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**PRINCIPAL INVESTIGATOR:** Drs. Fiona Crawford/Joseph Ojo

**CONTRACTING ORGANIZATION:** The Roskamp Institute, Inc.
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**14. ABSTRACT**

For many years TBI has been known to be a risk factor for later life neurodegenerative diseases, such as Alzheimer's Disease Related Dementias (ADRD), but the precise nature of how TBI leads to or precipitates these conditions (or different pathological substrates) is not understood. We have spent years developing and characterizing mouse models of r-mTBI that demonstrate lifelong behavioral and neuropathological features of human TBI and are thus relevant models in which to generate data that will translate to human patients. In our mouse model, we observe dramatic increases in reactive astrocyte cells, and these increases last for life. We thus hypothesized that repetitive mTBI induces significant and persistent changes to reactive astrocyte populations after injury and that these reactive astroglial responses are critical to TBI neurodegeneration and, in the context of other potentially pathogenic proteins such as tau or amyloid, distinct ADRD proteinopathies can be triggered. Here we propose neuropathological analyses of human autopsy cases of TBI, ADRD, and from the brains of normal mice and mice producing potentially pathological tau and amyloid proteins, at a range of timepoints following repetitive mild TBI.
This year, we began exploring the role of astrocytes in inducing and driving pathogenesis by utilizing novel mechanistic approaches in our mouse models, involving adoptive cell transfer of TBI reactive glial cells into naïve mice, and utilizing *ex vivo* functional assays to closely study whether reactive astrocytes in the injured brain can damage other healthy un-injured neural and vascular cells. We also began to apply our state of the art single cell genomic analyses to identify astrocyte specific changes at the gene level. These studies are almost completed, and we have generated data on three mouse models interrogated.

**15. SUBJECT TERMS: TBI, ADRD, Astrocytes, Neuropathology, Transcriptomics, Single cell profiling, Animal models**

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
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INTRODUCTION: ***Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.***

In this project we will interrogate the role of astrocytes in inducing and driving TBI related neurodegeneration by utilizing neuropathological tools to characterize astrocyte lesions in mouse models and autopsy tissue of TBI and ADRD cases. We will also use our novel mechanistic approaches involving adoptive cell transfer and *ex vivo* functional assays to clarify the means through which astrocytes contribute to TBI-neurodegeneration and precipitation of ADRD. We will conclude by applying our state of the art single cell gene profiling to interrogate astrocytes from our mouse models and autopsy cases to identify astrocyte specific changes at the gene and protein level, and to identify novel single cell targets to interrupt the pathobiological consequences of TBI.

1. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

TBI, ADRD, Astrocytes, Neuropathology, Transcriptomics, Single cell profiling, Animal models, Ex Vivo Models.

2. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

**What were the major goals of the project?**
*List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

**HYPOTHESIS**
*Repetitive mTBI induces significant and persistent changes to reactive astrocyte populations after injury. These reactive astroglial responses are critical to TBI neurodegeneration and, in the context of other potentially pathogenic proteins such as tau or amyloid, distinct ADRD proteinopathies can be triggered.*

***MAJOR TASK 1:(a)*** *Delineation of the chronic effects of repetitive mTBI on astrogliopathy and related proteinopathy in young and aged wild type, humanized tau and humanized amyloid mouse models at 24hrs, 3 months and 9 months post-injury* ***(Subtasks 1-2; 4-12).*** *(1b) Investigation of astroglial pathological lesions in autopsied brains from human TBI, age-related tau astrogliopathy (ARTAG), and ADRD cases* ***(Subtask 3 and 12).*** Y1Q1 – Y3Q1 [80% completed]
***MAJOR TASK 2:*** *Evaluating pathobiological effects of reactive/injured astrocytes derived from mouse models in Aim 1, on the induction and spreading of age-related proteinopathies and neurodegeneration* ***(Subtask 1-6)***. Y1Q3 – Y3Q3 [50% complete]
***MAJOR TASK 3:*** *(a) Generation of single cell genomic/secretome profiles in astrocyte populations obtained from mouse models described in Aim 1* ***(Subtask 2-5)***. *(b) Single cell genomic profiling of astrocyte populations in autopsied brains from TBI, ARTAG, ADRD cases* ***(Subtask 1 and 5)***.
Y1Q3 – Y3Q4 [90% complete]

**PROJECT MILESTONES/DELIVERABLES**
**1:** Detailed timeline and dynamics of astroglia lesions (using histopathology) in response to injury, ageing, and proteinopathies in animal models, and correlation with neuropathological staging in human r-mTBI/ARTAG/ADRD autopsy cases. [Month 1-27]

**2:** To determine whether reactive astrocytes from the injured brain milieu of our TBI models are sufficient to induce and drive age-related proteinopathies and neurodegeneration. [Month 7-32]
**3:** Novel gene and secreted protein expression profiles of astroglia populations in TBI mediated neurodegeneration, normal ageing and ADRD pathogenesis in mouse models and autopsy cases. [Month 7-36]

| DETAILED S.O.W | Timeline | |
|---|---|---|
| **Major Task 1** | Months | |
| Subtask 1: Obtaining ACURO approval (Roskamp Institute approval for TBI procedures is already in place) and Human Cadaver use approval. | 1-3 | |
| Subtask 2: Breeding of cohorts for studies | 1-6 | |
| Substask 2a: Breeding of cohorts for Aged mice TBI studies (36 mice per genotype) at 24hrs, 3 & 9 months post-TBI | 3-6 | |
| Subtask 2b: Breeding of cohorts for Young mice TBI studies (36 mice per genotype) | 3-6 | |
| Subtask 3: Histopathological analyses of astroglia lesions in autopsy cases. N=10/group; 100 samples. Samples are coded and de-identified. | 1-9 | |
| Subtask 4: Administering injuries to Young cohort - 3 months old at injury. For each of the genotypes there will be two groups - 18 r-mTBI young and 18 r-sham young. | 6-9 | |
| Subtask 5: Euthanasia of Young cohort in subtask 4. | 6-16 | |
| Subtask 6: Sectioning and histopathological staining of Young cohort tissues with astroglia, tau and amyloid antibodies. | 13-18 | |
| Subtask 7: Stereological analyses and Image quantitation of brain sections from Young cohort (staggered over time) | 13-21 | |
| Subtask 8: Administering injuries to Aged cohort (i.e. 12 months old at injury). Animals will receive injuries as in subtask 4. | 15-18 | |
| Subtask 9: Euthanasia of Aged cohort in subtask 8 | 15-25 | |
| Subtask 10: Sectioning and histopathological staining of Aged cohort tissues with astroglia, tau and amyloid antibodies. | 19-24 | |
| Subtask 11: Stereological analyses and Image quantitation of brain sections from Aged cohort (staggered over time) | 19-27 | |
| Subtask 12: Interpretation of data/consultation with clinical neuropathologists | 18-27 | |
| **Major Task 2** | | |
| Subtask 1: Breeding of cohorts for Young Donor mice for TBI studies in Subtask 2 and 3 (12 mice per genotype) | 7-9 | |
| Subtask 2: Administering injuries to Young 'Donor' cohort - 3 months old injury. Animals will receive injuries as in Major task 1 (subtask 4). For each of the genotypes there will be 6 r-mTBI and 6 r-sham . | 9-12 | |
| Subtask 3: Euthanasia of Young 'Donor' cohort from Subtask 1 at 12 months of age, isolation of astrocytes by magnetic cell sorting and injection into naïve 'Recipient' non-transgenic mouse brain. | 21-24 | |
| Subtask 4: Ex vivo experiment to determine pathobiological effects of exposing conditioned media derived from adult astrocyte cultures obtained from our transgenic TBI models, and their impact on healthy neural cell populations. | 21-27 | |
| Subtask 5: Euthanasia of inoculated 'Recipient' naïve non-transgenic mice, collection of brain tissue, sectioning and histopathological staining with astroglia, tau and | 24-27 | |

| | | |
|---|---|---|
| amyloid antibodies. | | |
| Subtask 6: Stereological analyses and Image quantitation of brain sections from inoculated 'Recipient' cohort (staggered over time). Interpretation of data and consultation with clinical neuropathologists | 28-32 | |
| **Major Task 3** | | |
| Subtask 1: Laser capture microdissection of astroglia from autopsy cases for single cell array for gene expression profiling. Same 10 groups and tissue sections from Major task 1 (subtask 3). N=8 cases/group; 80 samples. | 7-15 | |
| Subtask 2a: Laser capture microdissection of astroglia from Young Cohort (Major Task 1 – subtask 4) mice for single cell array for gene expression profiling (staggered overtime). There will be 36 mouse brains per genotype for analyses. | 15-24 | |
| Subtask 2b: LC-MS/MS analyses of astroglia secretome from Young Cohort (Major Task 1 – subtask 4) mice for unbiased proteomic expression profiling (staggered overtime). There will be 36 astrocyte supernatants/secretome collected from each mouse brain per genotype for analyses. | 15-24 | |
| Subtask 3a: Laser capture microdissection of astroglia from Aged Cohort (Major Task 1 – subtask 8) mice for single cell array for gene expression profiling (staggered overtime). There will be 36 mouse brains per genotypes for analyses. | 25-33 | |
| Subtask 3b: LC-MS/MS analyses of astroglia secretome from Aged Cohort (Major Task 1 – subtask 8) for unbiased proteomic expression profiling. | 25-33 | |
| Subtask 4: Validation of select gene transcript profiles altered in astroglia (and protein profiles in the secretome), in both Young/Aged cohorts. rt-PCR, in situ hybridization, flow cytometry, ELISA, co-immunolabeling, etc. | 21-33 | |
| Subtask 5: Data analysis and interpretation and correlation studies | 34-36 | |

**What was accomplished under these goals?**

*For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

The most important activities during this reporting period involved completing our transcriptomics analyses of the young and aged cohorts for the longitudinal mouse study. We also continued experiments for *ex vivo* studies involving exposure of r-mTBI/sham astrocyte secretome to neural cells, and initiated the inoculation studies (which are currently ongoing).
Major Tasks from the SOW and the corresponding data/results from each relevant section is provided below.

