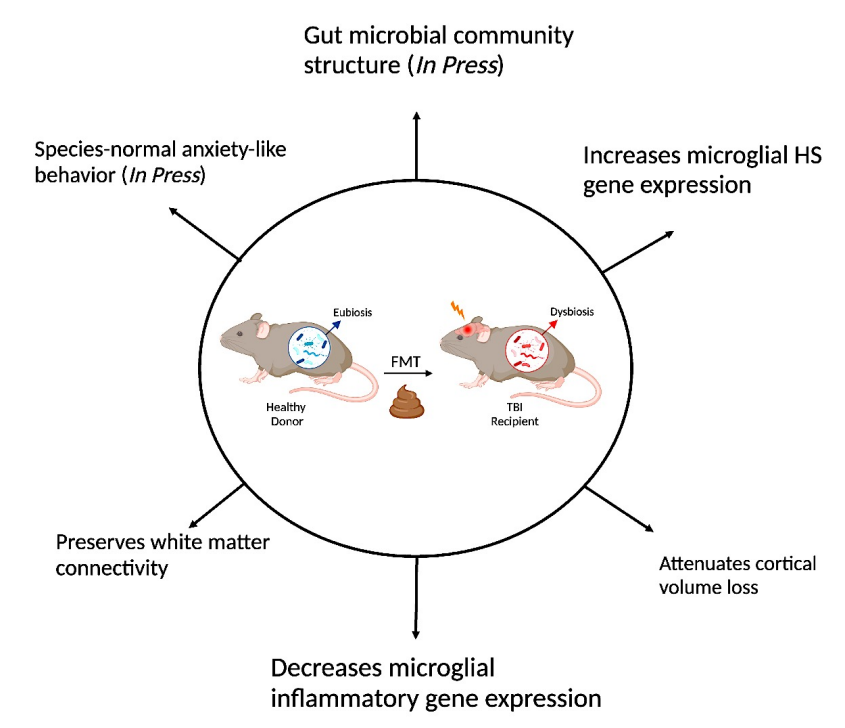


POST-INJURY FECAL MICROBIOTA TRANSPLANT DECREASES LESION SIZE AND NEUROINFLAMMATION AFTER TRAUMATIC BRAIN INJURY

Booker T Davis IV, PhD, MS, Zhangying Chen, MS, Mecca B.A.R. Islam, MS, Madeline Timken, Daniele Procissi PhD, and Steven J. Schwulst, MD

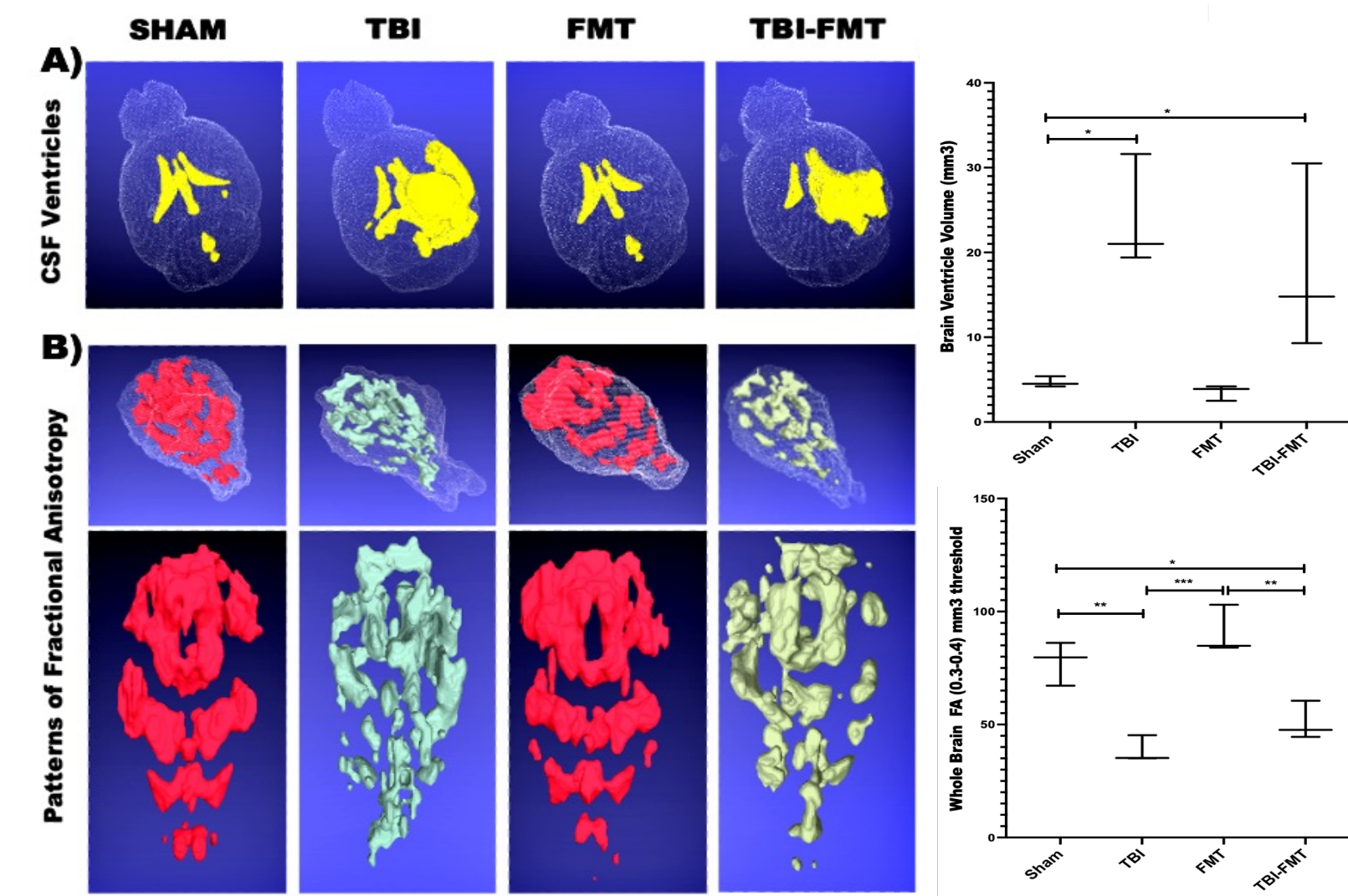
¹Division of Trauma and Critical Care ²Department of Surgery, Feinberg School of Medicine

