AWARD NUMBER: W81XWH-18-1-0727

TITLE: Novel Target and Lead Compound to Reverse TBI-Induced Alzheimer's-Related Dementia

PRINCIPAL INVESTIGATOR: Dr. Vikhyat Bebarta

CONTRACTING ORGANIZATION: University of Colorado Denver
Aurora, CO

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Fort Detrick, Maryland 21702-5012

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Novel Target and Lead Compound to Reverse TBI-Induced Alzheimer's-Related Dementia

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6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
It is estimated that >1.4 million Americans each year suffer a traumatic brain injury (TBI), defined as a blunt or penetrating injury to the headthat alters brain function. Importantly, TBI is also a major health issue in the U.S. military, with estimates of TBI prevalence as high as 23%ofreturning service members (including both moderate/severe and mild concussive TBI). Mild (concussive) and moderate/severe TBI haveboth been linked to immediate and delayed development of long-term disabilities; predominantly reduced working memory, difficulty learningnew information, execute function and reasoning. Interestingly, a similar array of cognitive deficits are observed in patients withAlzheimerâ€™s disease (AD) and AD related dementias (ADRD). Relevant to the current Program Announcement, there is an emergingconsensus that traumatic brain injury (TBI) is associated with increased risk of future AD and ADRD and military personnel are increasinglyliving with TBI and associated risk of cognitive decline. Thus, it is critical to improve our understanding of the etiology of TBI-induceddementia and cognitive dysfunction with the goal of identifying new therapeutic targets to improve quality of life for the thousands to millionsof military and civilian individuals living with TBI.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	UU	15	19b. TELEPHONE NUMBER *(include area code)*

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1. Accomplishments: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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.

Aim 1: Prevention of TBI-induced AD/ADRD. We will optimize dosing and timing regimen for preventing TBI-induced memory deficits and test the hypothesis that TRPM2 inhibition reduces TBI-induced APP/Aβ accumulation. Electrophysiology, molecular and neurobehavioral methods are established in the laboratory.

Aim 2: Reversal of TBI-induced AD/ADRD. We will test the hypothesis that TRPM2 channel inhibition at delayed time points reverses TBI and Aβ-induced synaptic dysfunction. We will optimize delayed administration (30-90 days after TBI) of our novel TRPM2 inhibitor to reverse synaptic plasticity deficits and recover memory function in the late chronic phase. We will test the hypothesis that TBI and Aβ-induced LTP dysfunction

Milestone #1: Complete dose-response of tat-M2NX reversal of TBI-induced memory deficits.

Methods: Dose-response of tat-M2NX: TBI in male and female mice will be induced by CCI (and sham controls), as described in specific methods of Aim 1. Treatment groups of 10 mice will include 4 doses of tat-M2NX (0.5, 2, 10, 20 mg/kg). Drug will be administered by intravenous injection of tat-M2NX or control tat-SCR 2 hours after recovery from TBI. The contextual fear conditioning paradigm will be used as a hippocampal-dependent memory task[45] 7 days after recovery from TBI. Additional analysis will include extracellular field recordings of the schaffer collateral-CA1 synaptic field to determine effects of hippocampal synaptic plasticity (LTP).

Criteria for Success: Completed dose-response relation in male and female mice with significant effects on memory function and LTP. Significant reduction in TBI-induce memory deficits and LTP deficits observed in a minimum of one experimental group. Statistical significance ($p<0.05$) to be determined using 1-Way ANOVA with post-hoc analysis for comparison of multiple groups. Power analysis was performed using our preliminary data, providing group sizes of 8-10 to generate data with alpha<0.05 and 80% Power. Additionally, a non-efficacious dose to be determined. All experiments performed in a blinded and randomized manner.

Anticipated Completion: Year 1

- This milestone is 100 % completed.

Aim 1: Prevention of TBI-induced AD/ADRD. We will optimize dosing and timing regimen for preventing TBI-induced memory deficits and test the hypothesis that TRPM2 inhibition reduces TBI-induced APP/Aβ accumulation. Electrophysiology, molecular and neurobehavioral methods are established in the laboratory.

