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

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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. We have begun analyzing astrocyte lesions in tissue from both mice and humans. Quantitative analyses is currently ongoing. 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 uninjured 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, pNFkB and NLRP3 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 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					
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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:

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

2. ACCOMPLISHMENTS:

What were the major goals of the project?

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 [70% 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 [30% 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 [80% 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	
Subtask 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?

The most important activities during this reporting period involved histopathology and transcriptomics analyses of the young and aged cohorts for the longitudinal mouse study, including performing human tissue immunostaining with astrocyte markers. We also began the ex vivo studies involving exposure of TBI/sham astrocyte secretome to neural cells, and initiated the adoptive transfer studies. Major Tasks from the SOW and the corresponding data/results from each relevant section is provided below.

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

Major Task 1

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. 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/TGF β , GFAP/GLT-1/S100 β and GFAP. **COMPLETED**

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 (50% 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. **90% 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 (20% COMPLETED)**

Data/Results

Subtask 3

Human TBI neuropathology of astrocyte lesions/markers

Table below shows group classification and history of de-identified patients used for the study.

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 here have evidence of 2 or more repetitive mild traumatic brain injuries with several decades post-survival.

Methods - Immunofluorescence protocol was used as previously detailed (see Ojo et al., 2018).

Qualitative images of markers analyzed are shown in Fig 1-3 below.

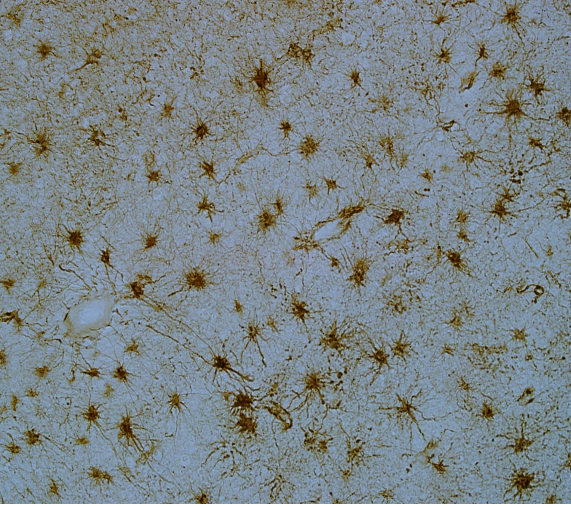


Fig.1 GFAP-DAB staining in human tissue. Magnification 20X.

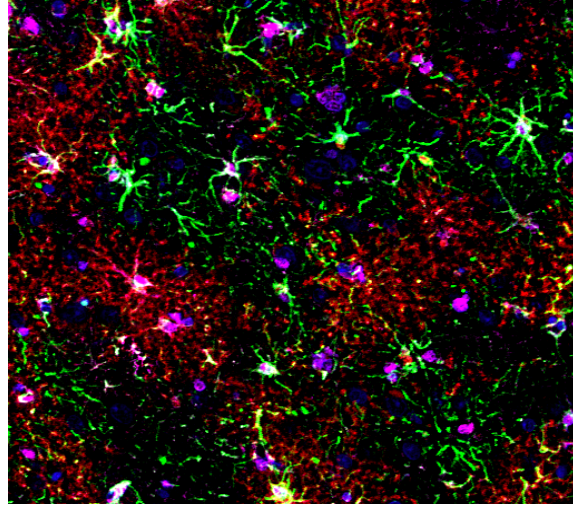


Fig.2 Immunofluorescence staining human tissue. GFAP (green), GLT1 (red) and S100b (purple). Magnification 20X.

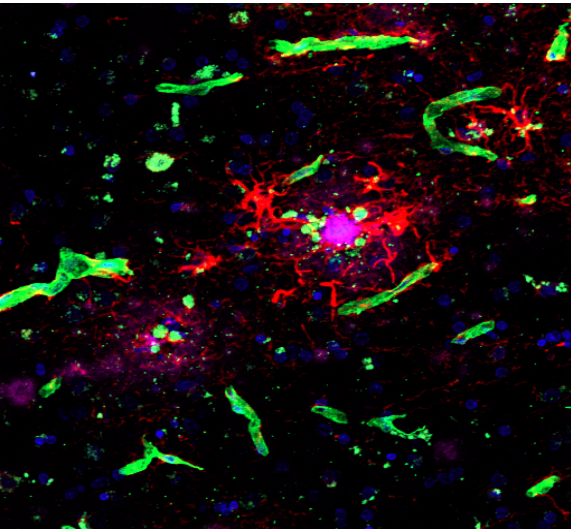


Fig.4 Immunofluorescence staining human tissue. GFAP (red), 4G8 (purple) and Laminin (green). Magnification 20X.

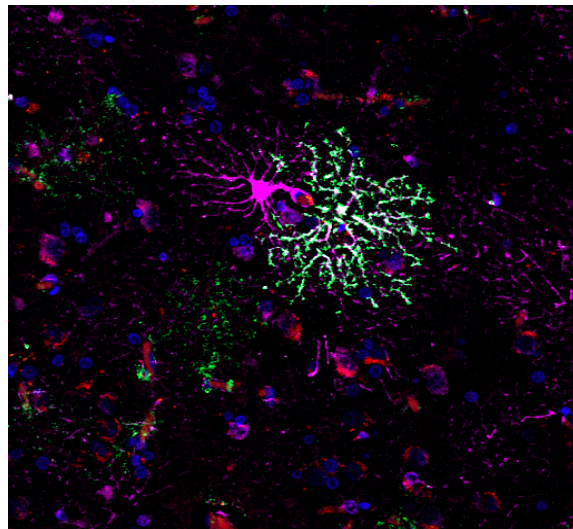


Fig.3 Immunofluorescence staining human tissue. GFAP (purple), MC1 (red) and AQ4 (green). Magnification 20X.

QUANTITATIVE ANALYSES

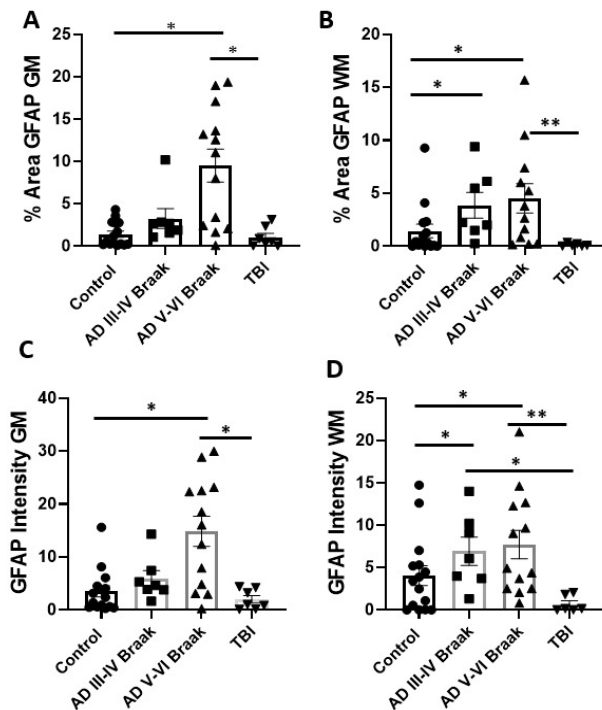


Figure 1. Percentage area [A-B] and intensity (au) [C-D] of positive Glial fibrillary acidic protein (GFAP) in grey (GM) and white matter (WM) of human brain derived from the medial temporal lobe. Control (n=15), AD Braak stages III-IV (n=7), AD Braak stages V-VI (n=13), and Traumatic brain injury (TBI) (n=7). $p < 0.03$ *; $p < 0.002$ **; $p < 0.001$ *. Data are presented as mean $\pm$ SEM.**

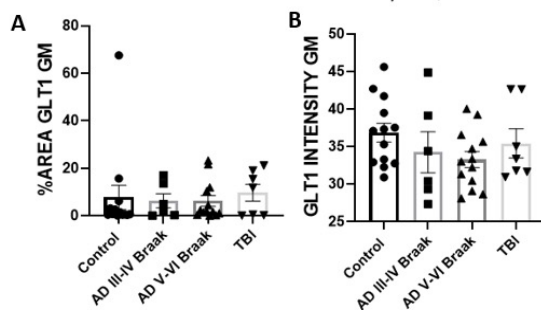


Figure 3. Percentage area [A] and intensity (au) [B] of Glutamate transporter 1 (GLT1) in grey matter (GM) of human brain derived from the medial temporal lobe. Control (n=15), AD Braak stages III-IV (n=7), AD Braak stages V-VI (n=13), and Traumatic brain injury (TBI) (n=7). $p < 0.03$ *; $p < 0.002$ **; $p < 0.001$ *. Data are presented as mean $\pm$ SEM.**

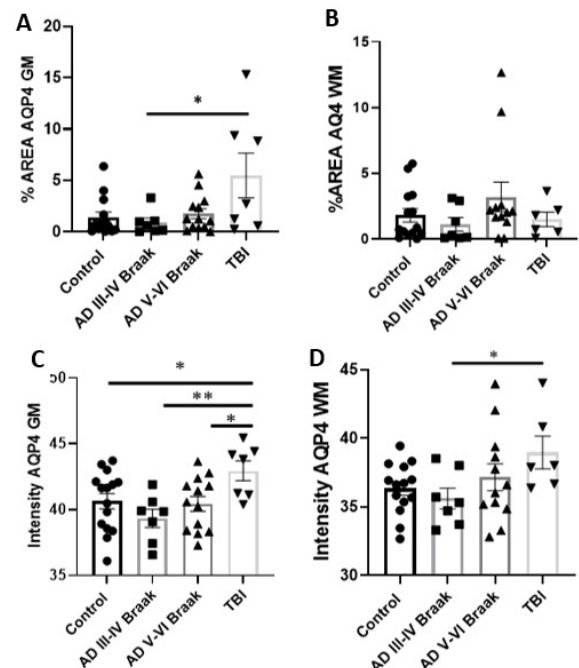


Figure 2. Percentage area [A-B] and intensity (au) [C-D] of Aquaporin 4 (AQP4) in grey (GM) and white matter (WM) of human brain derived from the medial temporal lobe. Control (n=15), AD Braak stages III-IV (n=7), AD Braak stages V-VI (n=13), and Traumatic brain injury (TBI) (n=7). $p < 0.03$ *; $p < 0.002$ **; $p < 0.001$ *. Data are presented as mean $\pm$ SEM.**

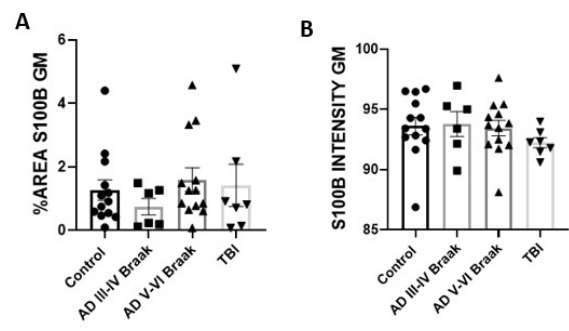


Figure 4. Percentage area [A] and intensity (au) [B] of S100B in grey matter (GM) of human brain derived from the medial temporal lobe. Control (n=15), AD Braak stages III-IV (n=7), AD Braak stages V-VI (n=13), and Traumatic brain injury (TBI) (n=7). $p < 0.03$ *; $p < 0.002$ **; $p < 0.001$ *. Data are presented as mean $\pm$ SEM.**

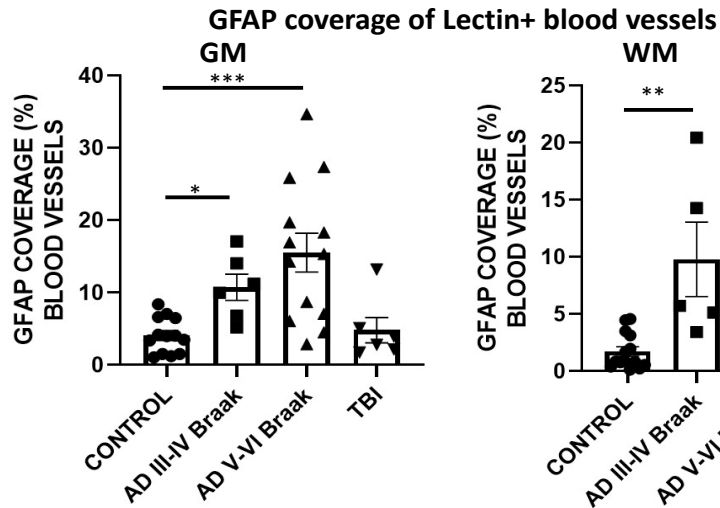


Figure 5. GFAP coverage of Lectin+ blood vessels in the grey and white matter of human brain from the medial temporal lobe. Control (n=15), AD Braak stages III-IV (n=7), AD Braak stages V-VI (n=13), and Traumatic brain injury (TBI) (n=7). $p < 0.03$ *; $p < 0.002$ **; $p < 0.001$ ***. Data are presented as mean $\pm$ SEM.

