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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 all rat cohorts which underwent TBI or sham operation and measured in-vivo and ex-vivo cerebrovascular function data. Our data show impaired cognitive function at 3 and 6 months following TBI with some regional association between cognitive and in vivo cerebrovascular function. Altered pial arterial smooth muscle function post-angiotensin II was seen post-TBI. Induction of diabetes using streptozotocin did not lead to greater cognitive impairment in TBI rats.

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

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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. Data have been analyzed and compared between groups, including correlation analysis. Results have been written and published in J of Neurotrauma (PMID 35593008).
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 discrimination ratio represents the ratio of attention to the familiar versus novel object, where a value of 0.5 indicate chance performance. The 3 and 6-month data show impairment in NOR at 3 months that persist up to 6 months, with similar trend (not statistically significant) for NOL and TOR (Fig. 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 enhances the value of this experimental animal model in recapitulating what has been observed in human epidemiologic studies.

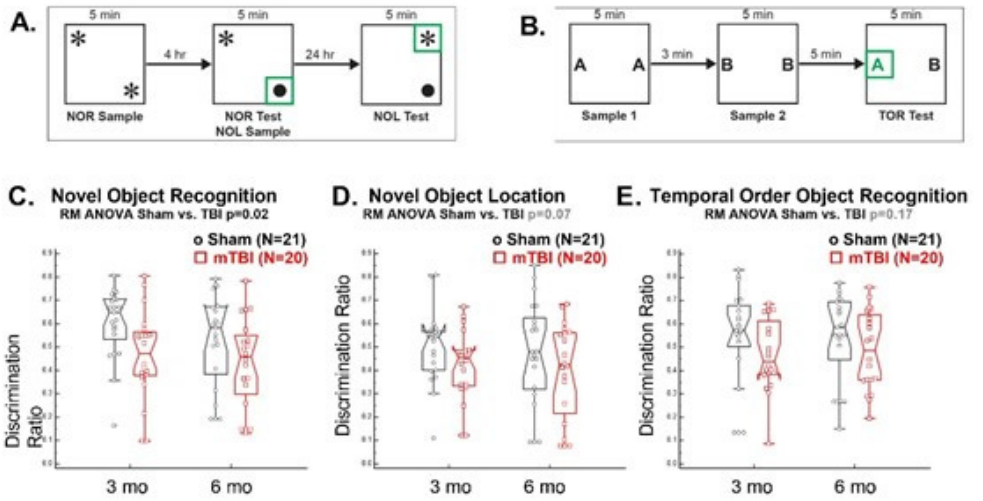


Fig. 1. Chronic cognitive impairment following mTBI. A. Schematic of object recognition tasks. Novel object recognition (NOR) tests short term memory by replacing an object (*) with (●) after a 4-hour delay. Novel object location (NOL) tests long term memory by shifting the position of the familiar object (*) after a 24-hour delay. B. Temporal order object recognition (TOR) tests working memory by presenting pairs of objects. C. There was impaired novel object recognition at 3 and 6 months in rats subjected to mTBI versus sham controls. D-E. There were similar trends but not statistically significant differences in novel object location and temporal order object recognition

When all rat cohorts are included (**N=89**) (sham, TBI, sham or TBI without or with LPS, sham or TBI without or with STZ), repeated measures ANOVA showed significantly worse results for TBI treatment for NOR (p=0.003), NOL (p=0.016) and TOR (p=0.026). Our results confirm that mTBI results in persistent chronic cognitive deficits.

We performed multivariable modeling putting as input variables (TBI-sham), (LPS pretreatment-no LPS) and (STZ treatment-no STZ) into the model to assess independent contributions

of these variables to cognitive function outcomes. We show that TBI is independently associated with impaired 6 month TOR (p=0.04) but not NOL (p=0.15) or NOR (p=0.44). Contrary to our hypotheses, LPS pretreatment is independently associated with impaired 6 month TOR (p=0.03), borderline for NOL (p=0.05) but not NOR (p=0.12). In multivariable modeling, treatment with STZ to induce diabetes was not independently associated with worse TOR (p=0.92), NOR (p=0.85) or NOL (p=0.57).

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

Accomplishments: We have completed in vivo imaging of all rat cohorts. Results in Figure 2 show no significant difference in global and regional cerebral blood flow between TBI and sham rats, with similar results for regional cerebral blood volume. However, there was significant correlation between cognitive function and regional CBF, where the 6 month NOR significantly correlated with CBF in lateral and medial hippocampus and S1BF. NOL correlated with medial hippocampus NOL (Figure 3). Our results are consistent with previous observations in human concussion patients that CBF is altered in the acute phase of mild TBI but normalizes within 30 days. Our study is not able to

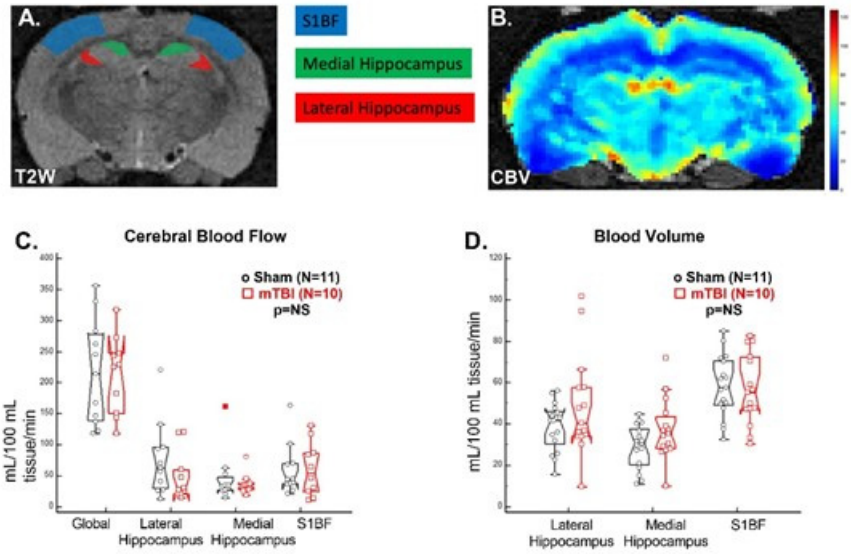


Figure 2. In vivo resting cerebral perfusion. A. Representative T2-weighted anatomic image. The colored areas represent regions of measurement. B. Parametric regional cerebral blood volume map. C. Global and regional resting cerebral blood flow did not differ between mTBI and sham rats at 6 months. D. Regional resting blood volume also did not differ between mTBI and sham rats.

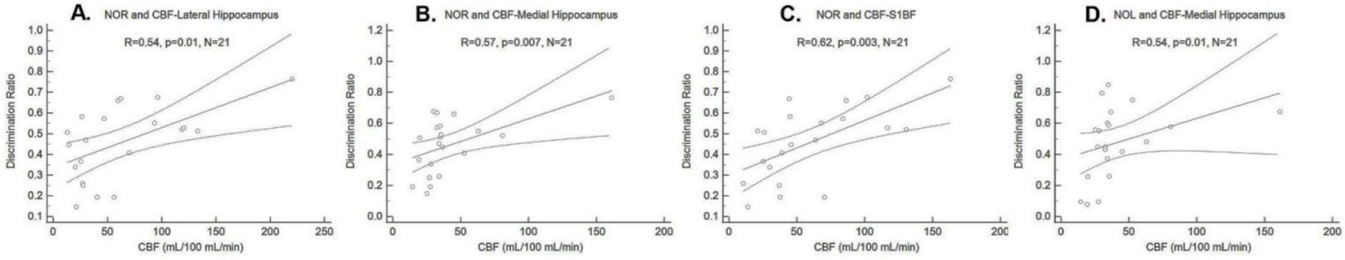


Fig. 3. There was significant correlation between 6 month NOR discrimination score and regional CBF in lateral hippocampus, medial hippocampus and S1BF; there was significant correlation between 6 month NOL and medial hippocampus CBF.

answer whether the direct association between cognitive function and regional CBF is causal in nature, which needs further investigation. Persistent abnormality in cognitive function but no

significant difference in CBF and CBV at 6 months suggest that the effects of vascular dysfunction are likely relevant in the early stage of the injury.

Of interest, the relationship between NOR to regional cerebral blood flow in hippocampal regions is greater in mTBI than sham rats (see table below).

A. Cognitive function and cerebral blood flow

	Sham		mTBI		All	
	R	p-value	R	p-value	R	p-value
NOR-CBF global	0.28	0.28	0.13	0.65	0.17	0.35
NOR-CBF medial hippocampus	0.60	0.05	0.61	0.06	**0.57**	**0.007**
NOR-CBF lateral hippocampus	0.52	0.10	**0.72**	**0.02**	**0.54**	**0.01**
NOR-CBF S1BF	0.51	0.11	**0.87**	**0.001**	**0.62**	**0.003**
NOL-CBF global	0.28	0.28	-0.12	0.66	0.11	0.55
NOL-CBF medial hippocampus	**0.65**	**0.03**	0.36	0.31	**0.54**	**0.01**
NOL-CBF lateral hippocampus	0.40	0.23	-0.33	0.35	0.23	0.32
NOL-CBF S1BF	0.33	0.32	0.13	0.73	0.23	0.31
TOR-CBF global	0.09	0.73	-0.19	0.50	0.01	0.94
TOR-CBF medial hippocampus	0.22	0.51	0.09	0.80	0.18	0.44
TOR-CBF lateral hippocampus	0.45	0.16	-0.07	0.85	0.24	0.30
TOR-CBF S1BF	-0.02	0.95	0.08	0.83	0.12	0.62

Phenotypic characterization of mild TBI animal model: We previously reported that there is currently no consensus classification schema for TBI severity. Our midline FPI model now adds structural and functional characterization of a TBI model that we believe would be consistent with current mild TBI classification by VA and DOD standards. Our model relates to non-catastrophic TBI with acute physiologic disruption that recovers within a few days with absence of gross histopathologic damage and lack of cavitation months post injury. In this cohort we found that brain injured animals had righting reflex recovery times of 522±34 (range 245-755 seconds) with sham animals recovering within 15 seconds, injured animals having apnea of 21±3.7 seconds). 6-month brain MRI T2* weighted images showed no evidence of gross bleeding and/or microhemorrhage, while structural images showed no evidence of ventriculomegaly of the third ventricle. Post-mortem brain exam showed lack of gross morphologic features of injury by H&E staining with no evidence of blood extravasation in subcortical white matter. These additional imaging and structural findings 6 months post injury support the mild TBI nature of our animal model and further validates this model for study of mild TBI, a less commonly studied form of TBI.

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

Accomplishments: Vasoreactivity data were obtained for all rat cohorts. Results show no significant difference in baseline resting pial arterial myogenic tone, endothelium-dependent dilation and smooth muscle-dependent dilation between mTBI and sham rats (Figure 4).

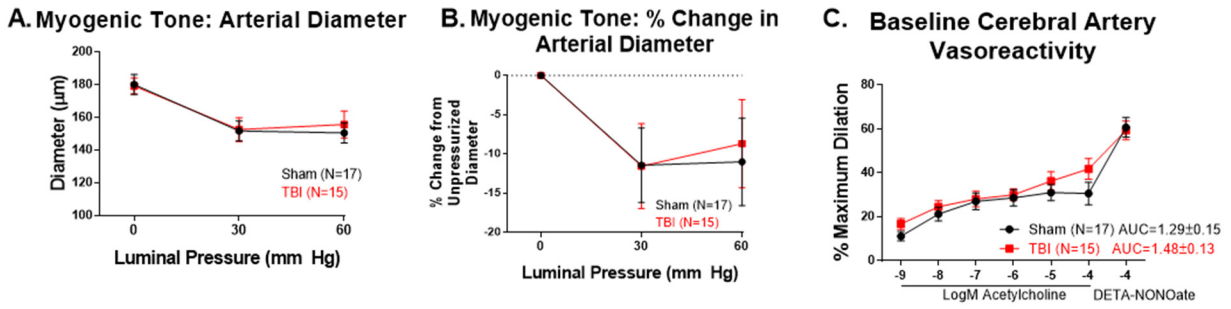


Fig. 4. **Myogenic tone and baseline vasoreactivity**. A-B. There was no significant difference in response to increasing intraluminal pressure between TBI and sham rat cerebral arteries. C. Dilator responses to increasing doses of acetylcholine and DETA-NONOate were also not significantly different between TBI and sham rats.

These results inform us that unstimulated, resting endothelial and smooth muscle cerebral arterial function is not impacted by mild TBI at 6 months but results following agonist stimulation with vasoactive stressors (see below) indicate that reliance only in resting, unstimulated arterial function may not be sufficient to uncover chronic vascular functional change.

We expanded our analyses to the whole cohort and performed multivariable modeling of baseline and post-stimulation vasoreactivity outcomes to determine independent associations with TBI, LPS pretreatment and STZ treatment. Similar to above findings, we find that TBI is not associated with worse *baseline* vasoreactivity outcomes (endothelial function, dilator response to max acetylcholine or AUC, or smooth muscle function, dilator response to DETA NONOate). However, TBI is associated with worse dilator responses (max Ach) following exposure to Aβ42 (p=0.03), angiotensin II (p=0.01) and high glucose (p=0.02). **This suggests that TBI leads to poor vascular resilience when exposed to vascular stressors.**

Table 1 Gene	Gene name	Gene Function	logFC	logCPM	LR	PValue	FDR
Gstt3	Glutathione S-transferase theta-3	Conjugation of reduced glutathione	16.974	6.78825427	19.392753	1.064E-05	0.1386714
Aldh3a2	aldehyde dehydrogenase 3 family member A2	fatty oxidation	16.988	6.80245614	19.066047	1.263E-05	0.1386714
Mag	myelin associated glycoprotein	myelination process	17.19	7.00448094	19.049525	1.274E-05	0.1386714
Slc30a3	solute carrier family 30 member 3	zinc accumulation in synaptic vesicles	18.473	8.2877259	18.213496	1.975E-05	0.1612478
Lrfn5	leucine rich repeat fibronectin III domain containing 5	presynaptic differentiation	17.496	7.31073704	17.668265	2.63E-05	0.1639674
Tns3	tensin 3	actin remodeling	16.486	6.30089171	16.932594	3.873E-05	0.1639674
Mtss1	MTSS I-BAR domain containing 1	interaction with the actin cytoskeleton	16.789	6.60412259	16.706678	4.363E-05	0.1639674
Inpp4a	inositol polyphosphate-4-phosphatase type I A	Protein Coding gene	16.635	6.44961188	16.640284	4.518E-05	0.1639674
Fdx1l	ferredoxin 2	heme A and Fe/S protein biosynthesis	17.237	7.05166895	16.154377	5.838E-05	0.1906922

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

Accomplishments: We collected *brain microvessels* using laser microdissection from TBI and sham rats (N=5) each and performed RNAseq analyses. Top 10 differentially expressed genes are shown in Table 1 above. Note that the false discovery rate values of ~0.1 is an acceptable threshold for initial screening of genes for future validation.