Aim 2: Reversal of TBI-induced AD/ADRD. We will test the hypothesis that TRPM2 channel inhibition at delayed time points reverses TBI and Aβ-induced synaptic dysfunction. We will optimize delayed administration (30-90 days after TBI) of our novel TRPM2 inhibitor to reverse synaptic plasticity deficits and recover memory function in the late chronic phase. We will test the hypothesis that TBI and Aβ-induced LTP dysfunction

Milestone #2: Acute inhibition of TRPM2 reduces APP/Aβ accumulation.

Methods: TBI in male and female wild-type and TRPM2-/- mice will be induced by CCI. Optimal dose determined in milestone #1 will be administered iv to sham and TBI injured mice 2 hours after recovery. Immunohistochemistry (IHC) will be performed to assess APP and Aβ levels in the injured and non-injured hemisphere. Localization of accumulated APP and Aβ will be assessed by double labeling with cell-type specific markers (neurons=NeuN; astrocytes=GFAP; blood vessels=Glut1). A separate cohort of mice will be used to analyze Aβ levels using commercially available ELISA kit to detect Aβ. Analysis will be performed in mice at varying time points after TBI (1, 3, 7, 30, 90 days). IHC and ELISA analysis will be performed by an investigator blinded to treatment conditions.

Criteria for Success: Significant reduction in TBI-induce APP/Aβ accumulation observed at one time point. Statistical significance ($p<0.05$) to be determined using 1-Way ANOVA with post-hoc analysis for comparison of multiple groups. Power analysis was performed using our preliminary data, providing group sizes of 8-10 to generate data with alpha<0.05 and 80% Power.

Anticipated Completion: Year 2

- This milestone is approximately 100 % completed.

Aim 1: Prevention of TBI-induced AD/ADRD. We will optimize dosing and timing regimen for preventing TBI-induced memory deficits and test the hypothesis that TRPM2 inhibition reduces TBI-induced APP/Aβ accumulation. Electrophysiology, molecular and neurobehavioral methods are established in the laboratory.

Aim 2: Reversal of TBI-induced AD/ADRD. We will test the hypothesis that TRPM2 channel inhibition at delayed time points reverses TBI and Aβ-induced synaptic dysfunction. We will optimize delayed administration (30-90 days after TBI) of our novel TRPM2 inhibitor to reverse synaptic plasticity deficits and recover memory function in the late chronic phase. We will test the hypothesis that TBI and Aβ-induced LTP dysfunction

Milestone 3: Optimize timing regimen of delayed tat-M2NX treatment.

Methods: TBI in male wild-type mice will be induced by CCI. Optimal dose determined in milestone #1 will be administered iv to sham and TBI injured mice at delayed time points (30, 90 days) and 24 hours after injection analyzed for memory (contextual fear conditioning) or synaptic plasticity (LTP). An additional cohort of mice will be used to determine the durability of tat-M2NX efficacy by initiating injections at 30 days and analyzing memory and synaptic plasticity function at 90 days. Dosing regimens of every 7, 14 or 30 days will be tested.

Criteria for Success: Significant recovery of TBI-induced deficits in memory and LTP observed. Statistical significance ($p<0.05$) to be determined using 1-Way ANOVA with post-hoc analysis for comparison of multiple groups. Power analysis was performed using our preliminary data, providing group sizes of 8-10 to generate data with alpha<0.05 and 80% Power.

Anticipated Completion: Year 3

- This milestone is approximately 75 % completed.

