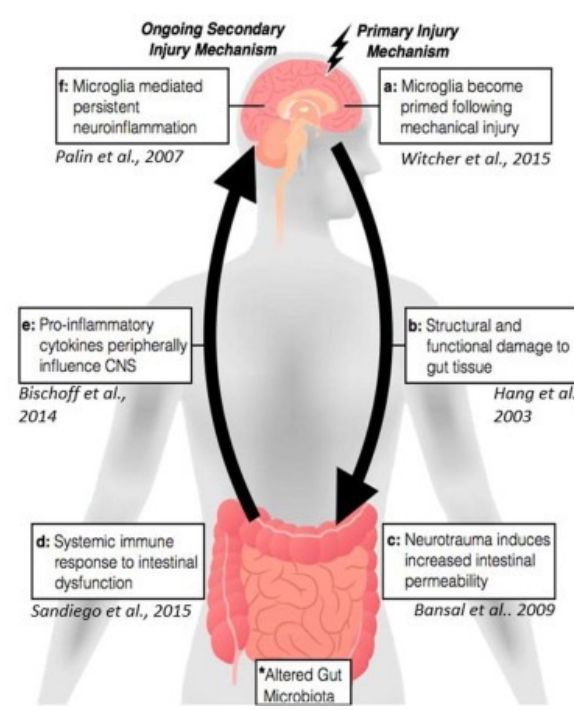


FECAL MICROBIOME TRANSFER REVERSES DYSBIOSIS AND FUNCTIONAL DEFICITS AFTER TRAUMATIC BRAIN INJURY IN MICE

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Background



Traumatic brain injury (TBI) is an underrecognized public health threat. Survivors of TBI often suffer long-term neurocognitive deficits leading to the progressive onset of chronic neurodegenerative disease. Recent data suggests that the gut-brain axis is complicit in this process. However, no study has specifically addressed whether fecal microbiota transplantation (FMT) can attenuate neurologic deficits after TBI.

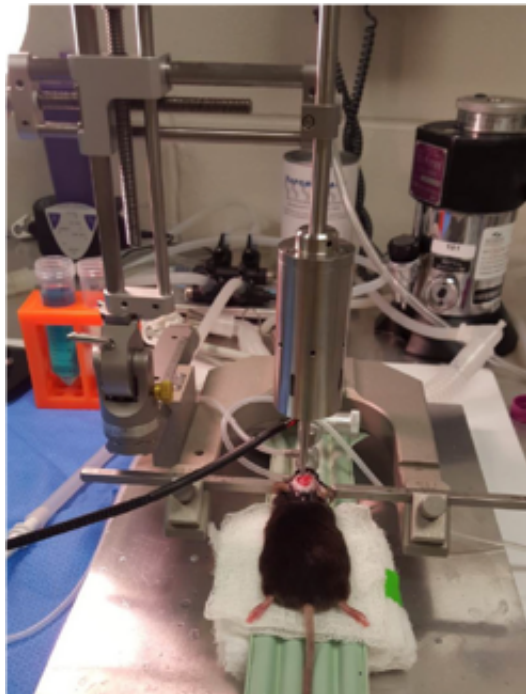
Research Objectives

To determine whether Fecal Microbiota Transplantation restores gut microbiota dysbiosis and relieves neurological deficits after Traumatic Brain Injury

