Traumatic Brain Injury Induces Regional Hypothalamic-Pituitary-Adrenal Axis Dysregulation in Rats

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Introduction

- Annually, 2.8 million traumatic brain injuries (TBI) are reported in the United States.
- Up to 50% of all TBI patients report mental health disorders (MHD) within the first-year post-injury [1,2], yet the underlying pathophysiology is unknown.
- Our previous publications demonstrate that TBI leads to chronic changes in neurotransmission, anxiety-like behavior, dysregulation of the hypothalamic-adrenal-pituitary (HPA) axis in response to stress, and decreased baseline glucocorticoid (GC) levels out to 56 days post-injury (DPI) [3,4].
- Despite chronic deficits, little neuropathology is detected in the paraventricular nucleus of the hypothalamus (PVN), basolateral amygdala (BLA), and central nucleus of the amygdala (CeA). However, acute and chronic neuropathology is present in the hippocampus (Hipp), especially in the dentate gyrus (DG) [3,4,5].
- GC receptor (GR) signaling in astrocytes and microglia has been implicated in the pathological sequelae following TBI [7].
- Few studies have evaluated region-dependent changes glial activation and glucocorticoid expression in chronic TBI-induced HPA axis dysregulation related to increased risk for MHD.

Hypothesis

TBI leads to mental health disorders due to changes in region-dependent glial activity and glucocorticoid receptor expression

HPA Axis

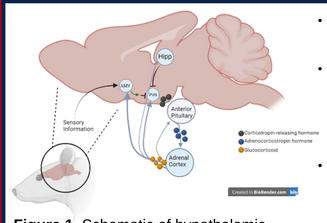

Figure 1. Schematic of hypothalamic-pituitary adrenal axis in rodents.

- BLA integrates sensory information through the CeA which stimulates the PVN through disinhibition of GABAergic neurons.
- PVN releases corticotropin releasing hormone (CRH) to stimulate the anterior pituitary to release adrenocorticotropin releasing hormone (ACTH), which ultimately triggers the release of glucocorticoid (GC) from the adrenal cortex.
- Region-specific GRs in the amygdala, Hipp, PVN, and pituitary, are responsible for returning GC levels to homeostasis (see blue arrows arising from the adrenal cortex (Figure 1).

Methods

- Young adult male and naturally cycling female Sprague-Dawley rats were subject to either diffuse axonal injury (DAI) via midline fluid percussion injury (FPI), or sham surgery (n=5-6 per group) and brain regions were prepared for protein quantification or histology at 7 and 56 DPI.
- In one brain hemisphere, biopsies from the hypothalamus, hippocampus, and amygdala were flash frozen for GR protein quantification using automated capillary westerns.
- The other hemisphere was prepared in a multiblock, sectioned, and stained for GFAP and IBA1 by Neuroscience Associates Inc. (Knoxville, TN).
- IBA1 and GFAP in the PVN, DG, and amygdala were analyzed using ImageJ Skeleton Analysis (Figures 5-7) or pixel density (Figure 3&4), respectively.
- Statistics: For molecular data, an unpaired, Student's t-test was performed comparing sham and FPI rats. When differences in normality were detected, data were logarithmically transformed for statistical analysis (raw data are depicted in graphs). For histology, a three-way ANOVA was performed using sex, FPI and DPI as factors. If no sex differences or interactions were detected, the data were consolidated for a two-way ANOVA. Differences in single factors were considered significant at p<0.05 and interactions at p<0.1. Graphical data is represented as mean + standard error of the mean (SEM). *p <0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

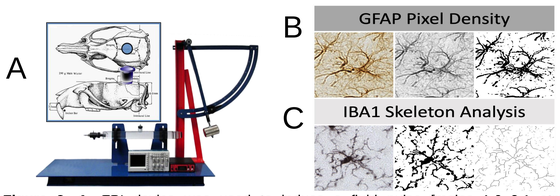

Figure 2. A. FPI device was used to induce a fluid pulse [males=1.9–2.1 atm and females=1.8–2.0 atm] by releasing the pendulum (16.5° for males and 16° for females) onto the fluid-filled cylinder. FPI rats had a righting reflex time between 6-10 minutes. **B.** ImageJ Pixel Density analysis of GFAP. Cropped example of the changes from original micrograph to 8-bit image to binary for quantification of GFAP stained pixels (dark). **C.** ImageJ Skeleton Analysis of IBA1. Cropped example of the changes from original micrograph to binary to skeletonized for analysis of microglial morphological parameters (number of microglia, branch length, end points).

