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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 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, we observed a progressive decline in cerebrovascular mural cell expression following 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. Furthermore, mural cell expression is reduced in Alzheimer's disease (AD) human brains and to a lesser extent in human TBI brains compared to control samples. 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

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Table of Contents

Page

1. **Introduction……4**
2. **Keywords……4**
3. **Accomplishments……..…….....4**
4. **Impact……...……..8**
5. **Changes/Problems...….……..8**
6. **Products…….……..…..……...9**
7. **Participants & Other Collaborating Organizations……..9**
8. **Special Reporting Requirements……10**
9. **Appendices……11**

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 1 has been completed. We have determined the expression of mural cells in isolated cerebrovasculature from human brain specimens encompassing four groups, 1) control (no history of TBI or AD diagnosis), 2) TBI (documented history of TBI), 3) AD (diagnosis of AD), and 4) TBI and AD (history of TBI and a diagnosis of AD).

Major Goal 2 is nearly completed. We have determined mural cell expression and tau processing in isolated cerebrovessels from r-mTBI mice at 24 hours, 3 months, and 6 months post-last injury. We have completed the injury paradigm for another cohort of r-mTBI mice and will interrogate these animals in the same manner at 1 month post-last injury, which will establish a timeline for mural cell disruption following injury in a mouse r-mTBI model.

- **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: obtain local IACUC approval, obtain anatomical substances approval, obtain ACURO approval, evaluate mural cell expression in isolated cerebrovasculature from human TBI brain specimens, and examine the timecourse of mural cell expression and function in a mouse model of repetitive head trauma. **2) specific objectives.** Evaluate mural cell expression in cerebrovasculature from human TBI brain specimens, and examine the timecourse of mural cell expression and function after r-mTBI in mice.

3) significant results. 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 [1,2]. 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). In line with our prior *in vitro* studies, tau uptake was examined in freshly isolated cerebrovessels from r-mTBI mice 24 hours, 3 months, and 6 months after the last injury. Cerebrovessels were treated with recombinant human tau (5ng/ml) for 1 hour at 37°C 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 3 and 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 [3] and human TBI/CTE brains [4,5,6,7]. 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 [8,9] and to a lesser extent, normal aging [10]. 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. To our knowledge no one has examined mural cell expression in TBI brains, animal or human, following mild repetitive head trauma. We probed the cerebrovasculature of each cohort with known mural cell markers, alpha smooth muscle cell actin (αSMC-actin) and PDGFRβ (platelet-derived growth factor receptor beta) [11]. For αSMC-actin, there was an initial increase in expression after r-mTBI at 24 hours post-injury, followed by a progressive

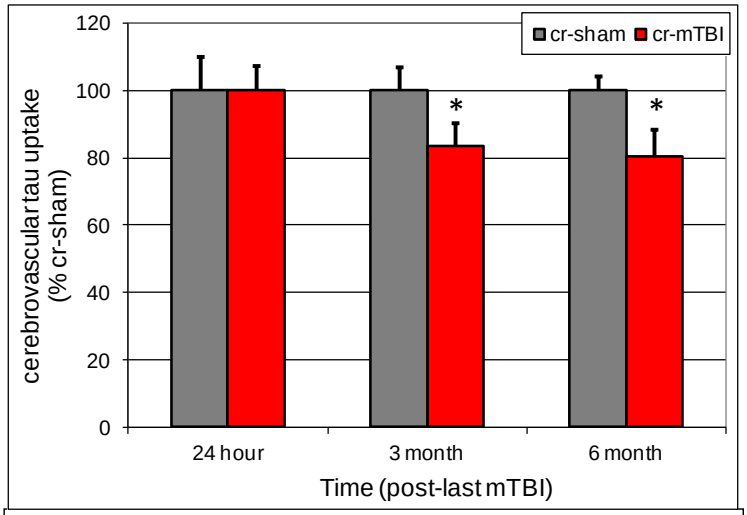
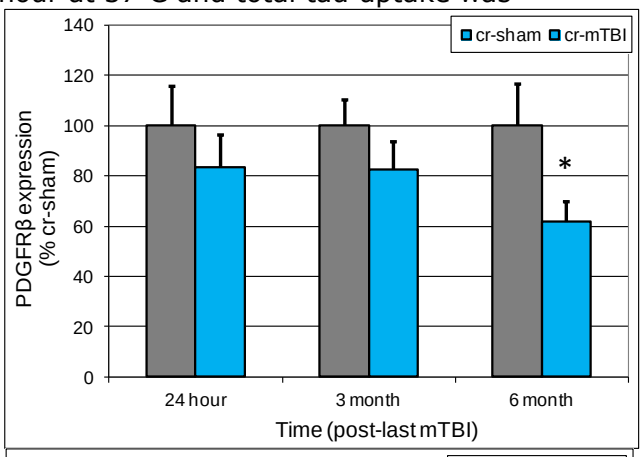


