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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
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Fort Detrick, Maryland 21702-5012**

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| 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 first 2 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 are consistent with our hypothesis of delayed cognitive dysfunction following TBI, but we advise caution in interpretation due to incomplete data. | | | | | |
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1. INTRODUCTION:

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:

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.

1. Obtain institution and DOD approval for live animal work.
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.
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.
4. Evaluate whether preconditioning with lipopolysaccharide will attenuate TBI-induced cerebrovascular dysfunction and inflammation and prevent TBI-mediated cognitive dysfunction.
5. Compare the ex-vivo responses of cerebral arterioles between uninjured and TBI rats following exposure to high glucose and A β 42.
6. Compare cerebrovascular function, vascular inflammation and cognitive function in streptozotocin-treated rats (diabetes model) which had antecedent TBI versus no injury.

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: Cohorts were divided into groups of 3 TBI and 3 uninjured animals (6 animals per cohort). Cohorts 1 and 2 180-day MRI imaging, circle of Willis and brain isolation have been completed. Cohort 3 injury was performed February 2018 and tissue harvest scheduled for September 2018. Cohort 4 injury was performed March 2018 and tissue harvest scheduled for September 2018. Blood draws and cognitive testing are proceeding as planned.

Identified challenges: To achieve a final cohort size of 6 rats, up to 10 animals are prepared for injury and initial evaluation. This approach ensures full cohorts for a 6-month time point, where additional animals are reassigned to other projects, as is common in pre-clinical TBI research. To date, 1 animal has been euthanized prior to the 6-month endpoint due to health concerns, as can be expected in similar studies.

Preliminary Scientific Findings: 3 and 6-month cognitive function data are available for cohorts 1-2 and 3-month data are available for cohorts 1-4. In this segment as well as the following segments, ***it is important to note that data collection is incomplete*** so it is possible for final data analyses to be other than what the preliminary analyses show (e.g. phenomenon of regression to the mean with larger sample sizes). Extreme caution should accompany interpretation of these preliminary data as these are presented more to show project progress rather than ultimate scientific results.

Cognitive function was assessed using 3 standardized measures: novel object recognition (NOR), novel object location (NOL) and temporal recognition (TR), 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.

Figure 1 shows the 3 and 6-month data. 3-month data show reduced temporal recognition in TBI rats versus sham, but not NOR and NOL. 6-month data show reduced NOR, NOL and TR in TBI versus sham rats, consistent with our hypothesis that **diffuse brain injury results in sustained 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 of, enhancing the novelty of our findings as well as the value of this experimental animal model. To provide a graphical

representation of each animal's progression of neurologic status (for those with 3 and 6-month data), **Figure 2** is provided.