Significant astrogliosis present in the DG, but not in the amygdala or PVN

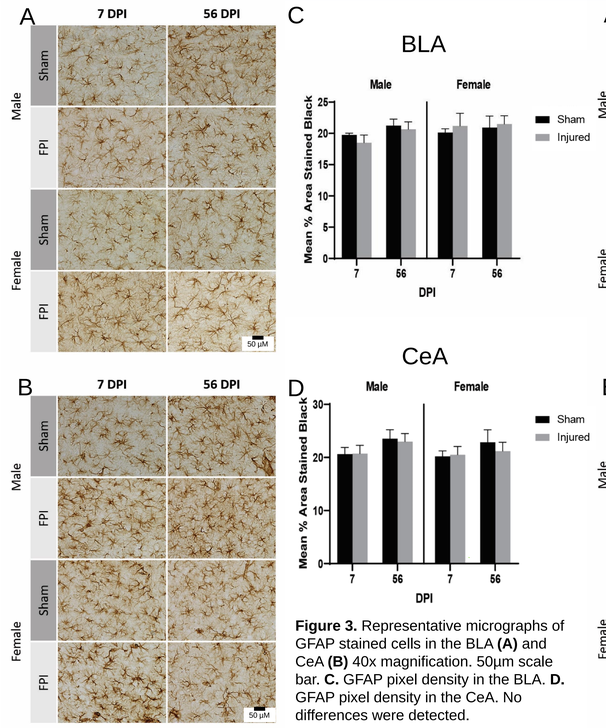

Figure 3. Representative micrographs of GFAP stained cells in the BLA **(A)** and CeA **(B)** 40x magnification. 50µm scale bar. **C.** GFAP pixel density in the BLA. **D.** GFAP pixel density in the CeA. No differences were detected.

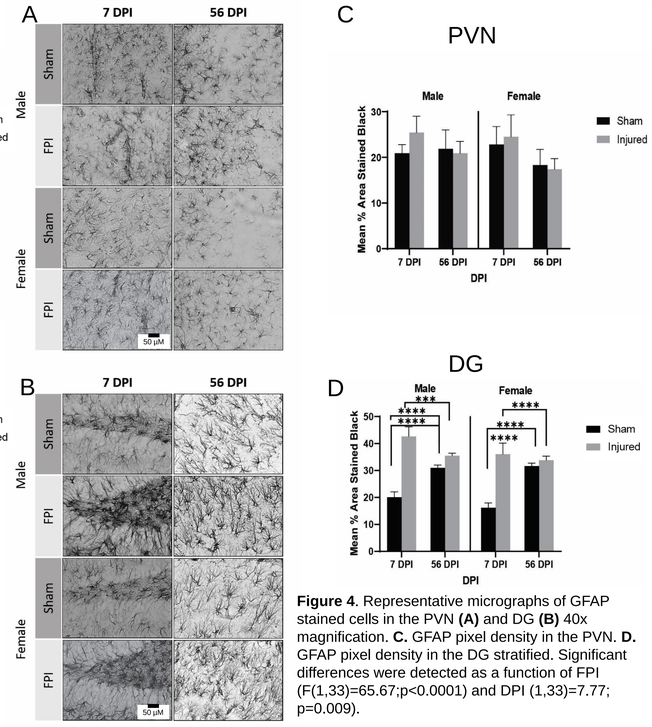

Figure 4. Representative micrographs of GFAP stained cells in the PVN **(A)** and DG **(B)** 40x magnification. **C.** GFAP pixel density in the PVN. **D.** GFAP pixel density in the DG stratified. Significant differences were detected as a function of FPI (F(1,33)=65.67;p<0.0001) and DPI (1,33)=7.77; p=0.009).

Robust microglia activation is seen at 7 DPI in PVN and DG

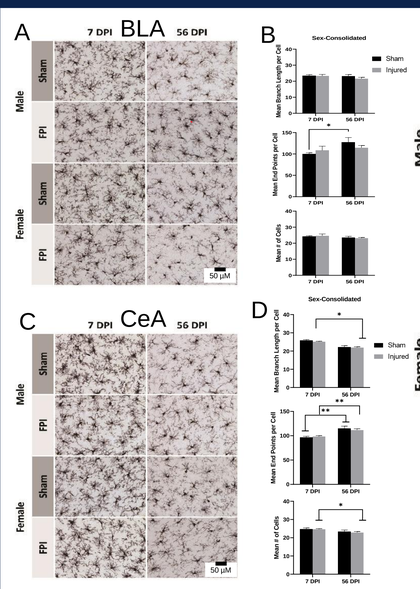

Figure 5. **A.** Representative micrographs of IBA1 stained cells in the BLA. 40x magnification. **B.** Microglia morphological parameters (branch length, end points) and number of microglia in the BLA. A significant difference was detected between 7- & 56-day shams (F(1,38)=4.813; p=0.0344) **C.** Representative micrographs of IBA1 stained cells in the CeA. **D.** Microglia morphological parameters (branch length, end points) and number of microglia in the CeA. Significance was achieved as a function of DPI for branch lengths (F(1,38)=44.46; p<0.0001), end points (F(1,38)=24.81; p<0.0001), and number of microglia (F(1,38)=6.341; p=0.0161).