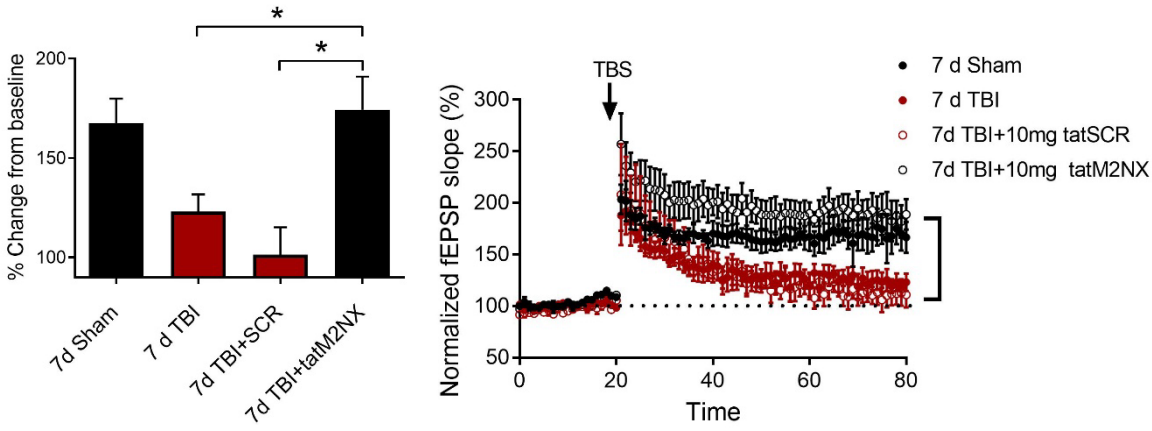
What was accomplished under these goals?

For this quarterly reporting period only 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.

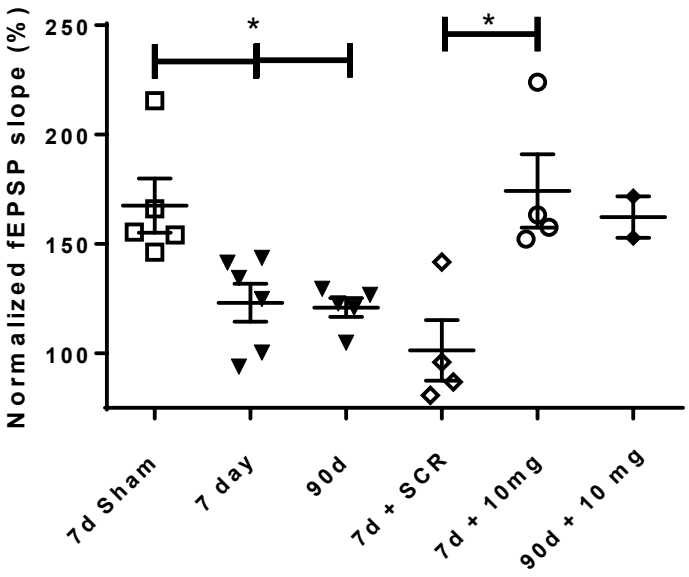
1) **Major Activities**

We have completed the study and manuscript is in preparation.

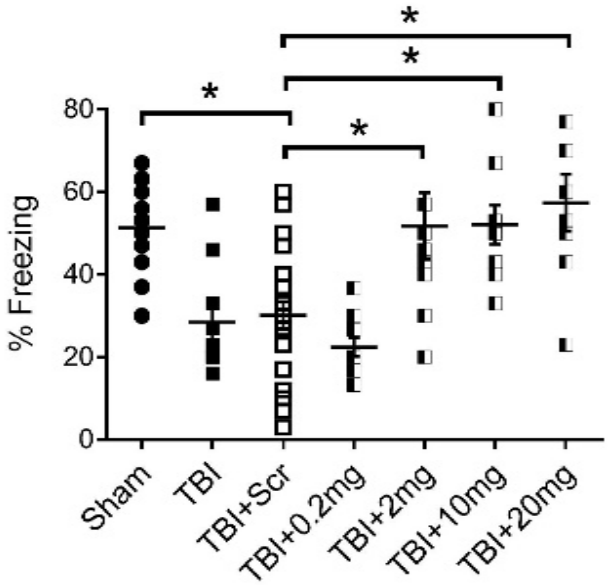
To test the effect of injury-induced by TBI on hippocampal synaptic plasticity (dementia-like memory deficits), extracellular field recordings of CA1 neurons were performed from acute slices from the hippocampus at 7 days after recovery from CCI and LTP was compared to sham-operated control mice. In sham control slices, a physiological theta burst stimulation (TBS; 40 pulses) resulted in LTP that increased the slope of fEPSP to 167.5 ± 12.4% of baseline after 60 min (n=5, P<0.05 compared to baseline). In contrast, recordings obtained from post-injury brain slices demonstrated diminished LTP; 117.3±8.2% (n=8, p<0.05) at 7 days. The acute administration (2 hrs after recovery from TBI) of 10 mg/kg control peptide (SCR) had no effect on TBI-induced LTP impairment. In contrast, the administration of 10 mg/kg tatM2NX completely prevented TBI-induced LTP deficits (see below). These data are consistent with our behavioral analyses.



