AWARD NUMBER: W81XWH-16-1-0724

TITLE: Tau Processing by Mural Cells in Traumatic Brain Injury and Alzheimer's Disease

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REPORT DATE: April 2020

TYPE OF REPORT: Final

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
April 2020	Final	09/30/2016 - 12/29/2019

4. TITLE AND SUBTITLE

Tau Processing by Mural Cells in Traumatic Brain Injury and Alzheimer's Disease

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0724

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Materiel 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

14. ABSTRACT One of the pathways responsible for the removal of solutes from the brain involves brain vascular mural cells. Previously, we found that mural cells associate with tau (which accumulates in the brain following traumatic brain injury, TBI) to a greater extent than other cerebrovascular cells. The purpose of the current proposal is to investigate mural cell status following repetitive mild TBI (r-mTBI) and determine the contribution of these cells to the tau pathology associated with head trauma. Consistent with other neurodegenerative disorders such as Alzheimer's disease (AD), we observed a progressive decline in cerebrovascular mural cell expression following r-mTBI in mice and in human TBI brain specimens. Moreover, isolated cerebrovasculature from r-mTBI and AD animals were less able to internalize tau than their respective controls. Taken together, we observed a correlation between mural cell disruption and tau processing in TBI and AD. To our knowledge, these are the first studies to observe perturbations in mural cell expression and functional tau processing in the context of brain trauma. In totality, our studies indicate mural cell disruption in TBI and AD may be an important factor in tau pathogenesis and neurodegeneration and could explain the association between head trauma and the development of AD.

15. SUBJECT TERMS
tau, traumatic brain injury, Alzheimer's disease, mural cells, metabolism, cerebrovasculature

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
Unclassified					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE	Unclassified	26	19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified			

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

One of the prominent pathological features of traumatic brain injury (TBI) is the accumulation of hyperphosphorylated and aggregated tau species. Several studies have indicated that tau pathology is propagated through extracellular tau spreading and it has been reported that extracellular tau levels in the brain correlate with clinical outcome in TBI. Recent work indicates extracellular tau is removed from the brain through paravascular pathways and our studies demonstrate that brain vasculature mural cells (pericytes and smooth muscle cells) are involved in the processing and elimination of extracellular tau. Consistent with other neurodegenerative disorders including Alzheimer's disease (AD), we observed a progressive decline in cerebrovascular mural cell expression following repetitive mild TBI (r-mTBI) in mice. Moreover, isolated cerebrovasculature from r-mTBI animals were less able to internalize tau than sham animals. To our knowledge, these are the first studies to observe perturbations in mural cell expression and functional tau processing in the context of brain trauma. We hypothesize that brain vascular mural cells serve as a pathway for processing and eliminating tau from extracellular brain fluids and disruption of these cells in TBI and AD leads to tau pathology and neurodegeneration. Specific Aims: Aim 1) Examine mural cell expression and function in human and murine TBI brains. Aim 2) Evaluate the impact of r-mTBI on tau internalization and degradation in cerebrovascular cells. Aim 3) Determine the role of platelet-derived growth factor receptor-beta (PDGFR-beta) signaling and inflammation in mural cell disruption following TBI.

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

tau, traumatic brain injury, Alzheimer's disease, mural cells, metabolism, cerebrovasculature.

3. **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.

Major Goal 1: Evaluate mural cell expression in cerebrovasculature from human TBI brain specimens.
Milestone: Determination of mural cell expression in human TBI and human AD brain specimens.

Major Goal 2: Examine the timecourse of mural cell expression and function after r-mTBI in mice.
Milestone: Generation of a timeline for mural cell disruption following r-mTBI.

Major Goal 3: Examine tau internalization in r-sham and r-mTBI cerebrovascular cells.
Milestone: Determination of tau internalization in r-mTBI cerebrovascular cells.

Major Goal 4: Evaluate tau degradation pathways in r-sham and r-mTBI cerebrovascular cells.
Milestone: Determination of tau degradation by r-mTBI cerebrovascular cells.

Major Goal 5: Examine the PDGF pathway in human TBI brains and murine brains following r-mTBI.
Milestone: Determination of PDGF pathway expression and function in the cerebrovasculature following r-mTBI.

Major Goal 6: Evaluate the effect of PDGF-BB stimulation on tau processing by mural cells after r-mTBI.
Milestone: Impact of PDGF-BB stimulation on tau accumulation in cerebrovasculature after r-mTBI.

Major Goal 7: Evaluate the impact of inflammation on tau processing by mural cells.
Milestone: Determination of inflammation on tau accumulation and PDGF pathway in mural cells.

Major Goal completion status: All of the Major Goals listed above have been completed.

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.

1) major activities. The major activities for this reporting period have focused on the major goals listed in the previous section which include: evaluate mural cell expression in cerebrovasculature from human TBI brain specimens, examine the timecourse of mural cell expression and function after r-mTBI in mice, examine tau internalization in r-sham and r-mTBI cerebrovascular cells, evaluate tau degradation pathways in r-sham and r-mTBI cerebrovascular cells, examine the PDGF pathway in human TBI brains and murine brains following r-mTBI, evaluate the effect of PDGF-BB stimulation on tau processing by mural cells after r-mTBI, and evaluate the impact of inflammation on tau processing by mural cells.

2) specific objectives. Determination of mural cell expression in human TBI and human AD brain specimens, generation of a timeline for mural cell disruption following r-mTBI, determination of tau internalization in r-mTBI cerebrovascular cells, determination of tau degradation by r-mTBI cerebrovascular cells, determination of PDGF pathway expression and function in the cerebrovasculature following r-mTBI, impact of PDGF-BB stimulation on tau accumulation in cerebrovasculature after r-mTBI, and determination of inflammation on tau accumulation and PDGF pathway in mural cells.

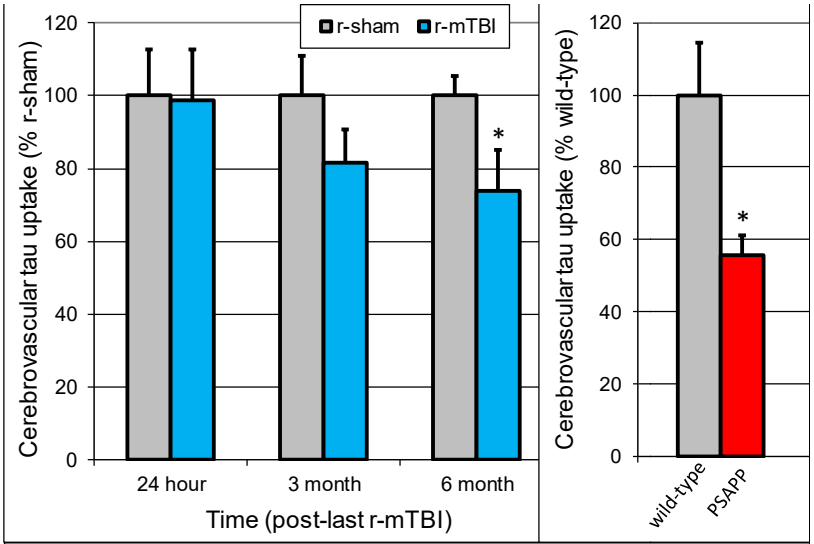


Figure A. Tau uptake in freshly isolated cerebrovessels from r-mTBI animals (24 hours, 3 months, and 6 months post-last injury). Cerebrovessels were exposed to 5ng/ml recombinant human tau (rhtau-441) for 1 hour at 37°C. Lysates were analyzed for total tau content by ELISA and normalized to total protein using the BCA assay. Values represent the percentage of each respective r-sham ± SEM (n=4). *P < 0.05 compared to respective r-sham as determined by ANOVA and Bonferroni post-hoc test.

3) significant results. ***AIM1:* Cerebrovascular tau uptake in r-mTBI *ex vivo*:** In our prior work, we observed an association between

tau and cells of the cerebrovasculature (i.e., smooth muscle and pericytes) *in vitro*. To investigate tau uptake in the cerebrovasculature following repetitive mild TBI (r-mTBI), we used a closed head model of concussive injury as previously characterized by our group. To study the human form of tau, we used transgenic human Tau (hTau) mice which express the six isoforms of human tau in the absence of endogenous mouse tau. For the present studies, 3-month old hTau mice were subjected to r-mTBI (2 injuries per week for 3 months) and, likewise, sham mice were subjected to 2 anesthesia exposures per week for 3 months (r-sham). Tau uptake was examined in freshly isolated cerebrovessels from r-mTBI mice 24 hours, 3 months, and 6 months after the last injury. In line with our prior *in vitro* studies, cerebrovessels were treated with recombinant human tau (5ng/ml) for 1 hour at 37ºC and total tau uptake was assessed in the lysates via ELISA. We observed a progressive decrease in tau uptake over time following the last injury compared to the respective r-sham group, as the ability to take in tau was significantly diminished at 6 months post-last injury (Figure A). Thus, in line with our overarching hypothesis, tau uptake by the cerebrovasculature (i.e., mural cells) is reduced following r-mTBI, which could lead to diminished tau elimination from the brain and may explain the increased tau pathology observed in our r-mTBI mouse model and human TBI/CTE brains. **Mural cell expression following r-mTBI in mice:** A potential explanation for the diminished functionality of the cerebrovasculature in processing tau may be depletion in mural cell density (i.e., mural cell loss) as previously observed in other neurodegenerative disorders. We examined mural cell expression in isolated cerebrovasculature from r-mTBI hTau mice at 24 hours, 3 months, and 6 months post-last injury, as above. We probed the cerebrovasculature of each cohort with known mural cell markers, PDGFRβ (platelet-derived growth factor receptor beta) and alpha smooth muscle cell actin (αSMC-actin). For PDGFRβ, in the r-mTBI group we observed a time-dependent reduction in PDGFRβ expression post-injury compared to r-sham mice,

