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TITLE: TBI-Induced Formation of Toxic Tau and Its Biochemical Similarities to Tau in AD Brains

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of the current study is to demonstrate that blast-induced traumatic brain injury (TBI) and Alzheimer's disease (AD) lead to similar biochemical changes in tau that increase its toxicity and contribute to cognitive and electrophysiological impairments. Specifically we will test the hypothesis that 1) blast-induced TBI leads to the production of a toxic form of tau that contributes to cognitive and electrophysiological impairments; 2) the formation of soluble tau aggregates contributes to cognitive impairments associated with both blast-exposure and AD; 3) an increase in tau phosphorylation contributes to cognitive impairments associated with both blast-exposure and AD. During the last year we have completed experiments related to the first point of the hypothesis, and started working on the second point. Specifically, we have found that the presence of tau is necessary for a preparation from shockwave-exposed mice to reduce 1) memory including contextual fear memory and spatial memory, and 2) long-term potentiation, a type of synaptic plasticity thought to underlie learning. We have also performed a dose response curve for the toxic effect of blasted tau onto memory and LTP.

15. SUBJECT TERMS
Contextual fear memory, spatial memory, synaptic plasticity, traumatic brain injury, Alzheimer's disease.

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1 INTRODUCTION

Although epidemiological studies find a strong link between TBI and an increased risk for dementia (i.e. AD), the molecular mechanisms responsible remain unclear. Evidence continues to accumulate highlighting the similarities between AD and post-TBI pathologies. A similarity between TBI and AD-related neurodegeneration exists at the histological level where both are characterized by the presence of aggregates of hyperphosphorylated forms of the microtubule associated protein, tau. Tau abnormalities and neurofibrillary tangles (NFTs), the classical histopathological hallmark of AD consisting of insoluble aggregated tau, have been reported in multiple animal models of TBI. NFTs like those in AD have been reported after a single TBI in humans. Evidence also exists in favor of a link between TBI and amyloid-β (Aβ), the amyloid precursor protein (APP) proteolytic fragment thought to act upstream of tau in AD [1] that deposits in amyloid plaques. After experimental TBI in animal models, Aβ accumulated in injured neurons and axons both acutely and chronically. Similar deposits of Aβ have been observed after a single TBI in humans. The proposed research project seeks to define the toxic molecular mechanism leading to TBI and AD.

2 KEYWORDS

Tau, contextual fear memory, spatial memory, synaptic plasticity, traumatic brain injury, Alzheimer's disease

3 ACCOMPLISHMENTS

a. What were the major goals?

Work performed during the third year of funding aimed to experiments described in Aim 2 "Test the hypothesis that the formation of soluble tau aggregates contributes to cognitive impairments associated with both blast-exposure and AD". Specifically, we have tested the oligomer-dependence of impairments in behavior and electrophysiology produced by tau purified from shockwave-exposed mice. Furthermore, we worked on Aim 3 "Test the hypothesis that a similar increase in tau phosphorylation contributes to cognitive impairments associated with both blast-exposure and Alzheimer's Disease." by examining whether tau hyperphosphorylation is responsible for the impairment of memory and synaptic plasticity in shockwave-exposed mouse brains.

b. What was accomplished under these goals?

To achieve the goal of demonstrating that oligomer-dependence of impairments in behavior and electrophysiology produced by tau purified from shockwave-exposed mice, the material derived from blasted mice and control animals was de-oligomerized using a reducing agent, DTT, and then re-oligomerized. Behavioral consisted of the following tasks: Novel open field environment test, radial arm

water maze, contextual fear conditioning, cued fear conditioning, visual platform task, sensory threshold. Electrophysiology consisted of investigating LTP. For behavior, we infused the extract of brain preparation onto the dorsal hippocampi bilaterally. Infusion of de-oligomerized tau from blasted mice did not impair spatial memory and contextual fear memory whereas re-oligomerized tau impaired the two types of memory. Control behavioral tasks including novel open field environment test, cued fear conditioning, visual platform task, and sensory threshold did not show any effect of the various preparations. These data confirmed our hypothesis that the oligomerized preparation (but not the de-oligomerized one) impairs memory as well as synaptic plasticity. Interestingly, these results were similar to the effect of tau extracted with similar techniques from the brain of AD patients which impaired both memory and LTP when the preparation was oligomerized (but not de-oligomerized) (Fa' et al, Sci Rep. 2016 6:19393), confirming the possibility that molecular similarities between tau prepared from shockwave-exposed mouse brains and human AD brains may underlie a common ability to produce cognitive impairment when infused into normal mice.