Laser captured (LCM) vessels from the Ca1 of the hippocampus were analyzed (DESeq2) in Sham and TBI rats at 6 mo. of age. Initial screening of the differentially expressed transcripts of TBI vs. Sham (Fig. 5A) revealed a list of genes often described as being associated with the process of neurodegeneration. One of the more significantly affected genes was plectin (Plec). Plec links the cytoskeleton to junctions found in the plasma membrane and plays an important role in maintaining the mechanical integrity and viscoelastic properties of tissues. Among other top hits were heat shock proteins, normally produced by cells in response to exposure to stressful conditions. Pathway analysis of the significantly upregulated genes (Fig. 5B) revealed: pathways of neurodegeneration-multiple-diseases, followed by cyclic AMP signaling and Alzheimer's disease. Pathway analysis of downregulated genes (Figure 5CB) also showed a significant association with Alzheimer's disease. These data indicate that TBI and neurodegeneration share overlapping biological changes, but what makes this very interesting is that most studies in Alzheimer's disease focus on neuronal health, and here we present data from vascular health, which may merit further investigation into Alzheimer's disease.

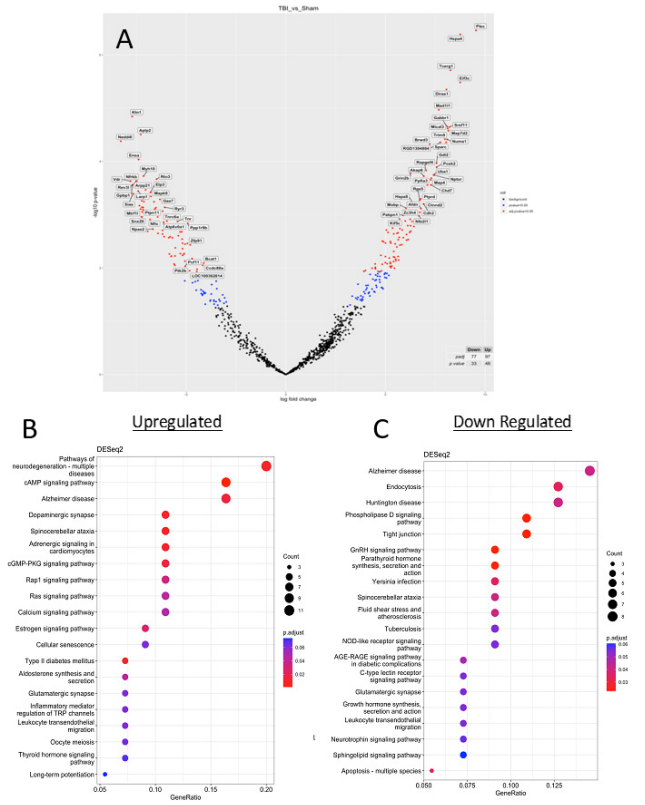


Fig. 5. A. Volcano plot of LCM CA1 vessels, sham versus mTBI. Significance is plotted versus fold-change on the y and x axes respectively. Pathway analyses of significantly upregulated genes (B) show top pathway being neurodegeneration-multiple-diseases, cAMP and Alzheimer's disease. Pathway analyses of downregulated genes (C) also showed significant association with Alzheimer's disease.

To evaluate neuroinflammation, we quantified activated astrocytes using GFAP staining and found no significant difference between sham and mTBI. There was also no significant difference between

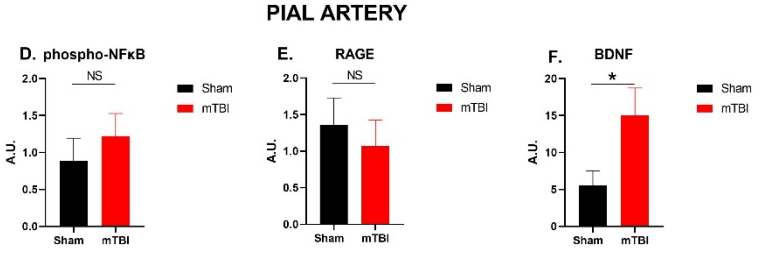


Figure 6. Tissue immunohistochemistry/immunofluorescence. Pial artery levels of phospho-NFkB and RAGE were not different. Surprisingly and unexpectedly, BDNF protein expression was higher in mTBI versus sham.

sham and mTBI in terms of markers of vascular inflammation as pial artery phosphorylated NFkB and RAGE were similar (Figure 6 D-E). Surprisingly, when we looked at BDNF expression, there was higher level in mTBI versus sham. The physiologic implication of this finding remains to be explored.

We performed immunohistochemistry assays of calpain-derived alpha II spectrin N-terminal fragment (SNTF), a proteolytic fragment of alpha-II spectrin, is a novel marker of TBI pathology and diffuse axonal injury in particular (Johnson VE, Acta Neuropathol 2016; 131:115-35, PMID 26589592). SNTF was detected in serum acutely following mild TBI in patients and was prognostic of poor clinical outcome. In this reference paper, SNTF immunoreactive axons were observed in human acute mild and severe TBI, additionally showing a subpopulation of degenerating axons undetected by accumulation of amyloid precursor protein (APP). After systematically optimizing the staining protocol for SNTF and verifying acceptable interobserver variability, we counted SNTF containing neurons per unit area in different cortical layers of the motor cortex. Results show that SNTF-containing neurons are significantly more abundant in TBI versus sham rats in Layer III (involved with communication among brain components) but not Layer V (external communication from brain) (Fig. 7) suggesting that axonal injury may be an important persistent mechanism of chronic cognitive dysfunction in TBI.

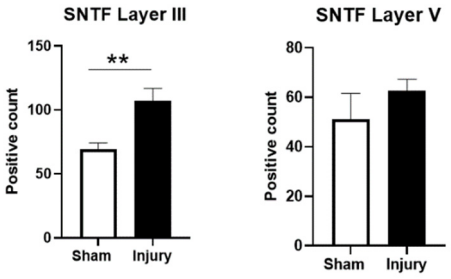


Figure 7. SNTF was higher in TBI rats versus sham rats in cortical layer III, but not in cortical layer V.

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: So far, we have not seen any difference in total antioxidant capacity between TBI and sham. We performed multi-cytokine assay of blood samples (N=10 each of TBI and sham). Our data show that at 6 months post-injury, there was significant elevation in IL-12 in TBI versus sham rats, with similar trend (although not statistically significant) with IL-5, IL-10 and TNF-a. This is an exciting finding as previous study implicated IL-12 abnormality in executive impairment post-acute TBI (https://www.liebertpub.com/doi/full/10.1089/neu.2016.4813) and our study suggests chronic impairment in this cytokine. IL-12 is produced by activated antigen-presenting cells (e.g. such as macrophages), promotes the development of Th1 responses and is an inducer of IFN-g production by T and NK cells. These findings can support new proposals to explore the hypotheses.

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

Accomplishments: Arteries from TBI and sham rats were processed and data collected. 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. Results for TBI versus sham showed no significant difference in baseline (vehicle-treated) cerebral artery superoxide, peroxynitrite and NO between TBI and uninjured rats. 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 vessels from TBI rats when exposed to Aβ42.

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

To sum, we found no significant difference in arterial smoothelin (mean±SD: 20.2±20% versus 21.0±20%, respectively, p=NS). We used semiquantitative means of measuring cerebral artery phospho-NFkB and RAGE protein expression using immunohistochemistry. So far, we found no significant difference in p-NFkB (1.60±1.1 vs. 1.10±1.2 AU TBI vs sham, p=0.3) or RAGE (0.96±1.1 vs. 1.5±1.3, p=0.3).

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 completed the LPS pretreated cohorts in terms of cognitive function, MRI imaging and vasoreactivity assessments. As detailed in prior reports, contrary to our hypothesis that LPS pretreatment would prime protective "hormesis"-type response in TBI, LPS pretreatment led to worse cognitive function. Potential translational significance is possible predisposition to worse long term cognitive dysfunction in soldiers or veterans with baseline systemic inflammatory state (e.g. obesity, vascular inflammation/atherosclerosis, periodontitis) who sustains a TBI. This effect should therefore be explored further in future proposals.

Additional multivariable modeling analyses of all rat cohorts show that LPS pretreatment is independently associated with worse baseline endothelial and smooth muscle function (max Ach p=0.02, Ach AUC p=0.02 and DETA NONOate p<0.001), but surprisingly no additional impairment following exposure to Aβ42, angiotensin II and high glucose.

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: As discussed above, we showed no significant difference in resting baseline cerebral arterial dilator response to acetylcholine (endothelium-dependent function) and to DETA-NONOate (smooth muscle-dependent function). Following arterial exposure to vascular agonists

known to impair vascular function (Aβ42, high glucose HG or angiotensin II), there was no difference in post-agonist dilator response between mTBI and sham rats following exposure to Aβ42 or HG (Figure 8 B1-3 and C1-3). However, there was significant difference between mTBI and sham following exposure to angiotensin II (Figure 8 A1-3), a key hormone in inducing hypertensive state. We believe this is a key finding in that even though there is recovery of cerebrovascular function 6 months following mTBI in the resting state, there may be persistent subtle vascular perturbation that could be unmasked in the presence of vascular stressor such as angiotensin II, suggesting normal resting but possible impaired vascular reserve.

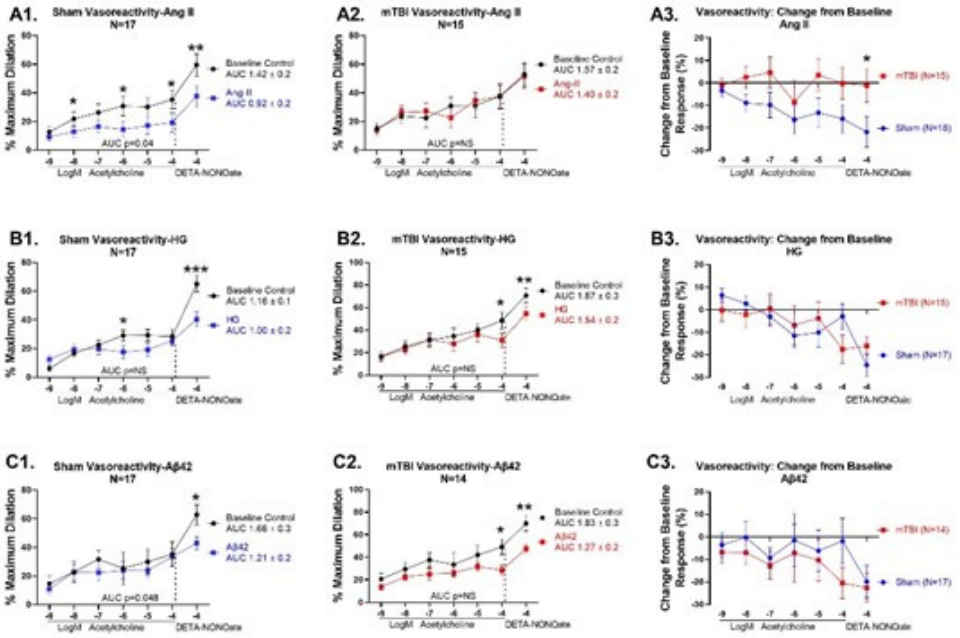


Fig. 8. Cerebral artery dilator responses at 6 months following exposure to vascular agonists. A. Sham rats showed reduced dilator response to acetylcholine and DETA-NONOate following exposure to angiotensin II (A1), which was not seen in mTBI rats (A2). There was significant reduction in dilation to DETA-NONOate in sham compared to mTBI (A3). B. Overall dilator response to acetylcholine (AUC) was not different following high glucose exposure in both sham and mTBI, while dilation to DETA-NONOate was reduced in both sham and mTBI (B1-B2). There was no difference in change in dilator response to high glucose between sham and mTBI (B3). C. There was marginal reduction in overall dilator response to acetylcholine (AUC) following Aβ42 in sham but not in mTBI, but dilation to DETA-NONOate was significantly reduced in both sham and mTBI (C1-2). There was no difference in change in dilator response to Aβ42 between sham and mTBI (C3).

As noted above, we performed multivariable modeling and found results consistent with Fig. 8 analyses.

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, measure cognitive function prior to 90 days, inject stretptozotocin at 90 days, measure cognitive function at 180 days, followed by in vivo and ex-vivo vascular function and neuropathological assessment.

Accomplishments: We have completed measuring cognitive function, in vivo and ex vivo cerebrovascular function in these cohorts. Our data on cognitive and vascular function were detailed in last annual and quarterly reports. Although there was a trend towards worse NOL and TOR scores in STZ treated rats, 2-way ANOVA showed no significant difference in NOR, NOL and TOR by STZ

treatment. We did not see any significant interaction between STZ treatment and TBI exposure, contrary to our hypothesis. For baseline cerebrovascular function, we found significant worsening of baseline endothelium-dependent ($P=0.002$) and smooth muscle-dependent function ($P<0.001$) in STZ-treated rats. The interaction term for TBI x STZ exposure was not significant.