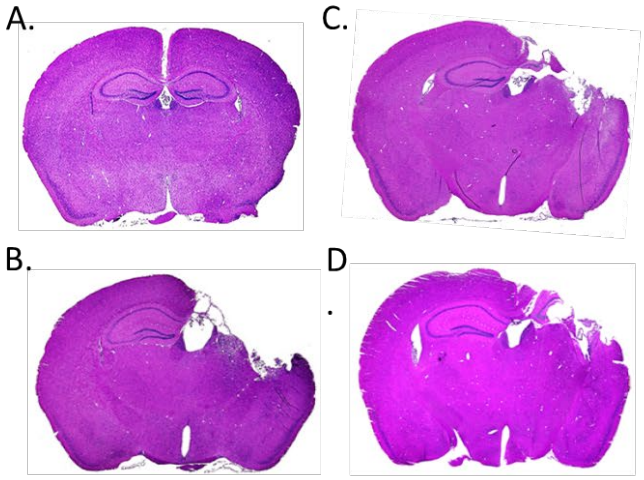
We performed experiments in male mice and assess LTP at 7 or 90 days after recovery from TBI. We injected tat-M2NX or control tat-SCR 24 hr before recording LTP (eg day 6 or 89 post-TBI). The figure below shows data obtained, demonstrating reversal of TBI-induced plasticity deficits at 1 week and 3 months. Experiments performed at 30 days in male and female are required to complete this milestone.

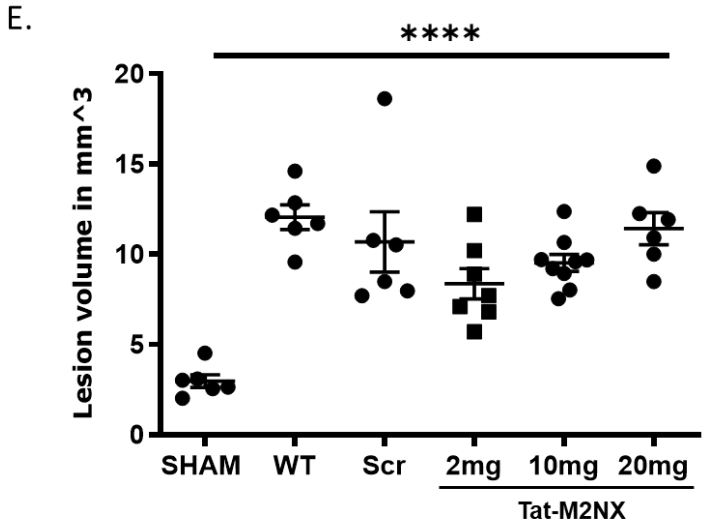


We performed experiments in male and female mice and did not observe differences in TBI-induced memory complications and therefore all experiments were performed in both male and female animals. We have completed our blinded and randomized dose finding experiment using the doses proposed (0.2, 2, 10, 20 mg/kg). We performed TBI surgery and administered tat-Scr or varying doses of tat-M2NX 2 hours after recovery. We analyzed memory function using the hippocampal-dependent neurobehavioral task contextual fear conditioning (CFC). Freezing behavior is an indication of intact spatial memory, with greater freezing representing better memory. We have successfully completed 8-12 animals at each dose, with our data indicating a dose-dependent response of tat-M2NX in improving memory function after TBI, with doses of 2 mg/kg and higher providing significant benefit. These data strongly indicate that acute administration of tat-M2NX reduces TBI-induced ADRD. 1-Way ANOVA with Tukey post-test comparison to adjust for multiple comparisons was performed. See figure below for summary of our data to date.