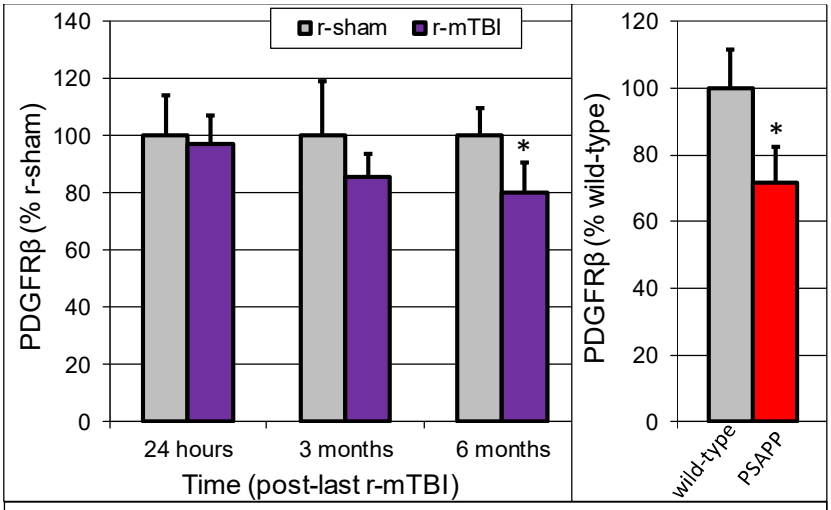


Figure B. Expression of PDGFRβ in isolated cerebrovessels from r-mTBI animals (24 hours, 3 months, and 6 months post-last injury), wild-type, and PSAPP mice (18 months of age). Brain vessels were analyzed for PDGFRβ content by ELISA and normalized to total protein using the BCA assay. Values represent the percentage of each respective r-sham or wild-type ± SEM (n=4). *P < 0.05 compared to respective r-sham or wild-type as determined by ANOVA and Bonferroni post-hoc test.

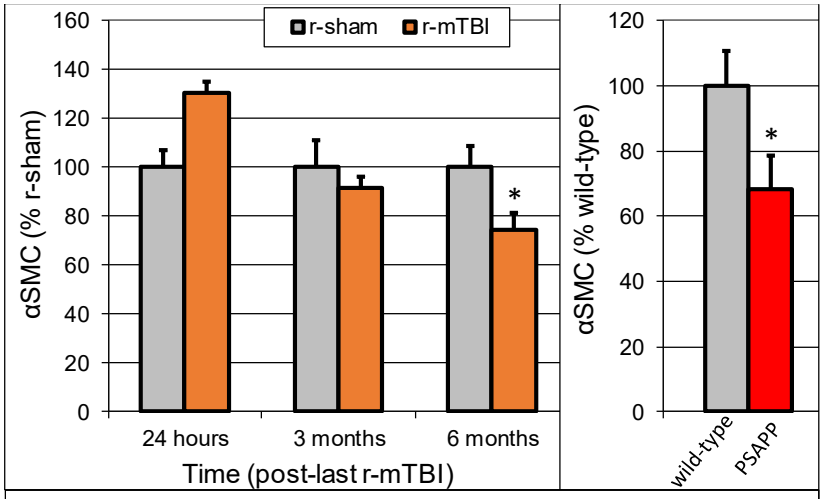


Figure C. Expression of α-SMC-actin in isolated cerebrovessels from r-mTBI animals (24 hours, 3 months, and 6 months post-last injury), wild-type, and PSAPP mice (18 months of age). Brain vessels were analyzed for α-SMC-actin content by ELISA and normalized to total protein using the BCA assay. Values represent the percentage of each respective r-sham or wild-type ± SEM (n=4). *P < 0.05 compared to respective r-sham or wild-type as determined by ANOVA and Bonferroni post-hoc test.

indicating a progressive reduction in mural cell density following r-mTBI (Figure B). For αSMC-actin, there was an initial increase in expression after r-mTBI at 24 hours post-injury, followed by a progressive decrease at 3 and 6 months post-injury (Figure C). These findings are consistent with our prior observations using the same r-mTBI paradigm in an aged wild-type cohort (12 months of age), where we also observed significant reductions following injury in both αSMC-actin and PDGFRβ via western blotting. These studies indicate the diminished tau uptake we observed in r-mTBI cerebrovessels above (Figure A) may be the result of reduced mural cell density in the cerebrovasculature post-injury. **Tau processing and expression of mural cell markers in AD mice:** In the above studies, we showed a progressive decrease in tau processing by r-mTBI cerebrovessels compared to r-sham animals and a corresponding reduction in the expression of αSMC-actin (alpha smooth muscle cell actin) and PDGFRβ (platelet-derived growth factor receptor beta). As one of the overarching goals of this project is to evaluate the interrelationship between TBI and the development of AD, we performed these same analyses in a mouse model of AD. The PSAPP transgenic model carries mutations in the presenilin 1 (PS1) and amyloid precursor protein (APP) genes, which recapitulates some of the pathological features of human AD. As with the r-mTBI studies above, we isolated fresh cerebrovessels from PSAPP mice at 18 months of age and examined tau processing and the expression of PDGFRβ and αSMC-actin. Similar to our observations following r-mTBI at 6 months post-last injury, we observed significant reductions in tau uptake and mural cell marker expression in the PSAPP mice compared to age-matched wild-type littermates (Figures A-C). Based on these findings, the brain vascular mural cell population appears to be disrupted following TBI and in AD, the result of which is reduced tau processing, which may describe the accumulation of tau in the brain that is observed in both of these disorders. **Mural cell expression in human TBI brain vasculature:** While mural cell loss has been observed in many neurodegenerative disorders in humans, no one has investigated the state of the mural cell population in humans following TBI. In line with the studies above in r-mTBI animals, we examined mural cell expression in cerebrovasculature isolated from frozen human cortical tissue (500mg) obtained from control subjects (no history of brain trauma) and TBI donors with an established history of brain trauma. In addition, as we are interested in the relationship between TBI and the development of AD, we examined human AD specimens (with and without a history of TBI). All tissue from each group came from the same

Table 1. Autopsied human cortical tissue (inferior frontal gyrus).

Group	Sample size	Age ± SEM (years)	Sex (M/F)	Years post-last injury ± SEM
Control	15	79.4 ± 1.9	8/7	
TBI	12	85.2 ± 1.9	6/6	38.1 ± 9.2
AD	15	82.5 ± 1.7	7/8	
AD + TBI	14	79.6 ± 2.3	7/7	41.5 ± 7.9

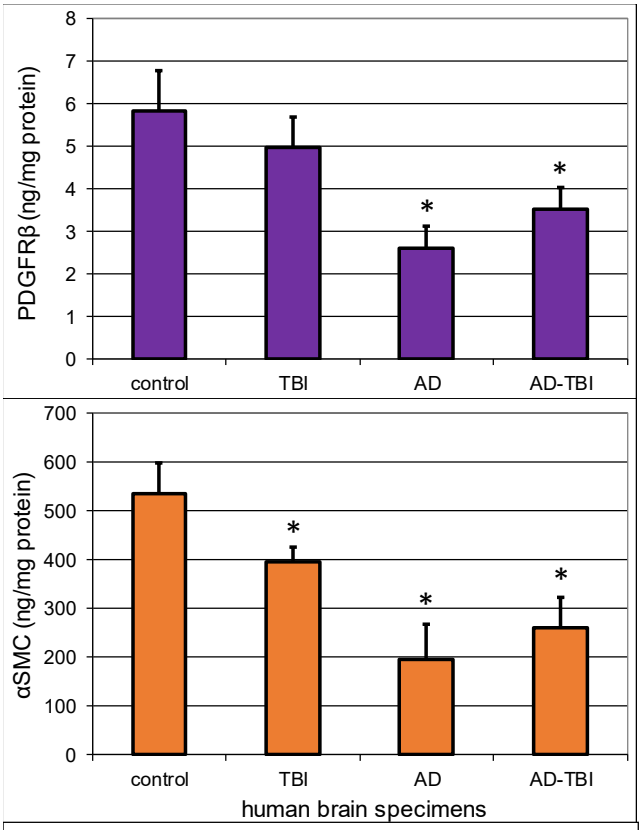


Figure D. Mural cell marker expression in cerebrovasculature isolated from human brain cortex derived from, 1) non-demented control subjects (no history of TBI or AD diagnosis), 2) TBI, 3) AD, and 4) TBI and AD. Lysates were analyzed for PDGFRβ (top) and αSMC-actin (bottom) by ELISA and normalized to total protein using the BCA assay. Values represent mean ± SEM (n=12-15) and are expressed as ng per mg of total protein. *P < 0.05 compared to control as determined by ANOVA and Bonferroni post-hoc test.

region of the brain cortex (inferior frontal gyrus). See Table 1 for additional information regarding this cohort. Using the same mural cell markers as the animal studies above, we observed a significant decrease (~50%) in both αSMC-actin and PDGFRβ in the human AD brain specimens compared to the control group (Figure D), consistent with prior reporting by other groups. For the TBI group, there was a subtle decrease in PDGFRβ levels compared to the control samples, and a significant decrease in αSMC-actin levels between the TBI group and control (Figure D). It is important to reiterate that in our mouse TBI paradigm, injuries are administered over a chronic period of time (3 months), akin to what professional athletes or soldiers may experience over the course of a career. All of the human TBI samples in this study experienced head trauma with a loss of consciousness (< 30 minutes), defined as mild to moderate, in line with our concussive mouse model. However, the human TBI cohort was exposed to 1-2 concussive events, which may not align with our mouse injury paradigm which intends to study TBI in a more chronic repetitive context. As such, a higher frequency of head injuries over one's lifetime may have resulted in diminished mural cell expression on par with the observations in our r-mTBI model and the human AD brain samples. That having been said, these findings are intriguing as they may improve our understanding of how different TBI paradigms and factors (e.g., severity, frequency, interval, age, etc.) can lead to different pathophysiological outcomes.