Background



Traumatic brain injury (TBI) is an underrecognized public health threat afflicting nearly 3 million Americans every year. TBI is highlighted not only by its high mortality rate, but also by long-term neurocognitive morbidity suffered by its survivors. We have shown in recent work that the gut-brain axis is complicit in the onset and progression of TBI-associated neurocognitive decline. Targeting the gut-brain axis may represent a novel therapeutic approach for TBI-related morbidity.

Fecal Microbiome Transplantation Decreases Lesion In TBI



A) Representative MRI scans of animals (60DPI) with injury cerebrospinal fluid (CSF) ventricles are denoted in yellow revealing attenuated cortical volume loss with FMT treatment. (N=3) B) Representative longitudinal and transverse images reveal the pattern of whole-brain fractional anisotropy (i.e. preserved white matter connectivity with FMT in TBI) C) MRI data highlights an enlargement of ventricles with TBI which is attenuated by FMT. (p<0.002). (Two-way ANOVA) D) Whole brain mapping highlighted differential atrophy.

Results

- Post TBI treatment with fecal microbiota transfer attenuated cortical volume loss and preserved white matter connectivity as compared to treatment with vehicle alone.
- Longitudinal and transverse MRI scans of mice denote a reduction of TBI induced ventriculomegaly and cortical volume loss of TBI (p<0.002). FMT also attenuated FA (connectivity) loss in FMT treated mice after TBI as compared to mice treated with vehicle alone (p=0.04).
- FMT does not change the overall proportion of infiltrating cell vs microglia between TBI groups
- TBI increased 2 predominant phenotypes of microglia based on greatest differentially expressed genes 1. Disease-associated (DAM) 2. Inflammatory (INMG)
- FMT significantly downregulated inflammatory transcripts in microglia after severe TBI and upregulated Heat-shock (hspMG) producing microglia

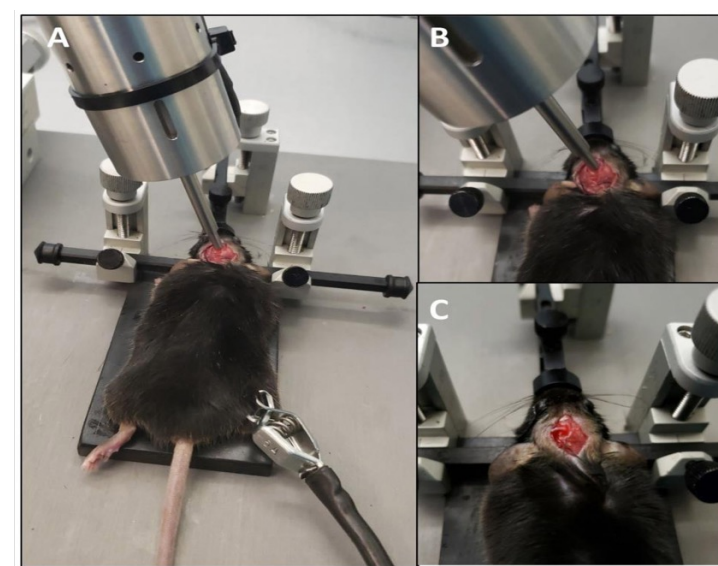
Research Objectives

To develop a basic understanding of the mechanisms within the Brain Gut Axis that impact TBI outcomes.