Summary

- >GFAP levels were significantly upregulated in AD brains but downregulated in TBI brains compared to healthy non-demented controls (Figure 1).
- >Aq4 levels were upregulated in TBI compared to healthy control cases (Figure 2).
- >No significant changes were observed in S100beta and GLT1 levels in TBI or AD cases compared to non-demented controls (Figure 3 and 4).
- >There was a greater area of astrocytic coverage around blood vessels in the brains of AD vs health control cases (Figure 5).

Subtasks 4-7: Mouse neuropathology for young cohorts

Only qualitative images from the young TauKI and GFAP-P301L mouse cohorts are presented as the analyses is currently ongoing.

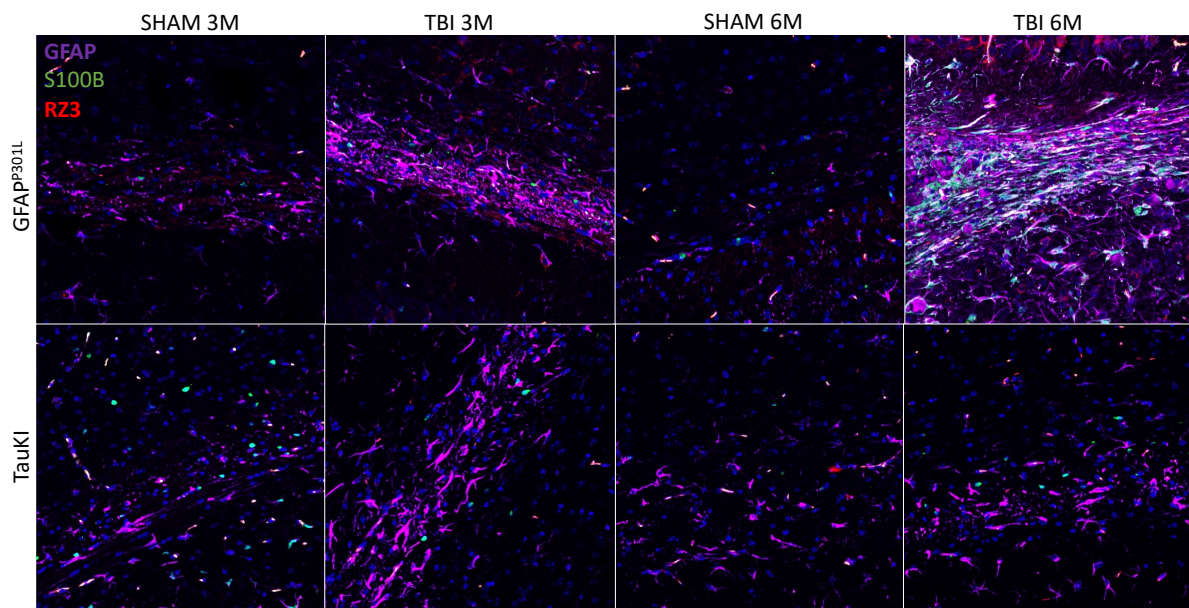


Figure 1. Immunofluorescence staining in the corpus callosum of GFAP^{P301L} and TauKI mice at 3- and 6 months post last injury. Astrocytes labelled with GFAP (purple) and S100B (green), and Tau with RZ3 (red)

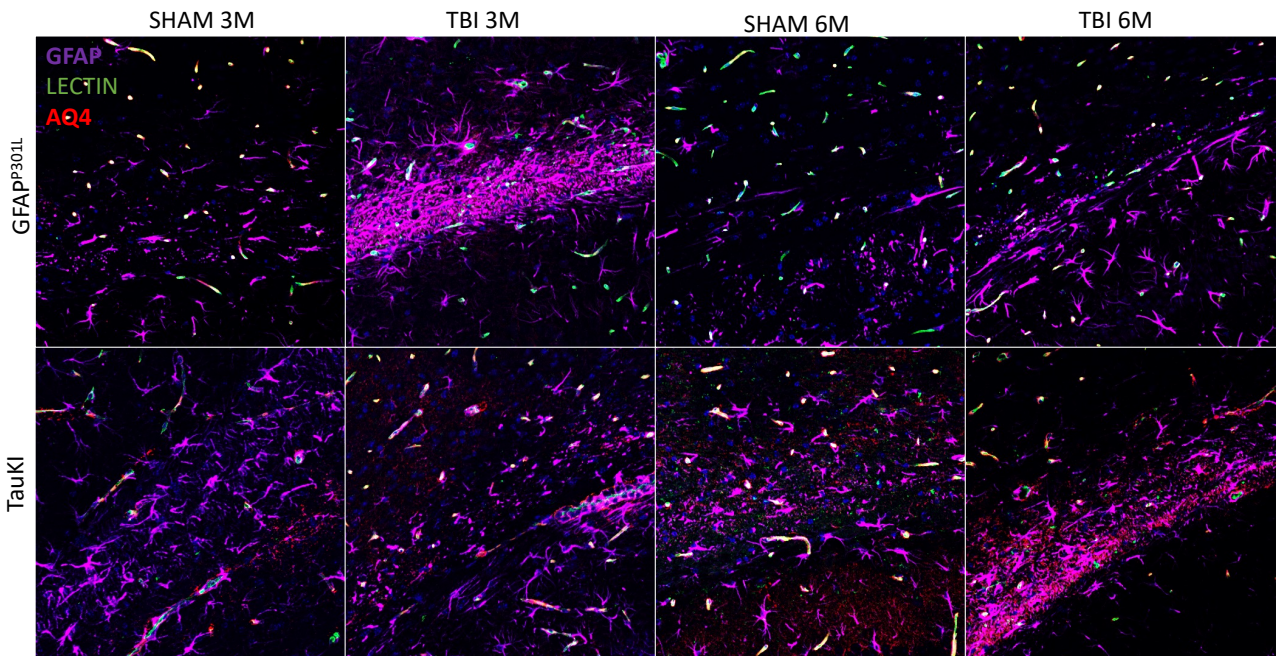


Figure2 . Immunofluorescence staining in the corpus callosum of GFAP^{P301L} and TauKI mice at 3- and 6 months post last injury. Astrocytes labelled with GFAP (purple), blood vessels with Lectin(green), and water channels with AQ4 (red).

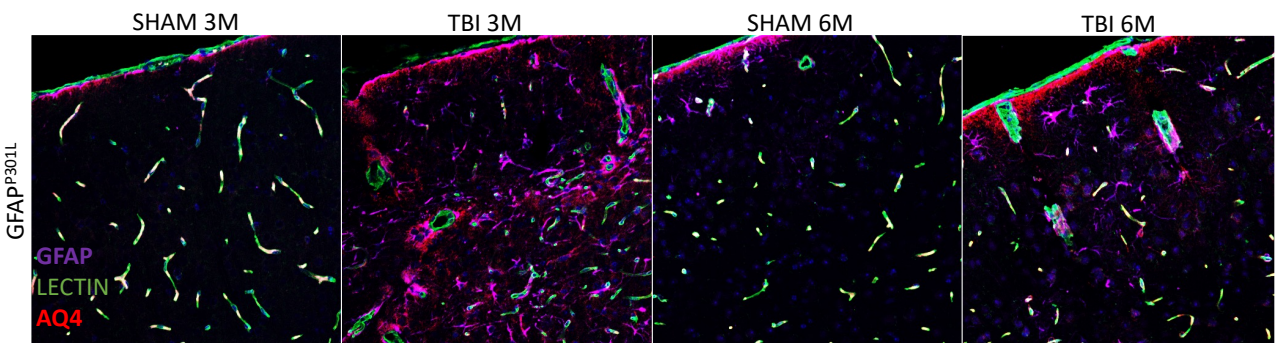


Figure3 . Immunofluorescence staining in the cortex of GFAP^{P301L} mice at 3- and 6 months post last injury. Astrocytes labelled with GFAP (purple), blood vessels with Lectin(green), and water channels with AQ4 (red).

Summary

Quantitative data have not been generated and finalized.

Our qualitative findings demonstrate prominent astrogliosis in the white and grey matter at 3 and 6 months in both young TauKI and GFAP-P301L models after r-mTBI (Fig 1-3).

S100b levels appear to be upregulated in the young GFAP-P301L model at 6 months post-injury (Fig 1).

AQ4 levels appeared to be upregulated at 3 months post-injury in the young GFAP-P301L model (Fig 3).

These data together implicates that astrocyte lesions play a prominent role in the white and grey matter regions following r-mTBI.

Major Task 2

We continued 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 successfully cultured astrocyte cells isolated from sham/injured 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 also explored MACS and recently immunopanning methods to improve purity of astrocyte cells. We previously conducted a pilot study for subtask 4 involving exposure of conditioned media (astrocyte secretomes) to healthy neural and microvascular cells. We are planning to carry out the main studies using the different transgenic mice in our study. These studies will be completed within the next quarter.

We have also conducted a preliminary study isolating a murine-CFDA-labelled ACSA2+ astrocytes using militenyl beads, and injected half a million cells into the brains of recipient mice. Recipient mice were euthanized after ~14days of injection, and we observe that the IC injected cells appears to readily migrate into the brain. Donor mice from the original planned study 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}). **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 (30% 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 Y3Q1.**

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 (80% COMPLETED).**

Subtask 5: Euthanasia of inoculated ‘Recipient’ naïve non-transgenic mice, collection of brain tissue, sectioning and histopathological staining with astroglia, tau and amyloid antibodies **PLANNED FOR Y3Q3.**

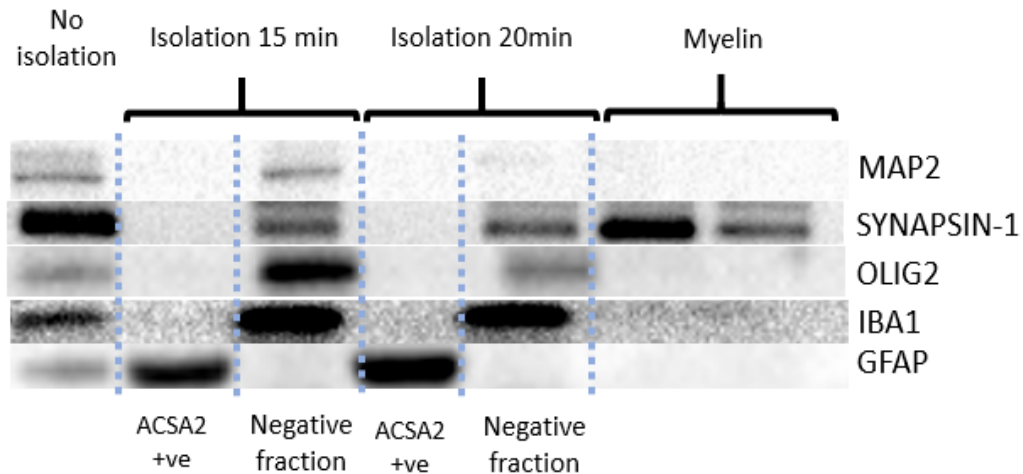
Data/Results

Subtask 3 and 5: Isolation of astrocytes by magnetic cell sorting (MACS) and injection into naïve ‘Recipient’ non-transgenic mouse brain. Euthanasia of inoculated ‘Recipient’ naïve non-transgenic mice, collection of brain tissue, sectioning and histopathological staining.