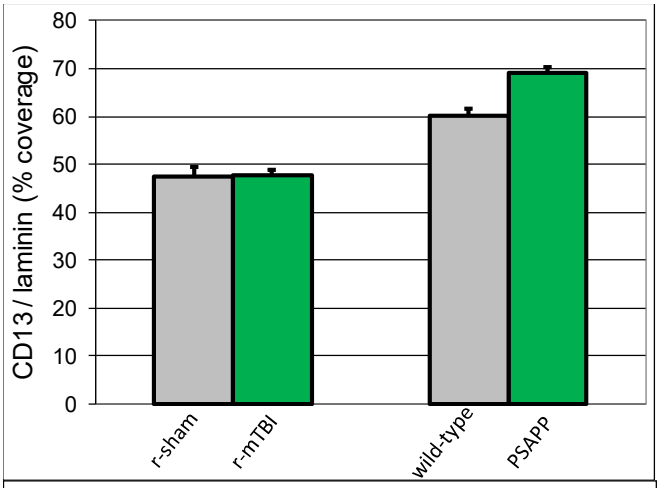


Figure E. Vessel coverage of the mural cell marker, CD13, in relation to the vessel marker, laminin, in r-sham and r-mTBI animals (6 months post-last injury) and wild-type and PSAPP animals (18 months of age) as determined by confocal microscopy. Values represent mean ± SEM (n=4-6) and are expressed as % coverage in comparison to laminin. *P < 0.05 compared to respective control as determined by ANOVA and Bonferroni post-hoc test.

<u>Mural cell density in r-mTBI and PSAPP mice using confocal microscopy:</u> As just described, we determined the expression of mural cell markers at various timepoints following head trauma in our mouse r-mTBI model and observed a progressive decrease in the expression of these markers (PDGFRβ and α-SMC-actin) in isolated cerebrovasculature from r-mTBI animals. These findings correlated with a decrease in tau uptake in isolated cerebrovessels at the same time points post-last injury. What remains to be determined is whether the changes in mural cell marker expression are due to a down regulation of these proteins or a decrease in the mural cell population. This is important in understanding the nature of the dysfunction, as it pertains to tau uptake, and may provide insight into the effectiveness of potential therapies. As such, we collected and prepared brains from animals at 6 months post-last injury

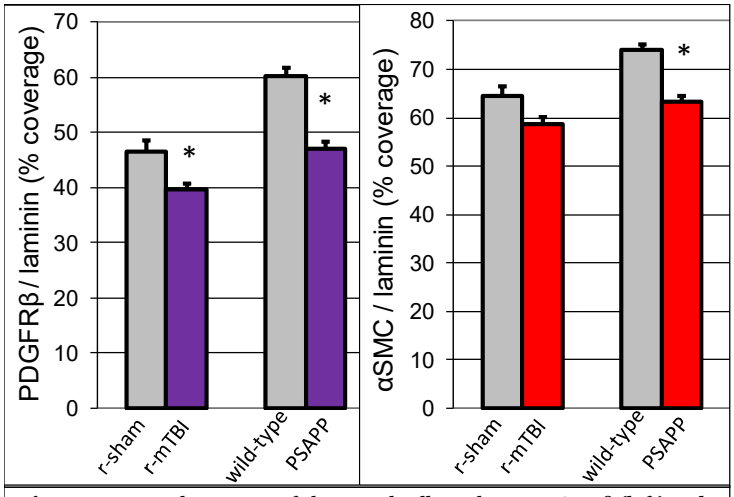


Figure F. Vessel coverage of the mural cell markers, PDGFRβ (left) and αSMC-actin (right), in relation to the vessel marker, laminin, in r-sham and r-mTBI animals (6 months post-last injury) and wild-type and PSAPP animals (18 months of age) as determined by confocal microscopy. Values represent mean ± SEM (n=4-6) and are expressed as % coverage in comparison to laminin. *P < 0.05 compared to respective control as determined by ANOVA and Bonferroni post-hoc test.

and PSAPP mice and examined several markers to assess the vessel density of the mural cell population in the brain following r-mTBI using confocal microscopy. As we have done in several aspects of this project, we compared these results to our analyses in the PSAPP mice, as previous reporting has indicated the mural cell population is depleted in AD animals. Using a common mural cell marker (CD13), we assessed the percentage of mural cell coverage in relation to vessel surface area, as determined by a known blood vessel marker, laminin. We did not observe significant changes in mural cell density following r-mTBI (6 months post-last injury) compared to r-sham animals, nor in the AD animals (PSAPP) compared to age-matched (18 months of age) wild-type mice (Figure E). Alternatively, we did see reductions in both PDGFR-beta and alpha-SMC-actin following r-mTBI and in the AD animals compared to their respective controls (Figure F), which is consistent with our biochemical assessments of these markers in the timeline studies above, and prior reporting from other groups interrogating AD animal models. As such, based on our CD13 analyses, there does not appear to be an overt depletion of the vascular mural cell population following r-mTBI. However, the degeneration we observed in tau uptake in the prior above studies could be due to changes in the expression of PDGFR-beta and alpha-SMC-actin. The PDGF pathway is tightly regulated and maintenance of this pathway is required for pericyte function. Specifically, while the PDGF pathway is primarily responsible for maintaining the brain vasculature, this pathway is also associated with a number of endocytic components of cell membrane, specifically caveolae. Altogether, while the mural cell population is largely maintained following brain injury, the PDGF pathway is disrupted, which may impact tau uptake and processing via caveolin-1, and contribute to the accumulation of extracellular tau in the brain following head trauma and in AD. Cerebrovascular caveolin-1 levels following head trauma and in AD in both animal and human brain specimens are described further in our studies below.

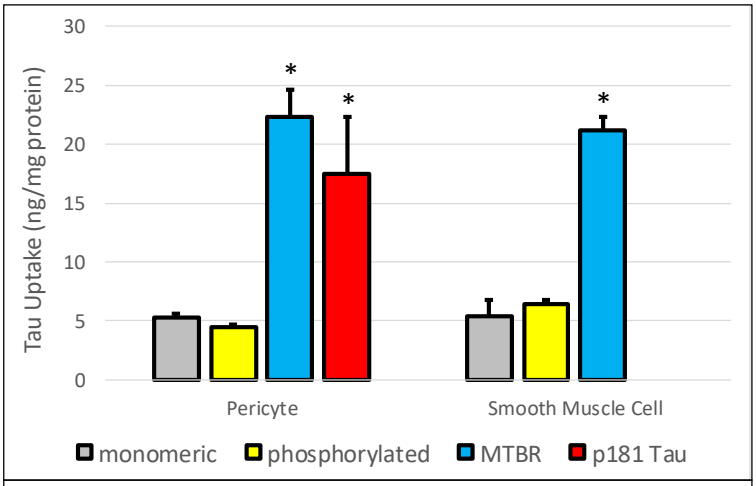


Figure G. Tau uptake in primary human pericyte and smooth muscle cell cultures. Cells were exposed to 50ng/ml tau (monomeric tau-441, DYRK1a phospho-tau-441, a tau fragment corresponding to the microtubule binding region MTBR, and phospho-tau p181) for 3 hours at 37°C. Lysates were analyzed for tau content by a tau species-specific ELISA and normalized to total protein using the BCA assay. Values represent mean ng tau per mg protein ± SEM (n=3). *p < 0.05 compared to respective monomeric tau as determined by ANOVA and Bonferroni post-hoc test.

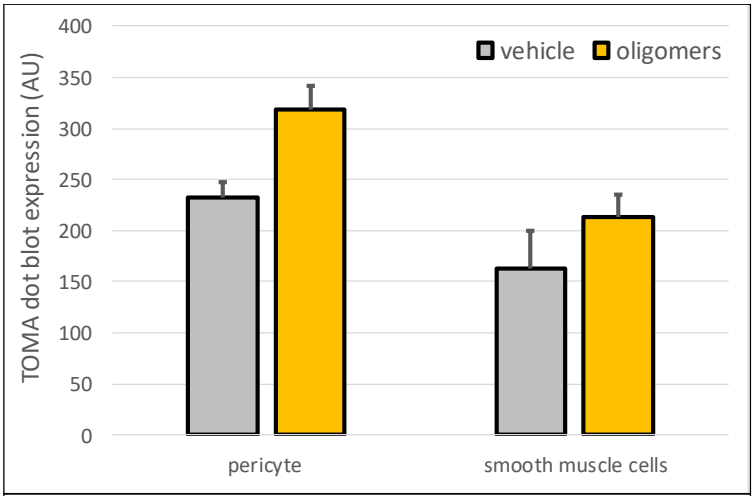


Figure H. Tau uptake in primary human pericyte and smooth muscle cell cultures. Cells were exposed to 50ng/ml oligomeric tau for 3 hours at 37°C. Lysates were analyzed for oligomeric tau content by dot blot using the TOMA antibody. Values represent mean tau expression ± SEM (n=3). *p < 0.05 compared to vehicle (water) as determined by ANOVA and Bonferroni post-hoc test.

