3-5 day old and 2 month old piglets. Taken together, we report axonal injury occurs in both piglet ages that may be associated with a high risk of concussion in adult humans and associated with higher loading conditions than falls in newborns. Angular accelerations from our 3-5 day and 2 month old piglets were mass-scaled to the adult human brain mass to yield values of 3154 and 4180 rad/s² respectively. For young adults, a range of 892-1169 rad/s² was
reported for head angular acceleration during soccer heading impacts in both sexes (Bretzin, et al., 2017). The mass-scaled angular accelerations induced in our study were much higher than values reported during the heading of a soccer ball. At high risk levels of concussions, we expect to observe larger magnitudes of axonal injury more consistent with the mild to moderate region of the TBI spectrum.

Fortunately, well-correlated pig brain development timelines to humans allows us to draw valuable insight into biomechanical changes across age in the piglet that may be relevant to the human child. For both pigs and children, we observed that MOI (or resistance to rotational motion) about the axis corresponding to SAG rotations was largest at all ages, compared to COR or AXI. Because SAG rotations correlated with the largest volume of axonal injury in the piglet, we speculate that SAG rotations would cause the most severe axonal injury in the human child compared to other axes if important white matter tracts in pigs and humans have similar spatial orientation. Future studies should test this hypothesis with human study data. In clinical research studies, the MOI of a patient’s head could be estimated by measuring the length of head along each x, y, z axis and assuming that the head is an ellipsoid. We generally expect head rotations about an axis with a larger MOI to induce more axonal injury than rotations about an axis with a smaller MOI. A subject who sustains a head rotation about an axis with high MOI should be closely monitored for neurocognitive symptoms and referred to a neurologist for a check-up in the near-term future. In cases where the head direction is known, such as during sports games and vehicular accidents, a rough estimate of MOI may be considered for prognosis and treatment options or potentially whether the subject should return to play. The formula for calculating the MOI about axis z is

\[ MOI_z = \frac{1}{5} M(a^2 + b^2), \]

where \( M \) is mass of the ellipsoid, \( a \) is the radius along the x axis
and $b$ is the radius along the $y$ axis. $MOI_y = \frac{1}{5}M(a^2 + c^2)$ and $MOI_x = \frac{1}{5}M(c^2 + b^2)$, where $c$ is the radius of the ellipsoid along the $z$ axis.

Limitations

We note several limitations in our study. First, the sample sizes for pigs and humans are small and we assumed normality in our analyses. Samples sizes per direction are also uneven. We recommend more data be collected to validate the importance of MOI in predicting direction-sensitive axonal injury in pigs and humans. Second, we estimated the MOI of the piglet head for both ages using finite element models based on magnetic resonance imaging of a single animal per age. There was inherent biological variance in the MOI from animal to animal that was not accounted for in this study, however we found the brain mass coefficient of variation to be less than 10% for both ages. Future studies could include individual MOI to evaluate increased accuracy of RotWork correlations with axonal injury. Third, in order to calculate critical velocity and acceleration values specific to both piglet age groups for BRIC and Revised BrIC, we used mass-scaling laws (Ommaya, et al., 1967), which assume similar brain shape and composition across species. We acknowledge the complexity and challenge to the translation of our work, performed in pigs, to humans due to differences in brain size, shape and tissue properties. There are two major limitations in the use of mass scaling of BRIC critical values – one involves the scaling of adult to pediatric populations and two mass scaling ignores differences between human and pig species. The conclusions drawn from Fig. 3 are limited because the published sample size is small, and more acute histopathological data are needed to definitively assess whether MOI is useful for predicting direction-sensitive axonal injury in humans. Few human studies report axonal injury volumes immediately following a single TBI, without complications from ischemia.
or previous brain injuries. Finally, our study was limited to acute axonal injury, and future studies should investigate if RotWork is predictive of sustained (chronic) axonal injury.

Conclusions
Rotational kinematic injury metrics that include MOI, such as Rotational Work (RotWork), strengthen the predictive relationship between head movement and acute axonal injury by accounting for the directional-dependence of axonal injury. SAG rotations have the largest MOI and RotWork in pigs and, due to the white matter tract orientation, have the largest AIV. If tracts are aligned similarly in humans and because children also have a large MOI in the SAG direction, we would expect that SAG rotations may create more severe acute axonal injury in children. For the same MOI or Rotational Work, we find that newborn brains have more axonal injury than pre-adolescent brains. We conclude that head injury kinematic metrics should use rotational kinematics and MOI to enhance directional-sensitivity of brain injury predictions, and future studies should identify age-specific metric thresholds for brain injury in children.
BIBLIOGRAPHY (CHAPTER 2)


CHAPTER 3:  
Changes in Event-Related Potential Functional Networks 
Predict Traumatic Brain Injury in Piglets

Abstract

Traumatic brain injury (TBI) is a leading cause of cognitive and behavioral deficits in children in the US each year. None of the current diagnostic tools, such as quantitative cognitive and balance tests, have been validated to identify mild TBI in infants, adults and animals. In this study, we report a novel, quantitative tool that has the potential to quickly and reliably diagnose TBI and which can track the state of the brain during recovery across multiple ages and species. Using 32 scalp electrodes, we recorded involuntary auditory event-related potentials (ERP) from piglet brains and generated functional ERP networks. Patterns of the observed changes in these networks were used to distinguish brain–injured piglets from non-injured. This novel approach is the first application of auditory ERP functional networks to the prediction of TBI. The resulting tool is a robust, objective and predictive method that offers promise for detecting mild TBI.

Introduction

Traumatic brain injury (TBI) can be defined as alterations in brain function resulting from an external force (Eucker, et al., 2011) such as blunt or penetrating trauma to the head (often causing focal or localized injury) or indirect acceleration and deceleration forces or blasts (often causing diffuse injury). An estimated $221 billion (combined acute and chronic care) is spent to treat TBI annually (Coronado et al., 2012) and concussion or mild TBIs constitutes approximately 85% of all TBI sustained in the United States. TBI is known to cause substantial physical, cognitive, and emotional impairments in both adults and children.
TBI is the leading cause of disability and death in children in the United States and the two age groups that are at highest risk for TBI are infants and toddlers (0-4 years) and young adults (15-19 years) (Faul, et al., 2010). In 2012, the CDC reported that 329,290 children (age 19 or younger) were treated for sports and recreation-related TBI. Given the significant cost and impact of mild pediatric TBI in the US, there is an urgent need for accurate diagnostic tools to identify mild TBI in the young brain.

Currently, there are limited biomarkers available for the diagnosis and prognosis of various severities of TBI in children (Davis et al., 2017). Clinicians typically use balance or eye-tracking, and cognitive tests on children, such as the Immediate Post-Concussion Assessment and Cognitive Test (ImPACT) or CogSport. However, there is little data to support the use of these neuropsychological tests in the assessment of concussion in children aged 5-12 years (Davis et al., 2017). Neuropsychological tests are also significantly affected by administration environment, practiced effect, feigned poor performance at baseline and age (Moser, et al., 2011; Vaughan, et al., 2014). Despite active research on diagnostic abilities of CSF or serum-based biomarkers for mild to severe TBI in adults (Berger et al., 2005; Nakhjavan-Shahraki, Yousefifard, Oraii, Sarveazad, & Hosseini, 2017; Papa et al., 2016; Paziana & Korley, 2015), clinical utilization is in the very early stage. Reliable biomarkers for prediction of brain lesions in pediatric patients with head trauma are lacking (Dayan et al., 2017; DeFazio et al., 2014). Currently, there are limited biomarkers available for the diagnosis and prognosis of various severities of TBI in children. There is no consensus of reliable biomarkers among researchers or clinicians or any US FDA-approved biomarker for prediction of the outcome of TBI in children. Functional impairment following TBI may evaluated in subjects via symptom self-reports (such as Post-Concussion Symptom Scale) vestibular-
ocular reflex testing (such as Vestibulo-Oculomotor Screen) and serum biomarkers such as myelin basic protein, neuron-specific enolase (NSE) and S100β. Papa and colleagues reported that glial fibrillary acidic protein (GFAP) out-performed S100β in detecting intracranial lesions on CT following head trauma in children 10 years or younger. A systematic review and meta-analysis of the predictive capability of NSE for TBI in children found that serum NSE had 0.75 AUC and concluded that more studies were needed (Nakhjavan-Shahraki, Yousefifard, Orai, Sarveazad, & Hosseini, 2017). Serum or cerebrospinal fluid (CSF) protein-based biomarkers have the disadvantage of being an invasive measure compared to EEG-based measures. A multimodal framework that enables age-appropriate diagnosis and clinical assessment of pediatric concussion as well as prognosis of recovery is necessary.

Event-Related Potentials (ERPs) are electrical signals evoked in response to a sensory stimulus. They can be easily measured at the scalp using standard electroencephalogram leads. ERPs measure post-synaptic potentials generated from the cortical and sub-cortical neurons and consist of characteristic sequences of peaks and troughs called ERP components that indicate cognitive processing. Auditory ERPs, evoked in response to an auditory stimulus, are involuntary, do not require prior training of the subject and permit easy control of the stimuli. In addition, auditory stimuli allow investigation of the cognitive state of non-verbal subjects such as children under the age of 2 years. Auditory ERPs have been extensively studied over the last decade and there is a growing list of specific ERP components that are connected to various aspects of cognition. Measurement of ERPs is a fast, objective, involuntary and noninvasive method to study the effects of both diffuse and focal head injury on brain function. Auditory ERPs have been used to predict dyslexia in children (Molfese, 2000), and
several studies show persistent changes in the amplitudes and latencies of ERP components in TBI patients compared to controls (De Beaumont, et al., 2007; Dundon et al., 2015; Mazzini, et al., 2001). In this study, we recorded auditory ERPs using a 32 scalp electrode array in juvenile piglets to develop quantitative, unbiased predictors of brain state after injury.

Traditionally, the analysis of ERPs is component-dependent, where researchers infer the significance of the changes in the magnitude and latency of the ERP components from different channels located in regions associated with specific cognitive processing states in humans. However, such an analysis of ERP components is subjective, as different researchers select different methods to quantify different features of interest. To overcome this problem, we applied network theory and machine learning to develop a robust predictor of brain state after injury. Network analysis allows simultaneous investigation of neural activity recorded by all the electrodes, i.e. the entire ERP signal from all channels thereby eliminating component and method specific bias and provides an objective framework for quantification of ERP changes. A previously published study reported the time line of recovery of 4 week old piglets after sagittal rapid head rotation where histopathology was obtained at 3-8 hours, 1 day, 3-4 days and 5-6 days post-injury (Weeks et al., 2014). Weeks and colleagues showed that cerebral axonal injury volume was low at 3-8 hours (0.57%) then peaked at 1 day (1.15%) before decreasing on 3-4 days (0.69%) and 5-6 days (0.56%) post-injury (Weeks et al., 2014). This previous study demonstrated partial recovery of the 4 week old piglet from diffuse axonal injury within 6 days of a SAG injury, which justifies our choice of study time points of 1, 4 and 7 days after injury. In this study, we applied network analysis to auditory ERP
signals and generated functional networks before and after well-characterized diffuse and localized white matter injury in piglets.

The aim of this study was to evaluate whether significant alterations occur in ERPs following diffuse and focal brain injury in awake piglets. Our data, obtained on three different post-injury days, show patterns of neural recovery in the piglet brain and that network approaches to the analysis of ERPs can discriminate injured piglets from uninjured. In this preclinical study, we measured auditory ERPs before and after injury in awake subjects. Future studies will translate these methods to humans to create an objective and sensitive diagnostic tool to detect neurotrauma in the pediatric population.

Methods

Diffuse & Localized Injury in Piglets

All animal experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania. Twenty-two 4-week old, female Yorkshire piglets (equivalent to the young child) were used (Dickerson & Dobbing, 1967). Piglets were housed singly and kept on 12 hour sleep cycle. Prior to the start of the experimental protocol, piglets were acclimated to wearing nylon caps in their cages for a minimum of two 30 minutes sessions over two days. During these acclimation sessions, the piglets were held on the lap of the experimenter with nylon caps on their head as would occur during the ERP experiment.

Piglets were allocated to an injury or sham group. All piglets were anesthetized with 1.5% isoflurane, intubated and mechanically ventilated. Either a focal injury via Controlled Cortical Impact (CCI) or a diffuse injury by rapid non-impact rotation (RNR) was prescribed to the piglets in the injury group. CCI was performed on the right side of...
the brain along the coronal suture, halfway between the sagittal suture and orbital rim as described previously (Margulies et al., 2015). A single RNR was imposed in either the sagittal (SAG) or coronal (COR) plane with mean velocity of 131 rad/s by a HYGE pneumatic actuator (Eucker et al., 2011; Ibrahim et al., 2010; Maltese, 2012). Angular velocity of head rotation was recorded using an angular rate sensor (ATA Engineering, Model#: ars-06, Inc., Herndon, VA) and a data acquisition system in LabView (National Instruments, Austin, TX). Sham pigs received the same procedures, including anesthesia, except injury, including anesthesia which included intramuscular injections of ketamine (20 mg/kg), xylazine (2 mg/kg) and buprenorphine (0.02 mg/kg), induction and maintenance at 4% and 1.5% isoflurane respectively on day 0. ERPs were measured one day before injury and on 1, 4, and 7 days after injury. Sample sizes for each group of piglets were 9 for Sham, 7 for SAG, 3 for COR and 3 for CCI.

**Immunohistochemistry**

Immunohistochemical processing of a sub-sample of piglet brains was performed 8 days after injury. Brains were perfused and fixed with 10% neutral buffered formalin for 24 hours (Ibrahim et al., 2010). Sections were stained for beta-amyloid precursor protein. Positive beta-amyloid precursor protein staining, indicative of damaged axons, was identified by a neuropathologist blind to the animal injury group. The cumulative sum of positively stained area throughout all the examined brain slices (6 μm thickness) was used to calculate an Axonal Injury Volume (AIV), calculated as a percent of total cerebral volume.

**Data Acquisition and Auditory Stimulus**

A customized double platform, 4-wheel cart was used to house all data acquisition equipment. The E-Prime (2.0.10.353 SPI) software program, a 32-channel net (Electrical
Geodesics, Inc. HydroCel™ Geodesic Sensor Net) and Electrical Geodesics, Inc Net Amps 400 EEG amplifier were used to generate auditory stimuli and acquire subsequent neural responses. The sensor net was submerged into a potassium chloride-based electrolyte solution before placement on the piglet’s head. To ensure that both longitudinal recordings and recordings across animals could be compared to each other, the net utilizes the International 10-20 EEG system for positioning electrodes on the scalp of the piglet. Two anatomical landmarks were used to ensure reproducible positioning of the EEG electrodes – the nasion and inion. Two thin nylon caps were slipped over the net in order to maintain electrode-scalp contact throughout the experiment. The impedances of all channels were below 50 kΩ before recording began.

Two sequences of 100 clicks were played by a portable USB speaker (JLab B-Flex X-bass Hi-Fi Laptop Stereo Speaker) to each piglet for the standard (Std) and oddball (OB) paradigms each. The piglet’s head was positioned 19.03 ± 6.63 inches from the speaker, which was level with the piglet’s left ear. In both paradigms, the clicks were 2 ms in duration with inter-stimulus interval of 316 ms, giving a rate of approximately 3 clicks per second. The clicks in the standard paradigm were “white noise”, at 800Hz auditory frequency. Each sequence of 100 clicks in the OB paradigm consisted of a random combination of 70% standard (OB-Std) “white noise” clicks (same as used in the Std paradigm) and 30% target (OB-Trgt) “brown noise” clicks (auditory frequency of 1000Hz). Heffner and Heffner previously reported the hearing range of domestic pigs to be 0.042 to 40.5 kHz, a range similar to that of humans (typically 0.02 to 20 kHz), therefore all auditory clicks fell within the pigs’ auditory range (Heffner & Heffner, 1990). ERP recordings obtained during major head movement, while the piglets were sleeping or agitated and in the presence of background noise were discarded and repeated.
Paradigms were repeated until a minimum of two acceptable standard trains and oddball trains per piglet were obtained. The total testing time was typically 30 minutes per subject. The net was disinfected and rinsed daily after the recording session was complete. ERP data was acquired at a rate of 1000 samples per second and referenced to channel 33 (frontal lobe midline).

ERPs were initially filtered before analysis with a band pass filter from 0.3Hz to 30Hz (NetStation Tool version 5.1.2). Next, all ERPs were segmented into 316 ms long epochs (50 ms before and 266 ms after each individual click). Excessive peak-to-peak amplitudes, due to eye blinks, eye movements or minor head movements, were then removed. All 200 ERP epochs per channel were centered and normalized (z-scored) by the mean and standard deviation of the pre-click recording. After z-scoring to the pre-click duration, all ERP epochs were averaged to remove background EEG before further analysis.

*Traditional Analysis of ERP Amplitudes and Latencies for Selected Channels*

Amplitudes and latencies of ERP components were detected using a custom MATLAB® (R2015b, Mathworks, Inc.) script. Four distinct ERP components identified from previous studies in adult pigs, were selected for analysis. The first two peaks and troughs in the ERP signals from channels 15 (left auditory cortex), 16 (right auditory cortex), 17 (near frontal lobe midline), 19 (parietal lobe midline), 20 (occipital lobe midline), and 28 (near central midline) were selected (Fig.3.1). These channels were selected because they are commonly discussed in the ERP literature (Andrews, et al., 1990; Arnfred, et al., 2004; Heisz & McIntosh, 2013; Martoft et al., 2001; Rogers, et al., 2015) and are particularly relevant to the auditory signal processing. All six channels captured widespread neural activity - from the central, parietal, occipital and frontal brain regions. The latency ranges
for each peak were based on the information from a study performed in adult Gottingen mini-pigs (Arnfred et al., 2004). The four ERP components examined were: N40 (the negative peak found in the latency range of 20–60 ms post-stimulus), P60 (positive peak found in range of 40–80 ms), N120 (negative peak found in range of 90–150 ms) and P200 (positive peak found in range of 150–250 ms). If a peak with the correct polarity was not found in the specified latency range of the ERP for a specified channel, the peak was excluded from analysis. As a result, we only analyzed peaks that could be reliably identified.

Figure 3.1: Top view of electrodes in EEG sensor net placed on piglet head, with selected channels (shown in blue) used for analysis of ERP magnitudes and latencies. Selected channels included: channels 15 (left auditory cortex), 16 (right auditory cortex), 17 (near frontal lobe midline), 19 (parietal lobe midline), 20 (occipital lobe midline), and 28 (near central midline).
*Generation of Auditory ERP Functional Networks*

Functional networks are collections of nodes (channels of ERP signals) and edges or connections between the nodes. A single network edge between two channels was constructed by calculating the maximum absolute cross-correlation between the averaged, z-scored post-stimulus ERP signals from both channels (Fig. 3.2B). The collection of cross-correlations between all pairs of channels form the network, which is depicted as a 32 x 32 matrix (Fig. 3.2C) and a set of connections between electrode sensor locations (Fig. 3.2D).

Our ERP networks were fully connected with a network density of 1 defined as 496 edges among 32 nodes. Network density, the number of connected edges throughout the network out of the total number of possible edges, determines network topology. ERP networks as a function of network density were generated by progressively removing the weakest edges. This was done in order to focus on the properties of the networks with strong edges. In order to threshold each network (i.e., remove the weakest edges), network edges with values below the range of 48th – 99th percentile edge values were sequentially removed. The range of thresholds were applied to each network and subsequently a series of network metrics on each thresholded network were calculated in order to examine the effect of density on sham, SAG, COR and CCI networks.

We calculated five network metrics on the thresholded networks in order to quantify functional network influence, integration and segregation in the brain. The following network metrics were calculated: nodal strength, clustering coefficient, global efficiency, characteristic path length and modularity. Nodal strength (NS) were calculated as the sum of all connections or edges from a specified node. Clustering coefficient (CC) was
calculated as the geometric mean of edges about each node. Paths are unique sequences of edges between two distinct nodes and their lengths are measured by the sum of edges. The Characteristic Path Length (PL) was calculated as the average length of shortest paths between all pairs of nodes in the network. Global Efficiency (GE) was calculated as the average of the inverse of the shortest path lengths in the network. Modularity (Q) is a network measure of segregation found by measuring the extent of densely interconnected nodes within clusters located in a network. Q was calculated using the MATLAB® (The MathWorks Inc., Natick, Massachusetts) implementation of the Brain Connectivity Toolbox (Rubinov & Sporns, 2010) via the Louvain-Greedy algorithm with the modularity resolution parameter set as 1. Median values of NS and CC were calculated over all 32 electrodes to yield single, representative measures of nodal strength and clustering for each ERP network.

In order to examine the role that a single node plays in the network, we calculated NS, CC, Q, GE and PL on every network after removing all combinations of a single node. We found 32 different 31-node networks after individually removing one out of 32 nodes and found the mean metric over all 32 networks. Only one of 32 nodes was removed at a time; nodes were not removed sequentially. Statistical comparison of post-injury time points with pre-injury times were performed using the non-parametric Dunnett’s test for each injury type.
Discrimination of Injured and Uninjured ERPs

A total of 22 awake, female piglets were used to form the dataset for predicting TBI. Each post-click signal (epoch; 316 ms in duration) was classified as injured or not injured. Because two different paradigms of click trains composed of both white and brown noise were included in this analysis, there were three types of epochs (and data sets): Standard (Std), Oddball-Standard (OB-Std) and Oddball-Target (OB-Trgt). A single machine learning model was constructed for each type of epoch. Preprocessed ERP signals were used to construct the model.

In order to construct our machine learning models, a binary classification dataset was created, in which epochs were classified as uninjured if they were acquired before injury...
(Day 0) in the SAG and CCI groups. Sham epochs from all the acquired days were also classified as uninjured. ERP epochs were classified as injured if acquired between 1 and 5 days after injury in the SAG and CCI groups. The resulting data set was not balanced, with approximately 75% of all ERP epochs belonging to the uninjured class and 25% to the injured class. The data set was randomly split into three sets: training (38%), testing (38%) and validation (24%). The training set was used to tune the parameters of the model, the testing set was used to select the final model and the validation set was used to report the predictive capability of the model with metrics such as accuracy and area under ROC curve (AUC). All performance data presented in this report were determined from the validation set. Because the dataset was not balanced in the number of epochs from CCI (28%), SAG (24%) and Shams (48%), we preserved the relative proportions of each group in the training, testing and validation sets. CORs were not included in our training set because it is characteristically a mild TBI (in terms of total axonal injury volume) compared to CCI and SAG (Eucker et al., 2011a; Ibrahim et al., 2010). Instead, we evaluated the performance of CORs using the algorithm separately.

In order to improve the accuracy of the predictive model, the ERP data was then dimensionally reduced using XDawn, a novel dimensionality reduction algorithm that is similar to principal component analysis, which maximizes signal to noise ratio of ERPs (Barachant, et al., 2012; Rivet et al., 2009.; Rivet & Souloumiac, 2007) and outputs data with a reduced number of components in the channel dimension. The dimensionally reduced ERP data was then used to calculate covariance matrices for each ERP epoch using XDawnCovariance (XDawnCov); this step simplifies classification of data into injured and uninjured groups. A separate logistic regression model (Pedregosa et al., 2011) was applied to the output from Xdawn and XDawnCov for each of the three types
of ERP epochs (Std, OB Std and OB Trgt). To predict TBI, the output from both the Xdawn and XDawnCov logistic regression models was averaged and a single predictive model for each ERP paradigm dataset was constructed.

Each predictive model outputs two scores representing the probabilities that an epoch belongs to the injured and uninjured class, where the sum of both scores for the same epoch is 1. For each animal, a probability score for injury (InjScore) was computed as the geometric mean of probability scores for the injured class for all ERP epochs. The InjScore is a continuous value over the range of 0 (uninjured) to 1 (injured), thereby reflecting the risk of injury in an animal. In order to convert the continuous InjScore to a discrete classification (injured or uninjured), we applied the k-means clustering (Pedregosa et al., 2011) method (k=2) to each paradigm’s InjScores. All three paradigms were combined by taking the majority vote over all discrete InjScore classifications for every animal. We also analyzed the mean of all continuous InjScores from the Std, OB-Std and OB-Trgt paradigms for each animal. An ROC analysis was used to determine the critical value of InjScore above which animals should be classified injured. The InjScore threshold was taken where both the true positive rate was highest and the false positive rate was lowest.

**Statistical Analyses**

The non-parametric Dunnett’s test (Gao, 2008) was used for comparison of ERP latencies and magnitudes between Day -1 (PRE) and all post-injury days (POST 1d, 4d and 7d). For the analysis of network metrics as a function of network density, group differences between SAG, COR and CCI compared to Sham were found by performing two-tailed permutation tests at each density value and then correcting for multiple comparisons using the Benjamini-Hochberg correction procedure (Harris et al., 2016).
The level of significance was 0.05 for all statistical tests. All statistical tests were performed in R 3.4.1 (R Core Team, 2017) using the nparcomp (Konietschke, et al., 2015) and coin (Hothorn, 2008) packages.

Results

Histopathology

Histopathological assessment of axonal damage and/or contusion volume yielded a mean of 0.0% of total injured cerebral volume in Sham (n=3), 0.31% in SAG (n=2), 0.0% in COR (n=3) and 4.69% in CCI (n=1) animals respectively. COR should present mild alterations in ERPs compared to SAG and CCI.

Traditional Analysis of ERP Amplitudes and Latencies for Selected Channels

We examined Standard paradigm ERP signals for channels 15 (left auditory cortex), 16 (right auditory cortex), 17 (near frontal lobe midline), 19 (parietal lobe midline), 20 (occipital lobe midline), and 28 (near central midline). Figure 3.3 shows representative ERP signals from Sham and SAG, COR and CCI groups before injury and 7 days after injury or sham (pre-anesthesia only) from channel 19.
Figure 3.3: Representative Standard paradigm ERP- time history for sagittal rapid head rotation injury and sham from channel 19 (parietal lobe midline) before and after injury or anesthesia. Each panel shows the ERP amplitude (in μV) as a function of time (in ms). The stimulus or click occurs at 0 ms, indicated by the broken, black line. The shaded area indicates the window of the P60 component. Arrows indicate the latency of the P60 component.

The median values of the amplitudes and latencies of the N40, P60, N120 and P200 components from channels 15, 16, 17, 19, 20, and 28 were largely unchanged from pre-injury (PRE) levels and did not vary widely in response to SAG injury or study day (Fig. 3.4). For N40 latencies, the POST 7d time point only the SAG group was significantly longer (non-parametric Dunnetts test, p=0.01) than PRE for Channel 15. All other comparisons of N40 latencies and amplitudes from post-injury to pre-injury for channels 15, 16, 17, 19, 20, and 28 were not significantly different (p>0.05). Channel 19’s P60 amplitudes decreased significantly on all post-SAG days relative to PRE. A reduction in P60 amplitudes at 4d was only seen in the Sham group compared to PRE (Fig. 3.4). All significant changes in the latencies and amplitudes of N120 or P200 in the SAG group were also present in the Shams, from channels 16, 17, 20 and 28. In summary,
significant changes in amplitudes and latencies from PRE levels were observed in early peaks (N40 and P60) in the SAG group. For the traditional ERP analysis, statistical tests were not performed on data from COR and CCI because of small sample sizes (n=3). The latency and magnitude data from all analyzed channels were shown in A3.19-A3.41.

**Figure 3.4:** Median N40 latencies for channels 15 (left auditory cortex) and P60 amplitudes for channel 19 (parietal lobe midline) in Sham and SAG groups from the Standard paradigm.

*TBI Alters Auditory ERP Networks*

In the network neuroscience literature, it is common to threshold networks in order to create binary networks or remove edges that are believed to be noisy or unimportant (weak edges). We thresholded our networks to remove weak edges as removal of weak edges in the network reveals the largest differences in network properties, such as characteristic path length and clustering coefficient, between the sham and injured groups that may not be evident at a single network density value.
Nodal strength (NS) quantifies the number and strength of the connection that each electrode has to all other electrodes, where a higher nodal strength represents increased synchrony with ERPs from other electrodes. NS increased as network density increased because more edges implies higher sum of connections. Before injury, all NS curves from injury groups were not significantly different (p>0.28) from sham (Fig. 3.5). At POST 1d, there was a large reduction in NS values (p<0.012) in all injury groups by 31.9% (in SAG), 34.4% (in COR) and 43.4% (in CCI) relative to Sham. This suggests that local connectivity decreased 1d after all modes of injury relative to Sham. By POST 4d, NS of COR and CCI injury groups increased (p<0.03) relative to Sham, and by POST 7d all injury groups’ NS values were comparable to sham (p>0.2). By POST 7d, NS values from Sham, SAG, COR and CCI have magnitudes comparable to PRE NS values.

Figure 3.5: Changes in Nodal Strength with network density for all injury groups and study dates compared to Sham from Standard paradigm. Mean values and standard error bars are shown. Horizontal bars indicate significant difference of injury groups from sham at single density level using two-tailed permutation test with false detection rate correction for multiple comparisons, p<0.05.
Next, we look at Clustering coefficient (CC) that represents local clustering within a network and is measured as the average “intensity” of edges around a node. CC peaks at a lower network density of 0.125 (Fig. 3.6). High CC values indicate increased local connectivity or clustering around nodes. Before and after injury, CC values in the injury groups were not significantly different from Sham (p>0.1).