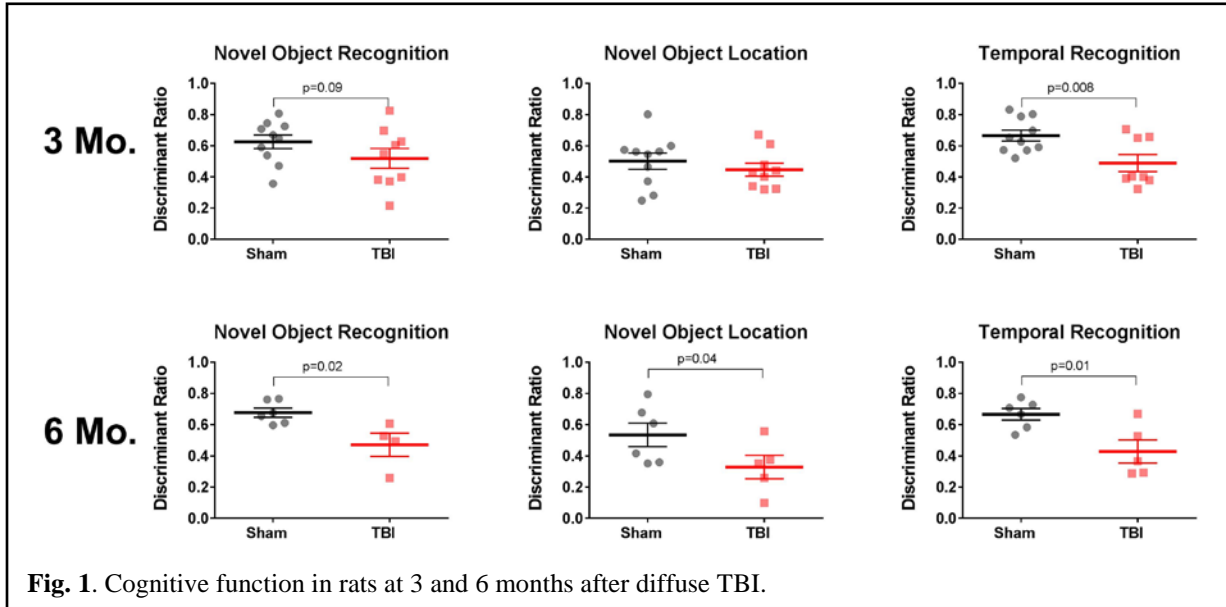


Fig. 1. Cognitive function in rats at 3 and 6 months after diffuse TBI.

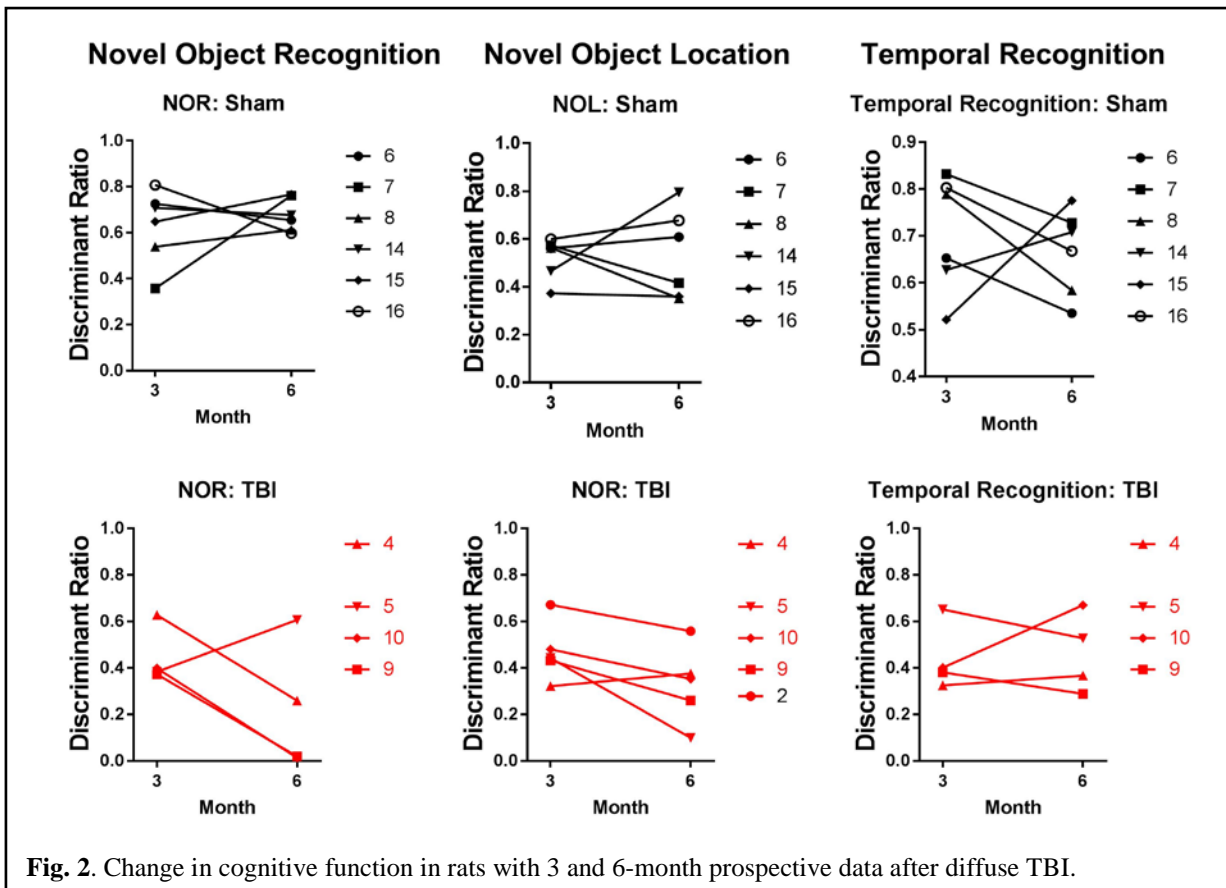


Fig. 2. Change in cognitive function in rats with 3 and 6-month prospective data after diffuse TBI.