AIM2: **<u>Tau internalization in mural cells *in vitro*</u>:** To examine the interaction of various tau species with brain vascular mural cells, we evaluated the cellular uptake of each tau species in pericytes and smooth

muscle cells *in vitro*. For these studies we tested 5 different tau species (50ng/ml) including monomeric tau441, DYRK1a phosphorylated-tau441, a tau fragment corresponding to the microtubule binding region (MTBR), phospho-tau p181, and oligomeric tau (TOMA). In Figure G, each species accumulated in both cell types significantly above the background levels indicating the mural cells internalize these tau species and may have a role in their processing and elimination. In comparing the uptake of each species, the accumulation of the MTBR and p181 tau species were significantly greater than the monomeric tau species. As such, degeneration in the mural cell population following head trauma may lead to an elevation of these species in the extracellular fluids of the brain, should they be present, as these cells would be less capable of degrading or eliminating these tau species, in particular. Due to the nature of this species, we could not apply the same analytical technique toward the evaluation of the tau oligomers, and instead used dot blotting with the TOMA (tau oligomer monoclonal antibody) antibody (Figure H). Interestingly, while the oligomers in the cell were slightly above the background (water vehicle) levels, they did not significantly accumulate in either of the mural cell cultures (Figure H). As such, it does not appear that these cells readily process the tau oligomers, which may be why these species often accumulate in the brain in TBI and other neurodegenerative disorders. **Tau internalization in r-mTBI cerebrovascular cells *ex vivo*:** To identify the influence of r-mTBI on the processing of various tau species by brain vascular mural cells, we isolated fresh cerebrovessels from r-sham and r-mTBI animals at 6 months post-last injury and examined tau uptake akin to the *in vitro* studies above. For monomeric, phosphorylated, cleaved tau, and oligomeric tau, we did not observe a significant difference in the uptake of these species between the r-sham and r-mTBI groups (Figures I and J). Thus, the processing of these tau species by the cerebrovasculature does not appear to be impacted by head trauma. Alternatively, we did

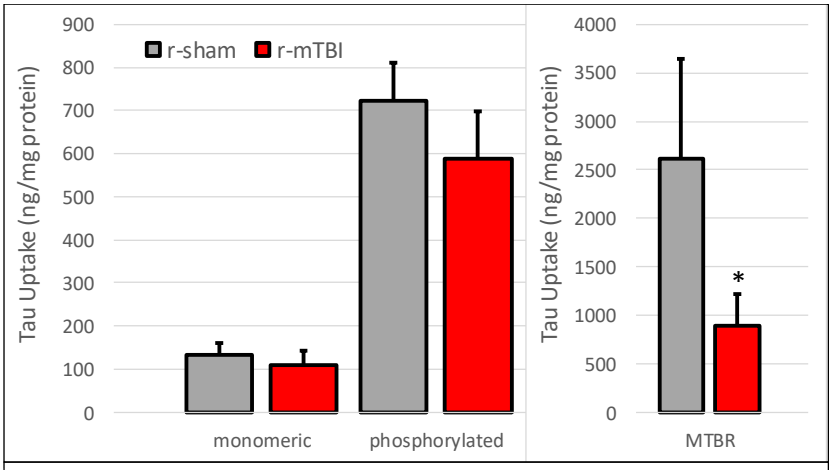


Figure I. Tau uptake in freshly isolated cerebrovessels from r-mTBI animals (6 months post-last injury). Cerebrovessels were exposed to 50ng/ml tau (monomeric tau-441, DYRK1a phospho-tau-441, and a tau fragment corresponding to the microtubule binding region MTBR) for 24 hours at 37°C. Lysates were analyzed for tau content by a tau species-specific ELISA and normalized to total protein using the BCA assay. Values represent mean ng tau per mg protein ± SEM (n=4). *p < 0.05 compared to respective r-sham as determined by ANOVA and Bonferroni post-hoc test.

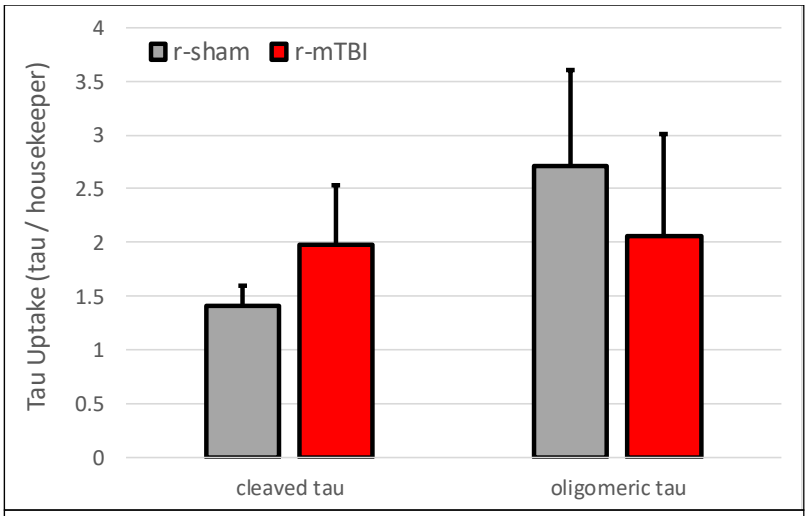


Figure J. Tau uptake in freshly isolated cerebrovessels from r-mTBI animals (6 months post-last injury). Cerebrovessels were exposed to 50ng/ml tau (cleaved tau C3 and oligomeric tau TOMA) for 24 hours at 37°C. Lysates were analyzed for tau content by immunoblotting and normalized to GAPDH (cleaved tau) or stain-free total protein (oligomeric tau). Values represent mean tau levels per housekeeping protein ± SEM (n=4). *p < 0.05 compared to respective r-sham as determined by ANOVA and Bonferroni post-hoc test.

observe a significant decrease (2.5-fold) in the uptake of the MTBR tau species in the r-mTBI cerebrovessels compared to r-sham (Figure I). As described in the *in vitro* studies, MTBR tau was highly interactive with each mural cell culture, and the disruption of the mural cell population post-injury may describe the lack of uptake or interaction of the cerebrovessels with MTBR tau in these *ex vivo* studies. In total, the MTBR species in particular may tend to accumulate in the brain due to reduced processing and elimination by the degenerating mural cell population following head trauma. **Caveolin-1 levels in mouse r-mTBI and AD cerebrovessels:** To further understand the mechanistic influence of r-mTBI and AD on tau internalization in cerebrovascular cells, we investigated the status of caveolin-1 in our cerebrovascular samples as diminished caveolin-1 levels have been shown to result in solute accumulation in the brain and promote neurodegeneration. In Figure K, caveolin-1 levels in isolated r-mTBI cerebrovessels significantly increased at 24 hours post-injury compared to r-sham, but were significantly decreased (2-fold) at 3 and 6 months post-injury (Figure K). Moreover, as we are interested in evaluating the association between TBI and the development of AD, we also found cerebrovascular caveolin-1 levels in PSAPP animals (AD mouse model) were significantly decreased (2-fold) compared to age-matched wild-type animals (Figure K). As caveolin-1 is responsible for the cellular uptake of a number of solutes, including tau, diminished caveolin-1 expression following head trauma may lead to reduced tau uptake and degradation by mural cells and subsequent tau accumulation in the brain post-injury. **Caveolin-1 levels in human TBI and AD cerebrovessels:** To complement the animal studies, we also examined caveolin-1 levels in cerebrovessels isolated from TBI and AD human brain specimens (described in Aim1). In line with the r-mTBI mouse studies, caveolin-1 levels were significantly reduced in the individual TBI and AD groups compared to control

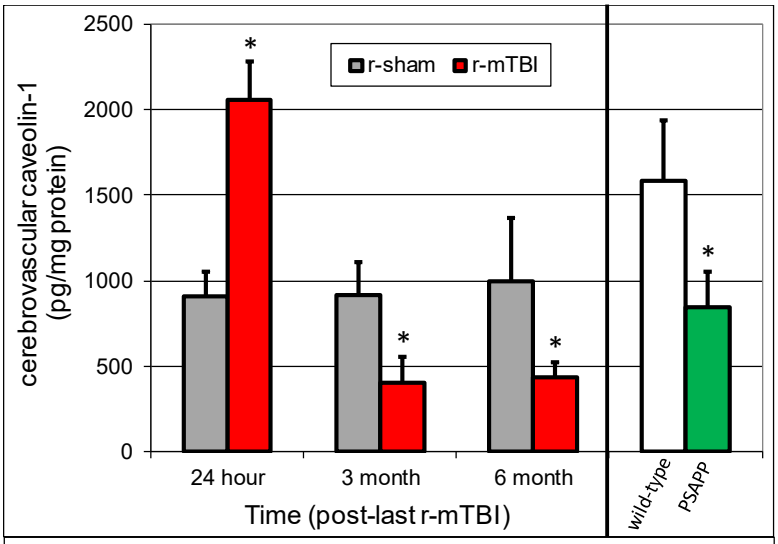


Figure K. Caveolin-1 expression in isolated cerebrovessels from r-mTBI (24 hours, 3 months, and 6 months post-last injury), wild-type, and PSAPP mice (18 months of age). Lysates were analyzed for caveolin-1 content by ELISA and normalized to total protein using the BCA assay. Values represent mean ± SEM (n=4-6). *P < 0.05 compared to each respective r-sham or wild type group as determined by ANOVA and Bonferroni post-hoc test.

