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: Oct2019

TYPE OF REPORT: Annual

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
Oct 2019	Annual	9/30/2018-9/29/2019

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			Unclassified	13	USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified			

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. **INTRODUCTION:** 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:** tau, traumatic brain injury, Alzheimer's disease, mural cells, metabolism, cerebrovasculature.
3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

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 except for the following studies, which will be completed in the next reporting period, 1) mural cell density using confocal microscopy in r-mTBI and AD mouse tissue samples, 2) uptake of tau species in human smooth muscle cells *in vitro*, and 3) uptake of tau species in isolated cerebrovessels from r-mTBI animals.

- **What was accomplished under these goals? 1) major activities.** The major activities for this annual reporting period have focused on the major goals listed in the previous section which include: examination of tau internalization and degradation in r-mTBI cerebrovascular cells, evaluation of the PDGF pathway in human TBI brains and murine brains following r-mTBI, determination of the effect of PDGF-BB stimulation on tau processing by mural cells after r-mTBI, and evaluation of the impact of inflammation on tau processing by mural cells. **2) specific objectives.** Evaluate the impact of r-mTBI on tau internalization and degradation in cerebrovascular cells, and determine the role of platelet-derived growth factor receptor-beta (PDGFR-beta) signaling and inflammation in mural cell disruption following TBI. **3) significant results.** Tau internalization in r-mTBI cerebrovascular cells: We have proceeded to examine the interaction of isolated tau species with pericytes and smooth muscle cells *in vitro*. In Figure A, we examined tau uptake in pericytes using monomeric and phospho-tau (pT181) species. We observed an increase in the uptake of both monomeric and phospho-tau by the pericytes

(~2-fold) compared to control conditions (no tau exposure), however only the monomeric tau reached statistical significance (Figure A). We will continue this line of investigation by examining various tau species in smooth muscle cells *in vitro*. Moreover, to identify the influence of r-mTBI on the processing of each tau species by these cells, we have administered the repetitive injury paradigm to a mouse cohort which will be used to assess cerebrovascular tau uptake in the context of head trauma. These few remaining studies will be completed in the next reporting period. 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 B, 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 B). 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 B). 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.

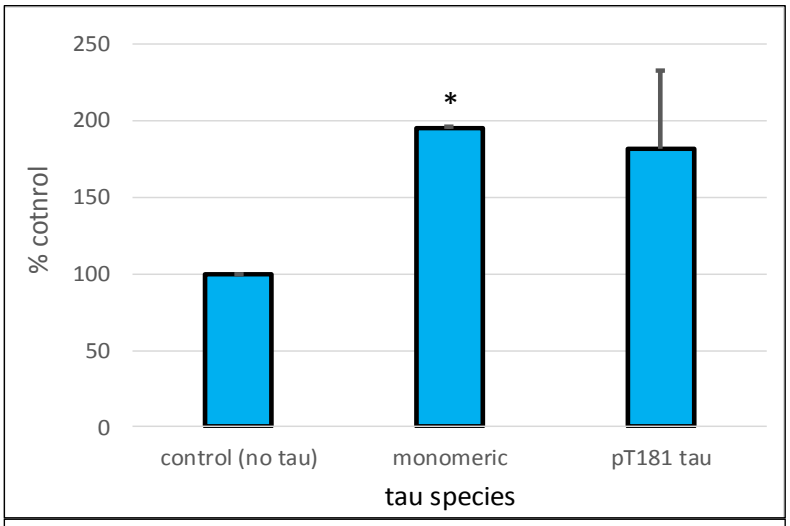

Figure A. Tau uptake in human pericytes *in vitro*. Pericytes were exposed to 50ng/ml monomeric recombinant human tau (rhtau-441) or pT181-isolated tau for 3 hours at 37°C. Lysates were analyzed for total tau content by ELISA and normalized to total protein using the BCA assay. Values represent the percentage of control (no tau exposure) ± SEM (n=4). *P < 0.05 compared to control as determined by ANOVA and Bonferroni post-hoc test.