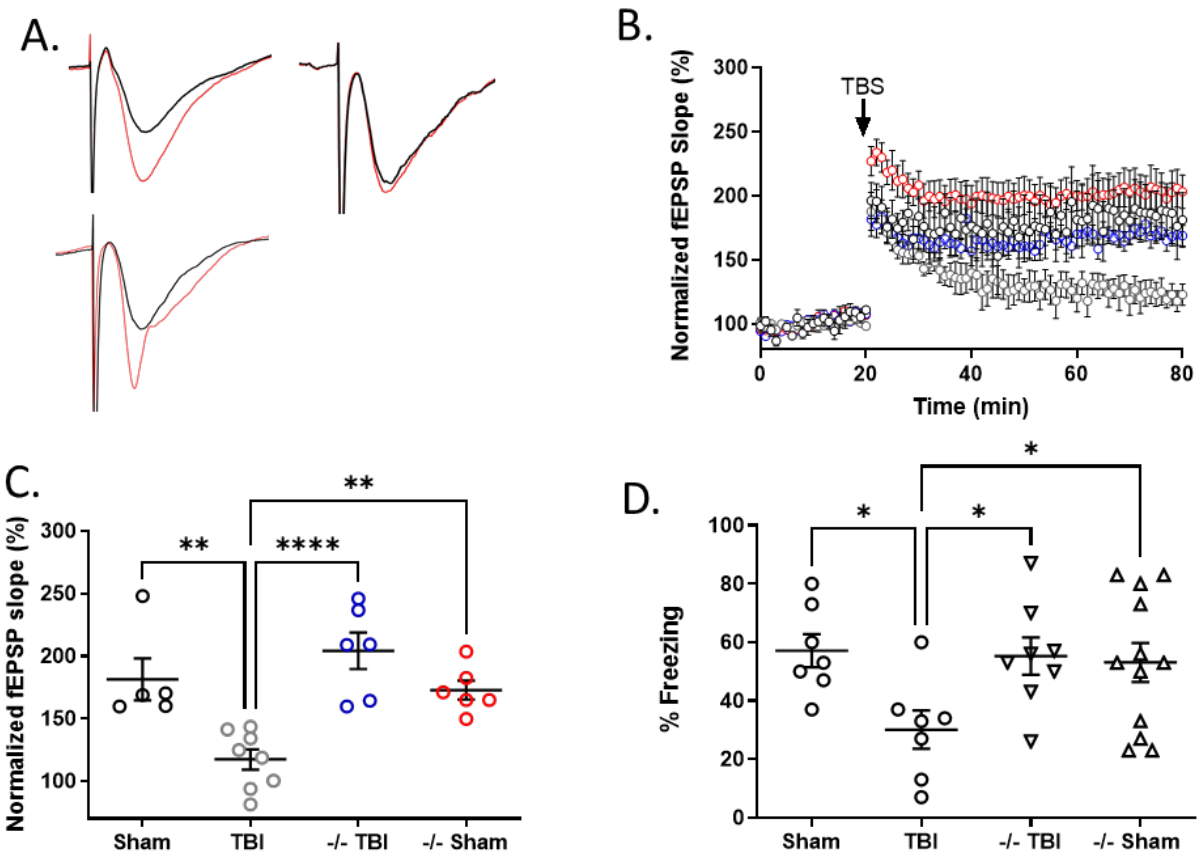


Counter to our hypothesis, we did not observe histological protection in mice administered tat-M2NX 2 hours after TBI. This is contrary to our observation of enhance cognitive recovery when administered acutely or at delayed timepoints.