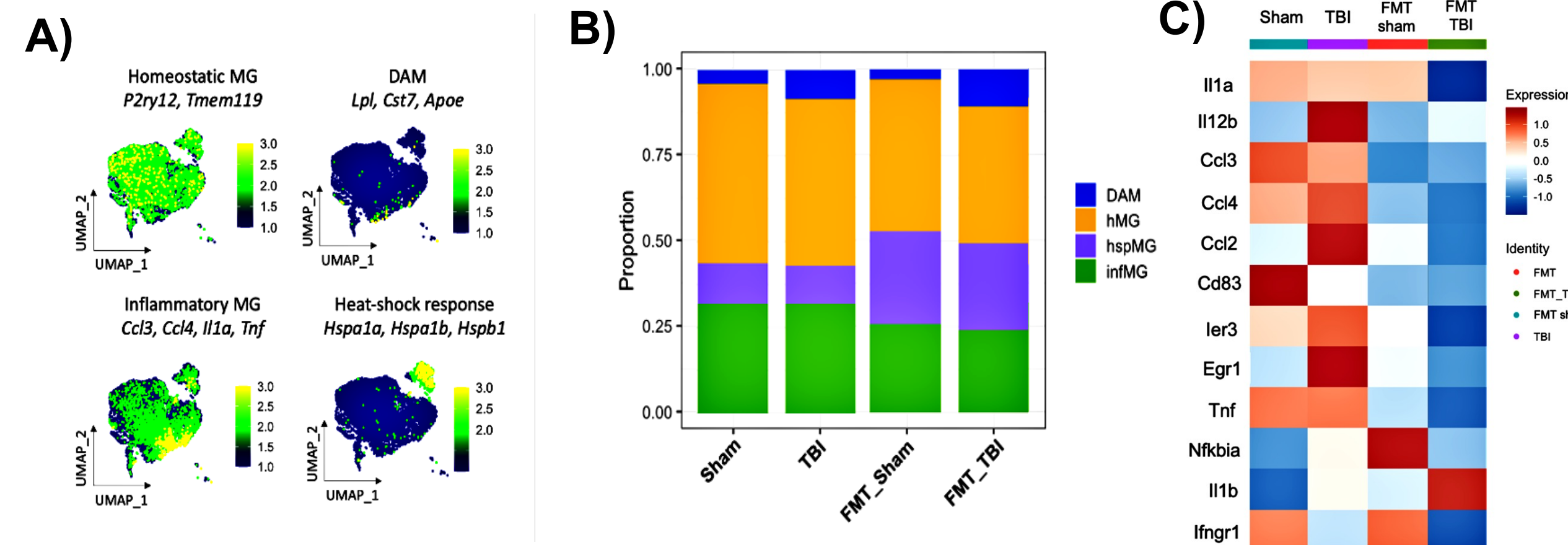
We hypothesized that fecal microbiota transplantation (FMT) would attenuate neuroinflammation and improve outcome after TBI.

Methods

Mice underwent FMT with an oral gavage of healthy mouse stool (1g:1ml sterile water) vs. vehicle alone immediately following injury and then once weekly for a total of 4 weeks post injury. At 60 days post mice underwent 3D contrast magnetic resonance imaging. 3D anatomical brain images were obtained using a gradient echo sequence (TR=56 msec, TE=2 msec) with isotropic resolution Images were assessed for ventriculomegaly and levels of fractional anisotropy (i.e. water diffusivity) which are surrogates for cortical volume and white matter connectivity respectively. At day 60, we extracted & sorted Cd45+/dim cells for Single Cell RNA Sequencing analysis. Statistical analyses were performed using Two-way ANOVA, followed by Tukey's post hoc test.



Microglial gene transcription decreased with FMT animals



(A) Microglia were grouped by gene expression according to function, revealing predominant microglia phenotypes within samples. The predominant phenotypes included microglia expressing 1. Homeostatic (HMG), 2. Disease-associated (DAM), 3. Inflammatory (INMG) and 4. Heat-shock (hspMG) related genes. B) Stacked bar charts demonstrating the proportion of DAM and hspMG microglia subclusters in injury and FMT. The microglia found within samples: FMT=2046, FMT_TBI=3435, Sham=2941, and TBI=3093. C) TBI results in a significant upregulation of inflammatory gene expression in microglia. Treatment with FMT markedly decreased inflammatory gene expression within the microglia of mice at baseline (sham-injury). This downregulation of inflammatory gene expression was further amplified after TBI.