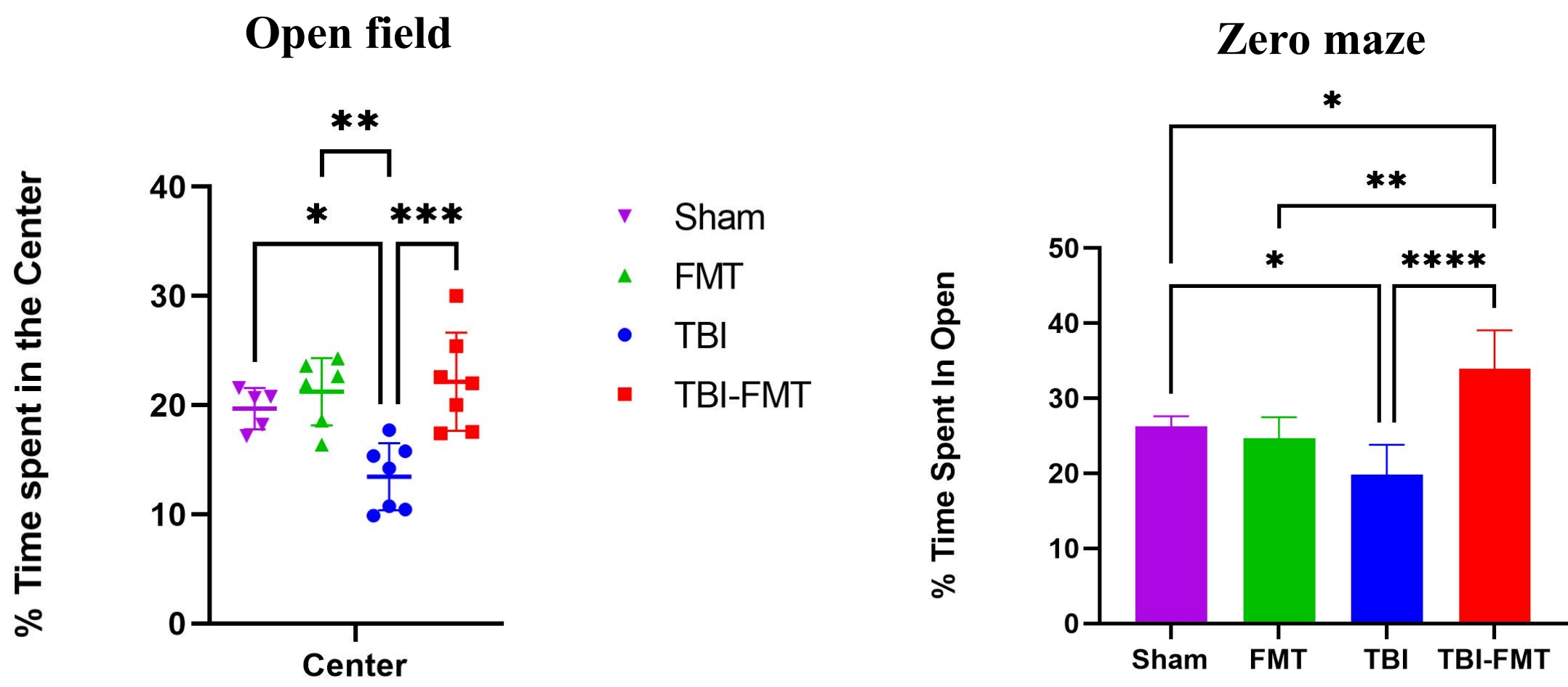
We hypothesized that FMT treated mice would demonstrate less severe fecal microbiome dysbiosis and attenuated neurocognitive deficits after TBI

Methods

14-week-old male C57Bl/6 mice were subjected to severe TBI (n=20) or sham-injury (n=20) via an open-head controlled cortical impact. Post-injury, mice underwent 4 weekly oral gavages with a slurry of healthy mouse stool or vehicle alone. Zero maze (ZM) and Open field testing (OF) were used to evaluate post-traumatic anxiety, exploratory behavior, and generalized activity at 45 day post-injury (DPI). 16S ribosomal RNA (rRNA) sequencing of fecal samples was performed to characterize the resultant gut microbiota at 60 days post-injury.