Primary astrocyte isolation by MACS

Method: Firstly, glial cells were isolated from brain homogenate using Percoll gradient centrifugation and isolated with or without militenyl ACSA2+ beads. Cell lysates from the positive and negative fractions were collected for further probing. Protein was extracted from cell lysates, run in a gel, and probed for specific markers using western blotting.

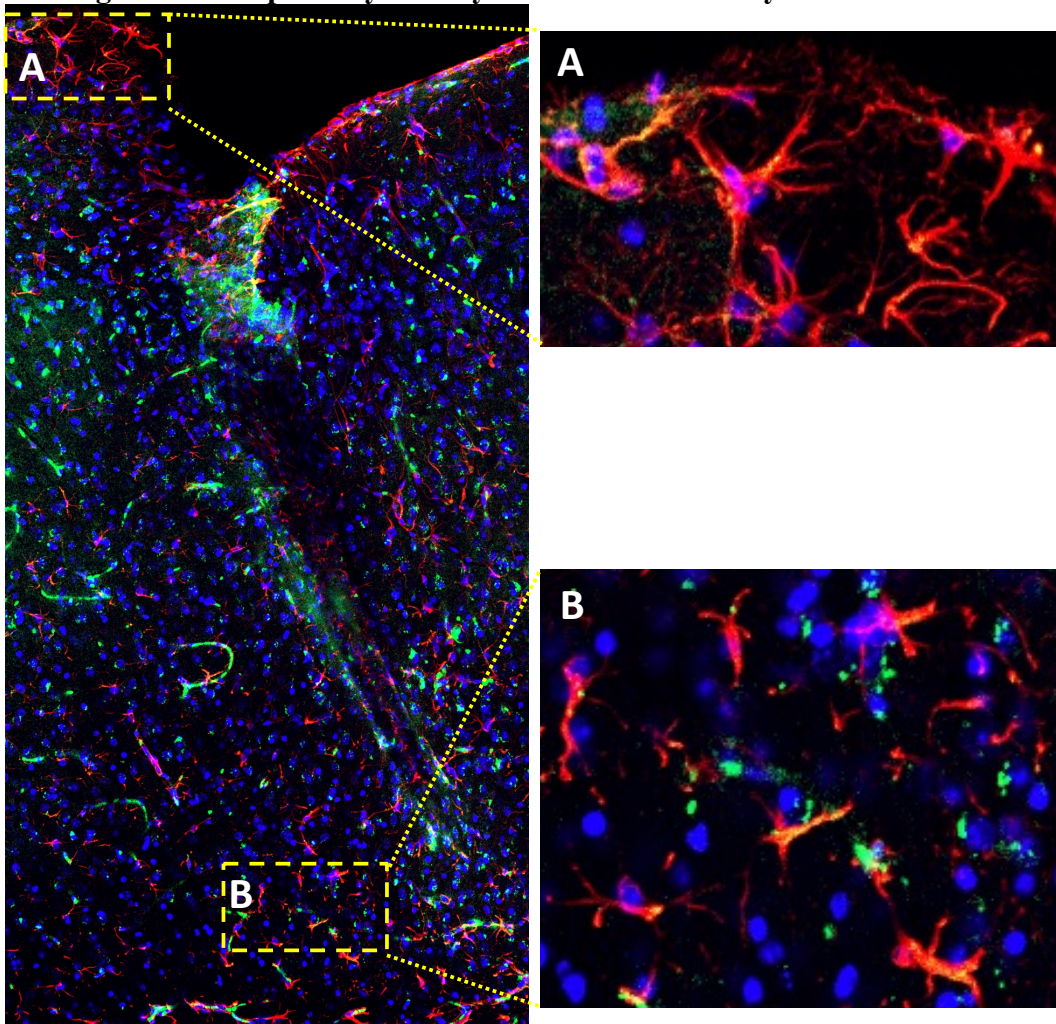
Immunoblotting: Ten micrograms of extracted protein were separated by 4-15% SDS-PAGE and transferred onto PVDF membranes. Membranes incubated with primary antibodies (Olig2 1:2000, MAP2 1:1000, IBA1 1:1000, GFAP 1:2000, Synapsin-1 1:1000) at 4°C overnight. Membranes were developed using enhanced chemiluminescence system.



Purity test of isolated of astrocytes through magnetic antibody, ACSA2: Cell preparations obtained from mouse brains were incubated with ACSA2 magnetic antibody for 15min and 20min (ACSA2 +ve). Magnetic separation was performed for 5 minutes. Purity of isolated astrocytes was compared to negative fraction of isolated samples, non-isolated sample and myelin. Markers for neurons (MAP2, Synapsin-1), oligodendrocyte (OLIG2), microglia (IBA1) and astrocytes (GFAP) were used to determined the purity of the samples through immunoblotting.

Summary: Results demonstrate purity of GFAP+ astrocyte cells isolated by MACS.

Labeling of isolated primary astrocyte cells with CFSE dye for cell inoculation studies.



Cell inoculation of CFDA-SE labelled primary astrocytes. A naïve recipient mouse was inoculated with 1.9×10^3 labeled astrocytes (3ul) into the cortex (AP -1, ML -1, DV 0.65). A and B, labelled astrocytes (in yellow) in the proximity of the inoculation site in the cortex of the mouse brain. (GFAP red, CFDA-SE green, DAPI, blue. 20X)"

Summary – Here we demonstrate that cells labelled with CFDA dye and injected into the cortex and hippocampus remain viable and are readily identifiable by the green fluorescent staining. These preliminary studies were conducted 14 days post-inoculation. We have now began the main study using astrocyte cells from TBI and sham donor mice from our 3 different mouse models. These cells have been injected into naïve WT mice which are currently aging and will be analyzed in the next 5-6 months.

Subtask 4: Ex-vivo studies using astrocyte conditioned media (ACM) exposed to healthy microglia.

Preliminary study to optimize conditions for the main chronic study.

METHOD: Mice on the C57BL6 background were exposed to r-mTBI (N=3) and sham injury (N=3). Brains were collected and processed under sterilized conditions 24 hours post last injury. Glial cells were isolated from brain homogenate using percoll gradient centrifugation and co-cultured at 37°C for one week. Astrocytes were separated from microglia through shaking at 37°C 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 also frozen for further probing. Protein was extracted from cell lysates of astrocytes, run in a gel, and probed for specific markers using western blotting. While 1/4th of ACM was exposed to media of healthy IMG microglia cell cultures for 30 min and 4hrs.

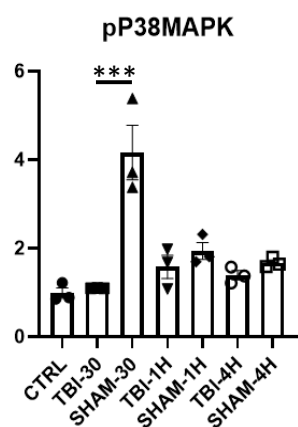


Fig.1 Phospho-p38MAPK levels in IMG microglia exposed to ACM from TBI and Sham mice (30min-4h exposure).

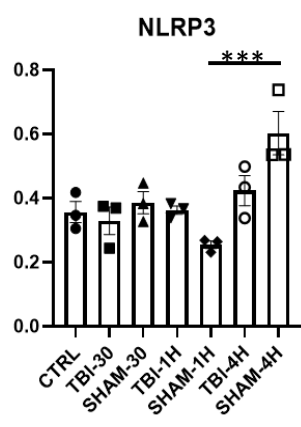


Fig.2 NLRP3 levels in IMG microglia exposed to ACM from TBI and Sham mice (30min-4h exposure).

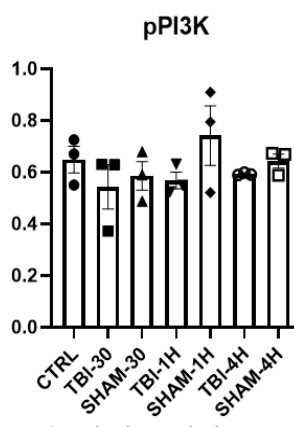


Fig.3 Phospho-Pi3K levels in IMG microglia exposed to ACM from TBI and Sham mice (30min-4h exposure).

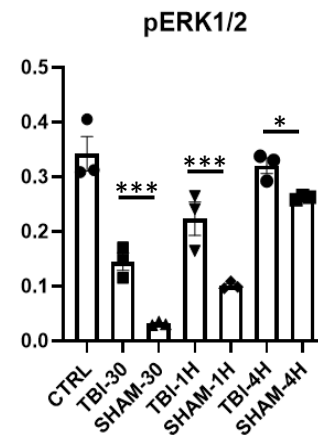


Fig.4 Phospho-ERK1/2 levels in IMG microglia exposed to ACM from TBI and Sham mice (30min-4h exposure).

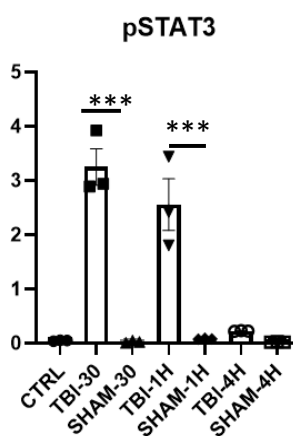


Fig.5 Phospho-STAT3 levels in IMG microglia exposed to ACM from TBI and Sham mice (30min-4h exposure).

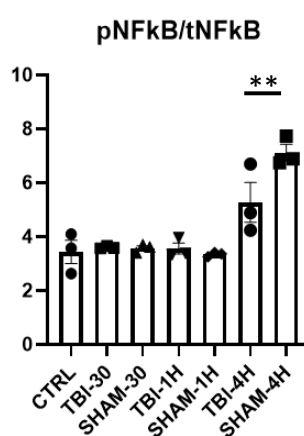


Fig.6 Phospho-NFkB levels in IMG microglia exposed to ACM from TBI and Sham mice (30min-4h exposure).

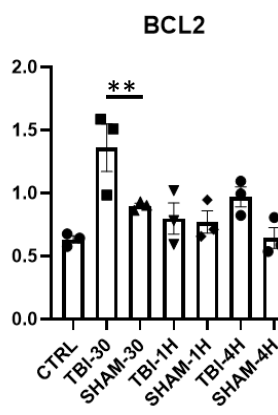


Fig.7 BCL2 levels in IMG microglia exposed to ACM from TBI and Sham mice (30min-4h exposure).

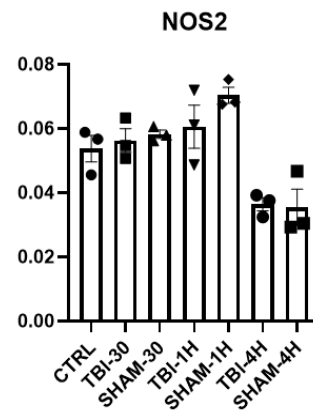


Fig.8 NOS2 levels in IMG microglia exposed to ACM from TBI and Sham mice (30min-4h exposure).

Summary: Conditioned media from TBI astrocytes elicited a response in healthy microglia cells typified by an increase in STAT3, BCL2 and pERK1/2. These studies were conducted to optimize the conditions required for the main chronic study using our three different mouse models. We have recently completed these ACM studies using the GFAP-P301L and APP-KI models. Analyses of exposed cell lysates are currently underway.

Major Task 3

Dr Mufson recently began LCM and gene array analyses on GFAP stained serial sections.

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 Y3Q1**

Subtask 2a: Astroglia transcriptomic from Young Cohort mice for single cell array for gene expression profiling. There will be 36 mouse brains per genotype for analyses.

90% COMPLETED

Subtask 2b: LC-MS/MS analyses of astroglia secretome from Young Cohort for unbiased proteomic expression profiling. **PLANNED Y3Q2**

Subtask 3a: Astroglia transcriptomic from Aged Cohort mice for single cell array for gene expression profiling. There will be 36 mouse brains per genotype for analyses.

90% COMPLETED

Subtask 3b: LC-MS/MS analyses of astroglia secretome from Aged Cohort for unbiased proteomic expression profiling. **PLANNED Y3Q2**

Data and Results

Single cell transcriptomics of astrocyte gene profiles (mouse models – young/aged cohorts)

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).

Data from Subtask 2a and 3a: Astroglia transcriptomics from Young (subtask 2a) and Aged (subtask 3a) Cohort mice for single cell (astrocyte) gene expression profiling.