Next, we tested the effect of the PP2A methylesterase PME and the PP2A methyltransferase LCMT overexpression on sensitivity to cognitive and electrophysiological impairments caused by tau purified from shockwave-exposed mice. In a series of experiments, we conducted analysis of the two-day radial arm water maze and contextual fear conditioning performance on PME transgenic animals infused with purified tau from either shockwave or sham-exposed mice at subtoxic concentrations of 4.59 µg/ml. We found that subtoxic doses of blast tau, but not sham tau, were capable of impairing memory tested both with the radial-arm water maze and the fear conditioning in the PME transgenic mice, but not in the non-transgenic littermates (Fig. 1A-B). Control behavioral tasks including novel open field environment test, cued fear conditioning, visual platform task, and sensory threshold did not show any effect in the

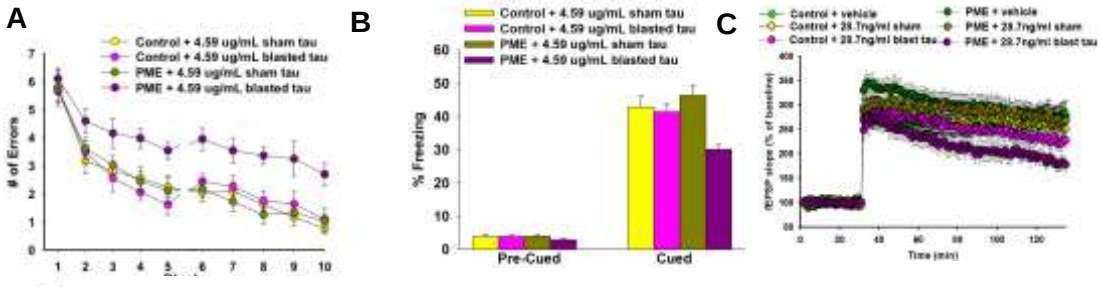

Fig. 1 PME overexpression enhances shockwave-exposed tau ability to lead to the impairing of memory and LTP. A) 2-day radial-arm water maze performance in PME transgenic mice infused with 4.59 µg/ml tau purified from shockwave-exposed or sham animals (*p<0.05, RM-ANOVA,). **B)** Percent of freezing during contextual Fear Conditioning test in PME transgenic mice infused with 4.59 µg/ml tau purified from shockwave-exposed, or sham control animals (*p<0.05, Bonferroni post-hoc comparisons). **C)** Amounts of LTP in slices from PME transgenic mice perfused for 20 min with 28.7 ng/ml shockwave exposed tau, or sham tau, prior to eliciting LTP (*p<0.05, Tukey's post-hoc test).

various groups of mice. We have also compared the ability of tau to interfere with LTP when bath applied to acute hippocampal slice preparations from PME transgenic mice at 28.7 ng/ml. We found an impairment of LTP in slices from PME transgenic mice treated with blasted tau, but not with sham tau (Fig 1C). Taken together, these data demonstrate that overexpression of the PP2A methylesterase, PME, alters sensitivity to exposure to tau oligomers extracted from blast injured brains.

