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TITLE: Targeting Toxic Oligomeric Protein Variants Generated After Traumatic Brain Injury to Decrease Risk of AD

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14. ABSTRACT
We analyzed brain tissue from a wild-type traumatic brain injury mouse model for presence of specific Alzheimer's disease and related dementia (ADRD) protein variant pathology. We determined the levels of 7 different ADRD related protein variants (2 beta-amyloid variants, 2 tau variants, 1 TDP-43 variant and 2 a-syn variants) in 10 different brain regions as a function of time following TBI. All protein variant levels were elevated shortly after injury even in the sham mice due to craniotomy necessary for the fluid percussion injury model. In general there was a short term increase in protein variant levels near the site of injury an craniotomy and a short term deficit in behavioral outcomes which resolved by 14 days after injury. However individual mice showed residual long term behavioral deficits and presence of toxic protein variants. We correlated the levels of different protein variants with behavioral outcomes for each mouse and found statistically significant correlations between long term accumulation of a specific oligomeric beta-amyloid variant and long term cognitive deficit and between a specific oligomeric tau variant and emotional disruption. Additional studies to confirm these results in a second TBI model using a milder TBI with additional co-stressors are underway.

15. SUBJECT TERMS
None listed.

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Table of Contents

1. INTRODUCTION

Traumatic Brain Injury (TBI) affects over 1.7 million people each year, including around 10-20 % of soldiers serving in Iraq and Afghanistan. Stress and injury from TBI induce changes in the brain that can disrupt cognitive functioning. Stress induces increased neuronal expression of both the Amyloid Precursor Protein (APP) and BACE-1 resulting in increased levels of amyloid-beta (Aβ) a known risk factor for AD, suggesting a potential mechanism for why patients that incur TBI are at greater risk of developing AD and at an earlier age. Neuronal axons are particularly vulnerable to the high sheer forces and mechanical deformation induced by TBI, which can damage protein transport mechanisms resulting in axonal accumulation of neurofilament proteins such as tau. While the mechanisms of progression and risk factors for TBI to convert to different neurodegenerative diseases are not well known, TBI is known to induce neuronal stress, which subsequently results in formation of toxic variants of different neuronal proteins including Aβ, tau, a-syn, and TDP-43. Our long-range hypothesis is that protein variants of Aß, tau, a-syn, and TDP-43 are generated in the brain following TBI and that the specific profile of the generated toxic protein variants determines the susceptibility of each individual for different neurodegenerative diseases. Our proposal objective is to develop a mouse TBI model that replicates key elements of the biomarker profile observed in early AD patients and to demonstrate that selectively targeting toxic protein variants is an effective therapeutic to minimize neuronal damage and subsequent risk of AD following TBI.

2. KEYWORDS

Traumatic brain injury, Alzheimer's disease, beta-amyloid, tau, TDP-43, alpha-synuclein, aggregation, misfolding, protein variants, mouse models, protein pathology,

3. ACCOMPLISHMENTS

Following are Milestones and progress to date for this project

Milestone 1 IACUC approval for animal studies and ACURO approval
Progress: Completed: We obtained both IACUC and ACURO approvals.

Milestone 2. Induce midline FPI TBI in two mouse models, wild type and ApoE4,4, utilizing FPI model. Both male and female mice will be utilized
Subtask 1 Induce TBI in wt mice with subsequent co-morbidity stressors (*n*=120)
Subtask 2 Induce TBI in ApoE4,4 mice with subsequent co-morbidity stressors (*n*=120)
Subtask 3 Perform cognitive, motor, sensorimotor behavioral assessments
Milestone Achieved: Induce TBI in male and female mice two mouse models and complete behavioral assessment
Progress: Subtasks 1 and 2: Induced midline fluid percussion brain injury at two severities (mild, moderate) or control sham surgery. These include male and female mice from two genotypes (wild type, Apoe4,4). Mice have been acclimated to piezoelectric sleep cages and baseline sleep measurements are being recorded.
Subtask 3: Behavioral testing is in progress.
We have made substantial progress and are on track to complete Milestone 2 early in year 2 as planned.

