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TITLE: **Probing the mechanistic role of vascular dysfunction and vascular inflammation in TBI-mediated cognitive dysfunction**

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CONTRACTING ORGANIZATION: **Carl T. Hayden Medical Research Foundation**
Phoenix, AZ 85012

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Fort Detrick, Maryland 21702-5012

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5e. TASK NUMBER

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Phoenix, AZ 85012

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Traumatic brain injury (TBI) is a major cause of mortality/morbidity among service-members/veterans and is linked to long-term development of aging related dementia disorders through still poorly-defined mechanisms. We are testing the hypothesis that an important etiopathologic basis of TBI-related cognitive dysfunction is cerebrovascular dysfunction and vascular inflammation resulting in chronic brain hypoperfusion. We are also testing the hypothesis that TBI confers susceptibility to later development of cardiovascular risk factor (specifically diabetes/hyperglycemia)-related cerebrovascular dysfunction leading to cognitive impairment. In Aim 1 we will measure the cognitive function of Sprague-Dawley rats exposed to TBI by fluid percussion injury and determine the relationship with cerebrovascular function (in vivo by MRI and ex vivo by circle of Willis artery vasoreactivity) and vascular inflammation. In Aim 2 we will determine whether TBI and diabetes-related metabolic derangements or β-amyloid confer synergistic deleterious effects on cognitive function, cerebrovascular function and inflammation. We completed the cohorts who underwent injury or sham treatment and obtained 6-month cognitive function and in-vivo and ex-vivo cerebrovascular function data, but our data remains preliminary and incomplete. Preliminary data so far show impaired cognitive function 6 months following TBI with some regional association between cognitive and in vivo cerebrovascular function, but we advise caution in interpretation due to incomplete data.

15. SUBJECT TERMS
Traumatic brain injury, cognitive dysfunction, endothelial function, dementia, inflammation

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	23	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

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

Traumatic brain injury (TBI) is a major cause of mortality and morbidity among service-members and veterans and has been linked to long-term development of aging related dementia disorders through still poorly-defined mechanisms. We are testing the hypothesis that an important etiopathologic basis of TBI-related cognitive dysfunction is through cerebrovascular dysfunction and vascular inflammation resulting in chronic brain hypoperfusion. We are also testing the hypothesis that TBI confers susceptibility to later development of cardiovascular risk factor (specifically diabetes/hyperglycemia)-related cerebrovascular dysfunction leading to cognitive impairment. In Aim 1 we will measure the cognitive function of Sprague-Dawley rats exposed to diffuse TBI by fluid percussion injury and determine the relationship with cerebrovascular function (in vivo by MRI and ex vivo by circle of Willis artery vasoreactivity) and vascular inflammation. In Aim 2 we will determine whether TBI and diabetes-related metabolic derangements or β-amyloid confer synergistic deleterious effects on cognitive function, cerebrovascular function and inflammation.

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

Traumatic brain injury, cognitive dysfunction, endothelial function, dementia, inflammation, cerebrovascular disease, vascular imaging

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Please see attachment.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1. Obtain institution and DOD approval for live animal work.
Accomplishment: Institutional and DOD approvals for live animal work were obtained during the first few months of the funding period.

2. Compare 180d in vivo cerebral blood flow and reactivity by MRI and ex-vivo by circle of Willis arteries between TBI vs. uninjured rats and determine the relationship of vascular function with measures of cognitive function and degree of neuropathology.
Subtask 1: Produce cohorts of uninjured and TBI rats (n=6 each)
Subtask 2: Draw blood and conduct cognitive testing at 3 and 6 months post injury

Accomplishments: We have completed the cohorts of sham and TBI rats in terms of cognitive behavioral testing (3 and 6 months), in-vivo MRI vascular perfusion testing and ex-vivo vasoreactivity testing. Serologic testing is scheduled for batch testing later and has not been started yet.
Identified challenges: Meticulous attention to scheduling and animal handoffs represented the greatest challenge since the project involves complex procedures performed at 3 institutions (University of Arizona College of Medicine-Phoenix, Barrow Neurological Institute, and Phoenix VA). Communication between the sites has been the greatest challenge, where regular in person meetings, clear email, and a shared project calendar system have been rewarded with continued success. Cohort survival rates are as expected and comparable to Dr. Lifshitz' previous cohorts in other studies.
Preliminary Scientific Findings:
Cognitive function was assessed using 3 standardized measures: novel object recognition (NOR), novel object location (NOL) and temporal order object recognition (TOR), which represent assessments of short-term, long-term, and working memory, respectively. The discriminant ratio represents the ratio of attention to the familiar versus novel object, where a value of 0.5 indicated chance performance. The 3 and 6-month data show impaired novel object recognition and temporal order object recognition at both 3 and 6 months and impaired novel object location at 6 months (Figure 1). This is consistent with our hypothesis that **diffuse brain injury results in sustained, chronic cognitive dysfunction**. This finding of cognitive dysfunction 6 months following mild-moderate TBI in this rat model is the longest follow up time that we are aware, enhancing the novelty of our findings as well as the value of this experimental animal model to recapitulating what