![Figure 3.6: Changes in Clustering Coefficient with network density for all injury groups and study dates compared to Sham from Standard paradigm. Mean values and standard error bars are shown. Horizontal bars indicate significant difference of injury groups from sham at density level using two-tailed permutation test with false detection rate correction for multiple comparisons, p<0.05.](image)

Global Efficiency (GE) quantifies functional integration, which is the ability to combine specialized information from distributed brain regions. GE estimates the ease with which brain regions communicate, and is commonly based on the concept of a path. As more network edges are added to a network and network density increases, GE in turn increases (Fig. 3.7). GE values were significantly different (p<0.05) on PRE for the COR and SAG groups compared to Sham, but not for CCI. All GE values were similar to
sham at POST 1d for all injury groups (p>0.14). On POST 4d, SAG, COR and CCI showed lower GE values (p<0.02) than Sham at network density of 1. SAG, CCI, and COR injuries also showed lower (p<0.049) GE values at POST 7d compared to Sham at network densities > 0.75. A higher global efficiency implies stronger connectivity or information transfer between the two most weakly connected nodes in the network.

![Image of global efficiency with network density for all injury groups and study dates compared to Sham from Standard paradigm. Mean values and standard error bars are shown. Horizontal bars indicate significant difference of injury groups from sham at density level using two-tailed permutation test with false detection rate correction for multiple comparisons, p<0.05.]

**Figure 3.7:** Changes in Global Efficiency with network density for all injury groups and study dates compared to Sham from Standard paradigm. Mean values and standard error bars are shown. Horizontal bars indicate significant difference of injury groups from sham at density level using two-tailed permutation test with false detection rate correction for multiple comparisons, p<0.05.

Characteristic Path Length (PL) in anatomical networks may represent potential routes of information flow between pairs of brain regions. PL was not significantly altered (p=0.12) in all of the three injury groups on any post-injury days compared to sham (Fig. 3.8). In addition, modularity (Q), or the ease with which channels and their edges can be clustered, increased as network density increased until a maximum value of 0.08 was reached, and then decreased as network density increased beyond 0.125 (Fig. 3.9).
However, before and after injury, modularity was not significantly different (p>0.17) in SAG, COR or CCI groups compared to Sham.

In injured pigs, changes in all network metrics before and after injury relative to Sham for both OB-Std and OB-Trgt (Appendix Fig. A3.1) were consistent with those seen in Std paradigm.

**Figure 3.8**: Changes in Characteristic Path Length with network density for all injury groups and study dates compared to Sham from Standard paradigm. Mean values and standard error bars are shown. Horizontal bars indicate significant difference of injury groups from sham at density level using two-tailed permutation test with false detection rate correction for multiple comparisons, p<0.05.
To examine the role of each node, we removed a single node from each 32-node network and determined five metrics from each network across all study time points and injury groups. The percent change in NS from the whole network to mean NS from 31-node networks was 6.25% for sham, SAG, COR and CCI (A3.9). By removing one node from the network, we note a decrease in NS on all time points from the full network due to the removal of connections associated with the missing node. There was no significant change in NS with injury type and/or time point after removal on a single node from networks. CC was reduced by approximately 3.1% when a single node was removed from the networks (A3.10). There was no significant change in CC by injury type or time point (pre vs post). There was no significant change in modularity with the removal of 1 node from each network across all injury types and time points (A3.11). Some piglets
have very large changes in $Q$ because $Q$ has very small values (on the order of 0.01-0.1). This level of variability is expected and similar to that observed in Fig. 3.9. A 6.4% reduction in global efficiency was noted on average with the removal of one node from the network (A3.12). No significant change in GE was observed across injury type and/or time points. A 0.5% increase in characteristic path length was observed when a single node was removed from the network across all injury types and time points (A3.13). Overall, removing a single node did not significantly affect the properties of ERP networks. A more detailed examination into which nodes change the network’s NS (A3.14), CC (A3.15), Q (A3.16), GE (A3.17) and PL (A3.18) when removed confirms that the removal of any single node (1-32) does not adversely change the network in any injury group or at any given time point.

Next, we will examine our ERP networks for which connections change after TBI. We analyzed fully connected networks (with network density of 1), thereby including information from both weak and strong connections, which may enable better discrimination between injured and uninjured networks. Figure 3.10 shows the channel locations of post-injury days’ network edge weight difference from PRE (POST-PRE) for all injury groups, where each 32 x 32 matrix represents the connectivity calculated between 32 channels across the pig brain. Each element within the 32 x 32 matrix indicates the degree of correlation between the two channels’ ERP time-varying signals. Connectivity between nodes is based on the value of edge weights, where higher edge weight values imply hyper-connectivity (≥1 shown in dark blue) and lower connectivity (≤1 white edges) imply hypo-connectivity. The matrices are symmetric about the diagonal; the main diagonal entries were assigned a value of zero because self-connected channels are irrelevant in this context. For each node pair, differences in
edge weights between PRE and each POST time point were calculated, and values averaged across animals in the group.

The Std paradigm ERP network difference (POST-PRE) matrix for Sham had a dramatic increased number of hypo-connected edges (with edge weight differences <= -1), from zero POST 1d to an average of 172 and 184 on POST 4 and 7d respectively (Fig. 3.10A). In contrast, there were no hypo-connected edges in the SAG, COR and CCI Std network on any POST day. The number of hyper-connected edges was defined as those with edge weights >= 1. Sham Std increased in the average number of hyper-connected edges with 30, 196 and 132 on POST 1, 4 and 7d respectively. In contrast, there were no hyper-connected edges after injury for SAG and COR Std networks. However, the CCI Std network had a very modest number of (4, 0 and 6) hyper-connected edges on POST 1, 4 and 7d respectively. We conclude that for the Std paradigm, sham animals demonstrated a trend towards increasing hypo-connectivity and hyper-connectivity over time, while the SAG, COR and CCI animals’ connectivity was unchanged by injury.

The OB-Std paradigm exhibited similar changes in the number of hypo-connected edges observed in the Std for all injury groups (Fig. 3.10 B, C). The number of OB-Std Sham hypo-connected edges increased from zero on POST 1d to 122 and 110 on POST 4 and 7d respectively. The number of hypo-connected edges in SAG, COR and CCI OB-Std was zero at all post-injury days, except for COR at POST 7d with just 4 hypo-connected edges. Similar to Std, the number of Sham OB-Std hyper-connected edges increased drastically from 10 on POST 1d to 298 and 344 on POST 4 and 7d respectively. For the OB-Std, the SAG group had few or no (0, 4, 0) hyper-connected edges on POST 1, 4 and 7d. There were no hyper-connected edges in OB-Std for COR. CCI hyper-
connected edges were zero, 12 and 8 for OB Std on POST 1, 4 and 7d respectively. Like the Std paradigm, connectivity remained relatively unchanged by injury.

The OB-Trgt paradigm is designed to be the most sensitive for detecting the ability to discriminate rare events. The shams responded similarly to the other paradigms. Specifically, the number of hypo-connected edges in OB-Trgt Shams was 0, 266 and 202 on POST 1, 4 and 7d respectively. The OB-Trgt SAG network now had 2, 0 and 0 hypo-connected edges on POST 1, 4 and 7d. COR OB-Trgt had 20, 30 and 36 of hypo-connected edges on POST 1, 4 and 7d respectively. For CCI, the number of hypo-connected edges was 0, 2 and 30 on POST 1, 4, 7d respectively. The number of hyper-connected edges for Sham OB-Trgt was 14, 182 and 256 for POST 1, 4 and 7d respectively. Also, the number of hyper-connected edges for COR OB-Trgt was 0, 2 and 0 on POST 1, 4 and 7d. In a notable departure from the other paradigms, SAG OB-Trgt had 0, 162 and 12 hyper-connected edges on POST 1, 4 and 7d respectively, and CCI’s OB-Trgt number of hyper-connected edges was 100, 124 and 36 on POST 1, 4 and 7d respectively.

When comparing POST to PRE injury edges, the number of hypo-connected and hyper-connected edges in the Sham group increased many-fold from POST 1d to POST 7d for all three paradigms. In contrast to sham, the number of hypo-connected edges after injury was a nearly zero for all time points across all three paradigms. Hyper-connectivity after injury varied by paradigm and injury type, and was only observed in the OB-Trgt paradigm was markedly evident at 4d POST for SAG, and at 1 and 4d post-injury for CCI. In sum, connectivity in sham animals became more extreme, with more hyper- and hypo- connected edges on POST compared to PRE days. SAG and CCI was hyper-connected after injury, but only for the sensitive OB-Trgt paradigm.
Figure 3.10: Median network matrices of post-injury day edges minus that of pre-injury day for Sham, SAG, COR and CCI injury groups for (A) Standard, (B) Oddball Standard and (C) Oddball Target paradigms. For each edge or element in 32x32 matrix, difference = POST-PRE.
Discrimination Between Injured and Uninjured ERPs

Our objective was to develop a robust metric for identifying TBI in a given animal using our ERP dataset. The Std predictive algorithm outperforms both OB Std and OB Trgt, which indicates that the Std paradigm may be the most informative individual paradigm for predicting injury. However, we obtained maximal predictive performance (82% accuracy, AUC = 0.82), when we combined the output of all three paradigms (Table 3.1).

<table>
<thead>
<tr>
<th></th>
<th>Accuracy</th>
<th>AUC</th>
<th>Precision</th>
</tr>
</thead>
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<td>1.00</td>
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<td>0.46</td>
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<tr>
<td><strong>OB Target</strong></td>
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<td>0.67</td>
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<td><strong>Majority Vote (All)</strong></td>
<td>82.35</td>
<td>0.82</td>
<td>0.86</td>
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Table 3.1: Accuracy, area under ROC curve and precision of TBI prediction algorithm performed on validation set from Standard, Oddball Standard, Oddball Target and majority vote over all three paradigms.

The mean InjScore from all paradigms for each injury group (Sham, SAG, COR or CCI) on days -1, 1 or 4 was calculated (Fig. 3.11). Sham/Pre-injury had the lowest mean InjScore (0.14), compared to the remainder of injury groups and days, while SAG at day 4 had the largest mean InjScore value (0.32), indicating a higher number of composite ERP epochs that were predicted to be injured. The mean InjScores for SAG at days 1 (p=0.022) and 4 (p=0.02) were significantly larger than that for Sham/Pre-injury. All post-CCI comparisons were not significantly different (p>0.19) from Sham/Pre-injury. The ROC analysis of InjScores against their true classifications revealed InjScores thresholds as 0.13 (Sensitivity: 55%, Specificity: 92%) for Std, 0.18 (Sensitivity: 54%, Specificity: 75%) for OB-Std, 0.19 (Sensitivity: 64%, Specificity: 75%) for OB-Trgt and 0.18 (Sensitivity: 54%, Specificity: 75%) for all paradigms. Although the InjScore may range from zero to one, InjScores from the combination of all paradigms that exceeded 0.19 were associated with being classified as injured.
Using our validation set, eight out of nine Sham or pre-injured animals were correctly predicted as uninjured. All CCI animals (n=2) were classified as having uninjured auditory ERPs on post-injury day 1, and an injured ERP signature on post-injury day 4. All SAG animals were classified as injured on both post-injury days 1 and 4 (n=2 each).

**Figure 3.11:** Mean and standard error bar charts of injury probability scores (InjScores) for all paradigms from xDawn/xDawnCov machine learning models. Black horizontal lines represent significant Wilcoxon ranksum test comparison with Sham/Pre-injury, p<0.05. The red, dotted line represents the threshold of InjScore determined from ROC analysis.

The mean InjScore for day 1 post-COR animals was not significantly different (p=0.29) from that of the uninjured group. Only one out of three COR animals was classified as injured on day 1 compared with 100% of SAG and 0% of CCI. The “uninjured” classification of animals experiencing rapid head rotations in COR was consistent with our finding of reduced axonal injury in COR. We also find no significant alterations in auditory ERPs at 1 day after a rapid COR head rotation.
Discussion

Auditory ERP components represent auditory information processing (Gosselin, et al., 2006), which involves the sensory and limbic systems. This requires temporal resolution on the scale of a few microseconds that may be adversely affected by TBI. While there are several neuroimaging modalities and promising CSF/serum-based biomarkers available, there is no widely adopted, clear standard of TBI detection and prognosis in the clinical treatment of children. ERPs provide the advantages of high temporal resolution, non-invasive and inexpensive recording of brain activity compared to CSF or serum-based biomarkers, DTI and fMRI. Changes in amplitudes and latencies are used to provide information on how the subject processes and responds to various stimuli or experimental manipulations (Bazarian et al., 2005). Several studies show concurrent decreases in magnitude and increases in latencies of auditory ERP responses in TBI patients compared to controls (De Beaumont et al., 2007; Dennis et al., 2015; Dundon et al., 2015; Mazzini et al., 2001). Auditory ERPs have been used to predict dyslexia (Molfese, 2000) and word-level reading abilities (Espy, et al., 2004) with high accuracy in 8 year old children. At the time of this publication, there are no studies that used auditory ERP networks to predict pediatric TBI.

Based on previous ERP studies conducted in humans, we expected TBI to cause smaller peak amplitudes and longer latencies in the majority of channels, however we only observed this in a few channels in our piglets. The N40 peak (from channel 15) over the left auditory cortex was the only channel with significantly longer latencies on POST 7d after SAG injury compared to PRE. Long-latency ERP components (after 150ms post-stimulus) in humans, such as P250 and P300, showed greater variability of occurrence in both TBI patients and controls compared to N100 and P150 components.
We similarly observed decreased probability of occurrence of later ERP components, N120 and P200 compared to N40 and P60 in the juvenile pig. Piglet brains provide a platform for better understanding the young child brain due to similar neuroanatomy and development (Buckley, 1986; Dickerson & Dobbing, 1967; A. C. Duhaime, 1998; Margulies et al., 2015). However, the characterization of auditory ERP components in pigs is not as well developed as it is in humans. To our knowledge, there are no reports detailing which waveforms in humans correlate to waveforms seen in piglets, or if the same waveforms in humans are present in piglets.

**Traditional Analysis of ERPs Poorly Discriminated TBI**

In our study, the traditional analysis of magnitudes and latencies from auditory ERPs poorly discriminated TBI in piglets. Kraus et al. recently used characteristic auditory brainstem ERPs to accurately predict severity of concussion in children, but did not validate their findings with a separate dataset (Kraus et al., 2016). Their study shows slower neural responses to speech and impaired ability of children to identify, group and track sounds in complex environments following concussion. Our study shows longer latencies and lower amplitudes, with partial recovery compared to the pre-injury baseline in select channels. Within the ERP literature, there are various ways to approach quantifying ERP component latencies and magnitudes – use template matching, peak detection and manually identify ERP components (subjective approach that varies by researcher), which may affect the reproducibility of ERP component findings previously reported. There is a paucity of auditory studies conducted in piglets and adult pigs. In our study, we assumed that ERP components in female 4 week old Yorkshire piglets were the same as those previously reported in a study that used adult (12-14 months) male Gottingen minipigs with limited sample size of six. A small sample size may imply
high variability in the magnitude and latencies of reported ERP components and explain why our traditional analysis of ERPs did not yield post-injury changes that were largely consistent with the human TBI literature. The difference in age may also account for poor alignment with literature, where developmentally immature pigs may present time-shifted ERP components compared to adult pig ERP components. There may also be age-specific differences in the magnitudes and latencies of ERPs after TBI. Our novel application of network analysis to ERPs overcomes the reliance on ERP peak detection from only a few channels. Instead, network analyses use all the data from all channels as a robust means of gaining insight into the effect of TBI on neural activity that may be relevant to the study of TBI in various species and ages. In this report, we demonstrate how ERP functional networks can be useful to identify and monitor deficits after TBI.

TBI Alters Auditory ERP Networks

Functional networks have shown immense potential as biomarkers for the detection of brain regions that are dysfunctional or have compromised structural plasticity. Previously, several studies used neuro-imaging modalities such as fMRI and EEG to construct functional brain networks that captured the dynamic structural and cognitive changes accompanying several neurological disorders (Dennis et al., 2015; Hillary et al., 2014, 2015). Douw and colleagues reported better cognitive performance correlated with increased local connectivity of MEG functional networks in the theta band and higher clustering coefficient in the delta and theta bands (Douw et al., 2011). Alzheimer's disease is a neurodegenerative disorder with progressive neuronal cell death. In a default mode (fMRI) network study on Alzheimer's patients, lower connectivity correlated with poorer working memory performance (Kikuchi et al., 2011). Another study reported increased path length and reduced global efficiency in structural cortical networks in
Alzheimer’s disease patients (Lo et al., 2010). This implies that slower transmission of information across the network and lower potential for local processing. Several studies have shown that functional connectivity changes with cognitive task demands (Esposito et al., 2006; Fornito et al., 2012; Sun et al., 2007) and learning (Albert et al., 2009; Lewis et al., 2009). Modularity provides biological insight in the analysis of structural brain networks where dense local connectivity of white matter tracts may enable swift, accurate processing between neighboring brain regions (Gallos et al. 2012), which may belie functional nesting (Bassett et al. 2010). Modularity in functional brain networks may allow evolutionary or developmental adaptation of one functional module without the loss of other modules. In fMRI functional connectivity studies, modules tend to be spatially contiguous and map to brain regions that perform specific functions (Power et al, 2011, Cole et al. 2014).

In contrast to adults, few researchers study pediatric TBI and those that do, use a subject population with highly variable ages, post-injury dates and brain injury types (Dennis et al., 2015; Espy et al., 2004; Kraus et al., 2016). Retrospective TBI studies in humans are often unable to control for consistency of the nature and severity of brain injury and utilize wide post-injury time ranges which are known to affect presentation of cognitive symptoms. These factors may explain the confounding results reported in human TBI functional network literature. There are very few animal studies of functional networks after TBI. TBI models in animals allow control of injury type, severity, and timing of study.

We expected electrodes over the auditory cortex to be most functionally prominent for the processing of auditory responses, however synaptic activity from the auditory cortex will be detected by electrodes on the visual cortex because of the poor
spatial resolution of the EEG and subsequently captures activity from nearby (on the order of centimeters) neurons. The removal of weak edges from the network does not significantly change the magnitudes of network metrics and the conclusions from comparison across injury type and study date. When we examine node-based metrics such as clustering coefficient (quantifies local connections), we observed that local connectivity changes were similar to global connectivity changes observed in Nodal Strength. Also, when we removed a single node from each network, the network metrics did not change. This implies that the networks are robust to removal of weak edges.

Networks are proxies for synchronization across the brain, which explains why we observed that the majority of nodes and edges are necessary to capture post-TBI changes to the ERP network. There are only 32 electrodes, each of which will detect synaptic activity from thousands (if not millions) of neurons within proximity because of scalp-skull conductance.

We found changes in network metrics, such as reductions in nodal strength at 1 day and global efficiency at 7 days post-injury, especially after SAG. In rats with focal brain injury, increases in nodal strength, local clustering coefficient and efficiency with simultaneous reduction in modularity in resting-state fMRI BOLD networks were reported 7 days after CCI, but not at 14 and 28 days post-injury relative to pre-injury (Harris et al., 2016). The difference in modularity post-TBI may be explained by the different injuries, and imaging modalities used, where fMRI yielded a larger number of nodes than our network, which permits increased sensitivity to modularity changes. Additionally, Harris et al. 2016 imaged rats under medetomidine sedation and did not include shams in their study.

Since there are only a few studies of functional networks in animals, we also compared our findings to TBI studies in humans, although the heterogeneity of analytical
approaches, clinical inclusion/exclusion criteria and severity of the TBI in human studies make comparison to studies of TBI in animals challenging.

At 1 day post-TBI, we find decreased nodal strength compared to Sham, some evidence of lower global efficiency and significant edge weight increases relative to PRE evoked by OB-Trgt paradigm. By 4d, only CCI had increased nodal strength compared to Sham and enhanced edge weights relative to its PRE. Global efficiency was reduced in all injured groups compared to Sham at 4d. At 1 week post-injury, nodal strength from the injured groups returned to Sham levels, but global efficiency in all injured groups maintained lower values compared to Sham. Thus, hypo- and hyperconnectivity varied in our study after injury by injury type and time point. Similarly, several studies have shown conflicting findings on the effect of TBI on functional networks – some proposing hyper-connectivity (Harris et al., 2016; Hillary et al., 2014, 2015; Sharp et al., 2011) and others hypo-connectivity (Kumar, et al., 2009; Mishra et al., 2014; Rigon, et al., 2015; Stevens et al., 2012) - after trauma in both humans and animals. We believe that the discrepancy in the effect of TBI on functional connectivity may be dependent on recovery time, type of stimuli, neuroimaging technique, regions analyzed and heterogeneity of TBI subject population. Several resting-state functional neuroimaging studies have reported distributed neural network functional disconnection in moderate-to-severe TBI samples using magnetoencephalography (Castellanos et al., 2011) and fMRI (Kasahara et al., 2010, 2011; Nakamura, et al., 2009). Reduction of task-related functional brain connectivity has been observed following mild TBI during working memory tasks (Kumar et al., 2009). A recent task-related fMRI study has demonstrated less inter-hemispheric functional connectivity in their TBI cohort, compared with healthy controls (Rigon et al., 2015). Evidence of disrupted functional EEG connectivity in the brain after combat-
related blast injury was reported in cohort 32 months after blast exposure (Sponheim et al., 2011). Despite an absence of deficits on neuropsychological indices, the blast injured group exhibited diminished EEG phase synchrony in the frontal lobe, which the authors suggest diminished interhemispheric coordination of brain activity as a result of blast injury. This study also found that correlations of EEG phase synchrony with frontal lobe white matter tract integrity which may indicate pathological change in the tissue.

Following a neuro-rehabilitation intervention, there was a significant decrease in connectivity in right inferior frontal gyrus concomitant with significant increase in composite cognitive score in TBI patients, which suggests that functional network reorganization parallels cognitive improvement (Pevzner et al., 2016). The axonal damage found in the piglet cerebrum 8 days after TBI may be the cause of acute hypo-connectivity observed, where loss of axonal connectivity causes a failure of communication or synchrony between cortical neurons. However, axonal damage was observed after SAG and CCI injuries, but not in COR. We speculate that the absence of axonal damage after COR may be due to intact but dysfunctional axons. Also, behavioral and/or cognitive deficits may be present at 7 days following COR, since hypo-connectivity was comparable to SAG and CCI injuries that both demonstrate such deficits (Sullivan et al., 2013).

Hypo-connectivity following TBI may be the result of cellular dysfunction (node failure) or white matter damage (edge failure). White matter degradation may explain the decreased connectivity observed across the ERP networks on day 1 after SAG injury compared to sham. The timeframe of axonal sprouting coincided with reductions in functional connectivity in Harris, 2016, however more research is needed to elucidate the mechanism of decreased connections. Decreased connectivity may also be due to
reductions in dendritic density and spine formation. Recent studies have found that functional connectivity patterns largely reflect that of underlying structural or white matter connectivity (Dennis et al., 2015; Honey et al., 2009; Mayer, et al., 2011; Sharp et al., 2011). However, it is unclear how much white matter tract integrity must be disrupted in order to affect functional connectivity, and how global and regional functional connectivity varies as a result. Our results align with several human fMRI studies that postulate that TBI causes weakening and removal of functional connections, which in turn may be due to loss of structural connectivity (Sharp et al., 2011; Vargas, et al., 2015). White matter degradation as measured by DTI and immunohistochemistry may be used to de-couple the complex functional-structural relationship and provide a source for altered functional networks observed in animal models. More research is needed to further our understanding of the relationship between the loss of white matter microstructure and functional disconnection.

Several studies have demonstrated that the direction of head motion strongly affects the extent of axonal injury in various animal models and ages (Atlan, et al., 2017, Browne, et al., 2011; Duhaime et al., 1987; Gennarelli et al., 1982; Smith, et al., 2003). By performing rapid head rotations about the sagittal and coronal planes, we varied TBI severity by modulating the amount of stretching along the white matter tracts in order to examine its effect on functional connectivity. In our neonatal and juvenile pig models, SAG rapid head rotations cause significantly higher magnitudes of AIV compared to COR (Sullivan et al., 2015). The SAG and COR AIV values found in this study were consistent with previously published reports (Sullivan et al., 2013). Mean AIV of 0.58% at 5-6 days post-injury was found when a single sagittal rapid head rotation (mean angular velocity of 127 rad/s) was prescribed to 4-week old piglets (Weeks et al., 2014).
While the minimum AIV was found at 5-6 day post-injury compared to 3-8 hours, 1 day and 3-4 days, maximal AIV of 1.15% was observed at 1day post-injury in 4-week old piglets (Weeks et al., 2014). Another previous study (Ibrahim et al., 2010) of axial rapid head rotations in juvenile piglets reported mean AIV of 0.20% for low (129 rad/s) angular velocities and 0.94% for moderate (194 rad/s) angular velocities at 6 hours post-injury. In both previous reports, injured piglets had significantly higher AIV than shams. The AIV found in this report align with previous reports and imply that our injured group (sagittal rapid head rotation) sustained significantly more axonal damage compared to sham. CCI had more focal axonal damage than SAG, but larger AIV compared to SAG and COR rapid head rotations. We speculate that any significant change in ERP networks is due to deficits in cognitive ability due to the magnitude and regional location of axonal injury and future studies should compare networks after CCI and RNR injuries.

Assuming that a 4 week old piglet has brain mass of 35.5 g, human newborn brain mass of 400 g and a human adult brain of 1400g, the mass scaled (using Ommaya scaling law) angular velocity (from 130 rad/s in piglets) was 58 rad/s and 38 rad/s for human infants and adults respectively. There are limitations to the use of mass scaling law for comparison of angular velocity and/or acceleration across species and age due to differences in skull, brain and neck mechanical properties and shape. However, mass scaling the piglet head kinematics to human adults and infants allows comparison with the rotational kinematic ranges associated with sports-related TBI events. Rowson reports 50% risk of concussion as 28.3 rad/s using impact data from American football games (Rowson et al., 2012). Sullivan and colleagues reported the range of sagittal head rotational angular velocity as 44-49 rad/s from parietal and occipital head impacts during low height falls in infants (Sullivan, Coats, & Margulies, 2015), which were smaller than the current study's mass-scaled equivalent, 72 rad/s. The angular velocity levels
prescribed to piglet in the current report led to ERP network changes and axonal injury that may be associated with a moderate to high risk of concussion in adult humans based on previously reported concussion thresholds. For newborns, our mass-scaled velocity also exceeded rotational kinematics associated with low height falls. For young adults, a range of 892-1169 rad/s$^2$ was reported for head angular acceleration during soccer heading impacts in both sexes (Bretzin, et al., 2017). Average angular acceleration (36,932 rad/s$^2$) from 4 week old piglets mass-scaled to an adult human brain mass yielded a value of 3188 rad/s$^2$, which is much higher than the average acceleration reported for heading a soccer ball. At moderate to high risk levels for concussions, we expect to observe significant changes to the ERP network after rapid head rotation.

**Accurate Discrimination between Injured and Uninjured ERPs**

The XDawn and XDawnCov techniques have both been successfully used to discriminate between ERP responses to different visual cues (Barachant et al., 2012; Rivet, 2009, 2011). These techniques were developed in the brain-computer interface field with the goal of augmenting human cognitive or sensory-motor functions. They were thus developed to run in real-time as the EEG was recorded. This study was a novel application of XDawn/XDawnCov technique to auditory ERPs and to the study of TBI. We believe that the fast processing speed of these techniques may be useful for delivering rapid diagnostic and prognostic information to clinicians and patients. XDawnCov was able to extract insight between injured and uninjured animals by looking at covariance matrices, which similarly captures synchrony across the brain, like the cross-correlation networks previously discussed.
Our injury prediction algorithm, combining Std and OB paradigms, was able to reliably classify injured animals with >80% accuracy. The application of graph theory methods to ERPs is useful to the assessment of TBI and deployment of effective neuro-rehabilitation treatments. This study is the first ERP network study on TBI in animals. This EEG-based biomarker may have direct application to humans, including infants, providing clinicians the ability to track recovery states of brain injury in subjects and to quantitatively assess whether a treatment is working appropriately. To summarize, we have developed an auditory EEG-based tool that detects mild TBI after trauma. It provides multiple advantages over those currently used and stands to inform TBI diagnosis and prognosis. It is non-invasive, portable, fast and inexpensive compared to other neuroimaging techniques.

Limitations
ERP components may not consistently occur in recordings in humans (Dundon et al., 2015) and was difficult to find parallel features in piglets due to a paucity of previous studies. The N40, P60, N120 and P200 ERP components were selected from a study on adult pigs and may not consistently present in ERP recordings from developmental pig brains. High variability in ERP signals among channels and animals made detecting group differences in latencies and magnitudes of ERP components challenging. Similar to other neuro-imaging modalities, the application of ERPs to the study of TBI has several limitations such as wide variability within and between subjects and sensitivity to subject alertness. Fully connected networks, as opposed to thresholded networks, were analyzed, in order to consider weak and strong connections which both may be critical to discriminating between uninjured and injured piglets. The removal of weak edges did not significantly affect comparisons of network metrics across injury type.
Because only 30% of stimuli in the OB paradigm were OB-Trgts, there were fewer ERP responses to average over for the OB-Trgt networks than for the OB-Std, and fewer OB-Trgts in our training group. A small group size may increase variance of the OB-Trgt ERP measurements if insufficient background EEG was removed from the measured EEG activity. Although our findings were statistically significant, our sample size was modest and our findings should be replicated in a larger subject cohort. Increasing the number and duration of ERP epochs recorded per animal may improve the overall predictive capability of the algorithm. By using a controlled TBI dataset, we successfully classified traumatic brain-injured from uninjured ERP epochs, although classification thresholds for ERPs from TBI patients may differ from those for patients with other neurological disorders, such as stroke or schizophrenia. The algorithm was trained on SAG and CCI animals, which sustained higher axonal injury volumes than COR. The results obtained in this study may be specific to auditory stimuli. Future studies may focus on classification thresholds for more subtle auditory ERP alterations.