Figure A. Tau uptake in freshly isolated cerebrovessels from chronic r-mTBI animals (24 hours, 3 months, and 6 months post-last injury). Cerebrovessels were exposed to 5ng/ml recombinant human tau (rhtau-441) for 1 hour at 37°C. Lysates were analyzed for total tau content by ELISA and normalized to total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



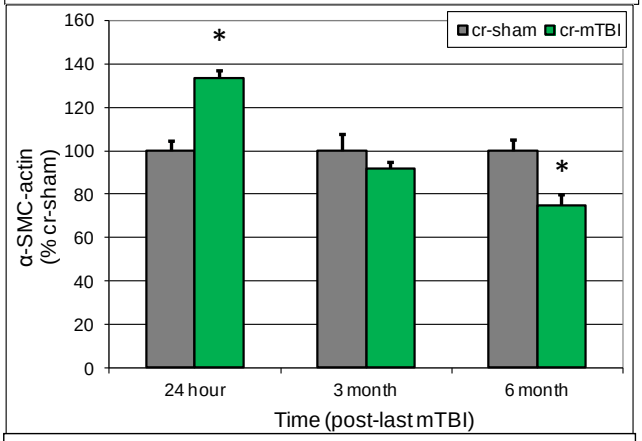


Figure B. Expression of PDGFRβ (top) and α-SMC-actin (bottom) in isolated cerebrovessels from chronic r-mTBI animals (24 hours, 3 months, and 6 months post-last injury). Brain vessels were analyzed for PDGFRβ and α-SMC-actin content by ELISA and normalized to total protein using the BCA assay. Values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

decrease at 3 and 6 months post-injury. For PDGFRβ, in the r-mTBI group we observed a time-dependent reduction in PDGFRβ expression post-injury compared to r-sham mice, indicating a progressive reduction in mural cell density following r-mTBI (Figure B). These findings are consistent with our prior observations using the same r-mTBI paradigm in an aged wild-type cohort (12 months of age) we also observed significant reductions following injury in both αSMC-actin and PDGFRβ via western blotting [12]. 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. Mural cell expression in human TBI brain vasculature: While mural cell loss has been observed in many neurodegenerative disorders in humans [8,9], 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 region of the brain cortex (inferior frontal gyrus). 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 C), 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 no difference in αSMC-actin levels between the TBI group and control (Figure C). Of note, there are still human specimens that have yet to be analyzed and included in this data set, primarily for the αSMC-actin studies, which we are currently in the process of evaluating. 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, defined as mild to moderate, in line with our concussive mouse model. However, the human TBI cohort was exposed to 1-3 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. As this proposal is focused on the interrelationship between TBI and AD, we will continue to explore TBI in a chronic repetitive context to identify the mechanisms following head trauma that precipitate the development of AD. To continue our evaluation of TBI and AD development, our upcoming studies will apply the same set of analyses on AD transgenic mice as those described for our r-mTBI model to evaluate tau processing and mural cell expression in the context of AD. **4) other achievements.** Nothing to report.