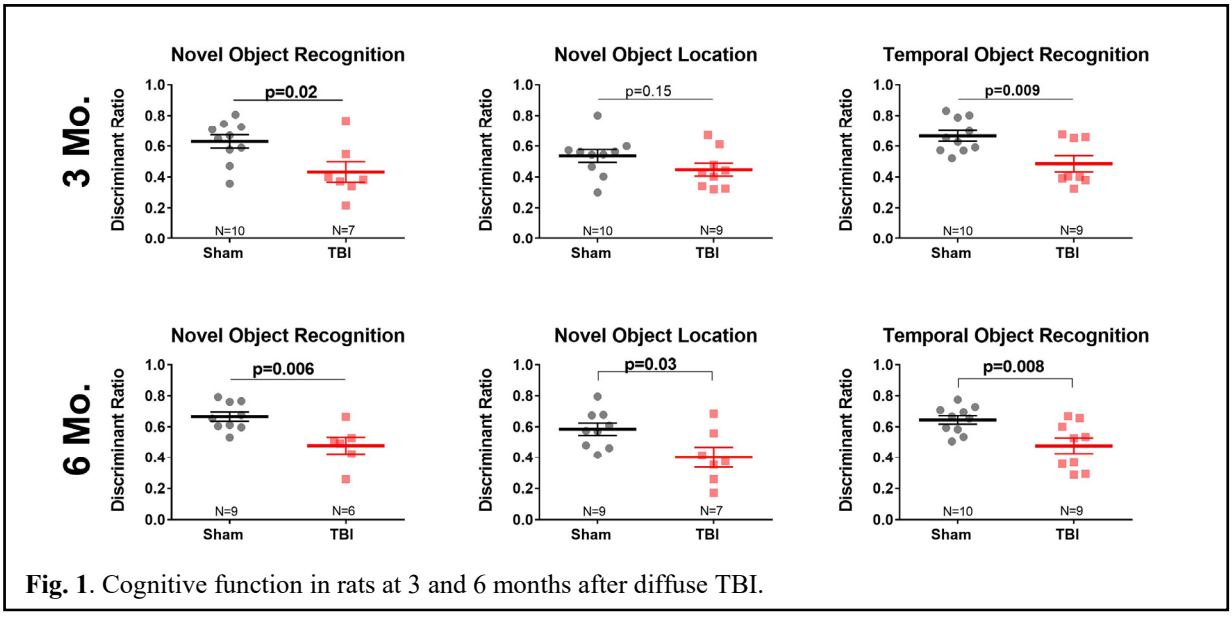


Fig. 1. Cognitive function in rats at 3 and 6 months after diffuse TBI.

has been observed in human epidemiologic studies. These data will be incorporated into analytical models in the final year of work.

Subtask 3: Conduct in vivo cerebral blood flow and cerebrovascular vasoreactivity using MRI in brain injured and uninjured rats.

Accomplishments: We have completed imaging of rat cohorts with TBI and sham injury and completing imaging data acquisition of LPS and saline rats treated prior to TBI injury.

Preliminary Scientific Findings: We observed blood volume differences in a region designated as

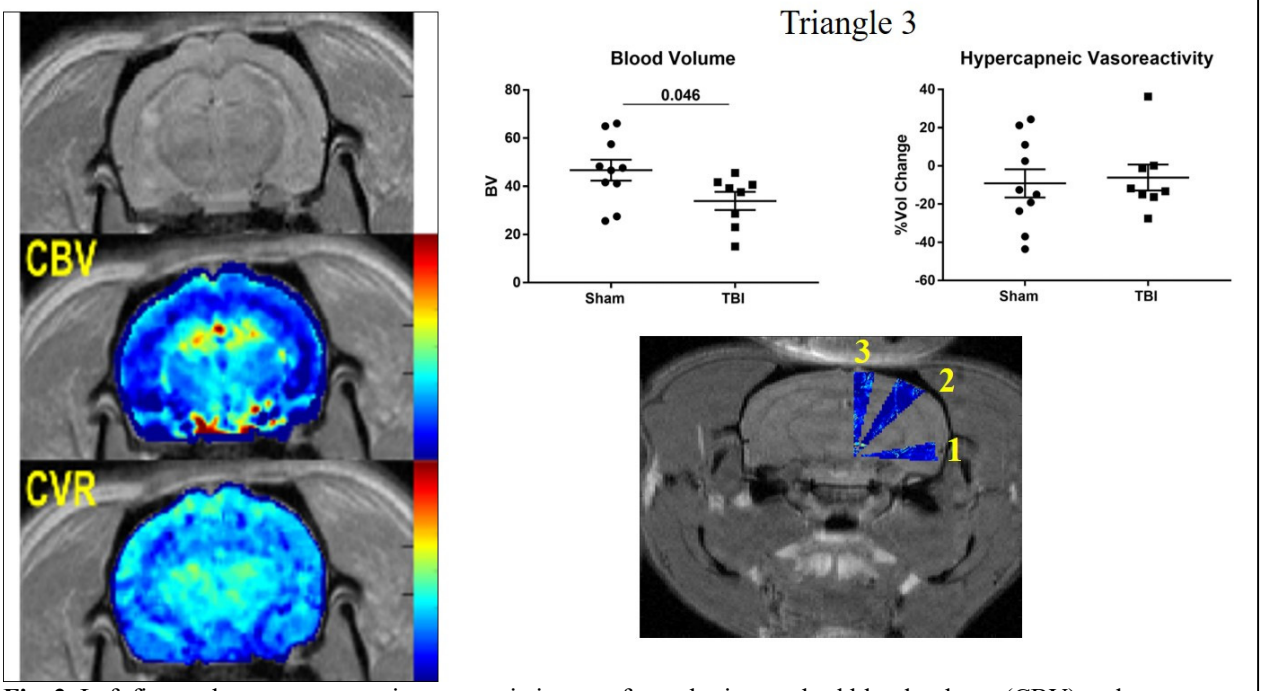


Fig. 2. Left figure shows representative anatomic image of a rat brain, cerebral blood volume (CBV) and hypercapneic vasoreactivity (CVR) quantification in our cohort. There appears to be regional variation but greatest difference in CBV was seen in triangle 3 region (area shown in bottom right) that represents deep brain, cingulate cortex, and lateral hypothalamus. There was significantly reduced CBV in TBI versus uninjured rats in triangle 3 with no difference in hypercapneic vasoreactivity.

Triangle 3 (Figure 2), which includes portions of deep brain, cingulate cortex, and portions of hypothalamus). In Triangle 3, there was significantly reduced cerebral blood volume (CBV) in TBI versus uninjured rats; the hypercapneic vasoreactivity (CVR) in this region was not different. The CBV and CVR in the hippocampus was not significantly different, contrary to what we hypothesized.

When we looked at post-hypercapnea cerebral vasodilation response (<1% CO_2, see note below), there was a trend towards impaired dilator response (% of pixels that did not show any positive change) in TBI versus uninjured in the medial hippocampus region that did not reach statistical significance (p=0.09, Figure 3). We observed that 6-month cognitive function (NOR) is inversely proportional to the magnitude of pixels that did not dilate in response to hypercapnea, showing the relationship between cognitive function and cerebrovascular function in this region (Figure 3).