Astrocyte transcriptomic data in young and aged wild-type cohort

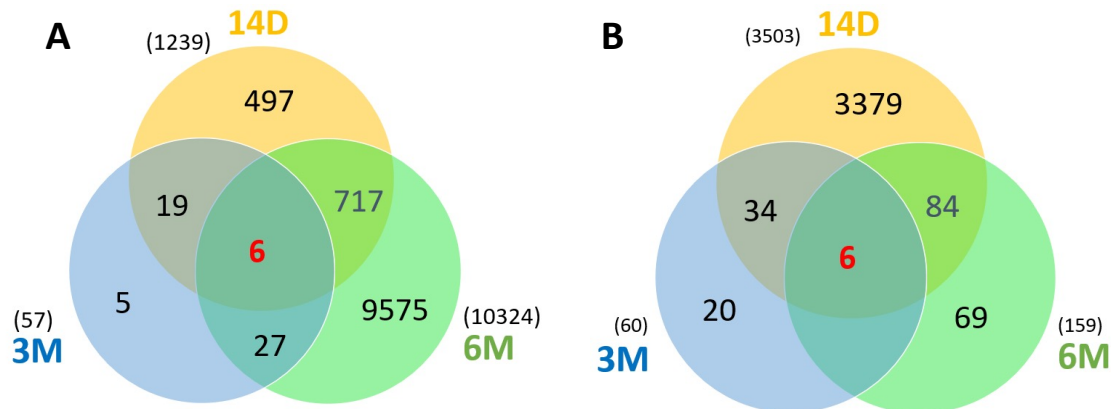


Figure 1. Venn-diagram of differentially expressed genes between TBI and Sham mice at 14 days, 3- and 6 months post last injury of the 3mo- and 12mo-old injured cohort (A and B, respectively).

YOUNG WT

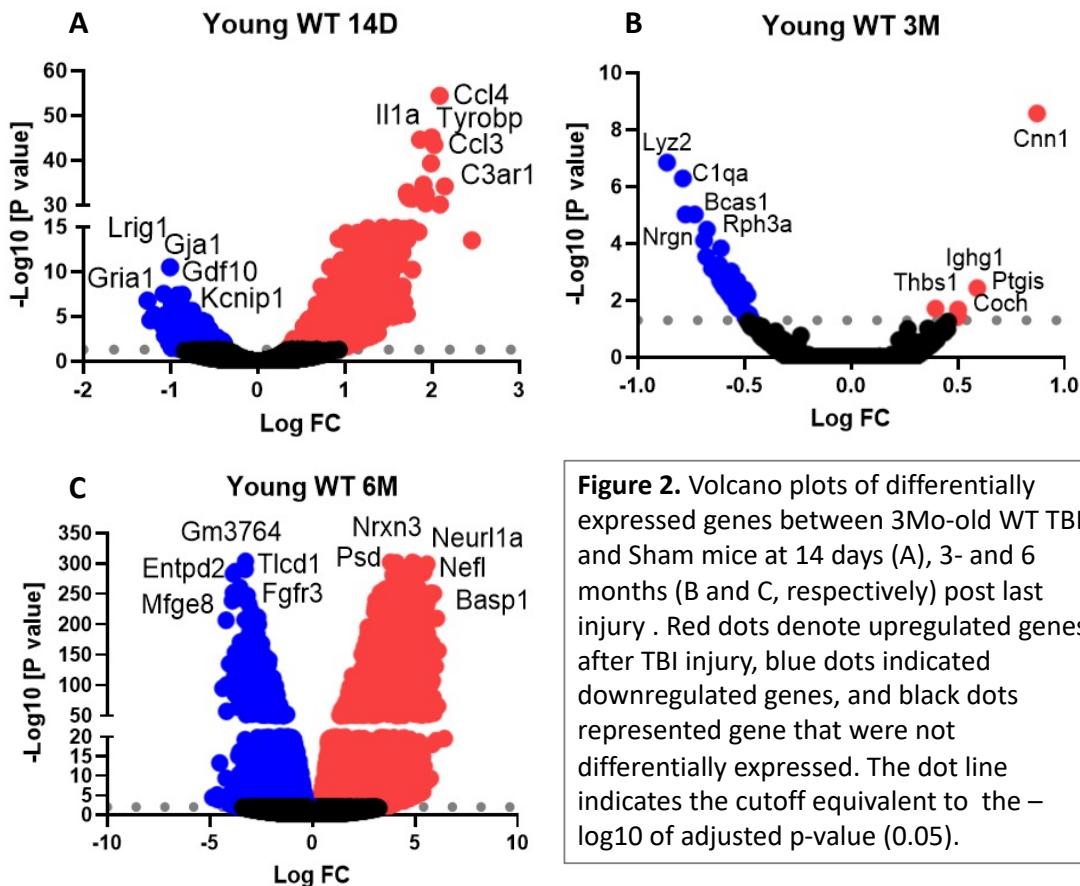


Figure 2. Volcano plots of differentially expressed genes between 3Mo-old WT TBI and Sham mice at 14 days (A), 3- and 6 months (B and C, respectively) post last injury. Red dots denote upregulated genes after TBI injury, blue dots indicated downregulated genes, and black dots represented gene that were not differentially expressed. The dot line indicates the cutoff equivalent to the $-\log_{10}$ of adjusted p-value (0.05).

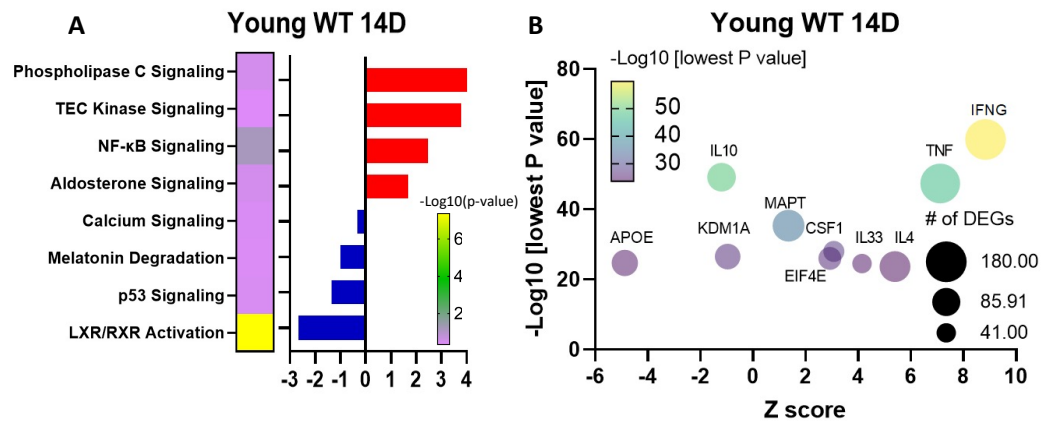


Figure 3. Pathway and Upstream regulator analysis on young WT 14days post last injury. A) Top 4 upregulated pathways in red and top 4 downregulated pathways in blue. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

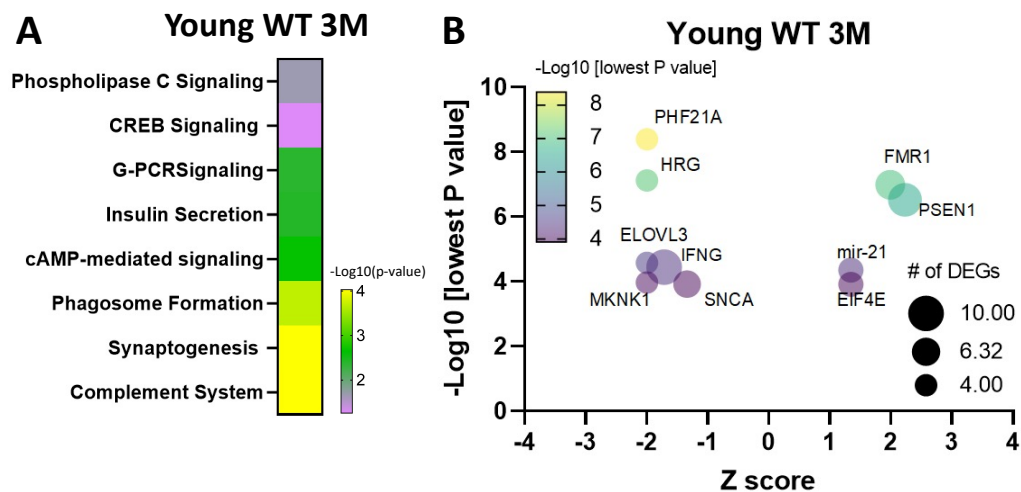


Figure 4. Pathway and Upstream regulator analysis on young WT 3 months post last injury. A) Top 8 dysregulated pathways. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

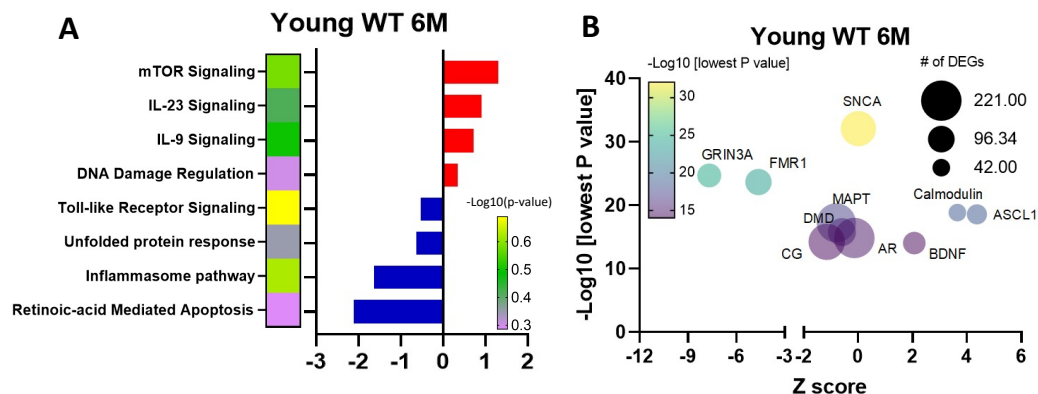
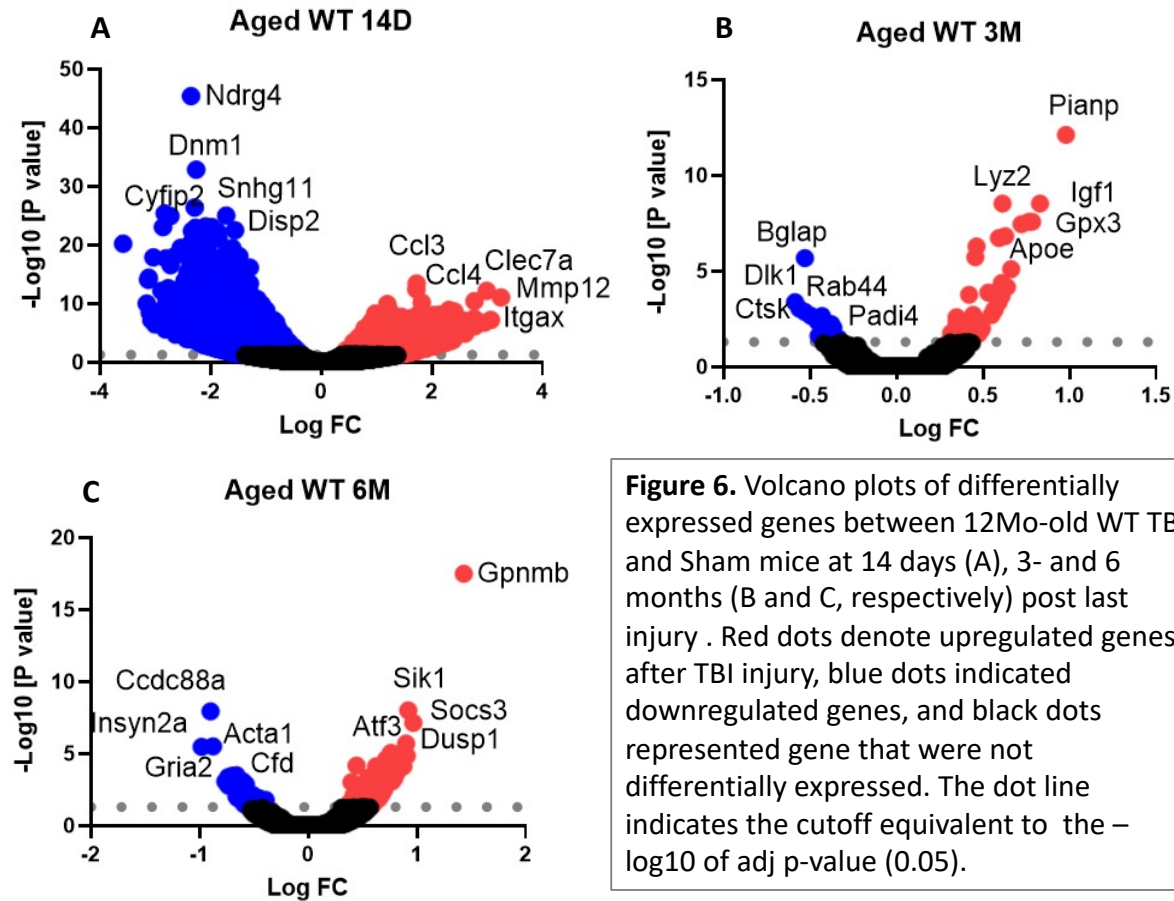


