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

PRINCIPAL INVESTIGATOR: Corbin Bachmeier, PhD

CONTRACTING ORGANIZATION: Roskamp Institute
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6. AUTHOR(S)

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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 such as Alzheimer's disease (AD), we observed a progressive decline in cerebrovascular mural cell expression following r-mTBI in mice. In particular, we observed significant reductions in an important mural cell ligand, PDGF-BB, post-injury. Moreover, isolated cerebrovasculature from r-mTBI animals were less able to internalize tau than r-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. 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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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. Major Goal 1 has been completed in full. Major Goal 2 has been completed except, as proposed, we will examine mural cell density using an additional technique, confocal microscopy. Major Goal 5 has been completed except, as proposed, we will determine caveolin expression in mouse and human TBI brains. Major Goal 6 is nearly complete as we have finished the laboratory studies and are now in the process of compiling the results.

- **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 the PDGF pathway in human TBI brains and murine brains following r-mTBI and, evaluation of the effect of PDGF-BB stimulation on tau processing by mural cells after r-mTBI. **2) specific objectives.** Examination of the PDGF pathway in human TBI brains and murine brains following r-mTBI and, evaluation of the effect of PDGF-BB stimulation on tau processing by mural cells after r-mTBI in mice. **3) significant results.** Tau processing and expression of mural cell markers in AD mice: In our prior work (Aim1), we examined tau uptake and the expression of mural cell markers in isolated cerebrovessels following r-mTBI at various timepoints post-last injury. We observed a progressive decrease in tau processing by r-mTBI cerebrovessels compared to r-sham animals (20% reduction at 6 months post-last injury). Correspondingly, we also showed a progressive reduction in the expression of αSMC-actin (alpha smooth muscle cell actin) and, to a lesser extent, 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 αSMC-actin and PDGFRβ. 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 (Figure A). 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. PDGF-BB expression in the brain following r-mTBI: To continue our evaluation of the PDGF pathway, as outlined in Aim3a of the proposed studies, we examined the PDGF-BB agonist in our TBI and AD samples, to complement our prior work evaluating the PDGFRβ receptor. These studies 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 B). We performed the same analyses in human TBI brain specimens and observed a similar 20% reduction in PDGF-BB (Figure C) compared to human control brain samples (i.e., no history of TBI). We are also in the process of examining PDGF-BB in additional human AD brain specimens and will be finalizing these studies shortly. In terms of AD animals, we examined the PSAPP mouse model of AD and found 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 (Figure B). 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 ligand is decreased in r-mTBI, while in AD, the receptor is substantially diminished. As such, the manner in which this pathway would be targeted therapeutically, could be quite different for each disease state. PDGF-BB secretion from

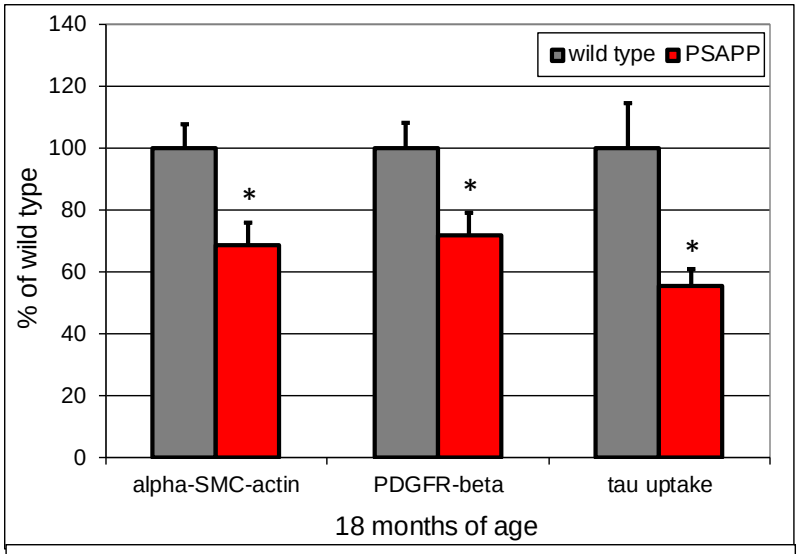

Figure A. Tau uptake in freshly isolated cerebrovessels from wild type and PSAPP animals (18 months of age). Cerebrovessels were exposed to 5ng/ml recombinant human tau (rhtau-441) for 1 hour at 37°C. Lysates were analyzed for total tau content, alpha-SMC-actin, and PDGFR-beta by ELISA and normalized to total protein using the BCA assay. Values represent the percentage of each respective wild type group ± SEM (n=6). *P < 0.05 compared to each respective wild type group as determined by ANOVA and Bonferroni post-hoc test.