Identified challenges: We did not anticipate the attenuated dilator response to hypercapnea in different brain regions even in uninjured rats (in Fig. 3 for example, ~50% of pixels in sham rats did not dilate following hypercapnea, an unusually large proportion). We were using the same protocol and anesthesia levels already validated extensively by our co-investigator Dr. Quarles, so this observation could not be explained by the known vasodilator effect of isoflurane. Dr. Quarles communicated on 8/26/19 that upon extensive investigation, they confirmed a technical error in calculation of flow rates being used leading to exposure of the rats to ~1% CO2 instead of the planned 5% CO_2 for the hypercapneic experiments. This is expected to cause blunting of dilator response to the unplanned reduced level of hypercapnea. This does not affect baseline CBV measurements but only affects hypercapneic response measurements. The team is undergoing discussions on how to address this hurdle, with solutions including additional animal cohorts *within limits allowed by our approved animal protocol* and extending the hypercapnic response measurements to include both ~1% and 5% CO_2, which provides for a comparison between states.

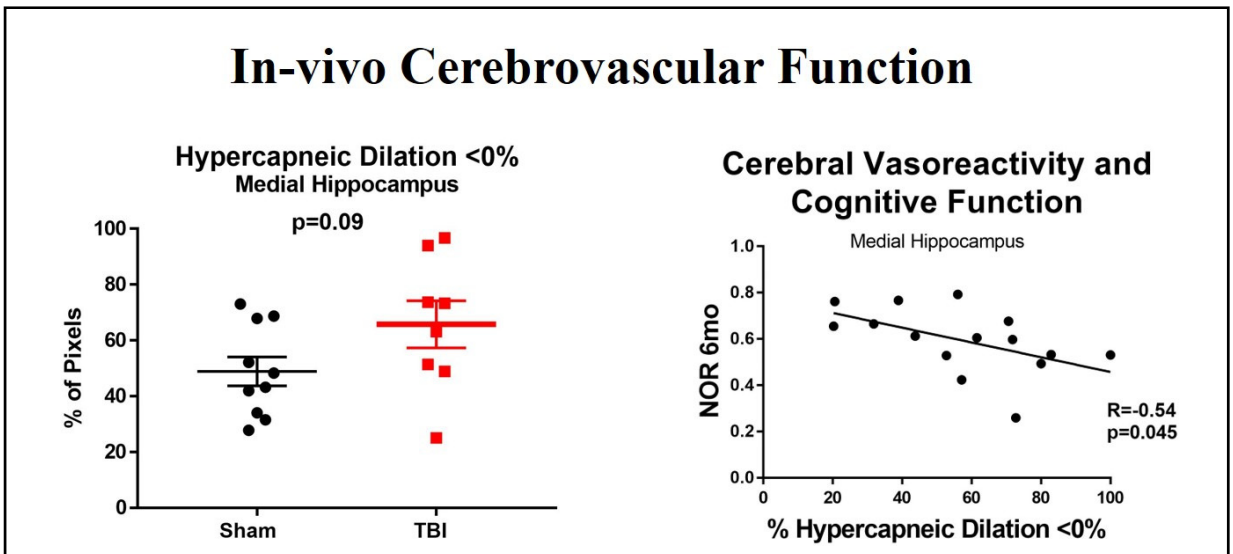


Fig. 3. A. In vivo MRI showing % of pixels in medial hippocampus with no dilator response to ~1% hypercapnea. There was a trend, not statistically significant, of greater impairment in hypercapneic response in TBI vs. uninjured rats. B. There was a significant inverse relationship between cognitive function in terms of NOR and the degree of lack of dilator response to hypercapnea in medial hippocampus.

Subtask 4: Conduct ex vivo vasoreactivity of isolated circle of Willis arteries from TBI and uninjured rats.

Accomplishments: Vasoreactivity data were obtained for sham and TBI rats as well as some LPS pretreated rats.

Preliminary Scientific Findings: Pial (circle of Willis) arterial myogenic tone was determined by progressive exposure of cannulated arteries to 30 and 60 mm Hg (physiologic pressures). Myogenic tone was not different between sham and TBI groups (**Figure 4**). Similarly, no difference was found in endothelium-dependent dilation and smooth-muscle dependent dilation in cerebral arteries of TBI

versus uninjured rats. Results of dilator response post-exposure to Aβ42 and high glucose are discussed in the following sections.

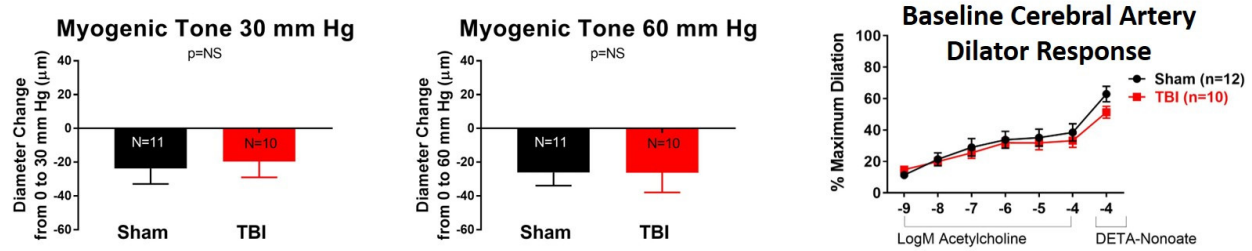


Fig. 4. Baseline myogenic tone in response to intraluminal 30 and 60 mm Hg pressures was not different between TBI and uninjured rats. Similarly, baseline dilator response to acetylcholine (endothelium-dependent) and DETA-NONOate (smooth muscle-dependent) did not differ between the two groups.

Subtask 5: Perform neuropathological assessment of brain hemispheres, including laser capture microdissection and initial gene expression assays.