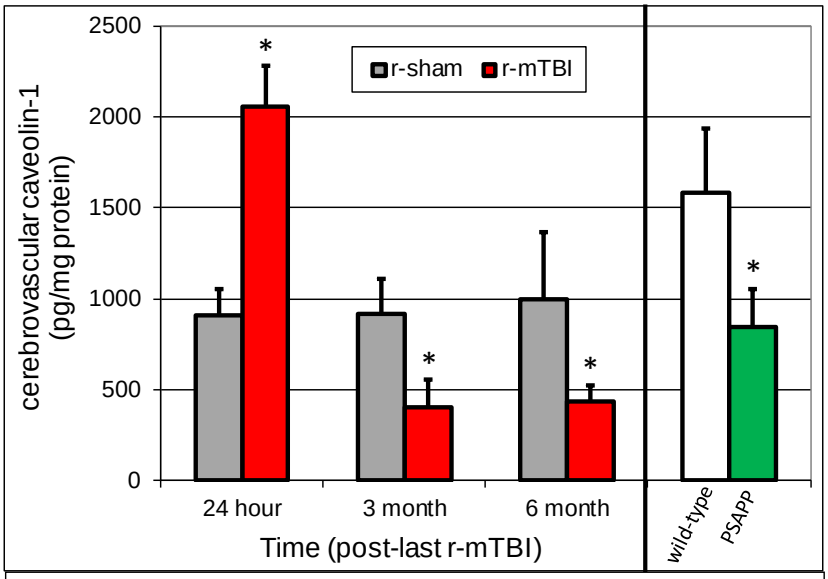

Figure B. Caveolin-1 expression in isolated cerebrovessels from r-mTBI (24 hours, 3 months, and 6 months post-last injury), wild-type, and PSAPP mice (18 months of age). Lysates were analyzed for caveolin-1 content by ELISA and normalized to total protein using the BCA assay. Values represent mean ± 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.

<u>Tau degradation by r-mTBI cerebrovascular cells:</u> 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 C). 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 D). While proteasome degradation was decreased overall following brain injury, no differences in proteasome activity was observed between the tau species (Figure D). 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.

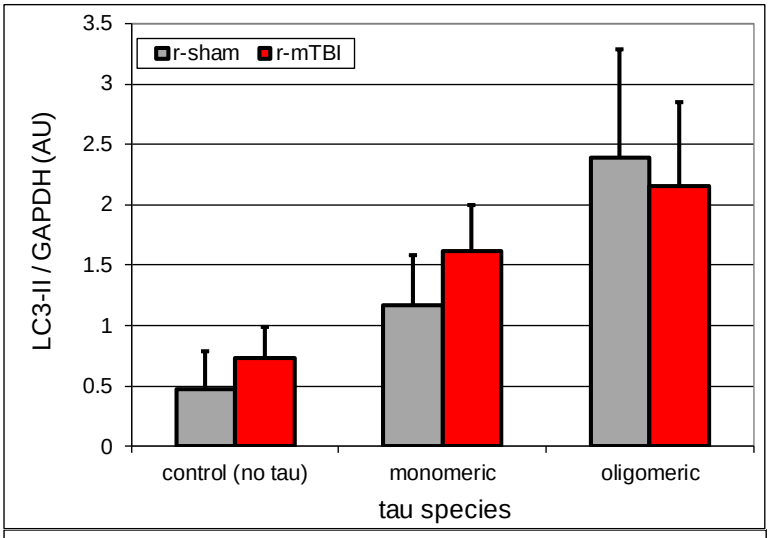

Figure C. Autophagic activity in response to tau exposure in isolated cerebrovessels from r-mTBI animals at 6 months post-last injury. Cells were exposed to water (control), monomeric, or oligomeric tau species (50ng/ml) for 24 hours at 37°C. Lysates were probed for LC3-II using immunoblotting and normalized 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.