In another series of experiments we tested the ability of LCMT overexpression to protect against the impairment of memory and LTP produced by blasted tau. Analysis of the two-day radial arm water maze and contextual fear conditioning performance on LCMT transgenic animals infused with purified tau from either shockwave or sham-exposed mice at concentrations of 22.9 µg/ml showed that toxic doses of blast tau were no longer capable of impairing memory tested both with the radial-arm water maze and the contextual fear conditioning in the LCMT transgenic mice, whereas memory was impaired in the non-transgenic littermates (Fig. 2A-B). Control behavioral tasks including novel open field environment test, cued fear conditioning, visual platform task, and sensory threshold did not show any effect in the various groups of mice. We have also compared the ability of tau to interfere with LTP when bath applied to acute hippocampal slice preparations from LCMT transgenic mice at 114.7 ng/ml. We found no impairment of LTP in slices from LCMT transgenic mice treated with blasted tau, whereas slices from non transgenic littermates showed an impairment (Fig 2C). Taken all together, these data demonstrate that overexpression of the PP2A methyltransferase, LCMT, alters sensitivity to exposure to tau oligomers extracted from blast injured brains.

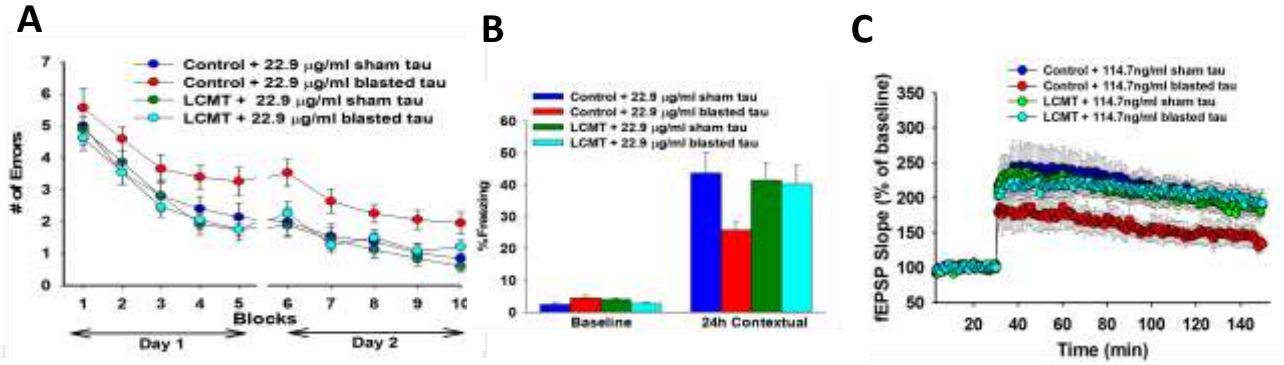

Fig. 2 LCMT overexpression protects against shockwave-exposed tau ability to lead to the impairing of memory and LTP. A) 2-day radial arm water maze performance in LCMT transgenic mice infused with 22.9 µg/ml tau purified from shockwave-exposed or sham animals (*p<0.01, RM-ANOVA,). **B)** Percent of freezing during contextual fear conditioning test in LCMT transgenic mice infused with 22.9 µg/ml tau purified from shockwave-exposed, or sham control animals (*p<0.05, Bonferroni post-hoc comparisons). **C)** Amounts of LTP in slices from LCMT transgenic mice perfused for 20 min with 14.7 0 ng/ml shockwave exposed tau, or sham tau, prior to eliciting LTP (*p<0.01, Tukey's post-hoc test).

We are currently testing the effect of LCMT overexpression on the production of toxic tau in shockwave-exposed mice. To this end we have already collected the brain tissue from LCMT overexpressing transgenics and control mice exposed to sham or blast injury. We have also extracted tau from these

samples using fast protein liquid chromatography (FPLC). In future experiments we will perform the behavioral and LTP experiments as detailed earlier. We will compare the behavioral and electrophysiological effect of tau extracted from shockwave-exposed LCMT overexpressing mice to that of tau extracted from shockwave-exposed non-transgenic mice that will be adminstred to WT mice.

c. What opportunities for training and professional development has the project provided

Nothing to Report"

d. How were the results disseminated to communities of interest?

Nothing to Report"

e. What do you plan to do during the next reporting period to accomplish the goals?

We will continue working on the hypothesis that a similar increase in tau phosphorylation contributes to cognitive impairments associated with both blast-exposure and Alzheimer's Disease. Specifically, we will continue investigating Aim 3C "Test the effect of LCMT overexpression on the production of toxic tau in shockwave-exposed mice" by examining whether tau derived from the brain of LCMT blasted mice is capable of impairing memory and synaptic plasticity in WT mice.