Future Work
ERPs have strong potential for real-time monitoring of athletes’ neural activity on game sidelines. Because auditory ERPs can be reliably measured during strenuous exercise (Makeig, et al., 2004) and EEG electrode caps are lightweight, early detection of concussions in athletes is feasible. Longer pig survival periods may also permit easier comparison with human TBI literature.

Future studies in pigs should consider acquiring histopathological data on altered synaptic junction proteins, which may indicate abnormal changes in neuroplasticity and thereby provide a pathological, structural mechanism for changes observed in EEG recordings following injury. Simultaneous measurement of ERPs and administration of
behavioral and cognitive tests should be performed in piglets 1, 4 and 7 days following injury in order to relate clinically relevant outcomes to changes in functional networks. The use of imaging modalities or other experimental methods that capture metabolic changes may also elucidate the cause of hypo-connectivity.

Conclusions
Network analysis provides an objective, quantitative framework for analyzing multi-source neural signals. We observed functional connectivity alterations in auditory ERP networks due to diffuse and local TBIs. Following diffuse injury, functional ERP networks in injured groups showed hypo-connectivity compared to the sham group, and enhancement of connectivity compared to pre-injury. We achieved 82% accuracy in the prediction of TBI from the functional connectivity networks of ERPs. Auditory ERPs provide a clinically adoptable method that provides fast, mobile and objective indications of TBI. This work has applications to sports-related concussions and strategies for early TBI detection, prevention and reduction of severity.


CHAPTER 4:
Frequency-Dependent Changes in Resting State EEG Functional Networks After Traumatic Brain Injury in Piglets

Abstract

Traumatic brain injury (TBI) is a major health concern in children, as it can cause chronic cognitive and behavioral deficits. The lack of objective involuntary metrics for the diagnosis of TBI makes prognosis more challenging, especially in the pediatric context, where children are often unable to articulate their symptoms. Resting state electroencephalograms (EEG), which are inexpensive, noninvasive, and do not require subjects to perform cognitive tasks, have not yet been used to create functional brain networks in relation to TBI in children or other animals; here we report the first such study. We recorded resting state EEG in awake piglets before and after TBI, from which we generated EEG functional networks from the alpha (8-12 Hz), beta (16.5-25 Hz), broad (1-35 Hz), delta (1-3.5 Hz), gamma (30-35 Hz), sigma (13-16 Hz) and theta (4-7.5 Hz) frequency bands. We hypothesize that mild TBI will induce persistent frequency-dependent changes in the 4 week old piglet at acute and chronic time points. Hyperconnectivity was found in several frequency band networks after TBI. This study serves as proof-of-concept that the study of EEG functional networks in awake piglets may be useful for the development of diagnostic metrics for TBI in children.

Introduction & Background

Traumatic brain injury (TBI) is the leading cause of death and disability in children in the United States (Bryan, et al., 2016; Faul, Xu, et al., 2010). Children have the highest incidence rates of emergency department visits associated with TBI, often due to sports
or recreation activities (Faul et al., 2010). Following pediatric TBI, typical symptoms include cognitive and behavioral deficits that are dependent on injury severity, location (diffuse or focal), and recovery duration. There is a great need for improved multimodal clinical assessment of pediatric TBI, because there are at present no standardized biomarkers for its diagnosis and prognosis either in the clinic or on the sidelines (Davis et al., 2017).

Electroencephalography (EEG) is a useful tool for examining the functional integrity of neuronal networks in health and disease. It is relatively inexpensive, noninvasive, and can provide a functional assay with high temporal resolution. In contrast to functional neuroimaging methods, a reasonable signal to noise ratio can be obtained even with minor subject movement, making it a particularly attractive means of evaluation in pediatric populations. However, conventional EEG is limited in its diagnostic and prognostic capability of pediatric TBI. Although there are EEG changes following severe TBI (Nadlonek, et al., 2015), the standard clinical interpretation of EEG signals is insensitive to mild TBI – most EEGs are within normal limits or show very subtle alterations (Nuwer, 2016; Schmitt & Dichter, 2015). Also, abnormalities in EEG signal amplitude and latencies do not consistently correlate with long-term symptoms (Schmitt & Dichter, 2015). As a way to overcome these limitations, network analyses of EEG recordings may provide a robust and objective platform for interpretation. Functional networks are commonly utilized in the study of neuropsychiatric disorders such as stroke and schizophrenia (Bartolomei et al., 2006; De et al., 2010; Nishida et al., 2013), and may similarly be helpful in determining the source of cognitive and behavioral deficits seen in TBI patients, including non-communicative children.
EEG recordings acquired while the subject is in a “resting state” are commonly analyzed using functional networks in order to study neurological disorders in humans (Boersma et al., 2011; Knyazev, et al., 2017; Mantini, et al., 2007; Musso, et al., 2010; Zheng et al., 2012). “Resting state” typically involves subjects sitting with eyes closed in a dark room while awake and free from any overt stimuli. Resting state EEG data is easier to collect and simpler for the subject than task-based procedures, which is appealing to the study of pediatric populations, for whom cognitive tasks can be more difficult to elicit consistently and reproducibly. At the time of this publication, there were few studies on developmental changes in resting state EEG functional networks (Boersma et al., 2011; Knyazev et al., 2017), but there are no reports on the effect of TBI on the pediatric human population.

Recently, the use of pigs in neuroscience research has grown because the pig brain is very similar to that of the human in anatomy and growth. Piglets share a similar rate of myelination and cerebral hemodynamics and metabolism with human neonates (Lind et al., 2007; Pareja, et al., 2016). Additionally, piglet brain development resembles the human post-natal developmental sequence as measured by EEG (Pareja et al., 2016; Saito, et al., 2005). EEG patterns in the piglet in the awake state and after cerebral insult were similar to those observed in children (Gavilanes et al., 2001; Iorioi et al., 2002; Saito et al., 2005). The use of the 4-week old piglet model permits investigation into the effects of TBI on EEG functional networks during early childhood (1-3 years old). We hypothesize that mild TBI will induce acute and chronic, frequency-specific changes in the resting state functional connectivity of juvenile piglet brains.

In this study, we examined resting state EEG functional networks across several frequency bands in awake, piglets before and after mild TBI. Our data shows significant
frequency-dependent changes in the characteristics of networks 1, 4 and 7 days after TBI, which may be helpful for the assessment of TBI in the pediatric population. The novel work presented here, addresses an important gap in the current EEG functional network literature on the study of pediatric TBI.

Methods

_Diffuse Traumatic Brain Injury in Piglets_

All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. Piglets were housed separately in cages and kept on a 12 hour light dark cycle. We studied 7 four-week old, female Yorkshire piglets (neurodevelopmentally equivalent to a human toddler) (Eucker, et al., 2011). The pathophysiology of TBI in piglets compares well with that of human children (Armstead, 2000, 2005) due to the similarities in gyral pattern, overall brain shape and distribution of gray and white matter (Buckley, 1986; Dickerson & Dobbing, 1967; Duhaime, 1998). Three piglets were randomly assigned to the injured group and four to the sham group. Each piglet in the injured group sustained a single, rapid, closed-head, non-impact rotation in the sagittal plane with mean peak angular velocity of 131 rad/s. The well-characterized diffuse white matter injury was induced via a HYGE pneumatic actuator (Eucker et al., 2011, Ibrahim et al., 2010, Maltese et al., 2012), and the angular velocity was recorded using an angular rate sensor (ATA Engineering Inc., Model#: ars-06, Herndon, VA) and a data acquisition system implemented in LabView (National Instruments, Austin, TX). Sham piglets received all of the procedures except the injury, including the anesthetic regimen. Anesthesia and analgesia were administered only on the day of injury. Buprenorphine (0.02 mg/kg) was delivered intramuscularly for analgesia prior to injury. Then, the following protocol was performed: premedication with
intramuscular injection of ketamine (20 mg/kg) and xylazine (2 mg/kg), induction with 4% inhaled isoflurane in 1.0 fraction of inspired oxygen via snout mask until lack of reflexive pinch response, maintenance at 1% inhaled isoflurane via endotracheal tube with fraction of inspired oxygen to 0.21 on the day of injury (day 0). Body temperature, blood pressure, oxygen saturation, heart rate, respiratory rate and end-tidal CO₂ were continuously monitored. A circulating water blanket was kept to maintain core body temperature between 36 and 38°C. For each animal, EEG data was acquired 1 day prior to injury and 1, 4 and 7 days post-injury.

**EEG Data Acquisition**

Before beginning EEG data acquisition, piglets were acclimated to wearing a nylon sleeve in their cages for a minimum of two 30-minute sessions over two days. Acquisition of resting-state EEG only occurred in non-agitated, silent, awake piglets using the 32-channel net (Electrical Geodesics, Inc. HydroCel™ Geodesic Sensor Net, Electrical Geodesics, Inc. Net Amps 400 EEG amplifier). The net was placed in potassium chloride electrolyte solution (11g dry potassium chloride, 1 L water and 5 mL baby shampoo) before placing it on the head. The International 10-20 EEG system (Nuwer et al., 1998) was utilized to ensure consistent positioning of the scalp electrodes (in the net) for each animal across days. We also used the nasion and inion as anatomical landmarks to ensure consistent sensor net placement across all animals. In order to maintain contact between electrode and scalp, we placed two thin nylon sleeves over the net. Before acquisition, the impedances of all electrodes were verified to be under 50 kΩ. We then recorded 1.5 minutes of resting state EEG data from immobile piglets in a silent room without any movement of nearby objects or investigators. EEG data was acquired at a rate of 1 kHz and referenced to channel 33 (located at the
midline in the center of the head). Recordings were discarded and repeated if background noise or head movement was observed.

Alpha band filtering was performed on the EEG signal using the Net Station Tools 4.6 software by applying a finite impulse response bandpass filter at 8-12 Hz (passband gain: -0.01 dB, stopband gain: -40 dB, rolloff: 0.99 Hz). Frequency band limits for the beta (16.5-25 Hz), broad (1-35 Hz), delta (1-3.5 Hz), gamma (30-35 Hz), sigma (13-16 Hz) and theta (4-7.5 Hz) bands were chosen based on the study by Modarres and colleagues (Modarres, et al., 2017). EEG recordings are influenced by electrical and physiologic artifacts, electrode placement, skull defects, anesthesia drugs and subject alertness (Nuwer et al., 1997; 1996; 2005). Cellular devices were not permitted in the study room during EEG recording and the 60 Hz (electrical noise) frequency was eliminated using a notch filter. After frequency filtering, artifact detection in and removal from EEG signals was performed using a user-defined algorithm in MATLAB®.

Our artifact detection algorithm removed variations in EEG voltage caused by bad channels (eye blinks and minor movements) and replaced these short segments with the mean EEG voltage without artifacts. We excluded the first and last second of each recording to remove any artifacts arising at the start and end of the data acquisition process. We then calculated the moving average (with span 80 ms) of the EEG signal. Within sequential 500 ms segments, the minimum (Min) and maximum (Max) moving average EEG values were computed. A segment of EEG signal was deemed to contain an artifact if its Max-Min value exceeded the threshold value of 20 μV, which was determined by manual inspection to robustly flag artifacts across all channels and animals.
**Average Spectral Power and Mean Frequency Analysis**

We then calculated average spectral power and mean frequency to illustrate changes in EEG features that were due to injury. Spectral power was calculated using the Fulop & Fitz method (Fulop & Fitz, 2006), in MATLAB® (R2015b, The MathWorks Inc., Natick, Massachusetts, United States), after applying a Hanning window to the 1.5 minute pre-processed EEG signal from all 32 electrodes. The average power of the post-processed (frequency-filtered and artifact removed) EEG signals was taken over the 1-35 Hz range of interest. We also calculated the mean frequency power-weighted average over the 1-35Hz range for every frequency band. Mean frequency was calculated as: \[ \sum_i f_i \cdot \text{Power}_{\text{norm},i} \], where \( f_i \) denotes the \( i \)th frequency value in the 1 – 35Hz domain and \( \text{Power}_{\text{norm},i} \) is the normalized power (power at \( f_i \) divided by the sum of power values across all frequencies). One scalar value of average power and the mean frequency were determined for each electrode at every frequency band; every animal contributed 32 average power and mean frequency measures.

**Construction of EEG Functional Networks**

Networks are collections of nodes and edges. The 32 electrodes can be regarded as representing the nodes and the synchrony between them as the edges. The resulting functional networks can provide insight into the interactions between the various brain regions over which electrodes are placed. Functional networks were constructed by calculating network edges as the maximum absolute cross-correlation of the amplitudes of the EEG signals between all pairs of electrodes (Fig. 4.1). One edge between two nodes represents the similarity between EEG signals from both nodes. Every 1.5 minute-long EEG recording was segmented into short (1000 milliseconds) segments and one network was built for each segment, yielding approximately 88 undirected, weighted
EEG functional networks per animal on a given study day (Fig. 4.1). All of the networks calculated were fully connected, i.e., had 496 edges among 32 nodes and were not thresholded for analysis in order to include the information from all edges. The broad band networks for all post-injury days were compared to the pre-injury day for sham and injury groups by constructing 32 x 32 matrices. Networks were calculated for the alpha (8-12 Hz), beta (16.5-25 Hz), broad (1-35 Hz), delta (1-3.5 Hz), gamma (30-35 Hz), sigma (13-16 Hz) and theta (4-7.5 Hz) bands.

**EEG Functional Network Metrics**

Network metrics were then calculated in order to quantify the integration (global communication and cooperation among brain regions) and segregation (formation of local and functionally specialized modules among brain regions) properties as well as the influence of key nodes and edges on the network. The five metrics calculated were: nodal strength, clustering coefficient, global efficiency, characteristic path length and modularity. Nodal strength was calculated as the sum of edges that each electrode has to all other electrodes. Clustering coefficient was calculated as the geometric mean of edges that formed triangles around each node. Nodal strength and clustering coefficient are node-based metrics meaning that there is a single value of nodal strength and clustering coefficient for each electrode; median values of nodal strength and clustering coefficient from all 32 electrodes were taken to yield a single, representative measure of nodal strength and clustering coefficient for each EEG network. Global efficiency was calculated as the average inverse of the shortest path length between all possible pairs of nodes in the network, where a path is the sequence of distinct edges taken to traverse nodes throughout the network. Characteristic path length was taken as the average shortest path among all possible pairs of nodes in the network. Modularity is the degree
to which a network’s nodes and edges may be separated or combined. Modularity was calculated using the MATLAB® Brain Connectivity Toolbox (Rubinov & Sporns, 2010) with the Louvain-Greedy algorithm with the modularity resolution parameter set as 1 (the classic value).

**Figure 4.1:** Diagram showing (A) resting-state EEG acquisition from sham and injured piglets and (B) network construction from all pairwise cross-correlations, which form an (C) adjacency matrix that indicates the strength of inter-electrode connections across the brain that may also be represented as a (D) weighted EEG functional network.

**Analysis of Core EEG Functional Network Topology**

Changes in the weighted EEG functional network metrics may be due to changes in the strength of edges, the number of connections or both. In order to determine the source of changes in networks, we examined whether network topology or the arrangement of
the core edges changes after injury. The method used was based on that performed by Chu and colleagues (Chu et al., 2012). Each weighted, cross-correlation network (captured from 1000 ms of EEG data) was converted to a binary network by statistical thresholding of its edges. One-sided empirical p-values were determined for each edge in the weighted network by counting the number of edges that were greater than the edge considered and dividing the count by the total number of edges in the network (Davison & Hinkley, 1997). Edges with significant p values (p < 0.05) formed a binary network representation of the strongest neural connections throughout the network over the 1000 ms epoch. For in a single animal, all binary networks were summed across time to obtain a weighted, consensus network over the 88 s duration of resting state EEG recording. We extracted the most persistent edges present over the total EEG recording duration by finding edges in the consensus network with weights above the 95th percentile. The strongest 5% of edges were calculated for all animals and frequency bands.

Statistical Analyses

We utilized a non-parametric Dunnett’s test (Gao, 2008) to compare all metrics from post-injury (POST 1, 4 and 7 d) days with those from the pre-injury (PRE) day, with a significance level of 0.05. The logarithm was taken of all network metrics (except modularity) before statistical tests were performed. Statistical tests were applied to all spectral power and network metrics for all frequency bands. All statistical analyses were performed in R 3.4.1 (R Core Team, 2017) using the nparcomp package (Konietzchke, et al., 2015).
Results

*Effect of TBI on Spectral Power*

We classified the EEG signals into human EEG frequency bands, specifically the alpha (8-12 Hz), beta (16.5-25 Hz), broad (1-35 Hz), delta (1-3.5 Hz), gamma (30-35 Hz), sigma (13-16 Hz) and theta (4-7.5 Hz). Across frequencies, there were large and significant alterations in the average spectral power in the injured group compared to the sham group (Fig. 4.2, summarized in Table 4.1). Generally, there was little change in the spectral power in the sham group. We focused our attention on those bands and time points with no change in sham, and significant changes after TBI. In the alpha frequency band (8-12 Hz), we observed no significant changes ($p>0.05$) in average spectral power in the sham group on any POST day compared to PRE (Fig. 4.2A). In the injured group, there was a significant increase ($p<0.0001$) in alpha power POST 1d and reductions POST 4 and 7d ($p<0.0001$) compared to pre-injury levels. There was no change ($p>0.05$) in average beta (Fig. 4.2B) power in the sham group on any day relative to PRE, however beta power increased significantly ($p<0.0001$) POST 1d compared to its PRE value and returned to its PRE value by POST 4d. Reductions ($p<0.01$) in broad band and delta (Fig. 4.2 C,D) average power values were observed in the sham group POST 1d relative to PRE. In the injured group, there were reductions in broad band ($p<0.01$) and delta power ($p<0.05$) POST 1, 4 and 7d relative to PRE. While no significant changes ($p>0.4$) in power were seen in the theta band (Fig. 4.2E) for sham, reduction ($p<0.03$) in power was seen in the injured group POST 1, 4 and 7d compared to PRE. In the sham group, a decrease ($p<0.01$) in gamma power (Fig. 4.2F) was noted POST 1d ($p=0.0004$), while increases were observed in the injured group POST 1 and 7d ($p<0.047$), in addition to a decrease POST 4d. Average power in the sigma band (Fig. 4.2G) decreased ($p<0.01$) POST 7d in the sham group compared to PRE, while
injury caused an increase (p<0.0001) in sigma power POST 1d. In summary, while 1d after TBI resulted in both increases and decreases in spectral power, we observe consistent and persistent 4d and 7d post-injury decreases in broadband, delta, theta and gamma spectral power measures that were not observed in the sham group.
Figure 4.2: Boxplots of average spectral power (on logarithmic scale) in sham and injured groups on days -1, 1, 4 and 7 across seven frequency bands. A non-parametric Dunnett’s test was performed for statistical comparison of post-injury days to pre. Black, horizontal bars indicate significant comparisons (p<0.05).
Table 4.1: Percent change in average power compared to pre-levels were analyzed at all frequency bands, and averaged across animals. Percent change in each metric indicates significant (p<0.05) non-parametric Dunnett's test significance.

**Effect of TBI on Mean Frequency**

Small, but significant injury and time-dependent changes in the mean frequency across all frequency bands were observed (Fig. 4.3, summarized in Table 4.2). Elevation of the mean alpha frequency (p<0.006) was seen on all days after sham treatment, while no change (p>0.05) was seen POST 1d and decreases (p=0.0036) were noted in the injured group POST 4 and 7d (Fig. 4.3A). The mean frequency in the beta band (Fig. 4.3B) was elevated (p<0.0001) POST 1, 4 and 7d compared to PRE in the sham group in contrast to the reductions (p<0.0001) on all POST days in the injury group. There were increases (p<0.05) in the broad band mean frequency (Fig. 4.3C) in sham and injured groups POST 4 and 7d relative to PRE; the injured group significantly increased (p<0.05) POST 1d while there was no change for sham (p>0.05). For the delta and theta bands (Fig. 4.3D, G), no significant changes (p>0.2) were observed in the sham or injured groups. In the gamma band (Fig. 4.3E), mean frequency increased (p<0.02) POST 1 and 7d and had no change (p>0.05) POST 4d in the sham group; injury caused uniform decreases (p<0.0001). In the sigma frequency (Fig. 4.3F), mean frequency in the sham group was reduced (p=0.004) POST 4d, while it increased (p<0.0001) POST 1
and 4d in the injured group. There was no change (p>0.05) in sigma mean frequency POST 7d for the sham and injury groups. In general, the injury effect magnitude was larger for spectral power than for mean frequency.

**Figure 4.3**: Boxplots of mean frequency in sham and injured groups on days -1, 1, 4 and 7 across frequency bands, (A) alpha, (B) beta, (C) broad, (D) delta, (E) gamma, (F) sigma and (G) theta. A non-parametric Dunnett’s test was performed for statistical comparison of post-injury days to pre. Black horizontal bars indicate significant comparisons (p<0.05).
Table 4.2: Percent change in mean frequency compared to pre-injury levels were analyzed at all frequency bands, and averaged across animals. Percent change in each metric indicates non-parametric Dunnett’s test significance (p<0.05).

Hyper-connectivity in Broad Band Networks During Recovery

Global resting state functional connectivity in the broad band network in shams was diminished compared to PRE on POST 1, 4 and 7d (Fig. 4.4). The majority of the sham group connections were classified as hypo-connected relative to PRE or had POST-PRE edge weight differences ≤ -5 (shown in white). Over the POST days, the sham group had a relatively modest decrease in the number of hypo-connected edges, with 992, 916 and 912 edges on POST 1, 4 and 7d respectively. In contrast, the number of hyper-connected edges relative to PRE (POST-PRE edge weight difference ≥ 5 and shown in dark blue) for sham increased from zero POST 1d to 54 and 56 on POST 4 and 7d respectively. The injured group had a dramatic transient increase in the number of hypo-connected edges, with 288, 26 and 2 POST 1, 4 and 7d respectively. After injury, diffuse hyper-connectivity increased markedly over POST time, with 368, 860 and 964 edges POST 1, 4 and 7d respectively. Injury induced a huge increase in the number of hyper-connected edges on all POST days that was more than 10 times the number of hyper-connected edges observed in the sham group. The injured group had substantially fewer hypo-connected edges than sham on all POST days.
Figure 4.4: Broadband network matrices for sham and injured groups showing median differences in edge weight after anesthesia or injury for all piglets. Each matrix shows the difference in connections between 32 electrodes across the piglet brain. The median difference in edge weights was calculated as post-injury–pre-injury across all animals.

**Nodal Strength**

First, nodal strength quantifies the number and strength of connection that each electrode has to all other electrodes, where a higher nodal strength represents increased synchrony of EEG signals with other electrodes. Shams had reduced (p<0.0001) nodal strength values for alpha networks POST 1, 4 and 7d compared to PRE (Table 4.3, Appendix Fig. A3.1). The injured group nodal strength was not significantly different (p>0.11) on any POST days relative to PRE in the alpha band networks. On all POST days, shams had smaller (p<0.0001) beta band nodal strength values, while the injured group presented elevated (p<0.0001) beta nodal strength values POST 1 and 7d compared to PRE; no change (p>0.05) was observed POST 4d. In broad band networks, sham showed a decrease (p<0.0005) in nodal strength POST 1d, and no change POST
4d \( (p=0.82) \) and 7d \( (p=0.82) \) (Fig. 5A). Broad band network nodal strength from injured animals did not change \( (p=0.1) \) POST 1d, but showed an increase \( (p<0.0005) \) POST 4 and 7d when compared to PRE. Sham delta band network nodal strength values decreased \( (p<0.0001) \) POST 1d relative to PRE and had no change POST 4 and 7d; in contrast, the nodal strength values from the injury group were not different \( (p>0.05) \) from PRE on POST 1d but were elevated \( (p<0.0001) \) POST 4 and 7d. There were significant changes in gamma network nodal strength for sham and injured on all POST days; the injured group showed uniform increases \( (p<0.0001) \) in nodal strength relative to PRE, while sham nodal strength decreased \( (p<0.0001) \) POST 1, 4 and 7d. Sigma nodal strength was reduced on all POST days in the sham group, while it increased \( (p<0.05) \) in the injured group on POST 1d and had no change POST 4 and 7d. In the shams, theta nodal strength decreased on POST 1, 4 and 7d and injured showed a reduction on POST 1d \( (p<0.0001) \), with no change \( (p>0.3) \) on POST 4 and 7d. Overall, there were widespread 6-30% reductions in nodal strength in sham networks across all frequency bands relative to PRE, whereas there were 7-20% elevations in nodal strength after injury.

<table>
<thead>
<tr>
<th>Network.Metric</th>
<th>Frequency</th>
<th>Sham.1d</th>
<th>Sham.4d</th>
<th>Sham.7d</th>
<th>Injured.1d</th>
<th>Injured.4d</th>
<th>Injured.7d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodal Strength</td>
<td>Alpha (8-12 Hz)</td>
<td>↓ -30.89%</td>
<td>↓ -8.46%</td>
<td>↓ -5.87%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
</tr>
<tr>
<td></td>
<td>Beta (16.5-25 Hz)</td>
<td>↓ -8.96%</td>
<td>↓ -6.85%</td>
<td>↓ -6.61%</td>
<td>↓ 14.34%</td>
<td>- 0.00%</td>
<td>↑ 3.59%</td>
</tr>
<tr>
<td></td>
<td>Broad (1-5 Hz)</td>
<td>↓ -21.67%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
<td>↑ 7.44%</td>
<td>↑ 8.81%</td>
</tr>
<tr>
<td></td>
<td>Delta (1-3.5 Hz)</td>
<td>↓ -23.94%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
<td>↑ 7.85%</td>
<td>↑ 8.82%</td>
</tr>
<tr>
<td></td>
<td>Gamma (30-55 Hz)</td>
<td>↓ -31.26%</td>
<td>↓ -9.22%</td>
<td>↓ -7.50%</td>
<td>↑ 11.90%</td>
<td>↑ 8.87%</td>
<td>↑ 12.94%</td>
</tr>
<tr>
<td></td>
<td>Sigma (13-16 Hz)</td>
<td>↓ -13.07%</td>
<td>↓ -10.31%</td>
<td>↓ -12.39%</td>
<td>↑ 21.55%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
</tr>
<tr>
<td></td>
<td>Theta (4-7 Hz)</td>
<td>↓ -31.72%</td>
<td>↓ -7.35%</td>
<td>↓ -6.03%</td>
<td>↓ -15.27%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
</tr>
</tbody>
</table>

**Table 4.3**: Percent change in mean values of nodal strength compared to pre-levels across all analyzed frequency bands. Percent change in each metric indicates non-parametric Dunnett’s test significance \( (p<0.05) \). Base 10 logarithm was applied to nodal strength before analysis.
Clusterin Coefficient

Second, clustering coefficient represents local clustering within a network that is measured as the average “intensity” of triangles around a node. When the average intensity of triangles about a node is low, the local connections are weak. In shams, broad band network clustering coefficient (Fig. 4.5B) decreased (p<0.0005) POST 1d relative to PRE then returned to PRE values by POST 4 and 7d (p=0.77); in the injured group, clustering coefficient did not change (p>0.05) POST 1d, but it increased (p<0.0005) POST 4 and 7 d compared to PRE. Clustering coefficient presented the same pattern of changes as nodal strength across all frequency networks and days with much larger changes, where 15-80% reductions were widely observed in the sham group and 16-75% elevations were observed in the injured group (Table 4.4, Appendix Fig. A3.2).

### Table 4.4: Percent change in mean values of clustering coefficient compared to pre-levels across all analyzed frequency bands. Percent change in each metric indicates non-parametric Dunnett’s test significance (p<0.05). Base 10 logarithm was applied to clustering coefficient before analysis.

