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TITLE: Role of Amyloid Precursor Protein in Alzheimer's Disease-Related Impairment of Synaptic Function and Memory Induced by Abnormal Tau Following TBI

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CONTRACTING ORGANIZATION: Columbia University Medical Center

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Role of Amyloid Precursor Protein in Alzheimer's Disease-Related Impairment of Synaptic Function and Memory Induced by Abnormal Tau Following TBI

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6. AUTHOR(S)

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Columbia University in the City of New York

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14. ABSTRACT

In the proposed research project we will test the hypothesis that tau derived from TBI brains produces a similar impairment in synaptic function and memory as tau from AD brains and thereby identify the mechanisms whereby tau derived from TBI leads to the development of abnormal synaptic function and memory that are associated with AD. Specifically, we will address the following aims: Aim 1) To test the prediction that tau derived from TBI brains produces changes in synaptic plasticity, neurotransmission, and neuronal excitability that are associated with AD. Aim 2) To test the prediction that the presence of amyloid-precursor protein (APP) is necessary for tau derived from TBI brains to produce the impairments of synaptic function and memory associated with AD. Aim 3) To test the prediction that phosphorylation at the APP-Thr668 site is necessary for tau derived from TBI brains to produce the impairments of synaptic function and memory that are associated with AD. During the last year we have worked on the first aim. We have found that similar to AD tau, administration of tau purified from shockwave-exposed mice onto wild-type mice markedly reduces certain forms of short-term plasticity. Additional, planned electrophysiological experiments are ongoing that will allow us to complete aim 1 and provide new insights into the similarities in tau changes between TBI and AD.

15. SUBJECT TERMS

Tau, amyloid precursor protein, neurotransmission, memory, traumatic brain injury, Alzheimer's disease

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1. INTRODUCTION:

In the proposed research project we will test the hypothesis that tau derived from TBI brains produces a similar impairment in synaptic function and memory as tau from AD brains and thereby identify the mechanisms whereby tau derived from TBI leads to the development of abnormal synaptic function and memory that are associated with AD.

2. KEYWORDS:

Tau, amyloid precursor protein, neurotransmission, memory, traumatic brain injury, Alzheimer's disease

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Work performed during this second year of funding aimed to address the subtask 3 of Aim 1 of the project "Compare the passive and active membrane properties of presynaptic CA3 and postsynaptic CA1 pyramidal cells in slices treated with tau derived from TBI brains and tau derived from AD brains", as well as Aim 2 "test the prediction that the presence of APP is necessary for tau derived from TBI brains to produce the impairments of synaptic function and memory that are associated with AD." Specifically, with respect to Aim 2, we had to compare the magnitude of long-term potentiation (LTP), three types of short-term synaptic plasticity including post-tetanic potentiation (PTP), paired-pulse facilitation (PPF), and synaptic fatigue (SF), as well as membrane passive and active properties recorded from slices from APP-KO mice and control mice perfused with tau derived from TBI brains and tau derived from AD brains. Additionally, we had to compare behavioral performance with the RAWM test and the contextual fear conditioning of APP-KO mice and control mice infused with tau derived from TBI brains and tau derived from AD brains.

What was accomplished under these goals?

We have found that administration of tau purified from shockwave-exposed mice (blast tau) onto slices from APP-KO mice no longer reduces long-term potentiation (LTP) whereas administration onto slices from wild type mice markedly reduces it (Fig. 1). This effect was similar to the effect of tau derived from AD patients (AD tau).

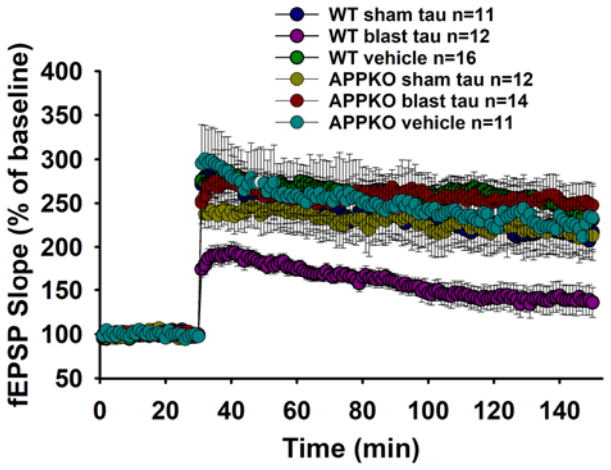

Figure 1: APP-KO slices display normal potentiation after perfusion with tau derived from shockwave exposed mice. Long-term potentiation (LTP) is not impaired following APP-KO hippocampal slice perfusion with of tau from shockwave exposed mice (blast tau) (n corresponds to number of slices per group of mice in this graph and the following ones; $p>0.05$, Two-way ANOVA). By contract LTP was significantly lowered in WT slices perfused with tau from shockwave exposed mice compared to sham tau slices ($p<0.05$, Two-way ANOVA). Sham-tau corresponds to tau derived from mice that did not undergo blast.