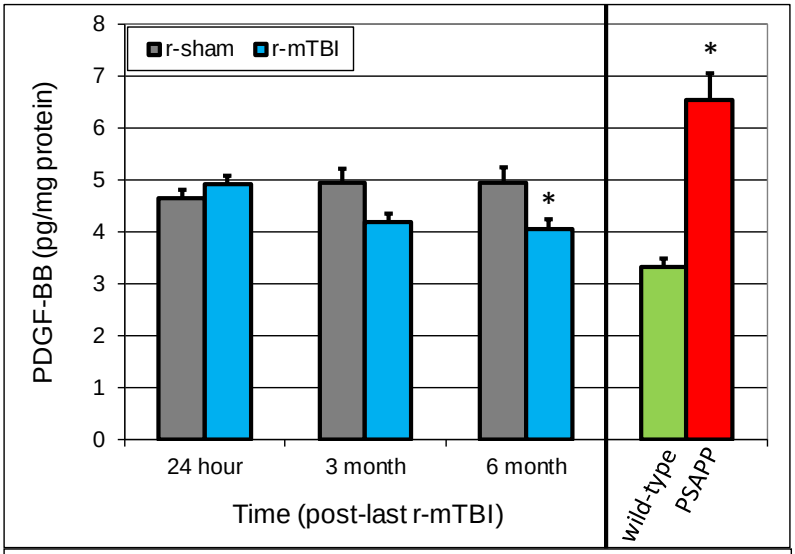

Figure B. Expression of PDGF-BB in brain homogenate from r-mTBI (24 hours, 3 months, and 6 months post-last injury), wild-type, and PSAPP mice (18 months of age). Brain samples were analyzed for PDGF-BB content by ELISA and normalized to total protein using the BCA assay. Values represent the mean amount (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.

freshly isolated cerebrovessels following r-mTBI: In the Aim3a 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, Aim3b 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 D), in line with the reduced PDGF-BB expression in the brain overall following r-mTBI (Aim3a Figure B 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 studies from the previous reporting period (Aim1b).

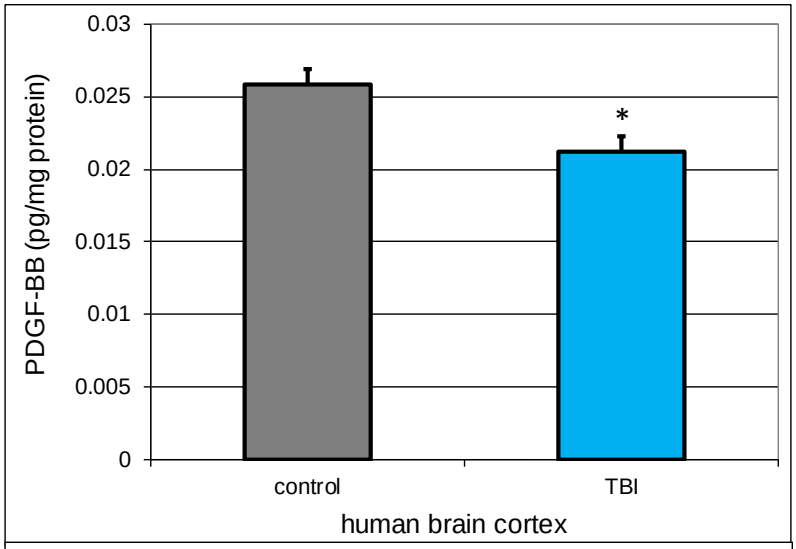

Figure C. Expression of PDGF-BB in brain homogenate from human control (i.e., no history of TBI) and TBI cortex. Brain samples were analyzed for PDGF-BB content by ELISA and normalized to total protein using the BCA assay. Values represent the mean amount (pg) of PDGF-BB per mg of total protein ± SEM (n=9-12). *P < 0.05 compared to control brains as determined by Student's t-test.

Mural cell stimulation with PDGF-BB following r-mTBI: As just described, the PDGF-BB ligand is diminished following head trauma, which may lead to reduced tau processing by mural cells and, potentially, tau pathogenesis in the brain. In response to this, Aim3b will evaluate the impact of PDGF-BB supplementation on tau processing in the cerebrovasculature from 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. We have recently completed these studies and are in the process of compiling the results of this dataset. It is anticipated the administration of PDGF-BB will rejuvenate the mural cell population following head trauma which could improve tau processing and mitigate tau pathogenesis in the brain post-injury.