Milestone 3. Characterize the toxic protein variant fingerprint generated in brain tissue of each of the different TBI cohorts
Subtask 1 Grow, purify and validate nanobodies.
Subtask 2 Assess reactivity of all wt mouse brain tissue samples with pool of nanobodies including cortex and hippocampus
Subtask 3 Assess reactivity of all Apoe4,4 mouse brain tissue with pool of nanobodies including cortex and hippocampus

Milestone(s) Achieved: Characterization of brain tissue samples from both mouse groups
Progress: Subtasks 1-3. We characterized the protein variant fingerprints of wt mice with either a single or double hit TBI without co-morbidity stressors for comparison with the mouse cohorts with co-morbidity stressors.

Milestone 4 Determine the 3-D and temporal protein variant profiles following injury in both mouse models
Subtask 1 Determine the protein variant profile in different spatial regions of the brain including cortex and hippocampus
Subtask 2 Determine the time dependence of the protein variant profiles following stressors
Milestone(s) Achieved: Determination of 3-D and temporal protein variant profiles including time dependence of additional co-morbidity stressors
Progress: Subtask 1 and 2. We determined the protein variant profile in 10 different brain regions of the sham, single and double hit wt mice as a function of time for comparison with the mouse cohorts with co-morbidity stressors. We showed that the protein variant fingerprint in mice with single or double hit TBI essentially returned to wt levels after 14 to 28 days, although individual mice showed persistent levels of different protein variants in different brain regions (see Fig 1 below). Similarly, individual mice also showed behavioral differences after 14 to 28 days after injury (see Fig 2 below)F.

