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Award Number: W81XWH-18-1-0497

TITLE: Does TBI Affect mtDNA Heteroplasmy?

PRINCIPAL INVESTIGATOR: Janna L. Harris, Ph.D.

CONTRACTING ORGANIZATION: University of Kansas Medical Center

Shawnee Mission, KS
66205-2522

REPORT DATE: Sept 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command

Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE:	2. REPORT TYPE:	3. DATES COVERED:
Sept 2019	Annual	15 Aug 2018 – 14 Aug 2019

4. TITLE AND SUBTITLE:
Does TBI Affect mtDNA Heteroplasmy?

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0497

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S):
Janna L. Harris, Ph.D.
Russell H. Swerdlow, M.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Kansas Medical Center
KUMC Research Institute
4330 Shawnee Mission Parkway
Shawnee Mission, KS
66205-2522

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

This collaborative project seeks to better understand the long-term consequences of traumatic brain injury (TBI) as they pertain to Alzheimer's disease (AD). We propose that a traumatic brain injury (TBI) accelerates the age-related accumulation of mitochondrial DNA (mtDNA) microheteroplasmic mutations, and that this will explain the recognized association between TBI and Alzheimer's disease (AD). The project's specific aims are (1) to test whether a young adulthood controlled cortical injury (CCI) accelerates the age-dependent accumulation of mtDNA mutations, (2) to test whether brain mtDNA heteroplasmy in aged mice subjected to a young adulthood CCI injury relates to behavioral function, brain metabolism, and brain structure, and (3) to test the relationship between age-related accumulation of mtDNA mutations and neurofibrillary tangles in a genetically engineered strain of mice (rTg4510). We anticipate that findings from this study will help to explain why sustaining a TBI during young adulthood increases one's late-life risk of developing AD.

15. SUBJECT TERMS
Traumatic Brain Injury, Alzheimer's Disease, Aging, Mitochondrial DNA, Mutations, Functional impairment, Memory, Magnetic resonance imaging, Magnetic resonance spectroscopy, Tau pathology

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	13	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

1. **INTRODUCTION:**

This collaborative project seeks to better understand the long-term consequences of traumatic brain injury (TBI) as they pertain to Alzheimer's disease (AD). To accomplish this, we will explore connections between TBI, brain aging, and mitochondrial DNA (mtDNA) mutations. Our central hypothesis is that TBI will accelerate the age-related accumulation of mtDNA microheteroplasmic mutations, and that this will explain the recognized association between TBI and AD. To test this, we will expose young adult mice to a TBI or sham intervention and let the mice age. In Aim 1, we will determine whether the TBI sustained during young adulthood accelerates the age-related accumulation of mtDNA heteroplasmy. In Aim 2, we will characterize these aging, brain-injured mice through behavioral testing, magnetic resonance imaging and spectroscopy, and measurements of mitochondrial enzyme activity, and we will correlate mtDNA heteroplasmy with these endpoints. In Aim 3, we will administer CCI to young adult rTg4510 mice (which accumulate neurofibrillary tangles), age the mice, quantify mtDNA heteroplasmy and neurofibrillary tangle burden, and test relationships between these two parameters. We anticipate that successful completion of these studies will help to explain why sustaining a TBI during young adulthood increases one's late-life risk of developing AD.

2. **KEYWORDS:**

Traumatic Brain Injury
Alzheimer's Disease
Aging
Mitochondrial DNA
Mutations
Functional impairment
Memory
Magnetic resonance imaging
Magnetic resonance spectroscopy
Tau pathology

3. **ACCOMPLISHMENTS:**

The major goals of the project are:

Aim 1: Test whether a young adulthood CCI injury accelerates the age-dependent accumulation of mtDNA.

Aim 2: Test whether brain mtDNA heteroplasmy in aged mice subjected to young adulthood CCI injury relates to behavioral function, brain metabolism, and brain structure.

Aim 3: Test whether neurofibrillary tangle burden in aged rTg4510 mice subjected to young adulthood CCI injury relates to mtDNA mutations.

The activities for this reporting period (months 1-12) are:

Aim 1:

Subtask 1 (months 1-3): Local IACUC Review/approval – COMPLETE.

Subtask 2 (months 4-6): ACURO Review/approval – COMPLETE.
Subtask 3 (months 6-10): Acquire mice, acquisition will be staggered – 61% complete. **this subtask is relevant to all major goals of the project.*
Subtask 4 (months 7-11): Administer CCI – 40% complete.
Subtask 5 (Months 8-12): Sacrifice mice & collect DNA 1 month after CCI – not complete; these experiments now scheduled to be complete by month 16.
Subtask 6 (Months 8-26): Age the mice – began in month 11, ongoing.