We have found that administration of tau purified from shockwave-exposed mice onto slices from APP-KO mice no longer reduces post-tetanic potentiation (PTP), a type of synaptic plasticity due to the release of calcium in the presynaptic terminal immediately after the tetanic stimulation that induces plasticity, whereas administration onto slices from wild type mice markedly reduces it (Fig. 2). This effect was similar to AD tau.

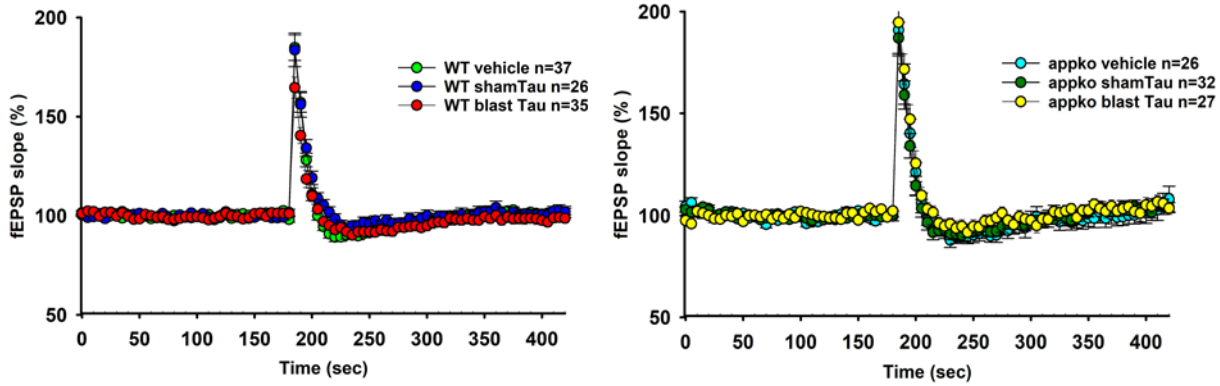

Figure 2: APP-KO slices display normal post-tetanic potentiation (PTP) after perfusion with tau derived from shockwave exposed mice. PTP is not impaired following APP-KO hippocampal slice perfusion with of tau from shockwave exposed mice ($p>0.05$, T-test). By contract PTP was significantly lowered in WT slices perfused with tau from shockwave exposed mice compared to sham tau slices ($p<0.05$, T-test).

We have found that, similar to AD tau, administration of blast tau onto slices from APP-KO mice does not increase the refilling rate (k) after the 100 Hz stimulation to restore the readily releasable pool (RRP), whereas administration onto slices from WT mice markedly increases it (Table 1).

WT + vehicle	7.986 ± 0.8545	APP-KO + vehicle	7.521 ± 0.9291
WT + sham tau	8.929 ± 0.8767	APP-KO + sham tau	5.795 ± 0.5242
WT + blast tau	20.87 ± 5.801	APP-KO + blast tau	5.197 ± 0.5121

Table 1: Rate of replenishment (k) in APP-KO and WT mice

No effect was observed after administration of blast tau onto slices from both APP-KO and WT mice on: i) paired pulse facilitation, a form of short-term plasticity due to calcium increase when two stimuli are closely spaced with each other, and ii) synaptic fatigue, a form of plasticity due to RRP depletion during a 100 Hz stimulation. These effects were similar to AD tau.

No effect was observed after administration of blast tau onto slices from both APP-KO and WT mice on measures of passive membrane properties including input resistance (Rin), membrane capacitance (Cm), membrane time constant (τ), and the resting membrane potential (RMP). These effects were similar to AD tau.

No effect was observed after administration of blast tau onto slices from both APP-KO and WT mice on measures of active membrane properties including the percentage of cells that generate action potentials (APs) upon depolarization, the full width at half maximum of APs, AP amplitude and threshold, Na^+ and K^+ channels by measurement of the amplitude of Na^+ and K^+ currents. These effects were similar to AD tau.

We have found that, similar to AD tau, administration of blast tau onto slices from APP-KO mice does not increase the mEPSC frequency and amplitude, whereas blast tau administration onto slices from WT mice markedly reduces it.

