The presence of tau pathology in the hippocampus at 6 months following experimental diffuse traumatic brain injury

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Introduction

- The CDC explains that traumatic brain injury (TBI) has resulted in 2.9 million emergency visits, hospitalizations, and deaths in the United States
- The brain's immune response to damage from TBI, activation of microglia, results in neuroinflammation. While defense against injuries and pathogens is essential, Newcombe et al., 2018 found that neuroinflammation has been linked to tau pathology formation, activated microglia release extensive protein kinases that phosphorylate tau protein.
- The tau protein is a primary structural component of the microtubules in neurons. When tau becomes hyper-phosphorylated, it becomes insoluble and sticks together, forming tau pathology (tauopathy), including neurofibrillary tangles (NFT) (see figure 2B).
- Increased tauopathies are linked to the progress of Alzheimer's disease, characterized by cognitive decline.
- AT8 immunohistochemistry labels hyper-phosphorylated tau.
- The dentate gyrus (DG) of the hippocampus is vulnerable to chronic neuroinflammation after TBI and is responsible for encoding cognitive information. Preliminary data indicate chronic neuroinflammation in this animal model for at least 1 month post-injury (Figure 2A).

Hypothesis

Chronic neuroinflammation after diffuse traumatic brain injury leads to tauopathy formation in the DG of the hippocampus.

Methods

Injury: Adult male and female Sprague – Dawley Rats were randomly assigned to a control (n=5/sex) or experimental TBI group (n=6/sex). The TBI group underwent midline fluid percussion injury (FPI) to mimic the effects of a diffuse TBI. Here, a craniectomy was performed and a hub attached to the craniectomy site. The hub was attached to the FPI device and the pendulum dropped to induce the injury. The control group underwent the same procedure without dropping the pendulum.

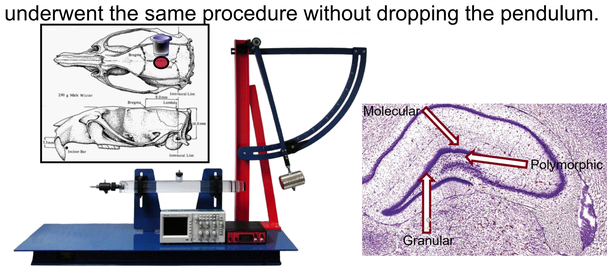


Figure 1. Fluid percussion device and DG anatomy

Histology: Rat brains were extracted at 6 months post-injury (168 days) and sent to Neuroscience Associates for immunohistochemistry for AT8. Brains were cryosectioned and stained in 1 of 2 MultiBrain® blocks, ensuring equivalent staining between sections. Few studies have evaluated pathology at 168 days post-injury (equivalent to 13-15 human years post-injury). Images of the DG were captured via a Zeiss microscope at three different levels of magnification: 10x, 20x, and 40x.

Data Analysis: ImageJ® pixel density was used to generate a rough estimate of the amount positive AT8 staining (representing tau pathology) by converting each image to black and white, adjusting the threshold to remove background, and measuring the percentage of black pixels in each of the images. A single image from the DG was used per rat. Percent black pixels were analyzed using a two-way ANOVA (injury x sex) with Tukey's post-hoc analysis in Graphpad® software. Bars represent the mean$\pm SEM$. *p<0.05

Preliminary Data and Controls

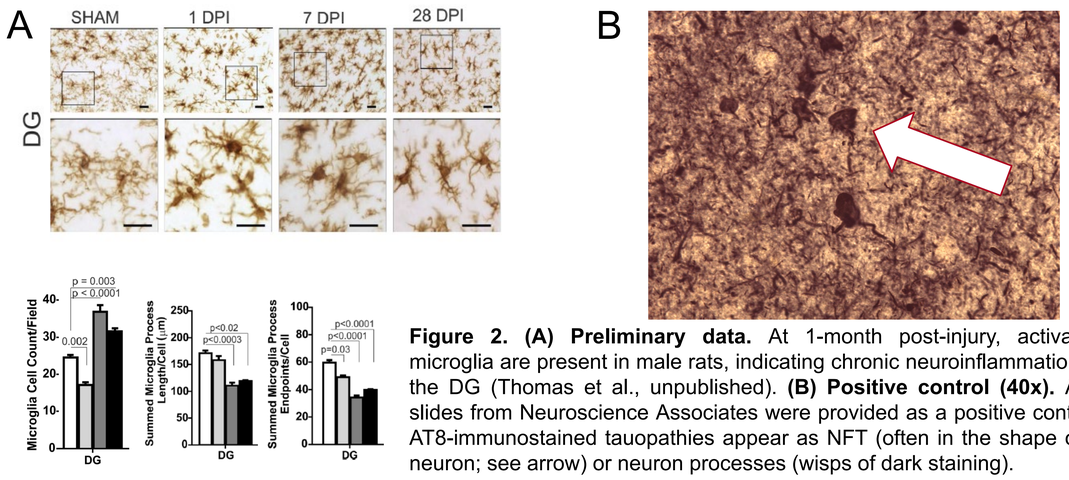
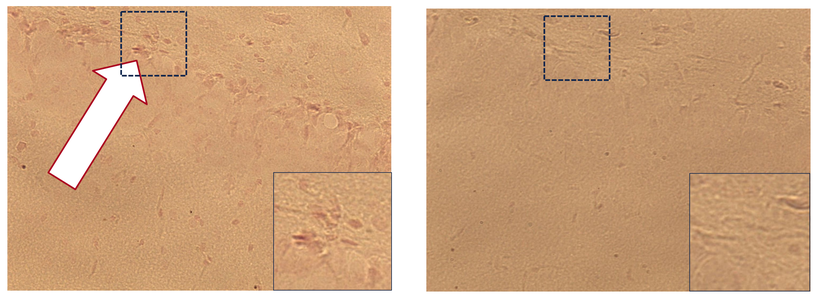