We note that Subtasks 3-6 were impacted by unexpected delays in year 1 due to a mouse parvovirus outbreak, as described below and in previous technical reports.

<u>Aim 2:</u>

Subtask 1 (months 1-3): Local IACUC Review/approval – COMPLETE.
Subtask 2 (months 4-6): ACURO Review/approval – COMPLETE.
Subtask 3 (months 6-10): Acquire mice, acquisition will be staggered – 61% complete. **this subtask is relevant to all major goals of the project.*
Subtask 4 (Months 7-11): Administer CCI – 40% complete.
Subtask 5 (Months 7-26): Assess time course of cognitive and motor function before and after CCI – began in month 10, ongoing.
Subtask 6 (Months 8-26): MRI/MRS 1 and 15 months after CCI – began in month 11, ongoing.
Subtask 7 (Months 8-26): Age the mice – began in month 11, ongoing.
Subtask 8 (Months 8-26): Sacrifice mice & collect brain hemispheres 1 and 15 months after CCI – began in month 11, ongoing.
Subtask 9 (Months 8-26): Perform mitochondrial enzyme assays – not complete; these experiments now scheduled to be complete by month 16.

We note that Subtasks 3-9 were impacted by unexpected delays due to a mouse parvovirus outbreak, as described below and in previous technical reports.

<u>Aim 3:</u>

Subtask 1 (months 1-3): Local IACUC Review/approval – COMPLETE.
Subtask 2 (months 4-6): ACURO Review/approval – COMPLETE.
Subtask 3 (by month 8): Acquire mice, acquisition will be staggered – not complete, this subtask is now scheduled for completion by month 13.
Subtask 4 (by month 9): Administer CCI – not complete, this subtask is now scheduled for completion by month 14.
Subtask 5 (months 10-24): Age the mice – this will now begin in month 14.

We note that Subtasks 3-5 were impacted by unexpected delays due to a mouse parvovirus outbreak, as described below and in previous technical reports.

Accomplishments under these goals:

1) Major activities:
- Prepared an animal protocol including all live animal work to be accomplished on this project. Approval obtained from KUMC IACUC on 1/14/2019.
- Prepared paperwork for ACURO review describing all live animal work to be accomplished on this project. Approval obtained from ACURO on 3/20/2019.
- Developed and customized data sheets to be used for keeping experimental records of all studies
- Established secure data storage on a University server with access by all authorized study personnel.
- Sourced and ordered supplies for mouse surgeries, behavioral studies, mtDNA sequencing
- Coordinated with the study team to develop a detailed schedule of in vivo experimental procedures for all animal cohorts.
- Developed methods to perform mtDNA sequencing:
    1. Method for mitochondrial extraction and isolation/enrichment.
    2. Method for mtDNA extraction from isolated mitochondria—high purity but still contains some nuclear DNA.
    3. Method for very high purity recovery of mtDNA while removing all nuclear DNA contamination.
    4. Method for high yield recovery of pure mtDNA even at picogram levels.
- Developed methods for multi-voxel chemical shift imaging in mice.
- Began behavioral, surgical, and acute post-surgical imaging studies in c57bl/6 mice.

2) Specific objectives:
- Obtain regulatory approvals
- Optimize experimental protocols and workflow
- Customize data sheets for record keeping
- Develop a staggered schedule for all experimental cohorts
- Acquire mice for all experimental cohorts
- Begin animal studies: behavioral, surgical, imaging.
- Begin aging the mice

Discussion of delay in our experimental timeline:
As noted above, for each aim of our project certain subtasks were affected by unexpected delays in year 1. These have been previously described in quarterly technical reports. In brief, the start of our *in vivo* animal work was delayed by a parvovirus outbreak in our University's animal facility. Our animals were not directly affected by the virus, but the decontamination of shared animal use areas, and the ensuing scheduling backup of multiple investigators using the shared resources, delayed the start of our experiments.

3) Significant results or key outcomes: The figures below present preliminary data we have generated during year 1 of the study.

