AWARD NUMBER: W81XWH-19-1-0535

TITLE: Astroglia Pathobiology in the Neurodegenerative Sequelae of Repetitive Mild Traumatic Brain Injury

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REPORT DATE: August 2020

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE: AUGUST 2020	**2. REPORT TYPE: Annual**	**3. DATES COVERED: 08/01/2019 – 07/31/2020**

4. TITLE AND SUBTITLE: Astroglia Pathobiology in the Neurodegenerative Sequelae of Repetitive Mild Traumatic Brain Injury.

5a. CONTRACT NUMBER
W81XWH-19-1-0535

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Roskamp Institute, Inc., 2040 Whitfield Avenue Sarasota, FL 34243-3922

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT: Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES: N/A

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. We have begun analyzing astrocyte lesions in tissue from both mice and humans, and observe qualitative changes to astrocyte morphologies, but no quantitative data has been yet finalized. 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 also 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. These studies are still ongoing, with the latter demonstrating that factors released by injured astrocytes can directly impact on health of other glial cell types (i.e. activate stat3 and pNFkB signaling etc..). As we are generating tissue samples from earlier timepoints from our mouse models, we have begun to apply our state of the art single cell genomic analyses to identify astrocyte specific changes at the gene level for comparison with autopsy cases. These studies are also ongoing and we plan to generate qualitative data on the younger cohort of mice in the next quarter.

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**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
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INTRODUCTION:

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 [40% complete]
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 [15% 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 [15% 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 amyloid antibodies.	24-27	
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 continuation of our mouse breeding regimen and administration of injuries to young and aged cohorts for the longitudinal mouse study, including performing human tissue immunostaining with astrocyte markers.

Our ORP Cadaver form submitted on the 7th of August 2019 has been approved (14th February 2020) for beginning our histological and single cell studies on human autopsy brain tissue. We currently have AD, TBI and control samples in our Roskamp BioBank. We began requesting for FTD-Tau and ARTAG human tissue samples from the NIH BioBank through Dr Purohit. We are still waiting for the approval of the request. We have now stained AD, TBI and control human

tissue samples with these following markers using immunofluorescence and immunohistochemistry – GFAP/AQ4/MC1, GFAP/Laminin/4G8, GFAP/IBA1/TGFß, GFAP/GLT-1/S100ß and GFAP.

We have completed the breeding and ageing of young and aged cohorts of TauKI and $APP^{NLF/NLF}$ mice, with a majority of these already exposed to their injuries and awaiting euthanasia. We began the breeding and ageing of transgenic mice (GFAP-Tau^{WT} and P301S mice) for the young cohorts, with half of both genotype mice exposed to their injuries.

Below Are The Tasks Initiated Or Completed During This Reporting Period:

Major Task 1

Subtask 1: Obtaining ACURO approval (Roskamp Institute approval for TBI procedures is already in place) and Human Cadaver use approval. **COMPLETED**

Substask 2a: Breeding of cohorts for Aged mice TBI studies (36 mice per genotype for TauKI, GFAP-Tau^{WT}, APP^{NLF}) at 24hrs, 3 & 9 months post-TBI. **ONGOING (70% COMPLETED)**

Subtask 2b: Breeding of cohorts for Young mice TBI studies (36 mice per genotype for TauKI, rTg4510, GFAP-Tau^{WT}, APP^{NLF}) **ONGOING (COMPLETED)**

Subtask 3: Histopathological analyses of astroglia lesions in autopsy cases. 10 Groups will consist of human AD (classified as Braak stage I-III and IV-VI), ARTAG (70-80yrs and >90years); FTD (40-50yrs and >60yrs) and TBI brains (40-50yrs and >60yrs) and two age-matched controls (40-60yrs, and >70yrs). N=10/group; 100 samples. Samples are coded and de-identified. **ONGOING (50% COMPLETED).**

Subtask 4: Administering injuries to Young cohort - 3 months old at injury. **(COMPLETED).**

Subtask 5: Euthanasia of Young cohort in subtask 4. **ONGOING (80% COMPLETED).**

Subtask 6: Sectioning and histopathological staining of Young cohort tissues with astroglia, tau and amyloid antibodies; and TUNEL, BrdU, EM preps. **ONGOING (15% COMPLETED).**

Subtask 7: Stereological analyses and Image quantitation of brain sections from Young cohort (staggered over time) **RECENTLY STARTED**

Subtask 8: Administering injuries to Aged cohort - 12 months old at injury. Animals will receive injuries as in subtask 4. **ONGOING (70% COMPLETED).**

Subtask 9: Euthanasia of Aged cohort in subtask 8. **ONGOING (50% COMPLETED).**

Major Task 2

We began optimizing two separate techniques required for studies in Major Task 2. These techniques involved optimization of conditions required for our adoptive cell transfer studies and generation of primary cell cultures for our ex vivo studies. We have now successfully cultured astrocyte cells isolated from sham/injured TauKI mice for colonization in a t75 flask. We have used a trituration/enzymatic degradation/Percoll centrifugation protocol under ascetic conditions to isolate a mixed glial cells population. The bottom astrocyte layer was separated from the top layer after 14days, and further allowed to colonize the flask. We have conducted a pilot study for subtask 4 involving exposure of conditioned media (astrocyte secretomes) to healthy neural and microvascular cells. We have also conducted a preliminary study isolating a murine-EGFP-labelled glial cell population using glia specific militenyl beads, and injecting half a million cells into the brains of recipient mice (using ICC vs intranasal routes). Recipient mice were euthanized after 2 months of injection, and we observe that the IC injected GFP+cells appears to penetrate the brain

more readily in larger populations than intranasal administered GFP+cells. Donor mice are currently being aged for these studies.

Subtask 1: Breeding of cohorts for Young Donor mice for TBI studies in Subtask 2 and 3 (12 mice per genotype for $GFAP^{GFP}$, hTau x $GFAP^{GFP}$, rTg4510 x $GFAP^{GFP}$, GFAP-TauWT x $GFAP^{GFP}$, APP^{NLF} x $GFAP^{GFP}$). **ONGOING (30% COMPLETED).**
Subtask 2: Administering injuries to Young 'Donor' cohort - 3 months old injury. Animals will receive injuries as in Major task 1 (subtask 4). **ONGOING (15% COMPLETED).**
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. **PLANNED FOR Y2Q2.**
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 (25% COMPLETED).**

Major Task 3
As mentioned above, we currently have AD, TBI and control samples in our Roskamp BioBank. We began requesting for FTD-Tau and ARTAG human tissue samples from the NIH BioBank through Dr Purohit, and are still waiting for the approval of the request. We plan to begin LCM and gene array analyses on GFAP stained serial sections once all the samples have been received. AD, TBI and control samples have been shipped to Dr Mufson.

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. **PLANNED FOR Y2Q2**
Subtask 2a: Laser capture microdissection of astroglia from Young Cohort TBI/SHAM mice for single cell array for gene expression profiling. There will be 36 mouse brains per genotype for analyses. **ONGOING [1st set of data in the next quarter]**

BELOW IS A SUMMARY OF THE MAIN FINDINGS FROM OUR STUDIES THIS YEAR

PART 1: ASTROGLIA ANALYSES IN AUTOPSY AND MOUSE TISSUE

Table 1 shows group classification and history of de-identified patients analyzed for astroglia markers.

Table 1: Group Classification and History

Disease status	Age (yrs)	CDR	Plaque count	Braak Stage
Low CDR/Plaque AD	93.00	0.30	5.21	4.80
High CDR/Braak AD	76.91	2.36	18.14	6.00
Non Demented	79.64	0.09	0.15	1.00
Repetitive MTBI	61.86	-	N/A	N/A

Note: TBI patients presented herein have evidence of 2 or more repetitive mild traumatic brain injuries with several decades post-survival.

Standard IF protocol was used for antibody optimization as previously detailed (see Ojo et al., 2018).

Human Tissue optimization for astrocyte specific antibodies

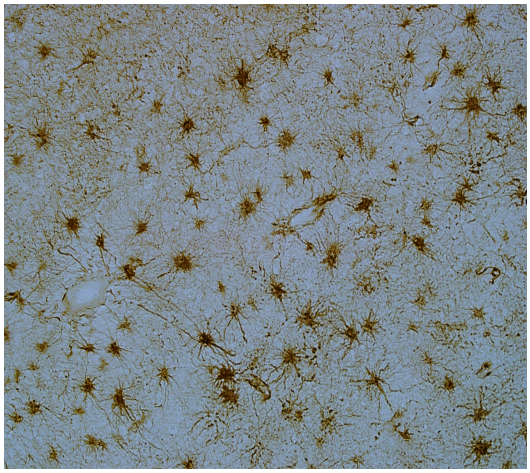


Fig.1 GFAP-DAB staining in human tissue. Magnification 20X.

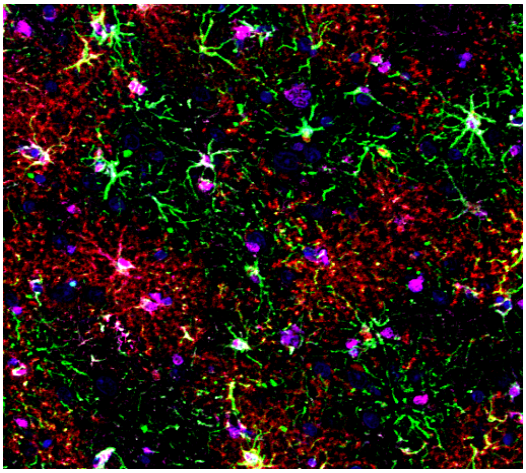


Fig.2 Immunofluorescence staining human tissue. GFAP (green), GLT1 (red) and S100b (purple). Magnification 20X.

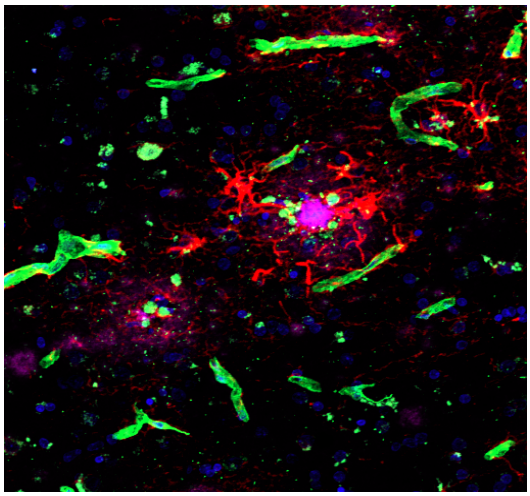


Fig.4 Immunofluorescence staining human tissue. GFAP (red), 4G8 (purple) and Laminin (green). Magnification 20X.

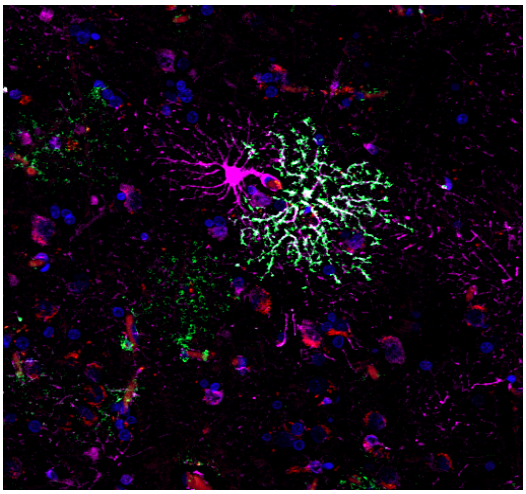


Fig.3 Immunofluorescence staining human tissue. GFAP (purple), MC1 (red) and AQ4 (green). Magnification 20X.