Figure 2. (A) Preliminary data. At 1-month post-injury, activated microglia are present in male rats, indicating chronic neuroinflammation in the DG (Thomas et al., unpublished). **(B) Positive control (40x).** AT8 slides from Neuroscience Associates were provided as a positive control. AT8-immunostained tauopathies appear as NFT (often in the shape of a neuron; see arrow) or neuron processes (wisps of dark staining).

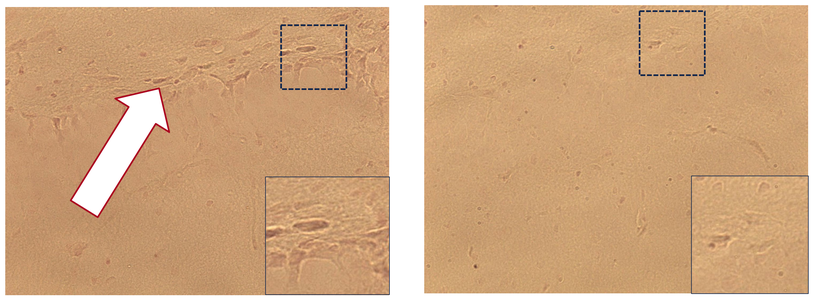
Male Injured vs. Male Sham



Male Injured 40x — Male Sham 40x

Figure 3. Comparison between injured male and sham male at 40x magnification. At 6 months post-injury, the DG in males appears to have has a higher occurrence of phosphorylated tau compared to the sham male. Arrow indicates tauopathy.

Female Injured vs. Female Sham



Female Injured 40x — Female Sham 40x

Figure 4: Comparison between injured female and sham female at 40x magnification. At 6 months post-injury, the DG in females appears to have has a higher occurrence of phosphorylated tau compared to the sham female. Arrow indicates tauopathy.

Pixel Density Analysis

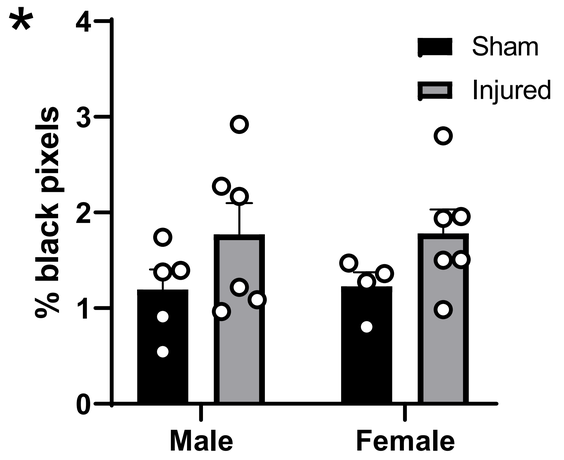


Figure 5: Hyper-phosphorylated tau is increased in the DG at 6-months post-injury. Overall, injury significantly increased the presence of AT8 staining ($F(1,17) = 4.504$; $p=0.049$). Sex did not influence the outcome. The percent of black pixels was 1.78% in the DG of injured rats compared to 1.21% in shams.

Conclusions

- The data indicate a higher occurrence of tau pathology formation in the rat DG at 6 months following diffuse TBI.
- These data do not support sex-differences as a factor influencing tau pathology.
- While the increase in tauopathies for rats in the dentate gyrus due to the midline fluid percussion injury is significant, when compared to the AT8 staining in a model of Alzheimer's disease (Figure 2B), the percentage of AT8 staining measured was relatively low.
- Despite the low levels of increased staining of hyper-phosphorylated tau, these preliminary data indicate that diffuse TBI can result in chronic tauopathy.

Future Directions

Repeat the experiments implementing the following changes.

(1) Take images from multiple slides where the DG is present for each rat to increase representation of staining for each animal.

(2) Have the data analyzed by an additional investigator blinded to the injury status to prevent experimenter bias.

(3) Evaluate tauopathy at earlier time points to determine whether tauopathy is increasing or resolving as a function of time.

(4) Evaluate microglia morphology at the same time points.

References and Funding

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This work was supported by grants from the NIH (R01NS100793) and PCH Mission Support. The authors thank Gokul Krishna, PhD, for valuable feedback on poster.