Figure 5. Pathway and Upstream regulator analysis on young WT 6 months post last injury. A) Top 4 upregulated pathways in red and top 4 downregulated pathways in blue. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

Aged WT



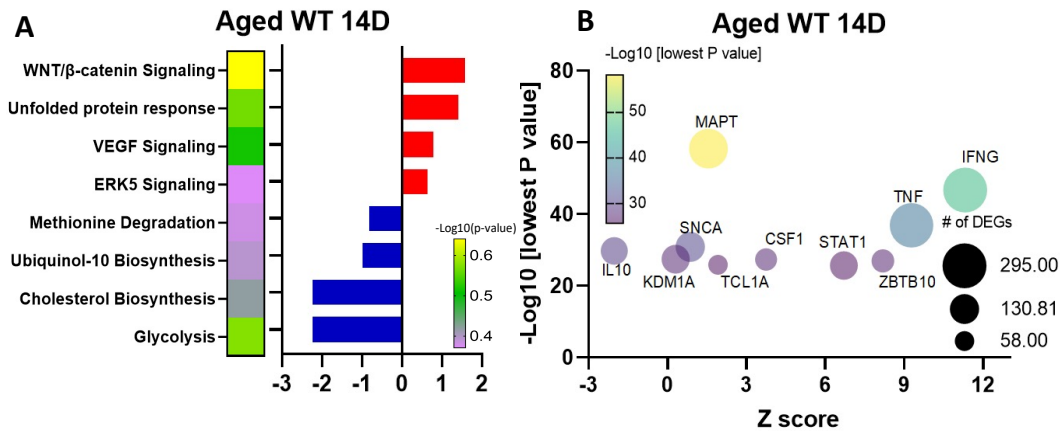


Figure 7. Pathway and Upstream regulator analysis on aged WT 14days post last injury. A) Top 4 upregulated pathways in red and top 4 downregulated pathways in blue. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

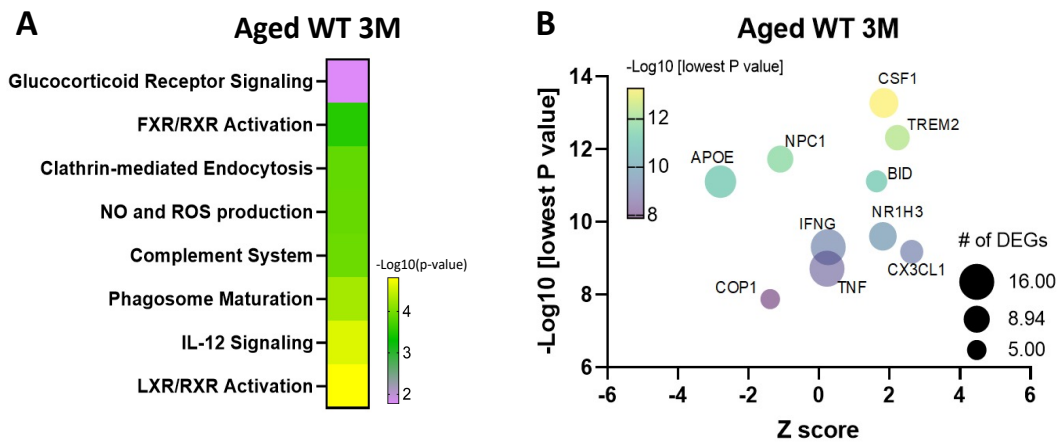


Figure 8. Pathway and Upstream regulator analysis on aged WT 3 months post last injury. A) Top 10 dysregulated pathways. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

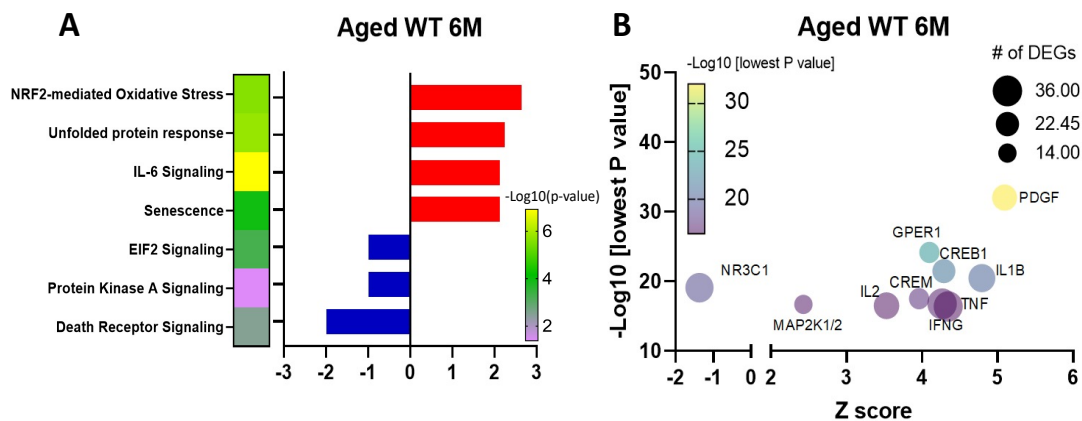


Figure 9. Pathway and Upstream regulator analysis on aged WT 6 months post last injury. A) Top 4 upregulated pathways in red and top 3 downregulated pathways in blue. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

Summary of astrocyte response in Young and Aged WT mice

In young WT mice following r-mTBI, we observed the greatest astrocyte transcriptomic response at 6 months post-injury (Fig. 1a and Fig. 2a-c). While in Aged WT mice, we observed the greatest astrocyte transcriptomic response at 14 days post-injury (Fig. 1b and Fig. 6a-c). This suggests that astrocytes from aged WT mice show a more robust reparative phenotype from injury compared to young WT mice.

Young injured astrocytes at 14days exhibited an increase in PLC, NFkB, TEC Kinase signaling, and a downregulation in LXR/RXR, calcium and p53 signaling (Fig 3a). While Aged injured astrocytes showed an increase in VEGF, ERK UPR and WNT/b-catenin signaling coupled with a downregulation in energy metabolic pathways (Fig 7a). Injured astrocytes from both Young (Fig 3b) and Aged (Fig 7b) WT mice at 14days exhibit a proinflammatory response with activation of classical pro-inflammatory upstream regulators (e.g., IFN γ , TNF α , etc.).

At 3 months similarities in astrocyte response were observed in Young (Fig 4a,b) and Aged (Fig 8a,b) WT mice typified by alterations in complement pathway and phagosome formation. But differences were also observed such as insulin, CREB, GPCR and phospholipase C signaling in young mice and clathrin mediated endocytosis, IL12, glucocorticoid receptor and LXR/RXR signaling in Aged WT mice.

At 6 months astrocyte response were distinctly different in Young (Fig 5a,b) and Aged (Fig 9a,b) WT mice. Injured young astrocytes exhibited an upregulation in mTOR, IL23 and IL9 signaling and DNA damage regulation, a reduction in TLR, UPR and inflammasome pathways (Fig 5a). While Injured Aged astrocytes exhibited an increase in NFR2 mediated oxidative stress, UPR, IL8 and cell senescence signaling, and a downregulation in ERF2 and PKA signaling (Fig 9a). Top upstream regulators in young injured astrocytes were SNCA, GRIN3A, FMR1, Calmodulin and ASCL1 (Fig 5b); while in Aged injured astrocytes were PDGF, CREB1, IL1B and GPER1 (Fig 9b).

Astrocyte transcriptomic data in young and aged TauKI cohort

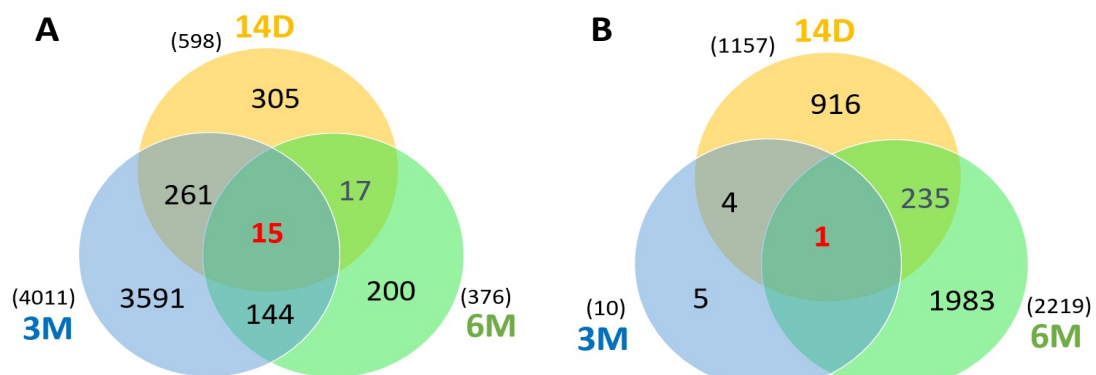


Figure 10. Venn-diagram of differentially expressed genes between TBI and Sham mice at 14 days, 3- and 6 months post last injury of the 3mo- and 12mo-old cohort (A and B, respectively).

YOUNG TauKI

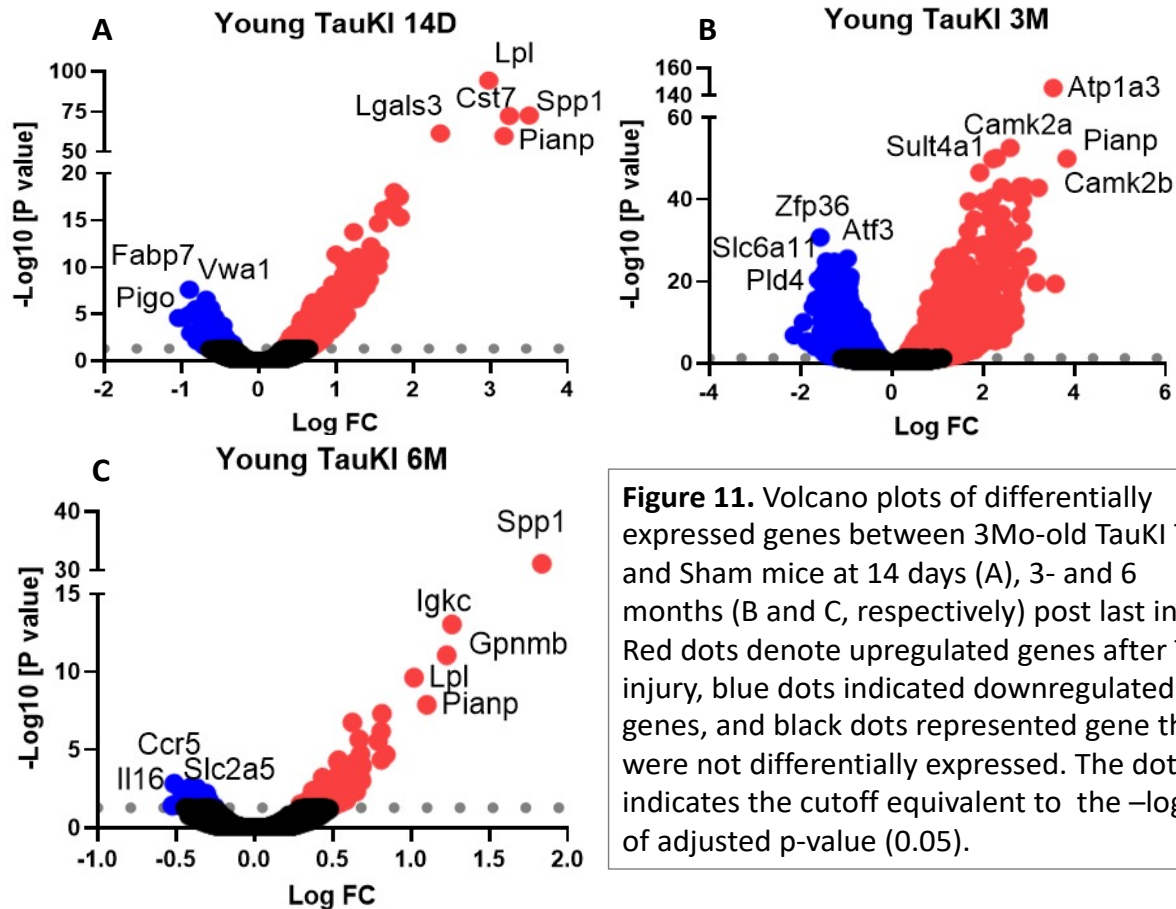


Figure 11. Volcano plots of differentially expressed genes between 3Mo-old TauKI TBI and Sham mice at 14 days (A), 3- and 6 months (B and C, respectively) post last injury. Red dots denote upregulated genes after TBI injury, blue dots indicated downregulated genes, and black dots represented genes that were not differentially expressed. The dot line indicates the cutoff equivalent to the $-\log_{10}$ of adjusted p-value (0.05).