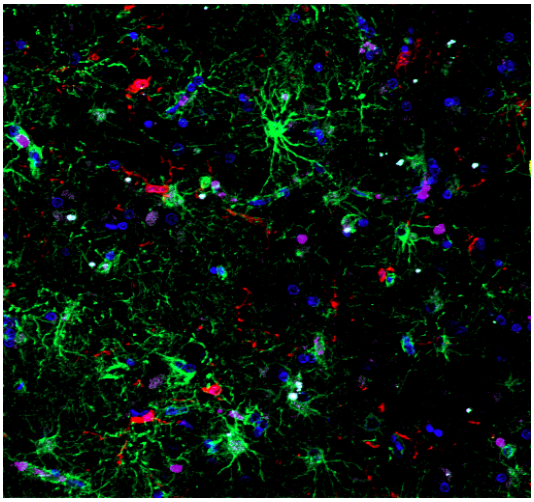


Fig.5 Immunofluorescence staining human tissue. GFAP (green), TGFB1 (purple) and IBA1 (red). Magnification 20X.

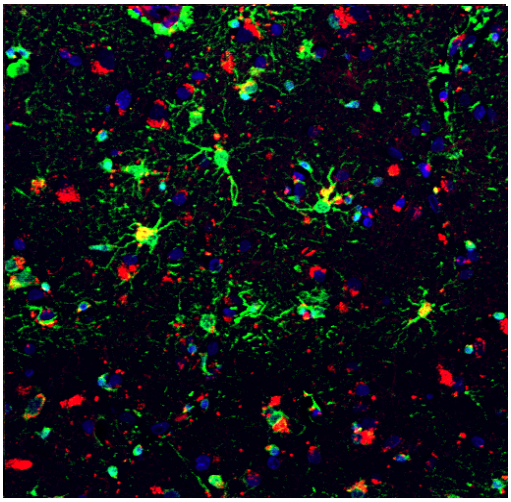


Fig.6 Immunofluorescence staining human tissue. GFAP (green) and MC1 (Red). Mag 20X.

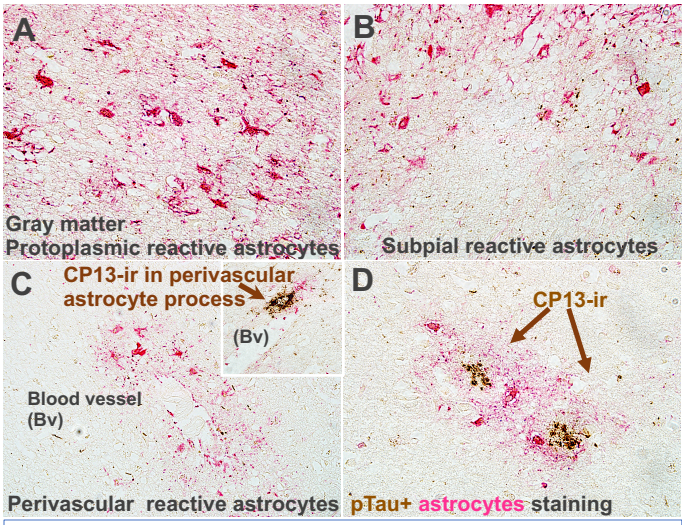


Fig. 7: Astroglia phenotypes in a TBI brain

PART TWO: Mouse Tissue Optimization For Astrocyte Specific Antibodies

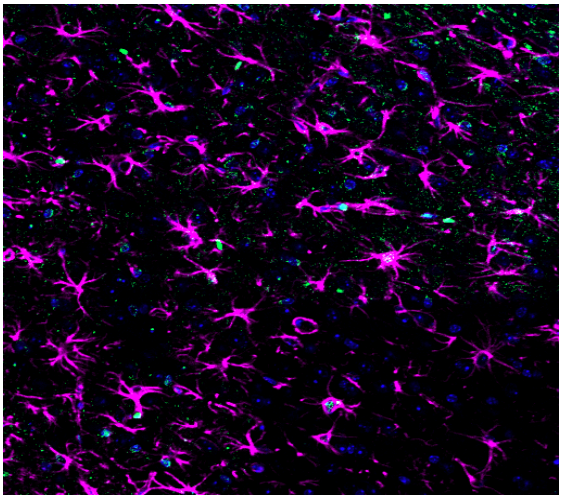


Fig.1 Immunofluorescence staining mouse tissue. GFAP (violet) and pSTAT3 (green). Magnification 20X.

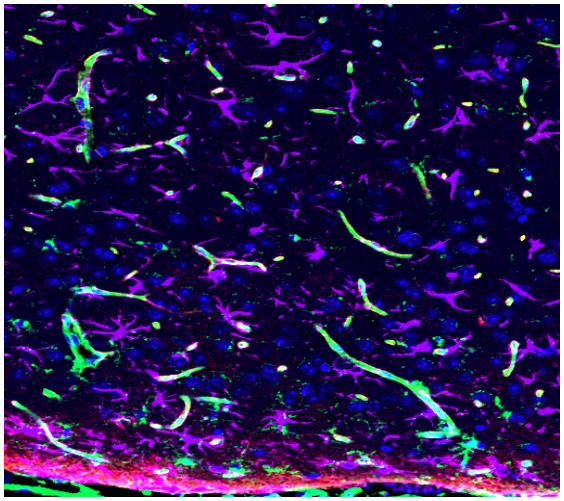


Fig.2 Immunofluorescence staining mouse tissue. GFAP (purple) and Lectin (green). Magnification 20X.

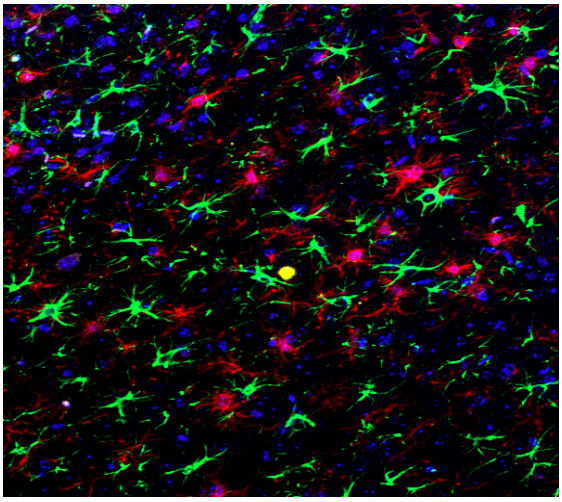


Fig.3 Immunofluorescence staining mouse tissue. GFAP (green), IBA1 (red). Magnification 20X.

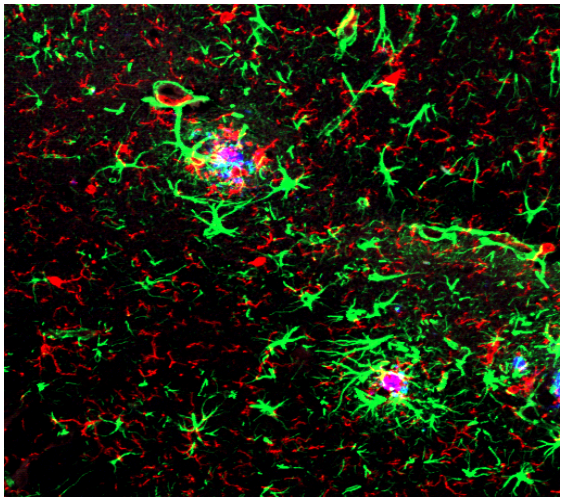


Fig.4 Immunofluorescence staining mouse tissue. GFAP (green), IBA1 (red) and methoxy-4 (blue). In purple colocalization of IBA1 and methoxy-04. Magnification 20X.

Astroglia Tau pathology and astrogliosis in GFAP-Tau mice exposed to r-mTBI.
PILOT STUDY (N=3)

We began to investigate tau pathology in mice engineered to produce human 4R tau in astrocytes. These mice begin to develop astrocyte p-tau pathology at 15 mos of age. Exposure to our r-mTBI injury paradigm at 3 mos of age, in a small pilot study, showed a significant upregulation of GFAP and S100B in the cortex and corpus callosum 24-48hrs after the last injury (**Fig. 5 ABC vs DEF or GHI**). pTau pathology was very remarkable and coincided with the pattern of GFAP staining in the cortex and corpus callosum (**Fig. 6 ABC vs DEF or GHI**). Microgliosis (IBA1+) was also prominent in this model after injury (**Fig. 7 AD vs BE or CF**). However, we have not explored a more chronic timepoint in this model (currently ongoing).

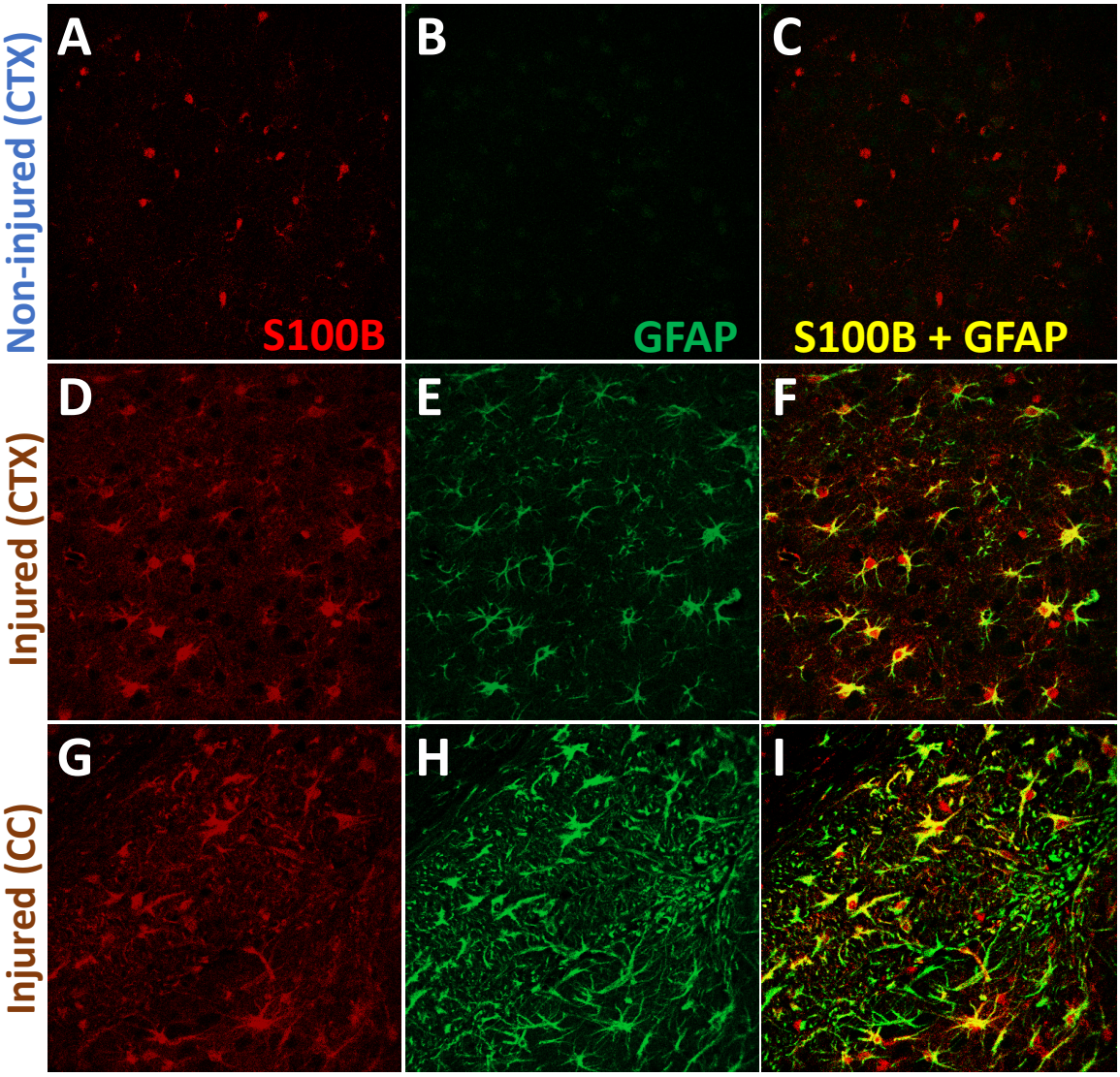


Figure 5: S100B-ir (ADG) and GFAP (BEH) in a GFAP-Tau model of r-mTBI. Cortex (CTX), Corpus Callosum (CC).