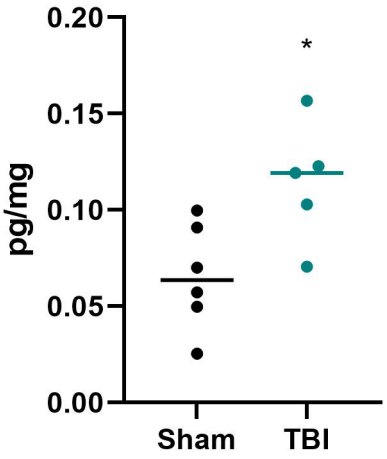




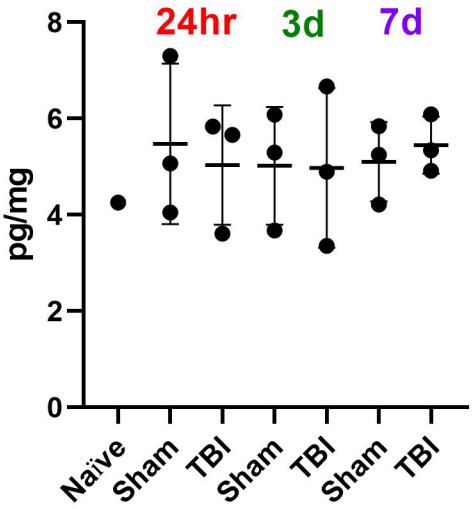
To confirm the role of TRPM2 channels on hippocampal function, we performed electrophysiology experiments to quantify hippocampal synaptic plasticity (LTP) in TRPM2 KO mice. Electrophysiologic data was collected using extracellular field recording in the CA1 area of acute hippocampal slices 7 days following TBI and compared to slices collected from sham-operated control mice in both WT and TRPM2 KO mice. There were no differences in paired pulse release of input-out characteristics in any of the conditions studied (Data not shown). To study LTP in control slices, a brief TBS (40 pulse TBS) resulted in LTP of 181.5 ± 16.82; (n=5). Figure below shows a significant impairment of LTP in WT mice (117.3± 8.18%, n=8) 7 days after TBI when compared to sham controls ($p<0.05$). In contrast, TBI did not alter LTP in TRPM2 KO mice compared to TRPM2 Sham mice (204.3 ± 14.64, n=6; 172.8 ± 7.542, n=6; respectively). These data suggest that TRPM2 KO mice displayed a preservation of hippocampal synaptic function when compared to WT TBI mice ($p<0.05$).

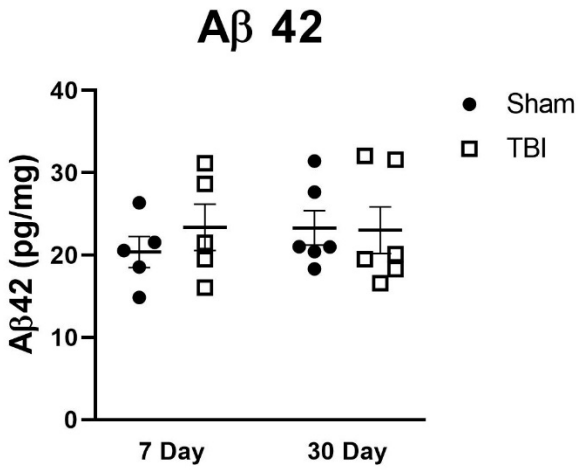


To assess the impact of TBI on circulating and brain level of neurotoxic amyloid beta-42 ($A\beta_{42}$) we performed ELISA measurements of soluble $A\beta_{42}$. We observed a significant increase in $A\beta_{42}$ in the serum 7 days after TBI, consistent with the clinical literature.



In light of our observation that $A\beta_{42}$ activates TRPM2 channels and impairs hippocampal synaptic plasticity. We next tested the impact of TBI on brain levels of soluble $A\beta_{42}$. Surprisingly, we did not observe an increase in $A\beta_{42}$ at any of the timepoints tested, see below. In light of the negative result and dis-proving our hypothesis, we repeated the experiment to confirm lack of change of $A\beta_{42}$ in the brain, this time extending our time window to 30 days. As observed previously, we did not observe differences in brain levels of $A\beta_{42}$ 7 or 30 days after TBI.





Overall, we complete the experiments outlined in the proposal, demonstrating impaired memory function following moderate TBI that was reversed by TRPM2 inhibition (tat-M2NX) or knockout. These data are being prepared as a manuscript for submission. Interestingly, we were able to disprove our hypothesis that TBI-induced impaired plasticity if via increased $A\beta_{42}$ in the brain. Nonetheless, we demonstrate a novel therapeutic approach targeting the TRPM2 channel to enhance cognitive recovery following TBI.