2-way ANOVA analyses on arterial responses following exposure to Aβ, high glucose or angiotensin-II in this cohort. In this larger cohort of rats as compared to the data reflected in Figure 7, we again showed that TBI resulted in altered dilator response (endothelium and smooth muscle dependent) when compared to sham following exposure to angiotensin-II, but STZ treatment showed borderline ($p=0.06$) significant difference in endothelium-dependent dilation (based on area under the curve) following angiotensin-II exposure. In this response, there was significant interaction term between TBI and STZ ($p=0.04$) suggesting that later development of diabetes in the setting of TBI may alter vasoreactivity related to angiotensin-II signaling. 2-way ANOVA also revealed that dilator response to acetylcholine (endothelial function) following exposure to Aβ42 is altered in TBI versus sham, but not with STZ treatment, while smooth muscle function response post-Aβ42 was altered by STZ treatment but not by TBI. Further mechanistic investigations are needed to explore the physiological implications of these findings. Vasoreactivity following exposure to high glucose was not altered by TBI or STZ treatment, unlike angiotensin II or Aβ42.

Multivariable analyses indicate that STZ treatment is independently associated with worse baseline cerebrovascular endothelial and smooth muscle function (max Ach, Ach AUC and DETA NONOate all $p<0.001$) and additional impairment in smooth muscle response following Aβ42 exposure ($p=0.01$). This confirms that STZ induced diabetes leads to impaired cerebral vasoreactivity, both in terms of endothelial and vascular smooth muscle function. Contrary to our hypothesis, the impairments induced by TBI and STZ do not appear to be additive or synergistic.

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 used to host 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. During COVID-19 era, there was suspension of the journal club.

Lifshitz and Migrino Lab: Ms. Hannah Emerson is a laboratory technician recruit who took over the responsibilities of Mr. Conor Young. Ms. Emerson was trained on animal handling, animal care, animal injections, tissue collection, Western blotting as well as planning of experiments. She was accepted to U of Arizona Veterinary Med School and will be able to use her training for this career

path. Mr. Connor Leighty is a Masters student who took over Ms. Emerson's responsibilities as part-time lab personnel and learned optimization and processing of tissue histopathology.

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.

An abstract and poster of our results was presented in the 2020 15th Annual NABIS Conference on Brain Injury. This conference was the last available prior to the COVID-19 cancellation of most in-person conferences. A poster was presented at the National Neurotrauma Society annual symposium held online July 2021. Two abstracts were presented for presentation at the 2021 Arizona Alzheimer's consortium scientific meeting, the largest scientific meeting in Arizona dealing with neurodegenerative conditions. An abstract was presented to the 2023 NNS annual symposium. Our manuscript was published at the J Neurotrauma PMID 35593008.

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.

N/A.

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).

The major impact of our project is the validation and characterization of this model of TBI, phenotypically classifiable as "mild" or non-severe. This classification is based primarily on the lack of overt neuropathologic changes, with chronic cognitive and vascular changes. Thus, we empirically show that even "mild" TBI could have medium-term pathophysiologic consequences. Although this observation was suggested by retrospective studies of human injuries, our results were the first comprehensive empirical evidence of the chronic effects of mild TBI on cognitive and vascular function that we are aware. This would form an empiric basis for rigorous prospective evaluations of soldiers, Veterans or civilians exposed to mild or moderate TBI or concussions to re-assess risk of future cognitive and vascular abnormalities, specifically when vascular challenges are introduced.

Our results validate the importance of the midline fluid percussion model by recapitulating observations in human subjects, and importantly, by showing the coexistence and associational relationship of chronic cognitive function and vascular function changes following TBI. Contrary to our hypothesis, the diabetes model we selected (STZ treatment) did not have additive or synergistic effects on cognitive function or in vivo cerebral blood flow effects to TBI, although some interactions were noted between TBI and STZ treatment in altering endothelium-dependent dilator response post-angiotensin II. Future studies will need to determine if other models of diabetes (e.g. animal models more closely mimicking type 2 diabetes) will or will not show interaction with TBI. The mechanistic pathway signaling from our laser microdissected RNAseq analyses (pending full analyses) will hopefully provide additional known or new pathways to pursue to identify potential druggable targets. We have leveraged our findings in this DOD project to extend the time of observation to 1 year post TBI and to specifically probe the mechanisms underlying TBI mediated neurovascular dysfunction in our ongoing VA RRD Merit study. Together with our existing DOD dataset, we will be able to tease out the mechanistic bases for vascular dysfunction in TBI-mediated cognitive dysfunction.

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.

This collaborative effort from various disciplines (neuroscience, cardiovascular disease, imaging) allowed a comprehensive evaluation of the medium-term consequences of mild TBI on multiple modalities that have previously not been measured simultaneously in the same cohort. Thus we will have the ability to tease out associational relationships among cognitive, vascular, imaging, genomic, functional, gross structural and microscopic outcomes of TBI. This framework serves as a practical foundational approach to expand on the findings of this project.

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.*

Nothing to report.

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. When we analyzed our synaptophysin immunohistochemistry images using standard processing, PI Dr. Lifshitz noted wide variability in staining conditions among different batches of tissues, raising rigor concerns about comparability of measured tissue signals. While we also attempted to be rigorous and consistent with our brain bregma selection, there was also concern about variability in regions selected. Out of these concerns, we decided to go back to our paraffin embedded brain sections and redo some of the IHC staining, measurement and analyses informed from these prior experiences.
2. We had personnel turnovers including the departure of Mr. Seth Truran for a private sector job and Ms. Hannah Emerson for Veterinary Medicine school. Each of them had specific expertise and roles in the tissue processing and evaluation. Their roles were replaced by additional personnel but this required additional training for these tasks.

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.

The full cost of the RNAseq of microglia and vessels was not fully anticipated so we had to make a decision on the practical number of samples to assay.

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

Nothing to report.

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).*

  Griffiths D, Law L, Young C, Fuentes A, Truran S, Karamanova N, Bell LC, Turner G, Emerson H, Mastroeni D, Gonzales R, Reaven PD, Quarles CC, Migrino RQ, Lifshitz J. Chronic cognitive and cerebrovascular function following mild traumatic brain injury in rats. J Neurotrauma 2022 May 20. PMID 35593008

  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).*

Abstract presented "Experimental TBI induces long term cognitive deficits and vascular pathology" at the 2020 15th Annual NABIS Conference on Brain Injury. Presented poster presentation 2021 National Neurotrauma Society Symposium (online) Griffiths, DR, LM Law, N Karamanova, S Truran, LC Bell, GH Turner, C Quarles, RQ Migrino, J Lifshitz. (2021) "Experimental Brain Injury Induces Long Term Cognitive Deficits and Vascular Pathology." J Neurotrauma https://doi.org/10.1089/neu.2021.29111.abstracts

Poster presentation 2021 Arizona Alzheimer's Consortium scientific session: "Association between cerebrovascular function and chronic cognitive dysfunction following mild-moderate traumatic brain injury in rats and lack of modulating influence by diabetes."

Poster presentation 2021 Arizona Alzheimer's Consortium scientific session: "Chronic pial cerebral arterial function and cognitive function following mild-moderate traumatic brain injury in rats".

Poster presentation 2023 National Neurotrauma Society Symposium Leighty CR, Griffiths DR, Giordano KR, Karamanova N, Green TRF, Rowe RK, Migrino RQ and Lifshitz J. Alpha II spectrin N terminal fragment (SNTF) detects evidence of cytoskeletal pathology in chronic experimental traumatic brain injury.

Dr. Lifshitz lecture: Department of Medicine, Banner University Medical Center - Phoenix 5/13/2022: *Innovation in Translational Neurotrauma Research*

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.*

In light of DOD grant support which provided Dr. Migrino and Dr. Lifshitz protected research time and allowed continued employment of research personnel, the DOD grant indirectly supported the publication of the following work :

1. Migrino RQ, **Karamanova N**, **Truran S**, Serrano GE, Davies HA, Madine J, Beach TG. Cerebrovascular medin is associated with Alzheimer's disease and vascular dementia. Alzheimer's Dement. 2020; 12:e12072. .

2. **Karamanova N, Truran S**, Serrano GE, Beach TG, Madine J, Weissig V, Davies HA, Veldhuizen J,Nikkhah M, Hansen M, Zhang W, D'Souza K, Franco DA and **Migrino RQ**. Endothelial Immune Activation by Medin: Potential Role in Cerebrovascular Disease and Reversal by Monosialoganglioside-Containing Nanoliposomes. J Am Heart Assoc. 2020;9:e014810.

3. Younger S, Jang H, Davies HA, Niemiec MJ, Garcia JGN, Nussinov R, **Migrino RQ**, Madine J, Arce FT. Medin oligomer membrane pore formation: a potential mechanism of vascular dysfunction. Biophysics J. 2020; 118:2769-2782.

4. Ahmad S, Truran S, Karamanova N, Kindelin A, Lozoya M, Weissig V, Emerson H, Griffiths DR, Vail T, Lifshitz J, Ducruet AF*, **Migrino RQ***. Nanoliposomes reduce stroke injury following middle cerebral artery occlusion in mice. Stroke 2022; 53:e37-e41. Epub 2021 Nov. 8. PMID 347435535.*equal senior authorship

5. YV Doust, RK Rowe, PD Adelson, **J Lifshitz**, Ziebell, JM. (2021) Age-at-Injury Determines the Extent of Long-Term Neuropathology and Microgliosis after a Diffuse Brain Injury in Male Rats. *Frontiers in Neurology* 12:722526.

6. Beitchman, JA*, **J Lifshitz***, NG Harris, TC Thomas, AD Lafrenaye, A Hånell, CE Dixon, JT Povlishock, RK Rowe. (2021) Intracranial Mechanics of Fluid Percussion Brain Injury in the Rodent. *Neurotrauma Reports* 2(1):59-75

7. Tjandra D, **Migrino RQ**, Giordani B, Wiens J. Use of blood pressure measurements extracted from the electronic health record in predicting Alzheimer's disease: a retrospective cohort study at two medical centers. Alzheimer's & Dementia 2022; 18:2368-2372. PMID 35429343
8. *Madine J*, Davies HA, **Migrino RQ**, Ruotsalainen SE, Wagner J, Neher JJ. Medin amyloid may drive arterial aging and disease in the periphery and brain. Nat Aging 2023; online ahead of print. PMID 37620584
9. Zhang Y, Karamanova N, Morrow KT, Madine J, Truran S, Lozoya M, Weissig V, Li M, Nikkhah M, Park JG, **Migrino RQ**. Transcriptomic analyses reveal proinflammatory activation of human brain microvascular endothelial cells by aging-associated peptide medin and reversal by nanoliposomes. Sci Rep 2023; 13(1):18802. PMID 37914766.

- **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
No change.

Name: Nina Karamanova DVM
No change

Name: Hannah Emerson
No change

Name: Robert Schaefer
No change

Name: Connor Leighty
No change

Name: Gail Farrell
No change

Name: Peter Reaven
No change

Name: L Matthew Law, PhD
No change

Name: Daniel Griffiths
No change

Name: Chengcheng Hu
No change

Name: Raymond Migrino MD
No change

Name: Jonathan Lifshitz PhD
No change

Name: Rayna Gonzales
No change

Name: C. Chad Quarles, PhD
No change

Name: Gregory Turner PhD
No change

Name: Alberto Fuentes
No change

Name: Diego Mastroeni, PhD
No change

Name: Jennifer Nolz
No change

Name: Laura Bell PhD
No change

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.

Migrino was awarded NIH R21, NIH R56, ABRC grants

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
Ashley Stokes, 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: Number of experimental groups: 3 (Group 1 TBI, Group 2 Sham control, Group 3 LPS>TBI group)** **Number of rats per group: 12 (total 36 with complete data)***		
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	98%
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	96%
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	100%
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	100%
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	95%
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	100%
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	95%
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	100%
Subtask 2: Conduct in vivo vascular function, ex vivo vasoreactivity, and neuropathology.	24-27	100%
Milestone(s) Achieved: Identification of role for inflammatory pre-conditioning in preserving vascular function after TBI	18	100%
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	100%
Milestone(s) Achieved: Determine whether ex vivo cerebral vessels isolated from injured rats have worse endothelial function when exposed to high	20	100%

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	100%
Subtask 2: Inject Streptozotocin (65 mg/kg, i.p.) at 90 days post-injury to induce type 2 diabetes mellitus.	22-30	100%
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	100%
Subtask 4: Conduct in-vivo cerebral flow (CBF) and cerebrovascular reactivity using MRI between brain-injured and uninjured rats.	27-33	100%
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	100%
Subtask 6: Perform neuropathological assessment of brain hemispheres, including laser capture microdissection and initial gene expression assays.	27-33	95%
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	95%

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

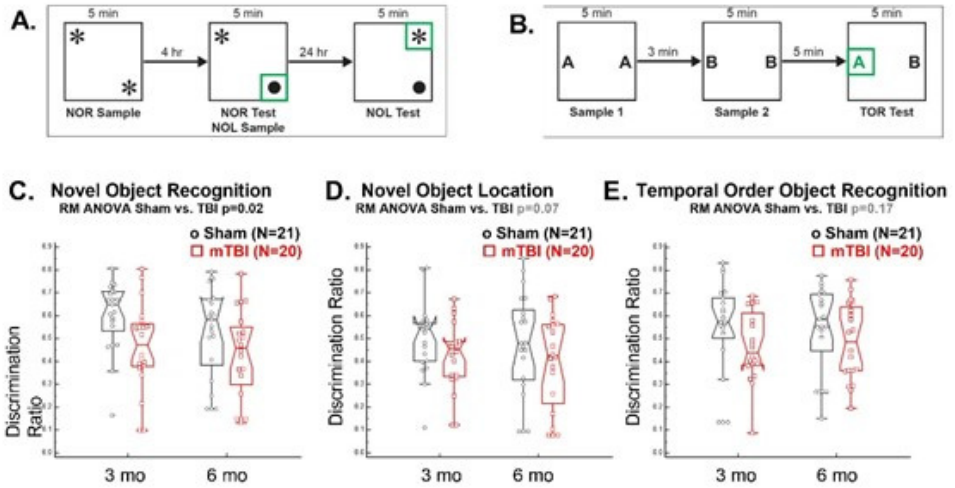
1A. Compare 6-month cerebral flow, and ***ex-vivo*** function of cerebral arteries from TBI versus uninjured rats and determine the relationship between vascular function with cognitive function.