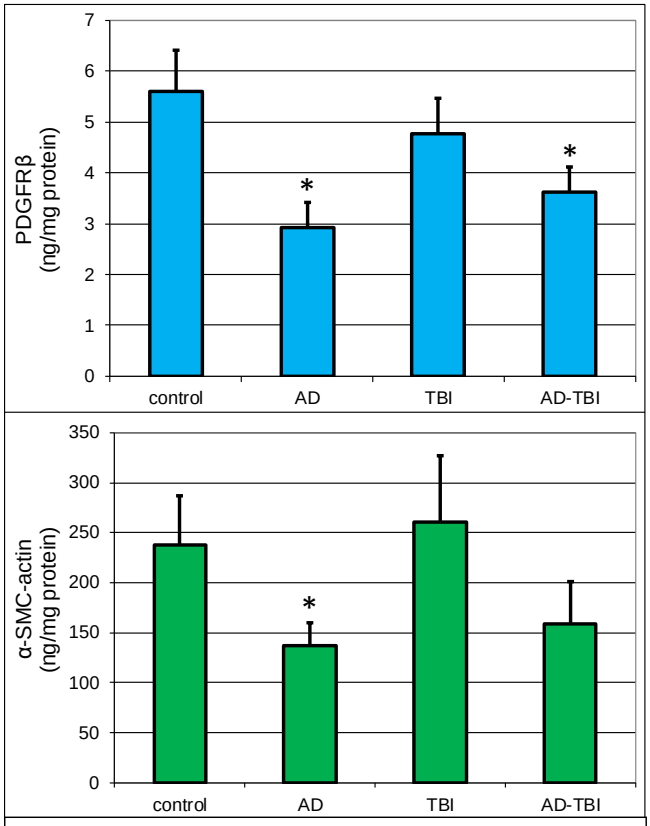


Figure C. Expression of PDGFRβ (top) and α-SMC-actin (bottom) in isolated cerebrovessels from human brain specimens. Brain vessels were analyzed for PDGFRβ and α-SMC-actin content by ELISA and normalized to total protein using the BCA assay. Values represent mean ± SEM (n=8-15). *P < 0.05 compared to respective control as determined by ANOVA and Bonferroni post-hoc test.

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

  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. 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 10, 2016 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?** Over the next reporting period, we will examine tau internalization in r-sham and r-mTBI cerebrovascular cells and evaluate tau degradation pathways in r-sham and r-mTBI cerebrovascular cells. Our preliminary studies indicate brain vascular mural cells have an interaction with tau. However, at this stage, the nature and purpose of this interaction is unclear. The objective of Aim2 is to investigate the processing of tau by mural cells and examine the impact of r-mTBI on these processes. Despite the obvious interaction between the mural cells and tau in our preliminary studies, it is not apparent what proportion of tau was associated with the cell surface and the level that actually entered the cell. Indeed, it has been shown that tau interacts with the surface of the cell [13] and can bind a number of molecules associated with the plasma membrane [14,15]. To reconcile this in cerebrovascular cells, Aim2a will examine tau internalization and cell surface binding in cerebrovascular cultures from r-sham and r-mTBI animals. If these cells internalize tau to a lesser extent following r-mTBI and a greater fraction of tau remains at the cell surface or in the extracellular environment, such events could lead to tau accumulation in the fluids of the brain and the formation of pathogenic species. Another important aspect to consider is whether the mural cells are involved in tau degradation and the extent to which r-mTBI impacts this process. We have hypothesized that the brain vascular mural cells interact with tau in order to degrade and remove tau from extracellular fluids within the brain. Several studies have shown that tau is eliminated through both proteasomal degradation [16,17] and autophagic clearance [18-20]. Our preliminary work demonstrated tau degradation by the 20S