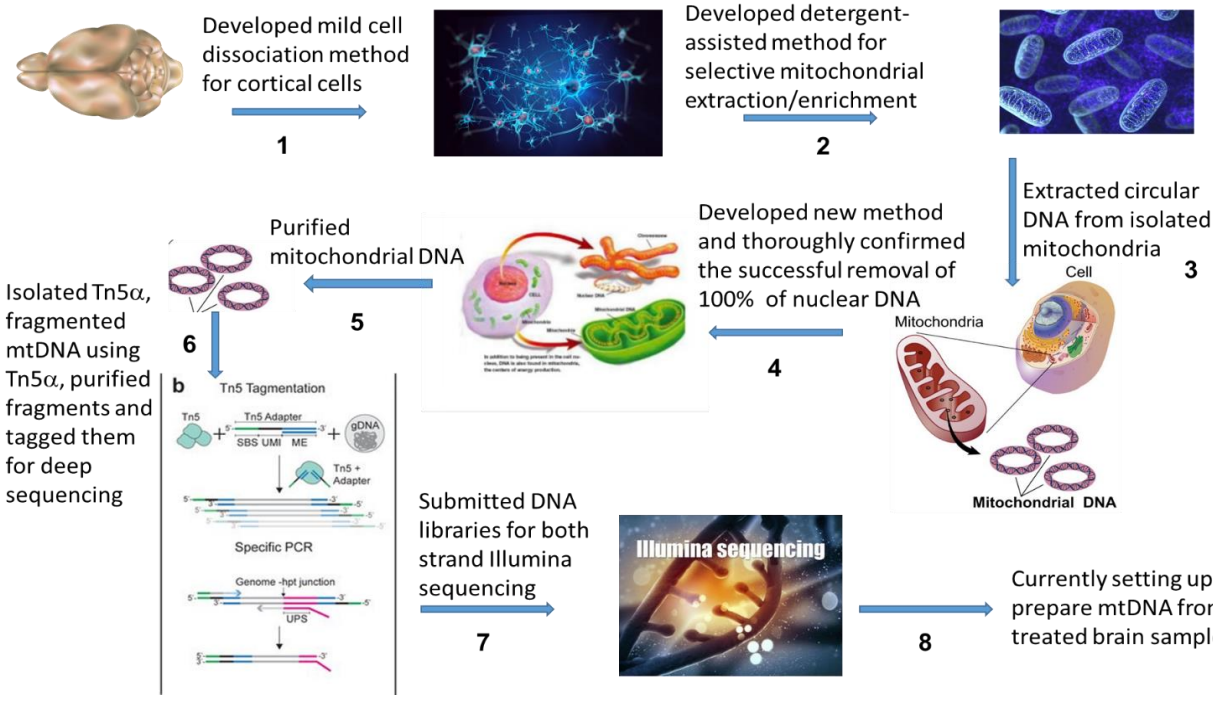


Figure 1. Summary of year 1 experiments performed by our mitochondrial genomics group. We developed and optimized methodologies for mitochondrial DNA (mtDNA) isolation from mouse cortex, and preparation of samples for deep sequencing. Samples were confirmed to be devoid of nuclear DNA contamination and without long-range DNA amplification, thereby avoiding errors introduced by DNA polymerase amplification.

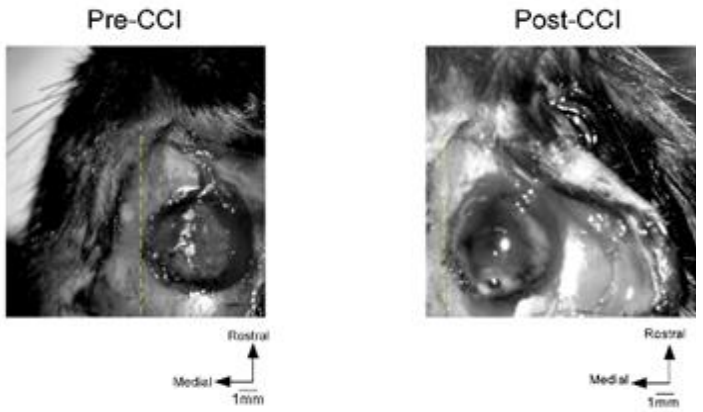


Figure 2. Controlled cortical impact (CCI) procedure. Craniectomy is made with a trephine drill to expose cerebral cortex of the mouse over the primary forelimb motor area. The cranium is carefully removed to expose cortex with underlying dura intact. A CCI was made with the Lecia Impact One device (2mm depth, 3.5 m/sec, and 100 msec duration). Mice were randomized to CCI or sham (no impact) groups; 32 surgeries were completed in year 1.

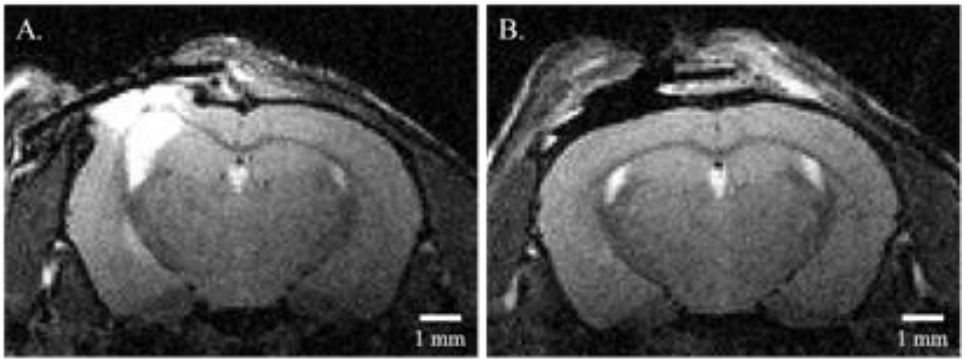


Figure 3. T2-weighted magnetic resonance imaging acquired 1 month post-surgery confirms the traumatic brain lesion in a controlled cortical impact mouse (A) and lack of damage in a sham control mouse (B).