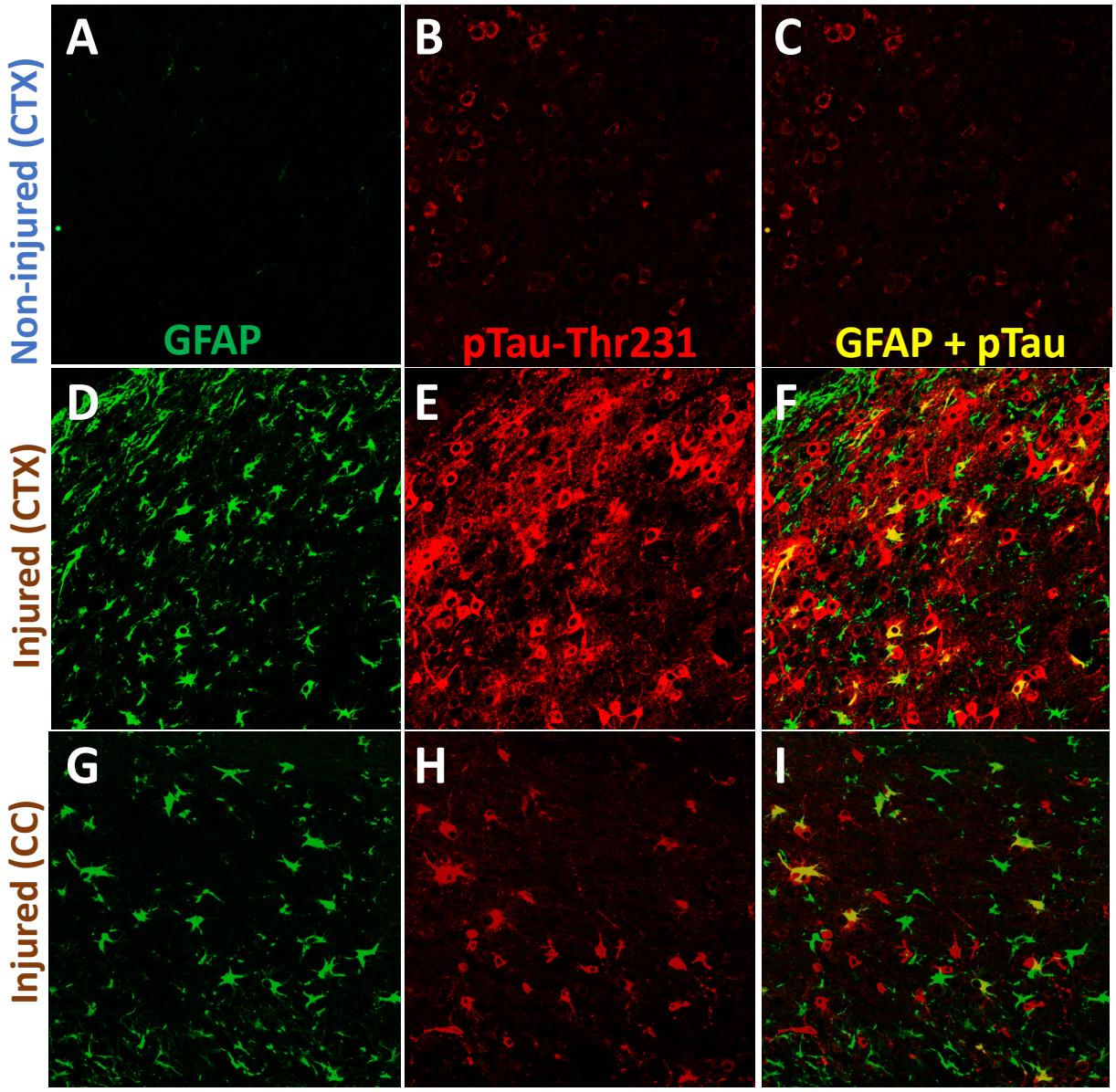


Figure 6: GFAP-ir (ADG) and pTau (BEH) staining in a GFAP-Tau model of r-mTBI. Cortex (CTX), Corpus Callosum (CC).

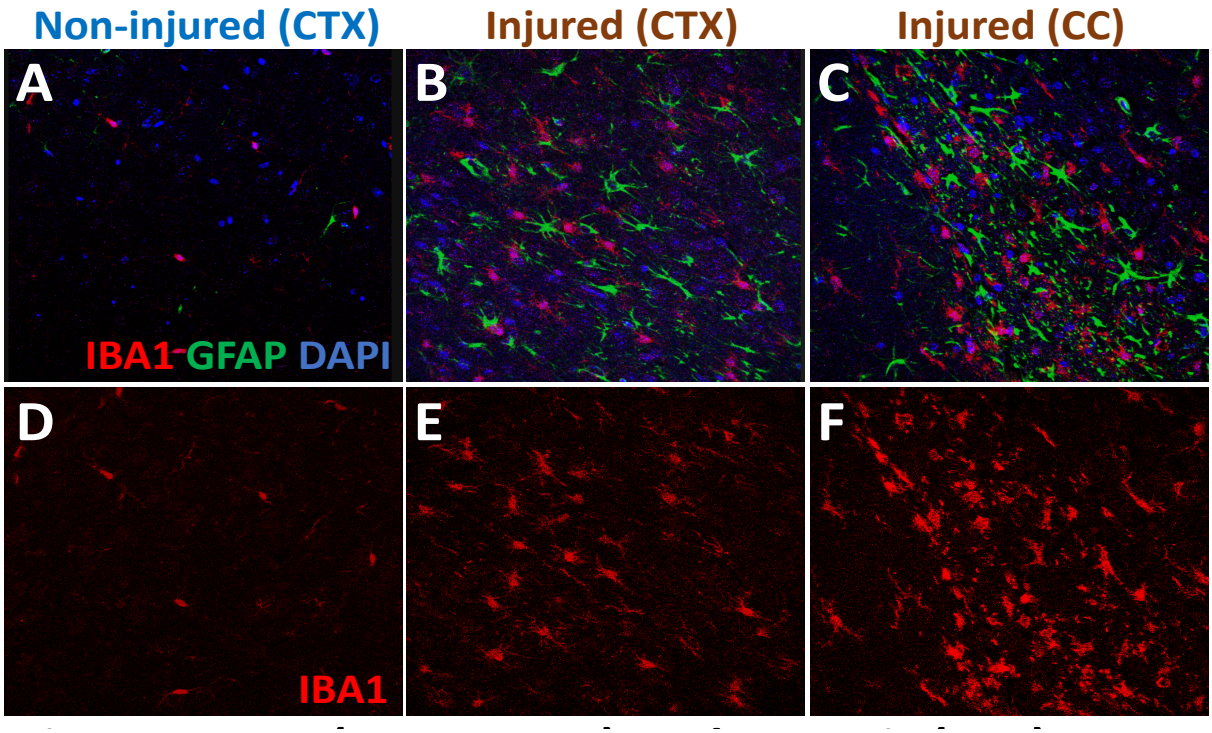


Figure 7: IBA1 (AD, BD, CF) and GFAP-ir (A-C) profiles in a GFAP-Tau model of r-mTBI. Cortex (CTX), Corpus Callosum (CC).

TauKI mouse model at 14 days and 3 months post rmTBI exposure. There is a notable increase in astrogliosis and microgliosis at both timepoints, which correlates with PHF1 increase and deficits in spatial learning.

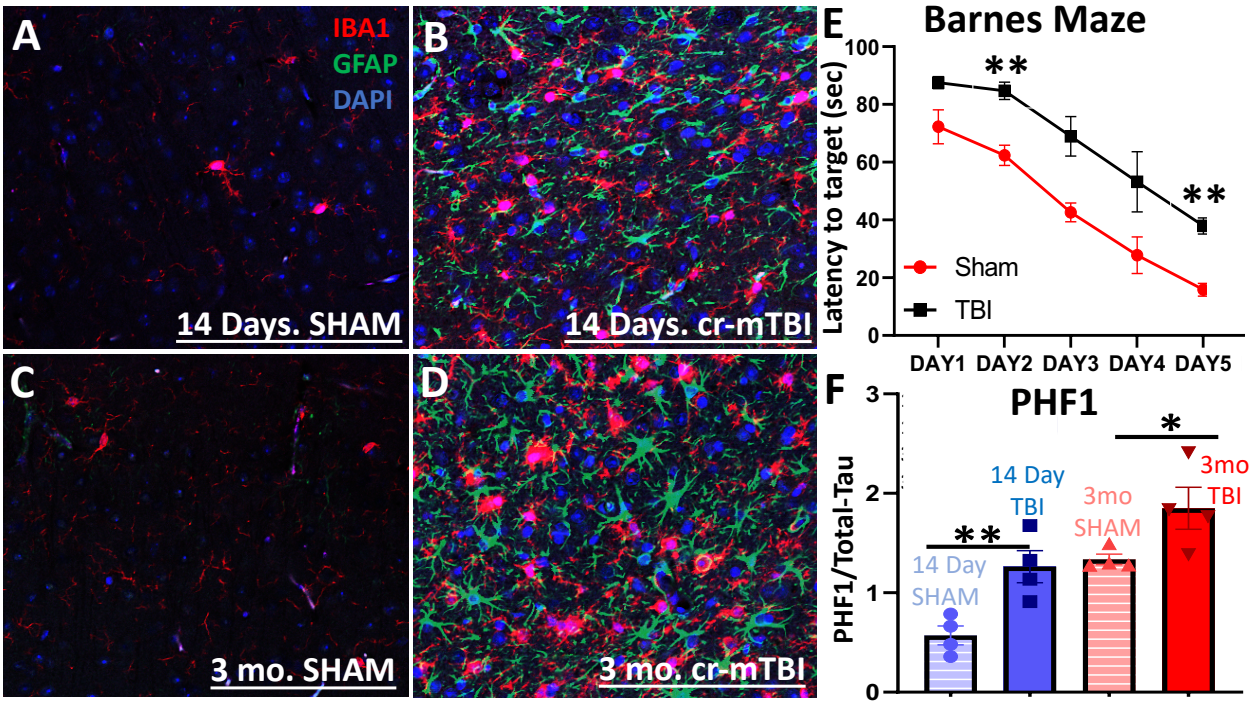
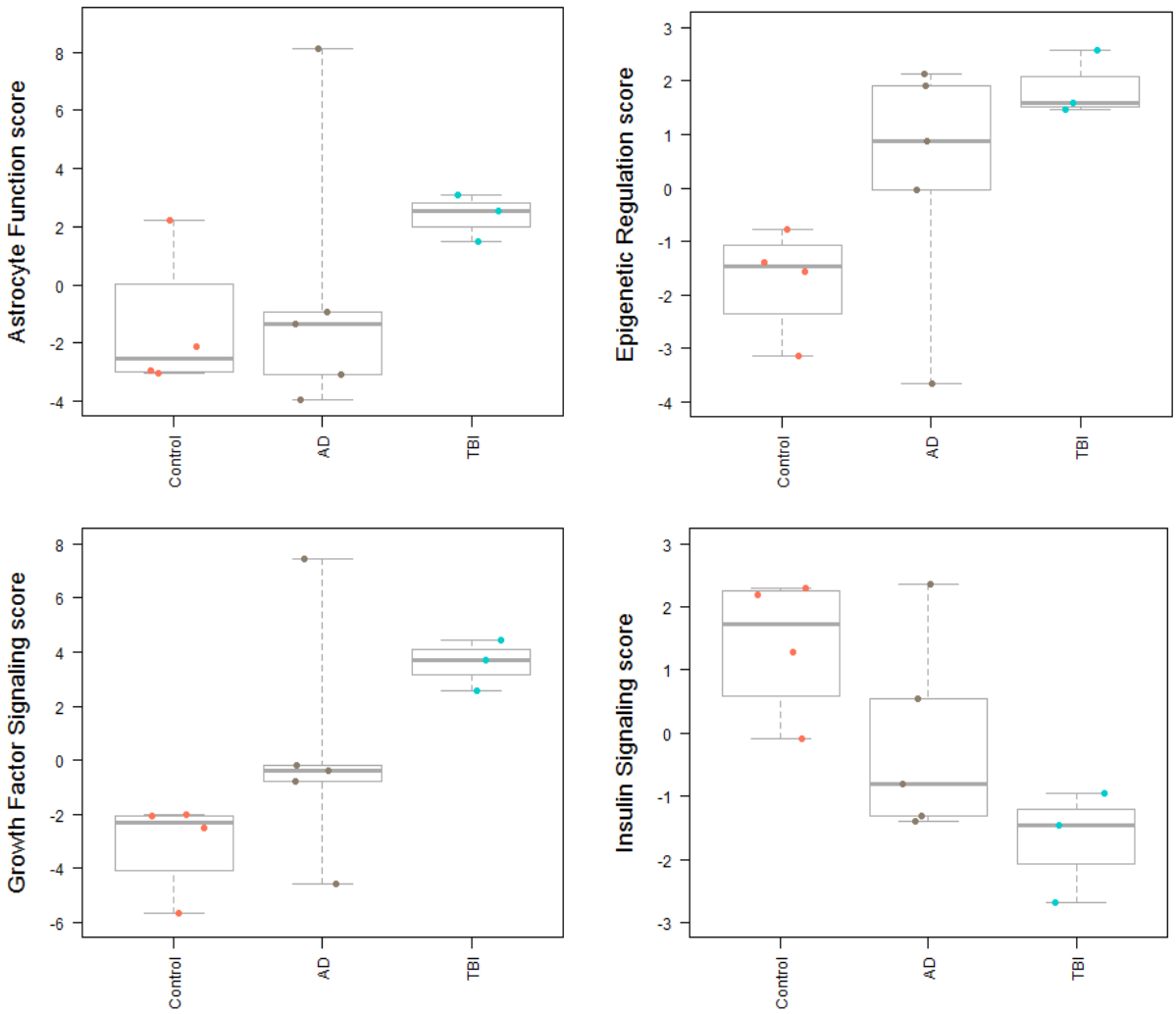