Accomplishments: Brain collection were completed for TBI and uninjured rats and being completed for LPS-pretreated rats. For each animal, one hemisphere was frozen (for laser capture microdissection and gene/protein assays) and the other hemisphere immersion-fixed in

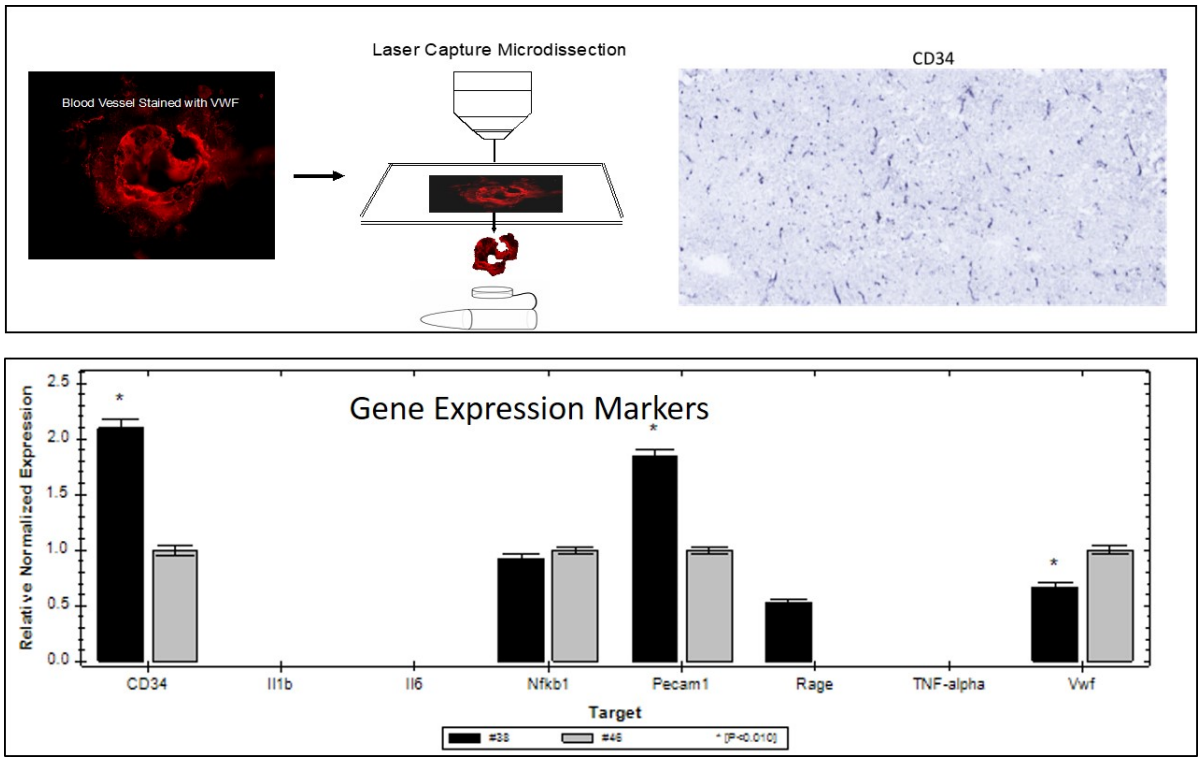


Fig. 5. Vessel laser capture microdissection. Top panel shows labeling of cerebral vessels and laser capture microdissesction. Below panel shows preliminary quality control results (1 injured in black, 1 uninjured in gray, 100 vessels each) showing that tissues collected had vascular/endothelial cell markers confirming they are vessels. There was low expression in cytokines IL-6, IL-1B and TNF-alpha, with RAGE expression in TBI but not uninjured rat (N=1).

paraformaldehyde (for immunohistochemistry and histology). We plan on batch processing the staining and analyses to enhance operational efficiency as well as to enhance rigor by minimizing potential confounding effect of variability from technical issues (e.g. variability in staining). As such, no data are available at this time. Laser capture protocols have been adapted and optimized for rat brain tissues (shown in **Figure 5**), and tissue batch processing is pending.

3. Identify potential mechanisms of TBI-induced cerebrovascular dysfunction by assessing vascular oxidative/nitrative stress and vascular inflammation following TBI and assess their relationship to cognitive dysfunction.

Subtask 1: Assess blood samples for markers of oxidative stress and inflammatory markers.

Accomplishments: Blood draws are complete for TBI and uninjured rats and being completed for LPS pretreated rats. As mentioned previously, we plan on doing batch processing and analyses of stored samples. As such, no data are available at this time.

Subtask 2: Evaluate oxidative and nitrative stress in circle of Willis arteries

Accomplishments: Arteries from TBI and sham rats have been processed. Separate circle of Willis arterial segments were isolated, treated with vehicle, Aβ42 or high glucose and exposed to hydroethidine (superoxide marker), dihydrorhodamine (peroxynitrite marker) and DAF-2 (nitric oxide marker) for immunofluorescence imaging. Preliminary results show no significant difference in baseline (vehicle-treated) cerebral artery superoxide, peroxynitrite and NO between TBI and uninjured rats (**Figure 6**). There was also no difference following exposure to high glucose. However, following exposure to Aβ42, there was significant increased production of peroxynitrite and trend towards increased superoxide in TBI vessels, suggesting increased predisposition to nitrative stress in TBI when exposed to Aβ42.

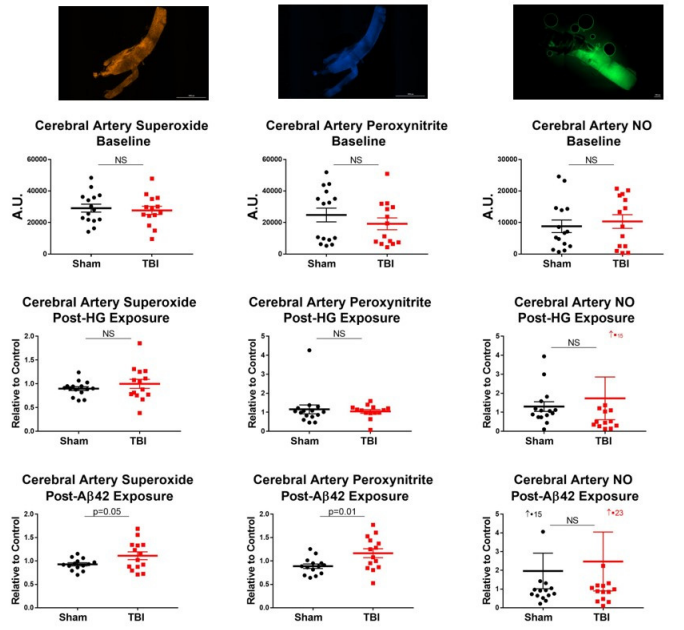


Fig. 6. Measures of oxidative and nitrative stress include superoxide, peroxynitrite and nitric oxide (NO). At baseline, there was no difference between TBI and sham rats. The same results were seen following exposure to high glucose. Following exposure to Aβ42, there was greater peroxynitrite and trend towards greater superoxide in TBI versus uninjured, suggesting greater predisposition to nitrative stress with Aβ42 exposure.