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

Accomplishments: Imaging of cohorts 1 and 2 were accomplished (**Figure 3**) while cohorts 3 and 4 are scheduled for September 2018. The analysis for computing cerebral blood volume (CBV) and cerebral vascular reactivity (CVR) maps for cohorts 1-2 is complete and we are in the process of finishing the kinetic analysis to get cerebral blood flow (CBF). Once all maps are generated, they will be registered to a standard rat brain atlas with predefined regions of interest. This will prevent biases due to manual segmental of major brain regions. Group data analyses are therefore pending.

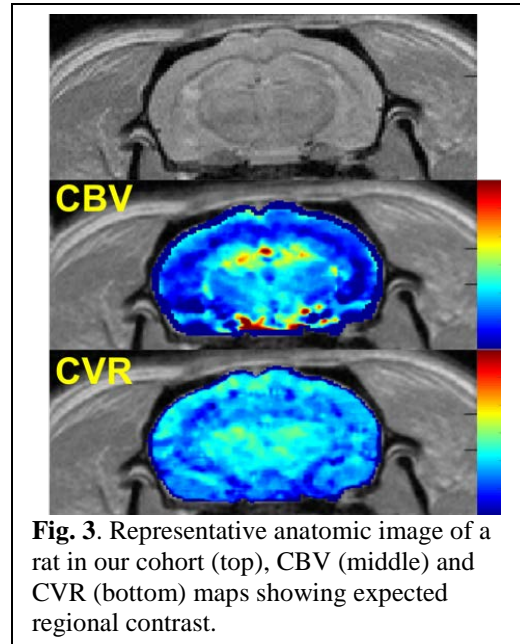


Fig. 3. Representative anatomic image of a rat in our cohort (top), CBV (middle) and CVR (bottom) maps showing expected regional contrast.

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

Accomplishments: Vasoreactivity data were obtained for cohorts 1-2 and cohorts 3-4 are scheduled for September 2018.

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). In preliminary analysis, myogenic tone was not different between sham and TBI groups (**Figure 4**). Following preconstruction with endothelin-1, baseline endothelial