Figure 8. Neurobehavior, glial and pTau changes in a TauKI cr-mTBI/sham model. A-D shows IBA1/GFAP in the cortex. E shows latency to target hole during the Barnes maze test, 3mo post-injury. F shows PHF1/DA9 immunoblotting data. Data=Mean±SEM. * P<0.05; ** P<0.01. N=5/6.

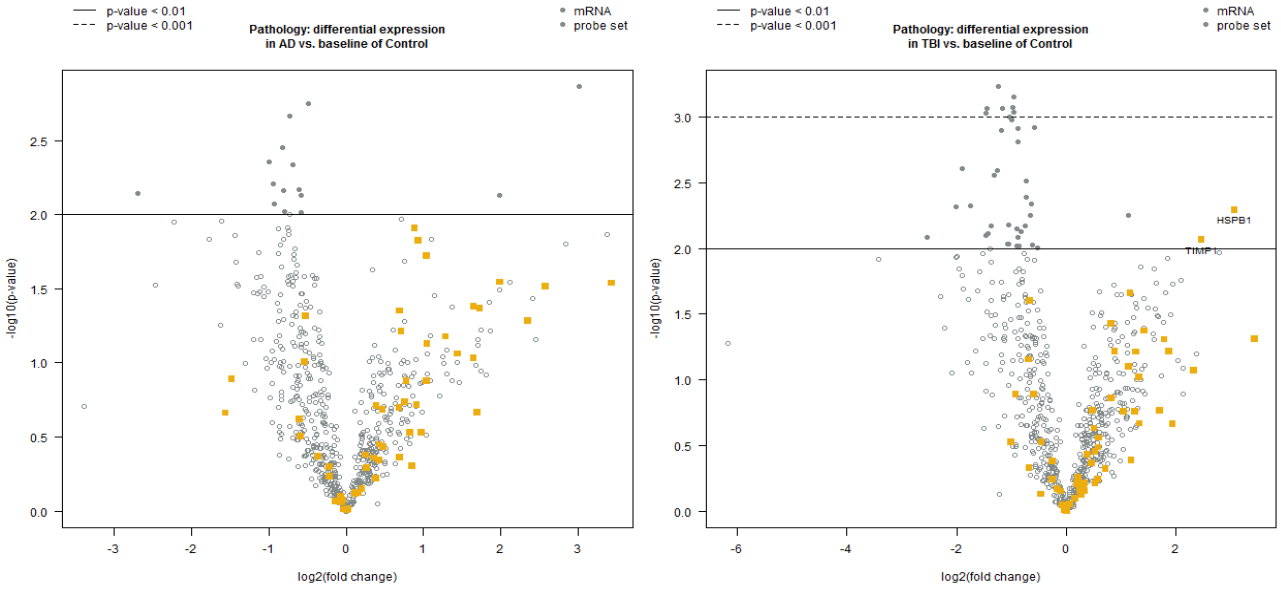
PART THREE: SINGLE CELL GENE ARRAY OPTIMIZATION

(Human pilot study [N=4/group] Control, TBI, AD cases)

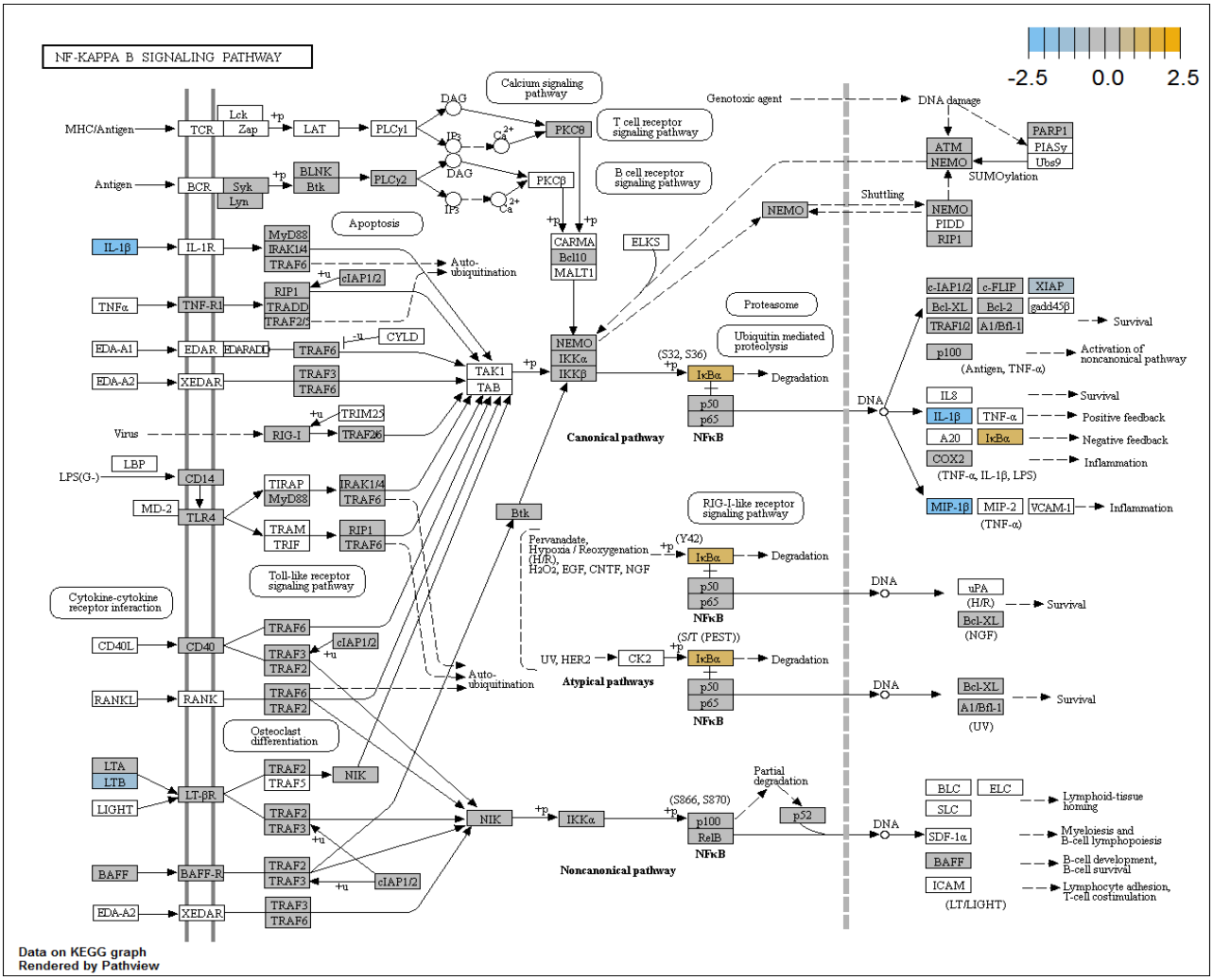
METHODS - Nanostrings gene transcript profiling: RNA extraction and NanoString quantification. Total RNA was extracted from frozen tissue samples using RNA Isolation Kit according to the manufacturer's protocol. Concentrations of extracted RNA were determined using the NanoDrop Spectrophotometer. Samples with RNA concentrations of <40 ng/μL, A260/A280 ratios <1.5 or A260/230 ratios <1.0 were considered as inadequate and were excluded from the analysis. A NanoString panel was designed, comprising 770 previously published inflammatory genes. Additionally, five housekeeping genes showing minimal alteration across samples were also included as controls. The custom-designed probes included a 100-bp region targeting the mRNA, with two sequence-specific, fluorescent-barcoded probes for each target (3' biotinylated capture probe and a 5' reporter probe). Probes and 100 ng total RNA were hybridised overnight at 65°C according to the manufacturer's protocol. A NanoString nCounter Digital Analyzer (NanoString Technologies, Seattle, Washington, USA) was used to count the digital barcodes representing the number of transcripts. The raw expression data were normalised using nSolver Analysis software. A normalisation factor was calculated by obtaining the geometric mean of the positive controls used for each sample and applied to the raw counts of the nCounter output data to eliminate variability that was unrelated to the samples. The resulting data were normalised again with the geometric mean of the housekeeping genes. Normalised data were $\log_2$-transformed for further analyses. **Statistical analysis** - The normalised $\log_2$-transformed mRNA expression data were analysed by unsupervised hierarchical clustering using Cluster V.3.0 and Java Tree view software. Heat maps showing high and low expression of genes in the subtypes were generated and categorized on the basis of these expression patterns. Statistical analysis was performed between TBI vs control groups using T-Test, and a p value <0.05 was considered significant. All p values were adjusted using a false discovery rate (FDR) correction of 5%. All tests were two-sided at the significance level $p<0.05$.