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.



Chronic cognitive impairment following mTBI. A. Schematic of object recognition tasks. Novel object location (NOL) tests long term memory. B. Temporal order object recognition (TOR) tests working memory. C. There was impaired novel object recognition at 3 and 6 months in rats subjected to mTBI versus sham controls. D-E. There were similar trends but not statistically significant differences in novel object location and temporal order object recognition

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: 11/29/2023

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 streptozotozin in TBI vascular and cognitive dysfunction

CY20-23 Goal – Establish mechanistic link between vascular and cognitive dysfunction in TBI
- ✓ Determine relationship and mechanisms of linkages

Comments/Challenges/Issues/Concerns
- Final report

Budget Expenditure to Date: November 29, 2023

Projected Expenditure: $1,300,000

Actual Expenditure: $1,300,000.00

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ORIGINAL ARTICLE PATHOPHYSIOLOGICAL MECHANISMS

Chronic Cognitive and Cerebrovascular Function after Mild Traumatic Brain Injury in Rats

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Abstract

Severe traumatic brain injury (TBI) results in cognitive dysfunction in part due to vascular perturbations. In contrast, the long-term vasculo-cognitive pathophysiology of mild TBI (mTBI) remains unknown. We evaluated mTBI effects on chronic cognitive and cerebrovascular function and assessed their interrelationships. Sprague-Dawley rats received midline fluid percussion injury ($n = 20$) or sham ($n = 21$). Cognitive function was assessed (3- and 6-month novel object recognition [NOR], novel object location [NOL], and temporal order object recognition [TOR]). Six-month cerebral blood flow (CBF) and cerebral blood volume (CBV) using contrast magnetic resonance imaging (MRI) and *ex vivo* circle of Willis artery endothelial and smooth muscle-dependent function were measured. mTBI rats showed significantly impaired NOR, with similar trends (non-significant) in NOL/TOR. Regional CBF and CBV were similar in sham and mTBI. NOR correlated with CBF in lateral hippocampus, medial hippocampus, and primary somatosensory barrel cortex, whereas it inversely correlated with arterial smooth muscle-dependent dilation. Six-month baseline endothelial and smooth muscle-dependent arterial function were similar among mTBI and sham, but post-angiotensin 2 stimulation, mTBI showed no change in smooth muscle-dependent dilation from baseline response, unlike the reduction in sham. mTBI led to chronic cognitive dysfunction and altered angiotensin 2-stimulated smooth muscle-dependent vasoreactivity. The findings of persistent pathophysiological consequences of mTBI in this animal model add to the broader understanding of chronic pathophysiological sequelae in human mild TBI.

Keywords: cerebrovascular function; dementia; endothelial function; magnetic resonance imaging; traumatic brain injury; vascular smooth muscle

Introduction

IT IS ESTIMATED that 61 million individuals worldwide experience traumatic brain injury (TBI) from all causes every year.[1,2] Nearly 20% of the more than 2.6 million U.S. service members deployed to Operation Enduring Freedom and Operation Iraqi Freedom have sustained at least one TBI event.[3] TBI may result in lifelong disability and survivors can face enduring motor, cognitive, and social impairments.[4] Secondary brain injury following TBI is caused by a combination of neuronal and

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vascular damage, proteolytic pathways, free radical damage, apoptosis, and inflammatory processes.[5] Cerebrovascular dysfunction plays an important role in severe, acute TBI with ischemic brain damage evident at autopsy in >90% of acute TBI mortalities.[6] The lifetime consequences of TBI include long-term cognitive dysfunction, which may be associated with chronic traumatic encephalopathy.[7,8]

Unlike repetitive and severe TBI, the long-term cognitive and vascular function in mild TBI (mTBI) remains poorly characterized. mTBI is the most common form of TBI among civilians and military service members, occurring in ~82% of military TBI.[9] Epidemiological data indicate that individuals with a TBI history have a higher risk of developing dementia.[10–12] Among World War II U.S. Navy and Marine veterans, non-penetrating head injury in early adulthood was associated with increased risk of Alzheimer's disease (AD) and other dementias.[13] Of interest, even mild head injury was found to be a predisposing factor for AD or dementia.[10,14] A longitudinal study of human mTBI (post-concussion) showed impaired cerebral blood flow (CBF) in the acute (1 day and 1 week) setting that closely correlated with neuropsychiatric symptoms, and global CBF recovered by 1 month.[15] The relationship between vascular and cognitive function was further supported by the finding that persistent impaired CBF at 1 month in the dorsal midinsular cortex was associated with slow recovery of neuropsychiatric symptoms. In separate studies, experimental severe TBI led to cognitive dysfunction[16–19] and impaired CBF[20] at 1-year post-injury; similar pre-clinical studies on chronic mTBI are not available. Thus, vascular dysfunction is likely related to long-term cognitive deficits, and therefore coexists in chronic mTBI, but empiric evidence of this relationship is still lacking.

Experimental models of mTBI provide a unique opportunity to investigate chronic effects as well as relationships between cognitive and cerebrovascular function. Midline fluid percussion injury (FPI) is a well-validated model of mTBI.[21] Mechanical forces of the fluid pulse reflect off the temporal ridge of the skull to primarily affect hippocampal area CA3, primary somatosensory barrel cortex (S1BF), and ventral posterior nuclei of the thalamus. The model relates to non-catastrophic TBI with acute physiological disruption that recovers within a few days with absence of gross histopathological damage and lack of cavitation months post-injury.[22,23]

The aims of this study were to determine the chronic effects of mTBI on cognitive and cerebrovascular function and to evaluate the relationship between the two.

Methods

Animals

The study was approved and supervised by the University of Arizona Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (~9–10 weeks old, ~300–325 g, Charles River Labs) were given 1 week to acclimate in their home cages. Rats were given standard chow and water *ad libitum* and were housed in a reverse light-cycle room. Experiments were conducted in accordance with University of Arizona, Department of Defense Instruction 3216.01, RIGOR and Animal Research: Reporting *In Vivo* Experiments (ARRIVE) guidelines concerning the care and use of laboratory animals. Adequate measures were taken to minimize pain or discomfort.

Midline fluid percussion injury

All rodent experiments were conducted in cohorts of uninjured (sham) and mTBI animals, randomly assigned to groups at the time of brain injury. The individual inducing the brain injury was different from the one assessing cognitive function, *in vivo* imaging data, or *ex vivo* vascular function. After data collection, the group assignment for each animal was decoded. Rats were subjected to midline FPI similar to methods described previously.[21,22,24] Briefly, rats were anesthetized with isoflurane for surgery, but they did not receive systemic analgesia before injury. Body temperature was maintained with a Deltaphase® isothermal heating pad (Braintree Scientific, Inc., Braintree, MA, USA). In a head-holder assembly (Kopf Instrument, Tujunga, CA, USA), a midline scalp incision exposed the skull. A 4.8-mm circular craniotomy was performed (centered on the sagittal suture midway between bregma and lambda) without disrupting the underlying tissue. An injury cap was fabricated from a Luer-Lock needle hub, which was cut, beveled, and scored to fit within the craniotomy. A skull screw was secured in a 1-mm hand-drilled hole into the right frontal bone. The injury hub was affixed over the craniotomy using cyanoacrylate gel and methyl-methacrylate (Hygenic Corp., Akron, OH, USA) was applied around the injury hub and screw. The incision was sutured at the anterior and posterior edges and topical bacitracin and lidocaine ointment were applied. Animals were returned to a warmed holding cage and monitored until ambulatory (approximately 60–90 min).

For injury induction, animals were re-anesthetized with isoflurane. The dura was inspected through the injury-hub assembly, which was then filled with normal saline and attached to the fluid percussion device (Custom Design and Fabrication, Virginia Commonwealth University, Richmond, VA, USA). Animals were randomly assigned to receive brain injury ($n=20$; mean: 2.29 ± 0.02 atm, range: 2.20–2.44 atm) or sham injury ($n=21$) by releasing (or not releasing) the pendulum onto the fluid-filled cylinder. Animals were monitored for the presence of a forearm fencing response and the return of the righting reflex as indicators of injury severity.[24] After injury, the injury-hub assembly was removed *en bloc*, integrity of the dura was observed, bleeding was

controlled with saline and gauze, and the incision was stapled. Brain-injured animals had righting reflex recovery times of 522 ± 34 sec (range 245–755 sec) and sham-injured animals recovered within 15 sec. No sham rat experienced apnea, whereas 16 of 20 injured rats experienced apnea (21 ± 3.7 sec, range 0–60 sec). After recovery of the righting reflex, animals were placed in a warmed holding cage before being returned to the housing room. Each rat was evaluated for post-operative health for 3 days. Appropriate interventions, including but not limited to injections of subcutaneous bolus of saline, and feeding with a wet mash of food and water, were performed if clinically indicated. Rats were euthanized if their body weights fell below 10% of their own pre-operative body weight; none met these criteria.

Cognitive function evaluation

The assessments were performed at 3 and 6 months post-injury by investigators blinded to treatment allocation. Object recognition tasks took place in a square arena (68.58 cm × 68.58 cm) (Fig. 1A,B). White noise (~46 dB in the arena) was used to mask environmental noises. The novel object recognition (NOR) task tested short-term recognition memory.[25] Rats were acclimated to the arena for 3 min. Rats were then presented with two objects (O1, O2) in opposite corners of the arena (5 min) in the sample trial. After a 4-h delay, rats were returned to the arena with one object (O2) having been replaced by a novel object (O3). Normal rats explored the novel object (O3) more than the familiar object.[25] The novel object location (NOL) task tested long-term spatial memory.[26] The test trial of NOR served as the sample trial. After 24 h, O3 was placed in the same place as the previous day and O1 was moved to an adjacent corner in the arena. Normal rats explored the item in the novel location (O1) more than the unmoved object. The temporal order object recognition (TOR) task tested temporal working memory by the ability to recognize the order of objects presented over time.[26] Two sample trials established the cognitive framework, followed by a test trial. In sample trials, rats explored two copies of an object for 5 min, followed by exploration of a separate pair of identical objects followed by the test trial where one of each item was present. Breaks between sample phases were 3 min before the two sample phases and 5 min before the test trial (5 min). Normal rats explored the initial object, rather than the more recent object.

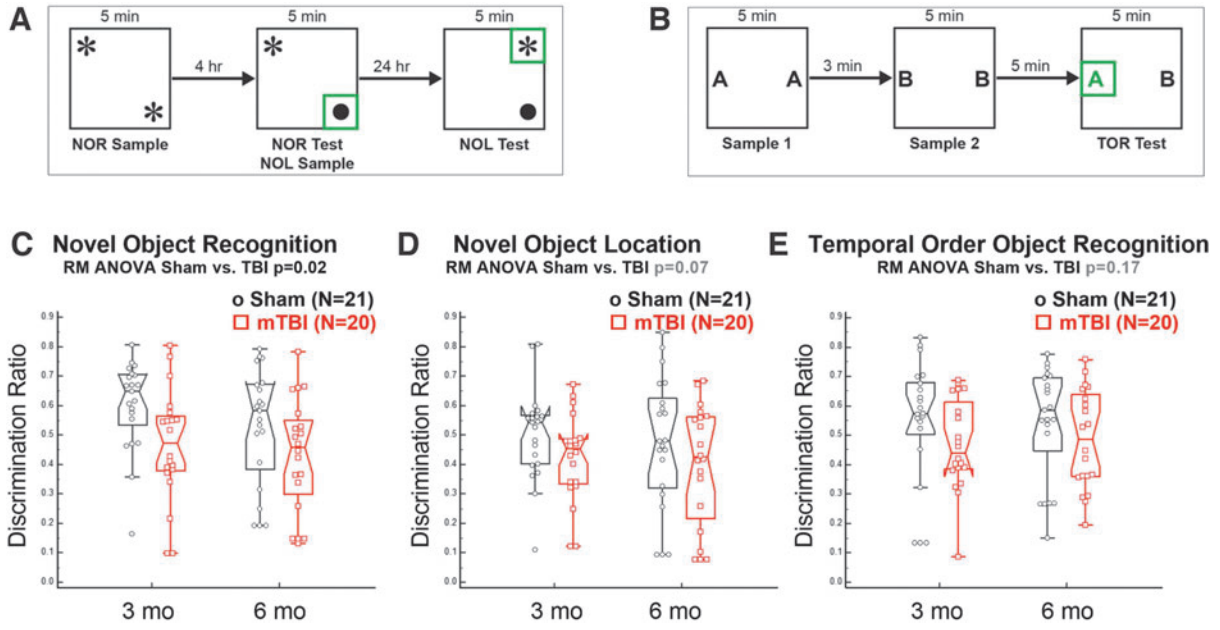


FIG. 1. Chronic cognitive impairment following mTBI. **(A)** Schematic of object recognition tasks. NOR tests short-term memory by replacing an object (*) with (●) after a 4-h delay. NOL tests long-term memory by shifting the position of the familiar object (*) after a 24-h delay. **(B)** TOR tests working memory by presenting pairs of objects. **(C)** There was impaired novel object recognition at 3 and 6 months in rats subjected to mTBI versus sham controls. **(D,E)** There were similar trends but not statistically significant differences in NOL and TOR between mTBI and sham rats. ANOVA, analysis of variance; mTBI, mild traumatic brain injury; NOL, novel object location; NOR, novel object recognition; TOR, temporal order object recognition. Color image is available online.

Exploration of an object was defined as the nose being within ~2 cm of the object. Differences in time spent exploring each object were recorded for all tasks and used to create a discrimination ratio (exploration time of the target object/exploration time of both objects). Normal rats explored the target object more than the original object, in this case resulting in discrimination ratio above 0.5.[26] A discrimination ratio of 0.5 indicated equal exploration of object, and equivalent to chance performance. Every trial was tracked and recorded using Ethovision software (Noldus, Leesburg, VA, USA).

