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

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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 head that 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%of returning service members (including both moderate/severe and mild concussive TBI). Mild (concussive) and moderate/severe TBI have both been linked to immediate and delayed development of long-term disabilities; predominantly reduced working memory, difficulty learning new information, execute function and reasoning. Interestingly, a similar array of cognitive deficits are observed in patients with Alzheimerâ€™s disease (AD) and AD related dementias (ADRD). Relevant to the current Program Announcement, there is an emerging consensus that traumatic brain injury (TBI) is associated with increased risk of future AD and ADRD and military personnel are increasingly living with TBI and associated risk of cognitive decline. Thus, it is critical to improve our understanding of the etiology of TBI-induced dementia and cognitive dysfunction with the goal of identifying new therapeutic targets to improve quality of life for the thousands to millions of military and civilian individuals living with TBI.

15. SUBJECT TERMS

None Listed

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1. Introduction

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 head that 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% of returning service members (including both moderate/severe and mild concussive TBI). Mild (concussive) and moderate/severe TBI have both been linked to immediate and delayed development of long-term disabilities; predominantly reduced working memory, difficulty learning new information, execute function and reasoning. Interestingly, a similar array of cognitive deficits are observed in patients with Alzheimer's disease (AD) and AD related dementias (ADRD). *Relevant to the current Program Announcement, there is an emerging consensus that traumatic brain injury (TBI) is associated with increased risk of future AD and ADRD and military personnel are increasingly living with TBI and associated risk of cognitive decline.* Thus, it is critical to improve our understanding of the etiology of TBI-induced dementia and cognitive dysfunction with the goal of identifying new therapeutic targets to improve quality of life for the thousands to millions of military and civilian individuals living with TBI. We have developed a new lead compound that inhibits a signal that is activated specifically during the brains response to injury, thus minimizing the likelihood of side-effects. Our data using our new compound shows that giving this new compound immediately after traumatic brain injury prevents the development of post-injury dementia. Remarkably, we have also observed the ability of this compound to reverse post-injury dementia symptoms when given at delayed time-points after the development of dementia. Therefore, the aims of the current study focus on *a new therapeutic target which has the potential to delay and even possible* ***reverse*** *injury-induced dementia symptoms, thus greatly improving the quality of life of soldiers, veterans and civilians living with the burden of brain injury.*

2. Keywords

Traumatic Brain Injury, Transient Receptor Potential, TRPM2, hippocampus, plasticity, LTP, cognition, dementia

3. Accomplishments

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 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).

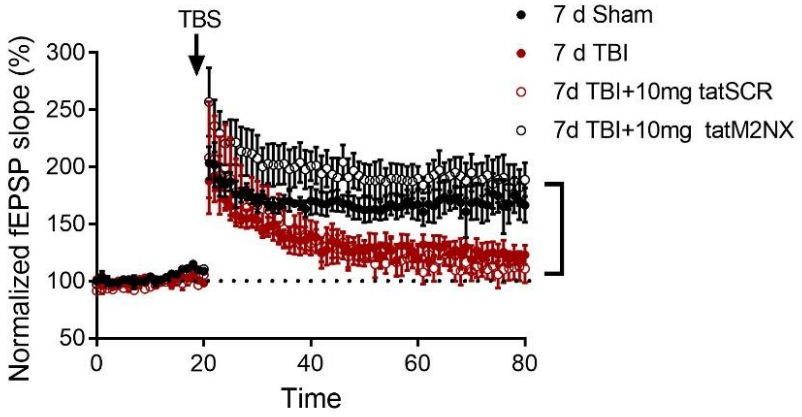
Criteria for Success: Completed dose-response relation in male and female mice with significant effects on memory function and LTP. Significant reduction in TBI-induce memory deficits and LTP deficits observed in a

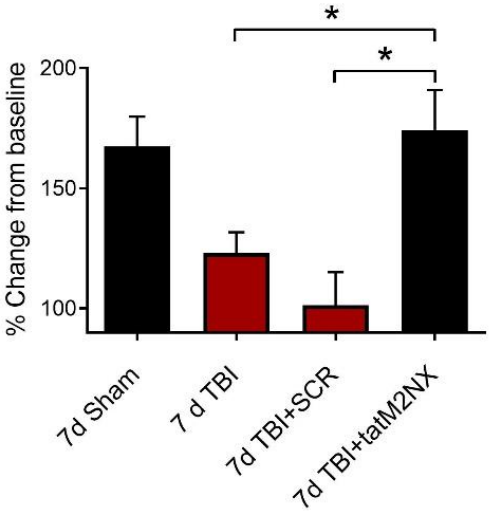
minimum of one experimental group. Statistical significance ($p<0.05$) to be determined using 1-Way ANOVA with post-hoc analysis for comparison of multiple groups. Power analysis was performed using our preliminary data, providing group sizes of 8-10 to generate data with alpha<0.05 and 80% Power. Additionally, a non-efficacious dose to be determined. All experiments performed in a blinded and randomized manner.