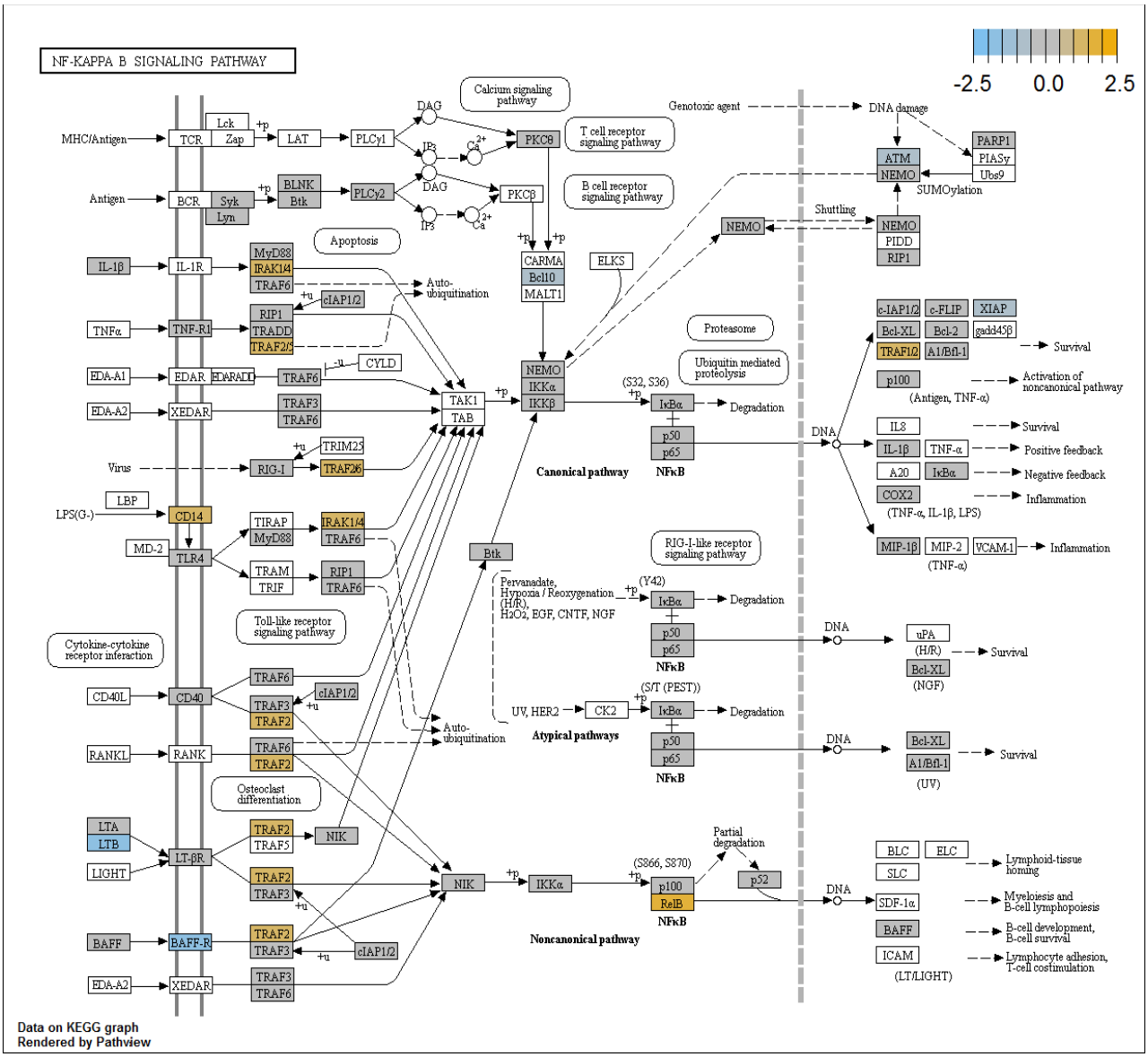


Gene enrichment analysis for astrocyte function, growth factor signaling, epigenetic regulation and insulin signaling scores in Control, TBI and AD human tissue

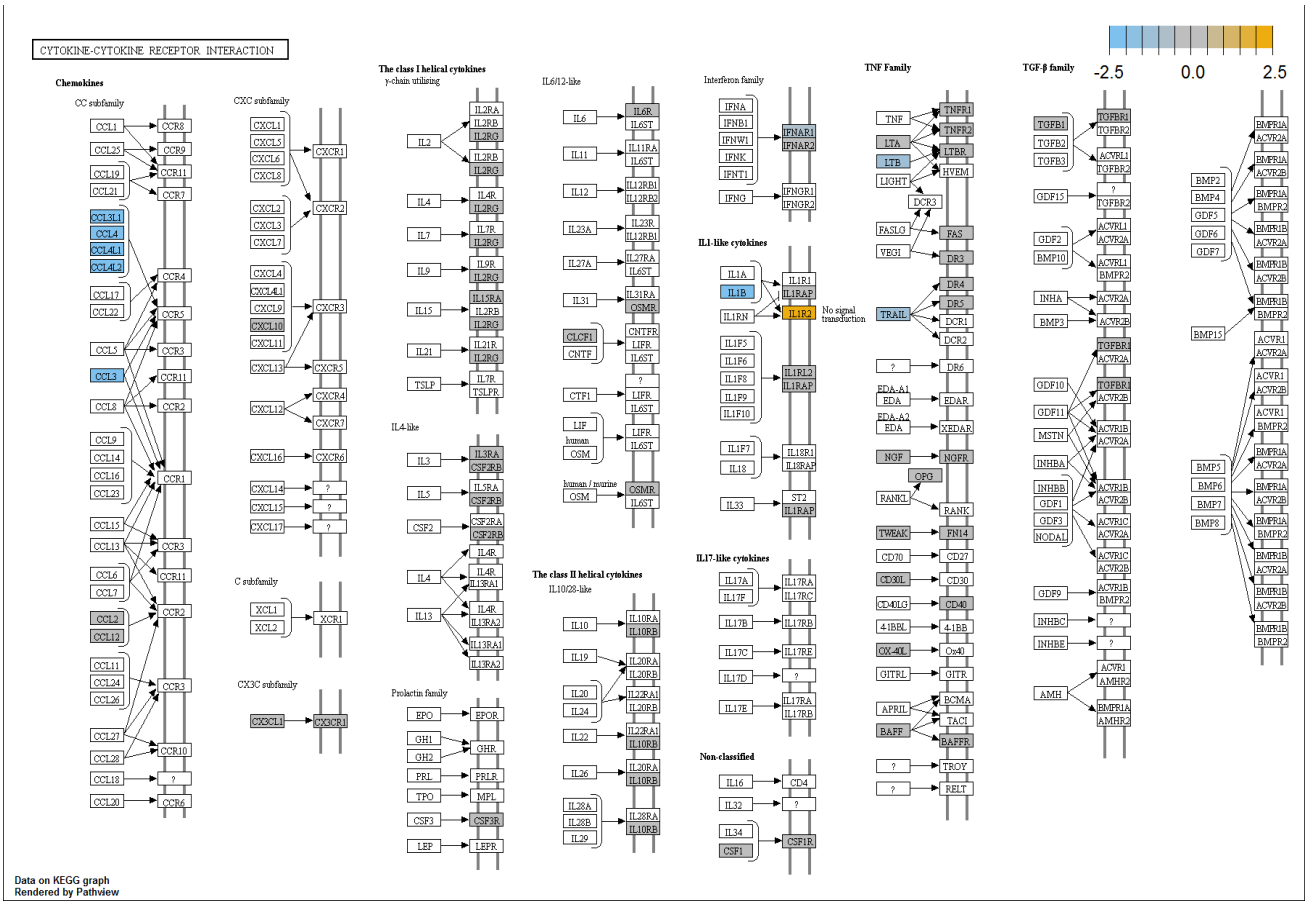


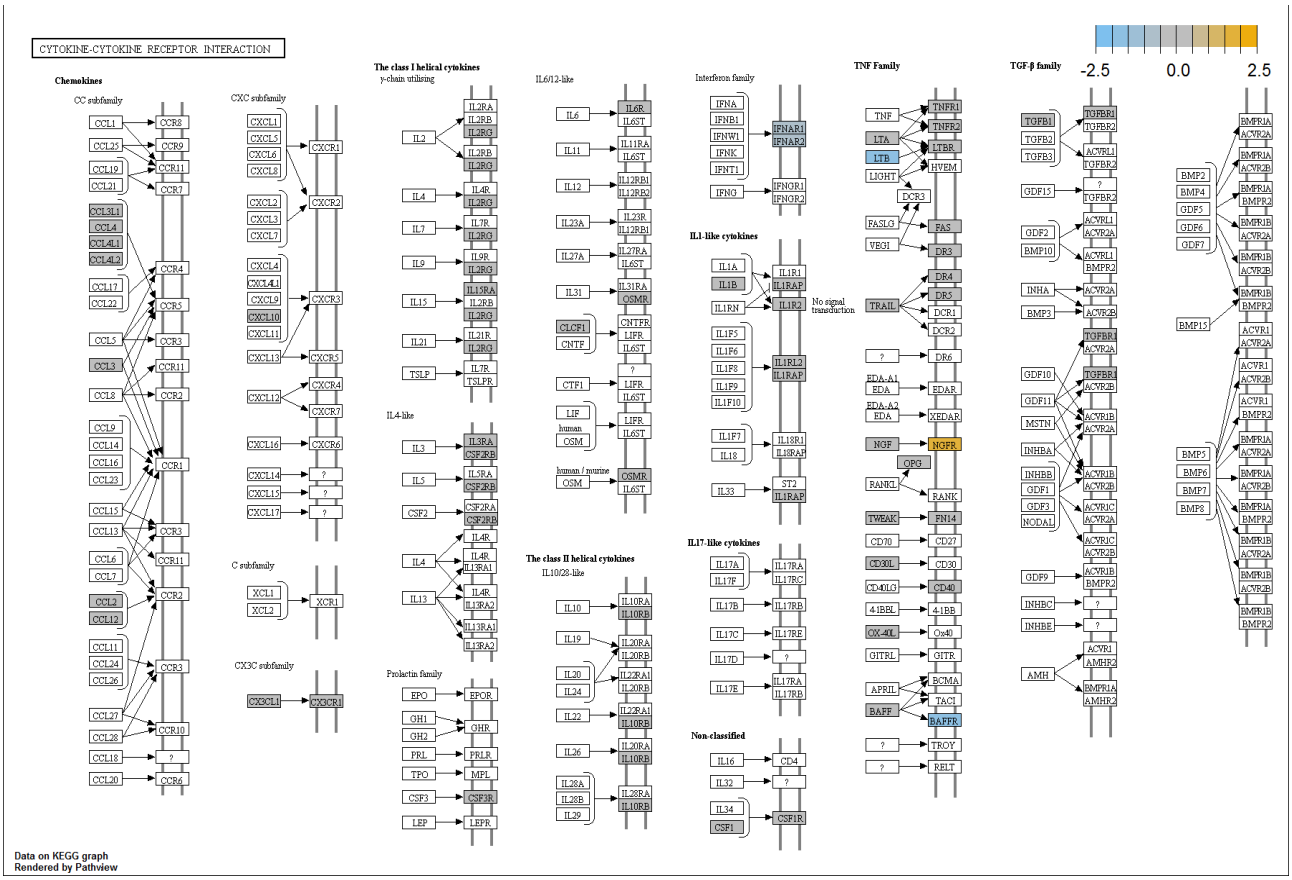
Volcano plot of significantly regulated genes in astrocytes from Control, TBI and AD human tissue