Subtask 3: Quantify inflammation through gene and protein expression analyses of inflammatory markers in circle of Willis arteries.

Accomplishments: Samples have been collected from TBI and sham and will be completed in LPS pretreated rats. Tissue preparations for IHC analyses are ongoing.

Subtask 4: Measure smooth muscle contractile proteins and eNOS gene and protein expression in TBI and sham groups.
Accomplishments: Following collection of circle of Willis arteries from cohorts 1-2, we now recognize that we will have insufficient arterial mass after vasoreactivity and oxidative stress assays to perform PCR gene expression assays. It is not possible to perform gene expression assays on these arteries. The same limitation holds for Western blot assay. We did collect arterial segments fixed in paraformaldehyde for immunohistochemistry that will allow protein assays by IHC or immunofluorescence. We plan on doing batch processing. No data are available at this time.

4. Evaluate whether preconditioning with lipopolysaccharide will attenuate TBI-induced cerebrovascular dysfunction and inflammation and prevent TBI-mediated cognitive dysfunction.
Subtask 1: Produce cohorts of uninjured and TBI LPS preconditioned rats with blood collection and cognitive function assessments.
Subtask 2: Conduct in vivo MRI vascular function, ex vivo vasoreactivity and neuropathology.
Accomplishments: We planned 4 additional cohorts (n=6 per cohort) to achieve the goals related to LPS pre-conditioning. The tissue from the cohorts have been collected or are currently in process through the established protocol. These animals received LPS injections 3 days prior to brain injury, with a rectal temperature monitor to track inflammation related hyperthermia.

5. Compare the ex-vivo responses of cerebral arterioles between uninjured and TBI rats following exposure to high glucose and Aβ42.
Subtasks 1-2: Test vascular function of cerebral vessels of TBI vs. uninjured when exposed to high glucose or Aβ.
Subtask 3: Assess oxidative and nitrative stress and inflammation following exposure to HG or Aβ.
Accomplishments: Unlike baseline cerebral artery responses where there was no difference, TBI cerebral arteries showed significant difference vs. uninjured vessels in smooth muscle-dependent dilator response to Aβ42 (but not endothelium-dependent response, **Figure 7**). There was no difference between TBI and uninjured responses following exposure to high glucose.

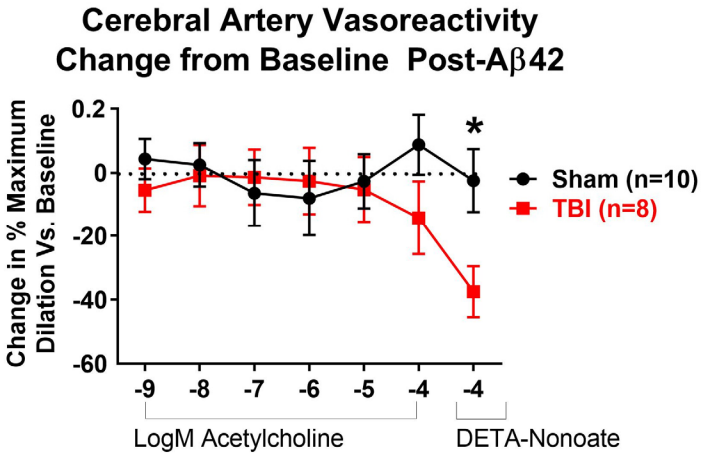


Fig. 7. Change in dilator response from baseline response following exposure to Aβ42 showed no difference in response to acetylcholine. However, TBI cerebral arteries showed greater significant reduction in dilator response to DETA-NONOate compared to uninjured rats, suggesting greater impairment in smooth muscle dependent function with exposure to Aβ42.

6. Compare cerebrovascular function, vascular inflammation and cognitive function in stretptozotocin-treated rats (diabetes model) which had antecedent TBI versus no injury.
Subtasks 1-6: produce cohorts of uninjured and TBI rats, inject stretptozotocin at 90 days, measure cognitive function, in vivo and ex-vivo vascular function and neuropathological assessment.
Accomplishments: In anticipation of this goal, we have planned and conducted preliminary investigations into Streptozotocin. To date, we have obtained a glucometer sensitive to rodent blood

sugar levels and mastered the techniques for repeated blood glucose measurement. In adverse cases, we have obtained injectable and implantable insulin to control glucose levels, as planned in the original proposal. Preliminary trials to induce diabetes in naïve rats has indicated that blood draws and glucose measurements must be taken following a 6 hour fasting to obtain consistent and predictable values not associated with food intake. Diabetes Cohorts 1-4 are planned for the beginning of project year 3.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The Translational Neurotrauma Research Program hosts a **monthly community journal club** to discuss neurotrauma from all aspects. We have attendees who represent the legal profession, physical therapy, drug companies, physicians, scientists, and trainees. Over the last academic year, the topics included: employment after TBI, addiction and TBI, psychosocial outcomes in pediatrics and TBI, environment and weather, tumorigenesis, biomarkers, the vegetative state, maternity, and transmissible disease.

Lifshitz Lab: Conor Young is the primary technician on this project. He joined the group in February of 2018, and currently coordinates all the physiology, behavior, transport, and tissue dissections for each cohort. He has become the primary point person for animal status on the project. In the next project year, he will continue to shepherd the animal cohorts through the behavior and physiology measurements, in addition to processing tissue for histology and IHC. Conor has also led the group in understanding the approaches for using LPS and Streptozotocin for the upcoming cohorts.

Migrino Lab: Michael Hansen, Research Technician, has acquired technical training and new skillsets in lab methods such as Western blot, tissue preparation, immunohistochemistry and microscopy, broadening his professional experience.

Quarles Lab: Alberto Fuentes is a masters student at Arizona State University who is analyzing the MRI data for this project. He has gained in rat brain image co-registration, atlas-based region of interest analysis, contrast agent-based perfusion modeling and cerebral vascular reactivity analysis.