Anticipated Completion: Year 1

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.

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.





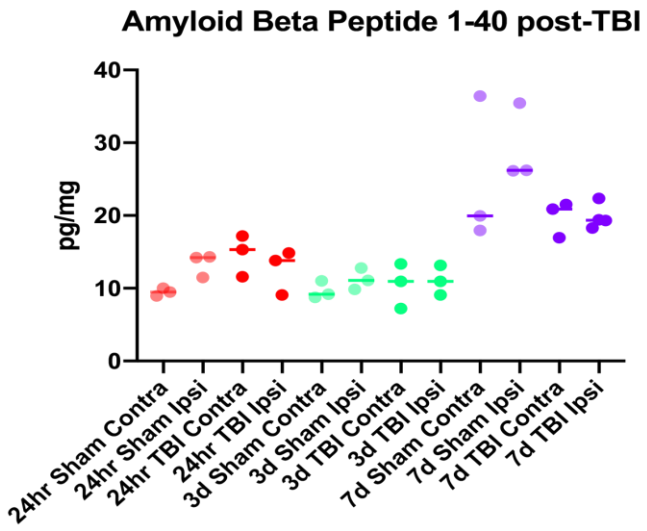
We have completed Milestone #1 in year 1, as outlined in our initial proposal.

1) **Specific Objectives for Milestone 2:**

   A. Major Task 1, Subtask 3: TBI in male mice will be induced by CCI (and sham controls), as described in specific methods of Aim 1. Treatment groups of 10 mice will 2 genotypes, WT vs TRPM2-/-. In addition a single dose of tatM2NX determined in Aim 1 will be used. IHC for APP and ELISA to detect Aβ will be performed on days 1,3,7,30,90 days after recovery from TBI.

2) **Significant Results**

We performed experiments in male mice and collected brain tissue at 24hr, 3 and 7 days after TBI. We observed significant increase in Amyloid β oligomers at 7 days after recovery from TBI, see figure below. These data demonstrate our ability to detect Aβ levels in the brain and show that chronic timepoints, as proposed, are needed to assess the role of TRPM2 and Aβ on TBI-induced ADRD.



We have met approximately 10% of Milestone 2.

Opportunities for training and professional development.

Nothing to report

4. Impact

What was the impact on the development of the principal disciplines of the project?

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. Changes/Problems

COVID-19 has significantly delayed progress. We have made significant advances in our research, but are behind our anticipated progress due to lack of access to the laboratory. Overall, we remain on track to accomplish the goals outlined, although likely will require a no-cost extension to complete.

6. Products

Journal manuscript containing data reported here is under preparation.

7. Participants & Other Collaborating Organizations

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.*

8. Special Reporting Requirements

Nothing to report

9. Appendices

Novel target and lead compound to reverse TBI-induced Alzheimer's related dementia

W81XWH-18-1-0727
AZ170060

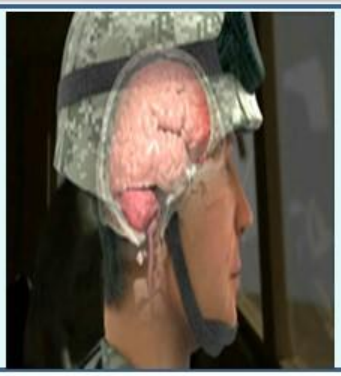
PI: Herson, PS **Org:** University of Colorado School of Medicine **Award Amount:** $776,154

Study/Product Aim(s)

- Analyze ability of acute and delayed administration of tat-M2NX to prevent and reverse TBI-induced cognitive deficits
- Optimize dose and timing of tat-M2NX administration
- Determine mechanism of TBI-induced cognitive deficits, with a focus on the role of Aβ-induced activation of TRPM2 channels and downstream mediators.

Approach

This application uses controlled cortical impact (CCI) model of experimental traumatic brain injury (TBI) to test a new approach to the treatment of TBI-induced cognitive deficits and Alzheimer's like dementia. Specifically, we will optimize our novel new TRPM2 inhibitor to prevent and reverse TBI-induced memory deficits.



Timeline and Cost (Direct+Indirect)

Activities CY	19	20	21
Prevention of TBI-induced AD/ADRD			
Inhibition of APP/Aβ accumulation			
Reversal of TBI-induced AD/ADRD			
Mechanism of TBI and Aβ deficits			
Estimated Budget ($K)	**$258,718**	**$258,718**	**$258,718**

Updated: October 1, 2020

Goals/Milestones

CY19 Goal – tat-M2NX efficacy
- Behavior and LTP recordings in mice treated with tat-M2NX

CY20 Goals – Reversal of TBI-induced AD/ADRD
☐Optimize timing regimen of delayed tat-M2NX treatment
25% Behavior and LTP measurements of delayed administration

CY21 Goal – Mechanism of TBI and Aβ-induced ADRD
☐ Pharmacology and biochemistry

Comments/Challenges/Issues/Concerns

- If timelines change, comment here. Nothing to report
- If off by more than one quarter in spending, comment here.