cells. Here we show that GSK3$\beta$ inhibitors also promote barrier tightness by affecting tight junction protein stability. Results using primary human brain microvascular endothelial cells and a novel vector based assay for the evaluation of occludin and claudin-5 protein stability showed a significant increase in the half-life of these proteins when GSK3$\beta$ was inactivated. Of the inhibitors tested, GSK3$\beta$ inhibitor SB216763 (5$\mu$M) and LiCl (5 mM) showed the best efficacy by preventing occludin and claudin-5 degradation by approximately 32% and 43% respectively. The effect on the tight junction proteins was further validated using transendothelial electrical resistance (TEER). Inhibition of GSK3$\beta$ produced a gradual and sustained increase in TEER (as high as 22% over baseline). This phenomenon was attributed to the effect on turn-over and not as result of transcriptional regulation since mRNA levels of occludin, claudin-5 were unchanged. These analyses led us to test whether GSK3$\beta$ inhibitors identified to stabilized tight junction proteins (such as SB216763) would offer protection of the BBB in our experimental CCI-TBI model. The results of BBB permeability by fibrinogen leakage, showed an approximately 70% decrease in barrier permeability at 24hrs when the selected inhibitor was present following moderate CCI-TBI.

**Conclusions**

We have developed a cellular assay using primary human brain endothelial cells to test the effect of experimental compounds on tight junction protein stability. Vector constructs which are introduced to the endothelial cell, are bicistronic for either claudin-5 or occludin fused to AcGFP that also contains an internal ribosomal entry site for transcription of the reference gene mCherry. The vectors also feature the Tet-off system in which doxycycline halts transactivation and allows the ratio of the fused tight junction protein and mCherry proteins to provide a measure for protein half-life. Using this assay in conjunction with CCI-TBI, we have identified specific GSK3$\beta$ inhibitors that are effective in aiding barrier integrity via reduction of claudin-5 and occludin protein turn-over.

**Acknowledgments**

Temple University development grant (to SHR)

**Keywords**

BBB, neuroinflammation, cerebrovascular, permeability, GSK3$\beta$

**C62**

## SCALING AXONAL INJURY AND UNCONSCIOUSNESS THRESHOLDS FROM INFANT TO TODDLER TO PRE-ADOLESCENT CHILDREN

Matthew R. Maltese, PhD, *Department of Anesthesiology and Critical Care Medicine, The Children's Hospital of Philadelphia*
Sarah Sullivan, BS, MS, *University of Pennsylvania*
Colin Smith, BSc, MBChB, MD, FRCPath, *University of Edinburgh*
Susan Margulies, BSE, MSE, PhD, *University of Pennsylvania*

**Introduction**

Currently, pediatric TBI rotational velocity and acceleration tolerances are scaled from the adult using age-dependent brain mass and modulus values. Recent data from infant, toddler and pre-adolescent piglets can be used to determine if mass and modulus are sufficient to determine load tolerances in the immature brain.

**Methods**

Diffuse TBI was produced via rapid axial head rotation in female pre-adolescent pigs (N = 10, 2 months, 19 to 26 kg), toddler piglets (N = 18, 4 weeks, 7–10 kg), and infant piglets (N = 20, 3–5 days, 2–4 kg). Uninjured shams (N = 4–5) were studied in each age group. All subjects were anesthetized with isoflurane (1–4%), and vitals were recorded. Prior to TBI, isoflurane anesthesia was discontinued, until pinch reflex was detected post-TBI, then anesthesia was resumed until sacrifice 6 hours post-TBI. Brains were perfusion-fixed, sectioned coronally, and 6 $\mu$m slices stained with $\beta$-APP to assess brain volume fraction experiencing axonal injury. Linear regression was used to evaluate the correlation between fractional axonal injury volume and either rotational velocity or acceleration. All protocols were approved by the IACUC of the University of Pennsylvania.