<table>
<thead>
<tr>
<th>Network Metric</th>
<th>Frequency</th>
<th>Sham.1d</th>
<th>Sham.4d</th>
<th>Sham.7d</th>
<th>Injured.1d</th>
<th>Injured.4d</th>
<th>Injured.7d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clustering Coefficient</td>
<td>Alpha (8-12 Hz)</td>
<td>-63.78%</td>
<td>-17.35%</td>
<td>-14.12%</td>
<td>-0.00%</td>
<td>-0.00%</td>
<td>-0.00%</td>
</tr>
<tr>
<td></td>
<td>Beta (16.5-26 Hz)</td>
<td>-33.82%</td>
<td>-24.15%</td>
<td>-23.44%</td>
<td>75.06%</td>
<td>-0.00%</td>
<td>20.28%</td>
</tr>
<tr>
<td></td>
<td>Broad (1-35 Hz)</td>
<td>-37.85%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>-0.06%</td>
<td>16.21%</td>
<td>19.37%</td>
</tr>
<tr>
<td></td>
<td>Delta (1-3 Hz)</td>
<td>-42.57%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>-0.00%</td>
<td>17.54%</td>
<td>20.99%</td>
</tr>
<tr>
<td></td>
<td>Gamma (30-55 Hz)</td>
<td>-78.90%</td>
<td>-23.02%</td>
<td>-18.84%</td>
<td>69.85%</td>
<td>53.92%</td>
<td>78.55%</td>
</tr>
<tr>
<td></td>
<td>Sigma (13-16 Hz)</td>
<td>-74.52%</td>
<td>-54.90%</td>
<td>-65.94%</td>
<td>320.63%</td>
<td>-0.00%</td>
<td>-0.00%</td>
</tr>
<tr>
<td></td>
<td>Theta (4-7 Hz)</td>
<td>-58.94%</td>
<td>-13.57%</td>
<td>-11.11%</td>
<td>-36.47%</td>
<td>0.00%</td>
<td>-0.00%</td>
</tr>
</tbody>
</table>

Global Efficiency

Third, global efficiency quantifies the functional integration in the brain, which is the ability to rapidly combine specialized information from distributed brain regions. We observed an increase (p<0.0005) in global efficiency from alpha band networks POST...
1d for both injury and sham (Table 4.5, Appendix Fig. A3.3). Global efficiency in shams returned to PRE values by day 4 (p=0.35 & p=0.51 on POST 4d and 7d, respectively), but remained significantly elevated (p<0.0005) in the injury group POST 4 and 7d. Global efficiency in beta band networks for the shams significantly increased (p<0.0005) POST 1, 4 and 7d compared to PRE, but did not change in the injured group on any POST day (POST 1d, p=0.055; POST 4d, p=0.055; POST 7d, p=0.59). Sham animals increased in broad band network global efficiency POST 1 and 7d (p<0.0001) compared to PRE, but had no change (p=0.61) POST 4d (Fig. 5C). In contrast, injured networks had reduced global efficiency values (p<0.0001) at POST 4 and 7d. Significant elevations (p<0.0005) in delta band global efficiency were observed in sham on all POST days relative to PRE, while the injured group had reduced (p<0.0001) global efficiency POST 4 and 7d, with the exception of POST 1d when an increase (p=0.045) was seen. Sham presented increases (p<0.02) in global efficiency in gamma band networks on POST 1 and 4d, but no change (p=0.36) on POST 7d. In the injured group, global efficiency increased (p=0.004) POST 1d then, decreased (p=0.0002) POST 4d before returning to PRE values POST 7d (p>0.05). Sigma band networks in the sham group had elevated (p<0.001) global efficiency at POST 1, 4 and 7d and injured had an increase POST 1d relative to PRE. Both the injured and sham groups had elevated (p<0.0005) theta network global efficiency values POST 1d compared to PRE, however only injured yielded significant increases in global efficiency POST 4 and 7d (p<0.0005). Out of all frequency bands, sham presented 6-2103 % increases (p<0.05), while only the injured group exhibited 71-225% reductions (p<0.05) in global efficiency in the broad, delta and gamma bands POST 4 and 7d relative to PRE.
Table 4.5: Percent change in mean values of global efficiency compared to pre-levels across all analyzed frequency bands. Percent change in each metric indicates non-parametric Dunnett’s test significance (p<0.05). Base 10 logarithm was applied to global efficiency before analysis.

**Characteristic Path Length**

We looked at changes in characteristic path length across different frequency bands. Measures of integration such as global efficiency and characteristic path length estimate the ease with which brain regions communicate and are commonly based on the concept of a path. Paths are sequences of distinct nodes and edges and represent potential routes of information flow between pairs of brain regions. The fewer number of edges it takes to traverse the network, the higher the global efficiency and smaller the path length. Alpha band network characteristic path length decreased (p<0.0005) POST 1d in the sham and injured groups compared to PRE, then decreased (p<0.02) only in the injured group POST 4 and 7d (Table 4.6, Appendix Fig. A3.4). No change in alpha characteristic path length was observed POST 4 and 7d (p=0.3, p=0.4 respectively) in shams. Beta band network characteristic path length decreased (p<0.003) in the shams POST 1, 4 and 7d, while there were no changes (p>0.30) in the injured group. We observed broad band network characteristic path length decreases (p<0.0001) in sham POST 1 and 7d relative to PRE, and increases (p<0.0001) in injured animals POST 4 and 7d relative to PRE (Fig. 4.5D). No change in broad band network characteristic path
length was observed in injured group POST 1d. Delta band network characteristic path length decreased (p<0.01) POST 1, 4 and 7d for shams, while it increased (p<0.008) in the injured group on all POST days. Sham had significant reductions (p<0.04) in gamma band network characteristic path length on POST 1 and 4d, while it increased (p<0.0001) injured POST 4d. In shams, there were significant decreases (p<0.0001) in the sigma band network characteristic path length POST 1, 4 and 7d; no changes (p>0.1) were observed in the injured group on any POST day relative to PRE. Theta characteristic path length decreased (p<0.0001) in sham POST 1d and in injured POST 1, 4 and 7d. In summary, the sham group exhibited 5–484% reductions in characteristic path length, while the injured group exhibited 10–272% elevations in the broad, delta and gamma bands.

Table 4.6: Percent change in mean values of characteristic path length compared to pre-levels across all analyzed frequency bands. Percent change in each metric indicates significant (p<0.05) non-parametric Dunnett’s test significance. Base 10 logarithm was applied to characteristic path length before analysis.

<table>
<thead>
<tr>
<th>Network Metric</th>
<th>Frequency</th>
<th>Sham.1d</th>
<th>Sham.4d</th>
<th>Sham.7d</th>
<th>Injured.1d</th>
<th>Injured.4d</th>
<th>Injured.7d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic Path Length</td>
<td>Alpha (8-12 Hz)</td>
<td>↓ -93.66%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
<td>↓ -167.62%</td>
<td>↓ -8.93%</td>
<td>↓ -47.25%</td>
</tr>
<tr>
<td></td>
<td>Beta (16.5-25 Hz)</td>
<td>↓ -122.22%</td>
<td>↓ -6.06%</td>
<td>↓ -6.11%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
</tr>
<tr>
<td></td>
<td>Brood (1-35 Hz)</td>
<td>↓ -68.66%</td>
<td>- 0.00%</td>
<td>↑ -10.52%</td>
<td>- 0.00%</td>
<td>↑ 59.90%</td>
<td>↑ 62.10%</td>
</tr>
<tr>
<td></td>
<td>Delta (1-3.5 Hz)</td>
<td>↓ -55.75%</td>
<td>↓ -5.86%</td>
<td>↓ -11.00%</td>
<td>↑ 10.69%</td>
<td>↑ 69.01%</td>
<td>↑ 92.13%</td>
</tr>
<tr>
<td></td>
<td>Gamma (30-35 Hz)</td>
<td>↓ -120.30%</td>
<td>↓ -8.87%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
<td>↑ 272.45%</td>
<td>- 0.00%</td>
</tr>
<tr>
<td></td>
<td>Sigma (13-16 Hz)</td>
<td>↓ -84.32%</td>
<td>↓ -101.22%</td>
<td>↓ -137.92%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
</tr>
<tr>
<td></td>
<td>Theta (4-7.5 Hz)</td>
<td>↓ -82.07%</td>
<td>- 0.00%</td>
<td>- 0.00%</td>
<td>↓ -123.02%</td>
<td>↓ -7.68%</td>
<td>↓ -28.63%</td>
</tr>
</tbody>
</table>

**Modularity**

Fifth, modularity measures the degree to which the network organizes its connections into segregated clusters of nodes and edges. For alpha networks, we observed an increase (p<0.0001) then decrease (p<0.05) in modularity POST 1 and 7d, respectively in the sham group (Table 4.7, Appendix Fig. A3.5). After injury, modularity decreased
(p<0.04) POST 1d and 4d relative to PRE; no changes (p=0.18) were observed POST 7d. Beta network modularity significantly increased (p<0.01) POST 1, 4 and 7d, while for the injured group, modularity decreased (p=0.002) POST 7d relative to PRE. There was an increase (p<0.0001) in broad band network modularity for sham POST 1d, while modularity decreased for the injured group POST 1 (p=0.01), 4 (p<0.0001) and 7d (p=0.003) relative to PRE (Fig. 5E). Delta network modularity increased (p<0.0001) POST 1d for sham, but decreased (p<0.0002) POST 4 and 7d, while modularity in the injured group decreased (p<0.0001) on all POST days. Gamma network modularity increased (p<0.01) POST 1 and 4d for the shams, while in the injured group, modularity decreased (p<0.0001) POST 4d after injury. Modularity in the sigma networks increased (p=0.04) POST 7d for the shams and decreased (p=0.0001) in the injured group POST 1d. In the sham group, increases (p<0.05) were observed in the theta network modularity POST 1 and 4d, followed by a reduction (p=0.05) POST 7d. Injury resulted in a reduction (p<0.0001) in theta network modularity POST 4d compared to PRE. In summary, significant elevations (by 30 - 564%) were observed POST 1d in the sham group across all frequency bands, while decreases (by 29 - 87%) or no changes in modularity were seen in the injured group POST 1, 4 and 7 days.

We used standard deviation (SD) for each network metric, frequency band and day to quantify session (day-to-day) variability across animals because SD provides a reliably measures the spread of the distribution of each animal’s metric. A 2-way ANOVA with repeated measures (to account for multiple measures from the same animal) was performed on the SD values by day and frequency band separately for the sham (p=0.5) and injured groups. Nodal strength variability was not affected by day in the sham or injured (p=0.291) group. For clustering coefficient, variability was not significantly
affected by day in the sham (p=0.513) or injured group (p=0.297). There was no effect of
days on global efficiency standard deviation in the sham (p=0.091) and injured (p=0.31)
group. The variability of characteristic path length was not significantly affected by day in
the sham (p=0.107) or injured (p=0.282) group. For modularity, there was no effect of
days on variability for the sham (p=0.145) and injured (p=0.534) group. The variability of
all metrics was significantly affected (p<0.01) by frequency band in both the sham and
injured groups. Day-to-day variability across animals did not significantly affect any
network metric, however was affected by frequency band.

<table>
<thead>
<tr>
<th>Network Metric</th>
<th>Frequency</th>
<th>Sham.1d</th>
<th>Sham.4d</th>
<th>Sham.7d</th>
<th>Injured.1d</th>
<th>Injured.4d</th>
<th>Injured.7d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modularity</td>
<td>Alpha (8-12 Hz)</td>
<td>↑ 172.83%</td>
<td>0.00%</td>
<td>↓ 64.42%</td>
<td>↓ 29.58%</td>
<td>↓ 37.35%</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>Beta (16.5-25 Hz)</td>
<td>↑ 30.91%</td>
<td>↑ 53.95%</td>
<td>↑ 34.04%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>↓ 41.49%</td>
</tr>
<tr>
<td></td>
<td>Broad (1-13 Hz)</td>
<td>↑ 175.13%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>↓ 47.10%</td>
<td>↓ 73.14%</td>
<td>↓ 51.91%</td>
</tr>
<tr>
<td></td>
<td>Delta (1-3.5 Hz)</td>
<td>↑ 90.34%</td>
<td>↓ 86.41%</td>
<td>↓ 85.17%</td>
<td>↓ 97.41%</td>
<td>↓ 87.53%</td>
<td>↓ 62.62%</td>
</tr>
<tr>
<td></td>
<td>Gamma (30-35 Hz)</td>
<td>↑ 120.87%</td>
<td>↑ 14.83%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>↓ 33.77%</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>Sigma (13-16 Hz)</td>
<td>0.00%</td>
<td>0.00%</td>
<td>↑ 32.20%</td>
<td>↓ 45.68%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>Theta (4-7.5 Hz)</td>
<td>↑ 564.74%</td>
<td>↑ 27.29%</td>
<td>↓ 56.17%</td>
<td>0.00%</td>
<td>↓ 36.84%</td>
<td>0.00%</td>
</tr>
</tbody>
</table>

Table 4.7: Percent change in mean values of modularity compared to pre-levels across
all analyzed frequency bands. Percent change in each metric indicates significant
(p<0.05) non-parametric Dunnett’s test significance.
Figure 4.5: Comparison of broad band resting state network metrics, (A) nodal strength, (B) clustering coefficient, (C) global efficiency, (D) characteristic path length and (E) modularity, by injury group and day. Each open circle represents a single 1s network and each color indicates an animal. The thick black horizontal bars are mean values over all animals, while the thin black horizontal lines at the top of panels represent significant comparisons of post-injury values relative to pre-injury values using a non-parametric Dunnett’s test (p<0.05).

In the injured group, alpha band nodal strength and clustering coefficient did not significantly change on 1, 4 or 7 days post-injury relative to pre, while in the sham nodal...
strength decreased on all days. Alpha band global efficiency increased 1 day in sham and injured groups, but presented no significant change at 4 and 7 day in the sham while increases were found in the injured group relative to pre-injury. A reduction in characteristic path length was only observed in the sham at 1 day and no significant changes at 4 and 7 days, while in the injured group reductions were found on 1, 4 and 7 days post-injury. An increase in alpha modularity was seen on 1 day post-anesthesia, then no change and a decrease at 4 and 7 days respectively in the sham group. Modularity decreased on days 1 and 4, but returned to pre-injury levels by day 7 for the injured group.

For the beta frequency band, nodal strength and clustering coefficient were reduced on all post-anesthesia days relative to pre in the sham group, but increased on days 1 and 7 in the injured group; no change was observed on post-injury day 4. Global efficiency in the beta band increased in the sham group and did not change in the injured group for all post-injury days. Characteristic path length in the beta band decreased on all days in the sham group but did not change on any post-injury day in the injured group. Beta modularity increased on days 1, 4 and 7 in the sham group relative to pre, however in the injured group modularity did not vary from pre-injury levels on days 1 and 4 then decreased on day 7.

Broad and delta band nodal strength and clustering coefficient decreased on 1 day in the sham group then returned to pre-anesthesia levels. In contrast, the injured group had pre-injury levels of nodal strength and clustering coefficient on 1 day then increased on 4 and 7 days post-injury. Broad and delta global efficiency had increases on all post-anesthesia days in the sham group, while the injured group increased on 1 day then decreased on 4 and 7 days post-injury. Characteristic path length from the broad and
delta band was reduced on all post-anesthesia days in the sham group and increased on all post-injury days in the injured group. Broad and delta band modularity increased on 1 day in the sham group then decreased or did not change on 4 and 7 days, while the modularity decreased on all post-injury days in the injured group.

Gamma nodal strength and clustering coefficient decreased on all post-anesthesia days in the sham group then increased on all post-injury days in the injured group. Gamma global efficiency increased on 1 and 4 days then returned to pre levels on day 7 in the sham group, while global efficiency increased on day 1 then decreased on day 4 before returning to pre-injury levels on day 7 in the injured group. Gamma characteristic path length decreased on days 1 and 4 then returned to pre levels by day 7 in the sham group, while the injured group had no change from pre-injury levels on days 1 and 7, but increased at 4 days. In the sham group, gamma modularity increased on 1 and 4 day before returning to pre-levels, while in the injured group modularity did not vary from pre levels on 1 and 7 days however did decrease on 4 d post injury.

Nodal strength and clustering coefficient in the sigma band decreased on all post-anesthesia days for the sham group and increased on 1 day then returned to pre-injury levels in the injured group. Sigma global efficiency increased on all post days in the sham group, but only increased 1 day post-injury before returning to pre-injury levels on 4 and 7 days. Sham group’s characteristic path length in the sigma band decreased on all post-anesthesia days, while the injured group did not change from pre-injury levels on any day. Sigma modularity stayed at pre levels on 1 and 4 days then increased at 7 day in the sham group, while modularity decreased on 1 day then returned to pre-injury levels at 4 and 7 days post-injury in the injured group.
In the sham group, theta nodal strength and clustering coefficient decreased on 1, 4 and 7 days post-anesthesia, while the injured group decreased on 1 day post-injury then returned to pre-injury levels on 4 and 7 days. Theta global efficiency increased on 1 day then returned to pre-anesthesia levels on 4 and 7 days in the sham group; global efficiency in the injured group increased on 1,4 and 7 days post-injury relative to pre. Characteristic path length in the theta band decreased on 1 day then returned to pre levels on 4 and 7 day post-anesthesia, while it decreased on all post-injury days in the injured group. Modularity in the theta band increased on 1 and 4 days post-anesthesia then decreased at 7 days in the sham group, while modularity did not vary from pre-injury levels on days 1 and 7 and decreased on day 4 in the injured group.

On post-injury day 1, nodal strength and clustering coefficient did not change from pre-injury levels in the alpha, broad and delta bands, increased in the beta, gamma and sigma bands and decreased in the theta band. Global efficiency increased in the alpha, delta, gamma, sigma and theta bands and did not change from pre-injury levels in the beta and broad bands on post-injury day 1. At 1 day, characteristic path length in the injured group increased in the delta band, decreased in the alpha and theta band and did not change from pre-injury levels in the beta, broad, gamma and sigma bands. Modularity in the inured group decreased in the alpha, broad, delta and sigma bands and did not change in the beta, gamma and theta bands.

Nodal strength and clustering coefficient in the injured group increased in the broad, delta and gamma bands at 4 days post-injury, while they did not significantly vary from pre levels in the alpha, beta, sigma and theta bands. Global efficiency increased in the alpha and theta bands, decreased in the broad, delta and gamma bands and did not change from pre-injury levels in the beta and sigma bands at 4 days post-injury.
Characteristic path length increased in the broad, delta and gamma bands, decreased in the alpha and theta bands and did not vary from pre-injury levels in the beta and theta bands. At 4 days post-injury, modularity decreased in the alpha, broad, delta, gamma and theta bands and did not change in the beta and sigma bands.

At 7 days post-injury, nodal strength and clustering coefficient increased in the beta, broad, delta and gamma bands, while they did not change from pre-injury levels in the alpha, sigma and theta bands. Increases in global efficiency were found in the alpha and theta bands, decreases in the broad and delta bands and no changes in the beta, gamma and sigma bands at 7 days post-injury. Characteristic path length increased in the broad and delta bands, decreased in the alpha and theta bands and did not significantly vary from pre-injury levels in the beta, gamma and sigma bands at 7 days post-injury. Modularity decreased in the beta, broad and delta bands and did not change in the alpha, gamma, sigma and theta bands.

Core Network Topology Across Frequency Bands

For every animal, the core network topology or arrangement of edges was calculated as the 95th percentile of the sum of all statistically significant one second binary networks over the entire duration of the EEG recording. The core edges represent the strongest connections throughout the brain in a given frequency band before and after TBI. Visual inspection of each piglets’ topology across all analyzed frequencies (Appendix Fig A3.6-A3.12) showed that the arrangement of edges between sham and injured did not differ. There was remarkably consistent alpha band network topology (averaged over all pigs) before and after injury (Fig. 4.6). The number of edges present in the core topology was also consistent between animals and on different days. The alpha band core topology had several edges in the left and frontal regions of the brain. We observed similar
conservation of core network topology before and after TBI in the beta band, but with distinct signature configurations (Fig. 4.7) of core edges that were symmetric about the midline. The averaged broad band core topology was similar in the sham and injured groups, with edges that were slightly more focused in the right hemisphere and across the frontal, temporal and parietal nodes (Fig. 4.8). Averaged delta core edges were similar in the sham and injured groups and focused in the right, occipital region (Fig. 4.9). Averaged gamma core networks had the majority of edges in the right hemisphere (Fig. 4.10) in both sham and injured groups. Averaged sigma core networks were similar in the sham and injured groups with symmetric distribution about midline and in all regions (Fig. 4.11). Theta edges were focused in the left hemisphere (Fig. 4.12) for both sham and injured groups. The total number and arrangement of core edges was dependent on the frequency band of interest, but did not change with injury or study day.

**Figure 4.6:** Averaged (across all piglets) core alpha band networks with the most consistent edges (95th percentile of frequency over the sum of 88 one-second networks) from sham and injured groups.
Figure 4.7: Averaged (across all piglets) core beta band networks with the most consistent edges (95th percentile of frequency over the sum of 88 one-second networks) from sham and injured groups.

Figure 4.8: Averaged (across all piglets) core broad band networks with the most consistent edges (95th percentile of frequency over the sum of 88 one-second networks) from sham and injured groups.
Figure 4.9: Averaged (across all piglets) core delta band networks with the most consistent edges (95th percentile of frequency over the sum of 88 one-second networks) from sham and injured groups.

Figure 4.10: Averaged (across all piglets) core gamma band networks with the most consistent edges (95th percentile of frequency over the sum of 88 one-second networks) from sham and injured groups.
**Figure 4.11:** Averaged (across all piglets) core sigma band networks with the most consistent edges (95th percentile of frequency over the sum of 88 one-second networks) from sham and injured groups

**Figure 4.12:** Averaged (across all piglets) core theta band networks with most the consistent edges (95th percentile of frequency over the sum of 88 one-second networks) from sham and injured groups

Discussion

“Resting state” typically involves subjects sitting with eyes closed in a dark room while awake and free from any overt stimuli. Resting state EEG data acquisition is easier to collect and simpler for the subject than task-based procedures, which is appealing to the
study of pediatric populations, for whom cognitive tasks can be more difficult or frustrating in unpredictable ways. However, identification of abnormalities in EEG recordings after mild TBI can be subtle compared to controls and may be subject to bias by the interpreter. While there are several resting state fMRI studies in adults that report both increases and decreases in functional connectivity after TBI (Hillary et al., 2011; Kasahara et al., 2011; Mayer, et al., 2011; Palacios et al., 2017; Rigon, et al., 2015; Sharp et al., 2011), hyperconnectivity was reported by the only resting state fMRI study on a pediatric TBI cohort (Risen, et al., 2015). Virji-Babul and colleagues reported increased local connectivity, but no change in global connectivity of resting state EEG networks in adolescent athletes with a sports-related concussion (Virji-Babul et al., 2014). There is a small number of resting state EEG studies of TBI in adults (Porter et al., 2017), however there are no reports on pediatric EEG functional networks following TBI. This report fills the gap in the TBI resting state EEG literature, by studying the effect of diffuse TBI in piglets. We hypothesized that TBI would induce acute and chronic changes in the properties of resting state EEG functional networks, that were also dependent on the frequency band of interest.

**TBI Changes Spectral Power & Mean Frequency for Different Frequency Bands**

We observed lower spectral power and reduction in frequency in the alpha and beta bands in our piglets 4 and 7 days following injury and this change was absent in shams. The suppression of alpha spectral power may be imply balance dysfunction (Slobounov, et al., 2012). Reductions in alpha and beta amplitudes have been associated with diminished cognitive function (Thatcher, et al., 1998). Mild, diffuse reduction in the alpha band mean frequency is a common EEG abnormality that is observed in TBI patients (Koufen & Dichgans, 1978; Haneef, et al., 2012; Schmitt & Dichter, 2015; Tebano, et al., 2015).
1988; von Bierbrauer, et al., 1992). Tebano and colleagues reported reductions of fast beta (20-35 Hz) mean frequency 3-10 days following mild TBI compared with normal controls (Tebano, et al., 1988). Their fast beta range corresponds to a portion of the beta (16.5-25) and gamma (30-35 Hz) bands used in this report. In our study, a significant change in mean theta frequency was not observed, which aligns with previously published studies that report inconsistent changes in the theta band in TBI patients (Haneef et al., 2012; Tebano et al., 1988). We saw no significant changes in the delta mean frequencies post-injury in our piglets, in contrast to the increases (Fenton, 1994, 1996; Gosselin et al., 2009; McClelland, Fenton, & Rutherford, 1994) and decreases (Moeller, et al., 2011) that have been observed in TBI patients during post-injury hours to weeks. In our study, we observed decreases in delta and theta average spectral power. Increased theta and delta power may be associated with postural instability (Thompson, et al., 2005).

When the brain engages in certain functions, such as directing attention or processing sensory stimuli, select oscillations are dominant (Buzsáki, et al., 2013). Theta and gamma activity are prominent in locomotion. Theta oscillations are linked to memory functions in the hippocampus. Delta oscillations appear to be implicated in many cognitive processes such as autonomic functions, high emotional involvement and behavioral inhibition (Harmony, 2013). Slow oscillations may involve many neurons in large brain areas, while brief time intervals of fast oscillations facilitate local integration due to limits of axon conduction delays. If an axon's myelin sheath is damaged, this may lead to increased axonal conduction delays and subsequent increased power in slow oscillation ranges. There is much less research on delta and beta range rhythms in infants and young children (Saby & Marshall, 2012). Alpha rhythm emerges around 3
months of age and is sensitive to visual input - it increases in amplitude when the eyes are closed (Saby & Marshall, 2012). Alpha is implicated in visual attention and processing. Theta oscillations are often observed during the transition from wakefulness to sleep in adults. An increase in theta power has been associated with processing of emotional information and memory-related tasks. In infants, an increase in theta power has been linked to executive control of attention. Studies of the gamma rhythm in infants and adults report its relation to active memory retrieval, where familiar stimuli evoke a greater gamma band response compared to unfamiliar stimuli (Saby & Marshall, 2012).

Oscillatory activity contributes to higher order information processing, for instance hippocampal theta (3-12 Hz) oscillations are concurrent with spatial learning deficits (Fedor, et al., 2010; Lee et al., 2013a). Hippocampal interneurons are vulnerable to cell death and altered function after TBI which may contribute to the changes observed in theta band. Following injury in rodents, there is a decrease in alpha, beta, delta and theta power (Dixon et al., 1987; Ishige et al., 1987; McIntosh, et al., 1987; Paterno, et al., 2016), which agrees with our results in the alpha, delta and theta bands at 4 and 7 days post-injury. Slower oscillatory rhythms can reset and bias computation in multiple cortical regions (Buzsáki, et al., 2013). Changes in EEG are observed in the alpha, beta, delta, theta and gamma bands and are not state-dependent because alterations are noted when a patient is at rest (Borich, et al., 2015; Virji-Babul et al., 2014), actively moving (Slobounov, et al., 2012) or asleep (Frieboes et al., 1999; Parsons, et al., 1997). The changes in average power and mean frequency from the alpha and beta bands induced by TBI in our piglets were generally consistent with findings reported in TBI patients, providing the basis for applying network analysis to our EEG data.
TBI Affects Resting State EEG Network Metrics in all Analyzed Frequency Bands

The resting state network represents the synchrony of background EEG activity across the brain. We observed changes in nodal strength, clustering coefficient, global efficiency, characteristic path length and modularity within the same animal on a single day due to the rapidly changing dynamics of neural activity on the time scale of seconds; this was comparable to previously published studies (Chu et al., 2012; Kramer et al., 2011). Additionally, we observed changes in network metrics between sham and injured subjects; these changes were dependent on the frequency band of interest. While alterations in average power and mean frequency were frequency band-dependent, only changes in characteristic path length and global efficiency were influenced by frequency band. There were significant changes in several resting state network metrics in the traumatic brain injured (sagittal rapid head rotation) group that were not observed in the shams. After TBI, there were reductions in modularity as well as elevations in nodal strength and clustering coefficient across all analyzed frequencies.

Nodal strength and clustering coefficient both increased in the beta, broad, delta, gamma and sigma bands as they both capture the heightened global and local connectivity throughout the brain. In contrast, we found that after TBI, alpha network connectivity did not change relative to pre-injury and the theta network connectivity decreased at 1 day post-injury. Several studies report hyper-connectivity in resting-state networks like that observed in the beta, broad, delta, gamma and sigma bands. Resting state functional magnetic resonance imaging (fMRI) connectivity was elevated in children with mild to moderate TBI (Risen, et al., 2015). Several resting-state studies in adults report hyper-connectivity after TBI using fMRI (Hillary et al., 2011; 2014; 2015) and EEG networks (Nunez, et al., 2015; Porter et al., 2017). Sharp and colleagues reported
overall increased default mode network connectivity in moderate/severe TBI patients with cognitive impairment 6 months after injury (Sharp et al., 2011). Porter and colleagues reported hyper-connectivity in the right inferior frontal gyrus and hypo-connectivity in the left inferior frontal gyrus in the chronic TBI group relative to controls calculated from resting state EEG recordings. An increase in local clustering was observed in adult rats at 7 days following mild and moderate controlled cortical impact injuries relative to pre-injury (Harris et al., 2016), which was similar to the current study findings of increases in nodal strength and clustering coefficient at 7d in the beta, broad, delta and gamma bands. Hyper-connectivity in EEG and fMRI resting state networks is evident after in children, adults and rats for various TBI severities and recovery durations. In summary, our studies are in agreement with the resting-state literature, where hyperconnectivity is observed following TBI.

Despite the prevalence of reports of hyperconnectivity in resting state functional network after trauma, it is important to note that some studies report hypoconnectivity following TBI (Mishra et al., 2014; Palacios et al., 2017; Rigon et al., 2015; Stevens et al., 2012). Stevens and colleagues reported region-dependent changes in resting state (fMRI BOLD) functional connectivity in a mild TBI cohort relative to age-matched controls. Palacios and colleagues reported region-dependent alterations in resting-state functional connectivity at 6 months after injury in a large and clinically well-defined mTBI sample. Our study found hypoconnectivity only in the theta band at 1 day post-injury, however without a more chronic time point (e.g. 1 month post-injury) it is challenging to compare across studies. Mishra and colleagues reported lower resting fMRI correlation coefficients between the ipsilateral parietal cortex and ipsilateral hippocampus in adult rats 4 months following TBI compared to shams. Mishra’s findings disagreed with our
findings of hyper-connectivity after injury in the beta, broad, delta, gamma and sigma bands, however differences may be attributed to the scanning being performed while the rat was under anesthesia, which may affect brain activity. We speculate that the brain region or the source of neural activity and its dominant frequency influences the extent of synchrony across cortical neuronal clusters. Virji-Babul and colleagues found that concussion in adolescent athletes did not alter resting state EEG global network efficiency, modularity or clustering coefficient (Virji-Babul et al., 2014), while our study found significant changes in all metrics and frequency bands.

We found variable frequency-specific changes in global efficiency and characteristic path length after injury. In alpha, beta, sigma and theta networks, global efficiency either increased or did not change post-injury, generally in contrast to increases observed in shams on the same day. For the broad, delta and gamma bands, reductions in global efficiency were observed for injured animals. Changes in characteristic path length were generally inversely proportional to those in global efficiency. In a study of sedated adult rats, post-CCI fMRI network global efficiency increased, while characteristic path length decreased compared to pre-injury (Harris et al., 2016). Harris’ findings agreed with our findings in the alpha, delta, gamma, sigma and theta bands at 1 day post-injury. The lack of anesthesia during EEG acquisition in our study may explain the opposite polarities of change in global efficiency and characteristic path length in the broad, delta and gamma bands at 4 and 7 days post-injury. The reduction in global efficiency and corresponding increase in characteristic path length may indicate loss of neural computational ability perhaps due to decreases in efficient communication throughout the network. The contrasting direction of change in between injured and sham groups makes global
efficiency and characteristic path length candidate metrics for identifying injured networks and for measuring recovery from TBI.