Describe the Regulatory Protocol and Activity Status (if applicable).
Describe the Protocol and Activity Status for sections a-c, as applicable, using the format described for each section. If there is nothing significant to report during this reporting period, state "Nothing to Report."

(a) ***Human Use Regulatory Protocols***

Not applicable

(b) Use of Human Cadavers for Research Development Test & Evaluation (RDT&E), Education or Training

Not applicable

(c) Animal Use Regulatory Protocols

TOTAL PROTOCOL(S):

State the total number of animal use protocols required to complete this project (e.g., 2 animal use research protocols will be required to complete the Statement of Work.). If not applicable, write "No animal use research will be performed to complete the Statement of Work."

PROTOCOL(S):

List the identifier and title for all animal use protocols needed to complete the project. Include information about the approved target number for statistical significance, type of submission, type of approval with associated dates, and performance status.

The following format shall be used:

Protocol (_ of _ total):

Protocol [ACURO Assigned Number]:

Title:

Target required for statistical significance:

Target approved for statistical significance:

Submitted to and Approved by:

Provide bullet point list of protocol development, submission, amendments, and approvals (include IACUC in addition to ACURO).

Status:

Provide bullet point list of performance and/or progress status relating to the above protocol and discuss any administrative, technical, or logistical issues that may impact performance or progress of the study (e.g. animal use protocol needs revision to minimize animal suffering, animal protocol modification to include additional staff) for the above ACURO approved protocol.

TOTAL PROTOCOL(S):

PROTOCOL (_ of _ total):
Protocol [ACURO Assigned Number]:
Title:
Target required for statistical significance:
Target approved for statistical significance:

SUBMITTED TO AND APPROVED BY:

STATUS:

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals and objectives?

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

We plan to assess lower doses of tatM2NX in LTP experiments to determine the minimal efficacious dose. In addition, we plan to begin measurements of Aβ accumulation and the impact of tatM2NX treatment.

2. Products: List any products resulting from the project during the reporting period. If there are no products to report for the current quarter, state "Nothing to report."

Examples of products include:

- *publications, conference papers, and presentations;*
- *website(s) or other Internet site(s);*
- *technologies or techniques;*
- *inventions, patent applications, and/or licenses; and*
- *other products, such as data or databases, biospecimen collections, germplasm, audio or video products, software, models, educational aids or curricula, instruments or equipment, data and research material, clinical or educational interventions, or new business creation.*

Nothing to Report

3. Participants & Other Collaborating Organizations

What individuals have worked on the project?
Provide the following information for: (1) Project Directors (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).

Provide the name and identify the role the person played in the project. Indicate the nearest whole person month (Calendar, Academic, Summer) that the individual worked on the project. Show the most senior role in which the person worked on the project for any significant length of time. For example, if an undergraduate student graduated, entered graduate school, and continued to work on the project, show that person as a graduate student, preferably explaining the change in involvement.

Describe how this person contributed to the project. If information is unchanged from a previous submission, provide the name only and indicate "no change."

Example:

Name:	*Paco S. Herson*
Project Role:	*PI*
Nearest person month worked:	*3*
Contribution to Project:	*Professor Herson supervised all experiments, analyzed data and maintained blinding code*

Name:	*James Orfila*
Project Role:	*Instructor*
Nearest person month worked:	*2*
Contribution to Project:	*Dr. Orfila performed all of the experiments, including TBI surgery and behavioral testing.*

4. Changes/Problems:

The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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:

a. Actual Problems or delays and actions to resolve them

Provide a description of current problems or issues that may impede performance or progress of this project along with proposed corrective action. Also 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.

For an award that includes the recruitment of human subjects for clinical research or a clinical trial, discuss any problems or barriers encountered, if applicable, and what has been done to mitigate those issues. Discussion may highlight enrollment problems, retention problems, and actions taken to increase enrollment and/or improve retention.

Nothing to Report

b. Anticipated Problems/Issues

Provide a description of anticipated problems or issues that have a potential to impede performance or progress. Also provide course of actions planned to mitigate problems or to take should the problem materialize.

Nothing to Report

5. Special Reporting Requirements:

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