**Results**

Regardless of age, axonal injury volume increased linearly with velocity and acceleration. Rotational velocity and acceleration of the infant and toddler piglets were scaled by brain mass and modulus to the pre-adolescent. Scaling loads revealed that brain mass and stiffness alone did not account for the enhanced axonal injury volume in the infant. Specifically, to produce the same axonal injury volume in the infant as the older piglets, median scaled velocity in the infant would have to be 1.4x to 1.9x lower than scaling by mass and modulus alone would predict, and median scaled angular acceleration 2.3x to 4.0x lower. Furthermore, return of the pinch reflex after TBI was 7.5x longer than shams in the infant piglets ($p = 0.0075$), but was indistinguishable from age-matched anesthetized shams in the toddler ($p = 0.1457$) and pre-adolescent groups ($p = 0.2288$), indicating more severe neurologic status of the infant group immediately following injury. Thus, both injury outcome metrics indicate an enhanced vulnerability in the infant brain, despite its smaller brain and stiffer modulus. When comparing only toddler and pre-adolescent age groups to each other (wherein brain modulus is similar), scaling velocity and acceleration by brain mass was sufficient to represent the age-dependent differences in axonal injury volume.

**Conclusions**

Mass and tissue modulus scaling laws are accurate between the toddler and pre-adolescent, but not between the either toddler or pre-adolescent and the infant. We find that the infant is 1.4x to 4.0x more vulnerable to axonal injury assessed at 6 hours, and this range is dependent upon whether acceleration- or velocity–based scaling is used. Also, we also find prolonged unconsciousness immediately following injury in the infant, that is not present in the toddler or pre-adolescent.

**Acknowledgments**

This research was supported by the US DOT NHTSA award DTNH22-07-00088, and the NIH award R01 NS039679.

**Keywords**

Traumatic Brain Injury, Pediatric, Biomechanics

**C63**

## COMPUTATIONAL ANALYSIS OF INJURED TISSUE FOLLOWING REPETITIVE MILD TRAUMATIC BRAIN INJURY

Virginia Donovan, B.S., *Cell, Molecular and Developmental Biology Program, University of California, Riverside*
Anthony Bianchi, B.S., *Center for Research in Intelligent Systems, University of California, Riverside*
Richard Hartman, Ph.D., *Department of Psychology, Loma Linda University*
Bir Bhanu, Ph.D., *Center for Research in Intelligent Systems, University of California, Riverside*

Monica J. Carson, Ph.D., *Center for Glial Neuronal Interactions, University of California, Riverside*
Andre Obenaus, Ph.D., *Department of Pediatrics, Loma Linda University*

**Introduction**
Mild traumatic brain injury (mTBI) has become an increasing public health concern as subsequent injuries can exacerbate existing neuropathology and result in neurological deficits. Experimental models of repetitive mTBI have explored injuries induced to the same location; however our study focuses on tissue level alterations following two contralateral mTBIs.

**Methods**
Controlled cortical impact (CCI; 0.5 mm deformation for 250 ms) was used to induce mTBI in adult male rats. An initial mTBI was induced to the right cortex of all animals, followed by a second injury delivered to rmTBI animals 3 (rmTBI 3d) or 7 (rmTBI 7d) days later. Animals underwent T2 weighted magnetic resonance imaging (MRI) 1, 4, 8, 14, 21, and 60 days after the initial injury. MR images were analyzed using a novel automated computational method that analyzed lesion composition (i.e. blood and edema) and volume. Animals were then perfused and histology for blood (Prussian Blue) and immunohistochemistry for neuroinflammation (IBA1: microglia; GFAP: astrocytes) were undertaken. Computational MR data were then compared to histological findings.

**Results**
Lesion volume was dramatically increased in rmTBI 7d animals compared to those that received a single or rmTBI 3d apart. Analysis of the first and second mTBI lesion volume revealed an increase in lesion volume at the site of the second injury within rmTBI 7d animals, in contrast to the rmTBI 3d group which exhibited a similar lesion volume to that of single mTBI controls. However, lesion volume increases in the rmTBI 7d animals was transient, as lesion volumes were similar to that of single animals by 14d post first injury. We then computationally evaluated the composition of the lesion for pixels containing blood, edema and normal tissue values based on quantitative T2 mapping. Comparison of lesion composition at 1d post last injury revealed increased edema within rmTBI 3d animals, while increased blood volume was observed in the rmTBI 7d group at the site of the second injury. By 14d post first injury, the rmTBI 3d animals exhibited similar lesion composition to those in the single group, while the rmTBI 7d animals continued to demonstrate increased blood deposition. Prussian Blue staining revealed increased extravascular blood at the injury site in rmTBI 7d animals compared to rmTBI 3d animals, consistent with our computational analysis. Immunolabeling for neuroinflammation revealed increased microglial activation and astrocyte reactivity within the rmTBI groups at the injury sites, primarily at the site of the second mTBI.