**Below Are The Tasks from the SOW Completed Or Initiated During This Reporting Period:**

**Major Task 1**

**Subtask 4:** Administering injuries to Young cohort - 3 months old at injury. **COMPLETED**
**Subtask 5:** Euthanasia of Young cohort in subtask 4. **COMPLETED**
**Subtask 6:** Sectioning and histopathological staining of Young cohort tissues with astroglia, tau and amyloid antibodies. **COMPLETED**
**Subtask 7:** Stereological analyses and Image quantitation of brain sections from Young cohort (staggered over time) **ONGOING (70% COMPLETED)**
**Subtask 8:** Administering injuries to Aged cohort - 12 months old at injury. Animals will receive injuries as in subtask 4. **COMPLETED**
**Subtask 9:** Euthanasia of Aged cohort in subtask 8. **COMPLETED**
**Subtask 10:** Sectioning and histopathological staining of Aged cohort tissues with astroglia, tau and amyloid antibodies. **COMPLETED**
**Subtask 11:** Stereological analyses and Image quantitation of brain sections from Aged cohort (staggered over time) **ONGOING (70% COMPLETED**

**Major Task 2**

**Subtask 3**: Euthanasia of Young 'Donor' cohort from Subtask 1 at 12 months of age, isolation of astrocytes by magnetic cell sorting and injection into naïve 'Recipient' non-transgenic mouse brain. **ONGOING (50% COMPLETED)**
**Subtask 4:** Ex vivo experiment to determine pathobiological effects of exposing conditioned media derived from adult astrocyte cultures obtained from our transgenic TBI models, and their impact on healthy neural cell populations. **ONGOING (50% COMPLETED)**

**Major Task 3**

Subtask 1: Laser capture microdissection of astroglia from autopsy cases for single cell array for gene expression profiling. **ONGOING (75% COMPLETED)**
Subtask 2a: Laser capture microdissection of astroglia from Young Cohort (Major Task 1 – subtask 4) mice for single cell array for gene expression profiling (staggered overtime). There will be 36 mouse brains per genotype for analyses. **COMPLETED**
Subtask 2b: Laser capture microdissection of astroglia from Aged Cohort (Major Task 1 – subtask 4) mice for single cell array for gene expression profiling (staggered overtime). There will be 36 mouse brains per genotype for analyses. **COMPLETED**

## FIRST AND SECOND QUARTER

### GENE ARRAY ANALYSES OF MICRODISSECTED ASTROGLIA IN AUTOPSY TBI TISSUE – MAJOR TASK 3

Table 1 shows group classification and history of de-identified patients analyzed for astroglia gene array. Sample sizes range from 10-12.

**Table 1:** Group Classification and History

| | CTE II | CTE III | CTE IV | p-value | Groupwise Comparison |
|---|---|---|---|---|---|
| Age at Death | 54.92±22.03 | 65.36±11.08 | 77.36±8.26 | 0.01 | IV > II |
| Age Began Playing | 12.00±3.36 | 12.73±1.95 | 11.36±2.25 | 0.37 | na |
| Years Played | 14.00±5.08 | 16.27±5.08 | 16.64±6.25 | 0.48 | na |
| Years Between Retirement and Death | 28.92±20.78 | 36.36±11.21 | 49.36±11.41 | 0.03 | IV > II |
| Age at Symptom Onset | 43.18±21.29 | 44.73±18.61 | 59.91±14.58 | 0.07 | na |

| | group | | | | | |
|---|---|---|---|---|---|---|
| | cte_2 | | cte_3 | | cte_4 | |
| | Mean | SD | Mean | SD | Mean | SD |
| age | 54.917 | 22.0349 | 65.364 | 11.0840 | 77.364 | 8.2616 |
| age_begun | 12.000 | 3.3575 | 12.727 | 1.9540 | 11.364 | 2.2482 |
| age_symonset | 43.182 | 21.2923 | 44.727 | 18.6069 | 59.909 | 14.5839 |
| calc_yrsrettodeath | 28.917 | 20.7779 | 36.364 | 11.2096 | 49.364 | 11.4129 |
| yrs_play | 14.000 | 5.0812 | 16.273 | 5.0812 | 16.636 | 6.2494 |

### METHODOLOGY

### LASER CAPTURE MICRODISSECTION (LCM)AND GENE TRANSCRIPT PROFILING:

A laser capture microdissection (LCM) instrument (Mufson lab) was used for these studies. LCM methodology results in accurate dissection of the astroglial cells of interest with minimal disruption of the surrounding tissue. For the proposed studies, GFAP-positive astroglia are captured per reaction, per condition for subsequent linear terminal continuation (TC) RNA amplification and microarray analysis. TC RNA amplification was developed in Dr. Ginsberg's laboratory. The method entails synthesizing first strand cDNA complementary to the RNA template, generating second strand cDNA complementary to the first strand cDNA, and finally *in vitro* transcription using the double stranded cDNA as template. This process displays high fidelity, reproducibility and increased signal sensitivity (4-fold) and flexibility compared to other RNA amplification protocols. Radiolabeled RNA probes will be hybridized to custom-designed

microarrays. Array platforms consist of 1µg of linearized cDNA purified from plasmid preparations adhered to high-density nitrocellulose (Hybond XL, GE Healthcare, Piscataway, NJ) using an arrayer robot (VersArray, Bio-Rad, Hercules, CA) (Ginsberg, 2005, 2008). Each cDNA and/or expressed sequence-tagged cDNA (EST) was verified by sequence analysis and restriction digestion. Human clones are employed on the custom-designed array. Approximately 864 cDNAs/ESTs organized into 22gene ontology groups are present on the current array platform. Dr. Ginsberg has already successfully and productively transferred this technology to Dr. Mufson's laboratory at Barrow Neurological Institute, which is where these experiments were carried out.

**Statistical procedures** (using GeneLinker Gold**)** for custom-designed microarray analysis have been described in detail previously (Ginsberg et al., 2014; 2016; 2017; Mufson et al., 2017). Gene expression differences will be assessed with respect to the hybridization signal intensity ratio of the total signal of all array genes. For each gene the signal intensity ratio is modelled as a function of study group, using mixed effects models with random effect to account for the correlation between repeated assays. Between subject versus within-subject variation will be analyzed by random intercept, fixed effect covariate, Kenward-Roger denominator degrees of freedom, unstructured covariance structure, and log-transformed expression levels. Significance will be judged at the level $\alpha$=0.01, two-sided; false discovery rate (FDR) based on an empirical null distribution due to strong correlation between genes is controlled at level $\alpha$=0.1. Spearman's coefficient will evaluate correlations of select transcripts. Serial section *in situ* hybridization will confirm changes in transcript expression.