Mastroeini Lab: Jennifer Nolz and Elaine Delvaux have acquired new skillsets in processing rat tissues specifically in identification and isolation of cerebral vessels using laser microdissection as well identifying specific gene transcripts appropriate for the study design and aims.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Results have yet to be disseminated beyond the investigators and key personnel on the project. The results remain preliminary. Dr. Migrino presented preliminary results to the DOD Meeting in Ft Detrick, MD in August 2019. Until further analysis of the complete cohort by the statistics personnel (Dr. Hu), data remain with the research team.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We will complete the planned cohorts, which include cohorts with LPS preconditioning and cohorts receiving Streptozotocin at 90 days. We will continue brain tissue preparation and processing, and do batch assays of blood and tissue analyses. Data will be organized with the oversight of our statistician (Dr. Hu) to begin modeling the main effects of injury. Dr. Hu has received an initial set of data to begin the regression modeling.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

We are just beginning to collect our data and there are more cohorts to be completed. An assessment of the impact of our findings cannot be made at this point.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

We are just beginning to collect our data and there are more cohorts to be completed. An assessment of the impact of our findings cannot be made at this point.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

We are just beginning to collect our data and there are more cohorts to be completed. An assessment of the impact of our findings cannot be made at this point.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

1. As stated previously, following collection of circle of Willis arteries from cohorts 1-2, we now recognize that we will have insufficient arterial mass after vasoreactivity and oxidative stress assays to perform PCR gene expression assays so we will not be able to perform gene expression assays on these arteries. The same limitation holds for Western blot assay. We did collect arterial segments fixed in paraformaldehyde for immunohistochemistry that will allow us to protein assays by IHC or immunofluorescence. We will also do gene expression assays on isolated parenchymal cerebral vessels.
2. We have adopted a clinically-relevant 6 hour fasting procedure prior to blood collection necessary to monitor the diabetes arm of the study.

3. Two stages of hypercapnic blood flow studies will be conducted in order to identify relationships between ~1% and 5% CO_2, which are necessary to determine the trajectory of cerebrovascular responses to TBI.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals

Our group does not routinely work with Streptozotocin. As previously stated, we have sought local expertise for guidance and consulted the literature. The initial preliminary animals guide our protocols for conducting the final cohorts of animals.

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Because of the preliminary and incomplete nature of the dataset, no publication has directly resulted from this project proposal.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Because of the preliminary and incomplete nature of the dataset, no publication directly from the project has been submitted.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

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

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Seth Truran
Project Role: Research Associate
Nearest person month worked: 1.89
Contribution to Project: Mr. Truran worked on logistical coordination, methodologic optimization of vascular procedure and tissue collection process required for the project.

Name: Nina Karamanova DVM
Project Role: Research Associate
Nearest person month worked: 3.36
Contribution to Project: Dr. Karamanova worked with Mr. Truran on logistical coordination, methodologic optimization of vascular procedure and tissue collection process required for the project.

Name: Karen D'Souza, PhD
Project Role: Research Technician
Nearest person month worked: 1.0

Contribution to Project: Dr. D'Souza worked with Mr. Truran on logistical coordination, methodologic optimization of vascular procedure and tissue collection process required for the project.

Name: John Hatfield
Project Role: Research Coordinator
Nearest person month worked: 0
Contribution to Project: Mr. Hatfield retired and is no longer on the project.

Name: Gail Farrell
Project Role: Research Coordinator-Backup
Nearest person month worked: 0.08
Contribution to Project: Ms. Farrell assisted as needed with local regulatory requirements for the project.

Name: L Matthew Law, PhD
Project Role: Post-doctoral fellow
Nearest person month worked: 2.4
Contribution to Project: Dr. Law is responsible for cohort planning, animal behavioral testing, and overall management of animal work. He is the primary communication for Dr. Lifshitz.

Name: Daniel Griffiths
Project Role: Research Technician
Nearest person month worked: 1.2
Contribution to Project: Mr. Griffiths performs the animal surgery and injury and is responsible for the day-to-day supply orders to conduct studies.

Name: Conor Young
Project Role: Research Technician
Nearest person month worked: 8.7
Contribution to Project: Mr. Young is a new technician assisting Dr. Law and Mr. Griffiths, while training on all animal procedures.

Name: Raymond Migrino MD
Project Role: Joint PI
Nearest person month worked: 2.4
Contribution to Project: As joint and corresponding PI, direction, supervision and logistical administration of the project with multiple partner scientists and institutions.

Name: Jonathan Lifshitz PhD
Project Role: Joint PI
Nearest person month worked: 1.2
Contribution to project: Supervision and organization of initiation of first animal cohorts including personnel supervision and administrative/regulatory

functions. As joint PI, logistical administration of the project with multiple partners.

Name: C. Chad Quarles, PhD
Project Role: Co-investigator
Nearest person month worked: 0.48
Contribution to project: Worked on imaging protocol optimization and validation in preparation for the first cohort of animals to be transferred to Barrow.

Name: Diego Mastroeni, PhD
Project Role: Co-investigator
Nearest person month worked: 0.6
Contribution to project: Developed and optimized protocols for Immuno-laser capture Microdissection on vascular cells.

Name: Jennifer Nolz
Project Role: Research Technician
Nearest person month worked: 1.5
Contribution to project: Methodologic optimization of vascular laser capture microdissection procedure and tissue sectioning and processing.