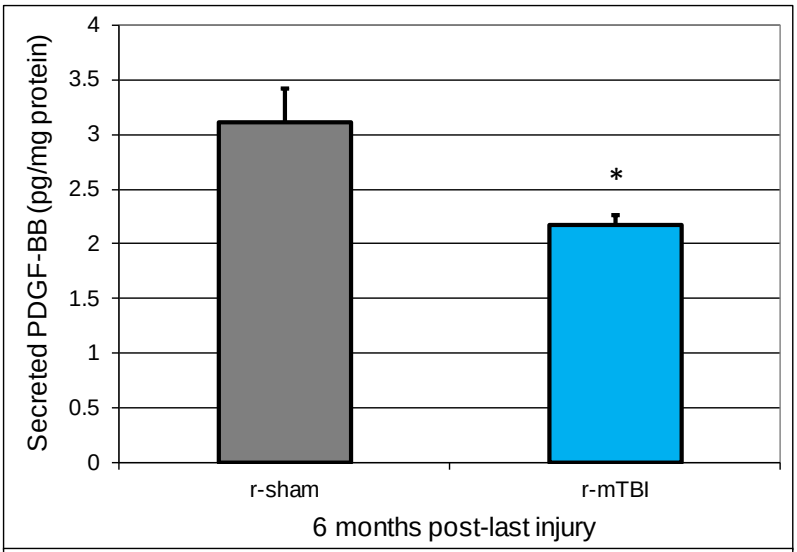

Figure D. Secretion of PDGF-BB in isolated brain vasculature from r-mTBI mice (6 months post-last injury). Freshly isolated cerebrovessels were incubated for 72 hours at 37°C and the extracellular media was collected and analyzed for PDGF-BB content by ELISA and normalized to total protein using the 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.

4) other achievements. Nothing to report.

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

  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. 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. 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. Corbin presented a poster on his work and had discussions with collaborators and other researchers for feedback on data and future directions.
- **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, 2017 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?** The majority of our efforts for the next reporting period will examine tau internalization and 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. 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 and autophagic clearance. 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. 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. 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, oligomers, and filaments, etc.) under r-sham and r-mTBI conditions. We have already begun the process of isolating

and characterizing various tau species for future testing in cell culture and isolated tissue. Thus far we have isolated three different tau species: phosphorylated tau (CP13), oligomeric tau (TOMA), and conformational tau (MC1), and are in the process of characterizing their relative purity. We hope to acquire a panel of tau species and compare their uptake and processing by brain vascular mural cells. When we have acquired the desired panel of tau species, the studies in Aim2 will evaluate the processing of tau in mural cell cultures and isolated r-mTBI brain vasculature, as proposed. 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. 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 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 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 are in the process of preparing a manuscript that will be submitted to a peer-reviewed scientific journal at some point in the next few months.
     - **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 was given at the 2017 CVB meeting, Melbourne, AUS, Nov. 28 to Dec. 1, 2017. Two additional poster presentations related to work in this project were given at the Joint Symposium of the International and National Neurotrauma Societies, Toronto, CAN, Aug 11-16, 2018.
   - **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 responsibilities of the PI on the following grant have been completed.

  NIH (R01AG041971)
  Influence of apoE on LRP1 function and beta-amyloid transport across the BBB.
  Bachmeier role: Principal Investigator
  2012-2018
  The goal of this project is to determine the influence of various apoE isoforms on LRP1 function and beta-amyloid clearance across the BBB.

  The following grant is now pending 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.

- **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

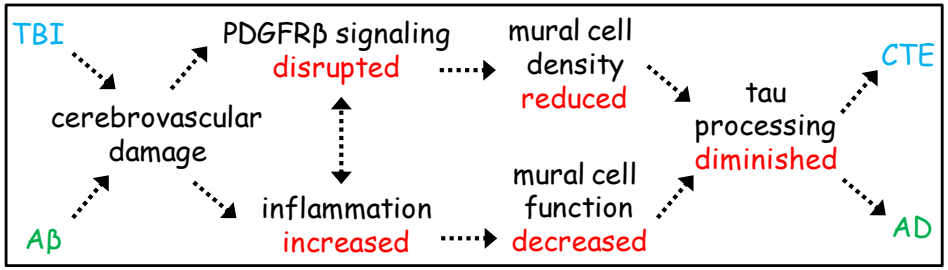
PI: Corbin Bachmeier **Org:** Roskamp Institute **Award Amount:** $799,904.00

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 have completed our examination of the PDGF pathway in TBI and AD human brain specimens and TBI and AD mouse brains, including the effect of TBI on PDGF-BB secretion in freshly isolated mouse cerebrovessels. We are in the process of evaluating the effect of PDGF-BB stimulation on tau processing post-injury, and have begun generating various tau species to test the role of brain vascular mural cells in tau internalization and degradation in the context of TBI.

Timeline and Cost

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

Updated: (10/29/2018)

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: $531,000.00

Actual Expenditure: $518,765.18