**Preliminary findings presented below from staged TBI groups vs controls – Major Task 3**

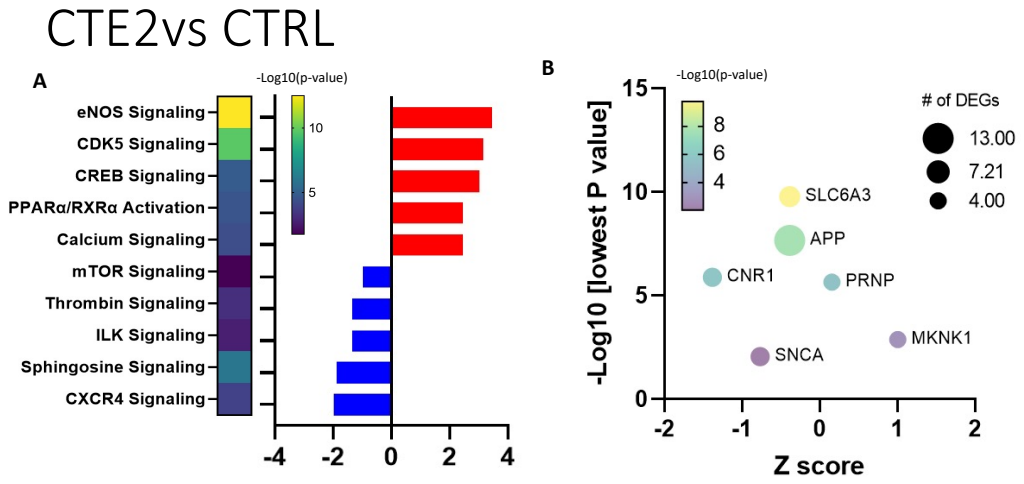



**Figure 1. Pathway and Upstream regulator analysis on cases of CTE (stage 2).** A) Top 5 upregulated pathways in red and top 5 downregulated pathways in blue. B) Bubble plot of the top 6 upstream regulators. DEGs=differentially expressed genes.

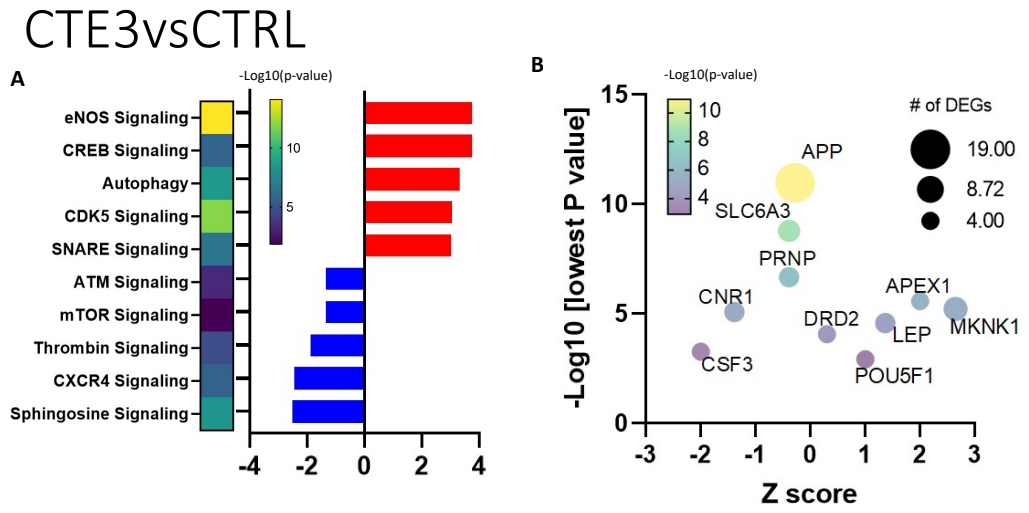



**Figure 2. Pathway and Upstream regulator analysis on cases of CTE (stage 3).** A) Top 5 upregulated pathways in red and top 5 downregulated pathways in blue. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

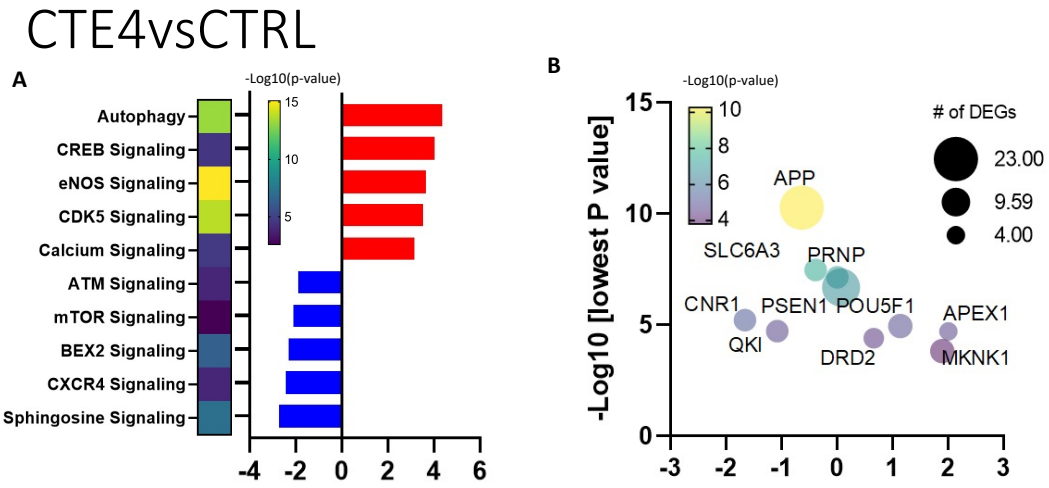



**Figure 3. Pathway and Upstream regulator analysis on cases of CTE (stage 4).** A) Top 5 upregulated pathways in red and top 5 downregulated pathways in blue. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

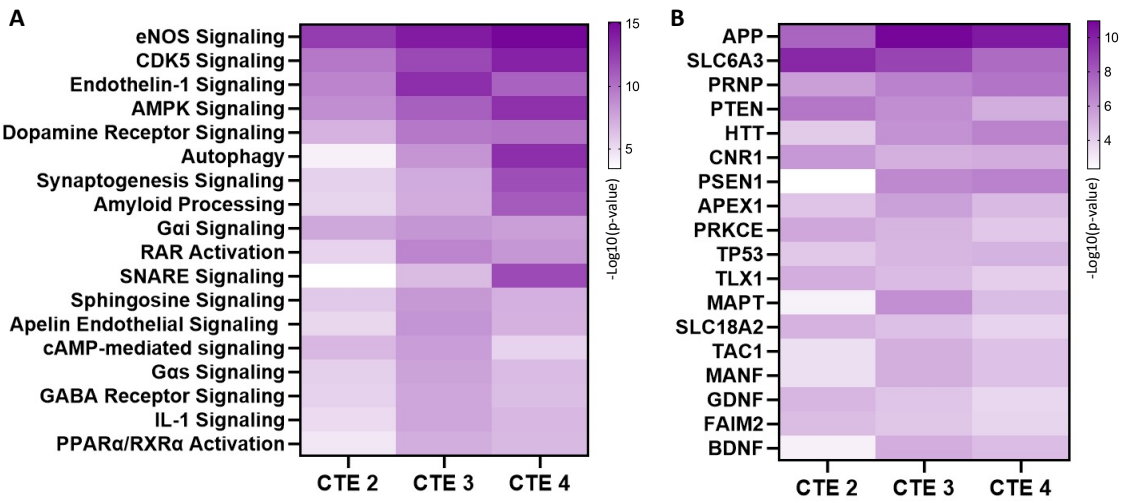



**Figure 4. Comparative pathway and upstream regulator analysis among CTE stages (2-4):** A) comparative analysis on the top 20 altered pathways B) comparative analysis on the top 20 upstream regulators

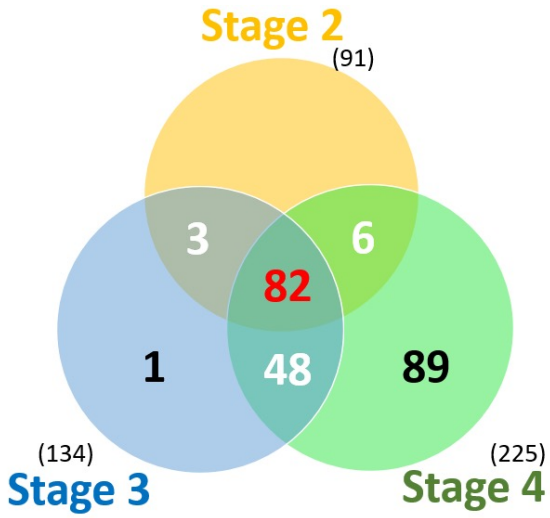



**Figure 5.** Venn-diagram of differentially expressed genes in astrocytes from CTE brains at the various stages (2-4).

| Ontology | Gene | P-value | Groupwise Comparison |
|---|---|---|---|
| AD related genes | ARMCX2 | 0.0005 | CTE-2<CTE-4 |
| | B2M | 0.0002 | CTE-2<CTE-4 |
| | BACE2 | 0.0004 | CTE-2<CTE-4 |
| | PRNP | 0.003 | CTE-2<CTE-4 |
| | PSEN2 | 0.0002 | CTE-2<CTE-4 |
| | SAA4 | 0.003 | CTE-2<CTE-4 |
| | MAPT6 | 0.03 | CTE-2<CTE-4 |
| | MAPT5 | 0.02 | CTE-2<CTE-4 |
| Ionic channels | DPP10 | 0.003 | CTE-2<CTE-4 |
| Cytoskeletal elements | CDH2 | 0.001 | CTE-2<CTE-4 |
| | LAMB2 | 0.002 | CTE-2<CTE-4 |
| Cell death related genes | AIFM1_PCD8 | 0.0008 | CTE-2<CTE-4 |
| | CCNG1 | 0.03 | CTE-2<CTE-4 |
| | CCNF | 0.02 | CTE-2<CTE-4 |
| | CCNB1 | 0.02 | CTE-2<CTE-4 |
| | CASP9 | 0.009 | CTE-2<CTE-4 |
| | CASP5 | 0.04 | CTE-2<CTE-4 |
| | CASP4 | 0.03 | CTE-2<CTE-4 |