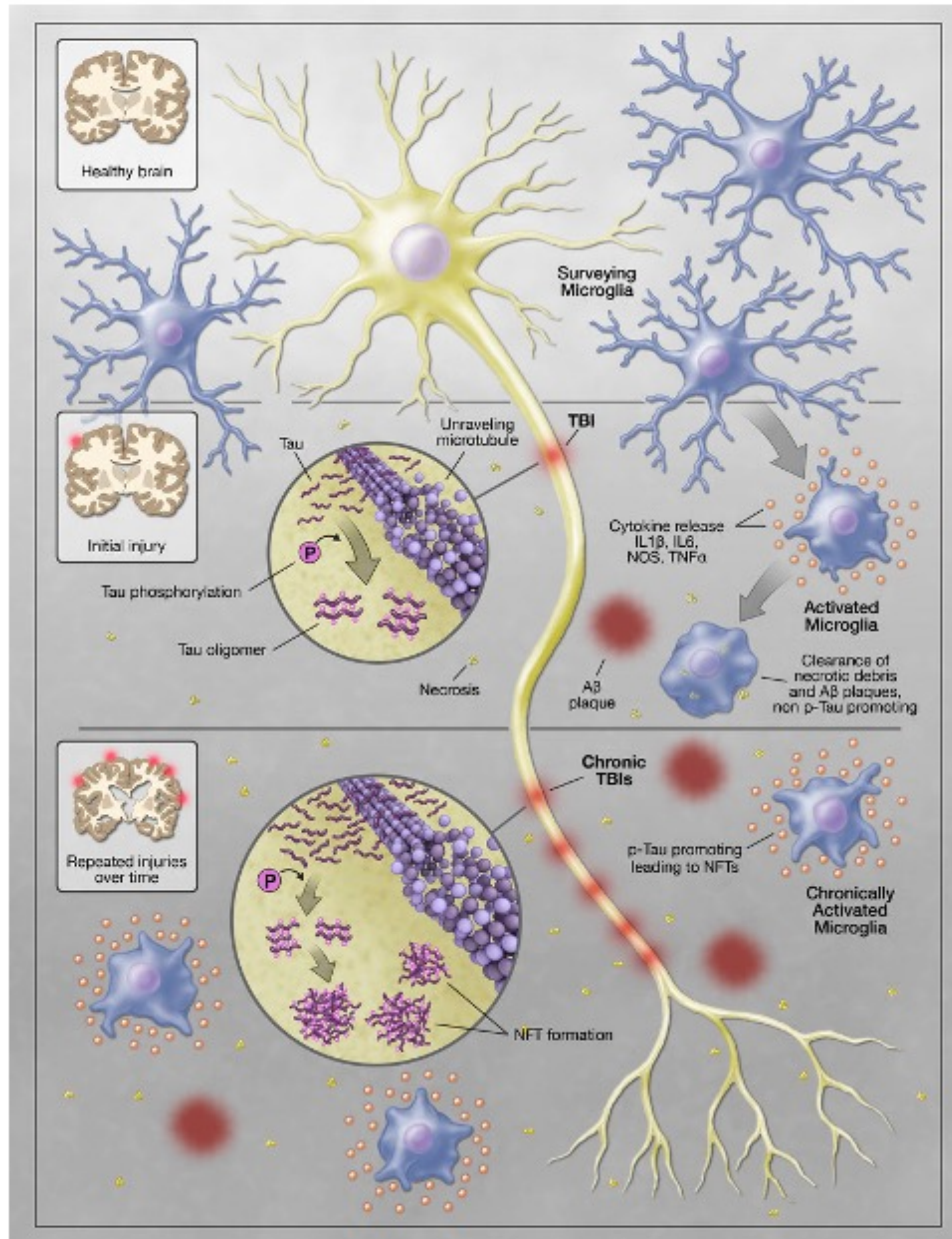
Limitations

- Limitations to CCI include the need for craniotomy and the expense of acquiring the impactor and actuating device.
- There is a dearth of consistent foundational literature highlighting TBI in females. As sex is a confounding factor in TBI, female mice are being assessed in a separate study.

Conclusions

- Fecal Microbiota Transplant attenuated cortical volume loss, improved white matter connectivity, attenuated microglial inflammatory gene expression, and increased heat shock protein gene expression within microglia after traumatic brain injury.
- These data highlight the potential of FMT as a novel therapeutic intervention for TBI. FMT is already an established, albeit non-traditional, therapy for other clinical disease entities; repurposing FMT for TBI treatment may represent a novel treatment paradigm for an injury process that is notorious for having few treatment options other than supportive care.

Background



Traumatic brain injury (TBI) has a bimodal age distribution with peaks occurring in the aged and in the young. Most data indicate worse outcomes in aged patients as compared to young patients. However, few studies specifically address age at the time of injury as an independent biologic variable.