4 IMPACT

a. What was the impact on the development of the principal discipline

Our studies have provided depth to the identification of tau as a culprit in TBI.

b. What was the impact on other disciplines?

Our studies indicate a very interesting similarity between TBI and Alzheimer's disease with tau being similarly affected in the two conditions and being held responsible for the cognitive problems linked with them.

c. What was the impact on technology transfer?

Nothing to Report"

d. What was the impact on society beyond science and technology?

Our studies are important as they are likely to impact the development of therapies against TBI and Alzheimer's disease.

5 CHANGES/PROBLEMS

No changes, nor problems

6 PRODUCTS

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. What individuals have worked on the project

b. Name:	Ottavio Arancio
Project Role:	Principle Investigator
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	1
Contribution to Project:	Dr. Arancio has supervised the whole project, insuring that the project is conducted in an efficient manner and with the highest scientific standards.
Funding Support:	National Institute of Health, Alzheimer's Drug Discovery Foundation

Name:	Russell Nicholls
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	2

Contribution to Project:	Dr. Nicholls has provided his expertise on tau biochemistry and behavioral assessment. He has purified tau from sham and blasted mice.
Funding Support:	National Institute of Health

Name:	Barclay Morrison
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	1
Contribution to Project:	Dr. Morrison has provided his expertise on traumatic brain injury and usage of blast tube.
Funding Support:	National Institute of Health, Paul Allen Family Foundation

Name:	Sowmya Sundaresh
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	6
Contribution to Project:	Sowmya has performed the behavioral experiments
Funding Support:	N/A

Name:	Lewis Brown
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	1234567

Nearest person month worked:	1
Contribution to Project:	Coordinated Proteomic experiments
Funding Support:	

Name:	Nicholas Kanaan
Project Role:	Co-Investigator (Subaward PI)
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	1
Contribution to Project:	Coordinated experiments, analyzed and interpreted data, and prepared reports.
Funding Support:	National Institute of Health, Brightfocus Foundation, Michael J. Fox Foundation

Name:	Collin Richards
Project Role:	Technician
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	9
Contribution to Project:	Prepared samples, ran assays, collected and analyzed data
Funding Support:	N/A

Name:	Chelsea Hamel
Project Role:	Technician
Researcher Identifier (e.g. ORCID ID):	1234567

Nearest person month worked:	3
Contribution to Project:	Prepared samples, ran assays, collected and analyzed data
Funding Support:	N/A

c. Has there been a change in the active or other support of the PD or key personnel during the last reporting period?

Dr. Ottavio Arancio

W81XWH-15-1-0550 (Arancio) 09/15/2015 - 09/14/2019 (NCE) 1.00 Calendar
DoD
TBI-Induced Formation of Toxic Tau and Its Biochemical Similarities to Tau in AD Brains
This project seeks to determine changes in tau status that are evoked by traumatic brain injury.

R01 NS092045 (Arancio/Nicholls) 02/15/2015 - 12/31/2019 1.20 Calendar
NIH/NINDS
The regulation of beta-amyloid sensitivity and Alzheimer's related impairments by PP2A
This project seeks to examine the ability of the serine/threonine protein phosphatase, PP2A, to control sensitivity to the pathological actions of beta-amyloid, a protein that accumulates in the brain of Alzheimer's disease patients.

R01 AG049402 (Arancio) 09/01/2015 - 03/31/2020 2.40 Calendar
NIH/NINDS
Extracellular tau oligomers and Alzheimer disease
This project seeks to establish extracellular soluble species of tau as major toxic species responsible for reduction of synaptic plasticity and memory in Alzheimer's disease.