**Table 1.** Groupwise analysis among human cases of CTE at the different stages (2-4).

## Summary of findings (discussion)

1) We observed a significant upregulation in autophagy, cdk5, CREB, calcium and eNOS signaling in CTE stages vs controls. These effects were more prominent in CTE4 vs control cases. See Figure 1-3 and Figure 4A.
2) We observed a significant downregulation in mTOR, CXCR4, sphingosine pathways in all CTE stages vs controls. See Figure 1-3 and Figure 4A.
3) Top upstream regulator in CTE2 vs Control cases was SLC6A3 (Figure 4B). Top upstream regulator in CTE3 vs control and CTE4 vs control was APP.
4) Venn diagram in Figure 5 shows 88 and 130 genes shared between 'CTE2 vs control & CTE4 vs control' and 'CTE3 vs control & CTE4 vs control' comparisons, respectively. Notable CTE4 vs control comparison also revealed a large number of unique significantly regulated genes (89) not shared between comparisons of CTE2 vs control and CTE3 vs control. See Figure 5
5) Groupwise analyses revealed a significant positive correlation in ADRD genes, genes related to cell death and cytoskeletal element with TBI staging.

## THIRD QUARTER

### MOUSE R-MTBI ASTROGLIAL HISTOPATHOLOGY – MAJOR TASK 1

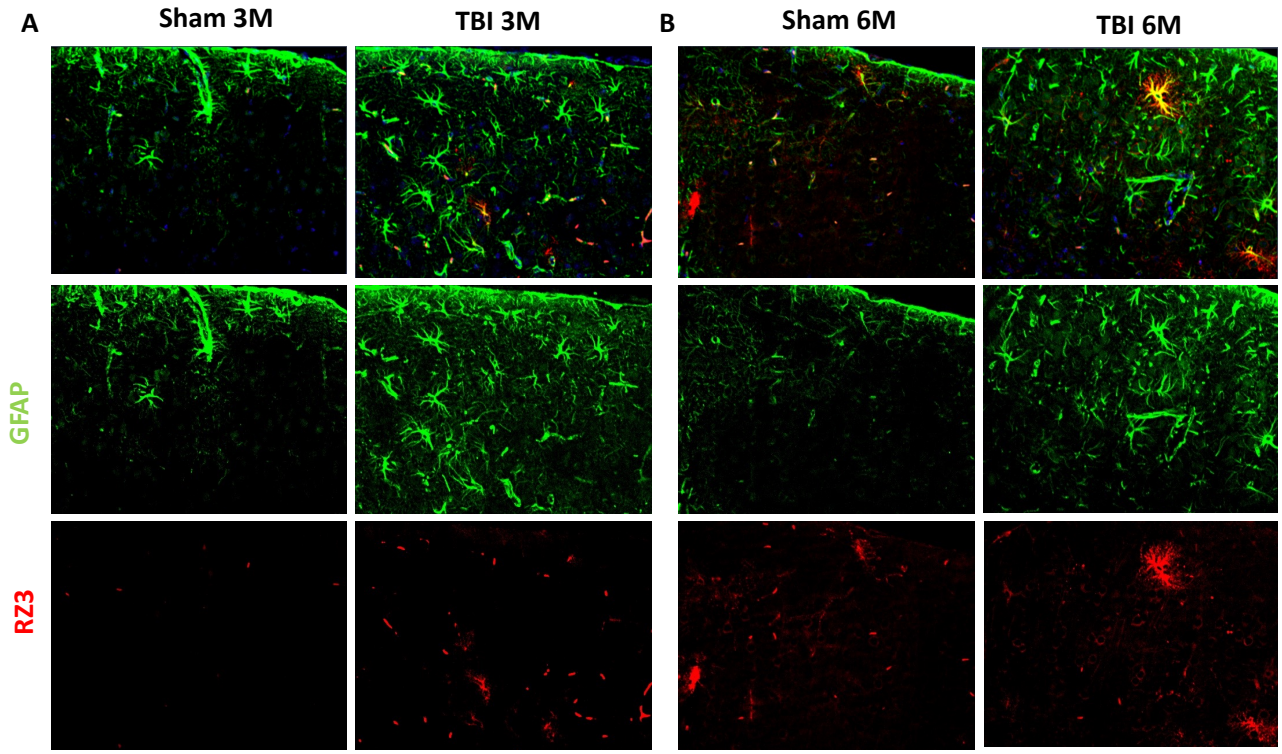



**Figure 1. Representative images of immunostaining on the cortex of young GFAP$^{p301l}$ mice after r-mTBI.** A) Cortex of GFAP$^{p301l}$ 3 months post last injury, B) 6 months post last injury. GFAP to visualize astrocyte activation and RZ3 for the presence of phosphorylated tau protein.

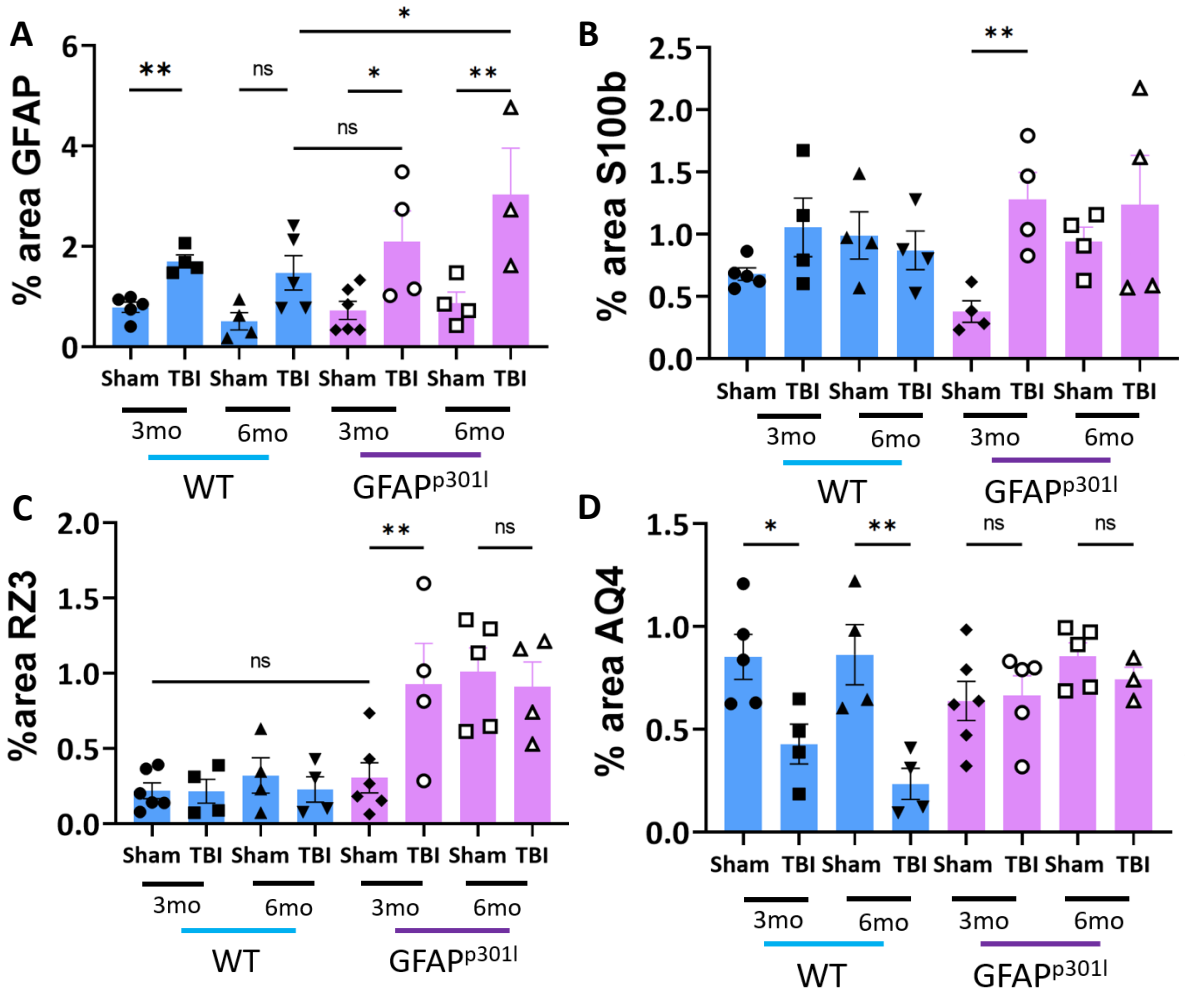



**Figure 2. Quantification of reactive astrogliosis, tau pathology and water channel abundance in the cortex of young WT and GFAP$^{P301L}$ mice after rmTBI.** Percentage area of GFAP (A), S100b (B) both markers of astrocytes. Percentage area of phosphorylated tau, RZ3 (C), and water channel AQ4 (D).