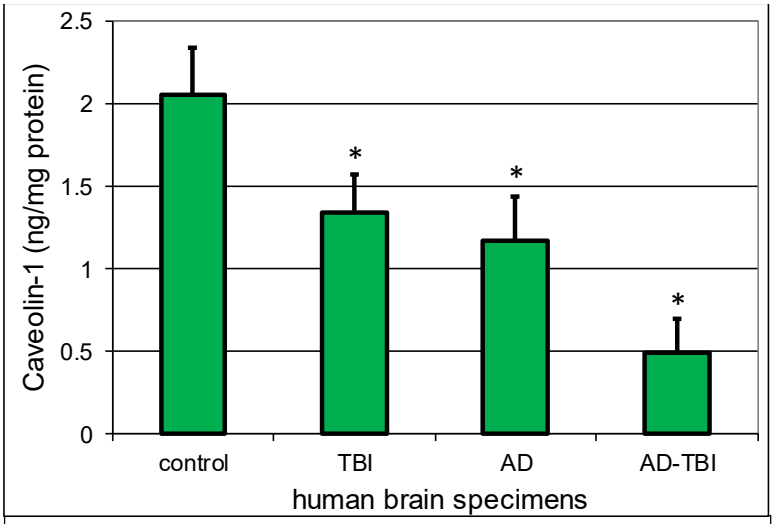


Figure L. Expression of caveolin-1 in cerebrovasculature isolated from human brain cortex derived from, 1) non-demented control subjects (no history of TBI or AD diagnosis), 2) TBI, 3) AD, and 4) TBI and AD. Lysates were analyzed for caveolin-1 content by ELISA and normalized to total protein using the BCA assay. Values represent the mean amount (ng) of caveolin-1 per mg of total protein ± SEM (n=12-15). *P < 0.05 compared to control brains as determined by ANOVA and Bonferroni post-hoc test.

samples, culminating in a 4-fold decrease in the samples with both TBI and AD (Figure L). **Tau degradation by r-mTBI cerebrovascular cells:** To examine tau degradation pathways by cerebrovascular mural cells following brain injury, we isolated fresh cerebrovessels from r-sham and r-mTBI mice at 6 months post-last injury and evaluated autophagy and proteasome activity following exposure to monomeric and oligomeric tau species. In terms of autophagy, no significant changes in LC3-II levels were observed between r-mTBI cerebrovessels and the r-sham group (Figure M). However, exposure to monomeric, and oligomeric tau in particular, resulted in elevated autophagy compared to control (no tau exposure). Alternatively, proteasome activity was significantly decreased in the r-mTBI animals compared to r-sham mice (Figure N). While proteasome degradation was decreased overall following brain injury, no differences in proteasome activity was observed between the tau species (Figure N). These studies indicate tau degradation via the proteasome may be diminished in cerebrovascular cells following head trauma, which may describe the elevated tau levels in the brain post-injury.

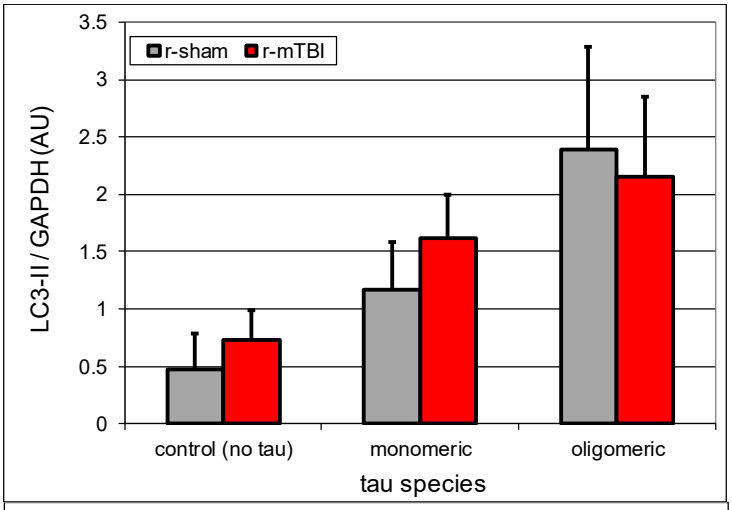


Figure M. Autophagic activity in response to tau exposure in isolated cerebrovessels from r-mTBI animals at 6 months post-last injury. Cerebrovessels were exposed to water (control), monomeric, or oligomeric tau species (50ng/ml) for 24 hours at 37°C. Lysates were probed for LC3-II using immunoblotting and normalized to GAPDH. Values represent mean arbitrary units (AU) of LC3-II/GAPDH ± SEM (n=3). *P < 0.05 compared to respective r-sham as determined by ANOVA and Bonferroni post-hoc test.

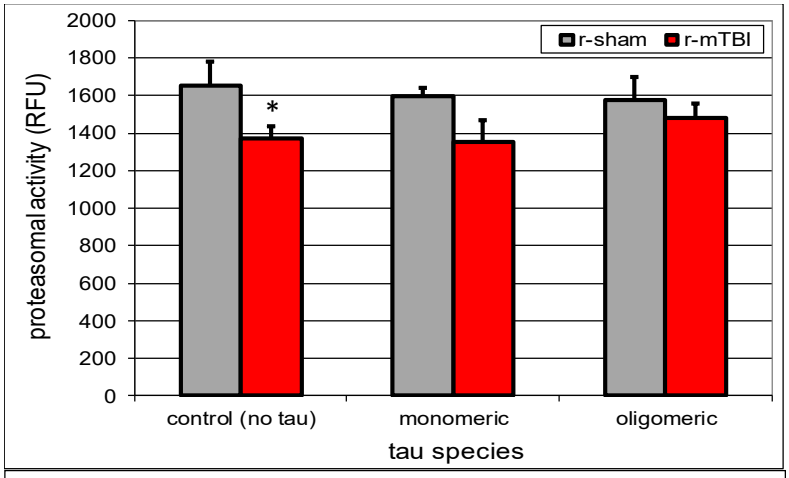


Figure N. Proteasomal activity in response to tau exposure in isolated cerebrovessels from r-mTBI animals at 6 months post-last injury. Cerebrovessels were exposed to water (control), monomeric, or oligomeric tau species (50ng/ml) for 24 hours at 37°C. The cells were analyzed for proteasomal activity using a 20S proteasome assay. Values represent mean activity ± SEM (n=3). *P < 0.05 compared to respective r-sham as determined by ANOVA and Bonferroni post-hoc test.

AIM3: **PDGF pathway in human TBI brains and murine brains following r-mTBI:** As outlined in Aim 3 of the proposed studies, we continued our evaluation of the PDGF pathway and examined the expression of the PDGF-BB agonist in our brain samples, to complement our above work evaluating the PDGFRβ receptor. These studies will provide a more complete assessment of the status of the PDGF pathway following TBI and in AD. In the mouse r-mTBI cohort, there was a progressive decrease in PDGF-BB expression in brain homogenate following r-mTBI, culminating in a 20% reduction at 6 months post-last injury compared to r-sham animals (Figure O). Interestingly, in the AD mouse model (PSAPP), the status of PDGF-BB was very different from our observations following r-mTBI. The levels of PDGF-BB in brain homogenate from AD mice were 2-times that observed in wild-type littermates. In addition, we performed these same analyses in human TBI brain specimens and human AD brain specimens. As in the prior animal studies, we investigated PDGF-BB expression in TBI brain homogenate and observed a similar 20% reduction in PDGF-BB (Figure P)

compared to human control brain samples (i.e., no history of TBI). In addition, we also examined a group of human AD brain samples and found no difference in PDGF-BB levels compared to the control group (Figure P). Lastly, we evaluated PDGF-BB in brain homogenate samples from patients with a history of TBI that were also diagnosed with AD (TBI-AD). In line with that observed in the TBI group, PDGF-BB levels in the TBI-AD group were significantly decreased compared to control brains (Figure P). Overall these findings are similar to our observations in the animals as PDGF-BB levels were significantly reduced in the brain following head trauma. With respect to the human and animal AD brains, we did not observe the same decrease in PDGF-BB levels as we did following TBI, and in the case of the AD animals, we observed a significant increase in PDGF-BB compared to age-matched wild-type brains (as described above). Thus, while the PDGF pathway is disrupted in both TBI and AD, the nature of the dysfunction is seemingly quite different. Based on our findings, the PDGF-BB ligand is decreased in TBI, while in AD, the receptor is substantially diminished. As such, the manner in which this pathway may be targeted therapeutically, could be quite different for each disease state.

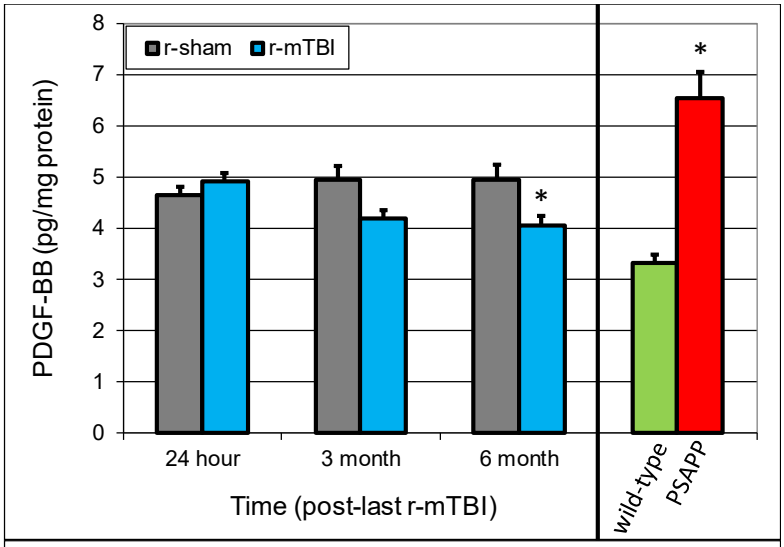


Figure O. Expression of PDGF-BB in brain homogenate from r-mTBI (24 hours, 3 months, and 6 months post-last injury), wild-type, and PSAPP mice (18 months of age). Brain samples were analyzed for PDGF-BB content by ELISA and normalized to total protein using the BCA assay. Values represent the mean amount (pg) of PDGF-BB per mg of total protein ± SEM (n=4-6). *P < 0.05 compared to each respective control as determined by ANOVA and Bonferroni post-hoc test.