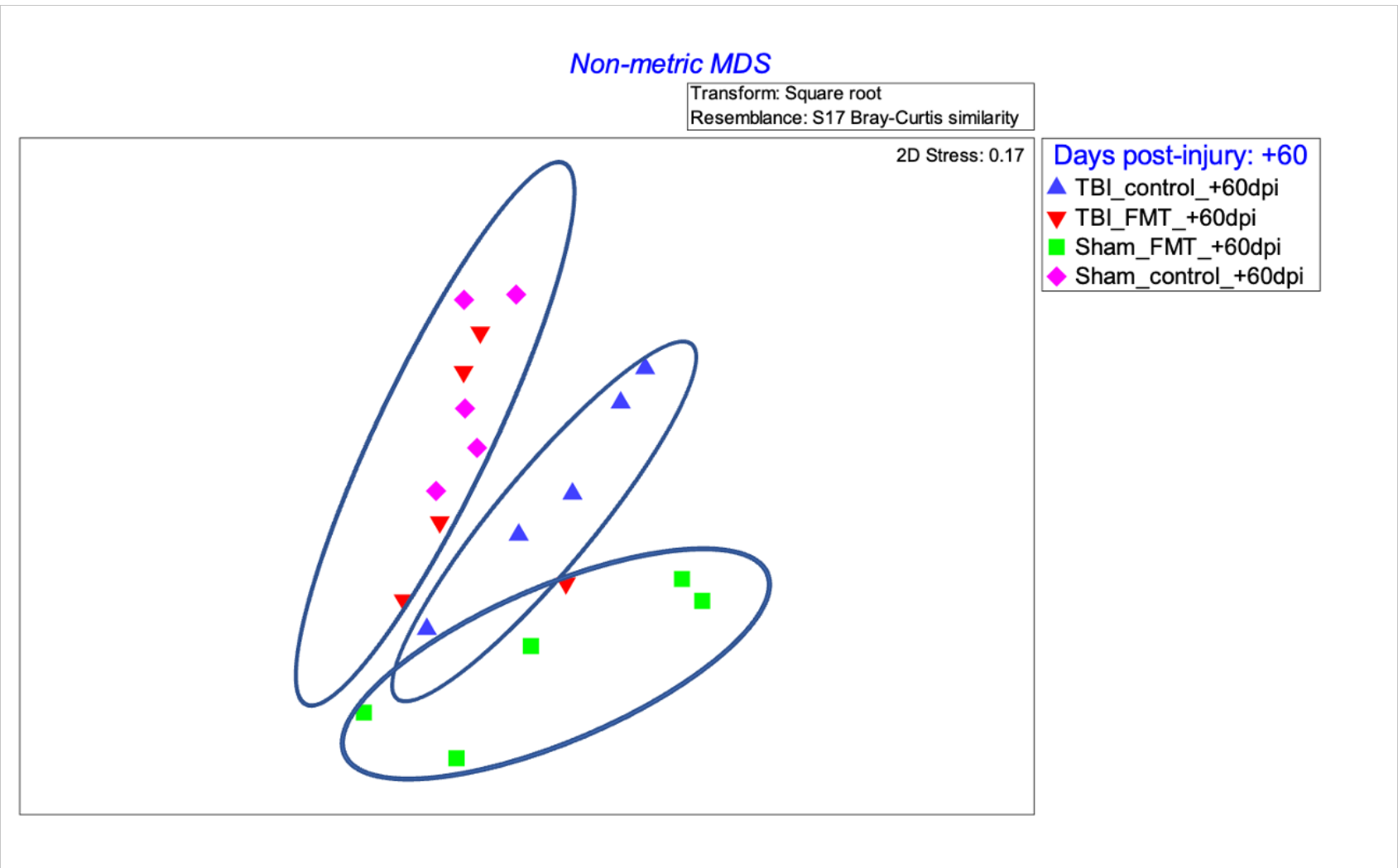


FMT Attenuates Neurocognitive Deficits in TBI



Behavior results in severe traumatic brain injury in young adult mouse brains. 45 days post TBI Wild type male mice were assessed for neurocognitive status using open field and zero maze behavioral testing. Open field (OF) and Zero Maze (ZM), revealed the rescue of

FMT Restores Microbiome Profile in TBI mice.



Mice were treated for 4 weeks with water or stool from healthy age-matched individuals. Analysis of similarity (ANOSIM) revealed a nonsignificant trend of dissimilarity between groups ($p = 0.1$). At day 60 we saw that TBI induced a noticeable shift in microbiome composition in between Sham-Control and TBI-Control mice (ANOSIM - 0.904). The FMT control microbial community was most dissimilar from baseline (ANOSIM - 0.988). FMT treatment maintained restored compositional similarity between Sham-Control and FMT-TBI animals (ANOSIM - 0.72).

Results

- Behavioral testing demonstrated a rescue of normal anxiety-like and exploratory behavior in TBI mice treated with FMT. These data suggest preservation of the connectivity between the hippocampus and prefrontal cortex in FMT treated TBI mice.
- FMT treated TBI mice spent a greater percentage of time (14.9 ± 1.1 , $p=0.004$) in the center regions of the Open Field as compared to untreated TBI mice ($12.93 \pm 2.2\%$).
- Untreated TBI animals also spent less time ($17.0 \pm 2.741\%$) in the open areas of ZM than treated TBI mice (27.3 ± 5.8 , $p=0.004$).
- Fecal microbiome analysis revealed a large variance between TBI and sham animals treated with vehicle, while FMT treated TBI mice had restoration of gut dysbiosis back to levels of control mice.