Rats were required to explore each item more than 5 sec during the sample trial(s). If a rat did not meet exploration criteria, a discrimination ratio was input (Supplementary Table S1). The imputed minimal discrimination ratio was calculated using the average lowest exploration time for the target object and the highest average exploration time for the specific rat's experimental condition.

Magnetic resonance imaging

Rats underwent brain magnetic resonance imaging (MRI) 6 months post-injury. Scanning was performed using a 7-T small animal, 30-cm horizontal-bore magnet and BioSpec Avance III spectrometer (Bruker, Billerica, MA, USA) with a 116-mm high-power gradient set (600 mT/m) and either a 70-mm rat volume quadrature coil or a 40-mm rat head volume quadrature coil depending on size and weight of the animal. Each animal was anesthetized and maintained under isoflurane anesthesia (1–2%) in medical air. A tail-vein catheter was placed for injection of ferumoxytol (Feraheme®) at a dosing scheme of 0.3 mg/kg for first-pass injection at 2.2 mL/min and 0.7 mg/kg for slow infusion at 1.1 mL/min using a Genie Touch (Kent Scientific: Torrington, CT, USA) power injector. Respiration was continually monitored via a pillow sensor positioned under the abdomen (SA Instruments, Stoney Brook, NY, USA). Normal body temperature (36–37°C) was maintained with a circulating warm-water blanket (Thermo Scientific, Rockford, IL, USA).

Anatomical T2-weighted MR images were acquired with rapid acquisition with relaxation enhancement (RARE) sequence (repetition time [TR]: 5,500, echo time [TE]: 12.5, flip angle [FA]: 90 degrees, averages: 4, field of view [FOV]: 30 · 30 mm, matrix size [MTX]: 150 · 150, slices: 50, slick thickness: 0.5 mm) for registration and localizing hippocampus. Relaxometry measurements were acquired with pre/post-contrast T1 maps with a RARE at variable TR (RARE-VTR) sequence (TE: 8.5; TR: 275, 500, 800, 1,100, 2,000, 4,000; FA: 90 degrees; RARE factor: 4; averages: 2; FOV: 30 × 30 mm; MTX: 150 × 150; slices: 6; slice thickness: 1 mm) and T2* maps with multi-gradient-echo (MGE) sequence (TR: 1.000; FA: 45 degrees; averages: 6; TE: 4, 8, 12, 16, 20, 24; FOV: 30 × 30 mm; MTX: 150 × 150; slices: 6; slice thickness: 1). First-pass imaging utilized an echo planar imaging (EPI) sequence (TE: 7.5, FA: 45 degrees, TR: 1,000, averages: 1, FOV: 30 × 30 mm, MTX: 64 × 64, slices: 6, slice thickness: 1 mm, repetitions: 240) allowing for 1 min of baseline image acquisition before injection and 3 min of post-injection image acquisition.

Magnetic resonance imaging analysis

The perfusion data sets were analyzed by investigators blind to treatment allocation using in-house code developed in MATLAB and the MATLAB Imaging Processing Toolbox for registration functions (Mathworks, Natick, MA, USA). Pre-processing steps included rigid registration and arterial input function (AIF) determination. The first time-point of the dynamic susceptibility contrast (DSC) data set was registered to the anatomical T2 images in order to apply the tissue regions of interest (ROIs). The remaining DSC time-points were registered across time to account for any potential movement during the scan. After registration, $\Delta R2^*$ time curves were computed using the conventional single-echo equation.[27] The AIF was automatically determined using a previously published algorithm.[28] Mean tissue curves were found in each of the predetermined ROIs within the brain as mentioned above. Finally, CBF was determined as the maximum value of the residue function—the end product of the deconvolution.[29] To compensate for any potential delay between the time curves, the AIF was discretized using a block-circulant method prior to the deconvolution and signal-to-noise (SNR)-based thresholding was used truncate the inverse matrix to help regularize this discretized AIF.[30] CBF and CBV were calculated from coronal slices. Regional measurements were made for S1BF and the medial and lateral hippocampus (bisected medial to the dentate gyrus). The length and width of the third ventricle (~3 mm posterior from bregma) were measured and compared to assess for ventriculomegaly. Evidence of gross bleeding and/or microhemorrhage was evaluated by gross visualization from T2*-weighted images.

Ex vivo cerebrovascular function assessment

Rats were euthanized at 6 months post-injury via sodium pentobarbital overdose and circle of Willis arteries were carefully dissected from the brain and placed immediately in HEPES buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). The methods were adapted from previous work.[31,32] Middle or posterior cerebral arteries were isolated and cannulated, and vessel luminal diameters were measured using videomicroscopes throughout the procedure by investigators blind as to treatment allocation. The vessels were pressurized to 30 mm Hg for 30 min and then 60 mm Hg for 30 min. Myogenic tone was measured based on dilator response to intraluminal pressure. For each rat, several arterial

segments (3–4) were cannulated and underwent vasoreactivity measurements at baseline and following 1-h exposure to 1 of 3 vascular agonists. Following stabilization, each vessel was pre-constricted with increasing doses of endothelin-1 (10^{-9} to 10^{-4} M, Sigma Aldrich, St. Louis MO, USA) until ~60% of last observed maximum diameter was achieved. Baseline dilator responses to acetylcholine (increasing doses from 10^{-9} to 10^{-4} M) were measured to assess endothelium-dependent vasodilation, followed by 10^{-4} M of nitric oxide donor diethylenetriamine NONOate (DETA NONOate, Cayman Chemicals, Ann Arbor, MI, USA) to evaluate smooth muscle-dependent dilation.

To assess arterial response to vascular agonists/stressors, baseline (control) response was followed by washout, and each artery was assigned to exposure for 1 h to one of the following treatments: angiotensin 2 (20 μM, Sigma Aldrich), β-amyloid 1-42 (Aβ42, 1 μM, Anaspec, Fremont CA, USA),[31,33] or high glucose (33 mM)[34] and a second measurement of dilator responses to acetylcholine and DETA NONOate was performed. For the arterial segments assigned to angiotensin 2, baseline intraluminal pressure prior to vasoreactivity experiments was increased 0 to 30, 60, and then 90 mm Hg. Post-treatment dilator responses were compared with baseline control response in both sham and mTBI rats and change in dilator responses (treatment minus baseline) was compared between sham and mTBI rats.

Statistical analysis

Sample size considerations: Both cerebrovascular and cognitive function outcomes represent study primary outcomes. Our separate preliminary pilot study ($n=3$) on circle of Willis arterial function 5 weeks following surgery showed a difference in dilator response to 10^{-4} M acetylcholine between mTBI and sham of -13.8% with a combined standard deviation (SD) of 8.9%. A sample size of at least $n=15$ per group would allow us to show a similar difference at 6 months post-surgery but with a more conservative SD of 13% ($\alpha=0.05$, $\beta=0.80$).

Cognitive function measures (NOR, NOL, TOR) were analyzed using repeated measures analysis of variance (ANOVA; two-factor study with repeated measures on one factor) with time (3 month and 6 month) as within-subject factor and treatment (mTBI, sham) as grouping/between-subjects factor. mTBI and sham MRI and *ex vivo* vasoreactivity data were compared using unpaired *t*-test for normally distributed data or Mann-Whitney U test for data that were not normally distributed. Shapiro-Wilk test was used to determine normality of distribution. Comparison of dilator responses before and following exposure to vascular agonists was done using paired *t*-test. Correlation analyses were performed using Pearson's method for normally distributed data or Spearman's method for non-normally distributed data. Significant *p*-value was set at $p<0.05$ (two-sided). Analyses were performed using MedCalc version 19.8 (MedCalc Software, Ostend, Belgium). Data in Figures 1 and 2 are represented as individual data points and as notched boxplot format (showing median, interquartile range [IQR], notch representing 95% confidence interval [CI] of the median, upper whisker as lesser of 75th percentile or maximum value, and lower whisker as greater of 25th percentile or minimum value). Pre-clinical data can be made available after official request to the corresponding author.

Results

mTBI leads to persistent cognitive impairment

Repeated measures ANOVA showed significant difference by treatment group factor (mTBI vs. sham) in NOR discrimination ratio ($p=0.02$), but not by time factor (3 vs. 6 months; $p=0.09$) (Fig. 1C). The NOR results indicate persistent impairment in cognitive function relating to short-term recognition memory[25] following mTBI. A similar trend by treatment group factor was seen in NOL and TOR, although the differences did not reach statistical significance (Fig. 1D,E).

Effects of mTBI on chronic resting CBF and CBV

A representative T2-weighted MRI brain structural image (Fig. 2A) and parametric mapping of CBV (Fig.2B) are shown, including delineation of brain regions analyzed. There was no significant difference in resting global or regional CBF between mTBI and sham rats at 6 months (Fig. 2C). There was also no difference in regional CBV between mTBI and sham (Fig. 2D). There was no gross bleeding and/or microhemorrhage noted among sham and mTBI rats and there were no significant differences in length (sham: 0.28 ± 0.004; mTBI: 0.27 ± 0.01 mm, $p=$NS) or width (sham: $0.03\pm0.0.002$; mTBI: 0.03 ± 0.004 mm, p=NS) of the third ventricle to suggest ventriculomegaly. Post-mortem brain examination showed lack of gross morphological features of injury and histopathology (hematoxylin and eosin staining) confirmed no evidence of blood extravasation in subcortical white matter (data not shown; serial sections for microhemorrhage were not performed).

Association between CBF and cognitive function

There were significant correlations between 6-month NOR and the following vascular parameters: lateral hippocampus CBF, medial hippocampus CBF, and S1BF CBF (Fig.3A–C and Supplementary Table S2A). There was a significant correlation between 6-month NOL and medial hippocampus CBF (Fig. 3D and Supplementary Table S2A). When stratified by treatment, the correlation between NOR and CBF was significant and greater in mTBI rats versus sham rats in the lateral hippocampus ($R=0.72$, $p=0.02$) and S1BF ($R=0.87$, $p=0.001$),

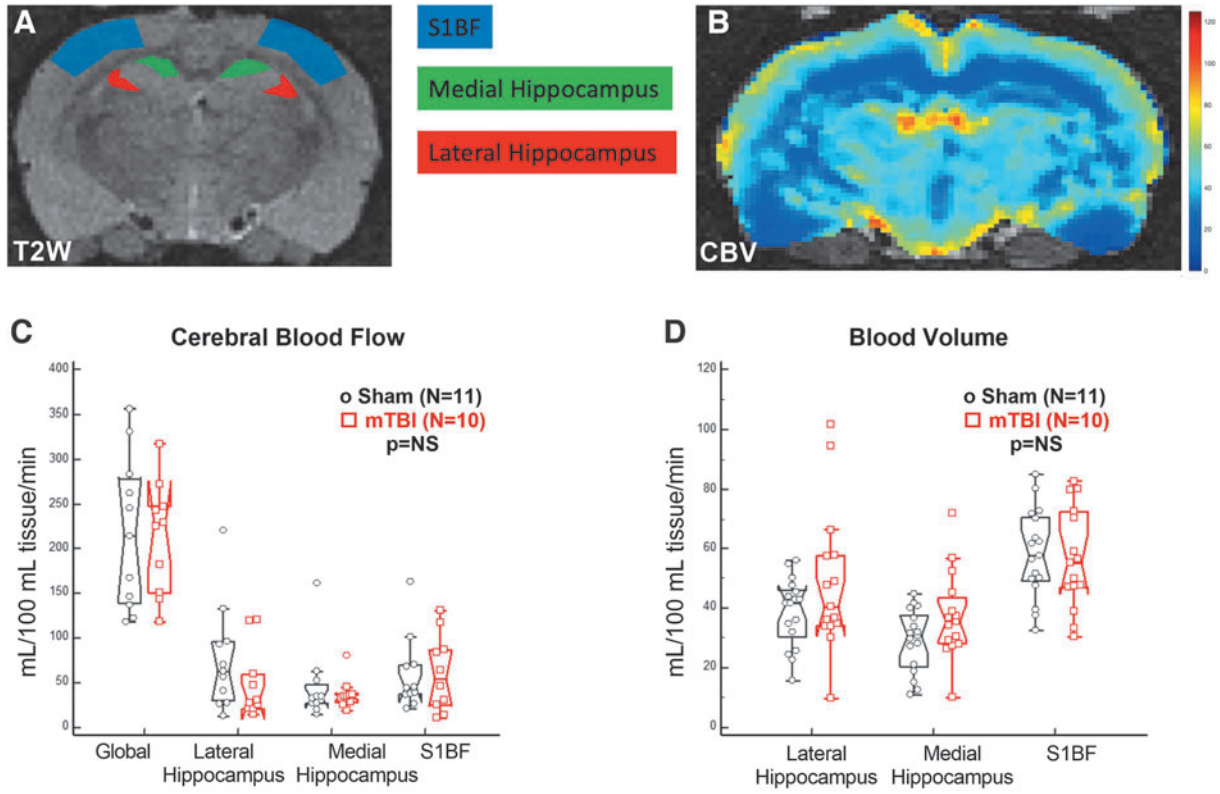


FIG. 2. *In vivo* resting cerebral perfusion. **(A)** Representative T2-weighted anatomical image. The colored areas represent regions of measurement. **(B)** Parametric regional cerebral blood volume map. **(C)** Global and regional resting cerebral blood flow did not differ between mTBI and sham rats at 6 months. **(D)** Regional resting blood volume also did not differ between mTBI and sham rats. CBV, cerebral blood volume; mTBI, mild traumatic brain injury; S1BF, primary somatosensory barrel cortex. Color image is available online.

whereas the association was of similar degree in the medial hippocampus (Supplementary Table S2A). Overall, there were no significant correlations between regional CBV and cognitive function measures (Supplementary Table S2B). The results demonstrate the association between cognitive function and resting regional cerebrovascular perfusion 6 months following mTBI or sham procedure.

Effects of mTBI on circle of Willis cerebral arterial function

Constriction responses to increasing intraluminal pressure in *ex vivo* cerebral arteries did not differ between mTBI and sham rats (Fig. 4A,B), signifying lack of difference in myogenic tone. There were also no differences in baseline (unstimulated) dilator response between mTBI and sham rats when arteries were exposed to increasing doses of acetylcholine, signifying lack of difference in baseline endothelium-dependent function, or when arteries were exposed to DETA NONOate, signifying lack of difference in baseline smooth muscle-dependent function (Fig. 4C).