<u>PDGF pathway in human TBI brains and murine brains following r-mTBI:</u> 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 prior 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 last annual report, we showed a progressive decrease in PDGF-BB expression in brain homogenate up to 6 months following r-mTBI in our mouse model of repetitive head trauma. Interestingly, in the AD mouse model, 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 brain homogenate and observed a similar 20% reduction in PDGF-BB (Figure E) 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 E). 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 E). 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 in prior reporting

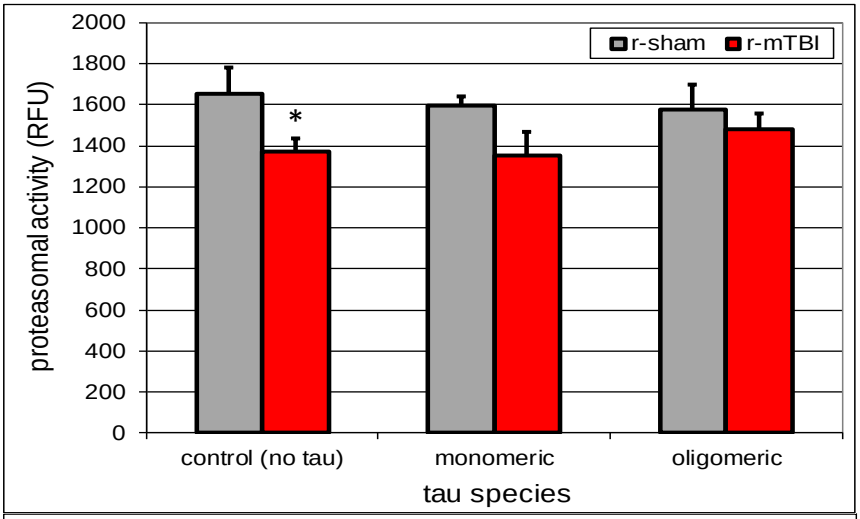

Figure D. Proteasomal activity in response to tau exposure in isolated cerebrovessels from r-mTBI animals at 6 months post-last injury. Cells were exposed to water (control), monomeric, or oligomeric tau species (50ng/ml) for 24 hours at 37°C. The cells were analyzed for proteasomal activity using a 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.

periods). 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. 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, in the prior annual report 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 (Aim3b). 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 37oC. 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 37oC 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 F). 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 F). 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. Impact of inflammation on tau processing by mural cells: As proposed in Aim3c, 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

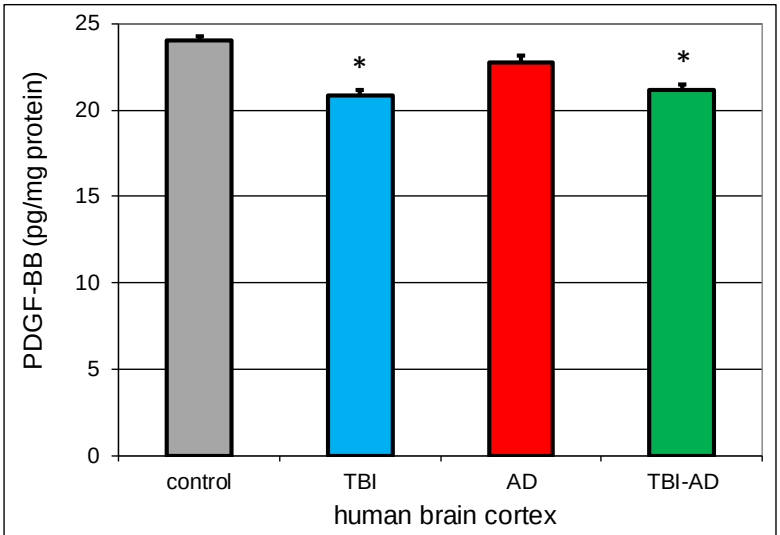

Figure E. Expression of PDGF-BB in brain homogenate from TBI and AD human cortical specimens. Brain samples were analyzed for PDGF-BB content by ELISA and normalized to total protein using the BCA assay. Values represent the mean amount (pg) of PDGF-BB per mg of total protein ± SEM (n=10-16). *P < 0.05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