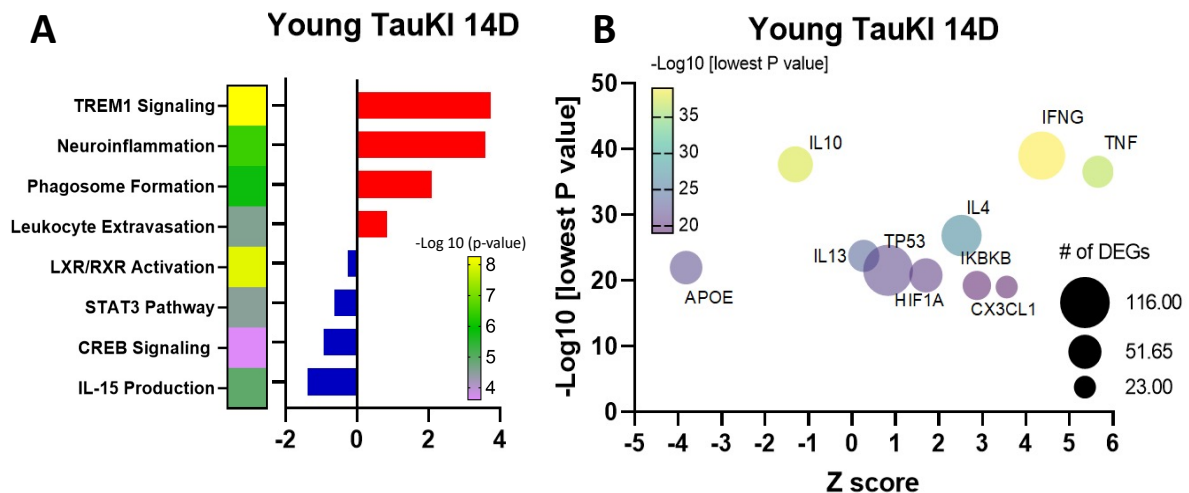


Figure 12. Pathway and Upstream regulator analysis on young TauKI 14days post last injury. A) Top 4 upregulated pathways in red and top 4 downregulated pathways in blue. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

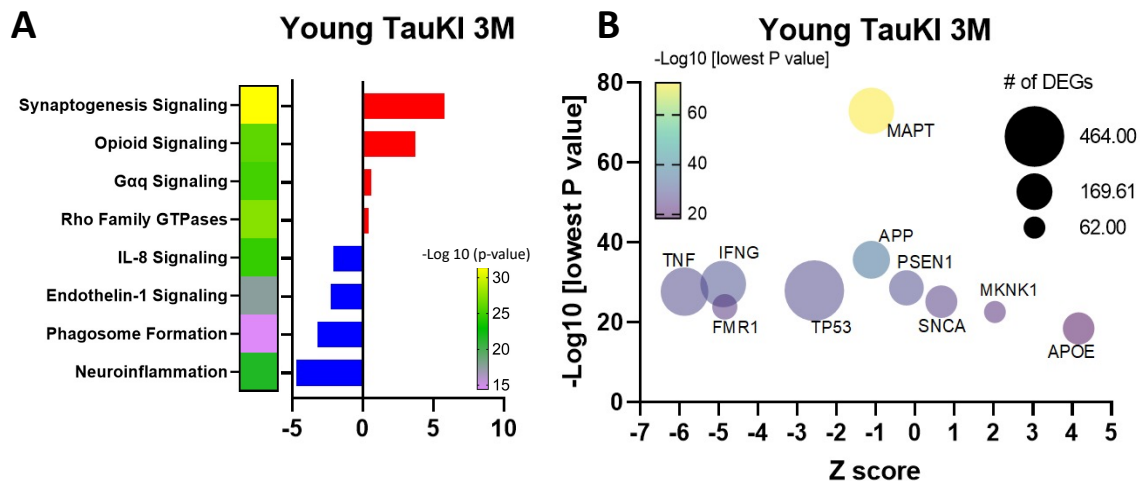


Figure 13. Pathway and Upstream regulator analysis on young TauKI 3 months post last injury. A) Top 4 upregulated pathways in red and top 4 downregulated pathways in blue. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

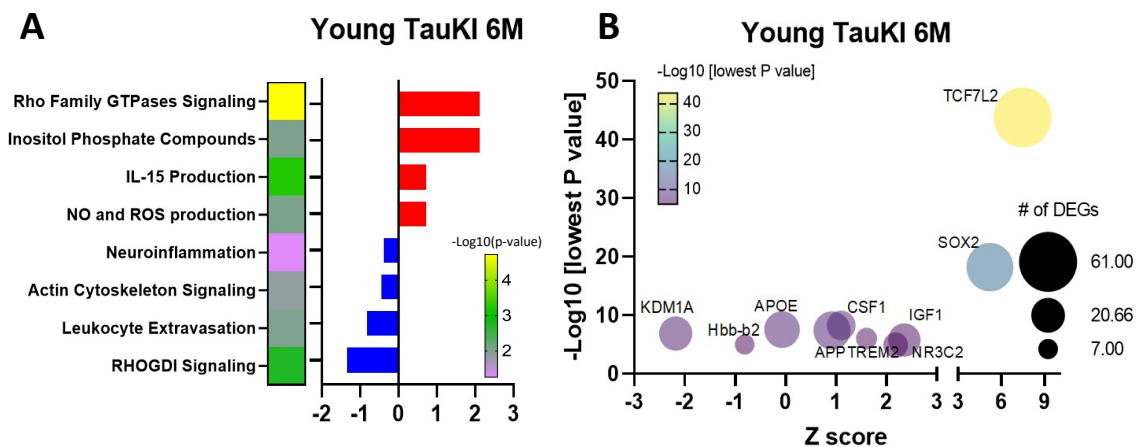
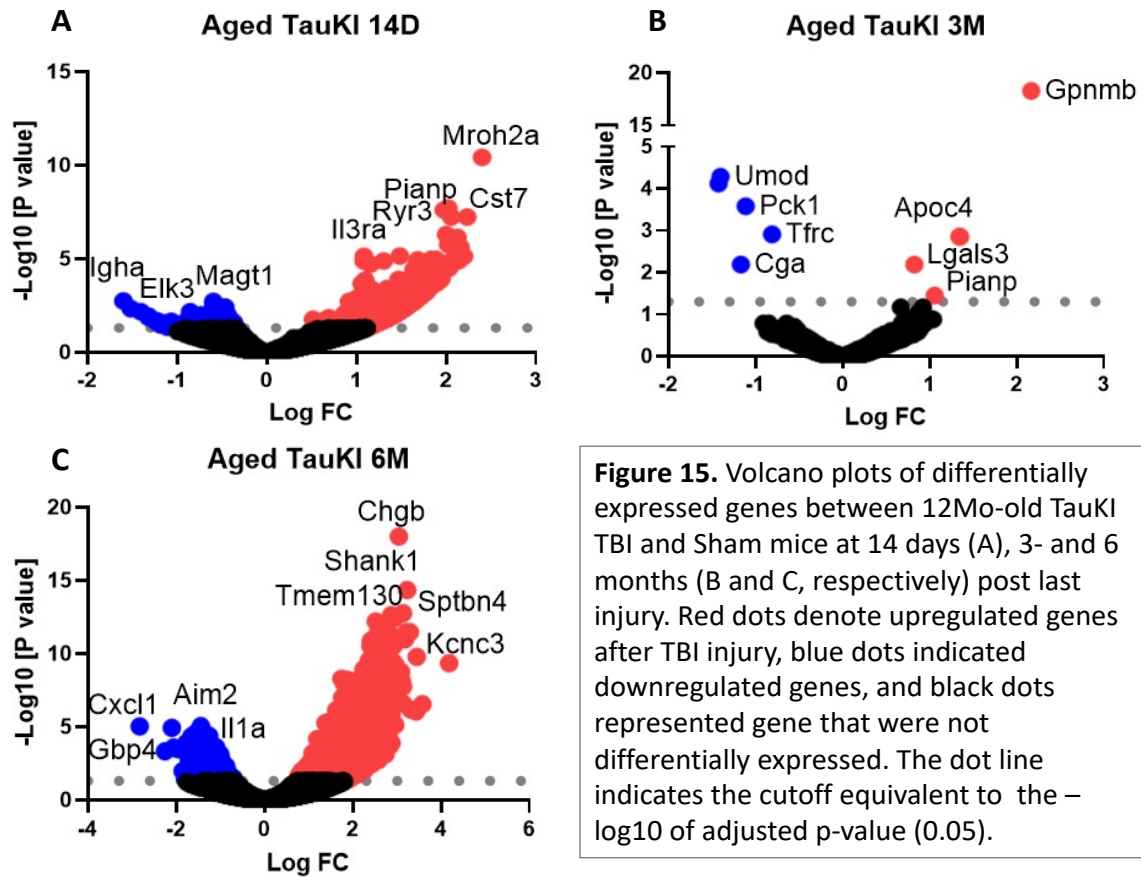


Figure 14. Pathway and Upstream regulator analysis on young TauKI 6 months post last injury. A) Top 4 upregulated pathways in red and top 4 downregulated pathways in blue. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

AGED TauKI



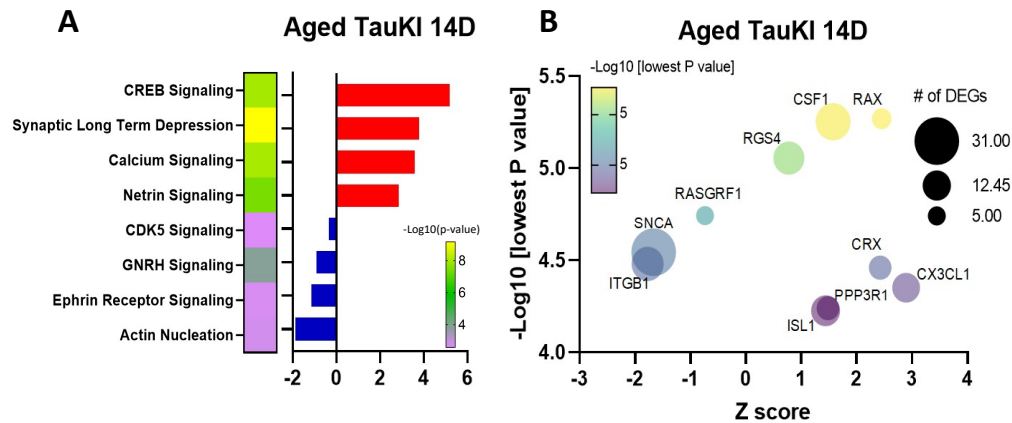


Figure 16. Pathway and Upstream regulator analysis on aged TauKI 14 days post last injury. A) Top 4 upregulated pathways in red and top 4 downregulated pathways in blue. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

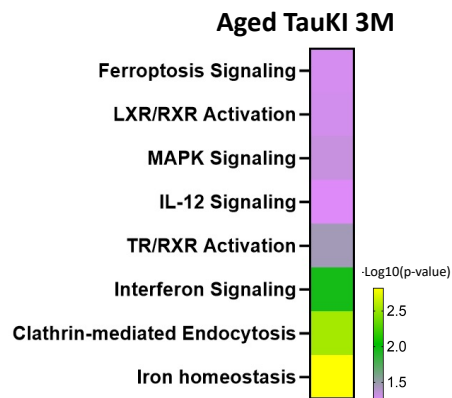


Figure 17. Pathway analysis on aged TauKI 3 months post last injury. Top 4 downregulated pathways.