Dilator responses to acetylcholine and DETA NONOate were compared at baseline (control) and following exposure to vascular agonists in sham and mTBI cerebral arteries. There was significant reduction in dilator response to acetylcholine and DETA NONOate following exposure to angiotensin 2 in sham rats (Fig. 5A1) but not in mTBI rats (Fig. 5A2). The change in dilator response to DETA NONOate following angiotensin2 exposure was significantly different between sham and mTBI with reduction in sham but not in mTBI, suggesting absence of smooth muscle-constriction response following angiotensin 2 exposure in mTBI versus sham. Based on area under the curve (representing combined response to increasing acetylcholine doses), exposure to high glucose showed no difference with baseline response in both sham and mTBI (Fig. 5B1,2), but high glucose resulted in significant decrease in dilator response to DETA NONOate in both sham and mTBI, with no significant difference in response between sham and mTBI (Fig. 5B3). Dilator response to acetylcholine was marginally reduced in sham following Aβ42 but not in mTBI (Fig. 5C1,2) but dilation to DETA NONOate

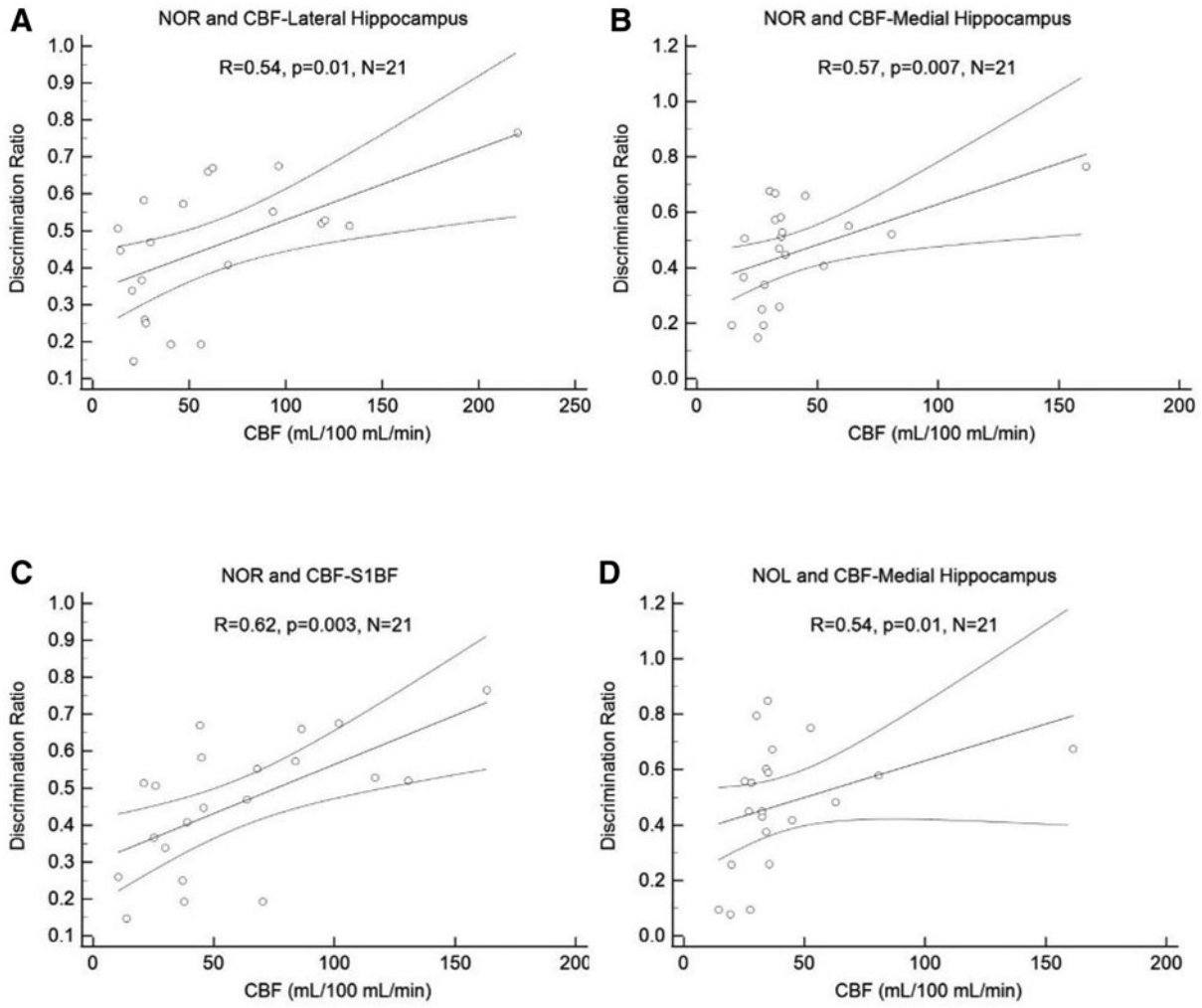


FIG. 3. Relationship between cognitive and vascular function. Six-month NOR was directly associated with CBF in the lateral hippocampus **(A)**, medial hippocampus **(B)**, and S1BF **(C)**. Six-month NOL was directly associated with CBF in medial hippocampus. Data are from sham and mTBI rats. CBF, cerebral blood flow; mTBI, mild traumatic brain injury; NOL, novel object location; NOR, novel object recognition; S1BF, primary somatosensory barrel cortex.

was reduced in both sham and mTBI. The Aβ42-induced change in dilator response was not different between sham and mTBI (Fig. 5C3). Overall, these results suggest reduced vasoconstrictor response post-angiotensin 2 exposure in mTBI versus sham cerebral arteries, but no difference between sham and mTBI with high glucose or Aβ42.

Association of ex vivo *cerebral arterial vasoreactivity with cognitive function and* in vivo *regional blood volume*

Cerebral arterial dilator response to DETA NONOate was inversely related to 6-month NOR (Fig. 6A, Supplementary Table S3). These data show the association between cerebral arterial smooth muscle function and cognitive function. There was no significant correlation between change in post- and pre-angiotensin 2 smooth muscle-dependent dilation response and each of the following 6-month outcomes: NOR ($p=0.5$), NOL ($p=0.96$), and TOR ($p=0.8$). These data suggest lack of association between cognitive function and arterial function following angiotensin 2 exposure. Arterial dilator responses to acetylcholine and DETA NONOate were inversely related to CBV in lateral hippocampus (Fig. 6B–D, Supplementary Table S3).

Acute righting reflex recovery and cognitive, imaging, and vasoreactivity outcomes

In brain-injured rats, correlation analyses showed no significant correlation between righting reflex recovery time post-injury with the following outcome measures: 3- and

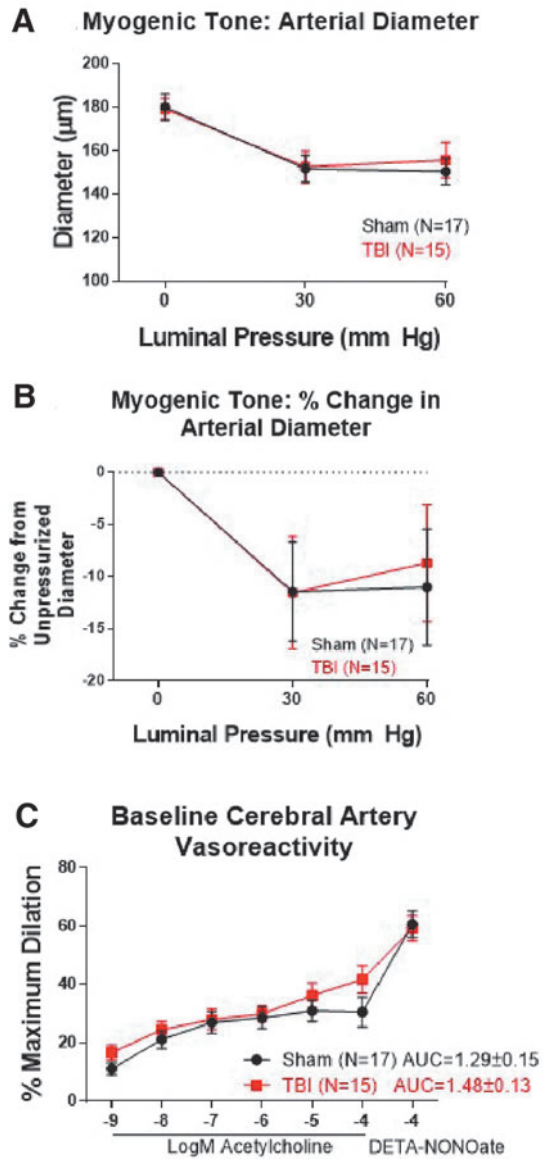


FIG. 4. Myogenic tone and baseline vasoreactivity. **(A,B)** There was no significant difference in response to increasing intraluminal pressure between TBI and sham rat cerebral arteries. Percent change in arterial diameter was calculated as (Diameter30 or 60 – Diameter0)/Diameter0 × 100%). **(C)** Dilator responses to increasing doses of acetylcholine and DETA NONOate were also not significantly different between TBI and sham rats. DETA NONOate, nitric oxide donor diethylenetriamine NONOate; TBI, traumatic brain injury. Color image is available online.

6-month NOR, NOL, TOR, 6-month regional and global CBF and regional CBV, 6-month baseline dilator response to acetylcholine and DETA-NONOate, and change in dilator response to acetylcholine or DETA NONOate following exposure to angiotensin 2, high glucose or Aβ42.

Discussion

The findings demonstrate novel pre-clinical evidence that mTBI from a midline FPI[21] results in persistent/chronic 3- and 6-month cognitive-behavioral impairment (NOR). It also shows for the first time that there are significant associations between regional CBF in the lateral and medial hippocampus and S1BF with cognitive function following mTBI. The 6-month resting *in vivo* regional CBF and CBV and *ex vivo* baseline cerebral arterial myogenic tone, endothelial and smooth muscle function did not differ between mTBI and sham rats, but the groups differed in smooth muscle response following exposure to angiotensin 2. The results provide evidence of chronic adverse consequences of mTBI, consistent with human epidemiological cross-sectional observations.[4]

TBI is a main cause of death and disability in the United States in people younger than 35 years[4] and individuals with TBI history have a higher risk of developing dementia.[11,12] Pre-clinical studies in severe TBI show late (1-year) development of cognitive impairment following injury,[16–19] but empiric evidence as regards chronic consequence of mTBI is lacking. Our results show that even mTBI leads to chronic persistent cognitive dysfunction (NOR test) present at 3 months and sustained at 6 months post-injury. This is consistent with clinical observations that even mild head injury was found to be a predisposing factor for some cases of Alzheimer's disease.[14] As cognitive function is multi-modal, the aspects of novel object testing only partially captures the range of cognitive function, where future assessments could include additional assessments. Our findings therefore validate the use of this injury model to explore mechanisms by which mTBI leads to late dementia.

Disturbed cerebrovascular function has been observed in humans following TBI in the acute (≤1 day) and subacute (1 week) period[5,35] with inverse relationship between CBF and cognitive function.[15] Vascular injury is known to contribute to severe TBI neuropathology with ischemic brain damage being found on autopsy in >90% of acute TBI mortalities.[6] Severe TBI acutely resulted in reduced local CBF and neurovascular uncoupling[36] with endothelial dysfunction.[37] Using the same FPI model, we previously showed regional morphological

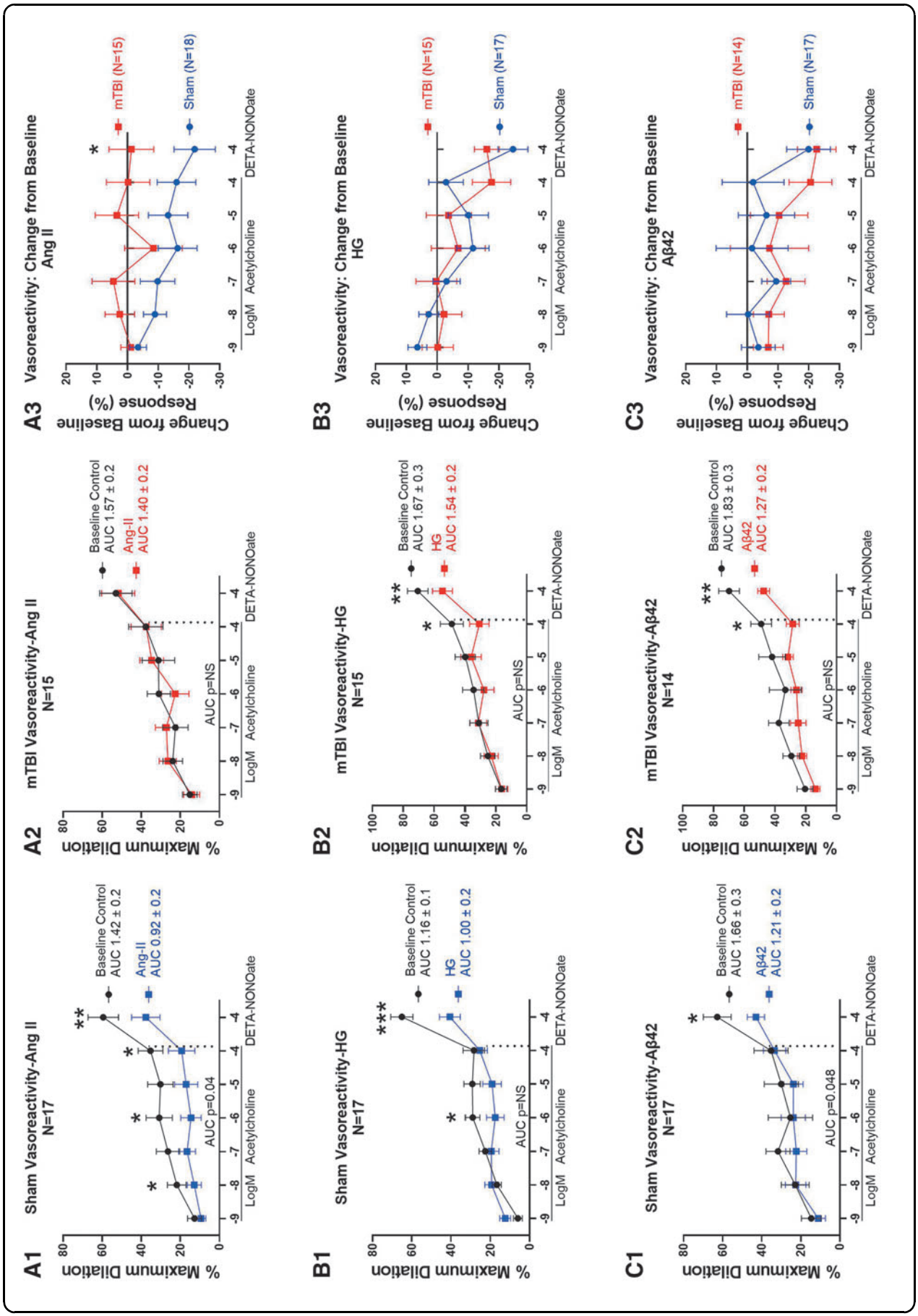
cerebrovascular changes (increased average cerebral arterial vessel volume and surface area) 7 days post-mTBI, likely representing an acute response to mechanical forces of injury.[23] In contrast to empiric data in the acute setting, data on cerebrovascular flow/function and their relationship to cognitive function in the chronic setting post-mTBI are lacking. Our findings show little difference in global and regional CBF and CBV 6 months post-mTBI in the rat model, similar to observations in human patients with concussion where CBF normalizes at 1-month post-injury.[15] These findings suggest that the vascular perturbations observed previously in the acute setting following mTBI recover in the chronic setting. At 6 months we found a direct relationship between regional CBF (lateral and medial hippocampus and S1BF) and cognitive function (NOR and NOL), similar to the association between CBF and neuropsychiatric function in the acute setting following concussion in humans.[15]