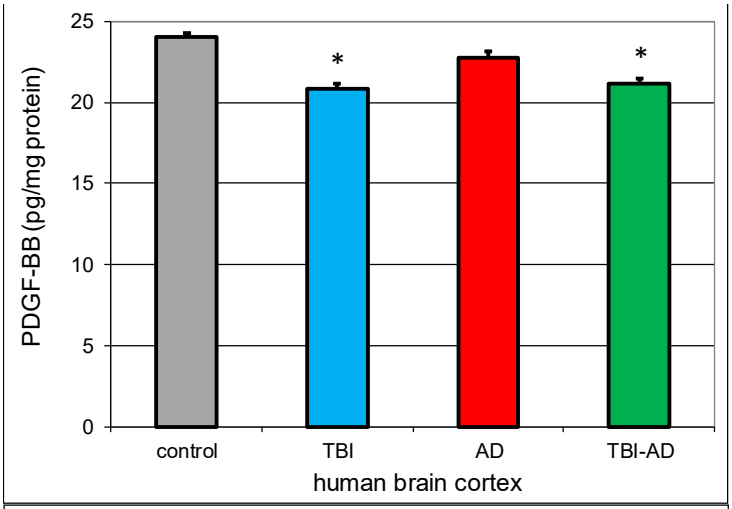


Figure P. Expression of PDGF-BB in brain homogenate from TBI and AD human cortical specimens. Brain samples were analyzed for PDGF-BB content by ELISA and normalized to total protein using the BCA assay. Values represent the mean amount (pg) of PDGF-BB per mg of total protein ± SEM (n=10-16). *P < 0.05 compared to control brains (i.e., no history of TBI or AD diagnosis) as determined by ANOVA and Bonferroni post-hoc test.

PDGF-BB secretion from freshly isolated cerebrovessels following r-mTBI: In the studies above, we evaluated the expression of PDGF-BB in the brain following r-mTBI. To complement these findings and gauge the functionality of cerebrovasculature following head trauma, we examined the secretion of PDGF-BB from freshly isolated cerebrovessels at 6 months following r-mTBI. Here, we isolated the cerebrovasculature from r-mTBI mice at 6 months post-last injury, which was incubated in media for 72 hours at 37°C. We collected the extracellular media and determined the levels of PDGF-BB secreted from the cerebrovessels. We found that cerebrovessels from r-mTBI animals secreted significantly less PDGF-BB (30% reduction) than r-sham cerebrovessels (Figure Q), in line with the reduced PDGF-BB expression in the brain overall following r-mTBI (Figure O results above). Thus, in the aftermath of head trauma, the brain vasculature secretes less PDGF-BB resulting in diminished levels of PDGF-BB in the brain. As this ligand is critical for mural cell health and function, the decreased availability

following r-mTBI may lead to perturbations in mural cell viability and could explain the decreased tau processing post-injury that we observed in our earlier studies (Aim1). **Effect of PDGF-BB stimulation on tau processing by mural cells after r-mTBI:** In addition to showing PDGF-BB levels are diminished following TBI, we revealed that PDGF-BB secretion is significantly reduced in freshly isolated brain vasculature from r-mTBI animals at 6 months post-last injury, compared to r-sham animals. Overall, the PDGF-BB ligand is depleted following head trauma, which may lead to reduced tau processing by mural cells and, potentially, increased tau pathogenesis in the brain. In response to this, we evaluated the impact of PDGF-BB supplementation on tau processing in the cerebrovasculature of r-mTBI animals. For these studies, fresh cerebrovessels were isolated from r-mTBI animals at 6 months post-last injury and treated with recombinant human PDGF-BB (1ng/ml) for 72 hours at 37°C. Next, in the same manner as the tau uptake studies performed in our prior work (Aim1), cerebrovessels were treated with recombinant human tau (5ng/ml) for 1 hour at 37°C and total tau uptake was assessed in the cerbrovessel lysates via ELISA, as performed in our previous tau uptake studies. In line with our prior observations, we observed a significant decrease in cerebrovascular tau uptake at 6 months post-last injury (Figure R). Upon treatment with recombinant human PDGF-BB, tau processing was restored as no difference in tau uptake was observed between PDGF-BB treated cerebrovessels from r-mTBI and r-sham mice (Figure R). These findings indicate administration of PDGF-BB can rejuvenate the mural cell population and improve tau processing following head trauma, which could mitigate tau pathogenesis in the brain post-injury.

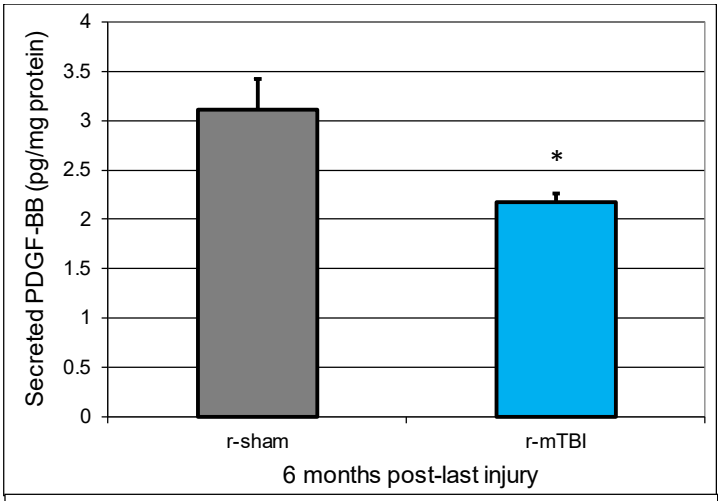


Figure Q. Secretion of PDGF-BB in isolated brain vasculature from r-mTBI mice (6 months post-last injury). Freshly isolated cerebrovessels were incubated for 72 hours at 37°C and the extracellular media was collected and analyzed for PDGF-BB content by ELISA and normalized to total protein using the BCA assay. Values represent the mean amount (pg) of PDGF-BB per mg of total protein ± SEM (n=4). *P < 0.05 compared to r-sham as determined by Student's t-test.

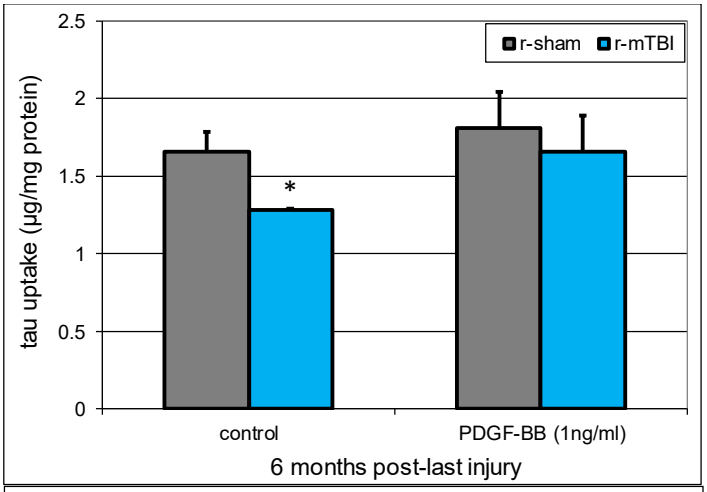


Figure R. Tau uptake in freshly isolated cerebrovessels from r-mTBI animals (6 months post-last injury) following treatment with PDGF-BB. Freshly isolated cerebrovessels were treated with recombinant human PDGF-BB (1ng/ml) for 72 hours at 37°C. Cerebrovessels were exposed to 5ng/ml recombinant human tau (rhtau-441) for 1 hour at 37°C. Lysates were analyzed for tau content by ELISA and normalized to total protein using the BCA assay. Values represent the mean amount of tau (µg) per mg of total protein ± SEM (n=3). *P < 0.05 compared to each respective r-sham as determined by ANOVA and Bonferroni post-hoc test.

Impact of inflammation on tau processing by mural cells: As proposed in Aim3, we evaluated tau processing by mural cells in the context of inflammation. We examined tau uptake in human pericytes and human smooth muscle cells *in vitro* following exposure to a known inducer of inflammation (lipopolysaccharide, LPS), in addition to treatment with TNFα and IL-1β, which are inflammatory cytokines commonly identified following TBI. In the pericytes, none of the inflammatory stimuli altered tau uptake when compared to control conditions (Figure S). In the smooth muscle cells, we observed a subtle decrease in tau uptake in the TNFα- and IL-1β-

treated cells compared to cells exposed to tau alone (Figure T), but these values did not reach statistical significance. The lack of an effect of the inflammatory stimuli on tau uptake in each of these cells could be due to the relatively acute nature of insult. That having been said, the reduced tau uptake in the presence of the TBI-related stimuli (TNFα and IL-1β) in the smooth muscle cells could lead to diminished cerebrovascular tau processing and describe the accumulation of tau in the brain following head trauma. **4) other achievements.** Nothing to report.