Following injury, we report that modularity across all analyzed frequency bands decreased or did not change, which was generally distinct from changes observed in same-day shams. After injury, there was a lower degree of segregation of the brain into smaller functional units. Similarly, a resting-state fMRI blood-oxygen-level dependent contrast imaging (fMRI BOLD) study in adult rats found a significant decrease in modularity 7 days after controlled cortical impact injury (Harris et al., 2016). Han and colleagues reported an increase in modularity of resting-state functional connectivity in an adult TBI cohort at 90 days post-blast compared to healthy controls. However, no change was observed after 6-12 months initial scan (Han et al., 2014). We speculate that a damaged brain network is less modular because more random connections form between previously defined functional modules. The reduction in functional segregation across the network implies a breakdown of information encapsulation among specialized brain systems, which may be associated with functional and cognitive deficits (Alexander-Bloch et al., 2010).

Synchrony among EEG signals from different frequency bands implies specialized neural communication, plasticity, formation of functional ensembles and consolidation of long-term memories. Temporal synchronization may also be important in information binding and computing in the brain. Hyper-connectivity may depend on demand and resource availability. We found increased synchrony in the beta, broad, delta, gamma and sigma frequency bands after TBI, which may be related to dysfunctional cortical activity. Several studies demonstrate that TBI alters physiological oscillatory rhythms (Fedor, et al., 2010; Lee et al., 2013; Pevzner, et al., 2016). Increased connectivity was
observed following TBI (Hillary et al., 2015). Luo and colleagues found abnormality in delta (1-4 Hz) frequency band of resting state MEG signals in military veterans following more than 6 months with mTBI compared to healthy controls (Luo et al., 2013). Douw and colleagues reported better cognitive performance correlated with increased local connectivity of MEG functional networks in the theta band and higher clustering coefficient in the delta and theta bands (Douw et al., 2011). The hyperconnectivity of EEG functional networks could play a crucial role in the monitoring of cognitive function before and after TBI-induced change in oscillations, which can impact behavioral function.

Human TBI studies are highly variable in their inclusion criteria, injury severity and type, and recovery duration, all of which have been shown to affect the presentation of cognitive symptoms and pathological outcomes. This heterogeneity across human EEG studies critically limits interpretation and comparison of results. Animal TBI research circumvents several of the aforementioned concerns by allowing control over injury severity and type, and recovery duration. The mass-scaled equivalent angular velocity from 130 rad/s in the 4 week old piglet was 58 rad/s and 38 rad/s for the human infant and adult respectively. Rowson reports 50% risk of concussion as 28.3 rad/s using impact data from American football games (Rowson et al., 2012). Our mass-scaled velocity exceeded Rowson’s concussion threshold. Sullivan and colleagues reported the range of sagittal head rotational angular velocity as 44-49 rad/s from parietal and occipital head impacts during low height falls in infants (Sullivan, Coats, & Margulies, 2015), which were smaller than the current study’s mass-scaled equivalent, 58 rad/s. The currently reported angular velocities prescribed to the 4 week old piglets led to EEG changes and axonal injury that may be associated with a moderate to high risk of
concussion in adult humans based on previously reported concussion thresholds. For young adults, a range of 892–1169 rad/s\(^2\) was reported for head angular acceleration during soccer heading impacts in both sexes (Bretzin, et al., 2017). Average angular acceleration (36,932 rad/s\(^2\)) from 4 week old piglets mass-scaled to an adult human brain mass yielded a value of 3188 rad/s\(^2\), which was much higher than the reported accelerations for heading a soccer ball. Significant changes to the EEG network were expected following TBI via rapid head rotation at prescribed levels.

**Core Network Topology Is Influenced By Frequency But Not TBI**

It is reported that each frequency band has a distinct network topological signature (Buzsáki et al., 2013), with engagement of a select combination of electrodes placed over the left, right, frontal, parietal, temporal or occipital brain regions. Stability of EEG functional networks was found in healthy humans for different frequency bands and across awake and sleeping states with a subject (Chu et al., 2012). While the spatial resolution of EEG is poor, we assume that each oscillation’s characteristic network arrangement is reflective of the regional specificity of the neuronal sources. The core network topology analysis examines the influence of topology on the diffuse hyperconnectivity observed after TBI. Hyperconnectivity may occur due to an increase in the number of total edges, increase in edge weights or both. We found that the presence and arrangement of the top 5% strongest edges was consistent among animals for each frequency band of interest in both groups. The core topology was dependent on the frequency, but did not change with injury or study day. This implies that the hyperconnectivity observed post-injury was not due to the addition of core edges, but to increases in synchrony or the strengthening of core edges. The lack of addition of core edges after injury could be related to the limited axonal regenerative ability of the brain.
The finding of functional hyperconnectivity does not imply clear changes in axonal conduction velocity since collective increases and/or decreases in axonal conduction velocity may not belie changes in synchronization across the brain. Aberrant changes to past or new synapses may also contribute to the observed hyperconnectivity, where selective pruning and/or strengthening of synapses across the brain may lead to increased brain-wide synchronization. We speculate that long-distance excitatory and/or inhibitory axons play a significant role in the global hyperconnectivity observed which may be due the diffuse nature of the TBI causing multifocal axonal injury. In order to minimize total energy spent in response to TBI, previously existing cognitive and structural architecture are utilized as opposed to creating new connections. Hyper-excitability was found in the CA1 in 2-3 month old Yorkshire pigs 7 days after a single coronal rapid head rotation (Wolf et al., 2017). The authors proposed that decreased axonal function leads to reduced input from afferent regions, which yields hyper-excitability in the post-synaptic neurons. Such an increase in the neuronal excitability or reduction in inhibition after injury in regions of the brain may account for their finding of elevation of synchrony across the network after TBI. EEG recordings at each scalp electrode is the cumulative sum of synaptic potentials from millions of neurons, as a macro-scale measure of brain activity there are limits on what we can conclude occurs at an individual neuron level.

Damage of a single axon could result in the shedding of as many as 7000 synaptic terminals. Diffuse synaptic loss can occur during both primary and secondary injury cascades. Reparative responses mounted by the brain after injury may involve membrane repair, somatic recovery and axonal outgrowth in the midst of hypoxia, ischemia and/or energy failure. Activation of proteases can then degrade the
cytoskeleton leading to impaired axonal transport and swelling within several hours of trauma (Merlo et al., 2014). We hypothesize that damage to synaptic junction proteins may indicate diffuse synaptic loss, disrupted plasticity or loss of long-term potentiation that may lead to functional hyperconnectivity. Synaptic junction proteins that are structural damaged in the pre and post synaptic densities may be important to examine because structural damage may lead to functional synaptic disconnection.

Synaptotagmin is family of calcium binding proteins located in the synaptic vesicles (Merlo et al., 2014). Proteolysis of Synaptotagmin-1 (Syt-1) may hinder vesicles from docking on pre-synaptic membrane terminal. Accumulation of Synaptotagmin-4 (Syt-4) after TBI reduces synaptic activity (Ansari, Roberts, & Scheff, 2008). Postsynaptic density complex protein 95 (PSD-95) is marker of synapse degeneration (Ansari, Roberts, & Scheff, 2008). F-actin is a microfilament that plays a significant role in mobility and contraction of cells during cell division and is vital to synaptotagmins (Wen, Li, Shen, & Chen, 2017). Synapsins are phosphoproteins that regulate the release of neurotransmitters into the pre-synaptic area. Oxidative stress induced by TBI may lead to synapsin-1 loss (Wen, Li, Shen, & Chen, 2017). Quantification of synaptic junction proteins after TBI using western blotting and/or histopathology would provide insight to the microscale neurophysiological underpinnings of functional EEG connectivity.

Limitations
We analyzed fully connected networks that is calculate network metrics from thresholded networks because strong and weak connections may be critical to distinguishing between injured and uninjured networks. More complex ways of calculating synchronization networks could have been applied such as phase lag index or mutual
information, instead of cross-correlation. We chose correlation because of its ease of calculation for application to our pilot study and common usage throughout TBI network literature. Comparing information from TBI studies in humans and piglets is difficult due to the inconsistency of data acquisition and analytical methods utilized such as the variability in the definition of resting state used across studies. In this report, we acquired EEG from awake, immobile piglets in a quiet room free of any overt stimuli or tasks. Comparison with other resting state studies depends on the definition of 'resting state' which in humans is believed to constitute a mental passive state. In animal models, like human studies, we cannot be certain that we capture such a passive state, only a mental state free of overt stimuli. In human studies, subjects may be instructed to focus on a cursor (Kramer et al., 2011; Luo et al., 2013), otherwise keep their eyes closed (Palacios et al., 2017), while in animal studies anesthesia is often administered (Harris et al., 2016; Mishra et al., 2014). It is relevant to note that we did not train our piglets to close their eyes, thus comparisons with human resting state studies may be imperfect. Attention and presentation of stimuli can significantly affect the features of the EEG, but it is less clear how much these factors modulate EEG network connectivity. A second limitation of this study is that measured changes from TBI were caused only by a single, rapid non-impact injury; future studies should include and compare both focal and diffuse forms of TBI, and repeated injuries.

Conclusions

Changes in resting state functional network connectivity may be applicable to the precise identification of diffuse TBI (compared to controls) in children when task-related networks are difficult to obtain. Decrease in spectral power and modularity, increases in nodal strength, clustering coefficient, global efficiency and characteristic path length may
suggest that mild TBI leads to neural network damage in both cortical and white matter tract regions. Altered functional connections may be a consequence of axonal disconnection and may also be related to brain function deficits. This study serves as proof-of-concept that EEG networks in piglets may be helpful for the development of biomarkers for TBI.


Abstract

Traumatic Brain Injury affects millions of children in the US each year, however child-specific research is needed to explain why children are more vulnerable to TBI. Additionally, the relationship between local tissue distortion and local altered pathophysiology following TBI remains unknown. Several severe clinical outcomes, such as global cerebral blood flow reductions, behavioral deficits and chronic axonal damage, are associated with sagittal rapid head rotations, but not with coronal and/or axial head rotations. We hypothesized that brain region and head rotation direction would influence tissue distortions. We subsequently developed a high-resolution finite element model of the 3-5 day old piglet head and simulated 32 rapid head rotations in the sagittal (n=5), coronal (n=6) and axial (n=21) planes. Out of the 32 injuries, we obtained hist-immunopathological staining for axonal damage on 9 injured piglet brains (n=3 for each plane), in addition to shams (n=2). We then examined the regional biomechanical and neuropathological responses to TBI in the neonatal pig and found that local tissue deformation and histopathology were head direction- and region-dependent, but poorly correlated at a local scale. Sagittal rapid head rotations induced higher numbers of injured axons in the corpus callosum and internal capsule; whereas axial and coronal head rotations induced the highest number of injured axons in the thalamus compared to the white matter regions, brainstem and striatum. A significantly higher injured volume fraction of brainstem was found in sagittal rotations, compared to that from axial and coronal rotations in neonatal pigs. Head rotation direction and brain region both affected brain tissue deformation, which in turn influenced the magnitude of regional, acute axonal damage.
Introduction

It is estimated that 1.1 million to 1.9 million U.S. children and adolescents (under the age of 18 years) sustain sports and recreation-related concussions every year (Bryan, et al., 2016). Pediatric traumatic brain injury (TBI) is a prevalent, costly problem (Coronado et al., 2012) that causes cognitive and functional impairment. Insufficient focus has been allocated towards the improvement of diagnostic, treatment and preventative measures specific to children, who exhibit distinct biomechanical and neuropathological responses to TBI compared to adults (Prange et al., 2002; Giza et al., 2007; Prins et al., 2003; Kochanek et al., 2010; Ibrahim et al., 2011). More children-specific TBI research is necessary for a better understanding of why the child is more vulnerable to TBI.

The concussed human brain typically experiences distributed white matter alterations (Benson et al., 2007; Kraus et al., 2007), such as during sports-related injuries, which have been previously reported in the piglet rapid head rotation TBI model. Structural white matter changes have been widely reported following TBI as measured by diffusion tensor imaging and histopathology in humans, rodents and pigs (Benson et al., 2007; Browne, Chen, Meaney, & Smith, 2011; Dennis et al., 2015; Hayashi, Ago, Nakamae, Higo, & Ogata, 2015; V.E. Johnson et al., 2016). Increased risk of Diffuse Axonal Injury (DAI) is associated with excessive brain tissue distortions. Concurrence of DAI with behavioral and cognitive deficits after TBI has also been reported in humans, pigs, rodents and non-human primates (Beaumont, Brisson, Lassonde, & Jolicoeur, 2007; Dundon et al., 2015; Gennarelli, 1994; Sarah Sullivan et al., 2013a). The relationship between head kinematics, local tissue deformation and local altered physiology (e.g. CBF, metabolism and structural damage) remains unknown.
Finite Element Modelling (FEM) is a numerical technique for calculating deformation of cerebral tissue under prescribed loading conditions. FEM is a critical tool for establishing biomechanical thresholds of functional and structural injury in the brain. There are many brain FEMs for the adult population across species, however few brain FEM exist for the pediatric population. Within the adult FEM literature, several publications (Chatelin et al., 2011; Ji et al., 2014; Pan, Pelegri, & Shreiber, 2011; Weaver, Danelson, & Stitzel, 2012; Wright & Ramesh, 2012) have shown that prediction of DAI from excessive deformation of finite elements is improved by consideration of white matter tract orientation. In order to robustly understand the biomechanical mechanisms of DAI, controlled study of the loading conditions and macro- and microscopic deformation of structures at specific time points after TBI is necessary. The well-characterized diffuse white matter injury in the piglet via rapid head rotation (Eucker, Smith, Ralston, Friess, & Margulies, 2011; Ibrahim, Ralston, Smith, & Margulies, 2010; Raghupathi & Margulies, 2002) is advantageous for the study of the biomechanics of pediatric TBI because the piglet shares similar brain anatomy and growth to humans. Piglets and human neonates have similar rates of myelination, ratios of white to gray matter, cerebral hemodynamics and metabolism (Lind et al., 2007; Pareja, et al., 2016).

Several studies report the directional-dependence of axonal injury in humans (Weaver et al., 2012), monkeys (Gennarelli, 1985; A.K. Ommaya & Hirsch, 1971; Ayub K. Ommaya, Corrao, & Letcher, 1973) and pediatric pigs (Eucker et al., 2011; Ibrahim et al., 2010). Sagittal (SAG) rapid head rotations in neonatal pigs caused global cerebral blood flow reductions and persistent axonal damage that was absent after Axial (AXI) and Coronal (COR) head rotations. The biomechanical mechanisms behind the persistent axonal damage specific to SAG rotations are unknown. Because we observe worse behavioral
deficits in SAG-rotated animals (Sarah Sullivan et al., 2013a, 2013b), we hypothesize that animals rotated in SAG plane will have a wider distribution of and/or higher density of axonal injury (AID) than those rotated in the AXI plane. We also hypothesize that the lateral ventricles plays an important biomechanical role in regional brain deformation during a rapid head rotation about the AXI, COR and SAG axes.

The goal of this study is to advance the knowledge of biomechanical causation of injury at the regional structure level. Specifically, we will elucidate the biomechanical relationship between regional deformation and structural impairment, which is in turn related to functional deficits. The regional distribution of acute axonal injury throughout the neonatal brain following a traumatic head event has never been reported. A thorough understanding of regional patterns of injury would permit clinicians to make more informed decisions on TBI patient treatments. It is important to identify tolerable and injurious strain values in the developing brain because the magnitude and rate of cerebral tissue distortion or deformation and its threshold may vary with brain development. In this report, we investigate the role of regional deformation on histopathology following rapid head rotation in the AXI, COR and SAG planes.

Methods
To determine the relationship between regional tissue deformation and regional axonal injury, we used histopathology data from piglets experiencing a sudden head rotation and computational model of the head. We used two sets of animals – one set with detailed histopathology (n=11) and simulated a larger set (n=32) of rapid head rotations using the FEM. Together we examined how head rotation direction affects axonal injury.
In the first set, we quantified regional histopathology indicating axonal damage in 9 injured and 2 sham animals. We then developed a high resolution finite element model of the 3-5 day old piglet brain by generating three meshes with different spacing. After performing a convergence analysis, we selected one mesh based on numerical accuracy and time efficiency. The predicted deformations of the best mesh were validated against experimentally measured ex vivo hemisection brain deformations. Next, we introduced lateral ventricles into the brain FEM to examine its biomechanical role in brain deformation and determine if the model should include ventricles. Next, the relationship between regional axonal damage and predicted brain deformation was examined in the first group of 11 animals to develop tissue deformation metrics associated with axonal injury. Finally, these deformation thresholds were used to estimate axonal injury from predicted tissue distortions in the second set of animals, from 32 rapid head rotation injuries in the SAG, AXI or COR directions.

Rapid Head Rotation Injury

All studies were approved by the University of Pennsylvania Institutional Animal Care and Use Committees (IACUC). Neonatal piglets sustained a single, non-impact, closed head, rapid head rotation about the SAG, AXI or COR axes (Table 5.1, Fig. 5.1). Diffuse white matter injury was induced using the HYGE rotational acceleration device while the piglets were under anesthesia. Sham animals experienced all procedures, including anesthesia, except for the rapid head rotation, were also examined. All piglets were female. The brain development of a 3-5 day old piglet corresponds to that of a human infant (<2 years) (Dickerson & Dobbing, 1967). The fixed tissue from a subset of the previously injured 3-5 day old piglets (n=9) were analyzed for a novel histopathological analysis to develop tissue deformation thresholds.
The piglet head rotated 90° during AXI and COR rotations while SAG spanned 60° because of limited cervical spine flexibility in this direction. The range of angular displacement of the HYGE device was limited to the physiological motion of the neck, such that hyperflexion and hyperextension was excluded. Our injury model captures the short, high deceleration aspects of impacts, but without a contact event.

<table>
<thead>
<tr>
<th>Head Rotation Direction</th>
<th>Mean ± standard error angular velocity (rad/s)</th>
<th>Mean ± standard error angular acceleration (rad/s²)</th>
<th>FEM Simulations Group Sample Size</th>
<th>Head Rotation Direction</th>
<th>Mean ± standard error angular velocity (rad/s)</th>
<th>Mean ± standard error angular acceleration (rad/s²)</th>
<th>Histopathology Group Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axial</td>
<td>170 ± 6.2</td>
<td>42,281 ± 2,916</td>
<td>21</td>
<td>Axial</td>
<td>180 ± 8.4</td>
<td>46,368 ± 4396</td>
<td>3</td>
</tr>
<tr>
<td>Coronal</td>
<td>200 ± 7.3</td>
<td>53,714 ± 5,507</td>
<td>6</td>
<td>Coronal</td>
<td>193 ± 13.0</td>
<td>54,339 ± 10,912</td>
<td>3</td>
</tr>
<tr>
<td>Sagittal</td>
<td>163 ± 1.6</td>
<td>49,925 ± 2,054</td>
<td>5</td>
<td>Sagittal</td>
<td>165 ± 2.1</td>
<td>48,829 ± 925</td>
<td>3</td>
</tr>
<tr>
<td>Sham</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Sham</td>
<td>0</td>
<td>-</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 5.1: Average and standard error peak angular velocities and accelerations sustained by 3-5 day old pigs’ heads about the axial, coronal and sagittal planes for all finite element model simulations and histopathology (a subset of all model simulations).
Figure 5.1: Diagram of piglet head rotations in the axial, coronal and axial directions. The center of rotation is indicated by a star.

Histoimmunochemical Staining of Pig Cerebral Tissue

Six hours following injury, pig brain tissue was removed from the skull and embedded in wax blocks after treatment with formalin (fixative), alcohol and xylene. Brains were cut into 16-20 serial 3 mm-thick coronal sections using a custom designed apparatus for anatomical consistency. Slices (6 μm thick) were then cut from sections 6, 8 and 10 to capture the following regions: corpus callosum, corona radiata, internal capsule, external capsule, thalamus, striatum and hippocampus (Fig. 5.2A). The slices were stained for beta-amyloid precursor protein (β-APP) with Leica Novolink Polymer Detection System – Leica, and Haematoxylin & Eosin (H&E). β-APP immunohistopathology identifies axonal transport disruption. The three coronal slices (6, 8 and 10) were collected from left and right hemispheres of each animal, yielding 6 slices per animal. Left and right images of stained slices, from the same section (6, 8 or 10), were imported into HistoloZee v.03 (Adler et al., 2014) and manually aligned to each other (Fig. 5.2B). Segmentations into the following seven regions were performed in HistoloZee: corpus callosum, corona radiata, internal & external capsule, hippocampus, thalamus and striatum.

Quantitative Analysis of Neuropathology

High-resolution images of histopathological slides were acquired using Leica Aperio Scanscope CS2 at 20x magnification. Images were discretized into rectangular sections (tiles) that were 600 pixels wide x 600 pixels high, encompassing 91,047 microns2. In order to generate a spatial map of axonal injury (Fig. 5.2C), each tile was allocated to a zero, mild, moderate or severe category of axonal injury by a neuropathologist. In order to determine the upper and lower bounds on the number of injured axons per tile, a small dataset composed of three slices (section 6-right hemisphere), each from an
individual animal (one from the SAG, AXI and COR plane) was used. We designed a graphic user interface (GUI), using MATLAB 2015b, to quantify the number of injured axons per unit volume or axonal injury density (AID), which was used by a neuropathologist to score histopathological images from 9 injured and 2 sham animals. Annotation (counting) of individual βAPP positive profiles was performed using an in-house MATLAB script by a neuropathologist. Once all 3 images were annotated, we performed k-means clustering (k=3) on the injured axon count from all tiles to determine the lower and upper bounds of AID for the low, moderate and severe categories across all animals. We assumed that the center of the lower and upper bounds for each cluster category was mean number of injured axons per tile. Tiles were classified as mild, if at least one injured axon was present per tile, while tiles without any discernable injured axons were classified as zero. After performing the k-means clustering analyses on the three brain slices, we used the following Axonal Injury Classification (AIC) system for each tile in all 9 animals (3 sections each): mild category of AID was 1 to 11 positive βAPP profiles per tile, moderate was 12 to 42 positive βAPP profiles and severe was greater than 43 positive βAPP profiles. Each AIC had an average of 21.97, 98.85 and 197.7 injured axons per mm3 of the brain for mild, moderate and severe respectively.
Figure 5.2: (A) Locations of three sections of neonatal piglet brain used for histopathology on a sagittal cross-section view. (B) Segmentations of histopathological slice 6 into corpus callosum (purple), corona radiata (green), internal (aqua) & external (red) capsules, and striatum (blue). (C) Scored histopathological brain section after being stained for beta-amyloid precursor protein (βAPP), with each 600 pixel² tile allocated to one of four categories of axonal injury densities: zero, mild, moderate or high numbers of βAPP profiles.

**High-Resolution Finite Element Model**

The finite element mesh was generated from high resolution Magnetic Resonance, Diffusion Tensor and CT images of a 3-5 day old pig that were previously performed by our lab (S. Sullivan et al., 2015). The pig head geometry were segmented into the following anatomical regions: skull, cerebro-spinal fluid (CSF), white matter (primarily the corona radiata and corpus callosum), cerebellum, thalamus, cerebrum and brainstem. For a 0.4 mm³ mesh size, the neonatal piglet head FEM was meshed into a combination of 3,411,396 tetrahedral and hexahedral elements using ScanIP (ScanIP with +FE Module 5.0.931, Simpleware LTD, United Kingdom). Abaqus Explicit v6.9-EF1 (Dassault Systèmes, Vélizy-Villacoublay, France) was used to simulate the rapid head rotation of
the FEM mesh. Distortion and enhanced hourglass control as well as reduced integration elements were utilized to reduce excessive mesh deformation.

A convergence analysis was performed in order to select the best of three mesh densities: one finer (0.25 mm3) and one coarser (0.8 mm3) than 0.4 mm3. The total number of elements for each mesh density were 9,559,315, 3,411,396 and 627,603. All meshes were prescribed the same material properties and loading conditions of a SAG head rotation with peak velocity 120 rad/s. The max principal strains over the 30 ms duration of the rotation was extracted at every brain element (or integration point). The mean max principal strains from the 0.4 and 0.8 mm3 meshes were compared with the 0.25 mm3 or the finest (most numerically accurate) mesh in order to establish numerical stability of the models. T-tests were performed on all pairwise comparisons of the white matter max principal strains from the 0.4 mm3 and 0.8 mm3 mesh with that of the 0.25 mm3 mesh. The best mesh with sufficient numerical accuracy and reasonable computational time (≤ 1 month per run) was utilized.

The cerebrospinal fluid (CSF) was modelled as a hydrodynamic material using Mie-Gruneisen equation with parameters consistent with water: \( \rho = 1.0 \text{ g/cm}^3, K = 2.2 \text{ GPa}, \eta = 0.727 \text{ mPa s} \) (Bloomfield et al., 1998). We selected this skull-brain interface based on previously published boundary condition validation (Coats, Eucker, Sullivan, & Margulies, 2012), in which FEM-predicted max principal strains were validated against measured displacements between brain and skull to approximate the response of the pia-arachnoid connective tissue, CSF and cortical vasculature sandwiched between the skull and brain. The lateral ventricles were prescribed the same material properties as the CSF. The CSF was sandwiched between the skull and brain, where skull and brain nodes were connected to the skull in one continuous mesh, effectively tied to the skull.
The brain tissue was modelled as a homogenous isotropic hyper-elastic material utilizing
the first order Ogden strain density function (Ogden, 1984):

\[ W = \frac{2\mu}{\alpha^2} (\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3) \quad \text{(Equation 1)} \]

where \( \mu \) is the shear modulus of the brain tissue, \( \alpha \) is a previously derived parameter
that represents the brain’s strain-magnitude sensitive, nonlinear characteristics (Prange & Margulies, 2002)
and \( \lambda_1-3 \) are the principal stretch ratios. Viscoelasticity was
modelled using a two-term Prony series function (Ogden, 1984):

\[ \mu(t) = \mu_0 \left[ 1 - \sum_{i=1}^{2} C_i \left( 1 - e^{-t/\tau_i} \right) \right] \quad \text{(Equation 2)} \]

where relaxation moduli, \( C_1=0.332, C_2=0.389 \) and time constants \( \tau_1=2.96 \text{ s} \) and \( \tau_2 =0.181 \text{ s} \) (Prange & Margulies, 2002). Based on our previous publications, brain tissue in
the FEM was prescribed a shear modulus of 553 Pa such that the mesh reflected the
properties of a mixture of white and gray matter regions, with a Poisson’s ratio of 0.49999
and density of 1.04E-6 kg/mm3 (Prange & Margulies, 2002; S. Sullivan et al., 2015). The
skull was modelled as a rigid material.

**Hemisection Finite Element Model**

Previously measured brain deformation during rapid head rotation at high velocities was
compared with simulated deformation from the newly generated, high resolution FEM of
the hemisection experiment (S. Sullivan et al., 2015). Briefly, in the experiment, the
heads of three 3-5 day old piglets were transected axially at the orbital ridge, seated into
a canister of PMMA that was prescribed a single axial rapid head rotation with peak
velocity of 120 rad/s. Several brain surface markers (n=89, 84 and 138 for animals 1, 2
and 3 respectively) were placed on the surface of the exposed brain and organized into
groups of three to create non-overlapping triads that spanned the cortical gray matter and deep white matter tracts. Through analysis of high-speed video footage of the rotation, we calculated max principal strains from a two-dimensional Lagrangian finite strain tensor for every triad (Fung, 1994).

These experimentally measured max principal strains were compared with distortions in an axially transected version of our high resolution FEM to provide an assessment of the FEM’s fidelity to mimic the high strains and strain rates of the hemisection study. The hemisection FEM was generated in ScanIP by axial transection of the whole piglet head FEM at the orbital ridge and without transecting the lateral ventricles. We compared the max principal strains from our hemisection FEM with those from the axial hemisection experiments previously outlined (S. Sullivan et al., 2015). Angular velocity-time history and boundary conditions from the head transection study were applied to its simulation component. In order to compare the hemisection FEM with experimental max principal strains, we scaled and aligned the cut surface of the hemisection FEM to that of the first frame of each experimental hemisection video for each animal. Then, we found the 10 nearest elements in the hemisection FEM to the triads’ locations for each animal using MATLAB (R2015b, The MathWorks Inc., Natick, Massachusetts, United States). For each triad, the mean max principal strain (from FEM hemisection) was calculated from its 10 nearest elements.

The max principal strains from the experimental hemisection data was fit to the max principal strains of the hemisection FEM for each animal using a linear regression in R (R Core Team, 2017). Studentized residuals were calculated as the residual divided by its estimated standard deviation and are well modelled by the t-distribution with n-k-1 degrees of freedom, where n is the number of observations used to fit linear regression
and k is the number of regression coefficients including the intercept. Outliers were designated as FEM observations with studentized residuals outside of the 95% confidence interval (p<0.05) (Jacoby, 2005). Outliers were removed from further analysis. The hemisection triads were divided into two groups- those located in the center of the piglet head and those near the circumference of the piglet head. The majority of central triads covered white matter regions. The modelling error was computed as the mean difference between the experimentally measured and hemisection FEM max principal strains. The modelling error in the central, periphery and all triads were calculated separately.