The lack of difference in resting CBF and CBV at 6 months between mTBI and sham rats does not necessarily rule out the causative role of vascular perturbations in chronic cognitive dysfunction in mTBI. The persistence of cognitive dysfunction, but not resting CBF and CBV abnormality, suggest that the vascular influence modulating chronic cognitive function could be predominant early in the injury process. Additionally, CBF and CBV were measured in a basal state devoid of functional challenges that could uncover latent vasoreactive deficits that would be relevant during the dynamic neurometabolic demands of NOR testing.

Results show no difference in pial cerebral arterial myogenic tone and resting endothelium-dependent and smooth muscle-dependent function between mTBI and sham. Persistent vasculopathy, however, is suggested by our finding of the variant dilator responses of mTBI cerebral arteries following exposure to angiotensin 2 when compared with sham arteries. Sham arteries exposed to angiotensin 2 demonstrate reduced dilation to DETA NONOate and acetylcholine compared with pre-exposure responses, whereas mTBI arteries showed no difference, demonstrating reduced vasoconstrictor response to angiotensin 2 in mTBI arteries. Angiotensin 2 is an important regulatory peptide mediating vascular oxidative stress, inflammation, and vasoconstriction via action on angiotensin 2 type 1 receptor in vascular smooth muscle cells.[38] Acute short-term exposure of epineural arterioles to angiotensin 2 resulted in decreased blood flow in normal peripheral nerves[39] and altered vasoreactivity response to angiotensin 2 was shown in diabetic animals when compared with normal controls.[39–41] The lack of difference in baseline dilator response to acetylcholine and DETA NONOate, but with differential response following exposure to angiotensin 2 between mTBI and sham parallels, but is opposite to, observations in another neurodegenerative condition. Isolated gluteal resistance arteries from patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencelopathy (CADASIL) showed similar vasoreactive responses to acetylcholine and spermine-NONOate as controls, but CADASIL arteries showed greater vasoconstrictor response to angiotensin 2 than controls.[42] Data on cerebral arterial response were not available for this cohort, which may be relevant as effects of angiotensin 2 were shown to vary in different arterial beds.[39] The pathophysiological consequence of the altered arterial vasoreactivity response following angiotensin 2 exposure in mTBI requires further mechanistic investigation. In contrast to angiotensin 2, no difference was noted between mTBI and sham following exposure to Aβ42, the amyloidogenic protein implicated in Alzheimer's disease that also induces cerebral arterial endothelial dysfunction,[33] or to high glucose, the metabolic abnormality in diabetes mellitus that also causes acute endothelial dysfunction.[34]

Our results showed significant correlation between 6-month NOR and baseline dilator response to DETA NONOate, suggesting the association between cognitive function and cerebral arterial smooth muscle function. The lack of correlation between measures of cognitive function and change in dilator response following angiotensin 2 exposure suggests this vascular perturbation could not explain the chronic cognitive dysfunction observed in this model.

FIG. 5. Cerebral artery dilator responses at 6 months following exposure to vascular agonists. **(A)** Sham rats showed reduced dilator response to acetylcholine (AUC) and DETA-NONOate following exposure to angiotensin 2 (A1), which was not seen in mTBI rats (A2). There was significant reduction in dilation to DETA-NONOate in sham compared with mTBI (A3). **(B)** Overall dilator response to acetylcholine (AUC) was not different following HG exposure in both sham and mTBI, whereas dilation to DETA-NONOate was reduced in both sham and mTBI (B1,B2). There was no difference in change in dilator response to HG between sham and mTBI (B3). **(C)** There was marginal reduction in overall dilator response to acetylcholine (AUC) following Aβ42 in sham but not in mTBI, but dilation to DETA-NONOate was significantly reduced in both sham and mTBI (C1,2). There was no difference in change in dilator response to Aβ42 between sham and mTBI (C3); $^{*}p < 0.05$, $^{**}p < 0.01$. Aβ42, β-amyloid 1-42; Ang II, angiotensin 2; DETA NONOate, nitric oxide donor diethylenetriamine NONOate; HG, high glucose; mTBI, mild traumatic brain injury. Color image is available online.



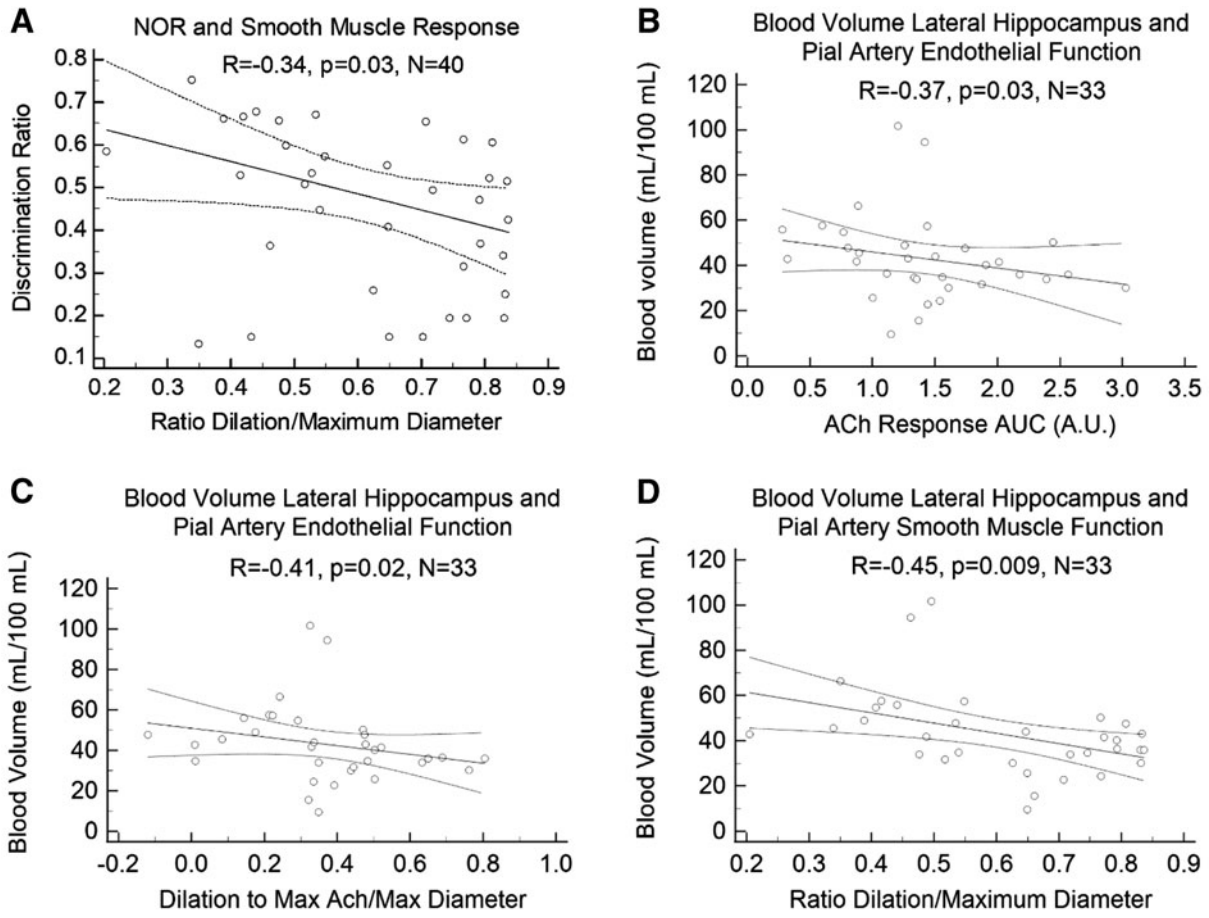


FIG. 6. Cerebral arterial function and cognitive function/regional resting blood volume relationships. **(A)** There is inverse relationship between 6-month novel object recognition score and dilator response to DETA NONOate (smooth muscle function). **(B–D)** Resting cerebral arterial dilator response to acetylcholine (AUC and maximum acetylcholine dose) and DET NONOate are also inversely related to *in vivo* resting blood volume in lateral hippocampus. DETA NONOate, nitric oxide donor diethylenetriamine NONOate.

Among the brain regions measured, only the lateral hippocampus resting CBV was related (inversely) to *ex vivo* cerebral artery endothelial and smooth muscle function. Whether this relationship represents enhanced influence by and/or greater vulnerability in this brain region to perturbations in large cerebral arterial function is unknown and needs to be explored further. A hippocampal subfield volumetry study in patients with normal cognition and patients with mild cognitive impairment showed region-specific vulnerability of hippocampal subfields to vascular injury with differential hippocampal subfield atrophy patterns seen between mild cognitive impairment from vascular disease versus non-vascular causes.[43]

Our study has several limitations. We only studied male rats, so chronic mTBI effects on female rats need to be studied in the future. We show novel cerebrovascular and cognitive function data 6 months following mTBI but lack data on early post-injury cerebrovascular function that could clarify the role of early vascular perturbation in the persistent cognitive impairment observed. Our *ex vivo* arterial studies did not include measurement of small vessel responses that are known to have physiological variance with large arteries[44] and are more intricately involved in neurovascular coupling.[45,46] We showed *in vivo* resting CBF and CBV but lack data on *in vivo* cerebrovascular reserve that could be shown by performing hypercapnic responsiveness or assessing neurovascular coupling. *Ex vivo* results following exposure of circle of Willis arteries to angiotensin 2 uncovered differential responsiveness between mTBI and sham rats and suggest the need for *in vivo* assessment not only of resting but also post-stress cerebrovascular function. Based on the associational relationships uncovered by this study, future efforts can follow to establish causal mechanisms using interventions that modulate vascular conditions

to establish the role of vascular dysfunction in chronic TBI-mediated cognitive impairment. The subtle magnitude of the chronic neurological and vascular changes observed may reflect the mTBI nature of the injury model and the intervening endogenous repair mechanisms, yet the chronic pathophysiology shows similarity to cross-sectional observations in human mTBI,[10,14] enhancing the utility of this model and the neurovascular findings.

Lastly, there currently exists no consensus classification schema for TBI severity,[47] and our injury model remains consistent with criteria for TBI using either Department of Defense or Veterans Affairs classification schemes[10]; future consensus classification may alter the designation of (or be informed by) our model. Future empiric testing is required to determine if abnormalities observed in our model are present in similar or greater magnitude in the acute period or in other injury models and whether angiotensin 2 administration alters memory in the setting of mTBI.

In conclusion, mTBI resulted in chronic 3- and 6-month cognitive dysfunction and altered cerebral arterial vasoreactivity response following exposure to angiotensin 2, without a change in 6-month resting CBF, CBV, or baseline endothelial or smooth muscle-dependent function. The results demonstrate persistent pathophysiological consequences of mTBI that could translate to human exposure to mTBI.

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The data are available upon reasonable request.

Authors' Contributions

RQM, JL, and CQ made substantial contributions to the concept and design. DRG, RQM, LML, AF, LCB, GT, and CCQ wrote the article draft and JL, PDR, and RG critically revised the article. AF, ST, NK, HE, and DM reviewed and approved the article. DRG, LML, CY, AF, ST, NK, GT, and HE acquired the data. RQM, JL, CCQ, LCB, and AF analyzed and interpreted the data. All authors reviewed and approved the version of the article submitted.

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Author Disclosure Statement

No competing financial interests exist.

Supplementary Material

Supplementary Table S1
Supplementary Table S2
Supplementary Table S3

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Supplement Table 1. Breakdown of animal outcome measures.

