

AWARD NUMBER: W81XWH-17-1-0583

TITLE: Neuropathology and Immune Biomarker Discovery in a Rat Model of Alzheimer's Disease, TgF344-AD, with Single or Repetitive Traumatic Brain Injury

PRINCIPAL INVESTIGATOR: Huntington Potter

CONTRACTING ORGANIZATION: Regents of the University of Colorado
Aurora, CO 80045

REPORT DATE: Sept 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE
Sept 2018

2. REPORT TYPE Annual

3. DATES COVERED
01 Sep 2017 – 31 Aug 2018

4. TITLE AND SUBTITLE

Neuropathology and Immune Biomarker Discovery in a Rat Model of Alzheimer's Disease, TgF344-AD, with Single or Repetitive Traumatic Brain Injury

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0583

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Huntington Potter, Timothy Boyd

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

E-Mail: Huntington.Potter@UCDenver.edu, Timothy.Boyd@UCDenver.edu

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

Regents of the University of Colorado
13001 E 17th Place F428
Aurora, CO 80045-2571

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Purpose: The major goals of this project are to develop better models to investigate mechanisms by which traumatic brain injury (TBI) is a risk factor for developing Alzheimer's disease (AD) and related neurodegenerative diseases, including chronic traumatic encephalopathy (CTE). To accomplish these goals, we are using wild-type control rats and transgenic (Tg) AD rat models to conduct and contrast two paradigms of closed-head TBI, and one paradigm of repetitive moderate blast TBI, after which cohorts of varying ages post-injury are studied for immune system changes within both the periphery and central nervous system (CNS) to determine their role in AD pathogenesis.

Scope: The study is limited to developing and characterizing these new TBI-AD models, and does not incorporate treatments or translational efforts for human clinical settings.

Major Findings: After receiving ACURA approval on 1-4-18 to begin animal work, we have been actively expanding the Tg AD rat colony, using all male progeny for breeding, to thereafter age and use within 12-month-old TBI cohorts. Meanwhile, we ordered 60 control rats that are now aged and scheduled for experimental procedures next month. The controlled cortical impactor was placed in the vivarium and validated, accessory equipment and supplies were obtained, and, to date, four 12-month-old rats have completed experimental procedures with biospecimens collected.

15. SUBJECT TERMS

Alzheimer's disease, AD, amyloid-beta, A β , amyloid, blood-brain barrier, BBB, chronic traumatic encephalopathy, CTE, closed-head injury, CHI, controlled cortical impact, CCI, gadolinium, Gd, mild cognitive impairment, MCI, myeloperoxidase, MPO, neurofibrillary tangles, NFTs, phosphorylated tau protein, p-Tau, repetitive moderate TBI, rmTBI, superparamagnetic iron oxide, SPIO, traumatic brain injury, TBI

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
REPORT U Unclassified	ABSTRACT U Unclassified	c. THIS PAGE U Unclassified	UU Unclassified	9	USAMRMC
					19b. TELEPHONE NUMBER (include area code)

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

	<u>Page</u>
1. Introduction.....	5
2. Keywords.....	5
3. Accomplishments.....	5
4. Impact.....	6
5. Changes/Problems.....	6
6. Products, Inventions, Patent Applications, and/or Licenses.....	6
7. Participants & Other Collaborating Organizations.....	7
8. Special Reporting Requirements.....	8
9. Appendices.....	NA

1. INTRODUCTION:

The purpose of this project is to develop better models for investigating potential mechanisms by which traumatic brain injury (TBI) is a risk factor for developing Alzheimer's disease (AD) and related neurodegenerative diseases, such as chronic traumatic encephalopathy (CTE). To accomplish the project's objective, we are using wild-type control rats and transgenic (Tg) AD rat models to conduct and contrast two paradigms of closed-head controlled-cortical impact (CCI) TBI (CCI/TBI), and one paradigm of repetitive moderate blast TBI (rmTBI), after which cohorts of rats of varying ages post-injury will be studied for immune system changes within both the periphery and the central nervous system (CNS) to determine their potential role(s) in AD pathogenesis. Specifically, immune system changes will be determined by examining changes in leukocyte populations, plasma protein biomarkers, and extracellular vesicle profiles. Neuroimaging will be used to examine one-month post-injury chronic neuroinflammatory effects in living animals, and those findings will be confirmed by postmortem immunohistochemical analyses. Notably, the scope of the study is limited to developing and characterizing these new TBI-AD rat models, and the project does not incorporate any treatment regimens or other translational efforts for using these results in a human clinical setting.