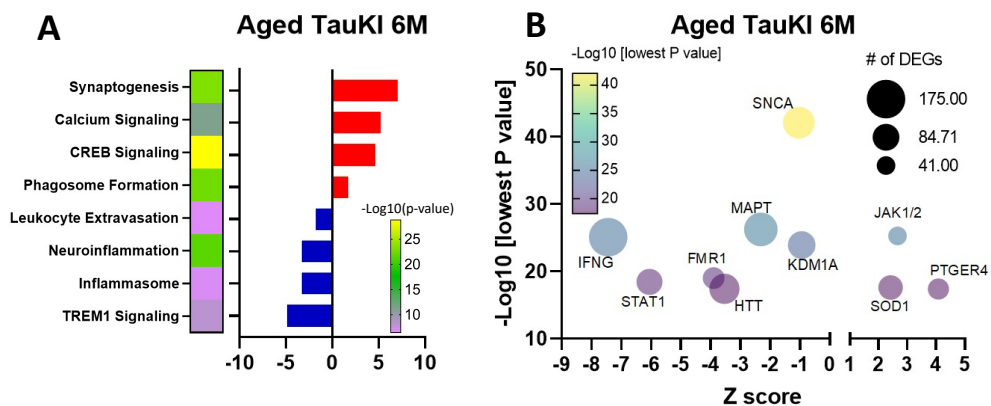


Figure 18. Pathway and Upstream regulator analysis on aged TauKI 6 months post last injury. A) Top 4 upregulated pathways in red and top 4 downregulated pathways in blue. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

Summary of astrocyte response in Young and Aged TauKI mice

In young TauKI mice following r-mTBI, we observed the greatest astrocyte transcriptomic response at 3 months post-injury (Fig. 10a and Fig. 11a-c). While in Aged TauKI mice, we observed the greatest astrocyte transcriptomic response at 6 months post-injury (Fig. 10b and Fig. 15a-c).

A greater transcriptomic response was observed in aged vs young mice at 14 and 6 months, whilst the reverse was observed at 3 months.

Young injured astrocytes at 14days exhibited an increase in TREM1, neuroinflammation and phagosome formation, and a downregulation in LXR/RXR, STAT3, CREB and IL15 signaling (Fig 12a). While Aged injured astrocytes showed an increase in CREB, calcium and netrin signaling coupled with a downregulation in CDK5, GNRH and Ephrin receptor signaling pathways (Fig 16a). Injured astrocytes from Young (Fig 12b) TauKI mice exhibited a proinflammatory response with activation of classical pro-inflammatory upstream regulators (e.g., IFN γ , TNF α , etc..) and downregulation in IL10. In Aged TauKI mice, injured astrocytes (Fig 16b) exhibit a proinflammatory response with an increase in CSF1, RAX and RGS4, and a downregulation in ITGB1 and SNCA.

At 3 months, injured young astrocyte exhibited an increase in synaptogenesis, Opioid, G α q and Rho signaling and NO/ROS production and, a downregulation in IL8, ET1 and neuroinflammation signaling and phagosome formation (Fig 13a). While Aged injured astrocytes showed an alteration in ferroptosis, MAPK, IL12, IFN signaling and iron homeostasis and clathrin mediated endocytosis (Fig 17). Injured astrocytes from Young (Fig 13b) TauKI mice exhibited an inhibition in proinflammatory upstream regulators (e.g., IFN γ , TNF α , etc..) and activation of APOE. Thus, suggesting that astrocytes from young injured TauKI mice exhibited an anti-inflammatory phenotype at the 3 month timepoint.

At 6 months astrocyte response were notably different in Young (Fig 14a,b) and Aged (Fig 18a,b) TauKI mice. Injured young astrocytes exhibited an upregulation in Rho and IL15 signaling and NO/ROS production, and a downregulation in LXR/RXR, STAT3, CREB and IL15 signaling (Fig 14a). While Injured Aged astrocytes exhibited an increase in calcium and CREB signaling, and phagosome formation, and a downregulation in neuroinflammation, inflammasome and TREM1 signaling (Fig 18a). Suggesting that aged astrocytes from TauKI mice at 6 months post-injury exhibited an anti-inflammatory phenotype. Top upstream regulators in young injured astrocytes were SOX2 and TCF7L2 which were both predicted to be activated (Fig 14b); while in Aged injured astrocytes the most significantly regulated upstream regulators were SNCA, MAPT, IFNG which were notably predicted to be inhibited (Fig 18b).

Astrocyte transcriptomic data in Young and Aged APP-KI cohort

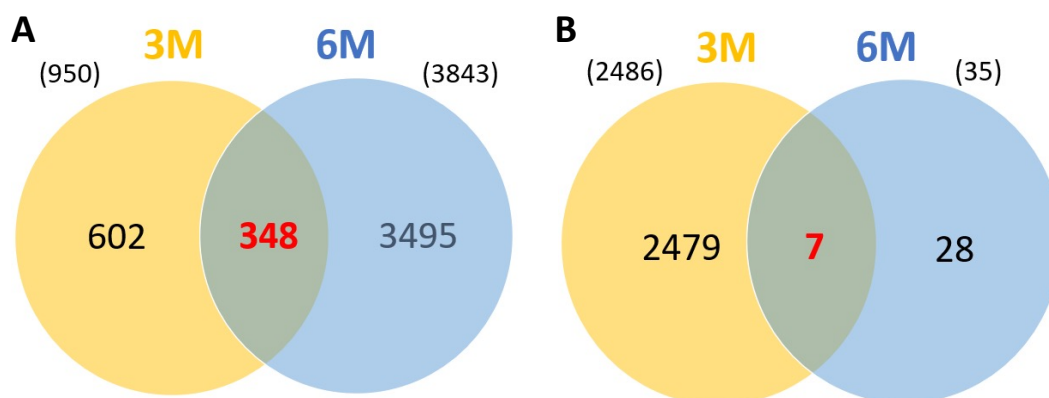


Figure 19. Venn-diagram of differentially expressed genes (TBI vs Sham) in 3mo- and 12mo-old cohort APPKI mice (A and B, respectively) at 3- and 6 months post last injury of the.

YOUNG APP-KI

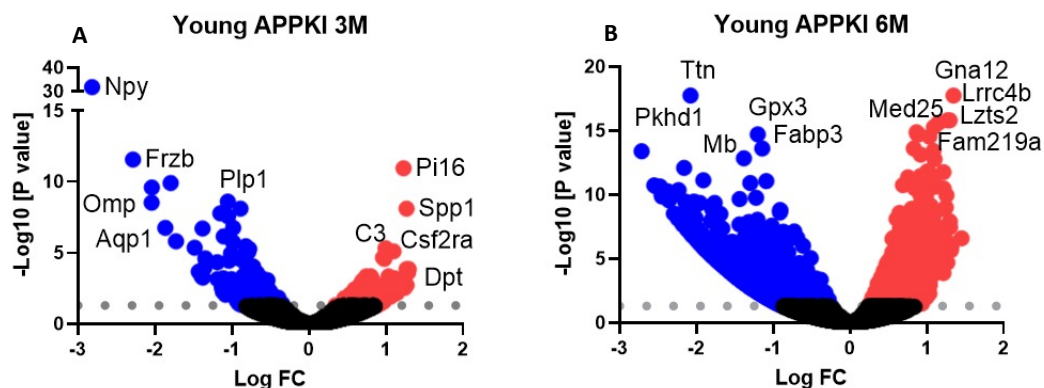


Figure 20. Volcano plots of differentially expressed genes in 3mo-old APPKI mice: TBI vs Sham mice at 3- (A), and 6 months (B) post last injury. Red dots denote upregulated genes after TBI injury, blue dots indicated downregulated genes, and black dots represented gene that were not differentially expressed. The dot line indicates the cutoff equivalent to the $-\log_{10}$ of adj p-value (0.05).

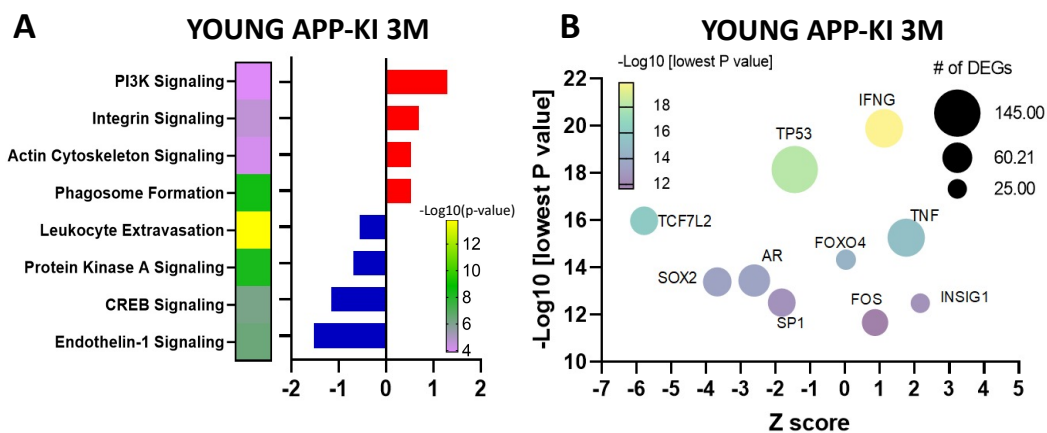


Figure 21. Pathway and Upstream regulator analysis on 3mo-old APPKI 3 months post last injury. A) Top 4 upregulated pathways in red and top 4 downregulated pathways in blue. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

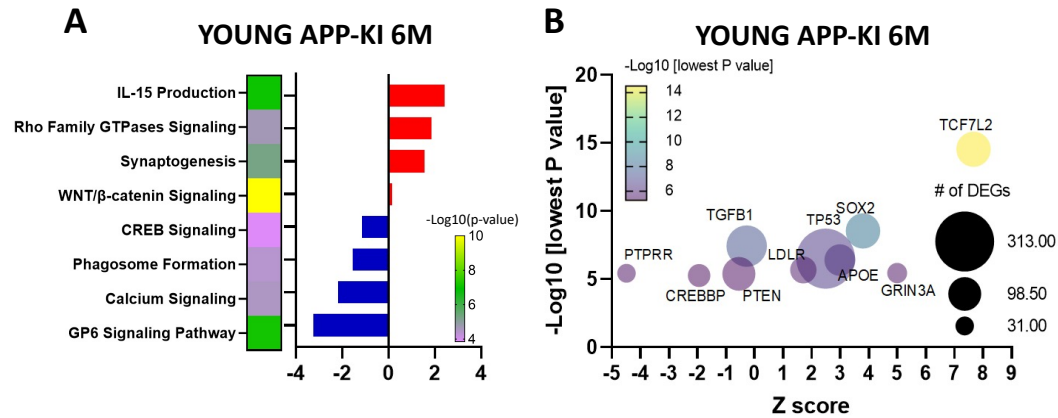


Figure 22. Pathway and Upstream regulator analysis on 3mo-old APPKI 6 months post last injury. A) Top 4 upregulated pathways in red and top 4 downregulated pathways in blue. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

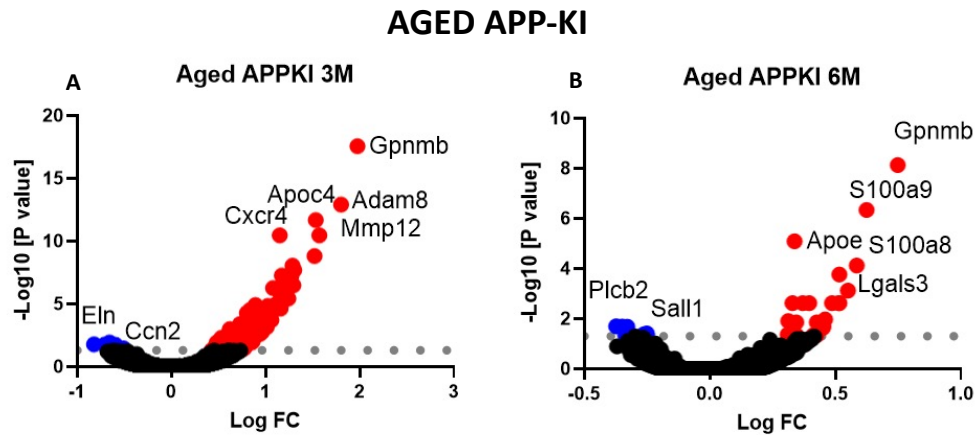


Figure 23. Volcano plots of differentially expressed genes in 12mo-old APPKI mice: TBI vs Sham mice at 3- (A), and 6 months (B) post last injury. Red dots denote upregulated genes after TBI injury, blue dots indicated downregulated genes, and black dots represented gene that were not differentially expressed. The dot line indicates the cutoff equivalent to the $-\log_{10}$ of adjusted p-value (0.05).