Note that Dr. Patricio Reyes (Neurologist, Phoenix VA) has opted not to continue his participation in the research project due to overwhelming clinical workload in his new capacity as Chief of Neurology at the Phoenix VA.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

No changes for Migrino, Lifshitz, Reaven, Gonzales, Turner.
Changes for Quarles not included in first annual report:
Dignity Health and ASU Collaborative Strategic Initiatives: PI: Quarles, 9/24/18-9/23/19 (1.2 calendar months)

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or

domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

The following 4 organization are partners in the current project, each identified by key personnel and funds awarded to each institution.
Organization Name: Phoenix VA Healthcare System
Location of Organization: Phoenix, AZ
Partner's contribution to the project (identify one or more): Collaboration

Organization Name: University of Arizona College of Medicine - Phoenix
Location of Organization: Phoenix, AZ
Partner's contribution to the project (identify one or more): Collaboration

Organization Name: Barrow Neurological Institute
Location of Organization: Phoenix, AZ
Partner's contribution to the project (identify one or more): Collaboration

Organization Name: Arizona State University
Location of Organization: Tempe, AZ
Partner's contribution to the project (identify one or more): Collaboration

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

STATEMENT OF WORK AND % COMPLETION OF WORK

Site 1: **Phoenix VA Healthcare System [PVAHCS]**
650 Indian School Rd
Phoenix, AZ 85012
PI: Ray Migrino, MD

Site 2: **University of Arizona, College of Medicine – Phoenix [UA COM-P]**
425 N. 5th St.
Phoenix, AZ 85004
PI: Jonathan Lifshitz, PhD

Site 3: **Arizona State University [ASU]**
727 E. Tyler Street
Tempe, AZ 85287-5001
PI: Diego Mastroeni, PhD

Site 4: **Barrow Neurological Institute [BNI]**
350 W. Thomas Rd.
Phoenix, AZ 85013
Christopher Quarles, PhD

Research-Specific Tasks:	**Months**	**% Completion**
Major Task 1: Obtain institutional and DOD approval for live animal work	1-3	
Milestone(s) Achieved: Obtain IACUC approval and DOD ACURO approval	3	100%
Specific Aim 1: In rats exposed to midline fluid percussion injury (FPI), evaluate the extent and mechanisms of cerebrovascular dysfunction and inflammation and establish their relationship with cognitive function.		
Major Task 2: Compare 180-day in-vivo cerebral flow (CBF) and cerebrovascular reactivity using MRI, and ex-vivo endothelial and smooth muscle-dependent function of isolated circle of Willis cerebral arteries from TBI versus uninjured rats and determine the relationship of vascular function with measures of cognitive function (novel object recognition tasks) and degree of neuropathology. Aim 1:		
Subtask 1: Produce cohorts (n=12) of uninjured and diffuse brain-injured rats using midline fluid percussion with inclusion criteria of acute neurological reflex suppression and transient motor impairments.	3-9	100%
Subtask 2: Draw submandibular blood monthly and conduct behavioral battery of cognitive testing (novel object recognition) at 3 months and 6 months post-injury.	4-12	100%
Subtask 3: Conduct in-vivo cerebral flow (CBF) and cerebrovascular reactivity using MRI between brain-injured and uninjured rats.	9-15	100%
Subtask 4: Conduct ex-vivo endothelial and smooth muscle-dependent function of isolated circle of Willis cerebral arteries from TBI versus uninjured rats.	9-15	100%
Subtask 5: Perform neuropathological assessment of brain hemispheres, including laser capture microdissection and initial gene expression assays.	9-18	25%
Milestone(s) Achieved: Defined relationship between vascular function (in vivo and ex vivo) and cognitive function, supported by neuropathology; publication of 1 peer reviewed paper.	18	50%
Major Task 3: Identify potential mechanisms of TBI-induced cerebrovascular dysfunction by assessing vascular oxidative/nitrative stress and vascular inflammation following TBI and assess their relationship to development of cognitive dysfunction		

Subtask 1: Assess blood samples for systemic markers of oxidative stress (malondialdehyde; superoxide dismutase; glutathione peroxidase) and inflammatory markers (IL-1B; IL-6; IL-8; C-reactive protein) by ELISA	10-16	0%
Subtask 2: Evaluate oxidative and nitrative stress in isolated circle of Willis cerebral arteries using immunofluorescence microscopy for NO, superoxide and peroxynitrite (using hydroethidium, diaminofluorescein-2 and coumarin boronate fluorescence, respectively)	10-16	40%
Subtask 3: Quantify inflammation using gene expression of RAGE, IL-1B, IL-6, IL-8 and protein expression of phosphorylated NFκB and RAGE in Circle of Willis arteries	10-16	30%
Subtask 4: Measure smooth muscle contractile proteins (MHC and smoothelin) and endothelial cell proteins relevant to vasoreactivity (total and phosphorylated endothelial and inducible nitric oxide synthases, eNOS and iNOS) by gene and protein expression between TBI and sham groups.	10-16	20%
Milestone(s) Achieved: Defined relationship between potential mechanisms of TBI-induced cerebrovascular dysfunction the development of cognitive dysfunction; publication of 1 peer reviewed paper.	18	25%
Major Task 4: Evaluate whether preconditioning with lipopolysaccharide (LPS) (a well-established and validated method to reduce brain injury through vascular protection and enhanced NO bioavailability) will attenuate TBI-induced cerebrovascular dysfunction and inflammation and prevent TBI-mediated cognitive dysfunction		
Subtask 1: Produce cohorts of uninjured and diffuse brain-injured and LPS-preconditioned rats (0.5 mg/kg i.p.) with blood collection and cognitive function assessments	18-27	75%
Subtask 2: Conduct in vivo vascular function, ex vivo vasoreactivity, and neuropathology.	24-27	75%
Milestone(s) Achieved: Identification of role for inflammatory pre-conditioning in preserving vascular function after TBI	18	40%
Specific Aim 2: To determine whether TBI and diabetes-related metabolic derangements or β-amyloid confer synergistic deleterious effects on cerebrovascular function, inflammation and cognitive function.		
Major Task 5: Compare the responses of ex-vivo circle of Willis arteries from uninjured and brain-injured rats without and with acute exposure to CVRF (high glucose) and β-amyloid (Aβ42) in terms of endothelial and smooth-muscle function, oxidative and nitrative stress and pro-inflammatory signaling.		
Subtask 1: Test vascular function of ex vivo cerebral vessels from TBI and uninjured rats when exposed to 1 hour of high glucose	10-16	100%
Subtask 2: Test vascular function of ex vivo cerebral vessels from TBI and uninjured rats when exposed to Aβ (Aβ40 or Aβ42) at two doses.	10-16	100%
Subtask 3: Expose ex vivo vessels to high glucose or Aβ for 1 or 24 hours and measure oxidative and nitrative stress (SO, NO, ONOO, eNOS) and inflammation (IL-6, IL-8, IL1B, NFκB, RAGE) by gene and/or protein expression.	11-17	50%
Milestone(s) Achieved: Determine whether ex vivo cerebral vessels isolated from injured rats have worse endothelial function when exposed to high	20	60%