**Comparison of FEM With and Without Lateral Ventricles**

To determine the role of the lateral ventricle (LV) on brain motion, a second whole brain FEM was developed using the same piglet head geometry (with 0.4 mm³ mesh density) but with the lateral ventricles (LV) (Fig. 5.3). The LV FEM mesh was similar in the total number of elements (3,452,513) and composition of tetrahedral and hexahedral elements to the FEM mesh without LV. The same material properties and loading conditions were applied to the LV FEM as to the FEM without LV. The lateral ventricles were prescribed the same hydrodynamic material properties as the CSF. A tied node interface between the lateral ventricle and the brain was used. In order to examine the role of that the LV plays in predicting brain deformation, we simulated 32 single rapid head rotations about either the SAG, COR or AXI axes for both FEMs with and without LV, and performed an element-by-element comparison of responses.

Due to the large numbers of elements and distinct meshes in both FEMs, we applied a regular sampling grid (1.3mm x 0.89 mm x 0.78 mm) to both FEMs after registration to each other to down-sample the number of comparisons between the model with LV and...
without LV. We then found the average deformation metric, such as max principal strain, over the 5 nearest elements to each grid point in both FEMs, yielding a one-to-one map from the LV FEM to the without LV FEM. A linear regression of deformation metrics, such as max principal strain, from the LV FEM against that of the FEM without LV was fit for each region (white matter, cerebrum, thalamus, brainstem and cerebellum). The linear regression was forced through the origin. Studentized residuals and their 95% confidence bounds were also calculated.

**Figure 5.3:** Left: Isometric view of lateral ventricles part in the piglet head finite element model. Right: Coronal cut through the FEM showing lateral ventricle elements (yellow) that are inferior to the corona radiata (orange).

*Registration of Finite Element Model to DTI and Pathology*

To determine white matter regions in the model, and tract-oriented deformation, we co-registered our FEM with Diffusion Tensor Image (DTI) on a 7T Siemens magnet with a 32-channel human head coil (FOV: 64×40×58mm3, resolution: 0.4×0.4×1mm3, TR = 400 ms, TE = 60 ms, 6 diffusion directions, 1 baseline scan, and diffusion b-value = 1,300 s/mm2) performed on one pig (Fig. 5.4). In order to calculate deformation along white matter tracts, FEM white matter element centroids were matched to DTI voxels to determine the orientation of white matter tracts at each element. Each DTI voxel was
evaluated for eigenvectors that defined the local principal white matter tract orientation. Briefly, the brain part of the FEM was registered with the DTI from the same animal in order to identify the nearest DTI voxels per white matter element. We performed manual registration of DTI with the FEM using custom MATLAB script (v2015b), where coronal slices of the FEM were scaled and translated along the y- and z- axes until optimal alignment of corona radiata, thalamus and overall brain morphology was achieved.

Figure 5.4: (A) Coronal cross-section of the Finite element model (FEM) of neonatal piglet brain, (B) Coronal cross-section of the Diffusion Tensor Image (DTI) of neonatal piglet brain with overlaid RGB colormap representing tissue tract orientation (where red: x-direction, green: y-direction and blue: z-direction), (C) Coronal cross-section of registered (overlaid) FEM and DTI.

Tract-oriented strain (TOS) was determined for each element (at its only integration point) from the projection of the rotated strain tensor onto the time-invariant unit, white matter tract orientation unit vector ($\hat{u}'$) (S. Sullivan et al., 2015). The peak max principal strain was defined as the maximum eigenvalue of the Lagrangian strain tensor ($E_{Global}$) across all time points for a given element. The magnitude of tensile strain oriented in the direction of the white matter tract was calculated by first transforming $E_{Global}$ out of the stationary global coordinate system into a coordinate system relative to the skull using rotational matrix $R(\theta(t))$ corresponding to the simulated head rotation.

$$E_{skull}(t) = [R(\theta(t))] [E_{Global}] [R(\theta(t))]^T$$

(Equation 3)
The rotational matrices for each direction were defined as follows:

Sagittal: \( R(\theta(t)) = \begin{bmatrix} \cos(\theta(t)) & 0 & \sin(\theta(t)) \\ 0 & 1 & 0 \\ -\sin(\theta(t)) & 0 & \cos(\theta(t)) \end{bmatrix} \)

Axial: \( R(\theta(t)) = \begin{bmatrix} \cos(\theta(t)) & -\sin(\theta(t)) & 0 \\ \sin(\theta(t)) & \cos(\theta(t)) & 0 \\ 0 & 0 & 1 \end{bmatrix} \)

Coronal: \( R(\theta(t)) = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos(\theta(t)) & -\sin(\theta(t)) \\ 0 & \sin(\theta(t)) & \cos(\theta(t)) \end{bmatrix} \)

The TOS as each time point was projected onto the white matter tract orientation (informed by the DTI) by:

\[
TOS(t) = \hat{u}' \cdot [E_{skull}(t)](\hat{u}')
\]

(Equation 4)

The peak TOS was calculated as the maximum TOS across all time over the duration simulated head rotation and for all white matter elements.

To match local tissue deformation to regional axonal injury, we also performed manual registration between the FEM and each histopathological section (n=3) from 9 animals. Because the spatial resolution of pathology images was higher (0.3mm3) than that of the FEM mesh (0.4mm3), we down sampled the AID data to obtain a single value of axonal damage for every element. For each FEM brain element, the mean AID level of all surrounding tiles within a 0.3 mm radius was calculated as its representative AID value. For the white matter region, we only considered FEM elements that were within both the histopathological and the FEM corona radiata and/or corpus callosum.
Comparing Regional Deformation Between Head Rotation Directions

Using the FEM with LV, we compared a subset of AXI (n=6; mean and standard error angular velocity 167.35±1.4 rad/s) simulations with velocity-matched SAG (n=5; mean and standard error angular velocity 163.74±1.6 rad/s) simulations in order to identify areas with high and low deformation for each direction. At each element, the AXI max principal strains were compared to those from SAG using a two-tailed Wilcoxon rank sum test. Adjusted p-values for all elements were calculated using Benjamini & Hochberg procedure (Benjamini & Hochberg, 1995) to correct for multiple comparisons (at 5% false positive rate). For elements with AXI strains that were significantly different from SAG strains, the difference in median max principal strain values (AXI - SAG) was used to classify elements as either AXI>SAG and AXI<SAG strains. The volume fraction of elements with significant AXI>SAG and AXI<SAG strains was calculated for all brain regions and represents the percentage of the region of interest with AXI strains exceeding SAG strains. This procedure was repeated for the comparison of AXI (n=11; mean and standard error angular velocity 192.22±3.7 rad/s) with velocity matched COR (n=7; mean and standard error angular velocity 199.83 ± 7.2 rad/s) simulations. This entire analysis was repeated, replacing max principal strain with TOS values.

Comparing Regional Axonal Injury Density with Predicted Deformation Metrics

For every (n=9) animal, FEM element deformation metrics (max principal strain, TOS, TOSR, and TOSxSR) were associated with mean AIC values from every histopathological slice (n=3 per animal). In the results, we describe significant differences between models with and without LV. Consequently, any data from the FEM with LV is described below. We performed a correlation between all FEM element
deformation metrics and corresponding mean AIC values for all elements in the brain (for max principal strains) and white matter (for tract-oriented metrics).

In order to determine max principal strain thresholds of injury for volume fractions in all regions of the brain, we calculated the volume fraction of AIC values exceeding Mild in the cerebrum in each of the 9 animals in our first cohort. We then found the max principal strain value where the strain volume fraction matched the AIC volume fraction. A range of volume fractions for strain was calculated over a range of max principal strains (0.05 – 1.2) for each of the 9 animals and used to identify the critical strain value. The critical max principal strain value was found by calculating the mean over all 9 strain values previously determined.

Next, using the FEM simulations of our second set of animals (n=32) with rapid head rotations, we determined the volume fraction exceeding the critical max principal strain value for each animal. Rotational Kinetic Energy (RotKE) was also calculated for each rapid head rotation and plotted against the volume fraction exceeding critical strain. RotKE was calculated as:

\[
RotKE = \frac{1}{2} I_{ii} VEL_{i}^2
\]  
(Equation 6)

where \( I_{ii} \) was the moment of inertia about axis \( i \) and \( VEL_{i} \) was max angular velocity about axis \( i \). We then performed a sensitivity analysis on the volume fraction of elements based on the critical strain value found to be 1.18. The upper and lower limits of the critical strain value were calculated as 1.18 + standard error (1.07) and 1.18 - standard error (1.29) and used to define the upper and lower bounds of volume fraction. The median percent change in volume fraction values were calculated for changes in strain values from 1.18 to 1.07 and from 1.18 to 1.29.
Statistical Analyses

For comparison of strain and other predicted tissue deformation metrics from the finite element model, their distributions were assumed to be normal. Pairwise comparisons using the Wilcoxon rank sum test was performed on histopathological data in order to identify differences in AID by region and direction, with significance level of 0.05. The Benjamini-Hochberg procedure (Benjamini & Hochberg, 1995) was used to correct for multiple comparisons. All statistical analyses were performed in R version 3.4.1 (R Core Team, 2017).

Results

Convergence of Finite Element Model Mesh

The finest mesh had the largest max principal strains (0.492 ± 0.000; mean ± standard error) during SAG head rotation (peak angular velocity = 120 rad/s), while the coarsest mesh (0.8 mm3) had the lowest mean (0.1373 ± 0.000; mean ± standard error) strain values (Fig. 5.5). The run time exponentially increased as mesh density increased, where a single run using the 0.8mm3 mesh took 8 hours to complete, the 0.4 mm3 took 3 weeks and the 0.24mm3 took 2 months. We selected the 0.4 mm3 mesh (mean strain = 0.361 ± 0.000; mean ± standard error) for further analyses over the 0.25 mm3 in order to prioritize time efficiency (≤1 month run time per simulation on CHOP cluster) as well as numerical accuracy of the FEM. With decreasing mesh size, the strain levels increased and in theory should increase until a plateau (convergence) is reached as reported in Maltese and colleagues (Maltese et al., 2012).
Figure 5.5: Bar plot comparison of mean and standard error max principal strains from the white matter part of the piglet head finite element model with 0.25, 0.4 and 0.8 mm³ meshes. The total time necessary for completion of one simulation are shown for each mesh density.

Hemisection Finite Element Model

The alignment of the hemisection FEM with the experimental hemisection triads for animals 1 (n=89), 2 (n=84) and 3 (n=138) are shown in Fig. 5.6. All experimental max principal strains had positive correlations with the FEM max principal strains (Fig. 5.7). Animal 1, 2 and 3 had slopes and standard errors: 0.83 ± 0.03, 0.88 ± 0.04 and 0.92 ± 0.03. All slopes were significantly lower than one (p=2.62e-05, 0.007 and 0.01 for animals 1, 2 and 3 respectively). For all animals, the studentized residuals within the 95% confidence interval were randomly and symmetrically distributed about zero indicating that a linear regression model appropriately captured the relationship between measured and predicted max principal strains (Fig. 5.8). For animals #1, 2 and 3, four,
five and four outliers, respectively, were found and removed from further analysis (Fig. 5.7, 5.8). Note that measured tissue triad strains can be quite large, approaching values of 1.0.

Figure 5.6: Top view of overlay of experimental hemisection triads (indicated by red plus signs) on the hemisection finite element model (blue) for (A) animal #1 (n=89), (B) animal #2 (n=84) and (C) animal #3 (n=138). Outlier triads are circled in black.
Figure 5.7: Scatter plot of matched max principal strains from the hemisection experiment and finite element model following axial rotations. Outliers are indicated in blue and were excluded from analysis. The identity line (slope 1, intercept=0) is shown as red, dashed line.
Figure 5.8: Scatter plot of studentized residuals from linear regression of hemisection experimental data against simulated max principal strains. Outliers outside of the 95% confidence interval (p<0.05) are indicated in blue.

The linear regression slope across all three hemisection experiments (combined) was 0.84 ± 0.02 for all triads (Fig. 5.9) and 0.771 ± 0.029 for central triads and 0.86 ± 0.02 for periphery triads, which were all significantly less than 1 (p < 4.8e-12). The mean difference in max principal strains (measured-predicted) was -0.093 ± 0.011 (mean ± standard error) for all triads, -0.132 ± 0.017 (mean ± standard error) for the central triads and -0.083 ± 0.011 (mean ± standard error) for the periphery triads. The mean error in max principal strains for central triads, encompassing majority white matter tracts, was significantly larger than that of the periphery triads, indicating that our brain material properties may have been too soft.
Figure 5.9: Scatter plot of matched max principal strains from the hemisection experiment and finite element model from all three animals (after excluding outliers). The identity line (dashed, red) and regression fit across all 3 animals (with y-intercept forced through the origin) (black) are shown.

**Biomechanical Role of the Lateral Ventricles**

In our point-to-point comparison, we observe that the FEM with LV has higher numbers of elements with larger max principal strains in the thalamus, white matter, cerebrum and cerebellum as the linear regressions have lower slopes than one (Fig. 5.10). In contrast, the FEM without LV had more elements with larger strain values in the brainstem, with a slope of 1.2. There were more elements with large positive residuals (without LV – with LV) over the low (0 – 1 mm/mm) range max principal strains for all regions except for the brainstem, where the presence of LV was a strain relief. For strain values greater than 1.2, the model with LV tended to yield higher strain values than without LV, creating a strain concentration.
Thalamus
\[ y = 0.81 \cdot x, \quad r^2 = 0.508 \]

White Matter
\[ y = 0.71 \cdot x, \quad r^2 = 0.585 \]

Cerebrum
\[ y = 0.87 \cdot x, \quad r^2 = 0.628 \]

Cerebellum
\[ y = 0.65 \cdot x, \quad r^2 = 0.636 \]
Figure 5.10: Scatter plot and linear regression fit (blue line) of max principal strain for the FEM without lateral ventricles to those from the FEM with lateral ventricles for all brain regions. Studentized residuals from each linear regression fit are shown on the right with their 95% confidence interval indicated by the red, dashed lines.

Comparison of TOS, TOSR and TOSxSR yield similar results as observed with max principal strain, where all slopes were significantly less than zero (p<2e-16) and the LV FEM presented larger TOS, TOSR and TOSxSR than the FEM without LV (Fig. 5.10). The magnitudes of slopes varied from 0.67 to 0.89. We concluded that it was essential to include LV in our further analyses.
Figure 5.11: Scatter plot and linear regression fit (blue line) of tract oriented strain, strain rate and strain rate for the FEM without lateral ventricles to those from the FEM with lateral ventricles for all brain regions. Studentized residuals from each linear regression fit are shown on the right with their 95% confidence interval indicated by the red, dashed lines.

Effect of Rotational Plane on Axonal Injury Density

We expected a wider distribution of βAPP profiles throughout the brain from SAG rotations compared to the AXI or COR direction. Increased dispersion of injured axons
may account for worse neurocognitive deficits and lower threshold to injury associated with SAG rotations compared with AXI. We also expected sagittal rotations to generate the highest number of injured axons in the corpus callosum and all throughout the brain.

Rapid head rotation injuries about all planes caused significantly higher axonal injury density (AID) than in sham (Fig. 5.12). For the corpus callosum and internal capsule, SAG (purple) yielded AID values more than 3 times those of AXI (green) and COR (blue). All pairwise comparisons of directions were significant (p<0.002) in the corpus callosum, except for AXI with COR (p=0.13). The corona radiata and striatum were significantly (p<1.2e-5) different in all comparisons except for AXI and SAG (p>0.21). The hippocampus was significantly different for comparisons to Sham (p<0.02). Statistical comparisons of the external capsule AID between Axial, Coronal and Sagittal injury groups and the Sham group were not performed because there was only one value for the Sham group. The internal capsule was significantly different for all pairwise comparisons (p<0.0001). AID in the thalamus exceeded 30 injured axons per cubic millimeters all injury planes compared to all other regions that were generally below 20 injured axons per cubic millimeters. All pairwise comparisons were significantly different for the thalamus and total white matter regions (p<0.0085).
Figure 5.12: Average and standard error of injured axon density (AID) from brain regions in histopathology slices from all injury groups. Pairwise comparisons using the Wilcoxon rank sum test were performed to compare AID between pairs of injury groups (Sham, Axial, Coronal and Sagittal rapid head rotations). Horizontal bars indicate all significant pairwise comparisons, where $p<0.05$ was the level of significance. Comparisons of External capsule AID between Axial, Coronal and Sagittal injury groups and the sham group were excluded from analysis because there was only one value for the Sham group.

SAG rapid head rotations yielded the largest AID values across all regions compared to COR and AXI, with the exception of the external capsule, hippocampus and striatum. All pairwise comparisons between injury groups were statistically significant ($p<0.05$). SAG, AXI and COR had significantly higher AID than sham, where SAG had the highest AID and COR the lowest (Fig. 5.13). There was left-right symmetry of AID in all injury groups and sections because no side-to-side comparison was significant ($p>0.05$) (Fig. 5.14, 5.15).
Figure 5.13: Average and standard error of injured axon density (AID) from sham, axial, coronal and sagittal groups. Pairwise comparisons using Wilcoxon rank sum test was performed to compare AID between all pairs of groups (sham, axial, coronal and sagittal rapid head rotations). Horizontal bars indicate all significant pairwise comparisons, where \( p < 0.05 \) was the level of significance.

Figure 5.14: Average and standard error in axonal injury density in the left and right histopathology slices (6, 8 and 10) categorized by injury group. A non-parametric Dunnett’s test was used to compare the left and right slices from each section, but no comparisons were significant \( (p > 0.05) \).
AXI and SAG induced the highest AID across all sections, compared to COR and Sham (Fig. 5.15). All pairwise comparisons (by Wilcoxon rank sum test) of angular velocities by direction were not significantly different (p>0.6). Section 8 has the highest number of injured axons compared to second 6 and 10, where section 10 has the lowest extent of axonal damage.

Figure 5.15: Heat maps showing the mean axonal injury density or number of injured axons per cubic millimeter calculated on all 9 animals by region and section.

Comparing Deformation in Axial & Sagittal Rapid Head Rotations

There was a wide distribution of elements where AXI max principal strains exceeded SAG strains (Fig. 5.16). These elements tended to be located in the lateral regions of the brain, while AXI<SAG elements tended to located closer to the midline.
Figure 5.16: Coronal sections of elements in the piglet brain finite element model comparing the median max principal strains from axial rapid head rotations with those from sagittal rotations with similar peak angular velocities. Top, left to bottom, left goes from front to back of piglet brain. Each point represents the centroid location of one element, where orange elements indicate axial strains that are significantly (p<0.05) higher than those from sagittal and blue elements indicate sagittal strains that are significantly (p<0.05) larger than those from axial rotations. Gray elements indicate locations where axial strains were not significantly (p>0.05) different from sagittal rotations. Statistical comparisons were performed using a two-sided Wilcoxon rank sum test, with significance defined as p<0.05.
Comparison of deformation metrics from AXI rapid head rotations with same-velocity SAG demonstrate region-dependence (Tables 5.2-5.4). The cerebrum, cerebellum and white matter regions had 1.3-5.7 times larger volume fractions of max principal strains where AXI>SAG compared to AXI<SAG. In contrast, the thalamus and brainstem had 3.7-5.2 times larger volume fractions of elements for AXI<SAG. There were similar trends and magnitudes of max principal strain rate volume fractions to those from max principal strains. There were 1.56 times higher volume fractions of TOS for AXI>SAG compared to AXI<SAG. TOSR and TOSxSR had similar volume fractions for AXI>SAG and AXI<SAG.

<table>
<thead>
<tr>
<th></th>
<th>Cerebrum</th>
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<th>Cerebellum</th>
<th>Brainstem</th>
<th>White Matter</th>
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</thead>
<tbody>
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<td>39.2</td>
<td>14.2</td>
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<tr>
<td>AXI&lt; SAG</td>
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<td>24.9</td>
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</tr>
</tbody>
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**Table 5.2:** Max Principal Strain Volume Fraction (%)

<table>
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<tr>
<th></th>
<th>Cerebrum</th>
<th>Thalamus</th>
<th>Cerebellum</th>
<th>Brainstem</th>
<th>White Matter</th>
</tr>
</thead>
<tbody>
<tr>
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<td>14</td>
<td>26.1</td>
<td>6.4</td>
<td>38.8</td>
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<tr>
<td>AXI&lt; SAG</td>
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<td>20</td>
<td>60.3</td>
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</tr>
</tbody>
</table>

**Table 5.3:** Max Principal Strain Rate Volume Fraction (%)

<table>
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<th>Tract-Oriented Strain Rate</th>
<th>Tract-Oriented Strain x Strain Rate</th>
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</thead>
<tbody>
<tr>
<td>AXI&gt; SAG</td>
<td>35.9</td>
<td>0</td>
<td>29.6</td>
</tr>
<tr>
<td>AXI&lt; SAG</td>
<td>23</td>
<td>0</td>
<td>21.5</td>
</tr>
</tbody>
</table>

**Table 5.4:** White Matter Tract Volume Fraction (%)

Matching the general spatial pattern observed throughout the brain, those elements with larger TOS in AXI compared to SAG simulations tended to be located more laterally in the white matter than the SAG simulations (Fig. 5.17).
Figure 5.17: Coronal sections of elements in the piglet white matter region of the finite element model comparing the mean tract-oriented strains from axial rapid head rotations with those from sagittal rotations with similar peak angular velocities. Top, left to bottom, left goes from front to back of piglet brain. Each point represents the centroid location of one element, where orange elements indicate axial tract oriented strains that are significantly (p<0.05) higher than those from sagittal and blue elements indicate sagittal tract oriented strains that are significantly (p<0.05) larger than those from axial rotations. Gray elements indicate locations where axial strains were not significantly (p>0.05) different from sagittal rotations. Statistical comparisons were performed using a two-sided Wilcoxon rank sum test, with significance defined as p<0.05.
Comparing Deformation in Axial and Coronal Rapid Head Rotations

There were high numbers of elements with larger max principal strains in the AXI rotations compared to the COR in the frontal and occipital slices (Fig. 5.18). The distribution of AXI<COR strains in the central slices was primarily confined to the thalamus, for instance there were groups of elements located near the midline. From rostral to caudal white matter, there were more elements with larger TOS in AXI than COR (Fig. 5.19). These elements were diffusely distributed throughout the corona radiata and corpus callosum.

Comparison of AXI and COR rapid head rotations yielded region-specific differences in max principal and tract-oriented strains and strain rates (Table 5.5). The volume fraction of cerebrum elements in the cerebrum with significantly larger max principal strains in AXI than COR was over 15 times as large as the cerebral volume fraction of elements with strains AXI>COR. The cerebellum and white matter regions presented 13-52 times larger volume fractions of max principal strains with AXI> COR than AXI<COR. The thalamus, brainstem and lateral ventricle had 1.6-2.3 times higher volume fractions for AXI<COR compared to AXI>COR. The max principal strain rate volume fractions across regions had similar regional distributions for AXI>COR and AXI<COR (Table 5.6). Within the white matter region, the volume fractions of elements with AXI>COR TOS, TOSR and TOSxSR were 1.4-1.8 times higher than those for AXI<COR (Table 5.7). In summary, there were higher numbers of elements in the cerebrum and white matter with larger max principal strains in the AXI compared to COR. This large number of white matter elements with larger strain in AXI compared to COR may account for the extensive axonal injury damage that we observed in the AXI histopathology compared to COR from this study.
Figure 5.18: Coronal sections of elements in the piglet brain finite element model comparing the mean max principal strains from axial rapid head rotations with those from coronal rotations with similar peak angular velocities. Top, left to bottom, left goes from front to back of piglet brain. Each point represents the centroid location of one element, where orange elements indicate axial strains that are significantly (p<0.05) higher than those from coronal and blue elements indicate coronal strains that are significantly (p<0.05) larger than those from axial rotations. Gray elements indicate locations where axial strains were not significantly (p>0.05) different from sagittal rotations. Statistical comparisons were performed using a two-sided Wilcoxon rank sum test, with significance defined as p<0.05.
Figure 5.19: Coronal sections of elements in the piglet white matter region of the finite element model comparing the mean tract-oriented strains from axial rapid head rotations with those from coronal rotations with similar peak angular velocities. Top, left to bottom, left goes from front to back of piglet brain. Each point represents the centroid location of one element, where orange elements indicate axial tract oriented strains that are significantly (p<0.05) higher than those from coronal and blue elements indicate coronal tract oriented strains that are significantly (p<0.05) larger than those from axial rotations. Gray elements indicate locations where axial strains were not significantly (p>0.05) different from sagittal rotations. Statistical comparisons were performed using a two-sided Wilcoxon rank sum test, with significance defined as p<0.05.
Comparing Regional Axonal Injury Density with Local Strain

Across all regions, including the cerebrum, thalamus and white matter, AID poorly correlated (R²<0.2) with max principal strains and TOS (Fig. 5.20, 5.21). The majority of elements had low AID values with a wide range of max principal strain values (1-3 mm/mm). TOS, TOSR and TOSxSR also poorly correlate with AID (Fig. 5.21). We conclude that on the local level strain does not correlate closely with axonal injury density. Next, we look at correlating regions of injury with regions of tissue distortion.
Figure 5.20: Scatter plot of axonal injury density and max principal strains from all elements across animals in the cerebrum, thalamus and white matter regions of the finite element model with lateral ventricles.

Figure 5.21: Scatter plot of axonal injury density against (A) tract oriented strain, (B) tract oriented strain rate and (C) tract oriented strain x strain rate from 9 simulations with lateral ventricles for all white matter elements.
To look at regions of injury, we determined the critical volume fraction max principal strain value from the 9 animals in which histopathology was available (0.8548, 1.1741, 1.2920, 1.3302, 1.2556, 1.4561, 1.7028, 0.8647, 0.7148). The mean of 1.18 was used to identify the volume fraction of FEM regions estimated for each of the 32 simulations that have at least mild axonal injury density. In the second set of animals we observe that there was a clustering of animals by direction by the magnitudes of max principal strain and RotKE for white matter (Fig. 5.22A). In the white matter region, the CORs had low RotKE relative to AXI and SAGs, however yielded similar (p>0.23) volume fraction values compared to SAG and AXI (Fig. 5.23A). AXI simulations presented a positive relationship between injured white matter volume fraction and RotKE. In the brainstem, SAG had significantly larger volume fraction values than AXI and COR (Fig. 5.22B, 5.23B). For the thalamus, COR yielded a wide range of volume fraction values over a small range of RotKE values, in contrast to SAG that presented a narrow range of RotKE over a similar range of volume fraction (Fig. 5.22C, 5.23C). AXI mean volume fraction values in the thalamus were significantly different (p<0.03) from that of the SAG and COR (Fig. 5.23C). The cerebellum had the lowest volume fractions of all regions for AXI, SAG and COR. Pairwise comparisons of volume fractions in the cerebellum among all directions were not significantly different (p>0.23) (Fig. 5.22D, 5.23D).

We performed a sensitivity analysis on volume fraction of elements based on the critical strain value of 1.18. The extremes of the mean of these max principal strains from all 9 animals were 1.12 (after removal of highest value 1.7028) and 1.24 (after removal of lowest value 0.7148). As the critical strain value increases from 1.18 (mean) to 1.24, the volume fraction of elements exceeding the critical strain value decreases. The volume fraction of damaged elements increased as the strain value decreased from 1.18 (mean)
to 1.12. The range of volume fractions for each animal in all FEM regions were indicated by error bars for every point (piglet), where the upper error bar limit indicates the volume fraction exceeding the strain value of 1.12 and the lower limit indicates that for 1.24 (Fig. 5.24 A-D). In all regions (the white matter, brainstem, cerebellum and thalamus), larger volume fractions at exceeding max principal strain of 1.18 have larger ranges of volume fractions between strains of 1.12 and 1.24 (longer error bars). Changing the max principal strain resulted in a uniform shift of the volume fraction values of all animals. The relationship between volume fraction and direction did not change when the max principal strain was changed from 1.18 (Fig. 5.25, 5.26). Varying the critical strain value from 1.18 to 1.12 or 1.24 did not significantly change the volume fraction value throughout any brain regions. The max principal strain criterion was robust to changes (from 1.18) did not affect the relationship between volume fraction, direction and brain region reported in Fig. 5.22, 5.23.
Figure 5.22: Scatter plot of the regional volume fraction of elements with max principal strains greater than critical strain value (1.18) against rotational kinetic energy calculated for 32 animals, in the (A) white matter, (B) brainstem, (C) thalamus and (D) cerebellum. The colors and shapes of points indicate the plane of head rotation.
Figure 5.23: Bar plot of the means and standard errors of volume fractions with max principal strain exceeding 1.18, grouped by injury plane, for the (A) white matter (B) brainstem, (C) thalamus and (D) cerebellum regions for 32 animals. Horizontal black bars indicate statistical significance (p<0.05) by pairwise comparison using t tests (with Bonferroni-Holm adjustment) among all pairs of injury planes.
Figure 5.24: Scatter plot of the regional volume fraction (VolFract) of elements with max principal strains greater than critical strain value (1.18) with upper error bar limits for VolFract for 1.12 and lower error bar limits for VolFract for 1.24 against rotational kinetic energy calculated for 32 animals, in the (A) white matter, (B) brainstem, (C) thalamus and (D) cerebellum. The colors and shapes of points indicate the plane of head rotation.
Figure 5.25: Bar plot of the means and standard errors of volume fractions with max principal strain exceeding 1.12, grouped by injury plane, for the (A) white matter (B) brainstem, (C) thalamus and (D) cerebellum regions for 32 animals. Horizontal black bars indicate statistical significance (p<0.05) by pairwise comparison using t tests (with Bonferroni-Holm adjustment) among all pairs of injury planes.
Figure 5.26: Bar plot of the means and standard errors of volume fractions with max principal strain exceeding 1.24, grouped by injury plane, for the (A) white matter (B) brainstem, (C) thalamus and (D) cerebellum regions for 32 animals. Horizontal black bars indicate statistical significance (p<0.05) by pairwise comparison using t tests (with Bonferroni-Holm adjustment) among all pairs of injury planes.