Altered genes in NF KAPPA B signaling pathway from Control vs TBI and Control vs AD human tissue


CYTOKINE-CYTOKINE RECEPTOR INTERACTION
Chemokines
CC subfamily
CXC subfamily
C subfamily
CX3C subfamily
The class I helical cytokines
γ-chain utilizing
IL4-like
Prolactin family
IL6/12-like
The class II helical cytokines
IL10/28-like
Interferon family
IL1-like cytokines
No signal transduction
IL17-like cytokines
Non-classified
TNF Family
TGF-β family
-2.5
0.0
2.5
Data on KEGG graph
Rendered by Pathview



Altered genes in cytokine-cytokine receptor interaction from Control vs TBI and Control vs AD human tissue

Probe Label	Log2 fold change	Gene.sets
GJA1-mRNA	0.888	Astrocyte Function, Neurons and Neurotransmission
NWD1-mRNA	0.94	Astrocyte Function
FKBP5-mRNA	1.04	Astrocyte Function
S100A10-mRNA	1.99	Astrocyte Function
SERPINA3-mRNA	3.44	Astrocyte Function
GBP2-mRNA	2.58	Astrocyte Function, Inflammatory Signaling
C4A-mRNA	1.65	Astrocyte Function
HSPB1-mRNA	1.73	Angiogenesis, Astrocyte Function, Cellular Stress, Growth Factor Signaling
ITGA7-mRNA	0.695	Astrocyte Function, Growth Factor Signaling, Matrix Remodeling
GRM3-mRNA	-0.523	Astrocyte Function, Neurons and Neurotransmission
CD44-mRNA	2.35	Astrocyte Function, Autophagy, Inflammatory Signaling, Matrix Remodeling, Microglia Function
ALDH1L1-mRNA	0.715	Astrocyte Function
PADI2-mRNA	1.29	Astrocyte Function, Epigenetic Regulation
VIM-mRNA	1.05	Apoptosis, Astrocyte Function
OSMR-mRNA	1.44	Astrocyte Function, Cytokine Signaling, Growth Factor Signaling
EMP1-mRNA	1.65	Astrocyte Function
AMIGO2-mRNA	-0.537	Astrocyte Function
CXCL10-mRNA	-1.48	Astrocyte Function, Cytokine Signaling, Inflammatory Signaling, Innate Immune Response, Microglia Function
CP-mRNA	1.04	Astrocyte Function
SERPING1-mRNA	0.781	Astrocyte Function
APOE-mRNA	0.762	Astrocyte Function, Cellular Stress, Lipid Metabolism, Microglia Function, Neurons and Neurotransmission
TIMP1-mRNA	0.915	Astrocyte Function, Matrix Remodeling
SOX9-mRNA	0.392	Astrocyte Function, Wnt
CD109-mRNA	0.687	Astrocyte Function
SLC1A3-mRNA	0.475	Astrocyte Function, Neurons and Neurotransmission
CCL2-mRNA	1.7	Astrocyte Function, Cytokine Signaling, Inflammatory Signaling, Innate Immune Response
LCN2-mRNA	-1.56	Astrocyte Function, Inflammatory Signaling
ENTPD2-mRNA	-0.602	Astrocyte Function
SRGN-mRNA	0.832	Astrocyte Function, Inflammatory Signaling
SPHK1-mRNA	0.973	Angiogenesis, Astrocyte Function
PTGS2-mRNA	-0.588	Astrocyte Function, Cellular Stress, Innate Immune Response, Lipid Metabolism, Microglia Function, NF-kB
HLA-E-mRNA	0.432	Adaptive Immune Response, Astrocyte Function, Inflammatory Signaling, Innate Immune Response, Matrix Remodeling
AGT-mRNA	0.478	Astrocyte Function
EGFR-mRNA	0.257	Adaptive Immune Response, Angiogenesis, Astrocyte Function, Cytokine Signaling, Growth Factor Signaling, Insulin Signaling
TGFA-mRNA	-0.366	Astrocyte Function, Microglia Function, Neurons and Neurotransmission
SOCS3-mRNA	0.695	Adaptive Immune Response, Astrocyte Function, Cytokine Signaling, Inflammatory Signaling, Innate Immune Response
GDPD2-mRNA	0.363	Astrocyte Function, Lipid Metabolism
S1PR3-mRNA	0.429	Astrocyte Function, Neurons and Neurotransmission
CLCF1-mRNA	0.858	Astrocyte Function, Cytokine Signaling
TM4SF1-mRNA	-0.218	Astrocyte Function
KCNJ10-mRNA	0.263	Astrocyte Function, Neurons and Neurotransmission
CD14-mRNA	-0.213	Apoptosis, Astrocyte Function, Autophagy, Growth Factor Signaling, Innate Immune Response, NF-kB
PTX3-mRNA	0.387	Astrocyte Function
B3GNT5-mRNA	0.207	Astrocyte Function
SLFN11-mRNA	0.117	Astrocyte Function
S100B-mRNA	0.157	Astrocyte Function, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response
APC-mRNA	0.122	Apoptosis, Astrocyte Function, Wnt
FBLN5-mRNA	-0.0663	Astrocyte Function, Matrix Remodeling
SLC6A1-mRNA	-0.0456	Astrocyte Function, Neurons and Neurotransmission
STEAP4-mRNA	-0.139	Astrocyte Function
PSMB8-mRNA	-0.0594	Adaptive Immune Response, Angiogenesis, Apoptosis, Astrocyte Function, Cell Cycle, Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling
TGM1-mRNA	0.031	Astrocyte Function
SLC10A6-mRNA	-0.0255	Astrocyte Function
CSF1-mRNA	0.0116	Astrocyte Function, Autophagy, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Microglia Function

Table 1: List of significantly regulated genes in control vs TBI cases

Probe Label	Log2 fold change	Gene.sets
HSPB1-mRNA	3.07	Angiogenesis, Astrocyte Function, Cellular Stress, Growth Factor Signaling
TIMP1-mRNA	2.47	Astrocyte Function, Matrix Remodeling
CD14-mRNA	1.17	Apoptosis, Astrocyte Function, Autophagy, Growth Factor Signaling, Innate Immune Response, NF-kB
SLC6A1-mRNA	-0.672	Astrocyte Function, Neurons and Neurotransmission
ITGA7-mRNA	0.826	Astrocyte Function, Growth Factor Signaling, Matrix Remodeling
APOE-mRNA	1.42	Astrocyte Function, Cellular Stress, Lipid Metabolism, Microglia Function, Neurons and Neurotransmission
SERPINA3-mRNA	3.44	Astrocyte Function
C4A-mRNA	1.79	Astrocyte Function
FKBP5-mRNA	0.888	Astrocyte Function
S100A10-mRNA	1.87	Astrocyte Function
VIM-mRNA	1.27	Apoptosis, Astrocyte Function
AMIGO2-mRNA	-0.683	Astrocyte Function
AGT-mRNA	1.14	Astrocyte Function
CD44-mRNA	2.32	Astrocyte Function, Autophagy, Inflammatory Signaling, Matrix Remodeling, Microglia Function
CP-mRNA	1.33	Astrocyte Function
TM4SF1-mRNA	-0.595	Astrocyte Function
ENTPD2-mRNA	-0.919	Astrocyte Function
GDPD2-mRNA	0.826	Astrocyte Function, Lipid Metabolism
GBP2-mRNA	1.7	Astrocyte Function, Inflammatory Signaling
GJA1-mRNA	0.482	Astrocyte Function, Neurons and Neurotransmission
PADI2-mRNA	1.04	Astrocyte Function, Epigenetic Regulation
OSMR-mRNA	1.26	Astrocyte Function, Cytokine Signaling, Growth Factor Signaling
EMP1-mRNA	1.34	Astrocyte Function
CCL2-mRNA	1.94	Astrocyte Function, Cytokine Signaling, Inflammatory Signaling, Innate Immune Response
SLC1A3-mRNA	0.509	Astrocyte Function, Neurons and Neurotransmission
HLA-E-mRNA	0.588	Adaptive Immune Response, Astrocyte Function, Inflammatory Signaling, Innate Immune Response, Matrix Remodeling
TGM1-mRNA	-1.01	Astrocyte Function
SLFN11-mRNA	-0.451	Astrocyte Function
CD109-mRNA	0.587	Astrocyte Function
SERPING1-mRNA	0.529	Astrocyte Function
PSMB8-mRNA	0.39	Adaptive Immune Response, Angiogenesis, Apoptosis, Astrocyte Function, Cell Cycle, Cytokine Signaling, Growth Factor Signaling, Inflammatory Signaling
CLCF1-mRNA	1.19	Astrocyte Function, Cytokine Signaling
FBLN5-mRNA	-0.253	Astrocyte Function, Matrix Remodeling
S100B-mRNA	0.457	Astrocyte Function, Growth Factor Signaling, Inflammatory Signaling, Innate Immune Response
STEAP4-mRNA	-0.674	Astrocyte Function
SOCS3-mRNA	0.717	Adaptive Immune Response, Astrocyte Function, Cytokine Signaling, Inflammatory Signaling, Innate Immune Response
NWD1-mRNA	0.223	Astrocyte Function
KCNJ10-mRNA	-0.257	Astrocyte Function, Neurons and Neurotransmission
CXCL10-mRNA	0.581	Astrocyte Function, Cytokine Signaling, Inflammatory Signaling, Innate Immune Response, Microglia Function
PTGS2-mRNA	0.332	Astrocyte Function, Cellular Stress, Innate Immune Response, Lipid Metabolism, Microglia Function, NF-kB
SPHK1-mRNA	0.531	Angiogenesis, Astrocyte Function
ALDH1L1-mRNA	0.197	Astrocyte Function
CSF1-mRNA	0.319	Astrocyte Function, Autophagy, Cytokine Signaling, Growth Factor Signaling, Innate Immune Response, Microglia Function
EGFR-mRNA	-0.151	Adaptive Immune Response, Angiogenesis, Astrocyte Function, Cytokine Signaling, Growth Factor Signaling, Insulin Signaling
PTX3-mRNA	0.328	Astrocyte Function
S1PR3-mRNA	0.25	Astrocyte Function, Neurons and Neurotransmission
GRM3-mRNA	-0.102	Astrocyte Function, Neurons and Neurotransmission
LCN2-mRNA	-0.462	Astrocyte Function, Inflammatory Signaling
SRGN-mRNA	0.286	Astrocyte Function, Inflammatory Signaling
B3GNT5-mRNA	0.163	Astrocyte Function
APC-mRNA	0.0686	Apoptosis, Astrocyte Function, Wnt
TGFA-mRNA	-0.0661	Astrocyte Function, Microglia Function, Neurons and Neurotransmission
SLC10A6-mRNA	-0.0255	Astrocyte Function
SOX9-mRNA	0.00631	Astrocyte Function, Wnt

Table 2: List of significantly regulated genes in control vs AD cases

PART FOUR: Pathological Profiles Of Cultured Primary Astrocyte Cells Obtained From Sham nnd TBI Brains

LPS exposure

Mice were exposed to mild TBI (N=1) and sham(N=1) once a day, for five days. Brains were collected and processed under sterilized conditions 24 hours post last injury. Glial cells (astrocytes and microglia) were isolated from brain homogenate using percoll gradient centrifugation and co-cultured at 37ºC for one week. Astrocytes were separated from microglia through shaking at 37ºC for three hours. Isolated astrocytes were cultured for another week until 80% confluency was reached. For the study, both TBI and sham astrocytes were plated into a 24well-plates. After four days, astrocytes were exposed to different concentrations of LPS (5-, 10-, 50-, and 100ng/ml) for 8 hours. After 8 hours, the astrocyte conditioned media (ACM) was collected and frozen until required. TBI and sham astrocytes were frozen and processed next day. Protein was extracted from cell lysates of astrocytes, run in a gel, and probed for specific markers using western blotting.