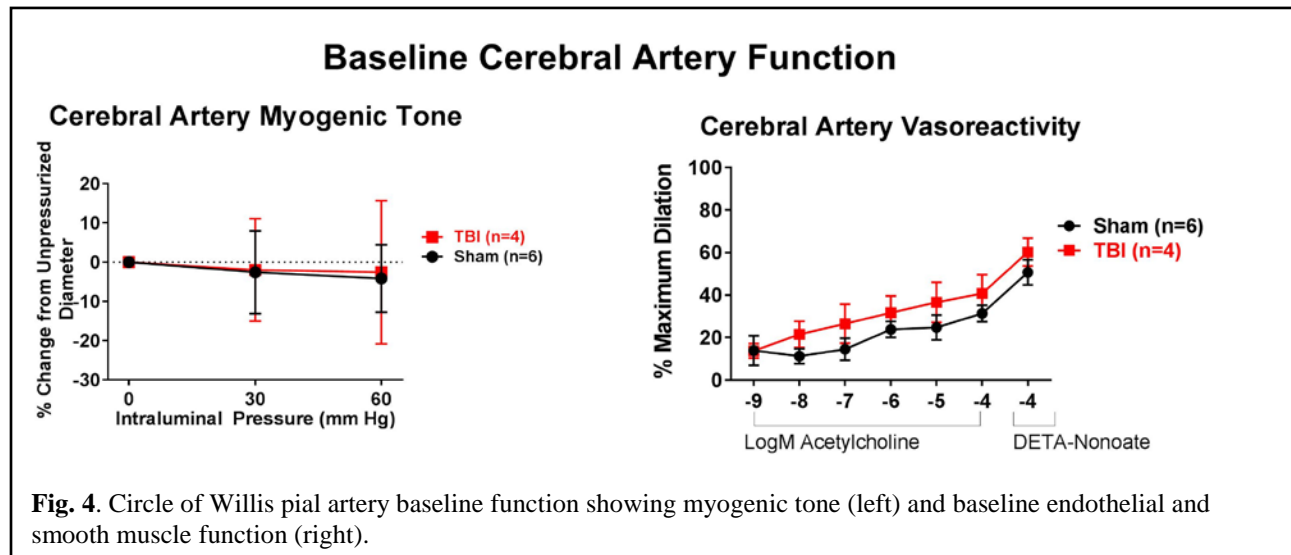
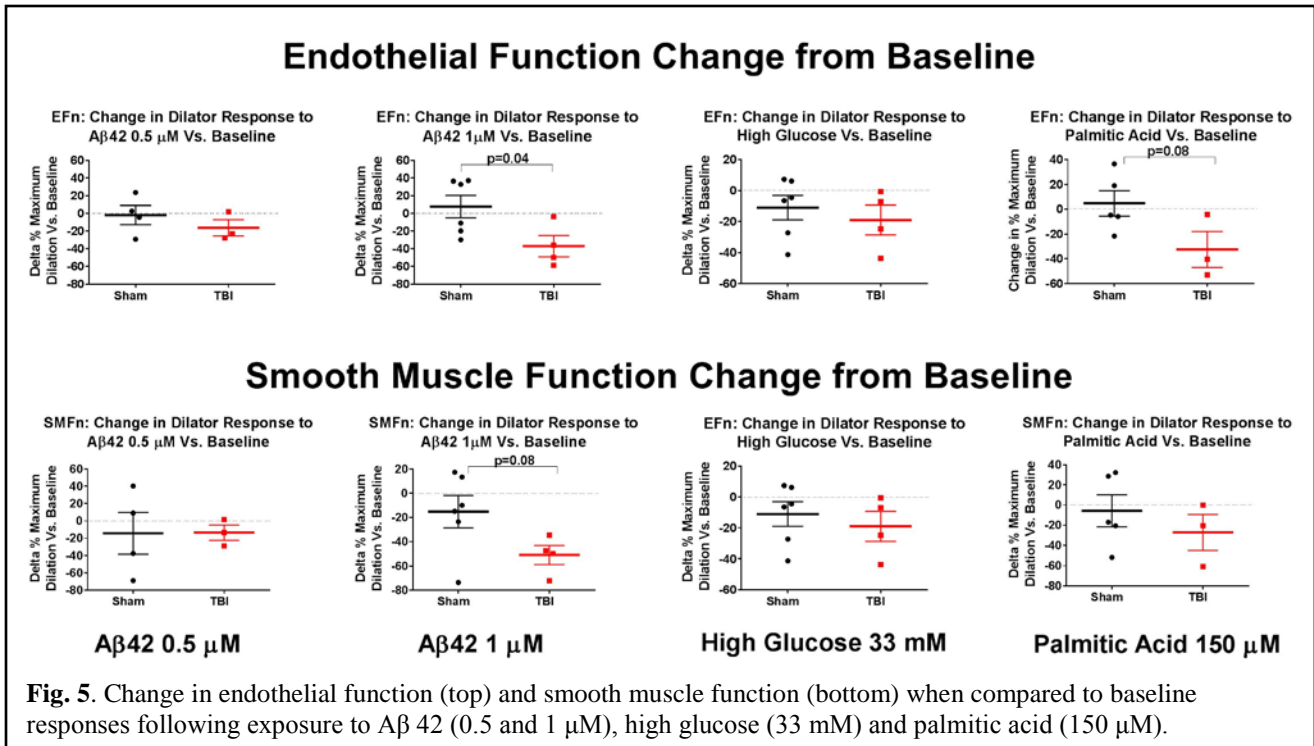