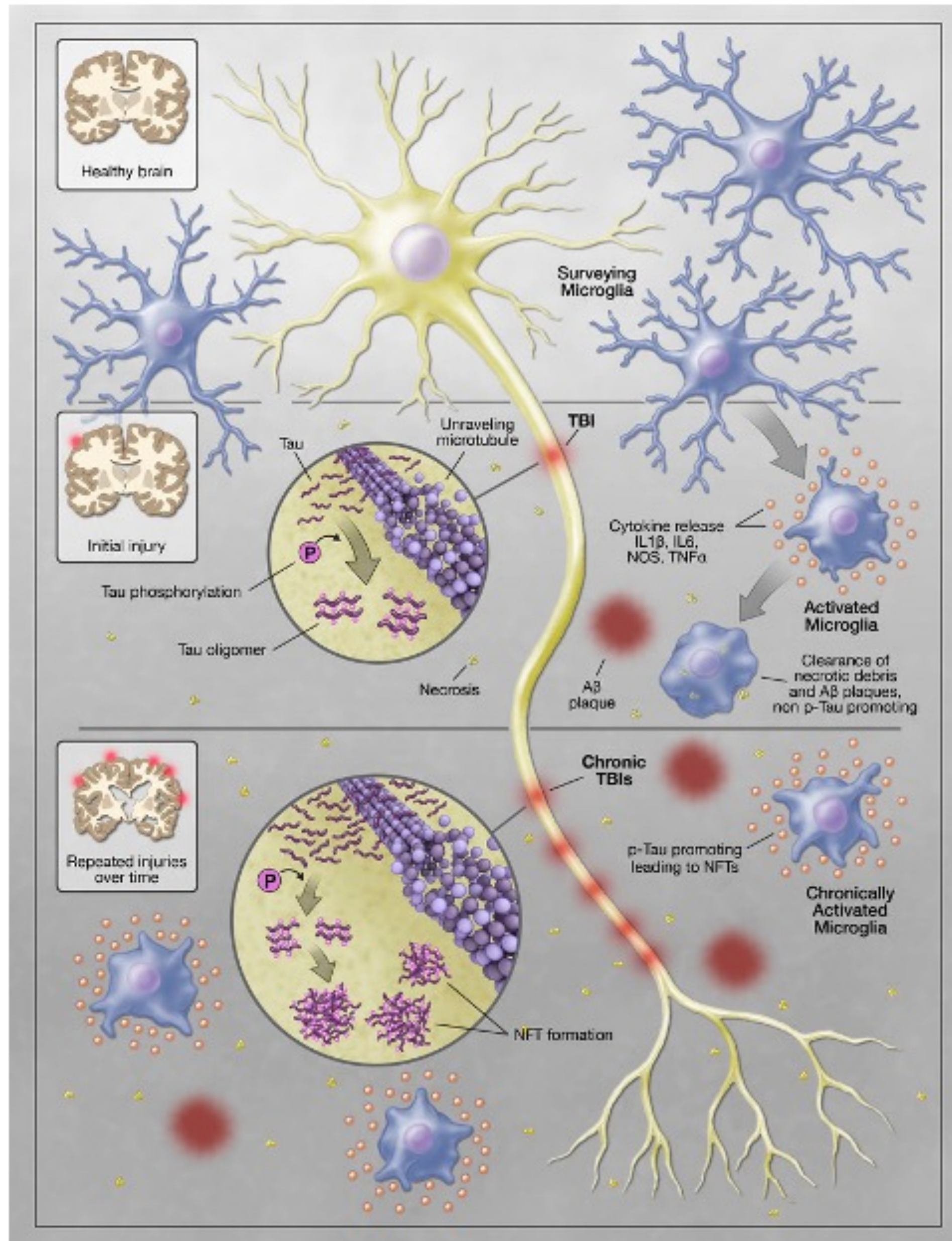
Limitations

- Limitations to CCI include the need for craniotomy and the expense of acquiring the impactor and actuating device.
- In-depth analysis of bacterial taxonomy is necessary to fully characterize species and phylum level changes within the fecal microbiome.

Conclusions

- Fecal microbiota transplant attenuated post-traumatic anxiety in TBI mice as compared to TBI mice treated with vehicle alone.
- This functional improvement correlated with correction of TBI-induced gut dysbiosis after TBI. These data suggest that restoring a pre-injury gut microbiota may be a promising therapeutic intervention after TBI.
- A more in-depth analysis of microbiome species and taxonomic variance necessary to identify mechanism of FMT effect on TBI related cognition

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.