2. KEYWORDS:

Alzheimer's disease, amyloid, chronic traumatic encephalopathy, controlled cortical impact, gadolinium, myeloperoxidase, tauopathy, repetitive TBI, superparamagnetic iron oxide, traumatic brain injury

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**
 - To develop new models of AD, CCI/TBI, and rmTBI.
 - Milestone target date of 36 months, as well as local IACUC approval at three months (already achieved) and ACURO approval at six months (already achieved).
 - To identify new peripheral immune system biomarkers associated with AD in CCI/TBI and rmTBI.
 - Milestone target date of 36 months.
 - To identify novel neuroimmune signaling biomarkers associated with AD in CCI/TBI and rmTBI.
 - Milestone target date of 36 months.
- **What was accomplished under these goals?**
 - Major activities: The most necessary activity for conducting the goals of this project is to breed and age the Tg AD rat cohorts. Therefore, we have been using all of the male Tg rat progeny as new breeders to boost our colony's Tg numbers. We did anticipate that obtaining our required cohort numbers would be a slow process until we finally have enough rats breeding at any given time to produce steady cohorts of Tg animals to age for our experimental time points at six and 12 months of age. After these animals are retired from breeding, they are then being aged for the 12-month-old TBI/sham cohorts. As a result of this necessary breeding strategy, we do not have any designated upcoming six-month Tg cohorts yet for TBI or sham procedures. Therefore, in the meantime, we have purchased and imported 60 male F344 background strain control rats, and they have now nearly aged to their six-month-old time point for both CCI/TBI and sham TBI procedures, and the majority of these purchased animals have been allocated and scheduled for upcoming experiments that we will carry out during the upcoming month.
 - Specific objectives: The overall objectives for all three major goals depend on our ability to perform the sham, CCI/TBI, and rmTBI procedures, and then to age the animals to appropriate time points, perfuse them, and collect tissues, including blood processing procedures for obtaining plasma and leukocyte populations. Thereafter, the tissues (brain and blood components) will be examined for both peripheral and CNS immune effects associated with the three TBI paradigms in an AD versus control setting, to determine the potential mechanism(s) by which TBI is a risk factor for AD and related neurodegenerative diseases, and to determine in the rat AD model the most informative TBI paradigm(s), age, and timing post-injury.
 - Significant results or key outcomes: We have placed the Leica Impact One device into the vivarium, have verified its functionality, and have shown that it delivers a mild-to-moderate CCI/TBI at the project's stated parameters. We have also obtained all of the other accessory materials and devices (recovery heating pad, temperature monitor, Type E foam padding,

anesthetics, etc.). Additionally, we have been able to conduct sham or CCI/TBI procedures using four 12-month-old rats, two of which were Tg rats. The rats have since been aged to appropriate post-injury time points, euthanized, and tissues removed and processed for histochemical analyses (i.e., whole brain fixation and paraffin-embedding/sectioning) and biomarker analyses (i.e., plasma aliquoted and biobanked for protein biomarkers and exosomal profiling, and buffy coat processed and cryopreserved for subsequent phenotyping).

- Other achievements: While working to get the blast device constructed, we encountered a significant hurdle when we were informed that the blast noise may affect surrounding animals in the vivarium. We have since met with investigators who are conducting similar blast studies in the research buildings where our animals are housed and who have some ETS-Lindgren noise-attenuation testing chambers. We are currently working to establish a collaborative agreement with them on this project and for future projects. The use of these testing chambers will be of immense benefit when validating our blast device and performing our rmTBI experiments.