glucose or Aβ as compared to uninjured rats; publication of 1 peer reviewed paper		
Major Task 6: Compare cerebrovascular function, vascular inflammation and cognitive function in rats with Streptozotocin-induced type 2 diabetes which had antecedent TBI versus uninjured rats **Aim 2: Number of experimental groups: 2 (Group 4 TBI>DM, Group 5 Sham>DM)** **Number of rats per group: 24, (total of 48 with complete data)***		
Subtask 1: Produce cohorts (n=24 each) of uninjured and diffuse brain-injured rats using midline fluid percussion with inclusion criteria of acute neurological reflex suppression and transient motor impairments.	21-27	20%
Subtask 2: Inject Streptozotocin (65 mg/kg, i.p.) at 90 days post-injury to induce type 2 diabetes mellitus.	22-30	0%
Subtask 3: Draw submandibular blood monthly and conduct behavioral battery of cognitive testing (novel object recognition) at 3 months and 6 months post-injury.	22-30	0%
Subtask 4: Conduct in-vivo cerebral flow (CBF) and cerebrovascular reactivity using MRI between brain-injured and uninjured rats.	27-33	0%
Subtask 5: Conduct ex-vivo endothelial and smooth muscle-dependent function of isolated circle of Willis cerebral vessels from TBI versus uninjured rats.	27-33	0%
Subtask 6: Perform neuropathological assessment of brain hemispheres, including laser capture microdissection and initial gene expression assays.	27-33	0%
Milestone(s) Achieved: Determined whether diabetic rats with preceding TBI have worse cognitive function and cerebrovascular function when compared to diabetic rats without preceding TBI or injured (TBI) rats; publication of 1 peer reviewed paper.	36	0%

Probing the Mechanistic Role of Vascular Dysfunction and Vascular Inflammation in TBI-Mediated Cognitive Dysfunction

W81XWH-17-1-0473

PI: Raymond Migrino/Jonathan Lifshitz **Org:** Carl T. Hayden Medical Research Foundation **Award Amount:** $1,300,000

Study Aims

Aim 1: In rats exposed to midline fluid percussion injury (FPI), evaluate the extent and mechanisms of cerebrovascular dysfunction and inflammation and establish their relationship with cognitive function.

Aim 2: To determine whether TBI and diabetes-related metabolic derangements or β-amyloid confer synergistic deleterious effects on cerebrovascular function, inflammation and cognitive function.

Approach

1A. Compare 6-month cerebral flow, and ***ex-vivo*** function of cerebral arteries from TBI versus uninjured rats and determine the relationship between vascular function with cognitive function.

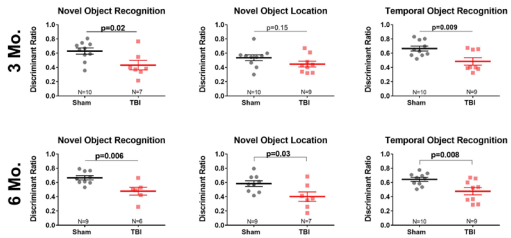
1B. Identify mechanisms of TBI-induced cerebrovascular dysfunction by assessing oxidative and inflammation following TBI.

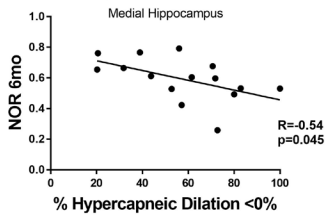
1C. Evaluate whether preconditioning with lipopolysaccharide will attenuate TBI-induced cerebrovascular dysfunction and inflammation and prevent TBI-mediated cognitive dysfunction.

2A. Compare the responses of cerebral arteries from uninjured and TBI rats without and with acute exposure to high glucose or β-amyloid.

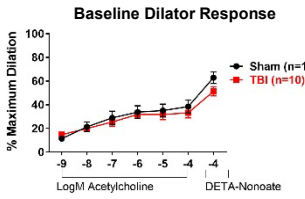
2B. Compare cerebrovascular function, vascular inflammation and cognitive function in rats with streptozotocin-induced type 2 diabetes which had antecedent TBI versus uninjured rats.

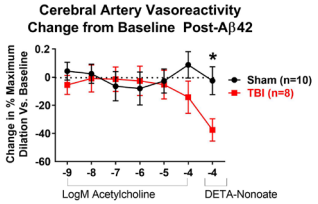
Cognitive Function Testing





There is significant reduction in cognitive function at 6 months in TBI vs. uninjured rats. There is an inverse relationship between NOR cognitive function and lack of cerebral artery dilator response to hypercapnea in medial hippocampus region.





Baseline cerebral artery endothelium and smooth muscle dilator response is not different between sham and TBI at 6 months. However, TBI vessels showed greater impairment in smooth muscle dilation response to Aβ42 vs. uninjured.

Timeline and Cost

Activities CY	17	18	19	20
Compare vascular function in TBI vs Sham				
Identify mechanisms of TBI vascular dysfunction				
Assess role of LPS in TBI pathophysiology				
Assess modulating role of metabolic risk factors in TBI and cognitive dysfunction				
Estimated Budget ($K)	**$50**	**$420**	**$420**	**$410**

Updated: 08/30/2019

Goals/Milestones

CY17 Goal – Project Initiation
- ■ Obtain institutional and DOD ACURO approval
- ■ Initiate first cohort of uninjured and TBI injured rats

CY18 Goals – Assess vascular function and cognition in TBI
- ■ Compare CBF and vascular function in TBI vs. sham
- ☐ Probe mechanisms of vascular dysfunction in TBI

CY19 Goal – Assess modulating roles of LPS and HG in TBI
- ☐ Probe effects of LPS and HG in TBI vascular and cognitive dysfunction

CY20 Goal – Establish mechanistic link between vascular and cognitive dysfunction in TBI
- ☐ Determine relationship and mechanisms of linkages

Comments/Challenges/Issues/Concerns
- LPS cohort completed and streptozotocin treatment anticipated end of next quarter

Budget Expenditure to Date; July 31, 2019

Projected Expenditure: $868,319.00

Actual Expenditure: $538,371.77