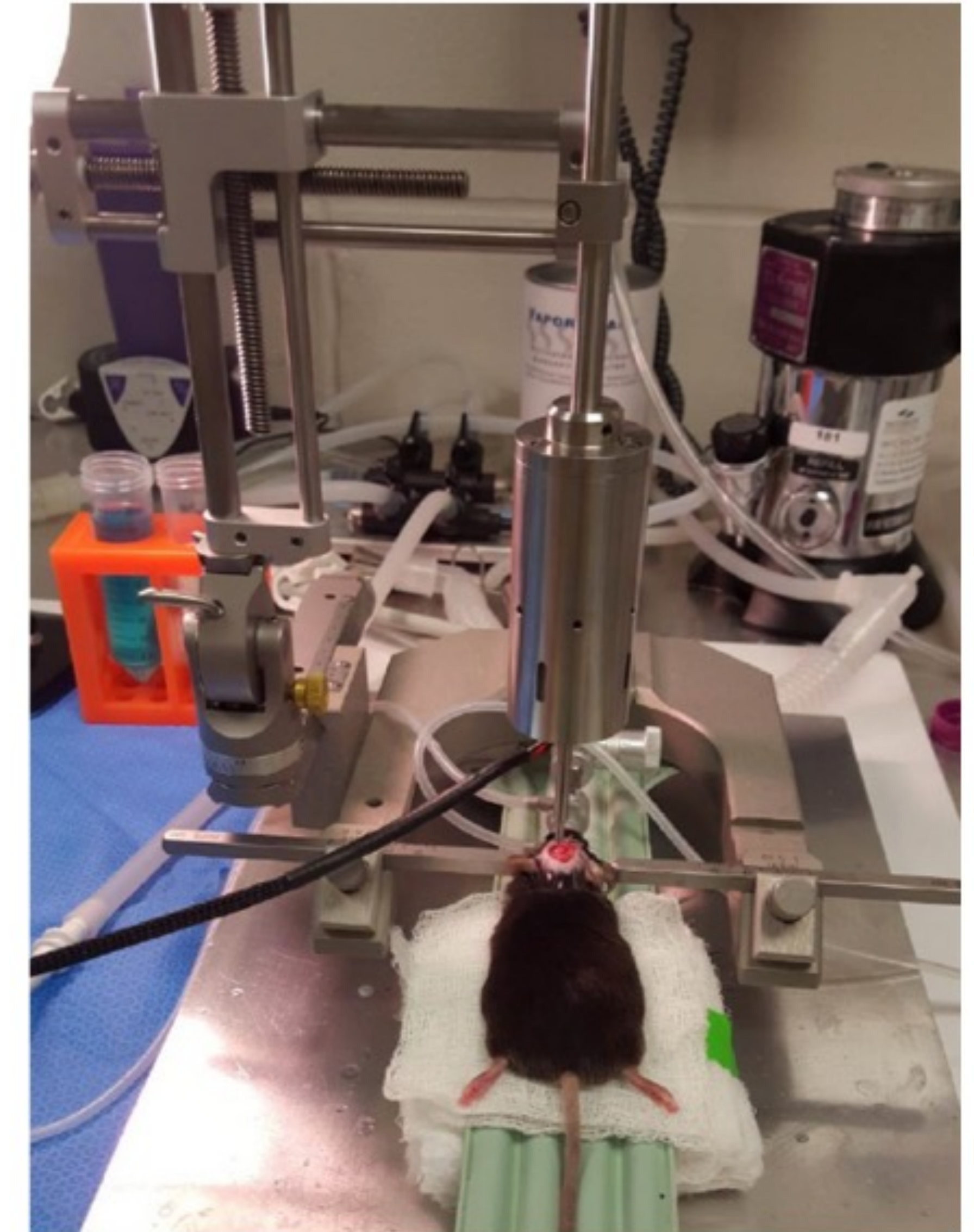
Research Objectives

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

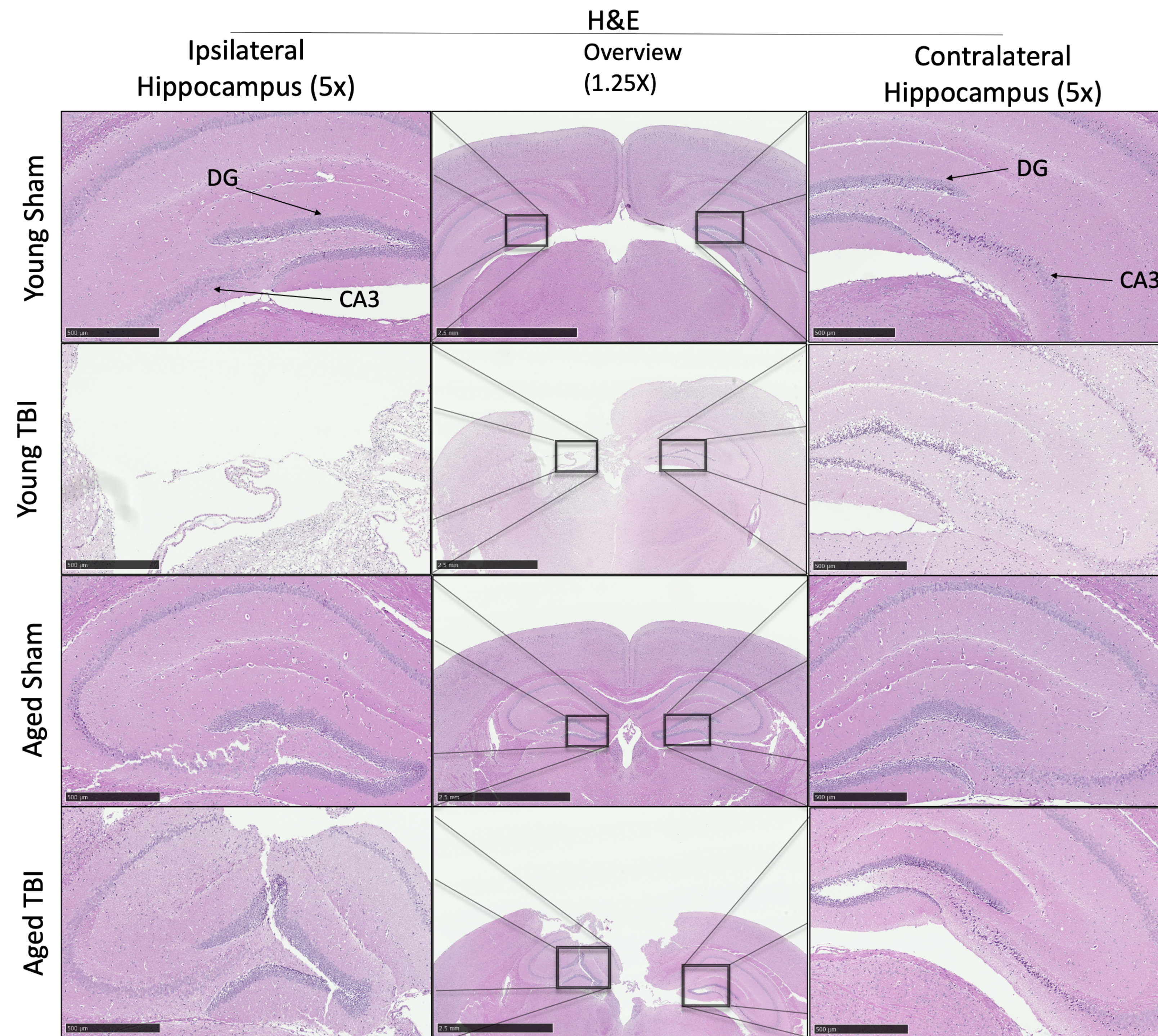
We hypothesized that aged mice would demonstrate a more severe histopathologic phenotype as compared to young mice after TBI

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.