Fig. 4. Circle of Willis pial artery baseline function showing myogenic tone (left) and baseline endothelial and smooth muscle function (right).

function was assessed by exposure to progressive doses of acetylcholine followed by exposure to NO donor DETA-NONOate to assess smooth muscle function. Preliminary results show no significant difference in endothelial and smooth muscle function between sham and TBI rats (**Figure 4**). Following baseline assay, some arteries were exposed to the following vascular stressors: $A\beta$ (0.5 and 1 μ M), high glucose (33 mM) and palmitic acid (PA; saturated fatty acid most common in Western diet). Results are shown in **Figure 5**. There was significant difference in change in dilator response

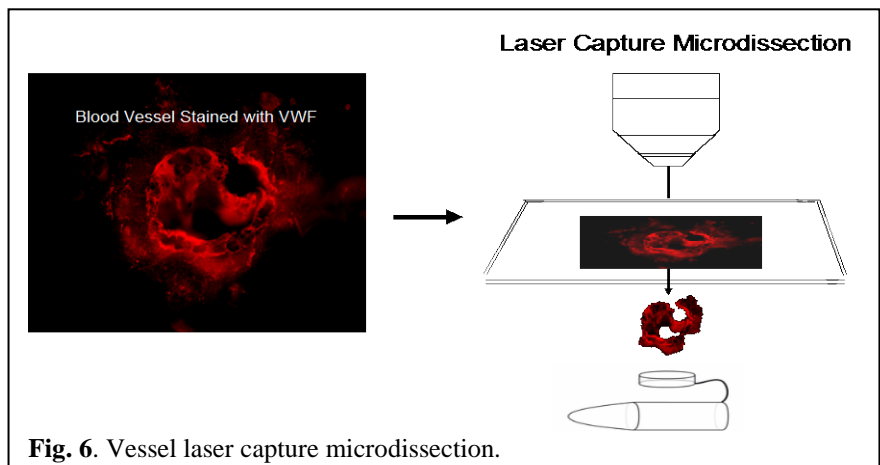
to acetylcholine when exposed to A β 42 1 μ M with greater impairment in TBI vs. sham rats. A trend towards the same results is showing with high glucose and PA, but not statistically significant.



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

Accomplishments: Brain isolations were completed for cohorts 1-2 and cohorts 3-4 are scheduled to be collected September 2018. The collaborative group has shared email communications as well met in person in quarterly meetings (last one was August 9, 2018) to plan the logistic and methodologic details for histopathology analyses. For each animal, one hemisphere was frozen (for laser capture microdissection and gene/protein assays) and the other hemisphere immersion-fixed in 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 for rat brain tissues (described below in **Figure 6**), and tissue batch processing is pending.

Laser Capture of VWF positive blood vessels. Immediately after detection of VWF-immunoreactivity,



sections were washed in 50mM Tris buffer and loaded onto a Leica AS-LMD laser capture microscope. After objective calibration, 300- blood vessels were captured (see image below) using 40X magnification, and dropped into an inverted microcentrifuge cap containing 50ul buffer RLT (RNeasy Micro Kit (Qiagen) and 1% β -Mercaptoethanol. Total RNA was extracted from laser captured microglia using RNeasy Micro Kit (Qiagen) per manufacturer's instructions. RNA was reverse transcribed using High Capacity cDNA Reverse Transcription Kit (Life Technologies/Applied Biosystems). The resulting cDNA was preamplified using TaqMan PreAmp Master Mix (Life Technologies/Applied Biosystems) then quantified by real time quantitative PCR in triplicate on an iCycler iQ (Bio-Rad) with TaqMan Gene Expression Assays, primers of VWF (Rn01492158_m1) and GUSB (Rn00566655_m1) (Life Technologies/Applied Biosystems). Both primer sets amplified at the expected CT-values 26 and 18 respectively. Detailed expression data are available if needed.

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 cohorts 1-2 and will be complete for cohorts 3-4 by September 2018. 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 cohorts 1-2 have been processed. Separate circle of Willis arterial segments were isolated, treated with vehicle, A β 42 or high glucose and exposed to hydroethidine (superoxide marker), dihydrorhodamine (peroxynitrite marker) and DAF-2 (nitric oxide marker) for immunofluorescence imaging. Representative images are shown in **Figure 7**. We plan to do batch reading of signal intensity using automated software so aggregate data is pending at this point.