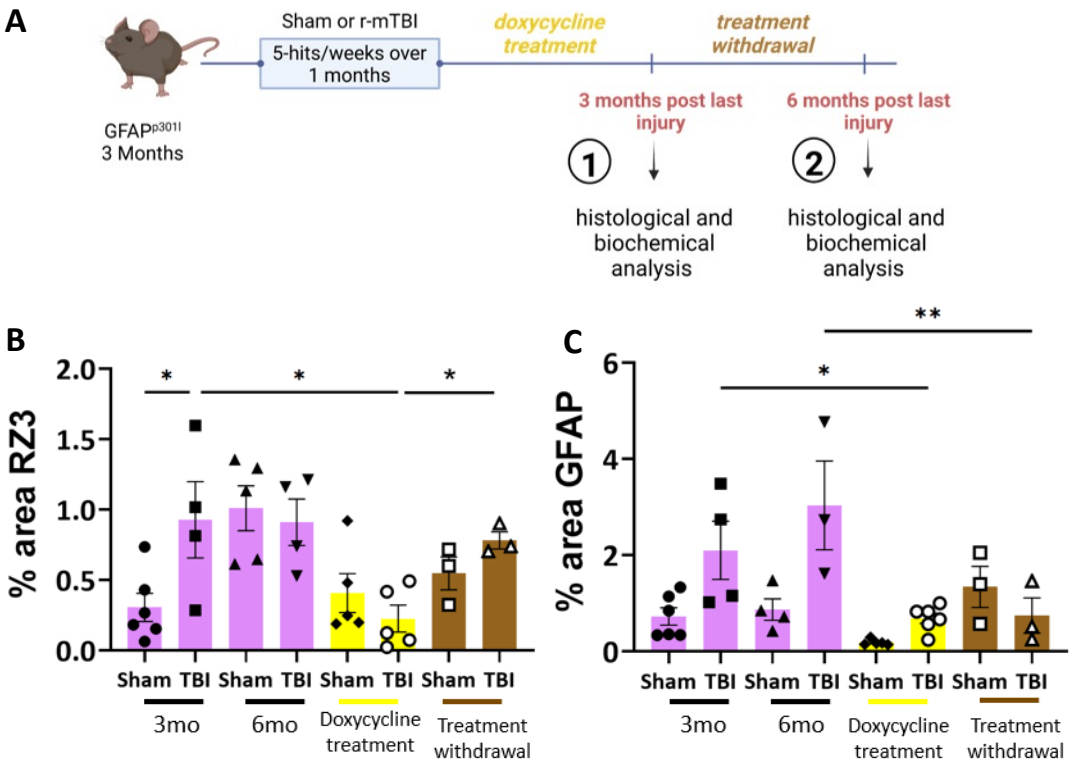



**Figure 3. Influence of conditional repression of astrocytic tau expression after r-mTBI in GFAP$^{p301l}$ mice.**(A) Experimental design. Percentage area of phosphorylated tau (RZ3) in B, and GFAP in C. Purple bars: Sham and TBI GFAP$^{p301l}$ mice 3 or 6 months post-last injury (3mo and 6mo, respectively). Yellow bars: Sham and TBI GFAP$^{p301l}$ mice treated with doxycycline for 3mo. Brown bars: Sham and TBI GFAP$^{p301l}$ mice 3mo after doxycycline treatment was withdrawn.

**Summary of findings (discussion)**

Histopathological analysis showed an increase in the expression of GFAP in the cortex of WT and GFAP$^{p301l}$ mice at three and six months post last injury in the r-mTBI groups compared to their controls. This is evidence of persistent astrocyte activation after r-mTBI in two different mouse models (Fig 1 and Fig2a). Additionally, at 6 months post-last injury, we observed a significant increase in GFAP expression of astrocytes from r-mTBI GFAP$^{P301L}$ mice compared to r-mTBI WT mice. Thus, indicating that the presence of mutant tau$^{P301L}$ in astrocytes augments their chronic response to repetitive mTBI.

We also analyzed S100b, a marker of astrocytes. For this marker, we only observed a significant increase in the r-mTBI GFAP$^{P301L}$ group compared to controls at 3 months post last injury (Fig.2b). This effect was not observed at 6 months post-injury. In WT groups we did not observe any injury dependent effects on s100b immunoreactivity at 3- or 6-months post-injury (Fig.2b).

We quantified the immunoreactivity of phosphorylated tau (p-tau, RZ3[p-Thr231]) in the cortex of WT and GFAP$^{p3011}$ mice. In the WT groups, no injury dependent effect was observed in RZ3 immunoreactivity (Fig.2c). In the GFAP$^{p3011}$ cohort, we revealed a significant increase p-Tau immunoreactivity after 3 months post-last injury (Fig.2c). At the 6 month timepoint, we observed a notable increase in RZ3 immunoreactivity in sham mice compared to 3 months timepoint. Paralleling the trend we saw in S100b, we also did not observe any injury dependent changes in pTau immunoreactivity at 6 months post-injury. Thus, indicating that at 6 months post-last injury the underlying injury dependent effects might be masked by the age-related increase in pTau levels between 3 and 6 months post-last injury.

Investigation of water channels (AQ4) immunoreactivity levels in the cortex of WT mice showed a persistent injury dependent reduction at 3 and 6months post-injury (Fig.2d). Intriguingly in the GFAP$^{P301L}$ cohort, there was no injury dependent effects at 3 or 6 months post-injury in AQ4 immunoreactivity levels (Fig.2d).

To investigate the contribution of tau astrogliopathy on histopathological changes to astrocytes following repetitive mTBI, we treated a separate cohort of mice with doxycycline to repress tau expression immediately after the last injury until 3 months post-last injury (Fig. 3a part 1). A separate cohort also received a similar treatment regimen described above, but also doxycycline was withdrawn after 3 months until 6 months post-injury (Fig. 3a part 2).

Histopathological analyses of cortices revealed that doxycycline treatment (study in Fig. 3a part 1) was able to mitigate the injury dependent increase in RZ3 (p-tau) immunoreactivity levels at the 3 month post-injury timepoint (Fig. 3b). Likewise, doxycycline treatment was also able to mitigate the injury dependent increase in GFAP immunoreactivity at the 3 month post-injury timepoint (Fig. 3c). Together suggesting protective effects of tau repression in astrocytes on histopathological outcomes.

In the doxycycline withdrawal study (Fig. 3a part 2), we revealed that both p-tau and GFAP immunoreactivity levels in the cortex, remained unchanged at 6 months between r-mTBI and sham mice (Fig 3b, Fig 3c). Signifying that long-lasting protective/reparative effects can be achieved by temporal repression of mutant tau expression in astrocytes after injury.

## FOURTH QUARTER

### CAMK2A Transcriptomic Profile (3M-6M) – Major Task 3

**Methodology** – RNA was extracted from ACSA2+ astrocytes after magnetic cell associated sorting with militenyl beads**. HiSeq sequencing:** RNA samples was prepared using the Qiagen RNeasy Kit, quantified and checked for integrity. RNA library preparation with PolyA and sequencing reactions were conducted at Genewiz (South Plainfield, NJ). The NEBNext Ultra RNA Library Prep Kit for Illumina will be used for RNAseq library preparations. Briefly, mRNA was first enriched with Oligod(T) beads and fragmented, with the 1st and 2nd strand cDNA subsequently synthesized. cDNA fragments was end repaired and adenylated, and universal adaptor ligated to fragments, followed by index addition and library enrichment with limited cycle PCR. Validated and quantified sequencing libraries was multiplexed and clustered on one lane of a Flow Cell. After clustering, the flow cell was loaded on the Illumina instrument, with samples sequenced using a 2×150 Paired End configuration. Raw sequence data generated from Illumina HiSeq was converted into Fastq files and de-multiplexed. **Bioinformatic analyses**: After quality checking of Fastq files, sequence reads was trimmed, and mapped to the Mus musculus GRCm38 reference genome. Data was inspected for outlying samples using unsupervised hierarchical clustering and principal component analysis. Combat batch correction was applied to combine the datasets and reduce systematic sources of variability. Differential gene expression analysis: This was conducted to determine relationships between gene expression levels and injury group. The covariates were included in all models to adjust for any potential confounding influence on gene expression between main group effects. This was conducted using the Wald test (in DESeq2Genes with FDR adj p<0.05 and log2 fold changes >1.5 classified as differentially expressed genes).