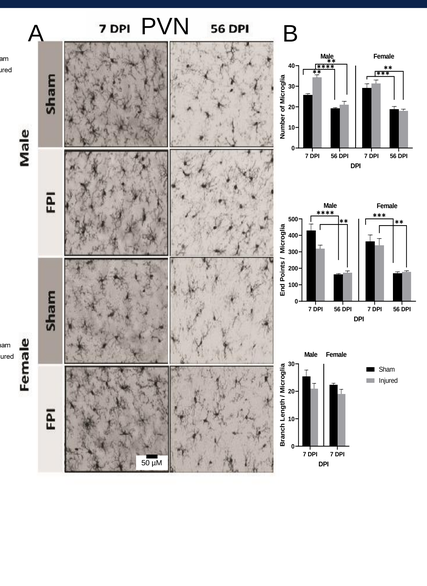

Figure 6. **A.** Representative micrographs of IBA1 stained cells in the PVN. 40x magnification. **B.** Number of microglia were significant as a function of DPI (F(1,34)=126.2; p<0.0001) and FPI*Sex (F(1,34)=5.368;p=0.027). Endpoints were also significant as a function of DPI (F(1,34)=113.9; p<0.0001) and FPI*DPI (F(1,34)=4.442; p=0.043). Analysis of branch length at 56 DPI is ongoing.

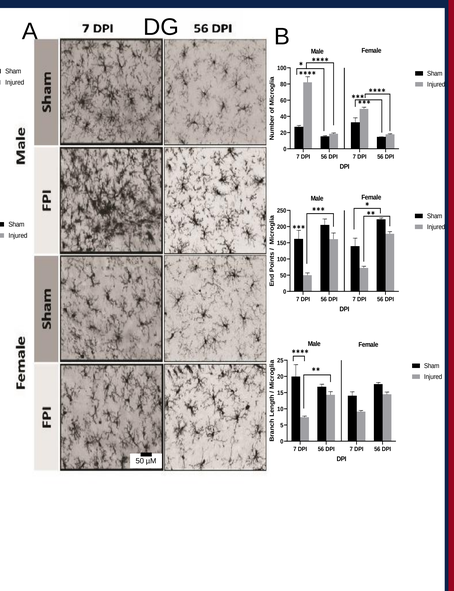

Figure 7. A. Representative micrographs of IBA1 stained cells in the DG. 40x magnification. **B.** Data indicate significant differences in number of microglia as a function of FPI (F(1,34)=73.32; p<0.0001) and DPI (F(1,34)=189.4; p<0.0001), with males having more cells present than females at 7 DPI. Endpoints/microglia were significant as a function of FPI (F(1,34)=33.05;p<0.0001) and DPI (F(1,34)=54.66; p<0.0001). Branch length/microglia was significant as a function of FPI (F(1,34)=32.49; p<0.0001), DPI (F(1,34)=9.825; p=0.0035), and FPI*DPI (F(1,34)=8.366;p=0.0066).

Females have increase GR protein at 56 DPI in the hypothalamus

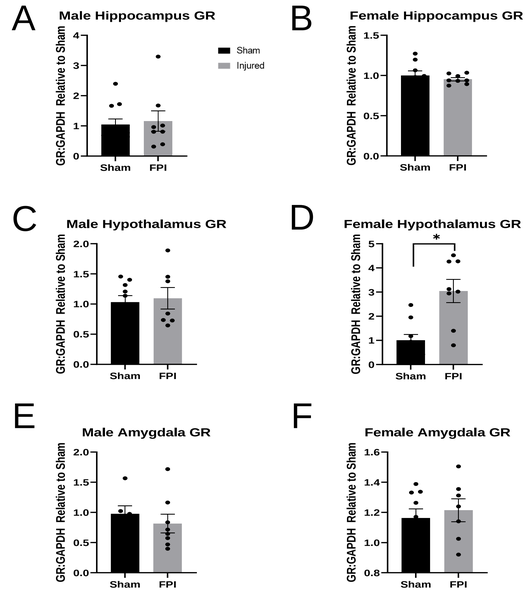

Figure 8. Significance was detected in female hypothalamus GR protein levels (**D.** p=0.0025). No significant differences were detected in the male hippocampus GR levels (**A.** p=0.7780), female hippocampus GR levels (**B.** p=0.3704), male hypothalamus GR levels (**C.** p=0.8868), male amygdala GR levels (**E.** p=0.2284), or female amygdala GR levels (**F.** p=0.757).

Conclusions

- Published chronic changes post-TBI in neurotransmission, HPA axis dysregulation, and anxiety-like behavior do not correspond to gliosis or neuropathology in the amygdala, indicating alternate mechanisms.
- In the DG, sub-acute astrocytes and microglial activation can modulate neuroplasticity and neuropathology can disrupt circuit function. indirectly influencing feedback regulation on the HPA axis.
- In the PVN, sub-acute microglial activation independent of neuropathology indicates an alternate mechanism regulating neuroinflammation.
- Increased GR protein levels in the hypothalamus of female, but not male, indicates TBI-induced chronic HPA axis dysregulation may be sex-dependent (and time-dependent).

Impact

Further understanding of the etiology leading up to late-onset HPA axis dysregulation following TBI could identify targets to stabilize feedback, attenuate symptoms, and improve efficacy of overall recovery.

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