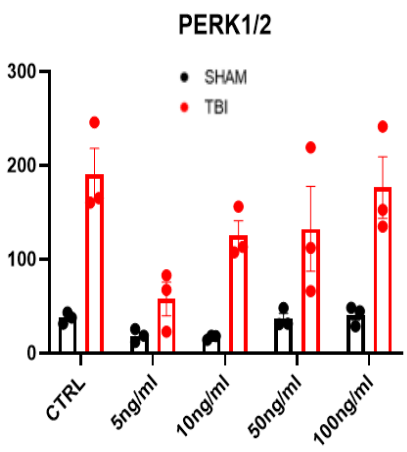


Fig.1 Phospho-ERK1/2 (PERK1/2) levels sham vs r-mTBI primary astrocytes after 8-hour LPS exposure (5, 10, 50, 100 ng/ml). Protein normalized to total ERK1/2 and b-actin.

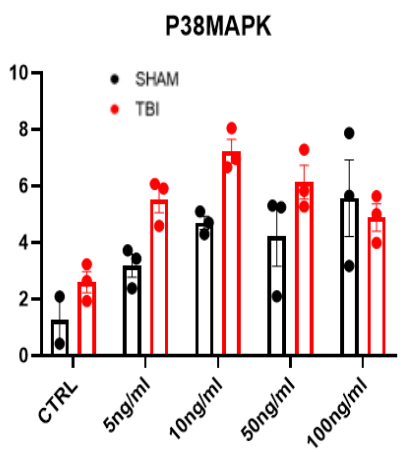


Fig.2 Phospho-MAPK-38 (P38MAPK) levels sham vs r-mTBI primary astrocytes after 8-hour LPS exposure (5, 10, 50, 100 ng/ml). Protein normalized to b-actin.

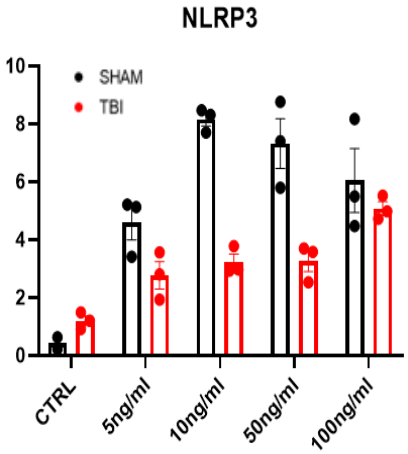


Fig.3 NLRP3 levels sham vs r-mTBI primary astrocytes after 8-hour LPS exposure (5, 10, 50, 100 ng/ml). Protein normalized to b-actin.

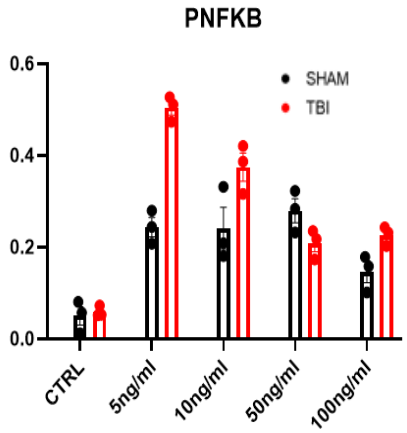


Fig.4 Phospho-NFkB (PNFKB) levels sham vs r-mTBI primary astrocytes after 8-hour LPS exposure (5, 10, 50, 100 ng/ml). Protein normalized to total NFkB and b-actin.

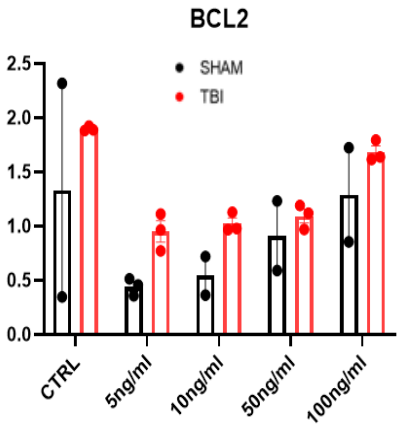


Fig.5 BCL2 levels sham vs r-mTBI primary astrocytes after 8-hour LPS exposure (5, 10, 50, 100 ng/ml). Protein normalized to b-actin.

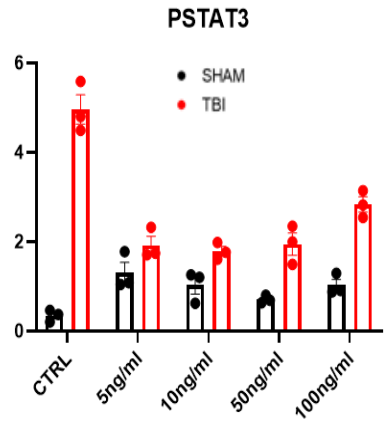


Fig.6 Phospho-STAT3 (PSTAT3) levels sham vs r-mTBI primary astrocytes after 8-hour LPS exposure (5, 10, 50, 100 ng/ml). Protein normalized to b-actin.

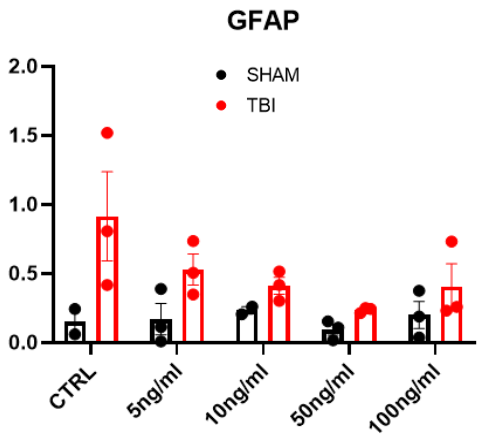


Fig.7 GFAP levels sham vs r-mTBI primary astrocytes after 8-hour LPS exposure (5, 10, 50, 100 ng/ml). Protein normalized to b-actin.

>Unexposed TBI astrocytes show an augmentation of pERK/MAPK, pSTAT3, NLRP3 and GFAP compared to sham astrocytes.
>Exposure to different concentrations of LPS induces differential responses as determined by the cell activation markers measured.
>Note: Timeline of measurement is 8 hours post exposure, therefore we do not expect prolonged responses of phospho-markers at this chronic timepoint.
>We have decided to remove the LPS exposures and focus primarily on sham vs TBI astrocytes (non-exposed to LPS) in our future secretome studies, as we can still clearly observe a TBI induced phenotype.

<u>PART FIVE</u>: Impact Of TBI Induced Astrocyte Secretome On Pathobiological Changes On Healthy Mouse Astrocyte (C8 D1a) Cell Lines

ACM exposure

CD81A cell line astrocytes were cultured until 80% confluency was achieved. CD81A astrocytes were seeded in 24well-plates. After two days, astrocytes were exposed to the ACM from TBI astrocytes exposed to 100ng/ml LPS. CD81A astrocytes were exposed to filtered (using 30KDa microcentrifuge filters) and unfiltered media for different periods of time: 30min, 1h, and 4h. CD81A astrocytes were frozen and processed the next day. Protein was extracted from cell lysates of astrocytes, run in a gel, and probed for specific markers using western blotting.

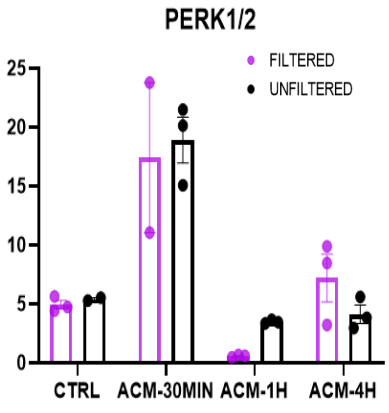


Fig.1 Phospho-ERK1/2 (PERK1/2) levels in CD81A astrocytes treated with Astrocyte Conditioned Media (ACM) from LPS-exposed (100ng/ml) r-mTBI astrocytes for 30min, 1hr and 4hrs. Protein normalized to total ERK1/2 and b-actin.

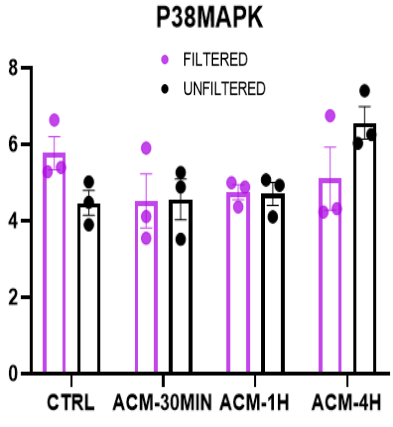


Fig.2 Phospho-MAPK-38 (P38MAPK) levels in CD81A astrocytes treated with Astrocyte Conditioned Media (ACM) from LPS-exposed (100ng/ml) r-mTBI astrocytes for 30min, 1hr and 4hrs . Protein normalized to b-actin.

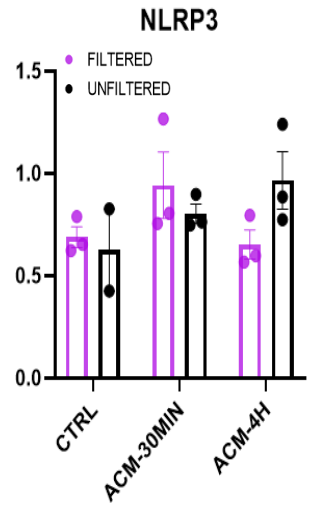


Fig.3 NLRP3 levels in CD81A astrocytes treated with Astrocyte Conditioned Media (ACM) from LPS-exposed (100ng/ml) r-mTBI astrocytes for 30min and 4hrs . Protein normalized to b-actin.

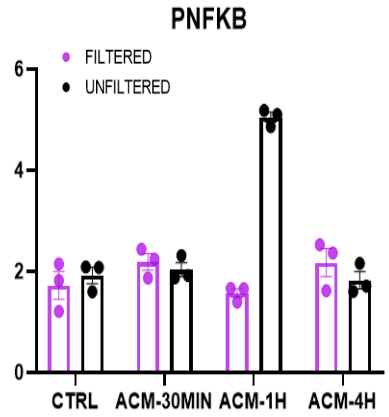


Fig.4 Phospho-NFkB (PNFKB) levels in CD81A astrocytes treated with Astrocyte Conditioned Media (ACM) from LPS-exposed (100ng/ml) r-mTBI astrocytes for 30min, 1hr and 4hrs. Protein normalized to total NFkB and b-actin.

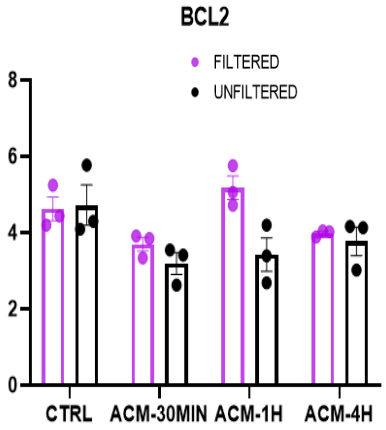


Fig.5 BCL2 levels in CD81A astrocytes treated with Astrocyte Conditioned Media (ACM) from LPS-exposed (100ng/ml) r-mTBI astrocytes for 30min, 1hr and 4hrs. Protein normalized to b-actin.