R01 AG050658 (Bartolini) 09/01/2016 – 05/31/2021 0.17 Calendar
NIH/NIA
Pathogenic role for formin mediated microtubule stabilization pathways in Alzheimer's disease
Test a unifying theory for the pathogenesis of Alzheimer's disease and examine the role for formins as potential targets in drug therapies aimed at rescuing Aβ and phospho--tau toxicity in Alzheimer's disease.
Role: Co-I

R56 AG056108 (Arancio/Verderio) 09/15/2017 – 08/31/2019 (NCE) 0.48 calendar
NIH/NIA
On the role of microglia-derived extracellular vesicles in amyloid-beta induced changes in synaptic function and network activity in Alzheimer's disease
The goal of this project is to determine whether Aβ-containing extracellular vesicles originating from microglia may result in synaptic and network activity dysfunction in AD, and whether the cellular prion PrPc protein mediates trans-synaptic propagation of these vesicles.

A2018816S (Arancio/Nicholls) 07/01/2018 – 0.24 calendar
06/30/2021 Brightfocus
Tau-induced impairments at hippocampal tripartite synapses
The goal of this project is understand how tau's pathological actions in the presynaptic, postsynaptic, and astrocytic constituents of hippocampal synapses contribute to synaptic dysfunction.

RF1 AG055125 (Arancio/Nicholls) 08/01/2018 – 07/31/2023 1.20 calendar
NIH/NIA
The role of methylation-sensitive PP2A isoforms in regulating the pathological response to tau
The goal of this project is to understand how PP2A regulates pathological responses to toxic forms of tau.

R56 AG058449 (Gosh/Arancio) 09/30/2018 – 08/31/2019 1.59 calendar
NIH/NIA
ECSIT protects against neurodegeneration and Alzheimer's disease through the regulation of mitochondrial function and oxidative stress
The goal of this project is to investigate the contribution of the mitochondrial protein ECSIT to the regulation of mitochondrial function, mitochondrial reactive oxygen species production, and mitophagy, in the context of AD pathogenesis and progression.

R01 NS104390 (Tang) 09/01/2018 – 06/30/2023 0.48 calendar
NIH (Arancio portion)
Cellular and Molecular basis for cognitive impairment associated with Glucocerebrosidase (GBA1) mutation
The goal of this project is to define how GBA1 plays a role in the reduction of memory in Parkinson's disease
Role: Co-I

R01 AG059854 (Teich) 09/15/2018 – 05/31/2023 0.01 calendar
NIH (Arancio portion) Years 4-5 only A
Translational Bioinformatics Approach to Rescuing Synaptic and Neurophysiologic Dysfunction in Alzheimer's Disease
The goal of this project is to use bioinformatics tools to defines genes and molecules that negatively influence synaptic function in Alzheimer's disease
Role: Co-I

R01CA222931 (Amengual) 09/21/2018 – 08/31/2023 0.60 calendar
NIH (Arancio's portion)
Development of first-in-class Histone Acetyltransferase (HAT) Activators for Precision targeting of Epigenetic Derangements in Lymphoma
The goal of this project is to determine whether and how the novel HAT activator, YF2, can be used to in the therapy diffuse large B-cell lymphoma (DLCBL)
Role: Co-I

Dr. Russell Nicholls
W81XWH-15-1-0550 (Arancio) 09/15/2015 – 06/30/2019 1.54 Calendar
DoD

TBI-Induced Formation of Toxic Tau and Its Biochemical Similarities to Tau in AD Brains
This project seeks to determine changes in tau status that are evoked by traumatic brain injury.

R01 NS092045 (Arancio/Nicholls) 02/15/2015 - 12/31/2019 3.00 Calendar
NIH/NINDS
The regulation of beta-amyloid sensitivity and Alzheimer's related impairments by PP2A
This project seeks to examine the ability of the serine/threonine protein phosphatase, PP2A, to control sensitivity to the pathological actions of beta-amyloid, a protein that accumulates in the brain of Alzheimer's disease patients.

R01 NS101134 (Mouradian/Nicholls) 05/01/2017 – 04/30/2022 1.80 Calendar
NIH/NINDS (Subaward – Rutgers) PP2A
Dysregulation in the Pathogenesis of alpha-Synucleinopathies
The goal of this project is to understand how PP2A activity and synuclein phosphorylation affect pathological actions of alpha-synuclein.