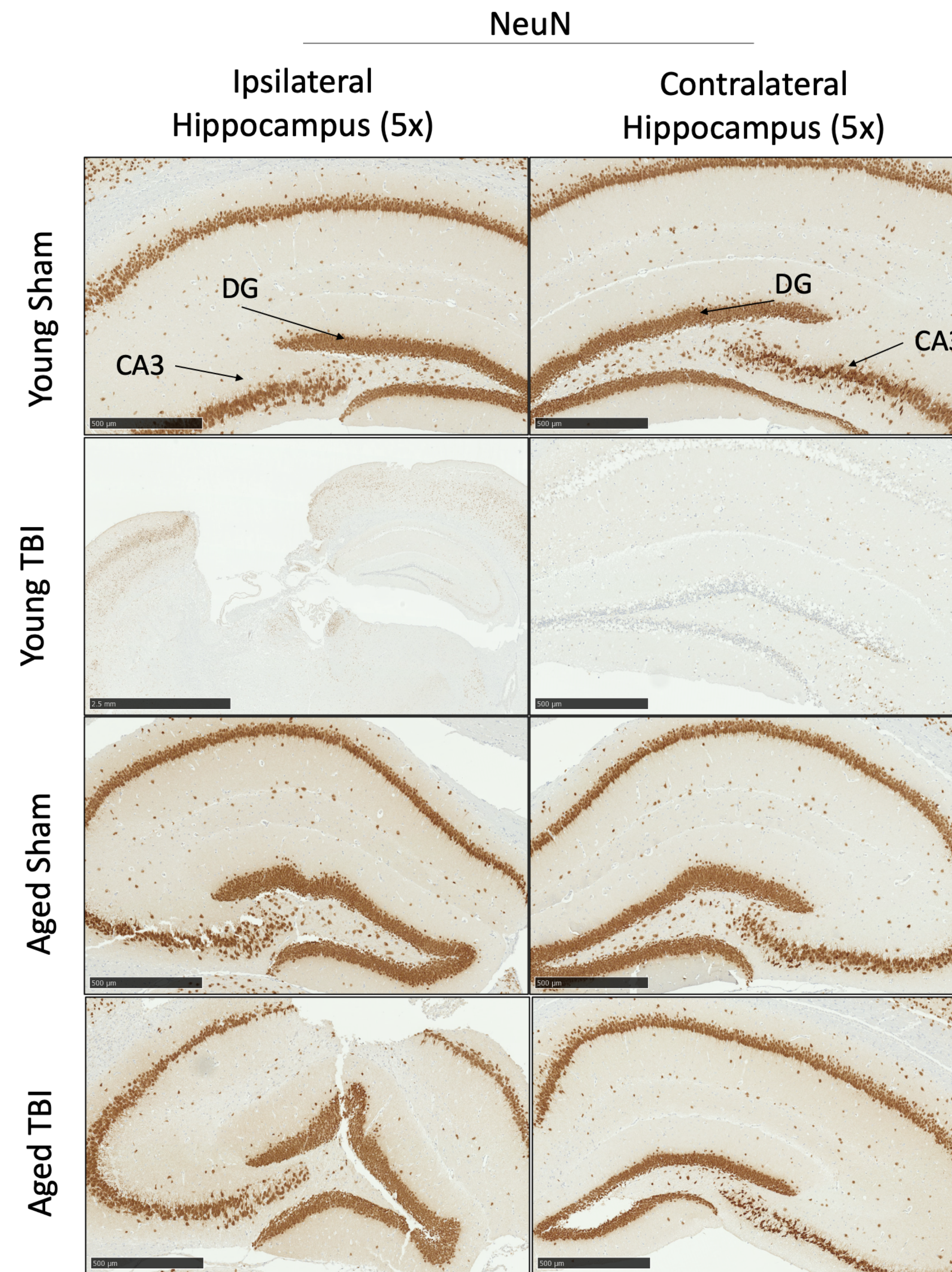


Controlled Cortical Injury induces TBI



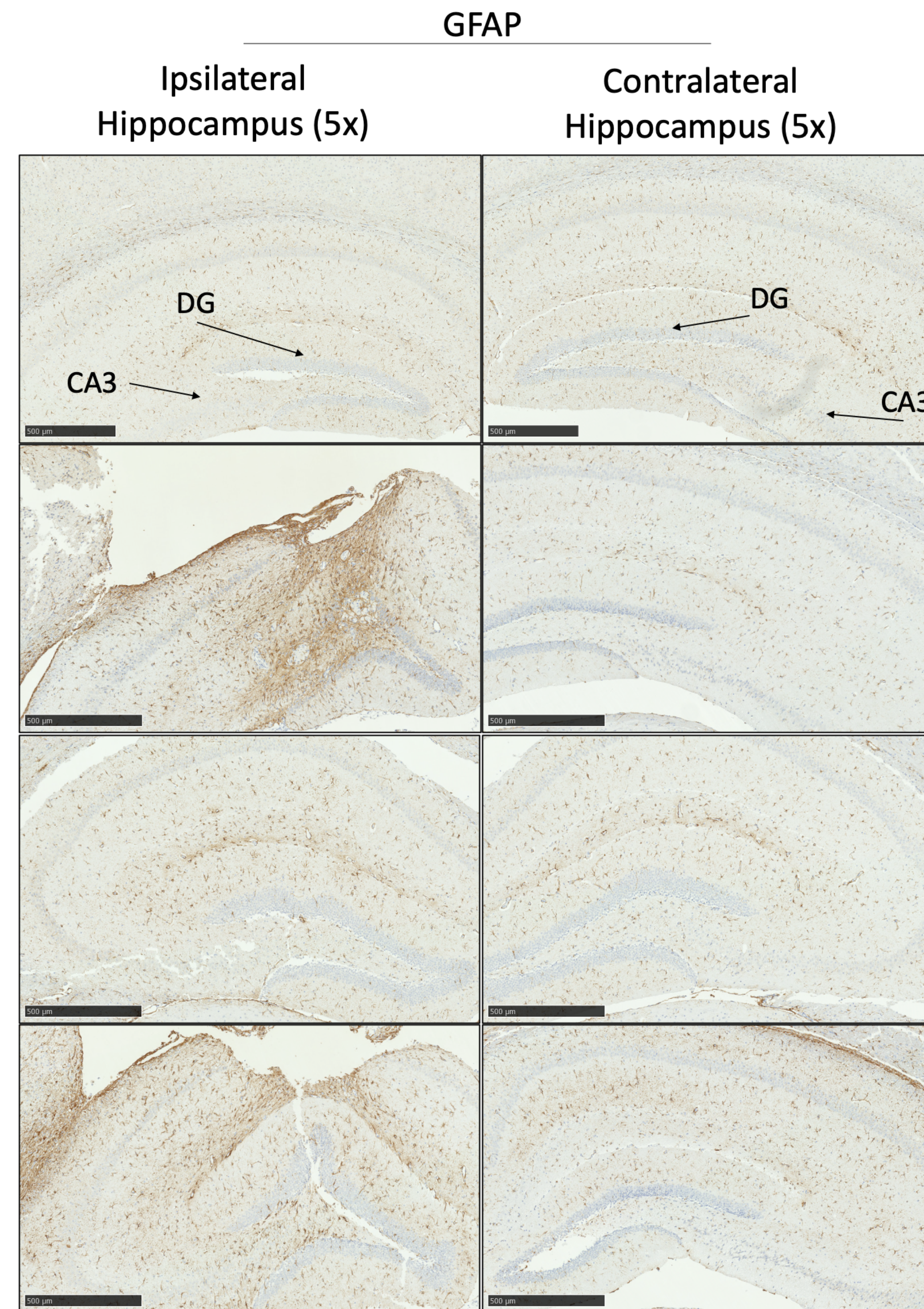
Controlled cortical injury results in severe traumatic brain injury in young adult and aged mouse brains. Coronal sections were stained using hematoxylin and eosin (H&E), neuronal nuclei antibodies (NeuN), as well as Glial fibrillary acidic protein (GFAP), and evaluated for damage by neurophysiologist.