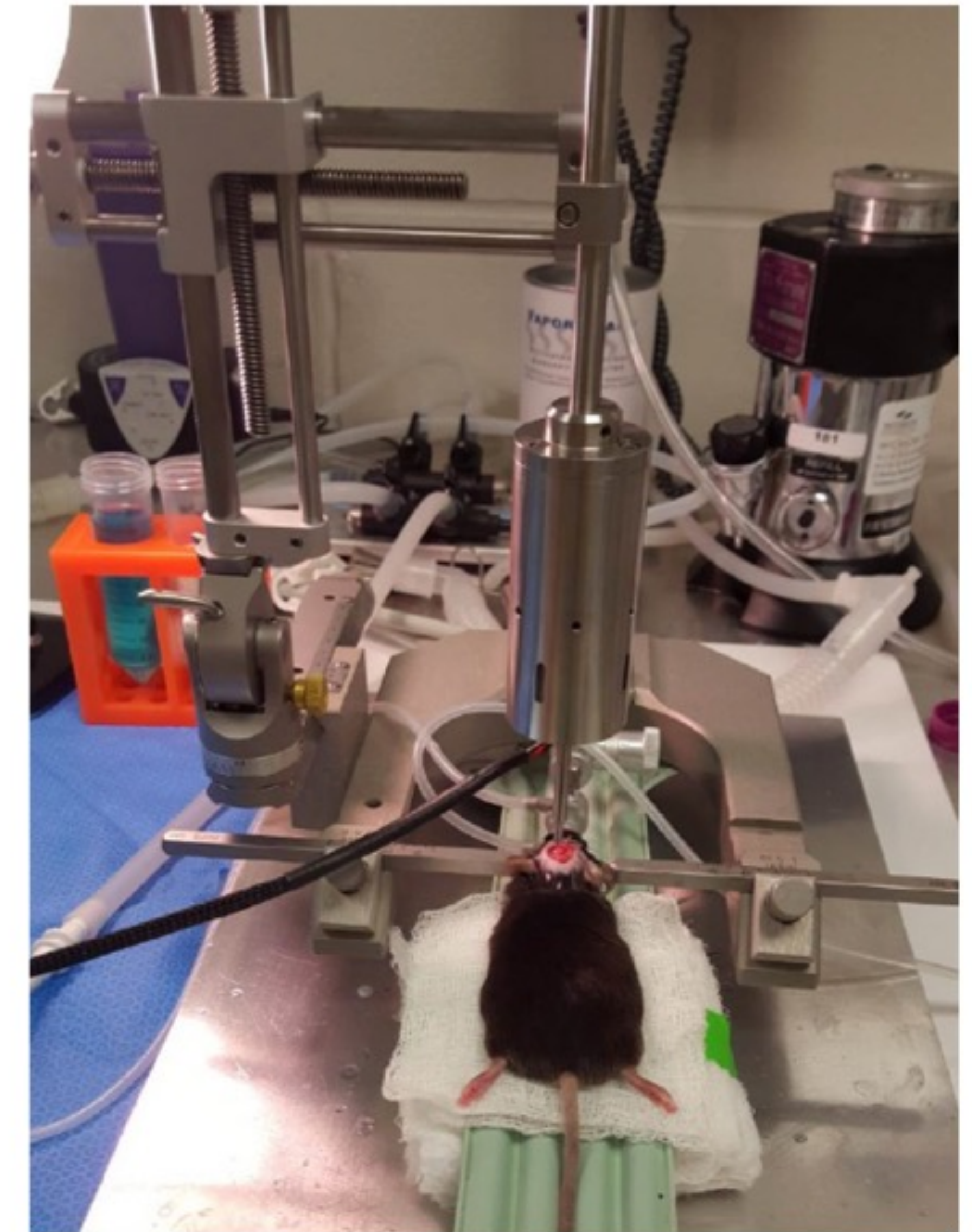
Research Objectives

To develop a basic understanding of the mechanisms that impact the differential TBI outcomes seen in different age groups.

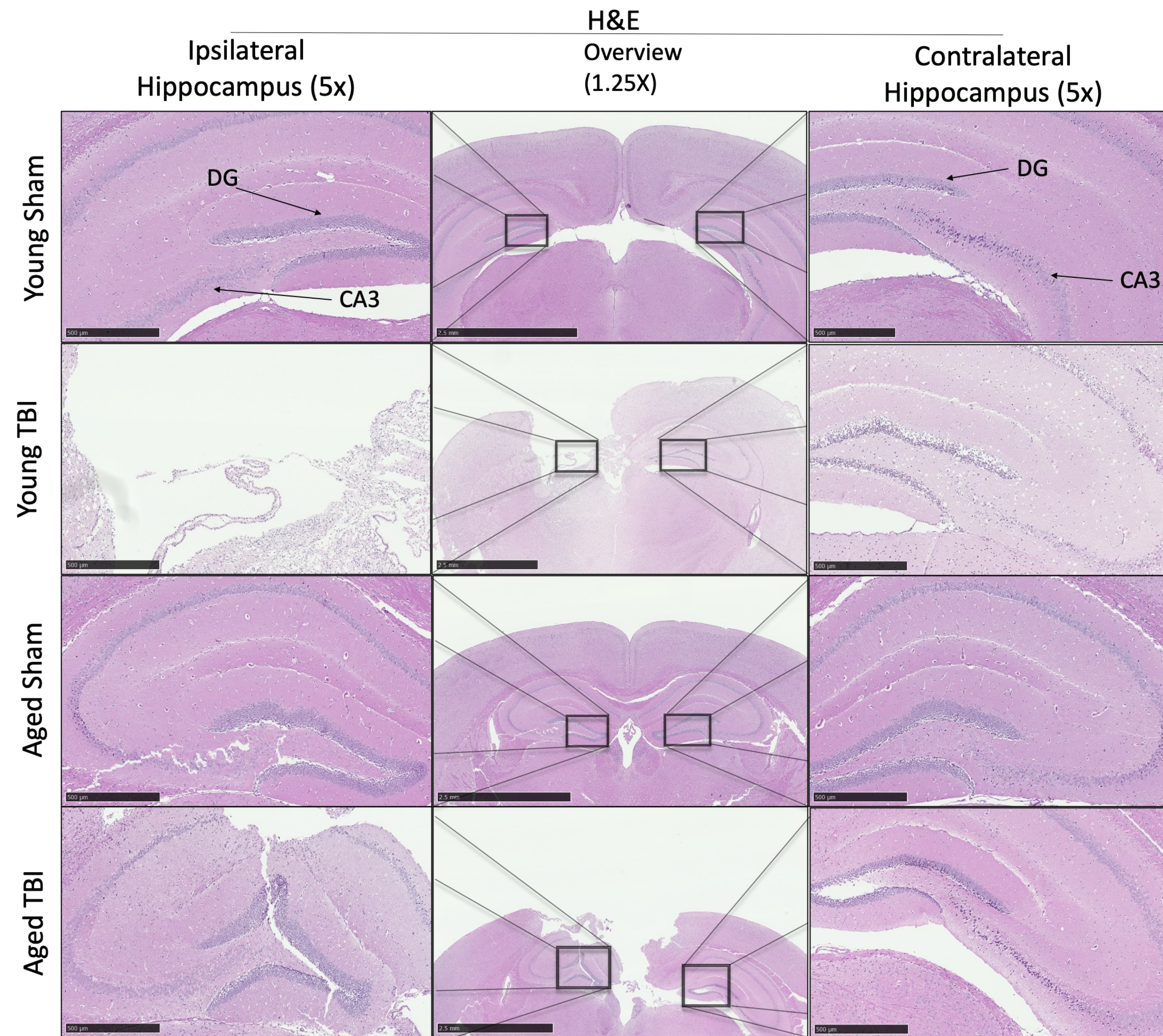
We hypothesized that aged mice would demonstrate a more severe histopathologic phenotype as compared to young mice after TBI