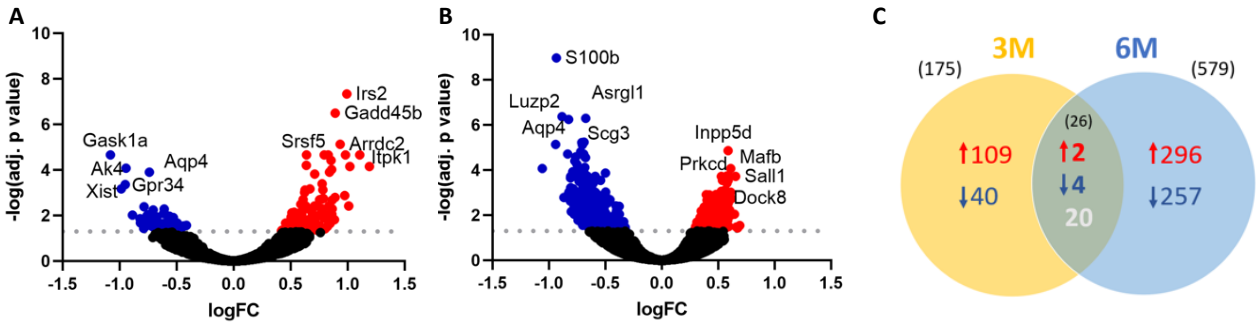



**Figure 1. Astrocytic differentially expressed genes in Camk2$^{P301L}$ 3 and 6 months after rmTBI.** Volcano plot of differentially expressed genes (DEGs) 3 months (A) and 6 months (B) post-last injury (TBI vs Sham). C. Venn diagram showing the overlap of astrocytic DEGs between both timepoints. Threshold for obtaining the DEGs: adj. p-value≥0.05 with its respective –log value≥1.3.

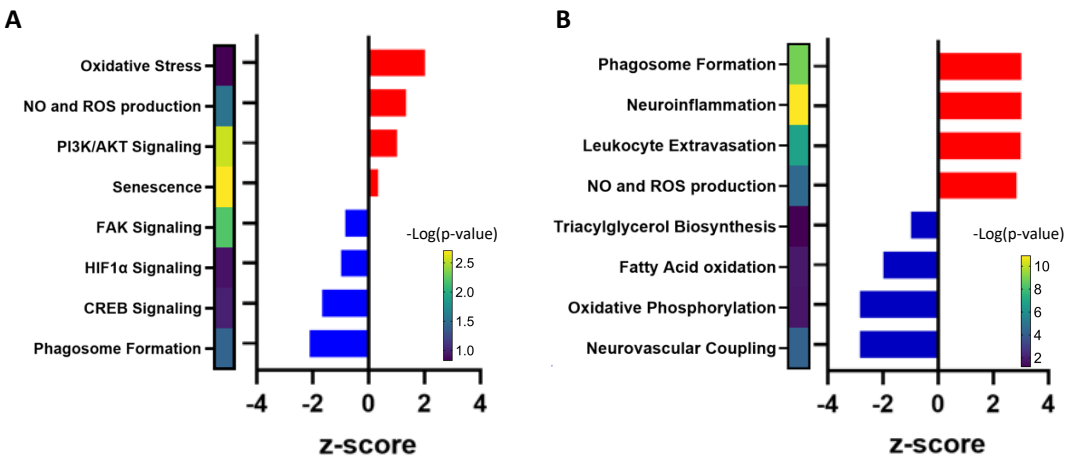



**Figure 2. Top astrocytic dysregulated pathways in Camk2$^{P301L}$ 3 and 6 months after rmTBI.** Top 4 upregulated (red) and top 4 downregulated (blue) pathways in astrocytes 3 months (A) and 6 months (B) post-last injury.

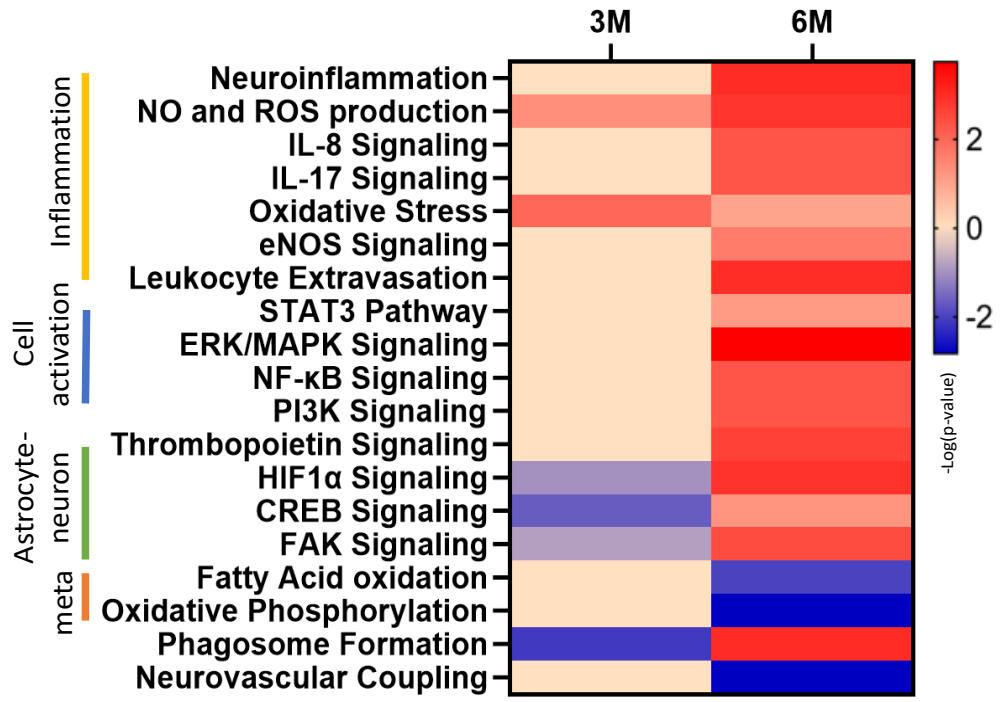



**Figure 3. Comparative analysis of astrocytic dysregulated pathways in Camk2$^{P301L}$ 3 and 6 months after rmTBI. 3M and 6M= 3 and 6 months post-last injury.** Threshold p-value ≥0.05 with its respective –log value≥1.3.

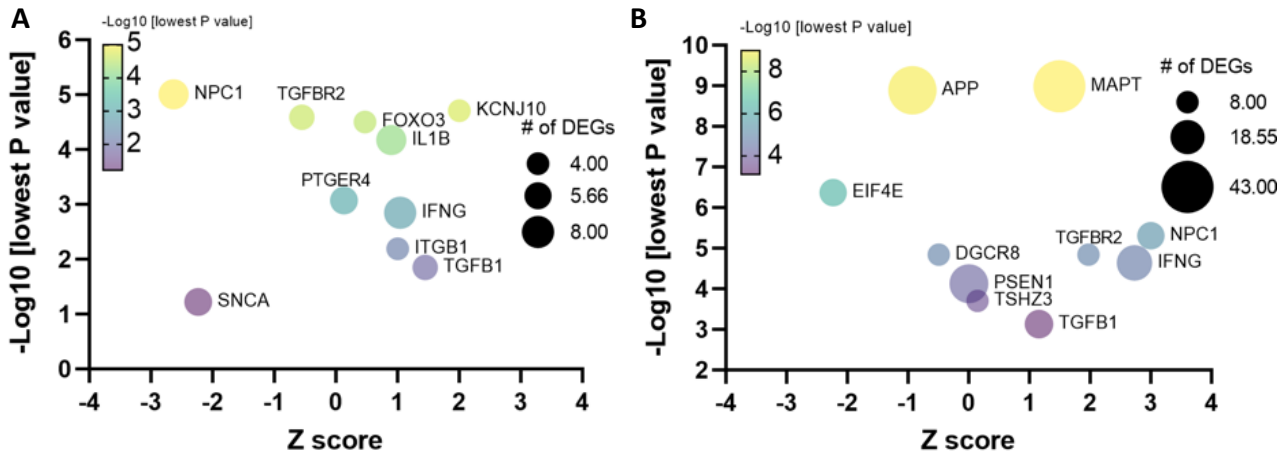



**Figure 4. Top upstream regulators of changes observe in Camk2$^{P301L}$ 3 and 6 months after rmTBI.** Top 10 upstream regulators at 3 months (A) and 6 months (B) post-last injury.