	Sham		mTBI	
	3 months	6 months	3 months	6 months
Cognitive Function Assessment	21	21	20	20
Imputed Score: NOR	1	3	2	4
Imputed Score: NOL	1	3	2	4
Imputed Score: TOR	3	3	1	3
MRI/Vasoreactivity		17		15

Supplement Table 2. Correlation Analyses Among Cognitive Function and MRI Outcome Measures

A. Cognitive function and cerebral blood flow

	Sham		mTBI		All
	R	p-value	R	p-value	R
NOR-CBF global	0.28	0.28	0.13	0.65	0.17
NOR-CBF medial hippocampus	0.60	0.05	0.61	0.06	**0.57**
NOR-CBF lateral hippocampus	0.52	0.10	**0.72**	**0.02**	**0.54**
NOR-CBF S1BF	0.51	0.11	**0.87**	**0.001**	**0.62**
NOL-CBF global	0.28	0.28	-0.12	0.66	0.11
NOL-CBF medial hippocampus	**0.65**	**0.03**	0.36	0.31	**0.54**
NOL-CBF lateral hippocampus	0.40	0.23	-0.33	0.35	0.23
NOL-CBF S1BF	0.33	0.32	0.13	0.73	0.23
TOR-CBF global	0.09	0.73	-0.19	0.50	0.01
TOR-CBF medial hippocampus	0.22	0.51	0.09	0.80	0.18
TOR-CBF lateral hippocampus	0.45	0.16	-0.07	0.85	0.24
TOR-CBF S1BF	-0.02	0.95	0.08	0.83	0.12

B. Cognitive function and cerebral blood volume

	Sham		mTBI		All
	R	p-value	R	p-value	R
NOR-CBV medial hippocampus	-0.44	0.08	0.17	0.54	-0.18
NOR-CBV lateral hippocampus	0.18	0.50	0.32	0.24	0.22
NOR-CBV S1BF	-0.12	0.66	0.41	0.12	0.18
NOL-CBV medial hippocampus	-0.28	0.27	-0.11	0.70	-0.23
NOL-CBV lateral hippocampus	0.14	0.60	-0.14	0.63	-0.07
NOL-CBV S1BF	0.03	0.90	0.12	0.68	0.07
TOR-CBV medial hippocampus	**-0.58**	**0.01**	0.28	0.32	-0.22
TOR-CBV lateral hippocampus	-0.02	0.94	0.34	0.22	0.10
TOR-CBV S1BF	-0.30	0.24	-0.10	0.72	-0.17

Values where p-value was significant are presented in bold font. CBF- cerebral blood flow, CBV-cerebral blood volume, NOL- novel object location, NOR-novel object recognition, TOR- temporal order object recognition, S1BF-primary somatosensory barrel cortex

Supplement Table 3. Correlation Analyses Among Vasoreactivity Outcomes and Cognitive/MRI Outcomes

Outcomes	Max. Acetylcholine Dilation	Dilation to DETA-NONOate
NOR (6 months)	R=-0.26 p=0.10	**R=-0.34** **p=0.03**
NOL (6 months)	R=-0.05 p=0.75	R=-0.13 p=0.44
TOR (6 months)	R=0.15 p=0.35	R=0.02 p=0.88
CBF Global	R=-0.08 p=0.68	R=-0.10 p=0.57
CBF Lateral Hippocampus	R=-0.24 p=0.29	R=-0.1 p=0.66
CBF Medial Hippocampus	R=-0.18 p=0.42	R=-0.30 p=0.18
CBF S1BF	R=-0.27 p=0.24	R=-0.35 p=0.12
CBF Thalamus	R=0.1 p=0.68	R=-0.30 p=0.18
BV Lateral Hippocampus	**R=-0.41** **p=0.02**	**R=-0.45** **p=0.009**
BV Medial Hippocampus	R=-0.165 p=0.36	R=-0.11 p=0.55
BV S1BF	R=-0.23 p=0.2	R=-0.23 p=0.19
BV Thalamus	R=-0.05 p=0.78	R=-0.12 p=0.50

Values where p-value was significant are presented in bold font. CBF- cerebral blood flow, CBV-cerebral blood volume, Dilation to DETA-NONOate- baseline dilation response to 10^{-4}M diethylenetriamine NONOate, Max Acetylcholine dilation- baseline dilation response to acetylcholine 10^{-4}M, NOL- novel object location, NOR-novel object recognition, TOR- temporal order object recognition, S1BF-primary somatosensory barrel cortex

Written Abstract DRAFT 5 (450 WORD MAX)

Collaborators: Mr. Conor Young BS; Dr. L. Matthew Law PhD; Dr. Nina Karamanova DVM; Mr. Seth Truran BS; Dr. Chad Quarles PhD; Dr. Raymond Migrino MD; Dr. Jonathan Lifshitz PhD

Traumatic brain injury (TBI) is a worldwide health concern with approximately 5.3 million Americans currently living with TBI-induced disability. For many, TBI-associated disabilities impair cognition, provoke seizures, and elevate risk for neurodegenerative disease. Oxidative stress, nitrative stress, and derangement of cerebrovascular physiology have been implicated in acute acquired neurological injury and aging-related dementia disorders. Experimental models of TBI provide a unique opportunity to investigate relationships between vascular disturbances and impaired cognitive performance. In this study, diffuse brain-injured (midline fluid percussion) adult male rats were assessed for long-term molecular, vascular, and cognitive pathologies. At 90 and 180 days post-injury, brain-injured and uninjured sham rats performed three cognitive tasks: a novel object recognition task (NOR) assessing short term memory, a novel location recognition task (NOL) assessing long term memory, and a temporal order recognition task (TOR) assessing working memory. Rats' brains were imaged at 182-187 days post-injury via dynamic susceptibility contrast monocrystalline iron oxide particle (MION) magnetic resonance imaging (MRI) to assess in-vivo cerebrovascular blood flow and vasoreactivity in response to hypercapnic challenge. At 189-193 days post-injury, brains were collected for histology and arteries (Circle of Willis, ventral circuit) were collected for ex-vivo vasoreactivity and markers of oxidative and nitrative stress, respectively. Brain-injured rats performed significantly worse than shams on the NOR and TOR tasks at 90 days post-injury. At 180 days post-injury, brain-injured rats performed significantly worse than sham on all three cognitive tasks. By MRI, brain-injured rats showed significantly less cerebrovascular blood volume compared to sham rats. Ex-vivo vasoreactivity was not significantly different in baseline cerebrovascular myogenic tone or endothelial reactivity between groups. Cerebral arteries of brain-injured rats exhibited significantly greater levels of superoxide and peroxynitrite in the presence of amyloid-beta-42 (Aβ42) compared to cerebral arteries of sham rats, indicating significantly higher response to oxidative and nitrative stress, which supports the potential for injury-induced vascular dementia. Further analysis will integrate the relationship between brain injury parameters, cognitive performance, and vascular function in order to elucidate the mechanistic role of vascular dysfunction in TBI-mediated cognitive dysfunction.

Funding: Department of Defense Grant AZ 160056

Title: Experimental brain injury induces long term cognitive deficits and vascular pathology

Authors and Institutions : DR Griffiths[1,2,4]; LM Law[1,3,4]; N Karamanova[1]; S Truran[1]; LC Bell[2]; GH Turner[2]; C Quarles[2]; RQ Migrino[1,4]; J Lifshitz[1,3,4]

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Introduction: Nearly 20% of ±2.6 million US service members deployed since 2003 sustained at least one traumatic brain injury (TBI). TBI-associated disabilities impair cognition, and elevate risk for neurodegenerative disease. Impaired cerebrovascular function has been implicated in acute acquired neurological injury and aging-related dementia disorders. Experimental models of TBI provide an opportunity to investigate relationships between vascular disturbances and impaired cognitive performance.

Methods: In this study, adult male rats received a sham or midline fluid percussion injury and were assessed for long-term vascular and cognitive pathologies. Cognitive performance at 90 and 180 days post-injury (DPI) was evaluated by novel object tasks. At 182-187 DPI rat brains were imaged via magnetic resonance imaging (MRI) to assess in-vivo cerebrovascular function. At 189-193 DPI, ex-vivo vascular function in cerebral arteries and brain histology were performed.

Results: Brain-injured rats performed significantly worse than shams on the short-term memory task at 90 and 180 DPI with trends of decreased working and long-term memory performance. By MRI, no statistical difference was found between regional cerebral blood flow (CBF) between sham and brain-injured rats. However, a correlation exists between increased regional CBF and impaired short-term memory. Ex-vivo vasoreactivity was not significantly different in baseline cerebrovascular myogenic tone or endothelial reactivity between groups. Cerebral arteries of brain-injured rats exhibited significantly greater levels of superoxide and peroxynitrite in the presence of amyloid-beta-42 (Aβ42) compared to cerebral arteries of sham rats, indicating significantly higher response to oxidative and nitrative stress, which supports the potential for injury-induced vascular dementia.

Conclusions: Further analysis will integrate the relationship between TBI parameters, cognitive performance, and vascular function in order to elucidate the role of vascular dysfunction in TBI-mediated cognitive dysfunction.

Acknowledgement of Support: Department of Defense Grant AZ 160056

Reimagine HEALTH: Health and Disease over the Lifespan
Deadline: Friday, December 17, 2021 at 5:00 p.m.

The University of Arizona College of Medicine – Phoenix Research Office, Arizona Biomedical Research Centre, and Flinn Foundation are co-hosting the 4th Annual reimagine Health Research Symposium to be held on Thursday, February 17, 2022.

All interested attendees including students, trainees, and principal investigators are encouraged to submit an abstract! Abstracts should focus on the categories listed below.

Abstract Categories

- Ageing and the Immune System
- Neurodevelopmental and Neurodegenerative Diseases
- Genomic Instability and Cancer over the Lifespan
- Gestation and the Environment
- Epidemiology, Public Health and Social Determinants of Health
- Disease Management

Abstract Format

Each abstract should be targeted to a medical/scientific audience. Please copy and paste your abstract into the text boxes within this form to follow our required format. The body of the abstract (excluding Title and Authors) should be 500 words or less.

- Title
- Author(s) and Institutions
- Introduction/Hypothesis/Scientific Premise
- Methods/Approach
- Results
- Conclusions/Discussion
- Acknowledgement of Support

Abstract Review Process

Submitted abstracts will be reviewed for applicability to the symposium theme, rigorous research methodology, impact to health care, and readability. The Research Symposium Committee will review abstracts for content and relevance to the program. You will be notified in early January 2022 about your abstract submission and if it has been accepted.

Accepted Abstracts / Posters

Accepted abstracts will be invited to submit a digital poster PDF, which will be due on Friday, February 4th, 2022 at 5:00 p.m., and shared during the duration of the symposium.

Symposium Registration

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Questions

Please contact the Research Office COMPHX-ResearchOffice@arizona.edu with any questions about the symposium or Call for Abstracts process!

Experimental brain injury induces long term cognitive deficits and vascular pathology

DR Griffiths[1,2,4]; LM Law[1,3,4]; N Karamanova[1]; S Truran[1]; LC Bell[2]; GH Turner[2]; C Quarles[2]; RQ Migrino[1,4]; J Lifshitz[1,3,4]

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Nearly 20% of ±2.6 million US service members deployed since 2003 sustained at least one traumatic brain injury (TBI). TBI-associated disabilities impair cognition, and elevate risk for neurodegenerative disease. Impaired cerebrovascular function has been implicated in acute acquired neurological injury and aging-related dementia disorders. Experimental models of TBI provide an opportunity to investigate relationships between vascular disturbances and impaired cognitive performance. In this study, adult male rats received a sham or midline fluid percussion injury and were assessed for long-term vascular and cognitive pathologies. Cognitive performance at 90 and 180 days post-injury (DPI) was evaluate by novel object tasks. At 182-187 DPI rat brains were imaged via magnetic resonance imaging (MRI) to assess in-vivo cerebrovascular function. At 189-193 DPI, ex-vivo vascular function in cerebral arteries and brain histology were performed. Brain-injured rats performed significantly worse than shams on the short-term memory task at 90 and 180 DPI with trends of decreased working and long-term memory performance. By MRI, no statistical difference was found between regional cerebral blood flow (CBF) between sham and brain-injured rats. However, a correlation exists between increased regional CBF and impaired short-term memory. Ex-vivo vasoreactivity was not significantly different in baseline cerebrovascular myogenic tone or endothelial reactivity between groups. Cerebral arteries of brain-injured rats exhibited significantly greater levels of superoxide and peroxynitrite in the presence of amyloid-beta-42 (Aβ42) compared to cerebral arteries of sham rats, indicating significantly higher response to oxidative and nitrative stress, which supports the potential for injury-induced vascular dementia. Further analysis will integrate the relationship between TBI parameters, cognitive performance, and vascular function in order to elucidate the role of vascular dysfunction in TBI-mediated cognitive dysfunction.

Funding: Department of Defense Grant AZ 160056

SNTF Chronic TBI NNS Abstract

Alpha-II Spectrin N-Terminal Fragment (SNTF) Detects Evidence of Cytoskeletal Pathology in Chronic Experimental Traumatic Brain Injury

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Military personnel are estimated to have a higher incidence of chronic traumatic brain injury (TBI) compared to civilians, and experience ongoing symptoms that negatively impact quality of life. Following diffuse axonal injury (DAI), proteolytic cleavage of axonal cytoskeleton protein, spectrin, leads to alpha-II spectrin N-terminal fragment (SNTF) generation. Visualization of accumulated SNTF can reveal morphological abnormalities, such as undulations and varicosities, that arise due to impaired axonal transport. We hypothesized that SNTF could be used as an experimental histological marker of enduring cortical and hippocampal DAI pathology following TBI.

Male Sprague Dawley rats received sham (n=11) or midline fluid percussion injury (n=11), and brains were collected 6 months post-injury. SNTF was detected in the somatosensory cortex and hippocampus using standard immunohistochemistry protocols (Anti-SNTF, Abcam, ABN2264) and brightfield microscopy, surveying the entire cortex. Injured brain tissue had elevated SNTF expression and visible axonopathies in pyramidal neurons of cortical layers III and V compared to sham.

Our findings add support for SNTF as a histological marker of chronic axonopathy after DAI. The pathology observed in SNTF-labeled neurons of cortical layer III and V pyramidal neurons may provide insights into the underlying mechanisms of symptoms associated with chronic DAI pathology, offering additional methods for monitoring its progression. Current research should focus on SNTF quantification and combining it with other immunohistochemical stains biomarkers to assess its utility and sensitivity.

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DAI, axonopathy, TBI, SNTF, chronic, Venn diagram