We have found that, similar to AD tau, infusion of blast tau onto the hippocampus of APP-KO mice does not impair associative memory tested with contextual fear conditioning, whereas blast tau infusion onto hippocampi of WT mice dramatically reduces fear memory (Fig. 3). This effect was similar to AD tau.

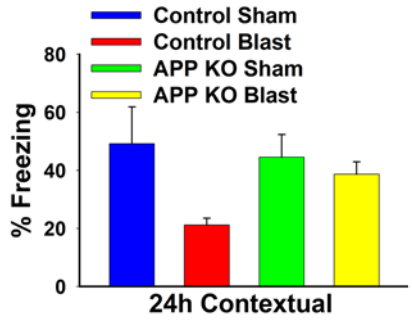

Figure 3: APP-KO mice display normal contextual fear memory after hippocampal infusion with tau derived from shockwave exposed mice. The amount of freezing is not reduced following APP-KO hippocampal infusion with blast tau ($p>0.05$, T-test). By contract freezing was significantly reduced in WT mice infused with blast tau compared to sham tau slices ($p<0.05$, T-test).

We have found that, similar to AD tau, infusion of blast tau onto the hippocampus of APP-KO mice does not impair spatial memory tested with the radial-arm water-maze, whereas blast tau infusion onto hippocampi of WT mice dramatically reduces memory (Fig. 4). This effect was similar to AD tau.

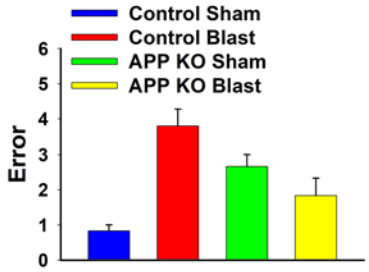

Figure 4: APP-KO mice display normal spatial memory after hippocampal infusion with tau derived from shockwave exposed mice. The number of errors in the last trial of the radial arm water maze is not increased following APP-KO hippocampal infusion with blast tau ($p>0.05$, T-test). By contract errors were significantly higher in WT mice infused with blast tau compared to sham tau slices ($p<0.05$, T-test).

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

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

We will continue testing the hypothesis that tau derived from TBI brains produces a similar impairment in synaptic function and memory as tau from AD brains and thereby identify the mechanisms whereby tau derived from TBI leads to the development of abnormal synaptic function and memory that are associated with AD". Specifically, we will work on aim 3 "test the prediction that phosphorylation at the APP-Thr668 site is necessary for tau derived from TBI brains to produce the impairments of synaptic function and memory that are associated with AD.", by." i) comparing the magnitudes of LTP in slices from APP-T/A KI mice and control mice perfused with tau derived from TBI brains and AD-tau; ii) comparing the behavioral performance with the RAWM test and the contextual fear conditioning of APP-T/A KI mice and control mice infused with tau derived from TBI brains and AD-tau.; iii) comparing the magnitude of changes in short-term synaptic plasticity, neurotransmission, and neuronal excitability, in slices from APP-T/A KI mice and control mice perfused with tau derived from TBI- and AD brains

4. IMPACT:

Our studies have confirmed a major role of tau in TBI. Furthermore, they have expanded the similarity to a role of amyloid precursor protein in the damage of the communication among cells and memory by tau from brains exposed to shockwaves..

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 with respect to LTP, short-term plasticity, memory, neurotransmission, and neuronal excitability.

What was the impact on technology transfer?

Nothing to report.

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:**

:

Changes in approach and reasons for change

No changes nor problems.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report.

Changes that had a significant impact on expenditures

During year one, we had difficulty finding a good post-doctoral candidate to work on this project, this situation has now be resolved with the hire of Andrea Paquola on 09/15/2022 which has allowed for a smoother operation and to increase our expenditures with respect to year 1.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

No changes.

Significant changes in use or care of vertebrate animals

No changes.

Significant changes in use of biohazards and/or select agents

No changes.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

- **Journal publications.**

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Ottavio Arancio
Project Role: PD/PI
Researcher Identifier: https://orcid.org/0000-0001-6335-164X
Nearest person month worked: 1 CM
Contribution to Project: Dr. Arancio is the principal investigator of this project and supervises overall aspects of this award. Dr. Arancio coordinates with Dr. Morrison.

Name: Barclay Morrison
Project Role: Co-Investigator
Researcher Identifier: 0000-0001-7676-0864
Nearest person month worked: 1 CM, 4%
Contribution to Project: Dr. Morrison provides expertise in blast TBI biomechanics and modeling.