A2018816S (Arancio/Nicholls) 07/01/2018 – 1.20 calendar
06/30/2021 Brightfocus
Tau-induced impairments at hippocampal tripartite synapses
The goal of this project is understand how tau's pathological actions in the presynaptic, postsynaptic, and astrocytic constituents of hippocampal synapses contribute to synaptic dysfunction.

R01AG055125 (Arancio/Nicholls) 08/01/2018 – 07/31/2023 3.96 calendar
NIH/NIA
The role of methylation-sensitive PP2A isoforms in regulating the pathological response to tau
The goal of this project is to understand how PP2A regulates pathological responses to toxic forms of tau.

Dr. Barclay Morrison
W81XWH-15-1-0550 (Arancio) 09/15/2015 - 09/14/2019 (NCE) 1.00 Calendar
DoD
TBI-Induced Formation of Toxic Tau and Its Biochemical Similarities to Tau in AD Brains
This project seeks to determine changes in tau status that are evoked by traumatic brain injury.

PI: Meaney/Morrison 07/2017 – 06/2022 1.92 calendar
Paul Allen Family Foundation 2,065,000
Reconstructing Concussion
The purpose of this grant is to uncover mechanisms and principles of concussive injury, repair and recovery at multiple scales from single cells to whole animals.

PI: Morrison 09/2017 – 12/2019 0.48 calendar
Department of Army 280,000
Long term potentiation deficits after repetitive primary blast
The purpose of this grant is to determine tolerance criteria to repetitive primary blast in organotypic brain slice cultures.

5R01EB009041 (Konofagou) 09/2014 – 08/2019 0.48 calendar
NIBIB

Optimization of ultrasound-facilitated blood-brain barrier opening
The purpose of this competitive renewal grant is to optimize ultrasound parameters for non-invasive opening of the BBB.
Role: Co-I

R44NS086118 (Graudejus) 09/2018 – 08/2020 1.20 calendar
NIH/NINDS
Lab-To-Marketplace: Commercialization of a stretchable microelectrode array
The purpose of this Phase II SBIR grant is to develop a commercially viable system for studying neurotrauma with stretchable microelectrode arrays for commercial sales for research purposes
Role: Co-I

Dr. Lewis Brown

No changes

Dr. Nicholas Kanaan
R01 AG044372 (Kanaan-PI) 9/30/14 – 4/30/19 3.0 calendar
NIA (NIH)
Tau-induced axonal degeneration in Alzheimer's disease and tauopathies
The main goal of this proposal is to identify the molecular mechanisms underlying axonal degeneration induced by AT8 phosphorylated tau using a viral vector rat model and a rat primary neuron model.

R01 NS082730 (Kanaan-PI, Brady-PI) 4/01/14-3/31/19 3 months
NINDS(NIH)
Tau Conformation in Tauopathies and Neuronal Function
This R01 is aimed at studying how tau conformation in various oligomeric forms affects its toxicity through axonal transport impairment and how tau conformation is regulated under normal biological conditions.

P01 AG14449 (Counts-PL, Kanaan-CoI) 7/01/97-6/30/19 1.2 months NIA
(NIH)
Neurobiology of Mild Cognitive Impairment in the Elderly
This PPG contains multiple projects that investigate the neurobiological substrates of cognitive decline in the elderly using the cholinergic basal forebrain as a model system for selective vulnerability.

W81XWH-15-1-0550 (Arancio-PI; Kanaan-CoI) 9/1/15-8/30/2018 0.6 months
DoD
TBI-Induced Formation of Toxic Tau and Its Biochemical Similarities to Tau in AD Brains
The purpose of this grant is to explore the molecular mechanisms that underlie the cognitive decline and mental health problems resulting from repetitive traumatic brain injuries

Gibby vs Parky Parkinson's Disease Research (Kanaan/Moore) 7/1/2018 – 06/30/2020 0.24 calendar
"LRRK2 and Tau in Parkinson's Disease"
Goal: The aim of this project is to study the role of LRRK2 mutations in the spread of tau in the brain in the context of animal and cell models of Parkinson's disease.

d. What other organizations were involved as partners?

None to Report

8. SPECIAL REPORTING REQUIREMENTS

Not applicable

9. APPENDICES

Nothing to report