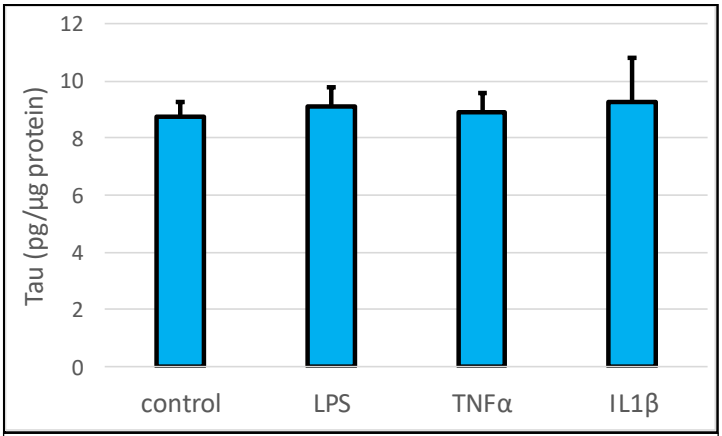


Figure S. Tau uptake in human pericytes *in vitro*. Cells were exposed to 10ng/ml LPS, TNF-alpha, or IL1-beta (or water for control) for 6 hours at 37°C followed by treatment with recombinant human tau (rhtau-441) at 10ng/ml for 1 hour at 37°C. Lysates were analyzed for total tau content by ELISA and normalized to total protein using the BCA assay. Values represent the mean ± SEM (n=3). *P < 0.05 compared to control as determined by ANOVA and Bonferroni post-hoc test.

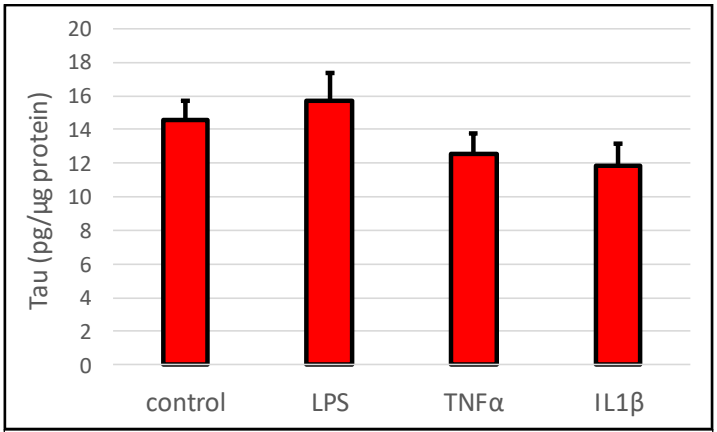


Figure T. Tau uptake in human smooth muscle cells *in vitro*. Cells were exposed to 10ng/ml LPS, TNF-alpha, or IL1-beta (or water for control) for 6 hours at 37°C followed by treatment with recombinant human tau (rhtau-441) at 10ng/ml for 1 hour at 37°C. Lysates were analyzed for total tau content by ELISA and normalized to total protein using the BCA assay. Values represent the mean ± SEM (n=3). *P < 0.05 compared to control as determined by ANOVA and Bonferroni post-hoc test.

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.

Year 1 – Training and Professional Development Opportunities

Training activities. Training opportunities were provided for Dr. Corbin Bachmeier and other members of the Roskamp Institute. A training session was given by Robert M. Umek, Ph.D, Director of External Scientific Affairs at Meso Scale Discovery (MSD) on April 14, 2017. Dr. Umek provided an introduction to the capabilities and function of the MSD platform for protein quantitation. He also presented a seminar on the applications of this technology titled "Challenges for Multiplex Biomarker Assays in Translational Research". We are interested in utilizing this instrument in our research as a new method for protein quantitation in biological samples. Dr. Corbin Bachmeier also participated in a training program through the Hope Center *In Vivo* Microdialysis Core at Washington University in St. Louis, MO on Aug 13-23, 2017. These sessions allowed for hands on training in the setup and surgical implementation of a microdialysis probe for the continuous sampling of interstitial fluid from the brain in live mice. Moreover, we received hands on training in the collection of cerebral spinal fluid (CSF) from mice. We are interested in applying these approaches and techniques to expand the capabilities of our research program and improve the quality and efficiency of our studies overall.

Professional development. Dr. Corbin Bachmeier attended a scientific meeting on Military Risk Factors for Dementia organized by the Alzheimer's Association on Dec 1, 2016 in Washington, DC, which is highly relevant to the current project. This meeting brought together thought leaders in AD military research with the purpose of identifying gaps in our knowledge of the risk factors leading to AD and related dementia in military service personnel. In addition, the goal of this meeting was to outline a future research roadmap, including emerging areas and studies needed for investigation. Dr. Corbin Bachmeier also attended the International Brain Injury Association's (IBIA) 12th World Congress on Brain Injury on Mar 29 – Apr 1, 2017 in New Orleans, LA. This meeting provided exposure to a broad range of topics and research areas, particularly those relevant to military personnel and our Veterans, and offered a unique opportunity to interact with scientists in a variety of disciplines from institutions around the world. Lastly, Dr. Corbin Bachmeier attended the National Neurotrauma Symposium on Jul 7 - 12, 2017 in Snowbird, UT. Dr. Corbin Bachmeier presented a poster on findings related to the current project and attended various symposia on the latest research in the neurotrauma field. In addition, at this meeting, there was an opportunity to discuss the current project with our collaborators and other researchers for feedback on our data and input on future studies.

Year 2 – Training and Professional Development Opportunities

Training activities. Training opportunities were provided for Dr. Corbin Bachmeier's research team from the Roskamp Institute. A training session was given by Dr. Andy Shih, Senior Scientist, Medical University of South Carolina, Charleston, SC, in January 2018. Dr. Shih provided hands on training in mouse skull thinning and the surgical implementation of a cranial window, with the purpose of applying laser speckle imaging to evaluate cerebral blood flow in mice. We are interested in utilizing these approaches and techniques to expand the capabilities of our research program and improve the quality and efficiency of our studies overall.

Professional development. Dr. Corbin Bachmeier attended multiple scientific meetings the past year, including the Joint Symposium of the International and National Neurotrauma Societies, Toronto, Canada, Aug 11-16, 2018, which is highly relevant to the current research project and military health in general. This meeting brought together thought leaders in TBI military research with the purpose of identifying gaps in our knowledge of the risk factors and long-term consequences of head trauma, including AD, in military service personnel and our Veterans. Dr. Corbin Bachmeier presented a poster on findings related to the current project and attended various symposia on the latest research in the neurotrauma field. In addition, at this meeting, there was an opportunity to discuss the current project with our collaborators and other researchers for feedback on our data and input on future studies. Dr. Bachmeier also attended the 12th International Conference on Cerebral Vascular Biology on Nov 28–Dec 1, 2017 in Melbourne, Australia, an area of research which is highly relevant to the current project. This meeting brought together leaders in the field of cerebral vascular biology to discuss a variety of topics including brain vascular abnormalities in TBI and AD, which is the focus of the current project. Dr. Corbin Bachmeier presented a poster on his work and had discussions with collaborators and other researchers for feedback on data and future directions.

Year 3 – Training and Professional Development Opportunities

Training activities. Recently, Dr. Corbin Bachmeier hosted a field-based meeting on TBI, which took place in St. Petersburg, FL on August 15-16, 2019. In this meeting, various scientists and clinicians gathered to develop a roadmap to address areas of need in TBI and accelerate therapies relevant to military and Veteran health. An important component of this meeting was the participation and feedback of Veterans and caregivers, who were instrumental in constructing a therapeutic strategy that prioritizes the needs of the military and Veterans with TBI. Additionally, Dr. Corbin Bachmeier was part of a team that organized follow-up virtual meetings on September 18 and 25, 2019 to continue our dialogue on these topics and further develop effective strategies to promote the care of the military and Veterans with TBI.

Professional development. This past year, Dr. Corbin Bachmeier attended the National Neurotrauma Symposium in Pittsburgh, PA from Jun 29 to Jul 3, 2019, which is highly relevant to the current research project and military health in general. This meeting brought together thought leaders in TBI military research with the purpose of identifying gaps in our knowledge of the risk factors and long-term consequences of head trauma, including AD, in military service personnel and our Veterans. Dr. Corbin Bachmeier presented a poster on findings related to the current project and attended various symposia on the latest research in the neurotrauma field. In addition, at this meeting, there was an opportunity to discuss the current project with our collaborators and other researchers for feedback on our data and input on future studies.

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.

In the month of November, of every year of this project, I was part of a team that organized a Veterans Day open house at the Roskamp Institute in Sarasota, FL. This event was available to military personnel, Veterans, researchers, and clinicians, with the purpose of honoring the military, our Veterans, and their service to our country. Alongside other scientists and clinicians, we had the opportunity share our research projects and findings with military personnel, Veterans, and the public at large. Furthermore, this gathering facilitated the exchange of ideas and feedback amongst researchers and, importantly, promoted dialogue and interactions between the military, Veterans, and the medical community.

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.

Nothing to Report.

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).