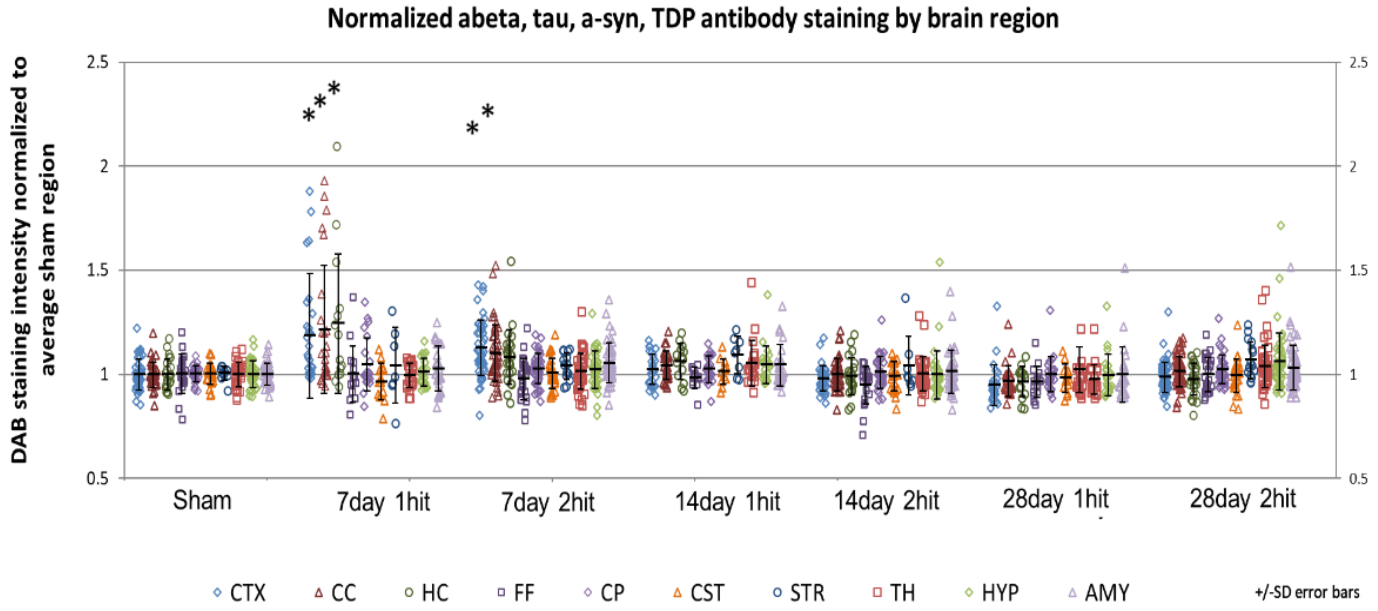

Figure 1 Combined Intensity values of all scFvs. Mouse brain tissue was stained with 7 different scFvs. Staining intensity was quantified by ImageJ and normalized to the average corresponding brain region for the sham mice. Brain tissue was collected at 7, 14, or 28 days after injury. Asterisks are $p < 0.05$ significance to corresponding sham region. Error bars are +/- SD
Brain regions: CTX, upper cortex; CC, corpus callosum; HC, hippocampus; CP, caudoputamen; FF, fornix fimbria fibrils; CST, cerebrospinal tract; STR, striatum; TH, thalamus; HYP, hypothalamus; AMY, amygdala/olfactory region