Discussion

Various TBI loading conditions such as during car crashes, falls and contact sports can induce axonal injury that is region and direction-specific. The development of effective tools for the diagnosis and prevention of mild TBI in the developing brain is complicated by cerebral maturation. Current neuroimaging methods and animal models provide
assessments of functional sequelae after concussion, which also provide evidence that the developing brain responds differently to TBI than the mature brain does (Choe et al., 2012; Shrey et al., 2011). We utilized a diffuse white matter injury model in the neonatal piglet to investigate the biomechanical sources of axonal injury regional dependence through integration of FEM and quantitative histopathology. We developed a converged, high resolution FEM of the neonatal piglet brain and verified agreement with previously measured data in order to gain confidence in its ability to predict tissue deformation. Predicted tissue deformation metrics were then correlated with regional AID from 3 coronal sections for 9 animals, which was used to develop new deformation thresholds that were applied to 32 simulated rapid head rotations.

Assuming that a 3-5 day old piglet has brain mass of 35.5g, human newborn brain mass of 400 g and an human adult brain of 1400g, we mass scaled (using Ommaya scaling law) (Ommaya, et al., 1967) the piglet mean angular velocity and acceleration (175 rad/s and 45,619 rad/s² respectively) to obtain head rotation kinematics of 78 rad/s and 9042 rad/s² for infants and 51 rad/s and 3937 rad/s² for adults. There are limitations to the use of mass scaling law for comparison of angular velocity and/or acceleration across species and age due to differences in skull, brain and neck mechanical properties and shape. However, mass scaling the piglet head kinematics to human adults and infants allows comparison with the rotational kinematic ranges associated with sports-related TBI events. For American football, Zhang and Rowson found an angular acceleration threshold for 50% chance of mTBI at 5900 and 6383 rad/s² respectively (Zhang et al., 2004; Rowson et al., 2012). The impact threshold for TBI during Australian football was reported as 8020 rad/s² using numerical models (Frechede and McIntosh, 2009). On average, our mass-scaled rapid head rotations did
not exceed any previously reported TBI angular acceleration threshold for adults. Rowson reports 50% risk of concussion as 28.3 rad/s using impact data from American football games (Rowson et al., 2012). A range of 892-1169 rad/s² was reported for head angular velocity during soccer heading impacts in both sexes (Bretzin, et al., 2017). Our mass-scaled velocity exceeded Rowson’s concussion threshold and the angular acceleration range associated with heading a soccer ball. Sullivan and colleagues reported the range of sagittal head rotational angular velocity as 44-49 rad/s from parietal and occipital head impacts during low height falls in infants (Sullivan, Coats, & Margulies, 2015), which were smaller than the current study’s mass-scaled equivalent. The range of sagittal plane angular accelerations for a study on infant head impacts from short falls were 2777–3632 rad/s² (Sullivan, Coats, & Margulies, 2015). The threshold for concussion for the infant was mass-scaled to infant brain mass (400 g) and found to be 10,000 - 15,000 rad/s² peak angular acceleration based on adult primate data (Duhaime et al., 1987; Ommaya et al., 2002) and instrumented football helmet data, respectively (Broglio et al., 2010; Rowson et al., 2012). At currently reported angular velocity levels, axonal injury was seen in both piglet ages that may be associated with a high risk of concussion in adult humans based on previously reported concussion thresholds. At high risk levels of concussions, we expect to observe larger magnitudes of axonal injury more consistent with the mild to moderate region of the TBI spectrum.

Regional Dependence of Axonal Injury Density

Rapid head rotation of piglets leads to neurocognitive deficits that may be due to the diffuse axonal pathology that is characteristic of the injury. Under normal physiological conditions, βAPP is carried throughout the axon via fast anterograde axoplasmic transport. However, excessive damage to axons causes the accumulation of βAPP
proximal to the site of axonal injury as soon as 6 hours post-injury in piglets (Eucker et al., 2011; Ibrahim et al., 2010). βAPP immunoreactivity is observed as dark, axonal spheroid plaques throughout the white matter tracts and is non-specific for a traumatic etiology (Hortobágyi et al., 2007). βAPP is the most sensitive immunohistochemical method of axonal damage detection as compared to neurofilament-68 staining (Ibrahim et al., 2010) and silver impregnation (Hortobágyi et al., 2007). In previous studies, a neuropathologist marked areas of βAPP positive profiles on coronal sections of brain tissue that were summed to yield a volumetric measure of axonal injury throughout the cerebrum (AIV) (Eucker et al., 2011; Ibrahim et al., 2010; S. Sullivan et al., 2015). However, AIV does not accurately capture the density of βAPP immune-reactive profiles (or the number of injured axons per unit volume) seen throughout various white matter regions.

In our study, we found that SAG rapid head rotations yielded the largest AID values in the corpus callosum, corona radiata, thalamus and internal capsule compared to COR and AXI, but not in the external capsule, hippocampus and striatum. SAG, AXI and COR had significantly higher AID than sham, where SAG had the highest AID and COR the lowest. We hypothesized and found extensive regional AID following SAG rotations compared to AXI, which may explain SAG’s chronic persistence of axonal injury (Sullivan et al., 2013a). The more extensive AID associated with SAG rotations may take longer to “resolve” than that in AXI, due to a patho-metabolic environment that may foster secondary (hypoxic/ischemic) injury of axons. A higher AID may also explain differences in neuro-cognitive deficits between SAG and AXI head rotations.

The population of axons with impaired axonal transport, which stained positively for βAPP, may only represent a subset of total damaged axons. A previous study found
distinct populations of injured axons – those with impaired axonal transport and those with neurofilament compaction in the immature rat after diffuse brain injury (DiLeonardi, Huh, & Raghupathi, 2009). There are reports of two different spatial patterns of βAPP staining – one likely corresponding to trauma to the white matter tracts and another to hypoxic or ischemic axonal injury. Because our objective was to relate axonal damage to the biomechanics of brain tissue deformation, we focused on the former βAPP staining pattern, which was oriented along the white matter bundles that typically presented axonal bulbs that have been previously reported in humans (Hayashi et al., 2015; Hortobágyi et al., 2007; Meythaler, Peduzzi, Eleftheriou, & Novack, 2001) and pigs (Browne et al., 2011; V.E. Johnson et al., 2016; Victoria E. Johnson, Stewart, & Smith, 2013; Raghupathi & Margulies, 2002; Sarah Sullivan et al., 2013a) after TBI. However, future studies may consider examining the relationship between hypoxic or ischemic axonal damage and strain, in order to evaluate whether a separate threshold of injury exists.

**Lateral Ventricles Affect Brain Deformation**

We compared predicted deformation across the brain with and without LV and found that inclusion of LV yielded lower max principal strains and TOS than with exclusion of LV. The increase in max principal strains is particularly evident in the SAG plane in the brainstem and thalamus (that are in close proximity to the LV). We speculate that the LV may dampen stress concentrations at the interface of neighboring brain regions such as between the white matter and thalamus. All subsequent analyses correlating regional deformation metrics with AID were performed with the FEM including LV, since this was more biofidelic.
Comparison of Axial and Sagittal Regional Deformation

The brainstem exhibits large max principal strains for SAG head rotations compared to AXI. The brainstem also had more elements with higher strains and strain rates in AXI than COR, which may account for more severe outcomes seen after AXI head rotations compared to COR. Our study shows direction and region-specific histopathology and predicted tissue distortion, which were poorly correlated. Injury-specific brain deformation thresholds may vary with region, which may account for the wide variability observed in our study (Cater, Sundstrom, & Morrison, 2006; Elkin & Morrison, 2013; Prange & Margulies, 2002; Vink, Mullins, Temple, Bao, & Faden, 2001; Yoshino, Hovda, Kawamata, Katayama, & Becker, 1991). Elkin and colleagues found region-specific mechanical behavior using a micro-indentation stress relaxation tests on the porcine brain in the coronal plane. The cerebellum, corpus callosum and brainstem were the softest regions measured, while the cortex and hippocampus were found to be the stiffest. Vink and colleagues provide evidence of significant variability in the localization and severity of tissue damage due to small shifts in craniotomy position in the lateral fluid percussion injury model performed in rats. Yoshino and colleagues reported local increases in metabolic rates of glucose in rats as early as 6 hours following mild FPI. This hypermetabolism was found in the ipsilateral hippocampus and cortex and lasted as long as 5 days. Hippocampal cell death from organotypical slice cultures was dependent on time and tissue strain but not on strain rate. Peak cell death that was maximally correlated with strain at long-term (3-4 days following injury) time points. All studies demonstrate that similar loading force profiles can induce distinct regional tissue distortion profiles, which may intern cause variability of injury outcome.
Poor Correlation Between Local Axonal Injury and Predicted Tissue Distortion

There was poor correlation between AID and max principal strain, TOS, TOSR and TOSxSR in our sample of 9 animals (3 sections each). There was high regional variability of βAPP staining throughout the piglet brain significantly affected correlations with predicted strains, where many elements with zero AID had relatively high strains and those elements with high AID had low strain values at specific matched locations. The spatial patterns of predicted deformation poorly associated with that of pathological axonal injury. The AID and strain may have poorly correlated because the spatial location βAPP staining (accumulation) may not match the location of the “breaks” along the axon, which implies that to the locations of peak max principal strains predicted by the model would not correspond to the axonal injury histopathology. It is also plausible that secondary injury may occur at 6 hours, however, the extent of secondary injury should be limited compared to post-injury time points at or beyond 1 day. Cullen and colleagues found alterations in plasmalemma permeability at 10 min in 3D neuronal-astrocytic co-cultures following mechanical loading (10/sec) (Cullen, Vernekar, & LaPlaca, 2011). Traumatic loading causes membrane permeability which may lead to cell death in cells that are unable to reseal after mechanoporation. Repair mechanisms of the plasma membrane may be energetically burdensome during secondary degradative events such as calcium-dependent activation of proteases, which digest the cytoskeleton and plasma membrane. Comparisons of individual piglet pathology with a single piglet head FEM was performed, which may also explain the reason that the location of strain values do not match the AID. The overall distributions of AID and strain are similar – within a region, however the element-to-element relationship does not hold. A previously published study on 3-5 day old piglets reported no regional changes in cerebral blood flow deficits despite finding global reductions after rapid head rotation.
(Eucker et al. 2011), which provides evidence that rapid head rotations induce truly diffuse axonal injury throughout the brain such that regional changes cannot be measured. Biological inter-animal variability in the histopathology may also account for poor correlation between AID and max principal strains across the nine animals. Inherent differences in among piglets may partially explain the poor correlation where animal-specific brain morphology and white matter tract orientation are not considered in the relationship between AID and predicted strains.

The quantification of histopathology from later time points may better correlate with simulated region brain deformation. Weeks and colleagues reported peak βAPP cerebral volumes at 24 hours following rapid head rotations (Weeks, Sullivan, Kilbaugh, Smith, & Margulies, 2014). A thorough investigation of regional βAPP patterns at 1, 4 and 7 days following injury could provide a more complete picture of the extent of brain deformation after rapid head rotations. The utilization of different types of immunological stains over post-injury time may also provide insight to the translation of brain deformation to pathological injury. The effect of secondary pathophysiological cascades on the histopathology was limited since our histopathology was collected at 6 hour following injury, however using different time points will introduce secondary injury as a confounding variable.

Large deformations may cause permanent structural changes (Cater et al., 2006; Elkin and Morrison, 2007) that may not be present within 6 hours of injury in βAPP immunostaining given that the peak time course of βAPP in neonatal pigs occurs at 24 hours. Smaller deformations may be associated with brief functional changes in synaptic activity, signaling pathways and membrane permeability (Meaney and Smith, 2011) that were not measured in this study.
Predicted Local Axonal Injury is Region and Direction-Specific

The calculation of a critical max principal strain value for inducing mild axonal injury was applied to each of 32 simulation in order to examine the relationship between predicted axonal injury across direction and region. The critical value of max principal strain (1.18) was large, likely consistent with our hemisection that FEM brain properties may be overestimating strain in the second animal set. Comparison of experimentally measured max principal strains to those from the hemisection FEM revealed that the central brain elements had a larger deformation error than elements closer to the periphery of the brain.

The Rotational Kinetic Energy (RotKE) of COR rapid head rotations was lower than SAG and AXI because of the piglet’s brain moment of inertia about the COR plane. The relationship of volume fraction exceeding the critical max principal strain value with RotKE was strongly influenced by region and direction. The axial piglets (where there is a wide range of RotKE) had a positive correlation between volume fraction and RotKE where greater RotKE resulted in higher volume fractions. However, the ranges for COR and SAG were relatively small so that volume fraction may not appear to change with RotKE. Volume fraction is region- and direction- dependent, for instance the white matter volume fraction ranged from 0-0.5 for AXI piglets, 0.03-0.25 in COR piglets and 0.03-0.04 in SAG piglets. However, in the brainstem only SAG piglets presented a non-zero range of volume fraction of 0.02-0.06 compared to AXI and COR rapid head rotations.

The sensitivity analysis on the critical strain value used for calculating volume fraction revealed that a 9% decrease in critical strain value produced an increase in regional volume fraction by 14-838 %, while a 9% increase in critical strain value causes a 13-100% reduction in volume fraction. Changes in strain from 1.18 to 1.07 and from 1.18 to
1.29 do not significantly change the regional volume fraction for each animal relative to other animals. Across all regions, the white matter and thalamic regions had larger values of volume fractions than those in the brainstem and cerebellum. Across all regions and injury planes, volume fraction values only significantly changed (from the critical strain value of 1.18) in the white matter and thalamic regions following a SAG head rotation. The volume fraction of damaged brain regions (at critical strain value 1.18) did not significantly vary from changes to the critical strain value following rapid head rotations in the AXI and COR directions. Persistent (6 days) axonal injury following SAG was previously observed in neonatal pigs that was absent in AXI injuries, when there were similar magnitudes of axonal injury at 6 hours (Sullivan et al., 2013a).

However, in the FEM white matter region, we interestingly noted lower volume fractions of element exceeding the critical max principal strain value following SAG rotation compared to COR and AXI, which may suggest that pathophysiological factors (causing enhanced hypoxic or ischemic axonal damage) may play a significant role in heightened magnitude of axonal injury that is characteristic of SAG rotations. The FEM white matter region primarily captures the corpus callosum and corona radiata structures, however it does not account for the internal capsule, where extensive pathological axonal injury was observed following SAG compared to other directions. Volumes of the corpus callosum and internal capsule constitute a small fraction of the total FEM white matter region compared to the corona radiata. In the brainstem, SAG was predicted to have extensive axonal injury compared to AXI and COR, which may explain the poor clinical outcomes, such as prolonged behavioral deficits and global cerebral blood flow reductions, observed in neonatal pigs following SAG injuries compared to rotations in other directions (Eucker et al., 2011; Sullivan et al., 2013b, 2013a).
Limitations and Future Work

As with all histopathological assessments of axonal injury, our current approach likely overestimates the number of injured axons since a single undulated axon within a 2-dimensional plane may be counted multiple times. In this report, we assumed that each βAPP profile indicated an individual axon that was not present twice within the same section or in another section. However, this should not affect the conclusions from these results since the overestimation of axons should be present across all injury groups.

There was variability in the quantity of each anatomical regions captured from each of the three sections due to variability in the size of the each piglet brain which contributed to inconsistent sectioning of the fixed brains. This can be seen in section 10 where more anterior regions of the hippocampi were present in some animals and absent in others. Future work may include sub-dividing the white matter in the FEM mesh into the corpus callosum and internal capsule in order to evaluate whether strong correlations of TOS and histopathology are present.

Based on comparisons of FEM max principal strains with those experimentally measured, the FEM brain material properties may be too soft, leading to more brain deformation. The skull-brain interface may also be too soft possibly leading to an overestimation of strains. The brain-skull interface was modelled as a low modulus solid elements (CSF) that were tied to the brain and skull, which were previously reported to represent brain-skull displacement similar to measured values in situ (Coats et al., 2012). Alternative brain-skull interfaces explicitly allowing for the sliding motion of the brain along the skull, such as a low coefficient of friction boundary condition, should be explored in future studies in order to examine their effect on brain deformation.

Previously, linear elastic axial connector elements with an elastic modulus of 3460 kPa
(the modulus for cortical veins) were placed on each brain surface node to the nearest skull (S. Sullivan et al., 2015). However, the connectors were assumed as linear elastic whereas the cortical vasculature in the pia-arachnoid complex are reported as nonlinear in the developing human and pig (Pasquesi & Margulies, 2017).

One piglet head FEM based on a MRI scan of a single animal was used to simulate several other 3-5 day old piglets. Inter-animal variability in the brain mass and shape may affect the deformation of brain tissue and is not accounted for in our study. Development of individualized FEMs for every piglet would address this issue and will be considered for future work.

Our dataset of animals with AID was modest and the findings should be replicated in a larger cohort of piglets. In addition, the study of very mild rapid head rotations (peak angular velocity of 50 rad/s) should be included along with a battery of behavioral tests to establish a wide spread of neuro-cognitive deficits as well as structural damage. A larger sampling of coronal sections throughout the brain per animal should be undertaken.

Conclusions
Sagittal rapid head rotations induce a substantially higher number of injured axons in the corpus callosum and internal capsule than axial and coronal rotations. The lack of inclusion of morphology such as the ventricles may substantially influence the magnitude of strains and/or strain rates obtained from piglet head finite element model rapid head simulations. The inclusion of lateral ventricles influences the magnitude of regional brain deformation observed, where larger max principal strains were seen in FEM with lateral ventricles. Predicted local tissue deformation and regional histopathology were both
direction- and region-dependent, but poorly correlated with each other. We conclude that sagittal rapid head rotations induce significantly higher strains in the brainstem compared to strains from axial and coronal rotations, which may explain the worst clinical outcomes that are characteristic of sagittal injuries in neonatal pigs.
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CHAPTER 6:
Conclusions and Future Directions

Introduction

Traumatic Brain Injury (TBI) affects millions of children each year and costs millions of dollars in healthcare (Faul, et al., 2010, Cheng, et al. 2016). The goals of this chapter are to comprehensively summarize this dissertation’s contribution to pediatric TBI literature and to propose future studies. We address these goals by presenting four subsections on Rotational Work, Auditory Event-related Potentials in Juvenile Piglets and Resting State Electroencephalogram in Juvenile Piglets, Region-Dependence of Histopathology and Strains in the Neonatal Piglet Brain and chapter cross-cutting concepts.

Rotational Work

The ability to predict pediatric TBI from head movement metrics during recreation and transportation is critical to the effective design of child safety equipment and the accurate assessment of brain injury risk. The severity of TBI is influenced by the magnitude and direction of head movement. To guide development of safety equipment that reduces sports-related head injuries, we sought to enhance predictive relationships between head movement and acute axonal injury severity. Previous studies have demonstrated correlation between rotational head kinematics and symptom severity in the adult. More recent studies have demonstrated brain injury age- and direction-dependence, relating head kinematics to white matter tract-oriented strains (TOS). Now, we have developed and assessed novel rotational head kinematic metrics as predictors of acute axonal pathology in two age groups of female, immature piglets (3-5 day old and 2 month old). We show that many previously published rotational kinematic injury
predictor metrics poorly predict acute axonal pathology induced by rapid, non-impact head rotations; and that inclusion of cerebral moments of inertia (MOI) in rotational head injury metrics refines prediction of diffuse axonal injury following rapid head rotations for two immature age groups. While Rotational Kinetic Energy (RotKE) was the best significant predictor in the neonatal pig, Rotational Work (RotWork) was the best significant predictor of traumatic axonal injury in both newborn and pre-adolescent piglets following head rotations in the axial (AXI), coronal (COR) and sagittal (SAG) planes. Specifically, COR head motions generated the lowest RotWork, compared with AXI and SAG rotations. An improvement over current metrics, we find that RotWork, which incorporates head rotation rate, direction and brain shape, significantly enhanced acute traumatic axonal injury prediction. For similar injury extent, the RotWork threshold is lower for the newborn piglet than the pre-adolescent. In this chapter, we evaluated the ability of RotWork to associate with axonal injury. Future studies should examine RotWork’s relationship with other clinically relevant outcomes such as behavioral tests, sleep patterns (actigraphy) and other changes in electrophysiology activity following injury. Structural and functional injury thresholds of RotWork could be determined for each pediatric age group. We speculate that age-specific responses to injury such as pro-inflammatory cascades may lead to an enhanced hypoxic/ischemic microenvironment leading to more axonal injury. Acute inflammation of white matter following TBI is affected by age where post-injury neuroinflammation was increased in old mice yet with fewer post-injury neurofilament-positive swellings were observed compared to young mice (Cheng et al., 2018). Another mechanism for age-dependent axonal injury following TBI may be cerebral blood flow deficits, which promote larger axonal injury volumes. Larger decreases in cerebral blood flow for the 5 day old piglet were noted compared to 4 week old piglets at 3 hours following fluid-percussion TBI.
(Armstead and Kurth et al., 1994). Ibrahim reported significant decreases in cerebral blood flow in 3-5 day old and 4 week old piglets at 6 hour after unscaled non-impact, rapid head rotation in the axial plane (Ibrahim et al., 2010). Future studies should focus on the biological and physiological mechanism responsible for the vulnerability in the newborn.

**Auditory Event-related Potentials in Juvenile Piglets**

None of the current diagnostic tools, such as quantitative cognitive and balance tests, have been validated to identify mild TBI in infants, adults and animals. In this study, we report a novel, quantitative tool that has the potential to quickly and reliably diagnose TBI and which can track the state of the brain during recovery across multiple ages and species. Using 32 scalp electrodes, we recorded involuntary auditory event-related potentials (ERP) from piglet brains and generated functional ERP networks. We has two primary goals: first, to determine the effect of head rotation on ERP networks and second, to propose a novel metric for diagnosis of TBI in the pediatric population. There is a lack of sensitive and objective diagnostics for TBI, the severity of which is dependent on head rotation direction. We examined SAG and COR injuries and found reductions in nodal strength following SAG and COR at 1d post-injury and an increase in nodal strength following COR at 4d post-injury. Patterns of the observed changes in ERP networks were used to distinguish brain–injured piglets from those non-injured. This novel approach is the first application of auditory ERP functional networks to the prediction of TBI. Our report on auditory ERPs was also the first to apply network analysis to ERPs in a large animal model of TBI. The resulting tool is a robust, objective and predictive method that offers promise for detecting mild TBI.
Auditory ERP networks allow discrimination between injured and uninjured piglets in a fast, objective manner that may be translated to detection of TBI in children on sports sidelines or in the clinic. The study of a large cohort of diffuse and focal TBI piglets could provide insight into the difference between injury types on functional connectivity, specifically which the critical connections are lost or strengthened across the brain. Such a study could lead to tools that are able to identify regions of the brain with highest risk of injury. We expect InjScore to increase with RotWork (that is generally higher in SAG compared to COR).

We expect similar post-diffuse TBI changes to occur in networks from visual ERPs as auditory ERPs assuming that subjects have similar visual and acoustic learning environments and practice. Bimodal (visual and auditory) processing after TBI may be selectively affected if certain regions, such as the auditory cortex, are damaged during a focal TBI while the visual cortex is intact. The testing of selective damage to the auditory compared to visual cortex could be achieved by administering a combined auditory-visual oddball paradigm where double flashes of light are used as infrequent (20%) stimuli among frequent auditory clicks (80%) or double clicks used as infrequent stimuli among frequent, single flashes of light. Subjects would indicate when an infrequent stimuli is presented (response rate). We hypothesize that damaged brain regions would present poor correlations of network metrics with the response rate post-TBI (relative to PRE).

Our ERP study did not find any difference in the shape and latencies between the standard (Std) and oddball (OB) paradigms in the juvenile pigs despite of previously reported differences in healthy and diseased humans (Bachiller et al., 2015; Beaumont, et al., 2007; Ghosh Hajra et al., 2016; Gosselin, et al., 2006; Kozlowska, et al., 2017). We believe that the lack of differences observed in the ERP epochs may be due to the
low number of OB ERP responses acquired and to the lack of the piglet attending to the stimulus. Attention can substantially affect the magnitude and latency of several auditory ERP components (Luck & Kappenman, 2012). When the brain engages in directing attention to sensory stimuli, specific neural oscillations dominate (Buzsáki, et al., 2013) and thereby influence the magnitude and latencies of ERP components. Prior training of the piglet to attend to the OB-Target (OB-Trgt) stimulus may lead to increases in the magnitude of select ERP components.

Training of piglets to attend to an auditory stimulus may prove difficult because of the total time necessary to achieve it may lead to ERP studies of piglets that are much older than 4 weeks. The calibration of a behavioral training regime to the 4 week old piglet developmental state will require extensive preliminary study among a large set of piglets and there may be intra-group variability on the piglet’s ability to learn how to attend to the stimulus. In order to measure ERPs in 4 week old piglets, the stimulus attention training would need to be performed on 2 week old piglets, ideally requiring 2 weeks of daily training sessions. We speculate that 2 week old piglets could be trained to move their left or right hoof (onto blue vs red patch) to indicate OB-Std or OB-Trgt stimuli respectively. However, the interval between each auditory stimulus would need to increase to accommodate the piglet’s behavioral response. Behavioral indications that involve head movement, such as nose pokes or head movements, are undesirable since they introduce artifact into the ERP recording.

Our study averaged over 200 ERP epochs with duration of 300 ms (50 ms before the stimulus). Future studies of ERPs in piglets should lengthen the duration of each ERP epoch (to 1200 ms, with 500 ms of pre-stimulus recording) and increase the number of stimuli played (to at least 500), in order to improve the signal-to-noise ratio of
the averaged ERP. This also permits easier comparison with the extensive human ERP literature and may elucidate the similarities and differences in neurocognitive processing of sound between children and piglets. Additionally, an increase in the number of ERP epochs permits further analysis on the most discriminant time point between injured and uninjured ERPs and may also increase the accuracy of previously discussed TBI prediction algorithm. The frequency at which the auditory stimuli are played could also be changed to approximately twice as slow (~2 Hz) in future studies to examine the effect of stimulus frequency on ERPs after TBI. There may be a difference in the frequency at which the piglet’s neural activity attunes to the auditory stimulus after injury.

Selecting a stimulus that is relevant to the subject (for instance playing their name) was reported to enhance the N100-P150 complex and alertness in a severe TBI cohort 6 and 12 months post-injury (Mazzini, et al., 2001). Future studies may also include using an emotionally-relevant auditory stimulus, such as an audio recording of the same pig grunting while feeding in order to ensure attention to the stimuli and potentially elicit novel, discriminant ERP components. The first 50 ms of each auditory ERP reflects processing performed by the brainstem at a fundamental stage of auditory processing that is conserved across mammals. There are reports of impaired neural encoding in the brainstem in adults following mild TBI compared to uninjured controls (Vander & Rieger, 2017). One study has shown that children with mild TBI exhibit a distinct small and slow signature neural profile using a frequency-following response elicited from the brainstem, which led to accurately predicting 95% of concussion cases (Kraus et al., 2016). We suggest study of brainstem auditory ERPs following TBI in piglets and infants because of its ease of data acquisition and translation to humans and to establish neuro functionality and location. The interpretation of neurocognitive events
underlying brainstem auditory ERPs in the piglet should be much less challenging than that of longer latency auditory ERPs.

We utilized graph theory tools that are not traditionally employed for the study of ERPs in piglets to study auditory cognitive processing in pigs after mild diffuse TBI. We found that injured animals presented hypoconnectivity of ERP networks at 1 day post-injury compared to sham. Extensive study of neuro-cognitive processes studies behind auditory sensing and discrimination in the piglet could provide much needed insight into neuro-pathological mechanisms behind TBI-induced changes that are observed in the human child.

This proposed research is directly translational to human children for prognosis and severity of brain injury. Investigations utilizing the piglet TBI model reduces the sample heterogeneity inherent to human studies, thereby elucidating the relationship between injury and network parameters. This work is novel because of its intersectionality between the pediatric TBI biomechanics and network neuroscience on auditory ERPs. ERPs are an objective, direct measure of brain injury that is non-invasive, which makes this approach suitable for assessment of concussions on the sidelines of a sports game. There is a paucity of work in the pediatric, functional network, TBI literature. Few studies relate functional network changes to structural damage and cognitive alterations resulting from TBI in a pediatric cohort that is homogenous in subject brain injury type, age and recovery time.

In future studies, the robustness of network dynamics in across different spatial and temporal scales of ERPs could be examined through increase in the number of channels, focus on recording from the brainstem region and longer post-injury recording
durations. Networks could be constructed using sub-sections instead of the entire ERP in order to identify the most discriminating features of the ERP for TBI. The use of these scales could elucidate the mechanism of recovery from TBI and the mechanism of hyper-connectivity due to TBI. Future studies could also explore acute and chronic electrophysiological changes following rapid head rotation about different planes in other piglet ages in order to evaluate the effect of development on TBI-induced responses.

**Resting State Electroencephalogram in Juvenile Piglets**

The lack of standardized biomarkers for the diagnosis of TBI makes prognosis more challenging, especially in the pediatric context, where children are often unable to clearly communicate their symptoms. Resting state electroencephalograms (EEG) address these problems by being inexpensive, noninvasive, and not requiring subjects to perform cognitive tasks. Resting state EEG have not yet been used to create functional brain networks in relation to TBI in children or other animals; here we report the first such study. We recorded resting state EEG in awake piglets before and after TBI, from which we generated EEG functional networks from the alpha (8-12 Hz), beta (16.5-25 Hz), broad (1-35 Hz), delta (1-3.5 Hz), gamma (30-35 Hz), sigma (13-16 Hz) and theta (4-7.5 Hz) frequency bands. We hypothesize that mild TBI will induce frequency-dependent changes in the 4 week old piglet at acute and chronic time points. Hyperconnectivity was found in several frequency band networks after TBI. This study serves as proof-of-concept that the study of EEG functional networks in piglets may be useful for the development of biomarkers for TBI in children. The integration of resting state EEG functional network metrics with RotWork lead to improved predictive capability of TBI in our piglet model. Future studies should expand resting state EEG
recording to piglets after COR and AXI rapid head rotations in order to examine the relationship between network metrics, RotWork and direction.