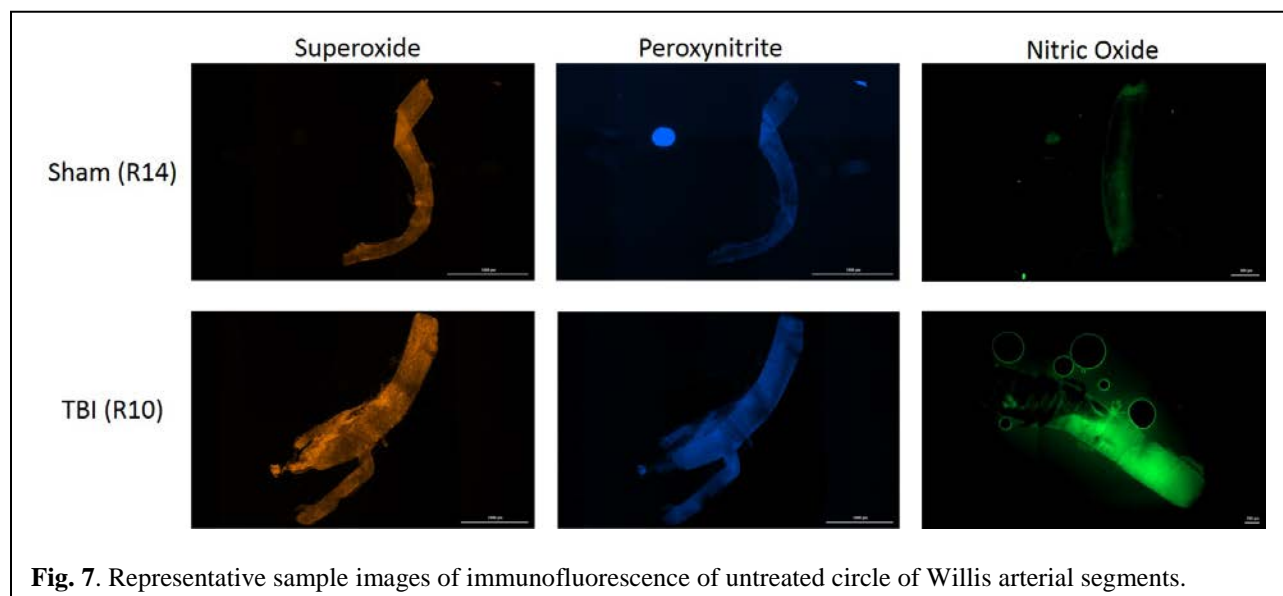


Fig. 7. Representative sample images of immunofluorescence of untreated circle of Willis arterial segments.

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

Accomplishments: Samples have been collected from cohorts 1-2, with cohorts 3-4 planned for September 2018. Quality control studies will be conducted in the next quarter to evaluate the samples and plan the analytical approach.

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. As such, 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 anticipate up to 4 additional cohorts (n=6 per cohort) to achieve the goals related to LPS pre-conditioning. These cohorts were introduced and discussed at our last in person quarterly meeting (August 9, 2018). The cohorts are planned for the upcoming quarter. Similar to cohorts 1-4, these animals will receive LPS injections 3 days prior to brain injury, with a rectal temperature monitor to track inflammation related hyperthermia. Results are anticipated in 9 months for LPS Cohorts 1-4.

5. Compare the ex-vivo responses of cerebral arterioles between uninjured and TBI rats following exposure to high glucose and A β 42.

Subtasks 1-2: Test vascular function of cerebral vessels of TBI vs. uninjured when exposed to high glucose or A β .

Subtask 3: Assess oxidative and nitrative stress and inflammation following exposure to HG or A β .

Accomplishments: The preliminary data and accomplishments were detailed above.

6. Compare cerebrovascular function, vascular inflammation and cognitive function in streptozotocin-treated rats (diabetes model) which had antecedent TBI versus no injury.