proteasome in brain vascular pericytes, suggesting a role for these cells in the elimination of tau. The studies in Aim2b will continue our investigation of tau degradation by the mural cells and, most notably, will examine the effect of r-mTBI on these degradation pathways. These studies are relevant as perturbations in lysosomal function or inhibition of autophagy has been shown to result in slower tau degradation and clearance [21]. It is worth mentioning that the degradation pathways we are investigating are also disrupted in AD and have been implicated in the accumulation of certain tau species and disease pathogenesis [22,23]. Lastly, as tau exists in a variety of forms under disease conditions, we will examine each of the above processes using various tau species (monomer, low molecular weight aggregates, and filaments) under r-sham and r-mTBI conditions. Notably, the internalization and degradation of exogenous tau species was recently interrogated in neuronal cultures, using the same approach we propose, and tau aggregates were internalized and trafficked to the lysosome for degradation [24]. The studies in Aim2 will evaluate the processing of tau in the same manner, but will investigate these effects in mural cell cultures and isolated r-mTBI brain vasculature. Completion of the studies in Aim2 will 1) provide fundamental information on the cellular processing of tau by mural cells, and 2) identify the impact of r-mTBI on cerebrovascular tau degradation pathways. In addition, we will examine tau internalization and degradation in AD transgenic animals and will compare our findings in TBI to that in AD to further our understanding of the association between TBI and AD. We anticipate tau internalization and/or degradation will be reduced in r-mTBI brain vasculature, which would lead to diminished processing of extracellular tau and may describe the abnormal accumulation of pathogenic tau species in TBI and AD brains.

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 of the PSAPP mouse cohort (animal model of AD) has been slow, due in part to increased mortality at

10 weeks of age and around 12 months of age. The proposed studies require a relatively small number of these animals, so we are continuing to breed and age this cohort, and do not anticipate this slowdown will significantly alter the progress of any studies. As an alternative, we are also considering the option of purchasing these animals from a commercial vendor if the current issues persist.

- **Changes that had a significant impact on expenditures**. Nothing to Report.
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
- **Significant changes in use or care of human subjects.** 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.** Nothing to Report.
  - **Books or other non-periodical, one-time publications.** Nothing to Report.
  - **Other publications, conference papers, and presentations.** An abstract related to work in this project was accepted by the 12th International Conference on Cerebral Vascular Biology (CVB). A poster presentation of this work will be given at the 2017 CVB meeting, which takes place from Nov. 28 to Dec. 1, 2017.
- **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 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 three changes in the active other support of the PI. These changes will not impact the effort of the PI on the current project.

  The responsibilities of the PI on the following grant have been completed.

  DOD CDMRP (GW1300045)
  Restoring brain lipid homeostasis as a therapeutic avenue for treating Gulf War Illness.
  Bachmeier role: Consultant (0.2 calendar)
  2014-2017
  The main goal of this project is to determine if therapeutic targeting of lipid metabolic and remodeling pathways can improve cognition and reduce astroglia pathology associated with Gulf War Illness (GWI) in a mouse model of Gulf War agent exposure.

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

  VA (I01RX002260)
  Treating GWI immune and metabolic disturbances by targeting lipid metabolism.
  Bachmeier role: Co-Investigator (1 calendar)
  2017-2020
  The main goal of this project is to target omega-3 and omega-6 PUFA and mitochondrial and peroxisomal lipid metabolism in an effort to better characterize the underlying inflammation and metabolic disturbances associated with GWI, which will ultimately facilitate development of novel treatments for GWI.

  DOD CDMRP (AZ160065)
  Lipidomics for identifying APOE4-Associated Biomarkers of AD-Related Cognitive Decline in TBI Patients.
  Bachmeier role: Consultant (0.2 calendar)
  2017-2020
  The goal of this project is to analyze intact blood lipids, phospholipid, triglycerides, and cholesterol esters which contain omega-3 and omega-6 fatty acids to determine if they can help diagnose TBI and identify subjects with AD related cognitive decline.

- **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:** Please see the Bibliography references cited list below.