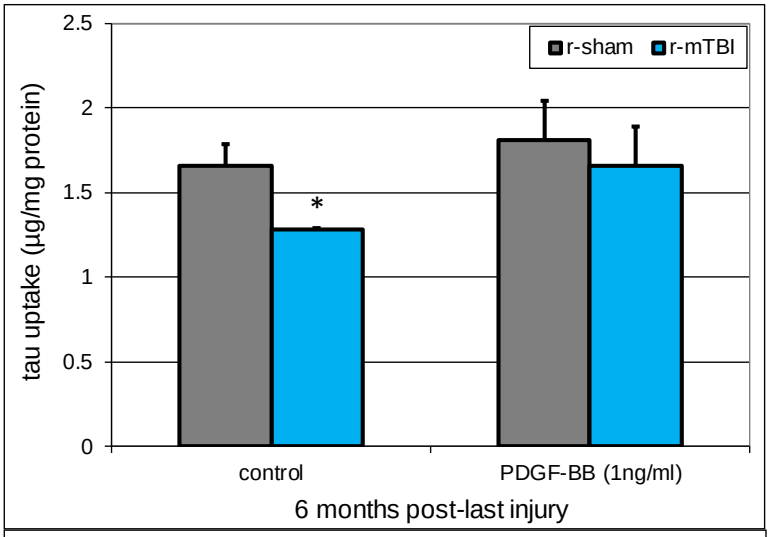

Figure F. Tau uptake in freshly isolated cerebrovessels from r-mTBI animals (6 months post-last injury) following treatment with PDGF-BB. Freshly isolated cerebrovessels were treated with recombinant human PDGF-BB (1ng/ml) for 72 hours at 37°C. Cerebrovessels were exposed to 5ng/ml recombinant human 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.

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 G). 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 H), 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.

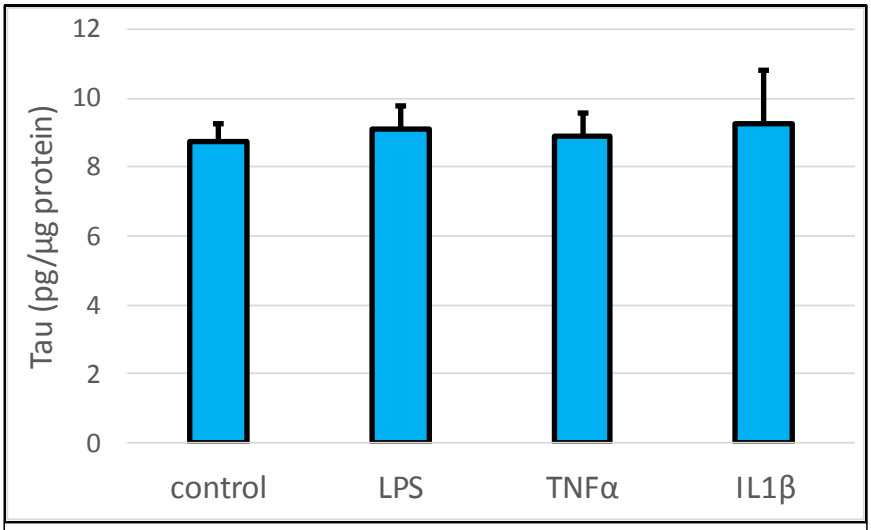

Figure G. Tau uptake in human pericytes *in vitro*. Cells were exposed to 10ng/ml LPS, TNF-alpha, or IL1-beta (or water for control) for 6 hours at 37°C followed by treatment with recombinant human tau (rhtau-441) at 10ng/ml for 1 hour at 37°C. Lysates were analyzed for total tau content by ELISA and 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.

4) other achievements. Nothing to report.

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

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, Corbin 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.