○ **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?**

- As described above, we have 60 F344 control rats that will be six months of age in the next few weeks, and they are scheduled to undergo both sham and CCI/TBI procedures. The rats that are not being used in the six-month-old experiments will be aged and used for the 12-month-old cohorts. We will then continue to order F344 control animals that we will age and use for the remaining cohorts. During this next reporting period, we also anticipate that the blast device will be functional and validated, and that we will begin using some of the control animals to fulfill the rmTBI cohorts. Meanwhile, we are continuing to diligently expand the Tg colony, and we will begin to age some of the retired breeders to fulfill the 12-month-old cohorts. With this efficient strategy in place, we anticipate that the latter part of next year's reporting and the beginning of our year three report will be focused primarily on the six-month-old Tg cohorts, when we have an ample supply of Tg litters being produced. We also will begin the immunohistochemical analyses of tissues in this upcoming reporting period, as well as phenotyping of the leukocyte populations.

4. **IMPACT:**

○ **What was the impact on the development of the principal discipline(s) 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:**

○ **Changes in approach and reasons for change**

- Nothing to Report.

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

- Nothing to Report.

○ **Changes that had a significant impact on expenditures**

- Nothing to Report.

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

- Nothing to Report.

○ **Significant changes in use or care of human subjects**

- Nothing to Report.

○ **Significant changes in use or care of vertebrate animals.**

- Nothing to Report.

○ **Significant changes in use of biohazards and/or select agents**

- Nothing to Report.

6. **PRODUCTS:**

- **Publications, conference papers, and presentations. Nothing to Report.**

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**
 - **We have started biospecimen collection of tissues and whole blood components of the animals that have undergone sham or CCI/TBI procedures, and these biospecimens are being banked until we obtain appropriate batch numbers in which to process them, as specified in the protocol.**

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	<i>Huntington Potter, PhD</i>
Project Role:	<i>PD/PI</i>
Researcher Identifier (e.g. ORCID ID):	<i>N/A</i>
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Oversees execution of the project</i>
Funding Support:	

Name:	<i>Timothy Boyd, PhD</i>
Project Role:	<i>Co-Investigator</i>
Researcher Identifier (e.g. ORCID ID):	<i>N/A</i>
Nearest person month worked:	<i>5</i>
Contribution to Project:	<i>IACUC protocol, procurement of materials needed for project, development of blast device, conducting CCI/TBI and sham procedures</i>
Funding Support:	<i>Insufficient DoD funds supplemented by philanthropy</i>

Name:	<i>Neil Markham, MBA</i>
Project Role:	<i>Senior Professional Research Assistant</i>
Researcher Identifier (e.g. ORCID ID):	<i>N/A</i>
Nearest person month worked:	<i>3</i>
Contribution to Project:	<i>Animal husbandry and genotyping</i>
Funding Support:	<i>Insufficient DoD funds supplemented by philanthropy</i>

Name:	<i>Athena Ching Jung Wang, PhD</i>
Project Role:	<i>Post-Doctoral Fellow</i>
Researcher Identifier (e.g. ORCID ID):	<i>N/A</i>
Nearest person month worked:	<i>2</i>
Contribution to Project:	<i>Animal husbandry, validation of required TBI procedural equipment, conducting CCI/TBI and sham procedures</i>
Funding Support:	<i>Insufficient DoD funds supplemented by philanthropy</i>

Name:	<i>Christina Coughlan</i>
Project Role:	<i>Research Lecturer</i>
Researcher Identifier (e.g. ORCID ID):	<i>N/A</i>
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Design of immunocytochemistry</i>
Funding Support:	

Name:	<i>Vanessa Adame</i>
Project Role:	<i>Research Assistant</i>
Researcher Identifier (e.g. ORCID ID):	<i>N/A</i>
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Animal husbandry and genotyping</i>
Funding Support:	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
 - Nothing to Report.
- **What other organizations were involved as partners?**
 - Nothing to Report.
 - **Organization Name: Nothing to Report.**
 - **Location of Organization:** Nothing to Report.
 - **Partner's contribution to the project** Nothing to Report.
 - **Financial support;** Nothing to Report.
 - **In-kind support.** Nothing to Report.
 - **Facilities.** Nothing to Report.
 - **Collaboration.** Nothing to Report.
 - **Personnel exchanges.** Nothing to Report.
 - **Other.** Nothing to Report.

7. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:** Nothing to Report.
- **QUAD CHARTS:** Nothing to Report.

8. APPENDICES: Nothing to Report.