Controlled Cortical Injury induces TBI



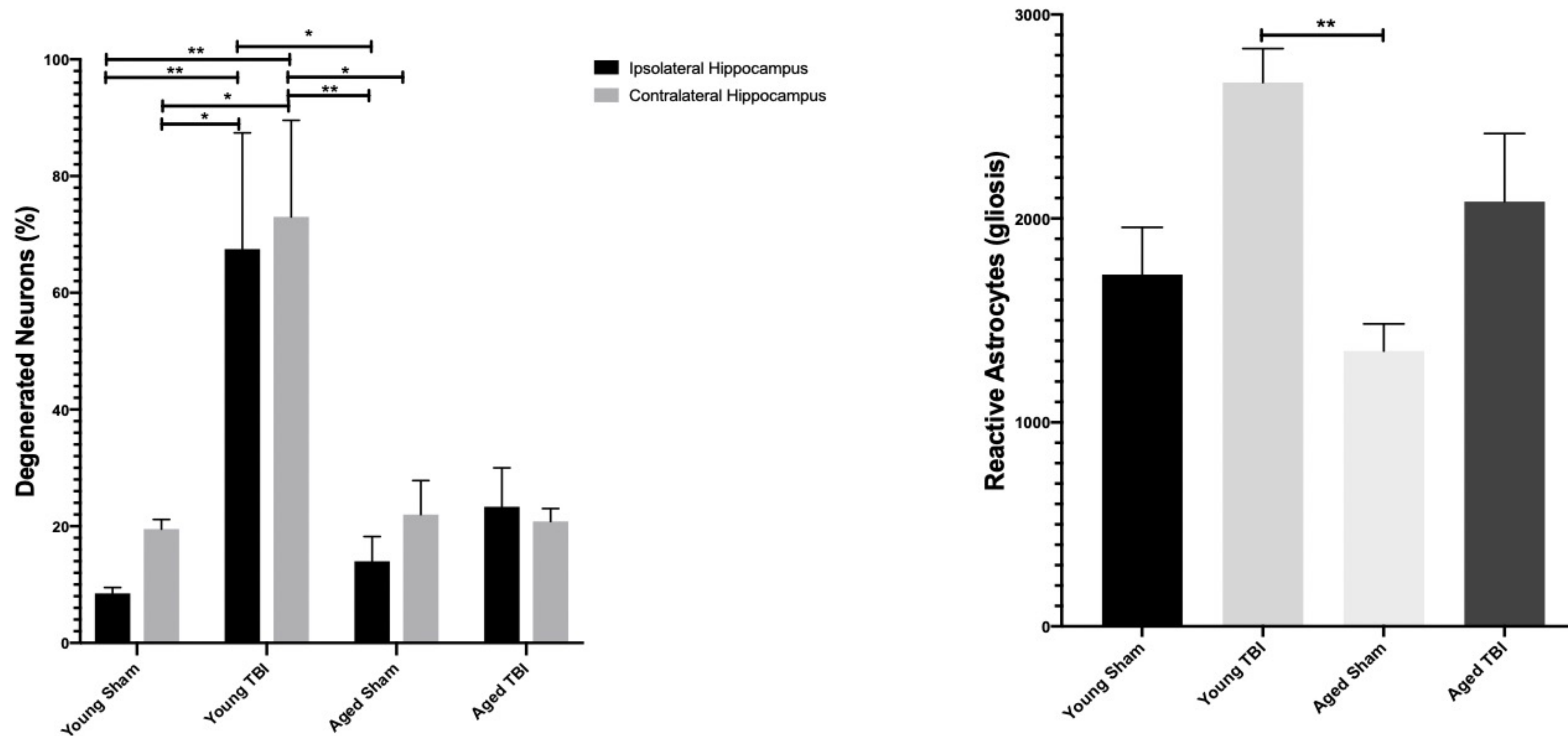
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Neuron loss and Gliosis Attenuated in aged TBI animals



(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

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