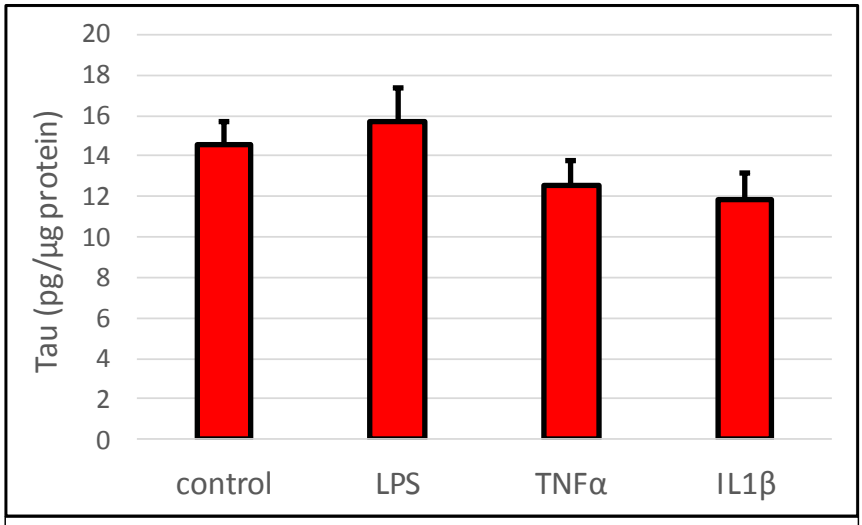

Figure H. Tau uptake in human smooth muscle cells *in vitro*. Cells were exposed to 10ng/ml LPS, TNF-alpha, or IL1-beta (or water for control) for 6 hours at 37°C followed by treatment with recombinant human tau (rhtau-441) at 10ng/ml for 1 hour at 37°C. Lysates were analyzed for total tau content by 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.

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. Corbin 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?** I was part of a team that organized a Veterans Day open house at the Roskamp Institute on November 9, 2018 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?** For this project, a no cost extension was granted on 9-13-2019, and the extension period will run through 12-29-19. This extension period will allow us to complete the last remaining proposed studies, namely the analysis of mural cell density in r-mTBI and PSAPP mice using confocal microscopy. In prior reporting periods, we determined the expression of mural cell markers at various timepoints following head trauma in our mouse r-mTBI model. We 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 have collected and prepared brains from animals at 6 months post-last injury and PSAPP mice. As proposed in Aim 1, we will examine several markers to determine 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 will compare these results to our analyses in the PSAPP mice, as previous reporting has indicated the mural cell population is depleted in AD animals. These comparisons will contribute to our overarching goal of determining the interrelationship between head trauma and the development of AD. All samples have been collected, sectioned, and probed for various mural cell markers, and are now undergoing quantitation for mural cell density. We are blinded to the sample identity and will not know the r-mTBI effect until all samples have been analyzed. We will complete the quantitation of these samples in the next reporting period. In terms of tau internalization, as indicated above, we have examined tau uptake in human brain pericytes *in vitro* and will perform the same analyses in smooth muscle cells *in vitro*. To determine the influence of TBI on the uptake of various tau species, we will also investigate tau uptake in cerebrovessels isolated from r-sham and r-mTBI animals. Each of these studies will be completed over the course of the next reporting period.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?** 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 brain 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?** 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:**
   - **Changes in approach and reasons for change.** Nothing to Report.
   - **Actual or anticipated problems or delays 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**. Nothing to Report.
   - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.** Nothing to Report.
   - **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:**
   - **Publications, conference papers, and presentations**
   - **Journal publications.** We have prepared a manuscript based on the work from this project and are awaiting the results of the confocal microscopy data, before submitting to a peer-reviewed scientific journal. In addition, we are preparing a second manuscript based on the tau internalization and degradation studies that will also be submitted to a scientific journal for peer-review.
   - **Books or other non-periodical, one-time publications.** Nothing to Report.
   - **Other publications, conference papers, and presentations.** An abstract and poster presentation related to work in this project was accepted by the National Neurotrauma Symposium, Pittsburgh, PA, Jun 29 to Jul 3, 2019, titled *Tau Processing by Mural Cells in Traumatic Brain Injury*.
   - **Website(s) or other Internet site(s).** We intend to display the results of this project on the Roskamp Institute website (www.rfdn.org), as the results become finalized, to disseminate our findings to the public at large and facilitate discussion on the interpretation of our results.
   - **Technologies or techniques.** 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.** Nothing to Report.
   - **Other Products.** Nothing to Report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

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/will be responsible for conducting/supervising all of the experiments for this proposal including the generation, analysis, and interpretation of the data.*
Funding Support:	

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:	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?** There have been two changes in the active other support of the PI. These changes will not impact the effort of the PI on the current project.