**Conclusions**
Repetitive mTBI is thought to result in cumulative injury leading to neuropathological and neurological abnormalities. We report that there appears to be a window of tissue vulnerability where a second mTBI 7d (but not 3d apart) after an initial injury results in increased tissue abnormalities (blood). The presence of an activated inflammatory response following rmTBI may play a role in defining this time window. Our findings are consistent with the concept of hemorrhagic transformation that has been reported in the clinical literature. Future work elucidating the underlying mechanisms responsible for the exacerbated injury and defining the temporal window in which injury can be worsened by subsequent injuries are needed. More importantly, use of automated computational analysis of MR images demonstrates the ability to extract tissue relevant quantitative information that may improve not only diagnosis but also allow for assessment of future pharmacological treatments.

**Acknowledgments**
Funding provided by DCMRP # DR080470 (AO) and NSF IGERT: Video Bioinformatics Grant DGE 0903667 (VMD).

**Keywords**
edema, blood, T2

**C64**

## EFFECTS OF GLUCOSE ON OXIDATIVE STRESS AFTER CORTICAL CONTUSION INJURY IN RATS

Katsunori Shijo, MD, PhD, *UCLA*
Sima Ghavim, BS, *UCLA*
Nobuhiro Moro, MD, PhD, *Nihon University*
David A. Hovda, PhD, *UCLA*
Neil G. Harris, PhD, *UCLA*
Richard L. Sutton, PhD, *UCLA*

**Introduction**
High glucose (Glc) levels after traumatic brain injury (TBI) are often thought to be detrimental, but we have previously found acute Glc treatments to be neuroprotective 24 h post-TBI. The current study was conducted to assess effects of acute Glc treatments on protein markers of oxidative stress.

**Methods**
Adult male Sprague-Dawley rats received Sham injury (n = 12) or left cortical contusion injury (CCI; n = 12) and injection of 50% Glc (2g/kg) or no treatment (NoTx) at 0, 1, 3 and 6 h post-surgery. At 24 h post-surgery rats were euthanized and brain tissue from left contused cortex, left peri-contusion cortex and left hippocampus was collected and stored at −80°C until homogenized. Total protein in samples was determined using RC-DC kits. Proteins analyzed using Western blots included glyceraldehyde-3-phosphate dehydrogenase (GAPDH), nitrotyrosine (NT) as an index of production of reactive nitrogen species (nitration of tyrosine residues), and 4-hydroxynonenal (4-HNE) as an index of polyunsaturated fatty acids exposure to peroxides and reactive oxygen species. Protein bands on enhanced chemiluminescent gel images (Fluoromax system, BioRad) were quantified by integrated optical density measurement using Quantity 1 software.

**Results**
GAPDH, NT and 4-HNE protein levels did not differ significantly between Sham-NoTx or Sham-Glc groups, so the levels in each brain region for each CCI group were expressed as a percent of the similarly treated Sham group. In all regions ipsilateral to injury, there were no effects of CCI or Glc treatments on the levels of GAPDH. The NT protein levels were significantly increased in the left contused cortex ($p = 0.001$), peri-contusion cortex ($p = 0.001$) and hippocampus ($p = 0.011$) of the CCI-NoTx group compared to Sham-NoTx controls. In the CCI-Glc group the NT levels in left contused cortex and peri-contusion cortex appeared reduced compared to CCI-NoTx, but these NT levels were significantly increased in the contused cortex ($p = 0.010$) and peri-contusion cortex ($p = 0.041$) as well as in the left hippocampus ($p = 0.025$) compared to Sham-Glc controls. The left peri-contusion cortex was the only region where reductions in NT approached significance in the CCI-Glc group compared to CCI-NoTx controls ($p = 0.052$). 4-HNE protein levels in the left contused cortex ($p < 0.001$), peri-contusion cortex ($p = 0.001$) and hippocampus ($p = 0.002$) were significantly increased in the CCI-NoTx group