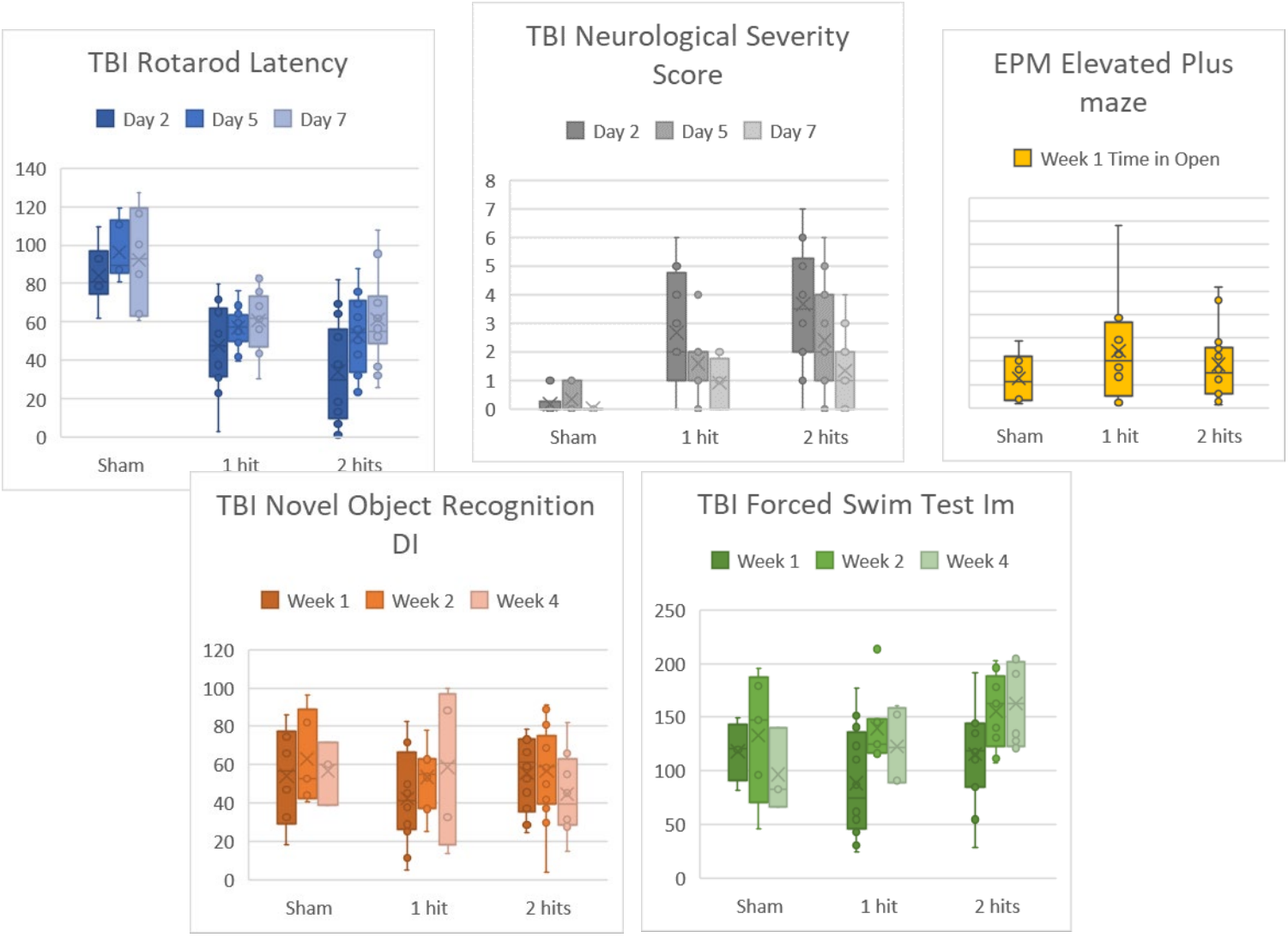

Figure 2. Behavioral tests done on cohorts after 2,5,7 days , or 1,2,4 weeks before tissue harvesting grouped by Sham, single hit or double hit.

Milestone 5 Correlate protein variant levels in specific brain regions with behavioral outcomes
Subtask 1 Correlation of wt mouse brain tissue with behavior
Subtask 2 Correlation of Apoe4,4 mouse brain tissue with behavior
Milestone(s) Achieved: Correlation of protein variant profiles in specific brain regions with different behavioral outcomes in both mouse models
Progress: Subtask 1 and 2. We determined the correlated the protein variant levels of 7 different protein variants in 10 different regions of the sham, single and double hit wt mice as a function of time for comparison with the mouse cohorts with co-morbidity stressors. We found numerous correlations between protein variant levels in different regions 14 days after injury, however 28 days after injury, only two protein variants had statistically significant correlations with specific behavioral deficits, an oligomeric variant of amyloid beta (C6T) and an oligomeric variant of tau (F9T). Both the C6T oligomeric amyloid beta and F9T tau variants are also excellent biomarkers of AD providing a direct mechanistic and pathological link between TBI and increased risk of AD (See Table I below)

Milestone 6 Determine therapeutic potential of selected nanobodies following TBI
Subtask 1 Construct and validate viral vectors expressing most promising Aβ, tau and TDP-43 nanobodies
Subtask 2 Inject vectors IP into wt and Apoe4,4 mice 2 weeks prior to TBI (*n*=320)

Subtask 3 Induce TBI and co-morbidity stressors
Subtask 4 Assess brain pathology and protein variant profiles
Milestone(s) Achieved: Demonstrate therapeutic potential of 3 different nanobodies to ameliorate neuronal damage and pathology following TBI and additional co-morbidity stressors

Table I.
Table I Long term Week 4 behavior and full staining cohort correlated to cognitive decline

R^2	P Value	behavior	staining	antigen	count (n)
0.436	0.0073	W4 FST	F9T CC	tau	15
0.429	0.0080	W4 FST	F9T HYP	tau	15
0.335	0.0486	W4 FST	F9T CST	tau	12
0.311	0.0384	W4 NOR	C6%a CC	β-amyloid	14
0.307	0.0320	W4 FST	C6%a HYP	β-amyloid	15
0.304	0.0330	W4 FST	C6tF HYP	β-amyloid	15
0.274	0.0452	W4 NOR	C6tF CTX	β-amyloid	15

4. IMPACT

It is still too early to determine impact of this project at this stage.

5. CHANGES/PROBLEMS Nothing to report

6. PRODUCTS

a) We presented a poster at the annual Arizona Alzheimer's Consortium Meeting, Tempe AZ. Sept. 20, 2022

Title: Traumatic brain injury generates Alzheimer's disease related protein variants in mouse model brain tissue
Authors Nicholas Panayi[1], Philip Schulz[1], Ping He[1], Rachel K. Rowe[2], and Michael R. Sierks[1]
1. Arizona State University. 2. University of Colorado Boulder.

b) A manuscript entitled "**Traumatic brain injury generates Alzheimer's disease related protein variants in mouse model brain tissue**" is in preparation for submission. The manuscript describes the correlation of AD pathology with behavioral deficits in a TBI mouse model as partially described above (See Figs 1 and 2 and Table I).

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Arizona State University: Dr. Ping He (Post-doctoral associate), Nicholas Panayi (PhD student0, Philip Schulz (Lab Director) and Dr. Michael Sierks (PI) have contributed to this project.

University of Colorado, Boulder: Dr. Rachel Rowe (co-PI)

8. **SPECIAL REPORTING REQUIREMENTS.** None that I am aware of. .

9. APPENDICES

None