Prior studies have demonstrated that mural cells are depleted in many brain disorders including Alzheimer's disease (AD). Due to the role of these cells in degrading and removing solutes from the brain, the loss of these cells in disease may explain the accumulation of toxic solutes that is observed in various brain disorders. Despite the significance of mural cells in the elimination of solutes from the brain and their diminished expression in neurological disease, to our knowledge, no one has investigated the state of the mural cells in TBI. In our preliminary studies, we observed a progressive decline in brain mural cells after injury in our mouse model of TBI. Moreover, we found that isolated brain vasculature from these same TBI animals were less able to internalize and process tau than animals that did not receive a TBI. To our knowledge these are the first studies to observe changes in mural cell expression in TBI and alterations in the functional processing of tau following injury. We also observed a significant decrease in mural cell markers in isolated vasculature from human AD brain specimens, with more modest reductions in PDGFRβ levels occurring in human TBI tissue, compared to control subjects. In the short-term, the current proposal will contribute to our existing knowledgebase by determining, 1) mural cell density in isolated cerebrovasculature from human TBI specimens and murine brains following r-mTBI, 2) internalization dynamics of tau in r-sham and r-mTBI cerebrovascular cells, 3) degradation pathways for tau in r-sham and r-mTBI cerebrovascular cells, 4) expression and function of the PDGFβ pathway in human TBI specimens and murine brains following r-mTBI, 5) impact of inflammation on tau processing by mural cells, and 6) the effect of PDGF-BB stimulation on tau processing by mural cells following TBI. As for the long-term contributions of this research, these studies will further our understanding of the relationship between TBI and the onset of AD. More specifically, it is anticipated that rejuvenation or reconciliation of the PDGF pathway (e.g., PDGF-BB stimulation) will stabilize the mural cell population and help regulate tau processing in the extracellular fluids of the brain following head trauma. Subsequent studies would further interrogate the PDGF pathway for viable therapeutic targets and the development of novel approaches to modulate the TBI phenotype and the onset of AD, which would ultimately benefit our Veteran and military populations and others afflicted with these disorders. In totality, our studies indicate mural cell disruption in TBI and AD may be an important factor in tau pathogenesis and neurodegeneration and could explain the association between head trauma and the development of AD.

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:*

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

Nothing to Report.

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.

The breeding and acquisition of the PSAPP mouse cohort (animal model of AD) had been slow, due in part to increased mortality at 10 weeks of age and around 12 months of age. However, we were able to acquire enough animals to complete our assessments of tau uptake, mural cell expression, and the PDGF-beta pathway, which will be compared to our results following r-mTBI, to evaluate the interrelationship between TBI and the development of AD.

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

Nothing to Report.

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).*

The following are manuscripts resulting from this project that are in submission:

Tau processing by mural cells following traumatic brain injury. J Ojo, M Eisenbaum, B Shackleton, C Lynch, U Joshi, N Saltiel, D Paris, B Mouzon, M Mullan, F Crawford, and C Bachmeier. *Mol. Neurobiol*. Federal support acknowledgement: Yes.

Influence of repetitive mild traumatic brain injury on mural cell tau elimination from the brain. M Eisenbaum, J Ojo, C Lungmus, U Joshi, M Mullan, F Crawford, and C Bachmeier. *Brain Inj*. Federal support acknowledgement: Yes.

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.*

Oral Presentations:

Tau Processing by Mural Cells in Traumatic Brain Injury and Alzheimer's Disease. Joseph Ojo and Corbin Bachmeier. Oral presentation. Department of Defense PRARP In-Progress Review, Frederick, MD on December 8, 2017.

Poster Presentations:

Tau Processing by Mural Cells in Traumatic Brain Injury. M. Eisenbaum, C. Lungmus, J. Ojo, F. Crawford, and C. Bachmeier. International Conference on Cerebral Vascular Biology, Melbourne, Australia on November 28 - December 1, 2017.

Tau Processing by Mural Cells in Traumatic Brain Injury. M Eisenbaum, C Lungmus, J Ojo, F Crawford, and C Bachmeier. Joint Symposium of the International and National Neurotrauma Societies, Toronto, Canada on August 11-16, 2018.

Tau processing by mural cells in traumatic brain injury. M. Eisenbaum, J. Ojo, C. Lungmus, U. Joshi, F. Crawford, and C. Bachmeier. Bay Pines VA Healthcare System Research Day, Bay Pines, FL on May 15, 2019.

Tau Processing by Mural Cells in Traumatic Brain Injury. M. Eisenbaum, J. Ojo, C. Lungmus, U. Joshi, F. Crawford, and C. Bachmeier. National Neurotrauma Symposium, Pittsburgh, PA on June 30 - July 03, 2019.

- **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.

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

In our prior work, we developed a technique for isolating various brain fractions in mice (i.e., homogenate, parenchyma, cerebrovasculature, and the soluble fraction) and adapted this method for use with human brain specimens. While a number of methods exist for separating cerebral microvessels and brain parenchyma, we have continued to refine this methodology and are now able to isolate the same brain fractions above, while using less starting material. These improvements will allow us to get more out of the existing brain material for current and future applications. We have now completed these studies and analyzed the cerebrovascular fractions from the mouse TBI samples and the human TBI specimens, as proposed in the current submission. We will share our latest techniques and observations with the scientific community by publishing our work in peer-reviewed journals.

- **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:	Corbin Bachmeier, PhD
Project Role:	PI
Researcher Identifier (e.g.ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Dr. Bachmeier has been responsible for conducting/supervising all of the experiments for this proposal including the generation, analysis, and interpretation of the data.
Funding Support:	N/A
Name:	Maxwell Eisenbaum, MS
Project Role:	Research Associate
Researcher Identifier (e.g.ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Mr. Eisenbaum has been conducting experiments and generating data for this proposal.
Funding Support:	N/A

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: Banner Sun Health Research Institute
Location of Organization: Sun City, AZ
Partner's contribution to the project: Human brain specimens were provided by Thomas Beach, M.D., Ph.D., Director of the Brain and Body Donation Program at the Banner Sun Health Research Institute.

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.*

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.*

Tau Processing by Mural Cells in Traumatic Brain Injury and Alzheimer's Disease

Log Number: AZ150052

Award Number: W81XWH-15-PRARP-CSRA

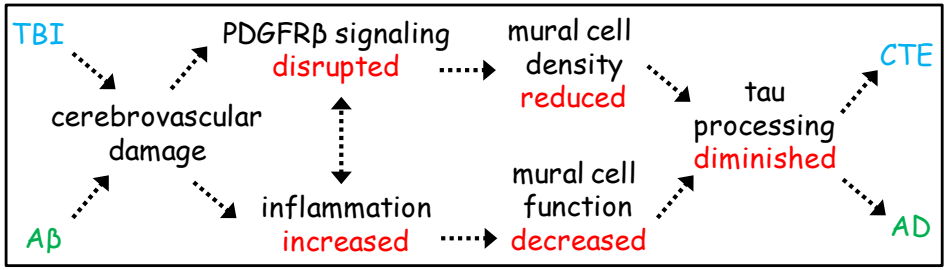
PI: Corbin Bachmeier **Org:** Roskamp Institute **Award Amount:** $799,904.00

Study/Product Aim(s)

- **Aim 1:** Determine mural cell expression and function following TBI.
- **Aim 2:** Examine the interaction between mural cells and tau.
- **Aim 3:** Evaluate the role of PDGFRβ signaling and inflammation in mural cell disruption.

Approach

1. Evaluate mural cell expression in isolated cerebrovasculature from human TBI brain specimens.
2. Examine the timecourse of mural cell expression and function in a mouse model of r-mTBI.
3. Examine tau internalization in r-mTBI cerebrovascular cells.
4. Evaluate tau degradation pathways in r-mTBI cerebrovascular cells.
5. Examine the PDGF pathway in human TBI brains and murine brains following r-mTBI.
6. Evaluate the effect of PDGF-BB stimulation on tau processing by mural cells following r-mTBI.
7. Evaluate the impact of inflammation on tau processing by mural cells.



Accomplishments: We found human pericytes and smooth muscle cells interact with various tau species *in vitro*, with the uptake of MTBR and p181 tau being particularly elevated. Alternatively, oligomeric tau was not substantially internalized by either mural cell type. *Ex vivo*, MTBR tau uptake by was substantially decreased in freshly isolated r-mTBI cerebrovessels (6 months post-last injury) compared to r-sham, while no difference in tau uptake was observed between r-mTBI and r-sham cerebrovessels for monomeric, phosphorylated, cleaved tau, or oligomeric tau.

Timeline and Cost

Activities CY	2016	2017	2018	2019
Aim 1				
Aim 2				
Aim 3				
Estimated Budget ($799K)	**$67K**	**$266K**	**$265K**	**$201K**

Updated: (4/15/2020)

Goals/Milestones

CY17 Goals – Mural cell status following TBI:

☐ Determination of mural cell expression in human TBI brain specimens.

☐ Timeline for mural cell disruption following r-mTBI in a mouse model.

CY18 Goals – Mural cell and tau interactions:

☐ Determination of tau internalization in cerebrovascular cells following r-mTBI.

☐ Determination of tau degradation by cerebrovascular cells following r-mTBI.

CY19 Goals – PDGFRβ signaling / inflammation in mural cells post-injury:

☐ PDGF pathway expression in the cerebrovasculature after r-mTBI.

☐ PDGF-BB stimulation and tau uptake in cerebrovascular cells after r-mTBI.

☐ Impact of inflammation on tau uptake and PDGF signaling in mural cells.

Comments/Challenges/Issues/Concerns

- If timelines change, comment here. No change to timeline.
- Comment, if off by more than one quarter in spending. Not off by more than one quarter in spending.

Budget Expenditure to Date

Projected Expenditure: $799,904.00

Actual Expenditure: $799,904.00