APPENDIX

Bibliography

[1] Mouzon B, Chaytow H, Crynen G, Bachmeier C, Stewart J, Mullan M, Stewart W and Crawford F. Repetitive mild traumatic brain injury in a mouse model produces learning and memory deficits accompanied by histological changes. *J Neurotrauma* 29:2761-73 (2012).

[2] Mouzon BC, Bachmeier C, Ferro A, Ojo JO, Crynen G, Acker CM, Davies P, Mullan M, Stewart W and Crawford F. Chronic neuropathological and neurobehavioral changes in a repetitive mild traumatic brain injury model. *Ann Neurol* 75:241-54 (2014).

[3] Ojo JO, Mouzon B, Algamal M, Leary P, Lynch C, Abdullah L, Evans J, Mullan M, Bachmeier C, Stewart W and Crawford F. Chronic Repetitive Mild Traumatic Brain Injury Results in Reduced Cerebral Blood Flow, Axonal Injury, Gliosis, and Increased T-Tau and Tau Oligomers. *J Neuropathol Exp Neurol* 75:636-55 (2016).

[4] McKee AC, Cantu RC, Nowinski CJ, Hedley-Whyte ET, Gavett BE, Budson AE, Santini VE, Lee HS, Kubilus CA and Stern RA. Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. *J Neuropathol Exp Neurol* 68:709-35 (2009).

[5] Omalu B, Bailes J, Hamilton RL, Kamboh MI, Hammers J, Case M and Fitzsimmons R. Emerging histomorphologic phenotypes of chronic traumatic encephalopathy in American athletes. *Neurosurgery* 69:173-83 (2011).

[6] Schmidt ML, Zhukareva V, Newell KL, Lee VM and Trojanowski JQ. Tau isoform profile and phosphorylation state in dementia pugilistica recapitulate Alzheimer's disease. *Acta Neuropathol* 101:518-24 (2001).

[7] Roberts GW, Allsop D and Bruton C. The occult aftermath of boxing. *J Neurol Neurosurg Psychiatry* 53:373-8 (1990).

[8] Sengillo JD, Winkler EA, Walker CT, Sullivan JS, Johnson M and Zlokovic BV. Deficiency in mural vascular cells coincides with blood-brain barrier disruption in Alzheimer's disease. *Brain Pathol* 23:303-10 (2013).

[9] Winkler EA, Sengillo JD, Sullivan JS, Henkel JS, Appel SH and Zlokovic BV. Blood-spinal cord barrier breakdown and pericyte reductions in amyotrophic lateral sclerosis. *Acta Neuropathol* 125:111-20 (2013).

[10] Bell RD, Winkler EA, Sagare AP, Singh I, LaRue B, Deane R and Zlokovic BV. Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. *Neuron* 68:409-27 (2010).

[11] Skalli O, Pelte MF, Peclet MC, Gabbiani G, Gugliotta P, Bussolati G, Ravazzola M and Orci L. Alpha-smooth muscle actin, a differentiation marker of smooth muscle cells, is present in microfilamentous bundles of pericytes. *J Histochem Cytochem* 37:315-21 (1989).

[12] Lynch CE, Crynen G, Ferguson S, Mouzon B, Paris D, Ojo J, Leary P, Crawford F and Bachmeier C. Chronic cerebrovascular abnormalities in a mouse model of repetitive mild traumatic brain injury. *Brain Inj* 30:1414-27 (2016).

[13] Pooler AM and Hanger DP (2010) Functional implications of the association of tau with the plasma membrane. *Biochem Soc Trans* **38**:1012-1015.

[14] Holmes BB, DeVos SL, Kfoury N, Li M, Jacks R, Yanamandra K, Ouidja MO, Brodsky FM, Marasa J, Bagchi DP, Kotzbauer PT, Miller TM, Papy-Garcia D and Diamond MI (2013) Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proc Natl Acad Sci U S A* **110**:E3138-3147.

[15] Georgieva ER, Xiao S, Borbat PP, Freed JH and Eliezer D (2014) Tau binds to lipid membrane surfaces via short amphipathic helices located in its microtubule-binding repeats. *Biophys J* **107**:1441-1452.