Methods

80-week-old (N=5) and 14-week-old (N=5) male C57Bl/6 mice were subjected to TBI via an open-head controlled cortical impact vs. sham-injury. Brains were harvested 30 days post-TBI and embedded in paraffin blocks. Sections were stained with H&E, NeuN, and GFAP and to assess for edema, neuronal degeneration, and gliosis, respectively. Sections were scored using the standardized NACC system by a neuropathologist blinded to the experimental group. Group analyses were performed using Two-way ANOVA, followed by Tukey's post hoc test.

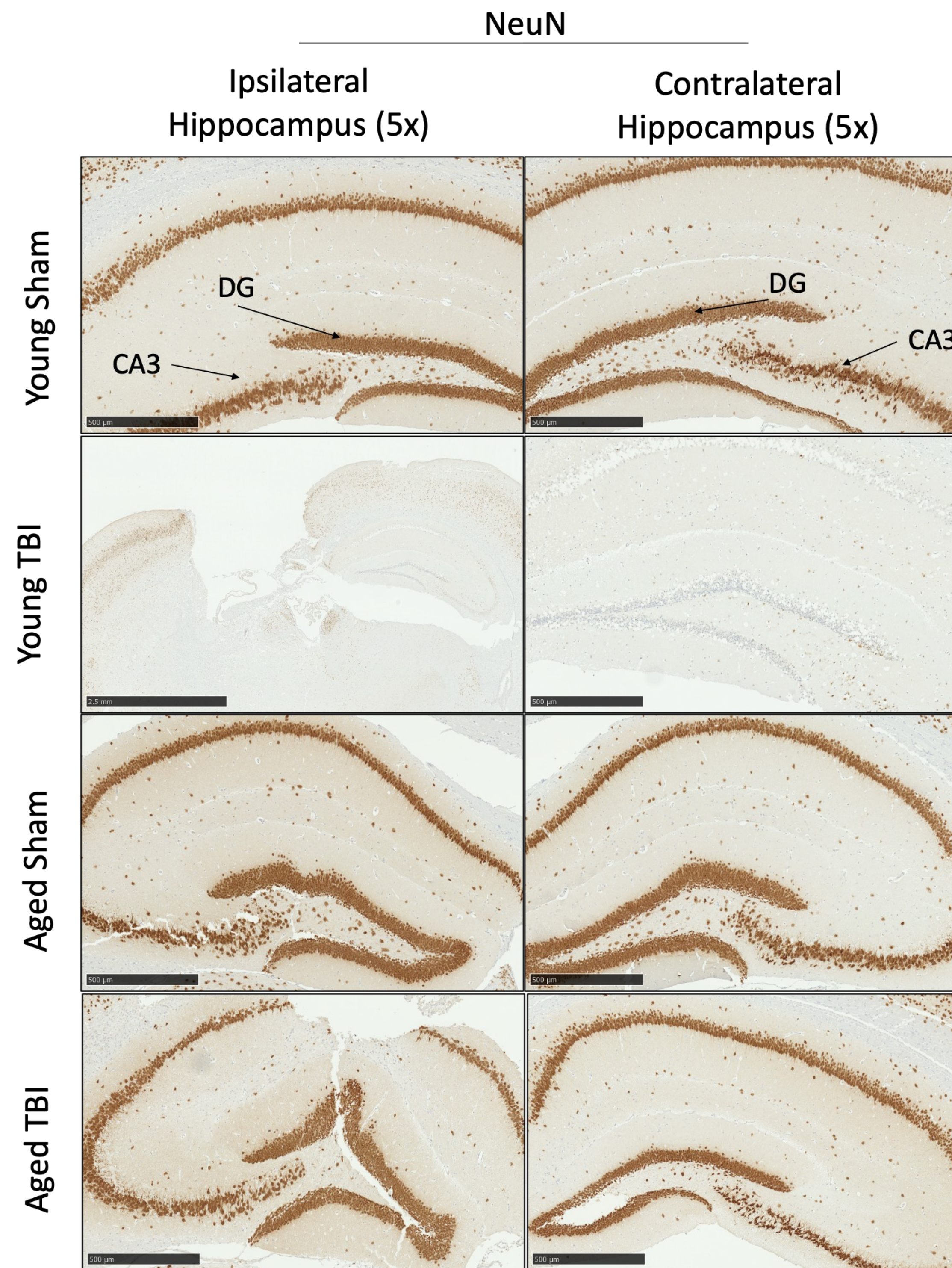


Controlled Cortical Injury induces TBI



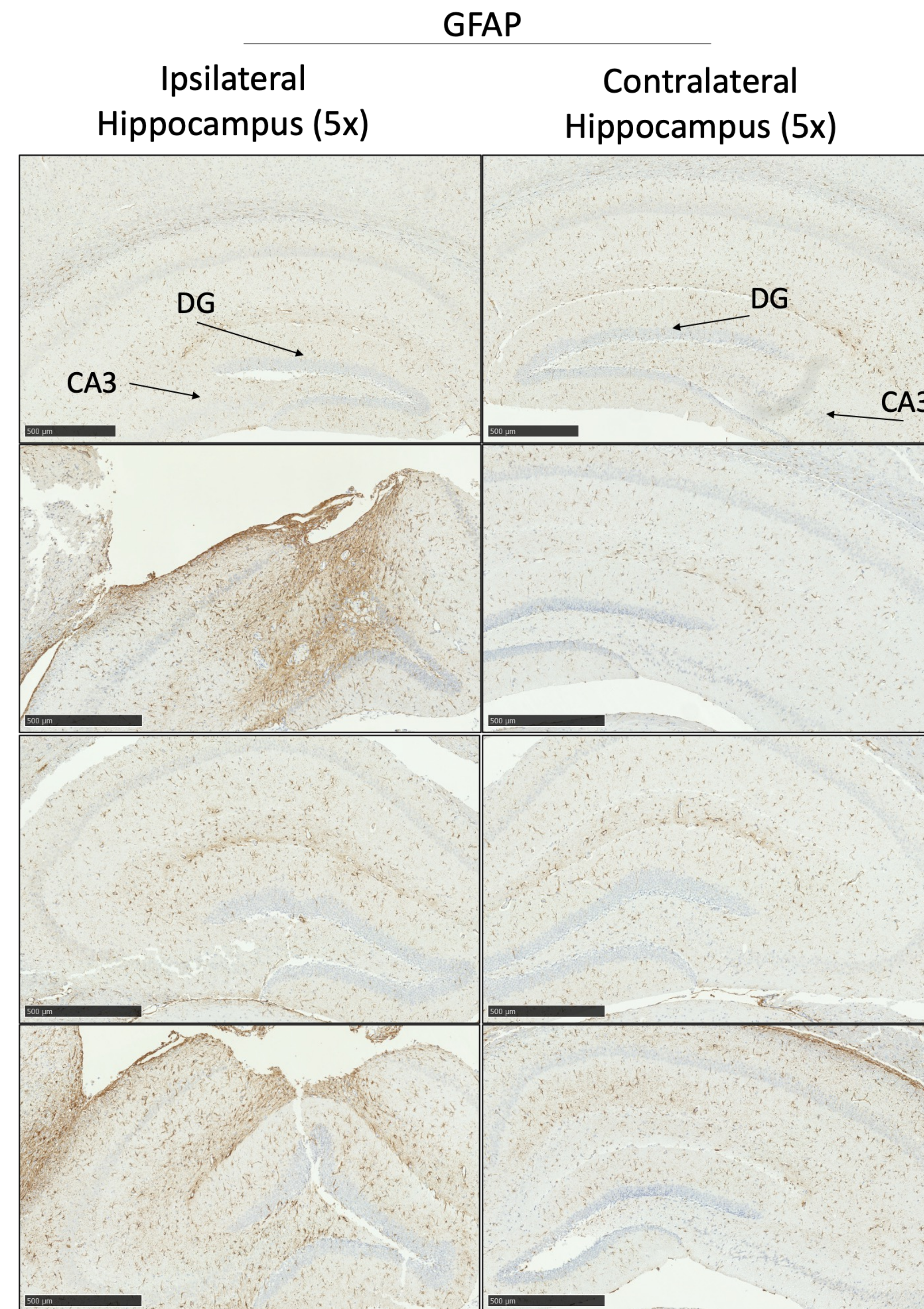
Controlled cortical injury results in severe traumatic brain injury in young adult and aged mouse brains. Coronal sections were stained using hematoxylin and eosin (H&E), neuronal nuclei antibodies (NeuN), as well as Glial fibrillary acidic protein (GFAP), and evaluated for damage by neurophysiologist.