Our EEG data set was based on recording durations of 90 s, which is comparable to the time of stabilization of human EEG functional networks found by Chu and colleagues at 100 s of recording (Chu et al., 2012). We suggest future experiments with longer resting state EEG recording durations, up to 10 minutes, for construction of possibly more robust networks. Longer EEG recording durations will also make comparison with results from rich human EEG literature easier. It will also permit the analysis of very low frequency oscillations, which were previously reported as altered following TBI (Modarres, et al., 2017).

Future resting state EEG studies could explore whether recording with the room lights off affects the EEG functional connectivity after TBI. Some studies in humans ask subjects to close their eyes (Honey et al., 2009; Palacios et al., 2017) and there is evidence that closing the eyes may affect the alpha oscillatory rhythm (Geller et al., 2014). Recording without extraneous visual stimulation could improve the quality of EEG signal. Piglets may fall asleep in a dark room during extended recording, however the study of EEGs during sleep states may reveal injury-specific changes because there is evidence that sleep is affected by TBI (Gosselin et al., 2009; Olson et al., 2016; Viola-Saltzman & Watson, 2012).

There is a paucity of resting state EEG functional network studies in the pediatric population following TBI. Knowledge gaps exist in the understanding of the relationship between functional networks, structural damage and cognitive impairment following TBI in children. By addressing these knowledge gaps, we can substantially improve pediatric
TBI tracking and intervention. The prediction of the type and the severity of TBI sustained in the pediatric population using functional connectivity patterns is also possible. The comparison of diffuse with focal injuries in the neonatal and juvenile piglet using resting state EEG networks would indicate in part the role of development on TBI-induced changes. There are very few papers that study resting state changes in the pediatric population and such a study would provide age-specific changes in functional connectivity after diffuse and focal TBIs.

White matter degradation as measured by DTI and synaptic junction protein immunohistochemistry may be used to de-couple the complex functional-structural relationship and provide a source for altered functional networks observed in animal models. The injury of a single axon could result in the shedding of approximately 7000 synaptic terminals. Diffuse synaptic loss can occur as a result of both primary and secondary mechanisms. Reparative responses mounted by the brain after injury may involve membrane repair, somatic recovery and axonal outgrowth in the midst of hypoxia, ischemia and/or energy failure. In the most severe case of injury, the axolemma can be focally perturbed, allowing for calcium influx and dysregulation. Subsequent activation of proteases can then degrade the cytoskeleton leading to impaired axonal transport and swelling within several hours of trauma (Merlo et al., 2014). We hypothesize that damage to synaptic junction proteins may indicate synaptic loss or disrupted plasticity or loss of long-term potentiation that may lead to functional hyperconnectivity in intact axons. Candidate synaptic junction proteins include those implicated in the structural damage to the pre and post synaptic densities and may lead to functional synaptic disconnection. Synaptotagmin is family of calcium binding proteins located in the synaptic vesicles (Merlo et al., 2014). Proteolysis of Synaptotagmin-1 (Syt-
1) may hinder vesicles from docking on pre-synaptic membrane terminal. Accumulation of Synaptotagmin-4 (Syt-4) after TBI reduces synaptic activity (Ansari, Roberts, & Scheff, 2008). Postsynaptic density complex protein 95 (PSD-95) is marker of synapse degeneration (Ansari, Roberts, & Scheff, 2008). F-actin is a microfilament that plays a significant role in mobility and contraction of cells during cell division and is vital to synaptotagmins (Wen, Li, Shen, & Chen, 2017). Synapsins are phosphoproteins that regulate the release of neurotransmitters into the pre-synaptic area. Oxidative stress induced by TBI may lead to synapsin-1 loss (Wen, Li, Shen, & Chen, 2017). Quantification of synaptic junction proteins after TBI using western blotting and/or histopathology would provide insight to the microscale neurophysiological underpinnings of functional EEG connectivity.

**Region-Dependence of Histopathology and Strains in the Neonatal Piglet Brain**

The relationship between local tissue distortion and local altered pathophysiology following TBI is unknown. We hypothesized and found that tissue distortions were dependent on brain region and head rotation direction in the neonatal pig. After examination of the regional biomechanical and neuropathological responses to TBI, we found significantly high numbers of injured axons per unit volume in the corpus callosum and internal capsule after SAG injury compared to AXI and COR rapid head rotations. We also noted poor correlations between local strain and axonal injury histopathology. SAG rapid head rotations induced significantly higher strains in the brainstem, which may account for worst clinical outcomes observed compared to AXI and COR rotations.

In light of the more accurate histopathology quantitative assessment of axonal damage in piglet brains post injury, there is much more axonal damage in piglets injured in the COR plane than previously reported. Behavioral testing and other
neurophysiological assessments should be conducted on neonatal and juvenile pigs after sustaining a single COR rapid head rotation, in order to more robustly compare with AXI and SAG data. The ranges of SAG and COR angular velocities used to induce TBI in the neonatal and juvenile pig should be expanded to enable velocity-matched comparison between all pairs of directions.

The poor correlation between strain and AID may be due to the mixture of two distinct spatial scales, because the AID tiles captured axons separated by microns and the FEM predicted strains in elements that were millimeters long. The strain calculated in one element may cover too large of a volume to be representative of the deformation that occurs at the micron-level. The use of elements with multiple integration points would permit the calculation of strain values throughout the elements, which could be matched to individual AID tiles. A finer mesh density (with smaller elements) may also lead to a stronger correlation between strain and axonal damage, however simulation time per run would exponentially increase.

There is need to develop injury thresholds for repeated TBIs and pre-existing biological conditions, because of a paucity of research in the pediatric population. The metabolic and neuro-pathological response to a second traumatic event shortly after mild TBI may differ from the first. Our neonatal piglet FEM (after validation of brain mechanical properties and brain-skull interface to low-rate hemisection data) could be used to simulate low rate (50 rad/s) head rotations in all directions. Piglets could be injured at 1 week apart and βAPP histopathology obtained at multiple time points following first and the second injury (days 1, 4 and 7). The relationship between the predicted tissue deformations from the FEM and the corresponding βAPP histopathology from different post-injury days could reveal the threshold of axonal damage with pre-
existing injury. Future studies can address how deformation thresholds in the (chemically-induced) hypoxic/ischemic brain may differ from normal tissue.

This study assumed that the cerebral tissue deformation threshold to injury in the corpus callosum and corona radiata were the same, however there is evidence of region-specific brain deformation thresholds, which may partially explain the poor correlation between axonal injury density and TOS observed in our study (Cater, Sundstrom, & Morrison, 2006; Elkin & Morrison, 2013; Prange & Margulies, 2002; Vink, Mullins, Temple, Bao, & Faden, 2001; Yoshino, Hovda, Kawamata, Katayama, & Becker, 1991). Future studies should expand the number of animals and sections from which histopathology was obtained, then generate an individualized FEM per animal, where the white matter part is segmented into morphologically distinct structures such as the corpus callosum, internal and external capsule and corona radiata. The co-registration between the individualized FEM and coronal histopathological sections may lead to stronger correlations between local histopathology and TOS. The regional quantification of immunostaining for microglial activation or other ischemia or hypoxia-specific markers in the current 9 animals with βAPP immunohistochemistry at 6 hours post-injury may reveal region-specific populations of ischemic axons. By examining the population of injured axons that are βAPP-positive but ischemia-negative, the effect of biomechanical deformation on strictly mechanically-induced axonal injury could be determined.

Chapter Cross-Cutting Concepts

Inhaled anesthetics such as isoflurane have a varied response on cerebral metabolic rate and CBF in adults (Olsen et al., 1992, Cucchiara et al., 1974, Todd et al., 1996, Kochs et al., 1993, Gelman et al., 1984) and children (Smith et al., 2005).
Isoflurane improved functional outcome and attenuated CA1 damage compared to fentanyl treatment post-TBI. Isoflurane may be neuroprotective by augmenting CBF, improving motor function and reducing excitotoxicity after TBI (Miura et al., 1998, O’Connor, Cernak, & Vink, 2003, Statler et al., 2000). Isoflurane has been reported to induce neurodegeneration via increased numbers of apoptotic cells 4 and 48 hours after CCI in adult rats (Hertle et al., 2012). Isoflurane was shown to affect motor function in rats 1-5 days after exposure compared to pre-injury time point (Statler et al., 2000). Baseline cerebral perfusion pressure was lower after isoflurane was administered compared to total IV anesthesia in immature (4 week) pigs, which may suggest reduced autoregulation (Bruins, Kilbaugh, Margulies, & Friess, 2013, Brady et al., 2009).

Anesthesia was administered to all piglets studied shortly before injury and to shams and may have considerable (acute and chronic) impact on the clinical outcomes (such as ERP and resting-state network metrics and axonal injury) following TBI.

For all ERP and Resting state EEG piglets studied, we recorded the total recovery time (from pre-med to full recovery in the cage) and for injured animals we also recorded injury recovery time (from time of injury to full recovery in the cage). The mean and standard error for injury recovery times were 79.86 ± 13.12 minutes for CCI, 46.25 ± 4.73 minutes for COR and 65.33 ± 7.97 minutes for SAG. COR had the shortest injury recovery time, while CCI had the longest recovery time. The mean and standard error for total recovery times were 187 ± 11 minutes for CCI, 183 ± 12 minutes for COR, 193 ± 11 minutes for SAG and 146 ± 59 minutes for shams. While shams had shorter total recovery times than CCI, COR and SAG, the injured group total recovery times were not significantly different from sham, suggesting that the injuries may be on the mild spectrum of severity. The mean cerebral axonal injury volume (AIV) 8 days after SAG
injuries (mean angular velocity of 130 rad/s) in the current study was 0.31%, which is consistent with Weeks and colleagues’ finding of mean AIV of 0.58% at 5-6 days post-injury was found when a single sagittal rapid head rotation (mean angular velocity of 127 rad/s) was prescribed to 4-week old piglets (Weeks et al., 2014). SAG induced nearly twice as high AIV as AXI AIV in the 4 week old pig with the same angular velocities. There was no bleeding (hemorrhage) found on any of the SAG-injured brains in our current study. COR rapid head rotations caused zero AIV and hemorrhage and subsequently constitute a mild TBI. A previous study (Ibrahim et al., 2010) of axial rapid head rotations in juvenile (4 week old) piglets reported mean AIV of 0.20% for low (129 rad/s) angular velocities and 0.94% for high (194 rad/s) angular velocities at 6 hours post-injury. High velocity AXI rotations caused significantly more subarachnoid bleeding and ischemia than shams, while low velocity animals were statistically similar to shams. In both previous reports, injured piglets had significantly higher AIV than shams. The AIV found in our current report align with previous reports and imply that our injured group (sagittal rapid head rotation) sustained significantly more axonal damage compared to sham. We conclude that the SAG rapid head rotations (mean velocity =130 rad/s) induced a mild-moderate level of diffuse axonal injury in the 4 week old piglet— one that is more severe than COR (mean velocity =130 rad/s), but milder than high-velocity AXI (196 rad/s).

In Chapter 2, we showed that in the 3-5 day old and 2 month old piglet, RotWork, MOI and AIV were highest for SAG rapid head rotations compared to AXI and COR. In Chapter 3, ERPs from SAG injured 4 week old piglets presented the highest InjScores and were thereby most reliably classified as ‘injured’. ERP networks from SAG injuries exhibited lower nodal strength and no change in global efficiency at 1 day post-injury.
compared to sham. At 4 and 7 days post-SAG, nodal strength returned to PRE levels and global efficiency was reduced relative to sham. Chapter 4 showed that resting state EEG networks had enhanced local and global connectivity after SAG injury in the beta, broad, delta, gamma and sigma frequency bands 1, 4 and/or 7 days post-injury compared to sham. The magnitude of change in connectivity across the resting state EEG network was largest on 1 day post-SAG compared to 4 and 7 days. In Chapter 5, SAG injuries induced significantly higher AID than shams in the corpus callosum, corona radiata, hippocampus, internal capsule, striatum and thalamus. 3-5 day old pigs rotated in the SAG direction had significantly longer unconscious durations than the sham group, as did AXI head rotations at similar angular velocities (Eucker, 2011). For the 2 month old pigs, SAG had comparable mean AIV (at 6 hours post-injury) to AXI over a range of angular velocities, but SAG was the only direction with significantly longer time to return of pinch reflex (return to consciousness) compared to sham (Maltese, 2012b). SAG is associated with prolonged cerebral blood flow reductions, behavioral deficits and apnea (Eucker, et al., 2011; Ibrahim, Ralston, et al., 2010; Sullivan et al., 2013a; Sullivan et al., 2013b). Across age, piglets that were injured by a SAG rapid head rotation generally presented worse acute axonal damage and other clinical outcomes compared to sham.

Chapter 2 showed that COR had the lowest AIV, MOI and RotWork of all three planes. In Chapter 3, we found that ERPs from COR injured piglets were more challenging to distinguish from ERPs from sham or uninjured animals as compared to SAG-injured piglets. ERP networks had reduced connectivity at 1 day post-COR and increased connectivity at 4 days before returning to sham levels on 7 days. This fluctuation in connectivity, that was absent in SAG-injured piglets, may indicate the
brain’s ability to recuperate from a mild diffuse head injury over time. Chapter 5 demonstrated that COR, similar to SAG, had significantly higher average AID than sham, however COR AID was also significantly lower than that of SAG. The predicted volume fraction of ‘damaged’ elements varied by region and head rotation direction, where COR induced lower volume fractions in the brainstem than SAG and the thalamus, white matter and cerebellum regions had the same volume fraction values for SAG and COR. Taken together, COR rapid head rotations yielded milder acute axonal damage than SAG, but more severe brain damage than sham. We speculate that COR induces mild behavioral outcomes compared to SAG.

SAG is associated with more severe clinical outcomes, which may be due to the high AID concentrated in the corpus callosum and internal capsule. Resting-state EEG networks in the broad, delta and gamma frequency bands had hyperconnectivity at 1 day that persisted to 7 days post-SAG injury. Extensive tissue deformation and predicted axonal injury volume fraction were predicted in the brainstem which may also explain the poor outcomes. SAG head rotations have the largest RotKE (and RotWork) compared to AXI and COR. Taken together, changes in rotational head kinematics, network metrics from resting-state EEG functional and auditory ERP networks may reflect the significant structural loss that is characteristic of SAG injuries. Prediction of TBI using auditory ERP networks produced InjScores for COR that were comparable to that of shams, which implies that auditory ERP networks may be insensitive to the reduced AID that is characteristic of COR injuries. The development of structural injury thresholds collectively based on rotational kinematics, electrophysiological functional networks and predicted tissue deformations could form a powerful clinical platform for predicting TBI and recovery in the neonatal and juvenile pig.
Conclusions

This body of work examined the biomechanical and neurophysiological mechanisms behind direction and region-dependent responses to traumatic brain injury in neonatal and juvenile pigs. The use of FEM, ERPs and resting state EEG are important for the prediction of direction and region-dependent axonal injury in the pediatric pig. We show that different rapid head rotation directions produce region-specific strains and axonal damage that in turn leads to direction-dependent neurophysiological responses.

Summary of Contributions

In summary, this dissertation’s primary contributions to academic TBI literature were as follows:

- Rotational Work is a novel rotational kinematic predictor of cerebral axonal injury and may be used on both the neonatal and pre-adolescent piglet populations.
- First resting state EEG functional network study in a pediatric TBI animal model. Critical contribution to the study of pediatric TBI using resting state EEG because there are few such studies in the literature.
- First and novel application of graph theory to the study of ERPs after TBI. Valuable contribution to the TBI literature as the first ERP study in piglets since there is a paucity of ERP animal studies following TBI.
- We validated that we can correctly classify auditory ERPs from piglets as injured with 82% accuracy.
- Hyperconnectivity of resting state EEG networks was found in juvenile pigs at acute and chronic time points following TBI, which is widely consistent with adult human TBI literature.
• Resting state EEG and auditory ERP functional networks are potential biomarkers for diagnosing and tracking recovery from TBI in children.

• The spatial distribution of beta-amyloid pre-cursor protein at 6 hours following rotational injury depends on the plane of head rotation.

• The magnitudes of max principal and tract oriented strains and strain rates calculated from simulations of rapid head rotations are significantly influenced by their regional location and head rotational injury plane.


Cucchiara RF, Theye RA, Michenfelder JD. The effects of isoflurane on canine cerebral metabolism and blood flow. Anesthesiology 1974;40:571–4


Appendix 3.1: Effect of Network Density on Oddball Paradigm ERP Network Properties
Appendix Figure A3.1: Oddball Standard and Target network metrics (nodal strength, clustering coefficient, global efficiency, characteristic path length and modularity) as a function of network density.

Appendix 3.2:

In the sham group, the edges from all animals on PRE had a large spread (Appendix Fig. A3.2). On POST 1d, there were four animals with lower edge weights than the mean CDF. All PRE and POST-SAG days, there were animals with CDFs greater than and below the mean CDF. For COR, all three animals are very consistent with each other in their distributions of edge weights on PRE and POST days. All three CCI animals had consistent CDFs on PRE and POST days, with the exception of POST 1d, where one animal had very large edges.
Appendix Figure A3.2: Empirical Cumulative Density Functions (CDFs) of Standard paradigm edges for individual animals (black line) and its injury group-day average (thick, red line) for all injury groups and study days

Appendix 3.3: Most discriminating time point between injured and uninjured classes is >175 ms post-clc

The original ERP epoch was reduced along the channel dimension so that differences between the injured and uninjured classes in the time dimension could be examined. We aimed to gain insight into which time point (feature) following the auditory stimulus was most predictive of injury. We hypothesized that the time feature that is most important to the prediction of injury would vary between the OB-Std and OB-Trgt ERP epochs to reflect the time needed for discrimination between the Std and Trgt clicks. A random forest machine learning algorithm was selected for its robustness, relatively good accuracy and straightforward assessment of feature importance (or feature selection). The Gini importance method is a common approach to feature selection.

A random forest with 250 trees was used on the spatially reduced OB-Trgt and OB-Std ERP training set. Dimension of reduced data set is total number of trials (~200 per animal) by time feature (300 ms with 250 ms after the stimulus). The Gini importance rank was returned for each time feature and represents how important each feature was to the overall accuracy of the random forest algorithm. A higher feature importance indicates that the feature increased the accuracy of the algorithm when distinguishing injured and uninjured epochs.

Appendix Figure A3.3 shows the ease of the random forest algorithm classifying OB-Trgt ERP epochs as injured at various time points. There was an increase in the feature importance measure from 175 to 250 ms following the click. Maximal feature importance occurred at 196 ms (post-stimulus) for OB-Trgt. The ease of distinction between the uninjured and injured classes is made clear by plotting the probability distributions of
select time features (Appendix Fig A.3.4). At the start of ERP epoch (before click stimulus), there is little distinction between injured and uninjured groups. This poor distinction between groups occurs until 175 ms after the click stimulus.

Appendix Figure A3.3: Bar chart of mean feature importance by reduced ERP time feature for Oddball-Target.

Appendix Figure A3.4: Oddball-Target reduced ERP time features for injured (green) and uninjured (blue) classes.

For OB-Std, there was increase in feature importance at 175 ms post-stimulus and the time feature with maximum feature importance was 181 ms.
Appendix Figure A3.5: Bar chart of mean feature importance for reduced ERP Oddball - Standard time features

There was an increase in feature importance for Std data starting at 175 ms and the maximum feature importance occurred at 231 ms post-stimulus.

Appendix Figure A3.6: Oddball-Standard reduced ERP 1time features for injured (green) and uninjured (blue) classes
Appendix Figure A3.7: Bar chart of mean feature importance by reduced ERP time feature for Standard paradigm

Across all paradigms, time features after 175 ms post-stimulus presented elevated feature importances, which suggests that late latency ERP components may be critical to distinguishing ERPs as injured. The early ERP components or pre-175 ms post-stimulus duration represents fundamental sensory processing that may be similar in injured and uninjured brains.
Below we present a more detailed look into which nodes change the network when a single node is removed from its network. Only 1 of 32 nodes was removed at a time; nodes were not removed sequentially.

Appendix Figure A3.9: Scatter plot of percent change of mean nodal strength from networks with 1 node (out of 32 nodes) removed compared to whole network for all studied time points. Each point represents the mean nodal strength over all combinations of 31-node networks from a single animal and the black horizontal bar indicates the median value over all piglets for each time point. The y-axis has a narrow range from 6.245-6.255.
Appendix Figure A3.10: Scatter plot of percent change of mean clustering coefficient from networks with 1 node (out of 32 nodes) removed compared to whole network for all studied time points. Each point represents the mean clustering coefficient over all combinations of 31-node networks from a single animal and the black horizontal bar indicates the median value over all piglets for each time point. The y-axis has a narrow range from 3.12 - 3.132.
Appendix Figure A3.11: Scatter plot of percent change of mean modularity from networks with 1 node (out of 32 nodes) removed compared to whole network for all studied time points. Each point represents the mean modularity over all combinations of 31-node networks from a single animal and the black horizontal bar indicates the median value over all piglets for each time point.

Appendix Figure A3.12: Scatter plot of percent change of mean global efficiency from networks with 1 node (out of 32 nodes) removed compared to whole network for all studied time points. Each point represents the mean global efficiency over all combinations of 31-node networks from a single animal and the black horizontal bar indicates the median value over all piglets for each time point. The y-axis has a narrow range from 6.2 - 7.4.
Appendix Figure A3.13: Scatter plot of percent change of mean characteristic path length from networks with 1 node (out of 32 nodes) removed compared to whole network for all studied time points. Each point represents the mean characteristic path length over all combinations of 31-node networks from a single animal and the black horizontal bar indicates the median value over all piglets for each time point.
Appendix Figure A3.14: Scatter plot of percent change (x-axis) in nodal strength from whole network when a single node is removed (y-axis). The black bars indicate the median value over all animals (represented by a single point) for each node removed.
Appendix Figure A3.25: Scatter plot of percent change (x-axis) in clustering coefficient from whole network when a single node is removed (y-axis). The black bars indicate the median value over all animals (represented by a single point) for each node removed.
Appendix Figure A3.36: Scatter plot of percent change (x-axis) in modularity from whole network when a single node is removed (y-axis). The black bars indicate the median value over all animals (represented by a single point) for each node removed.
Appendix Figure A3.47: Scatter plot of percent change (x-axis) in global efficiency from whole network when a single node is removed (y-axis). The black bars indicate the median value over all animals (represented by a single point) for each node removed.
Appendix Figure A3.58: Scatter plot of percent change (x-axis) in characteristic path length from whole network when a single node is removed (y-axis). The black bars indicate the median value over all animals (represented by a single point) for each node removed.

Appendix Figure A3.19: Median N40 amplitudes for channels 15 and 16 for all injury groups and time points from the Standard paradigm.
Appendix Figure A3.20: Median N40 amplitudes for channels 17 and 19 for all injury groups and time points from the Standard paradigm.

Appendix Figure A3.21: Median N40 amplitudes for channels 20 and 28 for all injury groups and time points from the Standard paradigm.
Appendix Figure A3.22: Median N40 latencies for channels 15 and 16 for all injury groups and time points from the Standard paradigm.
Appendix Figure A3.23: Median N40 latencies for channels 17 and 19 for all injury groups and time points from the Standard paradigm.

Appendix Figure A3.24: Median N40 latencies for channels 20 and 28 for all injury groups and time points from the Standard paradigm.
Appendix Figure A3.25: Median P60 amplitudes for channels 15 and 16 for all injury groups and time points from the Standard paradigm.

Appendix Figure A3.25: Median P60 amplitudes for channels 17 and 19 for all injury groups and time points from the Standard paradigm.
Appendix Figure A3.26: Median P60 amplitudes for channels 20 and 28 for all injury groups and time points from the Standard paradigm.
Appendix Figure A3.27: Median P60 latencies for channels 15 and 16 for all injury groups and time points from the Standard paradigm.

Appendix Figure A3.28: Median P60 latencies for channels 17 and 19 for all injury groups and time points from the Standard paradigm.
Appendix Figure A3.29: Median P60 latencies for channels 20 and 28 for all injury groups and time points from the Standard paradigm.
Appendix Figure A3.30: Median N120 amplitudes for channels 15 and 16 for all injury groups and time points from the Standard paradigm.

Appendix Figure A3.31: Median N120 amplitudes for channels 17 and 19 for all injury groups and time points from the Standard paradigm.
Appendix Figure A3.32: Median N120 amplitudes for channels 20 and 28 for all injury groups and time points from the Standard paradigm.

Appendix Figure A3.33: Median N120 latencies for channels 15 and 16 for all injury groups and time points from the Standard paradigm.
Appendix Figure A3.34: Median N120 latencies for channels 17 and 19 for all injury groups and time points from the Standard paradigm.

Appendix Figure A3.35: Median N120 latencies for channels 20 and 28 for all injury groups and time points from the Standard paradigm.
Appendix Figure A3.36: Median P200 latencies for channels 15 and 16 for all injury groups and time points from the Standard paradigm.

Appendix Figure A3.37: Median P200 amplitudes for channels 15 and 16 for all injury groups and time points from the Standard paradigm.
Appendix Figure A3.38: Median P200 amplitudes for channels 20 and 28 for all injury groups and time points from the Standard paradigm.
Appendix Figure A3.39: Median P200 latencies for channels 15 and 16 for all injury groups and time points from the Standard paradigm.

Appendix Figure A3.40: Median P200 latencies for channels 17 and 19 for all injury groups and time points from the Standard paradigm.
Appendix Figure A3.41: Median P200 latencies for channels 20 and 28 for all injury groups and time points from the Standard paradigm.

CHAPTER 4: APPENDIX

Appendix 4.1: Effect of TBI on EEG Network Metrics from Different Frequencies
Appendix Figure A4.1: Comparison of nodal strength across injury group and day for all frequency bands. Each open circle represents a single 1s network and each color indicates an animal. The thick black horizontal bars are mean values over all animals, while the thin black horizontal lines at the top of panels represent significant comparisons of post-injury values relative to pre-injury values using a non-parametric Dunnett's test (p<0.05).
Appendix Figure A4.2: Comparison of clustering coefficient across injury group and day for all frequency bands. Each open circle represents a single 1s network and each color indicates an animal. The thick black horizontal bars are mean values over all animals, while the thin black horizontal lines at the top of panels represent significant comparisons of post-injury values relative to pre-injury values using a non-parametric Dunnett’s test (p<0.05).
Appendix Figure A4.3: Comparison of global efficiency across injury group and day for all frequency bands. Each open circle represents a single 1s network and each color indicates an animal. The thick black horizontal bars are mean values over all animals, while the thin black horizontal lines at the top of panels represent significant comparisons of post-injury values relative to pre-injury values using a non-parametric Dunnett’s test (p<0.05).
Appendix Figure A4.4: Comparison of characteristic path length across injury group and day for all frequency bands. Each open circle represents a single 1s network and each color indicates an animal. The thick black horizontal bars are mean values over all animals, while the thin black horizontal lines at the top of panels represent significant comparisons of post-injury values relative to pre-injury values using a non-parametric Dunnett's test (p<0.05).
Appendix Figure A4.5: Comparison of modularity across injury group and day for all frequency bands. Each open circle represents a single 1s network and each color indicates an animal. The thick black horizontal bars are mean values over all animals, while the thin black horizontal lines at the top of panels represent significant comparisons of post-injury values relative to pre-injury values using a non-parametric Dunnett’s test (p<0.05).
Appendix 4.2: Core Network Topology from individual animals for all analyzed frequencies
Appendix Figure A4.6: Core alpha band networks to show the most consistent edges (95\textsuperscript{th} percentile of frequency over the sum of 88 one-second networks)
Appendix Figure A4.7: Core beta band networks to show the most consistent edges (95\textsuperscript{th} percentile of frequency over the sum of 88 one-second networks)
Appendix Figure A4.8: Core Broad band networks with the most consistent and strongest 5% of edges (found by summing over 88 one-second networks).
Appendix Figure A4.9: Core delta band networks with the most consistent and strongest 5% of edges (found by summing over 88 one-second networks).
Appendix Figure A4.10: Core gamma band networks with the most consistent and strongest 5% of edges (found by summing over 88 one-second networks).
Appendix Figure A4.11: Core sigma band networks with the most consistent and strongest 5% of edges (found by summing over 88 one-second networks).
Appendix Figure A4.12: Core theta band networks with the most consistent and strongest 5% of edges (found by summing over 88 one-second networks).
### Appendix Figure A4.13: Raw mean and standard deviation Global Efficiency for all 7 frequency bands and time points in the sham and injured groups.

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<th>Injury Group</th>
<th>Frequency</th>
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<th>PRE.sd</th>
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<th>POST1d.sd</th>
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### Appendix Figure A4.14: Raw mean and standard deviation Nodal Strength for all 7 frequency bands and time points in the sham and injured groups.

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Appendix Figure A4.15: Raw mean and standard deviation Clustering Coefficient for all 7 frequency bands and time points in the sham and injured groups.

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<th>POST4d.sd</th>
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Appendix Figure A4.16: Raw mean and standard deviation Characteristic Path Length for all 7 frequency bands and time points in the sham and injured groups.

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### Appendix Figure A4.17: Raw mean and standard deviation Modularity for all 7 frequency bands and time points in the sham and injured groups.

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