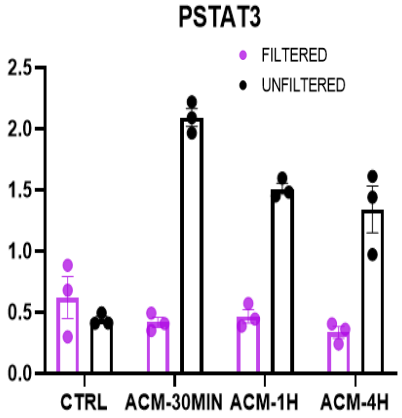


Fig.6 Phospho-STAT3 (PSTAT3) levels in CD81A astrocytes treated with Astrocyte Conditioned Media (ACM) from LPS-exposed (100ng/ml) r-mTBI astrocytes for 30min, 1hr and 4hrs. Protein normalized to b-actin.

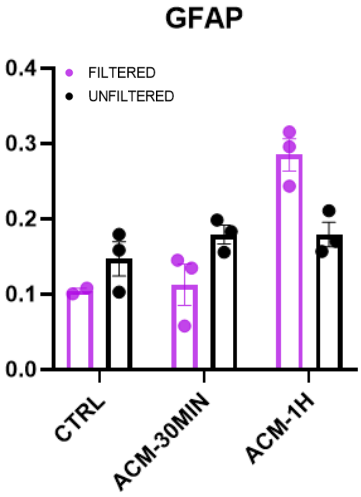


Fig.7 GFAP levels in CD81A astrocytes treated with Astrocyte Conditioned Media (ACM) from LPS-exposed (100ng/ml) r-mTBI astrocytes for 30min, 1hr and 4hrs. Protein normalized to b-actin.

- Differences are observed in pSTAT3 and pNFKB with filtered and unfiltered ACM.
- As sham groups have not been analyzed, we cannot draw any conclusions on the TBI mediated effects.
- Secretome exposure studies are also planned in microglia, endothelia and neuronal cell lines.
- We will use sham vs TBI astrocyte ACM (unexposed to LPS) for future work.

PART SIX: Adoptive Cell Transfer Pilot Study

In a pilot study, we purified CD11b+ microglia inoculum from donor reporter mice exposed to rmTBI/sham, and injected intranasally (IN) into naïve aged hTau recipient mice, demonstrated a dramatic increase in IBA1-ir (4-10fold) in the distant caudate, cortex and hippocampus, of mice receiving Cd11b+ cells from rmTBI vs sham and naïve mice. In our next set of study we will use astrocyte reporter Donor mice to enable tracking of astrocyte cells in vivo. This pilot study demonstrates that injured glial cells are able to induce an inflammatory response in a naïve mouse brain.

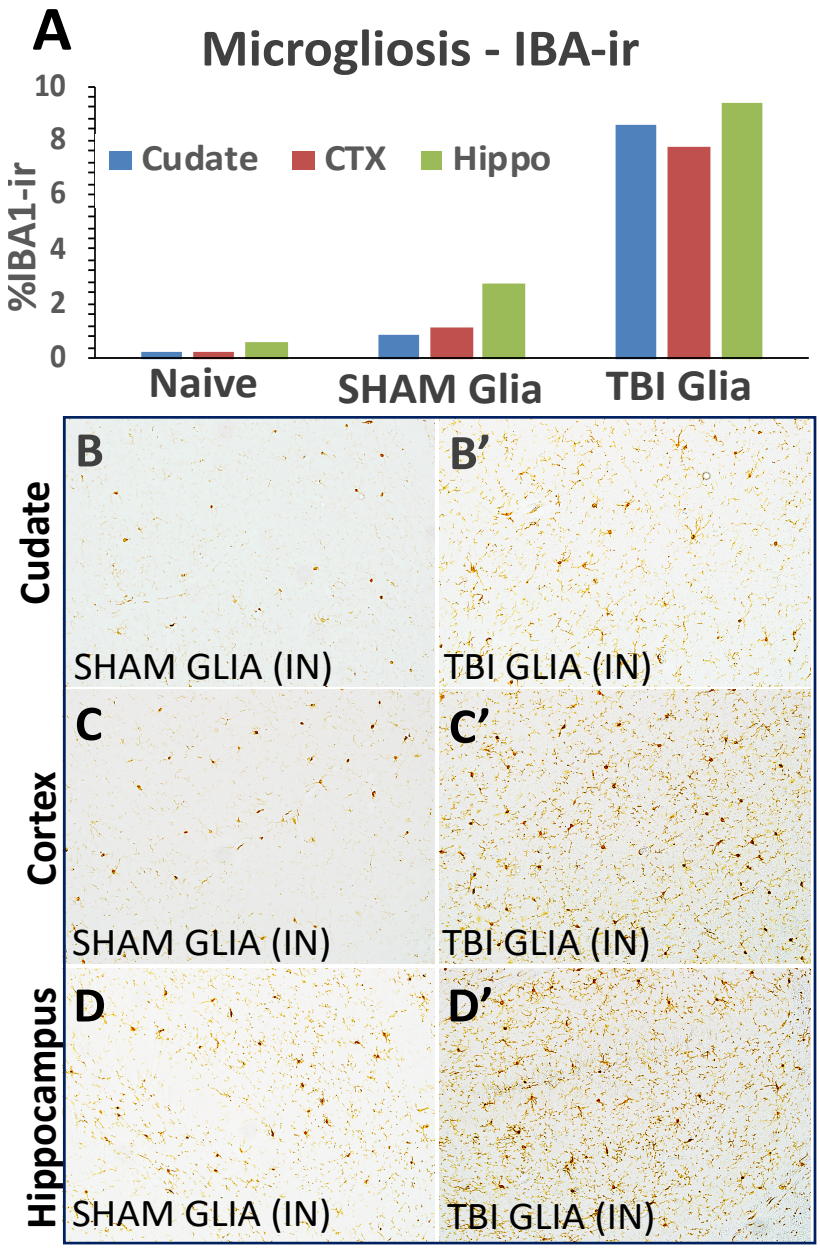


Figure 1: IBA1-ir in hTau mice injected with microglial inoculum from CX3CR1 reporter mice by (A) transnasal route. N=1/Group. BB'-DD' shows images of IBA1-ir.

Conclusion

- Optimized all astrocyte specific markers for human and mouse tissue staining.
- Performed pilot study of human autopsy tissue using nanostring gene profiling, and demonstrated unique and overlapping features in TBI and AD astrocyte gene profiles.
- Began analyses of mouse tissue by histopathology for astrocyte specific markers (under investigation). GFAP-Tau and TauKI mice show prominent astrogliosis post-injury. Tau is upregulated in GFAP-Tau mice acutely after (24-48hrs) injury.
- Began analyses of mouse astrocyte gene profiling in young mouse cohort (under investigation).
- In pilot studies we have showed that intranasal administration of glial cells from TBI brains can induce an inflammatory phenotype in naïve mouse brains in cortical and subcortical regions.
- We have optimized conditions for our planned ex vivo functional assay studies.
- We have demonstrated that TBI primary astrocytes exhibit an activated phenotype even after 21 days in culture compared to sham primary astrocytes.
- We are continuing to explore the effect of exposing TBI astrocyte secretome on healthy neuronal, glial and vascular cell function.

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 BEGIN:
Major Task 1
Subtask 3: Continue histopathological analyses of astroglia lesions in autopsy cases
Subtask 6: Begin sectioning and histopathological staining of Young cohort (TBI/SHAM mice) brain tissues with astroglia, tau and amyloid antibodies.
Subtask 7: Begin stereological analyses and Image quantitation of brain sections from Young cohort of TBI/SHAM mice.
Subtask 8: Continue administering injuries to Aged cohort of TBI/SHAM mice.
Subtask 9: Continue Euthanasia of Aged cohort of TBI/SHAM mice in subtask 8.

Major Task 2
Subtask 1: Breeding of cohorts for Young Donor mice for TBI studies in Subtask 2 and 3.
Subtask 2: Administering injuries to Young 'Donor' cohort - 3 months old injury.
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: 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.

Major Task 3
Subtask 2a: Laser capture microdissection of astroglia from Young Cohort TBI/SHAM mice for single cell array for gene expression profiling. There will be 36 mouse brains per genotype for analyses.

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 3 months. However, work is now progressing as normal and we aim to gain grounds in the work out over the next few quarters.

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.

Obtaining adequate numbers of human autopsy tissue sections for neuropathological analyses. We have applied to the NIH biobank (through Dr Purohit) after approval of our ORP Cadaver application.
We plan to request for more samples than we require, and anticipate obtaining sufficient numbers, however in the case where we are short in numbers for our required sample size, we may explore other brain banks if necessary.

Generating adequate numbers for mouse studies in a limited time frame.
We have these animals in house and have begun our breeding protocols to generate numbers required for the study. We have previous experience with working on projects involving large animal numbers (e.g. the completed DOD/VA CENC preclinical project involved 672 mice over 5 years) and therefore we are well equipped to generate adequate numbers from our current vivarium breeding program.

As mentioned above, due to COVID-19, there has been a small reduction in work output.
This set us back by around 3 months, but work is now progressing as normal and we aim to gain grounds over the next few quarters.

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

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.*

Nothing to report

- **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".

Example:

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 will commence in the next quarter. Samples have been shipped to Dr Mufson.

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: Drs Fiona Crawford / Joseph Ojo **Org:** Roskamp Institute, Sarasota, FL **Award Amount:** $718,000

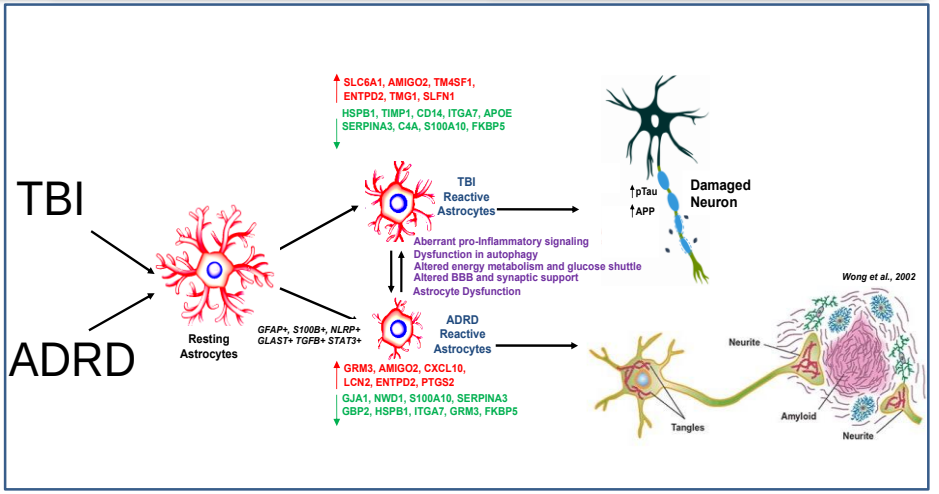
Study Aim 1: Delineation of the effects of chronic repetitive mTBI on astrocyte pathobiology and related proteinopathy in tau and amyloid bearing preclinical models at multiple time points post-injury, and validation in autopsied brains from human TBI/ADRD cases. **Study Aim 2:** Evaluating pathobiological 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: Finished histopathological staining of 42 autopsy cases for astrocyte markers. Generated pilot human astrocyte gene analyses data. Completed young mouse cohort breeding/injuries, and euthanized 70% of this cohort (most of the aged mouse cohort are still ageing). Began optimizing conditions for inoculation and ex vivo assay studies.

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

- Waiting for more human tissue from TBI cases to complete TBI cohort
- LCM needed replacement, and LCM work has been held back by 3 months.

Projected Expenditure: $196,426 **Actual Expenditure**: $202,721