Controlled Cortical Injury induces TBI



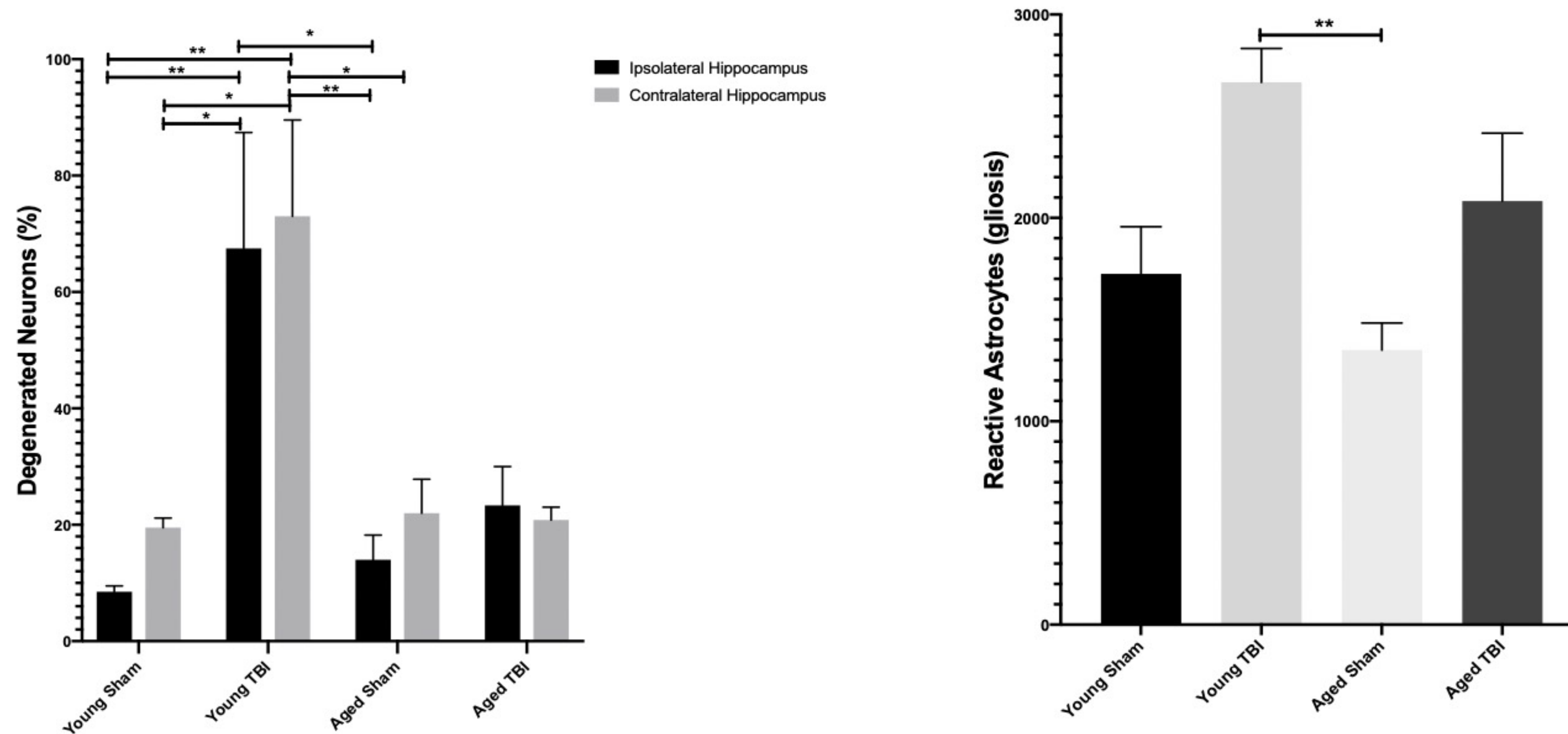
Controlled cortical injury results in severe traumatic brain injury in young adult and aged mouse brains. Coronal sections were stained using hematoxylin and eosin (H&E), neuronal nuclei antibodies (NeuN), as well as Glial fibrillary acidic protein (GFAP), and evaluated for damage by neurophysiologist.

Controlled Cortical Injury induces TBI



Controlled cortical injury results in severe traumatic brain injury in young adult and aged mouse brains. Coronal sections were stained using hematoxylin and eosin (H&E), neuronal nuclei antibodies (NeuN), as well as Glial fibrillary acidic protein (GFAP), and evaluated for damage by neurophysiologist.

Neuron loss and Gliosis Attenuated in aged TBI animals



(A) The average number of NeuN-positive cells in the dentate gyrus and CA3 regions per hemisphere were not different within the groups ($n = 3-5$); however TBI caused a significant decrease in the average number of NeuN-positive cells in young animals compared to young and aged sham ($p = 0.05, 0.001$). (B) GFAP was evaluated over whole coronal slice. Young mice demonstrated severe and extensive edema, neuron loss, and gliosis ($p = 0.01$) within the cortex, hippocampus, and subcortical grey matter where aged mice which demonstrated moderate and variable edema and neuronal loss. Scoring data in mice were analyzed by ANOVA (3-way, or 1-way) followed by multiple comparison tests to assess differences. P-values of 0.05 or less were considered statistically significant.

Results

- Aged mice demonstrated less cerebral edema and attenuated neuronal loss within the cortex and subcortical grey matter as compared to young mice. Histopathological injury was scored from 0-3, with “1” indicating mild and “3” representing severe injury.
- Astrocyte reactivity was elevated in young TBI animals. Hippocampal gliosis was severe in both TBI groups.
- Young mice demonstrated severe and extensive edema, neuron loss, and gliosis within the cortex, hippocampus, and subcortical grey matter (mean score of 3) as compared to aged mice which demonstrated moderate and variable edema and neuronal loss (mean score of 2; $p < 0.0001$).

Limitations

- Limitations to CCI include the need for craniotomy and the expense of acquiring the impactor and actuating device.

Conclusions

- Contrary to our hypothesis, aged mice demonstrated markedly less cerebral edema and neuronal loss compared to young mice.
- These data suggest different post-TBI pathophysiology in aged versus young brains. Different pathophysiology may imply that different treatment strategies are needed for aged vs. young TBI patients.

Special Thanks

Mecca B.A.R. Islam, MS,
Madeline Timken, BS,
Qinwen Mao, MD, PhD,
Karen Ho, MD, and
Steven J. Schwulst, MD

The committee: William H.
Pearce, MD Research Symposium

Funding:



National Institute of
General Medical Sciences

Thank You

Booker T Davis IV, PhD, MS

Booker.iv@northwestern.edu