Subtasks 1-6: produce cohorts of uninjured and TBI rats, inject streptozotocin 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. After LPS Cohorts 1-4 are underway, we will initiate preliminary trials to induce diabetes in naïve rats. Diabetes Cohorts 1-4 are planned for the end of budget year 2.

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: Genetics, Autoantibodies, Psychotherapy, Patients in Clinical Trials, Aggression, Endocrine, Nicotine and Cannabis, Microbiome, Databases, Concussion Dynamics, and Placebo.

Lifshitz Lab: Conor Young is the newly hired 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 quarter, he will begin to process the 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, was added to Migrino lab. He is undergoing technical training and acquiring 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 is gaining skills in rat brain image co-registration, atlas-based region of interest analysis, contrast agent-based perfusion modeling and cerebral vascular reactivity analysis.

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. 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 our cohorts 3-4 (TBI versus uninjured) in September 2018 which will be followed by planned 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.

4. IMPACT: *Describe 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. Adjustments had to be made with small animal imaging as the rat size at 6 months turned out to be larger than anticipated but Dr. Turner was able to finetune the setup to accommodate this larger size as well as make adjustments for contrast agent dosing.
3. The team has consulted with experienced preclinical investigators at University of Arizona on optimization of management during treatment of streptozotocin and nuances of biochemical assays and monitoring regimes as we anticipate this aspect of the proposal will be the most technically challenging and to ensure optimum survival of this rat cohort.

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 has expertise in working with LPS in vertebrate animals. We do not anticipate any complications. 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 will 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 |
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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: 1.54
 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: 3.22
 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: 0.90
 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: 0.84
 Contribution to Project: Mr. Hatfield assisted with local regulatory requirements for the project.

Name: Gail Farrell
 Project Role: Research Coordinator-Backup
 Nearest person month worked: 0.11

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:
NIH R01 CA158079 (PI: Quarles), 07/01/2017-06/30/2022 (3 calendar months)
NIH R01 CA213158-01 (PI: Quarles), 12/1/2016 – 11/30/2019 (1.2 calendar months)
Flinn Foundation #204 (PI:Quarles), 01/01/17 – 12/31/2018 (0 calendar months)
NIH R01 CA221938 (PI: Quarles), 12/1/2017 – 11/30/2020 (1.2 calendar months)
NIH U01 CA220378 (Co-Inv), 04/01/2017 – 03/31/2022 (0.6 calendar months)
ADHS18-198850 (PI: Quarles), 04/01/2018 – 03/31/2021 (3.6 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: Carl T. Hayden 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: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES: *N/A*

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

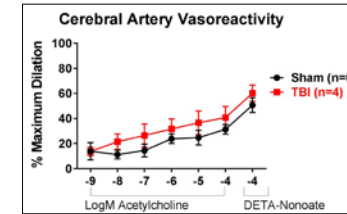
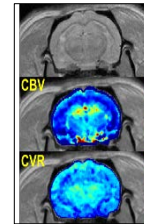
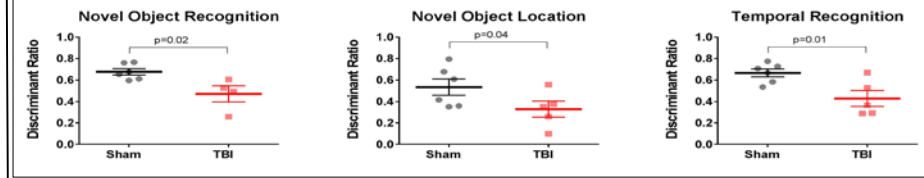
Aim 1: In rats exposed to midline fluid percussion injury (FPI), evaluate the extent and mechanisms of cerebrovascular dysfunction and inflammation and establish their relationship with cognitive function.

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



Experimental rat cohorts were started and 6-month cognitive function (top) and cerebrovascular function (in vivo, bottom left and ex vivo, bottom right) were obtained. Data are incomplete and very preliminary until all cohorts are completed.

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: 8/24/2018

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

- Maintain communication, consistency and rigor over time.

Budget Expenditure to Date; July 31, 2018

Projected Expenditure: \$435,600.00

Actual Expenditure: \$224,567.03