[16] David DC, Layfield R, Serpell L, Narain Y, Goedert M and Spillantini MG (2002) Proteasomal degradation of tau protein. *J Neurochem* **83**:176-185.

[17] Grune T, Botzen D, Engels M, Voss P, Kaiser B, Jung T, Grimm S, Ermak G and Davies KJ (2010) Tau protein degradation is catalyzed by the ATP/ubiquitin-independent 20S proteasome under normal cell conditions. *Arch Biochem Biophys* **500**:181-188.

[18] Wang Y, Martinez-Vicente M, Kruger U, Kaushik S, Wong E, Mandelkow EM, Cuervo AM and Mandelkow E (2009) Tau fragmentation, aggregation and clearance: the dual role of lysosomal processing. *Hum Mol Genet* **18**:4153-4170.

[19] Lee VM, Brunden KR, Hutton M and Trojanowski JQ (2011) Developing therapeutic approaches to tau, selected kinases, and related neuronal protein targets. *Cold Spring Harb Perspect Med* **1**:a006437.
[20] Kruger U, Wang Y, Kumar S and Mandelkow EM (2012) Autophagic degradation of tau in primary neurons and its enhancement by trehalose. *Neurobiol Aging* **33**:2291-2305.
[21] Hamano T, Gendron TF, Causevic E, Yen SH, Lin WL, Isidoro C, Deture M and Ko LW (2008) Autophagic-lysosomal perturbation enhances tau aggregation in transfectants with induced wild-type tau expression. *Eur J Neurosci* **27**:1119-1130.
[22] Upadhya SC and Hegde AN (2007) Role of the ubiquitin proteasome system in Alzheimer's disease. *BMC Biochem* **8 Suppl 1**:S12.
[23] Funk KE and Kuret J (2012) Lysosomal fusion dysfunction as a unifying hypothesis for Alzheimer's disease pathology. *Int J Alzheimers Dis* **2012**:752894.
[24] Wu JW, Herman M, Liu L, Simoes S, Acker CM, Figueroa H, Steinberg JI, Margittai M, Kayed R, Zurzolo C, Di Paolo G and Duff KE (2013) Small misfolded Tau species are internalized via bulk endocytosis and anterogradely and retrogradely transported in neurons. *J Biol Chem* **288**:1856-1870.

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

Log Number: AZ150052

Award Number: W81XWH-15-PRARP-CSRA

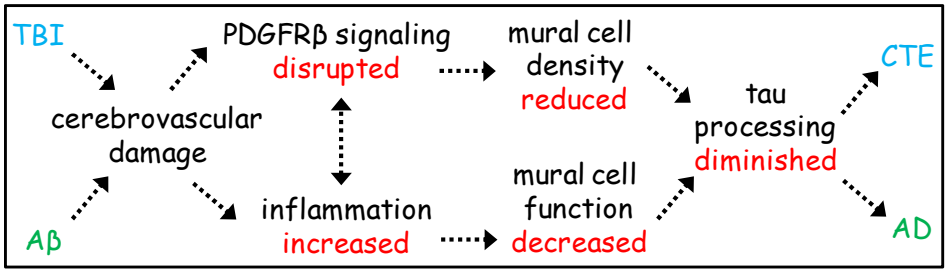
PI: Corbin Bachmeier **Org:** Roskamp Institute **Award Amount:** $799,904.00

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.



Accomplishment: We have now established a timeline for tau processing and mural cell expression following head trauma in our r-mTBI mouse model (24hr, 3mth, and 6mth). Furthermore, we have begun the injury paradigm for a cohort of mice which will examine the same parameters above at 1 month post-last injury. In addition, we have obtained human TBI brain specimens and isolated the cerebrovasculature from all 50 brain samples. Moreover, we have completed our evaluation of mural cell expression in these samples in the same manner as the animal TBI studies.

Timeline and Cost

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

Updated: (10/30/2017)

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: $263,288.00
Actual Expenditure: $288,588.83