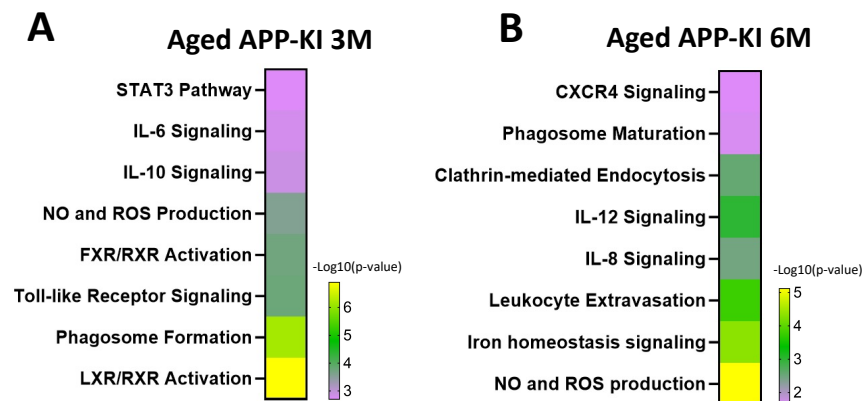


Figure 24. Pathway analysis on 12mo-old APPKI 3 and 6 months post last injury. A) Top 8 dysregulated pathways in aged APPKI 3mo post last injury B) Top 8 dysregulated pathways in aged APPKI 6mo post last injury.

Summary of astrocyte response in Young and Aged APP-KI mice

In young APP-KI mice following r-mTBI, we observed the greatest astrocyte transcriptomic response at 6 months post-injury (Fig. 19a and Fig. 20a,b). While in Aged APP-KI mice, we observed the greatest astrocyte transcriptomic response at 3 months post-injury (Fig. 19b and Fig. 23a,b). Thus, indicating that there was a more pronounced injury associated astrocytic response at 3 months in aged APP-KI mice compared to young mice. While astrocyte response in young injured mice was progressively more pronounced compared to shams at 6 months, the initial transcriptomic changes observed at 3 months in injured aged mice diminished compared to shams at 6 months.

Young injured astrocytes at 3 months exhibited an increase in P13K, integrin and phagosome formation, and a downregulation in PKA, ET1 and CREB signaling (Fig 21a). While Aged injured astrocytes showed alterations in TLR4, RXR, IL6, IL10 and STAT3 signaling and NO/ROS production (Fig 24a). Injured astrocytes from Young (Fig 21b) APP-KI mice exhibited a proinflammatory response with activation of classical pro-inflammatory upstream regulators (e.g., IFN γ , TNF α , etc..) and downregulation in TP53, TCF7L2 and SOX2. Due to the low number of significant transcripts in aged 3-month mice, no upstream regulators were generated by IPA.

At 6 months, injured young astrocyte exhibited an increase in synaptogenesis, Opioid, IL15, WNT/b-catenin and Rho signaling and, a downregulation in CREB, GP6, calcium and phagosome formation (Fig 22a). While aged injured astrocytes showed an alteration in CXCR4, NO/ROS production, IL8, IL12, IFN signaling and iron homeostasis and clathrin mediated endocytosis (Fig 24b). Injured astrocytes from Young (Fig 22b) APP-KI mice exhibited a predicted activation of upstream regulators, such as TCF7L2, SOX2, APOE, TP53, LDLR and inhibition of PTEN and TGFB1. Due to the low number of significant transcripts in aged 6-month mice, no upstream regulators were generated by IPA.

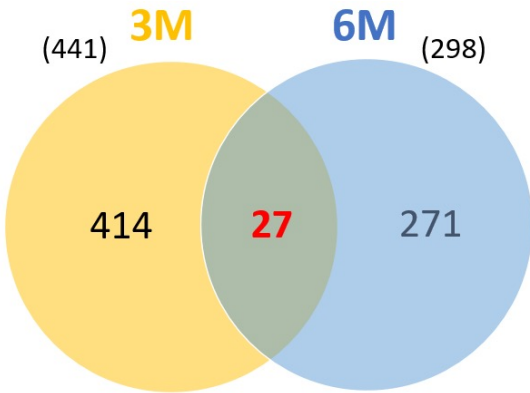


Figure 25. Venn-diagram of differentially expressed genes (TBI vs Sham) in 3mo-old GFAP^{P301L} mice at 3- and 6 months post last injury.

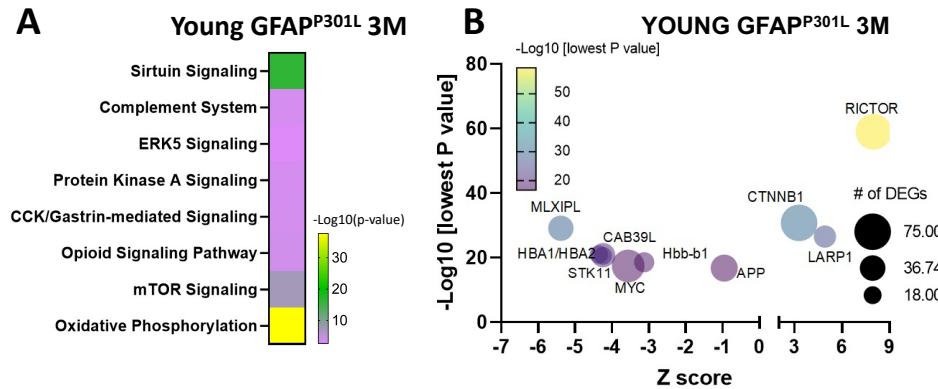


Figure 26. Pathway and Upstream regulator analysis on 3mo-old GFAP^{P301L} at 3 months post last injury. A) Top 8 dysregulated pathways in aged APPKI 3mo post last injury. B) Bubble plot of the top 10 upstream regulators. DEGs=differentially expressed genes.

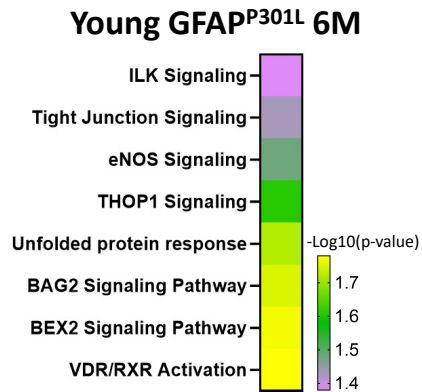


Figure 27. Pathway analysis on 12mo-old GFAP^{P301L} 3 months post last injury. Top 8 dysregulated pathways in aged APPKI 3mo post last injury.

Summary of astrocyte response in Young GFAP-P301L mice

In young GFAP-P301L mice, we observed 441 and 298 genes changing between r-mTBI vs sham mice 3 and 6 months post-injury, respectively (Fig. 25; volcano plot not shown). 27 of these significantly regulated genes were altered at both timepoints, while most (>80-90%) were distinctly different at each timepoint, indicating distinct types of astrocytic response/phenotypes at each timepoints.

Young injured astrocytes at 3 months an alteration in Sirtuin, complement, PKA, ERK5 and mTOR signaling and oxidative phosphorylation following IPA analyses (Fig 26a). These changes were driven by activation of upstream regulators such as ROCTOR, CTNNB1 and LARP1, and inhibition of upstream regulators MLXIPL, HBA1/2, MYC and CAB39L (Fig 26b). At 6 months astrocytic response were typified by an alteration in ILK, Tight junction, eNOS, THOP1, BAG2 and BEX2 signaling and VDR/RXR activation (Fig 27).

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

OVER THE NEXT QUARTER WE PLAN TO CONTINUE OR BEGIN:

Major Task 1

Subtask 7: Continue stereological analyses and Image quantitation of brain sections from Young cohort of TBI/SHAM mice.

Subtask 11: Continue stereological analyses and Image quantitation of brain sections from 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.

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 2b: Perform LC-MS/MS analyses of astroglia secretome from Young Cohort for unbiased proteomic expression profiling.

Subtask 3b: Perform LC-MS/MS analyses of astroglia secretome from Aged Cohort for unbiased proteomic expression profiling.

ALL plans are consistent with the original approved SOW.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Due to COVID-19, there has been a small reduction in work output at the Roskamp Institute. This has set us back by around 4-5 months. However, work is now progressing as normal and we aim to gain grounds in the work out over the next year.

Actual or anticipated problems or delays 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 4-5 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

Nothing to report

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

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:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report (In progress, planned for submission in Jan 2022)

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

*Neurotrauma meeting 2021 (Poster) – Camila Ortiz [Presenter]
Astrocyte transcriptomic profiles in human Tau and amyloid models after exposure to r-mTBI*

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

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?

Nothing to Report

What other organizations were involved as partners?

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 currently ongoing and planned completion will be in the next quarter.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

Please find the quad chart attached on the next page.

9. APPENDICES:

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

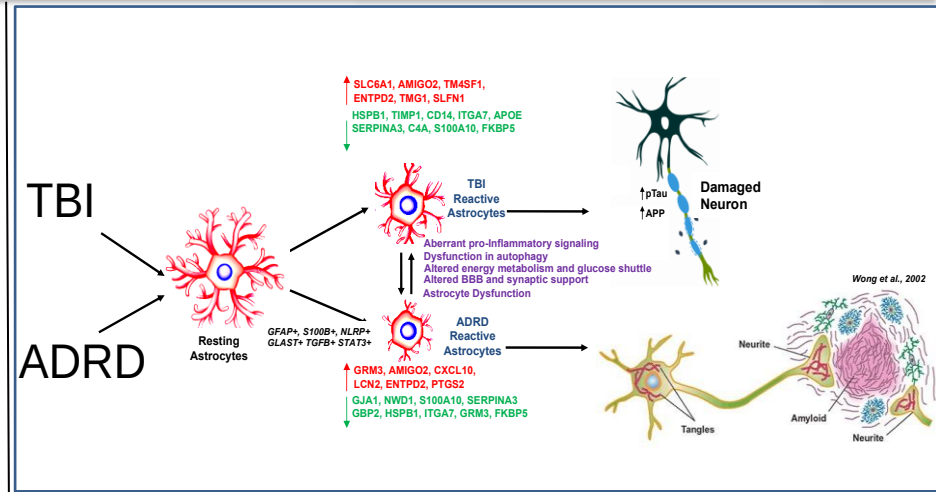
Org: Roskamp Institute, Sarasota, FL

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

- 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.
- 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.
- 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 and young mouse cohort for astrocyte markers. Generated astrocyte transcriptomic profiles in sham/TBI mice. Completed optimizing conditions for inoculation/adoptive transfer 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

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: \$488,570.00 **Actual Expenditure:** \$553,730.97.

Last updated: (September 2019)