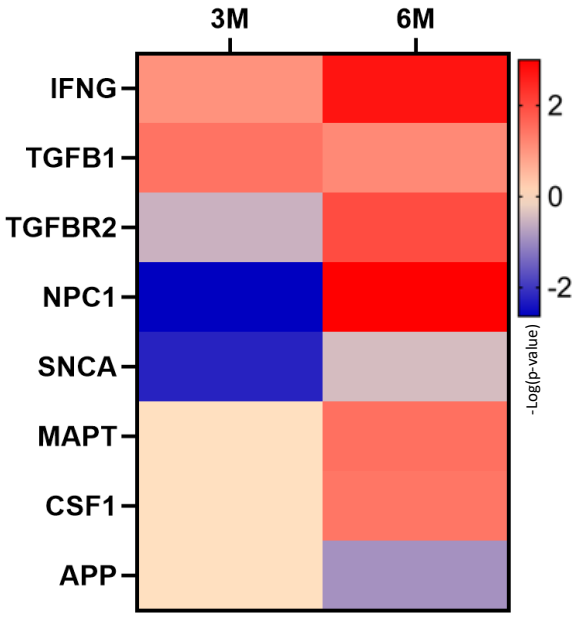



**Figure 5. Comparative analysis of upstream regulators in Camk2$^{P301L}$ 3 and 6 months after rmTBI.**

**BRIEF SUMMARY OF RESULTS**

Camk2$^{P301L}$ mice were subjected to our 20-hit model of r-mTBI at the age of 3 months. Astrocytes from TBI and Sham mice were magnetically isolated 3 and 6 months post-last injury. RNA was isolated and sent for sequencing. Bioinformatic analysis revealed 175 differentially expressed genes (DEGs) in astrocytes from TBI brains compared to sham brains at 3mo post-last injury.

At 6mo post last injury, the number of DEG's was ~3x higher (579) than at 3 month post last injury) (Fig. 1). IPA analysis of the DEG's at 3mo post last injury revealed upregulation of pathways involved in neuroinflammation and cellular activation. Additionally, pathways involved in astrocytic regulation of neuronal signaling were downregulated (Fig. 2A). At 6 mo post-last injury, astrocyte mediated neuroinflammatory pathways were upregulated to a greater extent than at 3 months post-injury. Furthermore, r-mTBI induced additional changes to astrocytes typified by the downregulation in lipid synthesis and fatty acid oxidation, and deficits in mitochondrial metabolism (Fig.2B & 3).

IPA analysis of upstream regulators responsible for mediating the changes observed in astrocytes at 3 or 6 months post-last injury revealed that out of the top 10 regulators, interferon-gamma (INFG) and TGFB1 were found in both cohorts sharing the same directionality (upregulation). Both are master regulators of pro- and anti-inflammatory immune response, respectively (Fig. 4 & 5)

**Ex-vivo study of astrocyte conditioned media exposed to healthy neural cells (Major Task 2)**

METHOD: GFAP-Tau and non-carrier mice were exposed to r-mTBI (N=3) and sham injury(N=3). Brains were collected and processed under sterilized conditions. Glial cells were isolated from brain homogenate using percoll gradient centrifugation and co-cultured at 37ºC for a few weeks. Astrocytes were separated from microglia through shaking at 37ºC for three hours. Isolated astrocytes were cultured for another week until 80% confluency was reached, during these processes astrocyte conditioned media (ACM) was collected and frozen until required for the subsequent study. TBI and sham astrocytes were exposed to LPS for 8hrs and frozen for further probing. Protein was extracted from cell lysates of astrocytes, run in a gel, and probed for specific markers using western blotting. ACM will be used for exposure to healthy neural cell lines in subsequent experiments that are ongoing. ***Data below only reveals effect of exposing sham vs r-mTBI astrocytes to LPS.***

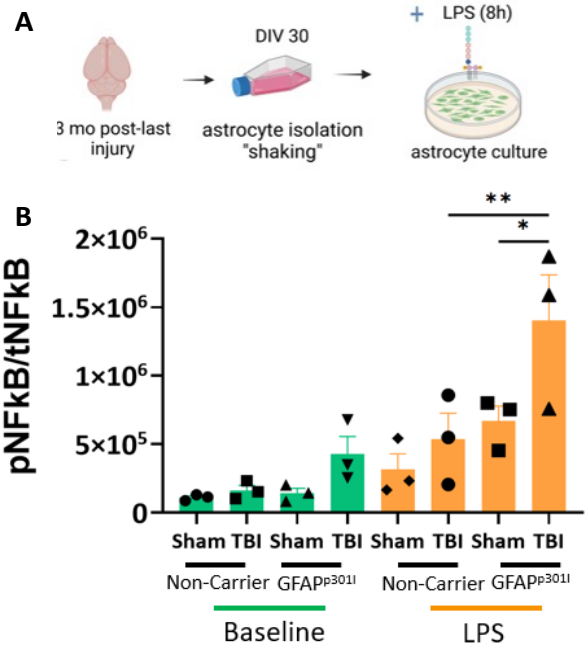



**Figure 6. Astrocytic cellular activation at baseline and after LPS exposure at 3 months after r-mTBI in GFAP$^{p301l}$ and Non-carrier controls.** (A) Experimental design. (B) Normalized levels of phosphorylated NFκB (p NFκB) to evaluate astrocytic activation.

**BRIEF SUMMARY OF RESULTS**

Primary astrocytes harvested from GFAP$^{p301l}$ and Non-carrier control mice were exposed to 50ng/ml of LPS for 8h (Fig. 6A). Phosphorylated NFκB (p NFκB) was used to evaluate cellular activation/response (Fig. 6B). In general, astrocytes exposed to LPS showed increased pNFκB levels compared to their baseline levels, regardless of their genotype (non-carrier or GFAP$^{p301l}$) or condition (Sham or TBI). It is important to highlight that astrocytes extracted from TBI GFAP$^{p301l}$ mice exhibited a very pronounced cellular activation compared to its sham counterpart and TBI non-carrier. These results provide an insight to the potential detrimental role of tau astrogliopathy on astrocyte homeostatic function, typified here by a primed and augmented response to the exposure of inflammatory stimuli.

**What opportunities for training and professional development has the project provided?**
*If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

| |
|---|
| *Nothing to Report* |

**How were the results disseminated to communities of interest?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

| |
|---|
| *Nothing to Report* |

**What do you plan to do during the next reporting period to accomplish the goals?**
*If this is the final report, state "Nothing to Report."*

*Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

**OVER THE NEXT QUARTER WE PLAN TO CONTINUE OR COMPLETE:**

**Major Task 1**
**Subtask 7/11:** Continue stereological analyses and Image quantitation of brain sections from Young/Aged cohort of TBI/SHAM mice.

**Major Task 2**
**Subtask 3:** Euthanasia of Young 'Donor' cohort from Subtask 1, isolation of astrocytes by magnetic cell sorting and injection into naïve 'Recipient' non-transgenic mouse brain.
**Subtask 4:** Finish ex vivo experiment to determine pathobiological effects of exposing conditioned media derived from adult astrocyte cultures obtained from our transgenic TBI models, and their impact on healthy neural cell populations.
**Subtask 5**: Euthanasia of inoculated 'Recipient' naïve non-transgenic mice, collection of brain tissue, sectioning and histopathological staining with astroglia, tau and amyloid antibodies.
**Subtask 6**: Stereological analyses and Image quantitation of brain sections from inoculated 'Recipient' cohort (staggered over time). Interpretation of data and consultation with clinical neuropathologists

**Major Task 3**
**Subtask 1:** Continue Laser capture microdissection of astroglia from autopsy cases for single cell array for gene expression profiling. Same 10 groups and tissue sections from Major task 1 (subtask 3). N=8 cases/group; 80 samples.
**Subtask 2a/b**: Perform LC-MS/MS analyses of astroglia secretome from Young/Aged Cohort for unbiased proteomic expression profiling.
**Subtask 3a/b**: LC-MS/MS analyses of astroglia secretome from Young/Aged Cohort for unbiased proteomic expression profiling.
**Subtask 4**: Validation of select gene transcript profiles altered in astroglia (and protein profiles in the secretome), in both Young/Aged cohorts. rt-PCR, in situ hybridization, flow cytometry, ELISA, co-immunolabeling, etc.
**Subtask 5**: Data analysis and interpretation and correlation studies
**ALL plans are consistent with the original approved SOW.**

***4.*** **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

**What was the impact on the development of the principal discipline(s) of the project?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and*

*research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

*Nothing to Report*

**What was the impact on other disciplines?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

*Nothing to Report*

**What was the impact on technology transfer?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

*Nothing to Report*

**What was the impact on society beyond science and technology?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

*Nothing to Report*

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Due to COVID-19, there has been a small reduction in work output at the Roskamp Institute. This has set us back by around 6-8 months. However, work is now progressing as normal and we aim to gain grounds in the work out over the next year. We recently requested for a 12 month NCE.

*Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*

**Actual or anticipated problems or delays and actions or plans to resolve them**

*Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

As mentioned above, due to COVID-19, there has been a small reduction in work output.
This set us back by around 6-8 months, but work is now progressing as normal and we aim to gain grounds over the next few quarters. We recently requested for a 12 month NCE.