Name: Hong Zhang
Project Role: Staff Associate
Researcher Identifier: N/A
Nearest person month worked: 5 CM, 39%
Contribution to Project: Ms. Zhang contributed to the electrophysiology experiments.

Name: Samantha Overcashier
Project Role: Technician
Researcher Identifier: N/A
Nearest person month worked: 4 CM, 32%
Contribution to Project: Ms. Overcashier contributed to the maintenance of the mouse colony.

Name: Andrea Paquola
Project Role: Postdoc
Research Identifier: N/A
Nearest person month worked: 8 CM, 70%
Contribution to Project: Ms. Paquola performed blast TBI injuries in mice, conducted follow up behavior studies, and westerns for tau.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Ottavio Arancio, PD/PI
Recently Completed Research Support since last submission.
PI: Arancio, Ottavio 1.2 Cal. Mo. 8/2018-8/2023
NIH/NIA Total: Project Number: RF1AG055125
Grant officer: Austin Jyan-Yu Yang
"The role of methylation-sensitive PP2A isoforms in regulating the pathological response to Tau"
Our poor understanding of the molecular mechanisms that underlie the cognitive and behavioral impairments that characterize Alzheimer's disease stands as a critical barrier to identifying effective preventative measures and treatments for Alzheimer's disease. This project seeks to address this gap in our understanding by examining the ability of enzymes that control the methylation of the serine/threonine protein phosphatase, PP2A, to control the pathological actions of tau – a central component in the molecular etiology of the disease. PP2A is a heterotrimeric enzyme that exists in multiple isoforms with different expression patterns and substrate specificities, and the activity and composition of these isoforms is regulated by multiple mechanisms including protein methylation. PP2A methylation is controlled by an evolutionarily conserved mechanism involving the competing actions of a dedicated methylesterase and a dedicated methyltransferase – PME-1 and LCMT-1 in mice respectively. In preliminary studies, we found that transgenic over expression of LCMT-1 protected mice from cognitive impairments caused by acute exposure to soluble tau aggregates, while PME-1 over expression increased sensitivity to these impairments. In this proposal, we will build on these observations by pursuing the following specific aims: 1) Determine which aspects of tau pathology are affected by increased PME and LCMT expression levels. 2) Test the hypothesis that inhibiting PME-1 protects against tau related impairments. 3) Test the hypothesis that PME and LCMT expression levels affect sensitivity to tau by altering the proportion of Ppp2r2a-containing PP2A enzymes. These aims will be addressed through a combination of behavioral, electrophysiological, and biochemical techniques in genetically modified mice. The data obtained from these experiments will identify the mechanisms whereby methylation of the catalytic subunit of PP2A controls the development of tau-related impairments in Alzheimer's disease, and test the possibility that interventions that target this pathway could constitute an effective therapeutic approach for their prevention or treatment.
Indicate overlap if any: No overlap.

Recently Awarded Research Support since last submission.
PI: Watterson, Daniel; Arancio, Ottavio effort covered in parent grant 06/2023-04/2024
NIH/NIA Total: Project Number: 3U01AG066722-04S1
Grant officer: Lorenzo Refolo
Aim 1. Perform secondary pharmacology analyses following FDA guidance, as a necessary prelude and a firm foundation for future GxP IND-enabling preclinical safety and toxicology research. Aim 2. Validate the efficacy of MW071 and MW109 in prevention/reversal of synaptic and memory impairments in AD-relevant animal models.
Indicate overlap if any: No overlap.

AZ220062 (DoD CDMRP) PI: Arancio, O. 9/23-8/26
0.36 Cal. Mo. Total:
Department of Defense
"Role of SUMO conjugation in Alzheimer's Disease related impairment of synaptic function and memory induced by abnormal tau following TBI"

The purpose of this grant is to stablish a role for SUMOylation in traumatic brain injury.
Aim 1: To test the prediction that increased SUMO1 conjugation contributes to synaptic and behavioral impairments as well as tau hyperphosphorylation and aggregation in blast and CCI exposure.
Aim 2: To test the prediction that increased SUMO2 conjugation protects against synaptic and behavioral impairments as well as tau hyperphosphorylation and aggregation by blast and CCI exposure.
Aim 3: Test whether the susceptibility to cognitive and behavioral impairment in blast exposure as well as AD is associated with changes in the level of global SUMOylation and/or tau SUMOylation.
Indicate overlap if any: No overlap.