  The following grants have become active since the last reporting period.

  VA Merit Award (I01BX004352)
  Influence of APOE genotypes on blood-brain barrier transport of DHA by mfsd2a in Alzheimer's Disease.
  Bachmeier role: Co-Principal Investigator
  2019-2023
  The purpose of this project is to determine the impact of apoE genotype on the transport of lipids across the blood-brain barrier by the mfsd2a protein in AD.

  DOD CDMRP (AZ180112)
  Influence of apoE genotype on tau elimination from the brain in traumatic brain injury and Alzheimer's disease.
  Bachmeier role: Principal Investigator
  2019-2022
  The goal of this project is to examine the interaction between apoE and tau and evaluate the role of apoE genotype on extracellular tau degradation and elimination from the brain in TBI and AD.

- **What other organizations were involved as partners?**
  - **Organization Name:** Banner Sun Health Research Institute
  - **Location of Organization:** Sun City, AZ
  - **Partner's contribution to the projec**t: 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:** Not Applicable.
   - **QUAD CHARTS:** Please see the Quad Chart below.
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. Reminder: Pages shall be consecutively numbered throughout the report. ***DO NOT RENUMBER PAGES IN THE APPENDICES.***

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**** **ADDITIONAL NOTES:**

MARKING OF PROPRIETARY INFORMATION: Data that was developed partially or exclusively at private expense shall be marked as "Proprietary Data" and Distribution Statement B included on the cover page of the report. Federal government approval is required before including Distribution Statement B. The recipient/PI shall coordinate with the COR/GOR to obtain approval. **REPORTS NOT PROPERLY MARKED FOR LIMITATION WILL BE DISTRIBUTED AS APPROVED FOR PUBLIC RELEASE.** It is the responsibility of the Principal Investigator to advise the COR/GOR when restricted limitation assigned to a document can be downgraded to "Approved for Public Release." **DO NOT USE THE WORD "CONFIDENTIAL" WHEN MARKING DOCUMENTS. DO NOT USE WATERMARKS WHEN MARKING DOCUMENTS.**

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

Log Number: AZ150052

Award Number: W81XWH-15-PRARP-CSRA

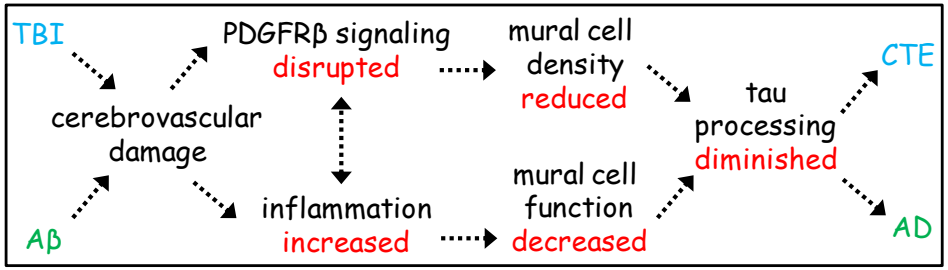
PI: Corbin Bachmeier **Org:** Roskamp Institute **Award Amount:** $799,904.00

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 PDGF-BB and caveolin-1 levels were significantly decreased in TBI and AD brains, which may describe the reductions in mural cell markers and cerebrovascular tau processing. Moreover, PDGF-BB treatment promoted mural cell function and tau internalization in cerebrovessels. Proteasome activity was significantly lower in r-mTBI cerebrovessels compared to r-sham mice, while no change in autophagy was observed between these groups. Inflammatory insult (LPS, TNF-alpha, IL-1beta) did not alter tau uptake in pericyte and smooth muscle cell cultures.

Timeline and Cost

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

Updated: (10/29/2019)

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: $797,494.72