**Changes that had a significant impact on expenditures**

*Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

*Nothing to report*

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

*Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

**Significant changes in use or care of human subjects**

*N/A*

**Significant changes in use or care of vertebrate animals**

*Nothing to report*

**Significant changes in use of biohazards and/or select agents**

*Nothing to report*

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  *Report only the major publication(s) resulting from the work under this award.*

  **Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  | |
  |---|
  | *Nothing to report (In progress, planned for submission in Jan 2023)* |

  **Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  | |
  |---|
  | *Nothing to report* |

  **Other publications, conference papers and presentations**. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

  | |
  |---|
  | *Neurotrauma meeting 2022 (Poster) – Camila Ortiz [Presenter]*<br>*Tau astrogliopathy after exposure to r-mTBI in mouse models* |

- **Website(s) or other Internet site(s)**
  *List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.*

  | |
  |---|
  | *Nothing to report* |

- **Technologies or techniques**

*Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.*

| |
|---|
| *Nothing to report* |

- **Inventions, patent applications, and/or licenses**
  *Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.*

| |
|---|
| *Nothing to report* |

- **Other Products**
  *Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:*
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

| |
|---|
| *Nothing to report* |

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**
*Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".*

*<u>Example:</u>*

*Name:* *Mary Smith*
*Project Role:* *Graduate Student*
*Researcher Identifier (e.g. ORCID ID):* *1234567*
*Nearest person month worked:* *5*

*Contribution to Project:* *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
*Funding Support:* *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

*Name:* *Fiona Crawford (no change)*
*Project Role:* *PI*
*Researcher Identifier (e.g. ORCID ID):* *N/A*
*Nearest person month worked:* *1.2*
*Contribution to Project:* *Dr Crawford directs all aspects of this project and provides supervision on the overall approach and data interpretation for the experiments outlined in this application. In particular, she will interact with all of the team members listed on this application and provide full oversight as they implement the work proposed in this application, including regular communication with the expert consultants.*

*Name:* *Joseph Ojo (no change)*
*Project Role:* *Co-PI*
*Researcher Identifier (e.g. ORCID ID):* *N/A*
*Nearest person month worked:* *2.4*
*Contribution to Project:* *Dr. Ojo works alongside Dr. Crawford in directing all aspects of this project and providing supervision on mTBI animal modeling, histopathological analyses and data interpretation. He is responsible for overseeing all aspects of animal manipulation and ensuring that the projects are executed in a timely fashion. He will also perform histopathological assessments in both humans and animal models as described in the proposal.*

*Name:* *Camila Ortiz (no change)*
*Project Role:* *Graduate Student*
*Researcher Identifier (e.g. ORCID ID):* *N/A*
*Nearest person month worked:* *12*
*Contribution to Project:* *Ms. Ortiz will be involved in animal handling and surgical procedures, as well as histopathological characterization and molecular analyses, such as western blotting and ELISA. She will assist in all surgical procedures (primarily years 1 and 2) and conduct histopathological analyses with the supervision of Dr. Ojo (primarily Years 2 & 3).*

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

| |
|---|
| *Nothing to Report* |

**What other organizations were involved as partners?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.*

*Provide the following information for each partnership:*
*Organization Name:*
*Location of Organization: (if foreign location list country)*
*Partner's contribution to the project (identify one or more)*

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

*Organization Name:* Barrow Neurological Institute
*Location of Organization: (if foreign location list country):* Arizona, US
*Partner's contribution to the project:* Collaboration (subaward site) – Dr Mufson uses his laser capture microdissection technique to capture astrocytes on autopsy tissue of TBI/ADRD cases, and conducts single cell array for novel TBI induced astrocyte gene profiles. This work is almost completed. Bioinformatic analyses is currently ongoing.

## 8. SPECIAL REPORTING REQUIREMENTS

**COLLABORATIVE AWARDS:** *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

**QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

*Please find the quad chart attached on the next page.*

## 9. APPENDICES:

*Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

*Please find the quad chart attached on the next page.*

# Astroglia Pathobiology in the Neurodegenerative Sequelae of Repetitive Mild TBI

Log Number AZ180123

W81XWH-19-1-0535

**PI's:** Drs Fiona Crawford & Joseph Ojo **Org:** Roskamp Institute, Sarasota, FL **Award Amount:** $718,000

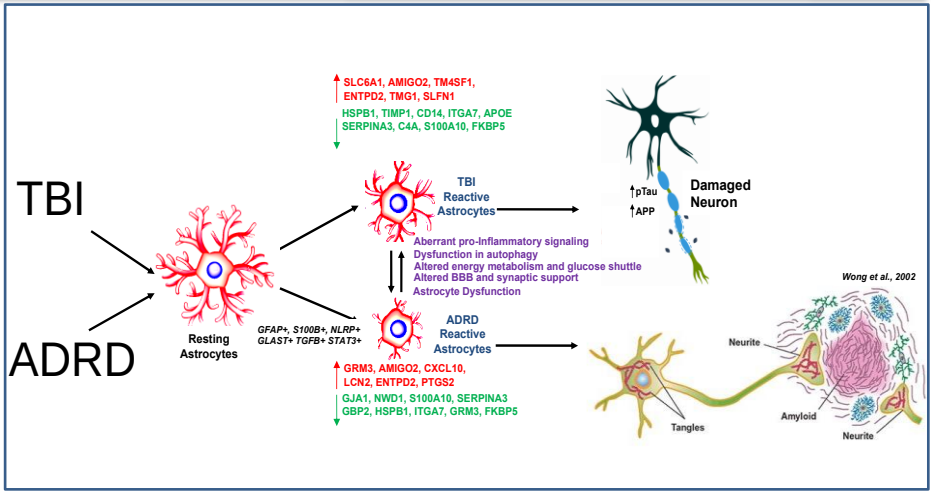
**Study Aim 1:** Delineation of the effects of chronic repetitive mTBI on astrocyte pathobiology and related proteinopathy in tau and amyloid bearing preclinical models at multiple time points post-injury, and validation in autopsied brains from human TBI/ADRD cases. **Study Aim 2:** Evaluating pathobiological effects of reactive/injured astrocytes derived from TBI models, on the induction and spreading of age-related proteinopathies and neurodegeneration. **Study Aim 3:** Generation of single cell omic profiles in different populations of astrocytes obtained from preclinical models and validation in autopsied brains from human TBI/ADRD cases.

**Approach**

i) Histopathological assessment of astrocyte pathobiology in TauKI, APP$^{NLF}$, and GFAP-Tau$^{WT}$ mouse models exposed to r-mTBI or sham injury, when young (3 mos) or aged (12mos) and analyzed at 24hr, 3 and 9mos post-injury. Histopathological assessment of astrocyte lesions in human ADRD/TBI cases.

ii) Adoptive transfer of TBI astrocytes into naïve mice (in vivo), and exposure of TBI astrocyte secretomes to healthy neural/vascular cells (ex vivo). Assessment of pathobiological changes induced by reactive astrocytes.

iii) Single cell omic profiles of astrocytes obtained from models above. Validation of distinct astrocyte cell population of interest and gene profiles using flow cytometry and cell sorting. Single cell gene profiles of astrocytes from autopsied human brains.




**Accomplishment**: (1) Complete transcriptomic analyses of astrocytes from different mouse models r-mTBI and autopsy tissue. (2) Interrogated ex vivo behavior of astrocytes from models above. (3) Began investigating spreading of tau astrogliopathy after r-mTBI

## Timeline and Cost

| Activities CY | 19-20 | 20-21 | 21-22 |
|---|---|---|---|
| MAJOR TASK ONE OR AIM 1 | | | |
| MAJOR TASK TWO OR AIM 2 | | | |
| MAJOR TASK TWO OR AIM 3 | | | |
| **Estimated Direct Budget (500K)** | **$129K** | **$205K** | **$166K** |

**Last updated:** (September 2019)

**Goals/Milestones**

**CY19 Goal**

☑ Obtain regulatory approval to begin animal and human specimen studies

**CY20 Goals**

☑ Breeding and administering injuries to different mouse models

☑ Begin histological assessment of human control/TBI/ADRD brains tissue

☑ Begin gene analyses of microdissected astrocyte from autopsy brain tissue

**CY21 Goals**

☑ Histological assessment of astrocyte and proteinopathy in mouse models

☑ Conduct adoptive transfer studies and ex vivo functional assay studies.

☑ Complete human histological and gene array analyses

**CY22 Goal**

☑ Gene/protein analyses of astrocyte from mouse models

☑ Validation experiments of gene array/proteomic findings in mice/humans

**Comments/Challenges/Issues/Concerns**

- Imaging for mouse histopathology staining impacted by COVID restrictions.
- LCM needed replacement, and LCM work has been held back by 3 months.

**Projected Expenditure**: $718,348 **Actual Expenditure**: $718,348