PI: Arancio, Ottavio No committed effort 03/2023-Present
Annovis Project Number: AB CU23-0703
Effects of Buntanetap Combined with a GLP-1 Analogue in a Mouse Model of Alzheimer's Disease
The Study's overall goal is to determine if the combination Buntanetap with Liraglutide (or alternatively another Glucagon-Like Peptide-1, GLP-1, analogue such as Dulaglutide) (here named "the combination") will prevent synaptic dysfunction and memory loss in a murine model of AD, the APP/PS1 animal (line 6.2). This mouse was obtained by crossing the APP mouse (a.k.a. Tg2576) in which the APP transgene is driven by the prion promoter, with the PS1 mouse (line 6.2) in which the mutated PS1 transgene is driven by the PDGF promoter. The electrophysiological and behavioral phenotype of this mouse has been characterized by the laboratory of Dr. Ottavio Arancio (Trinchese et al, Ann. Neurol, 2004).
Grants Officer: Maria Maccecchini, Annovis Bio
Indicate overlap if any: No overlap.

Dr. Barclay Morrison
Recently Completed Research Support since last submission.

PI: Morrison III, B. 25% FTE 3/20-8/23
NeuroTrauma Sciences, LLC
"Effect of NTS compounds in an organotypic slice culture model of TBI"
The purpose of this grant is to test the efficacy of novel compounds in an in vitro model of traumatic brain injury to reduce cell death as well as preserve neuronal function.
Aim1: To measure the effect of NTS compounds on post-traumatic cell death.
Aim 2: To measure the effect of NTS compounds on post-traumatic changes in electrophysiological function.
Indicate overlap if any: No overlap.

Grant Officer:Tom Parry
Chief Science Officer NeuroTrauma Sciences
2655 Northwinds Parkway, Suite 5C, Alpharetta, GA 30009

Recently Awarded Research Support since last submission.

AZ220062 PI: Arancio, O.
Co-I: Morrison III, B. 3% FTE 9/23-8/26
Department of Defense
"Role of SUMO conjugation in Alzheimer's Disease related impairment of synaptic function and memory induced by abnormal tau following TBI"
The purpose of this grant is to stablish a role for SUMOylation in traumatic brain injury.
Aim 1: To test the prediction that increased SUMO1 conjugation contributes to synaptic and behavioral impairments as well as tau hyperphosphorylation and aggregation in blast and CCI exposure.
Aim 2: To test the prediction that increased SUMO2 conjugation protects against synaptic and behavioral impairments as well as tau hyperphosphorylation and aggregation by blast and CCI exposure.
Aim 3: Test whether the susceptibility to cognitive and behavioral impairment in blast exposure as well as AD is associated with changes in the level of global SUMOylation and/or tau SUMOylation.
Indicate overlap if any: No overlap.

Pending Research Support since last submission.
PI: Morrison III, B. 16% FTE 9/23-8/23
NeuroTrauma Sciences, LLC
"Role of the mineralocorticoid receptor in NTS-105 protection after mild traumatic brain injury"
The purpose of this grant is to test the role of mineralocorticoid receptors in the neuroprotective effects of a novel compound in an in vitro model of traumatic brain injury.
Aim1: To measure the effect of injury on the expression of mineralocorticoid receptors after injury in organotypic hippocampal slice cultures.
Aim 2: To measure inflammatory markers after injury in organotypic hippocampal slice cultures and the effect of NTS-105.
Aim 3: To measure the effect of mineralocorticoid receptor antagonists and agonists on neuronal function after injury in organotypic hippocampal slice cultures
Indicate overlap if any: No overlap.

Grant Officer:
Tom Parry
Chief Science Officer
NeuroTrauma Sciences
2655 Northwinds Parkway, Suite 5C
Alpharetta, GA 30009

W911NF-23-S-0002 PI: Morrison III, B. 8% FTE 6/23-5/26
DEVCOM/ARL
"Long term potentiation deficits after repetitive primary blast"
The purpose of this grant is to determine tolerance criteria to repetitive primary blast at occupational levels in organotypic brain slice cultures.
Aim 1: To identify the critical interval between two blasts that leads to worse outcome and the recovery time course identify the critical interval between three blasts that leads to worse outcome and the recovery time course.
Indicate overlap if any: No overlap.

Grant Officer
Michael Kleinberger, Ph.D.
Team Leader, Injury Biomechanics
Soldier Protection Sciences Branch, RDRL-WMP-B
US Army Research Laboratory
Aberdeen Proving Ground, MD 21005

What other organizations were involved as partners?

No other organization has been involved as a partner.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES: *N/A*