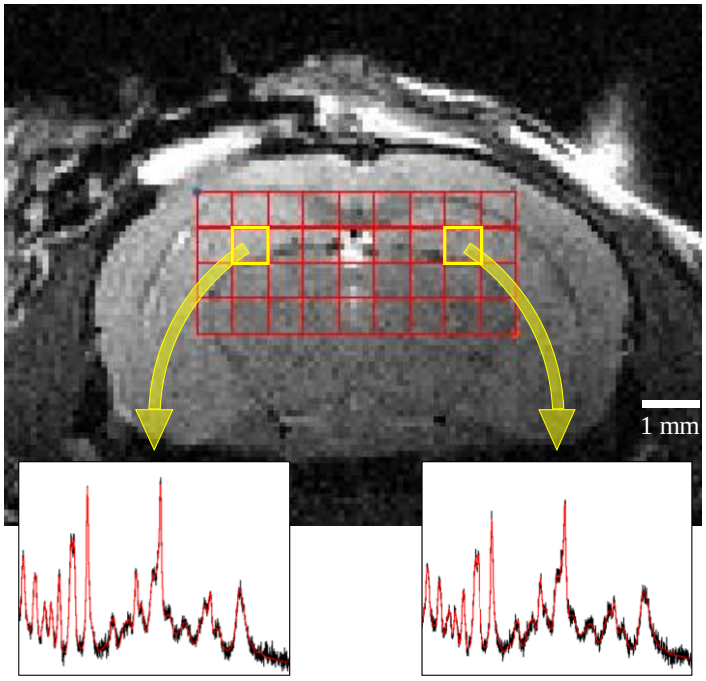


Figure 4. Multi-voxel Chemical Shift Imaging (CSI) based spectroscopic acquisition. We developed this imaging technique for use in mouse brain, which has several benefits over our previous single voxel protocol. CSI allows us to probe brain chemistry over a larger area, now encompassing both ipsi-lesional and contra-lesional hemispheres, in the same amount of scanning time. A 0.6 x 0.6 mm2 spectral grid enables more precise voxel positioning over the region of interest, as well as better voxel placement reproducibility. Inset boxes depict sample spectra from the ipsi-lesional (left) and contra-lesional (right) hippocampi.

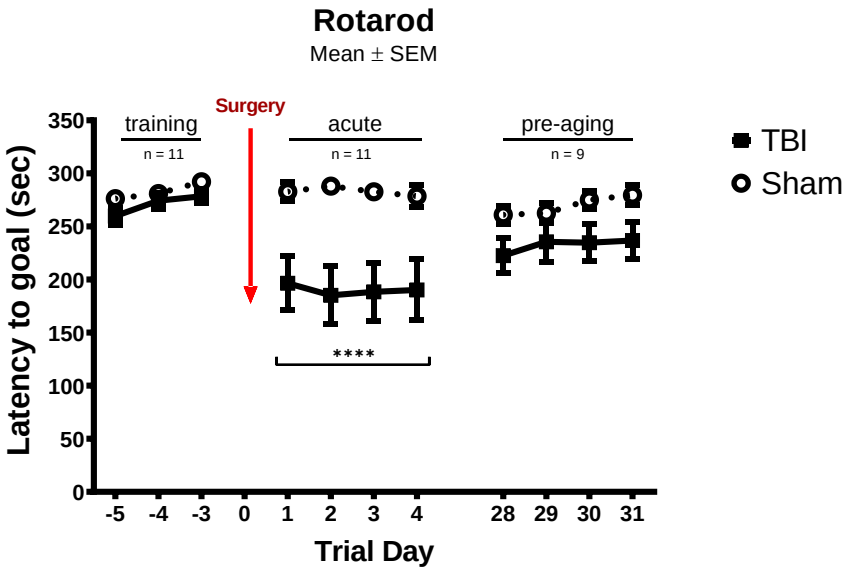


Figure 5. Preliminary results on the rotarod, a test of motor function, show acute functional impairment after traumatic brain injury (TBI) but not sham surgery. TBI mice showed partial recovery between the acute (1-4 days) and pre-aging (1 month) time points.

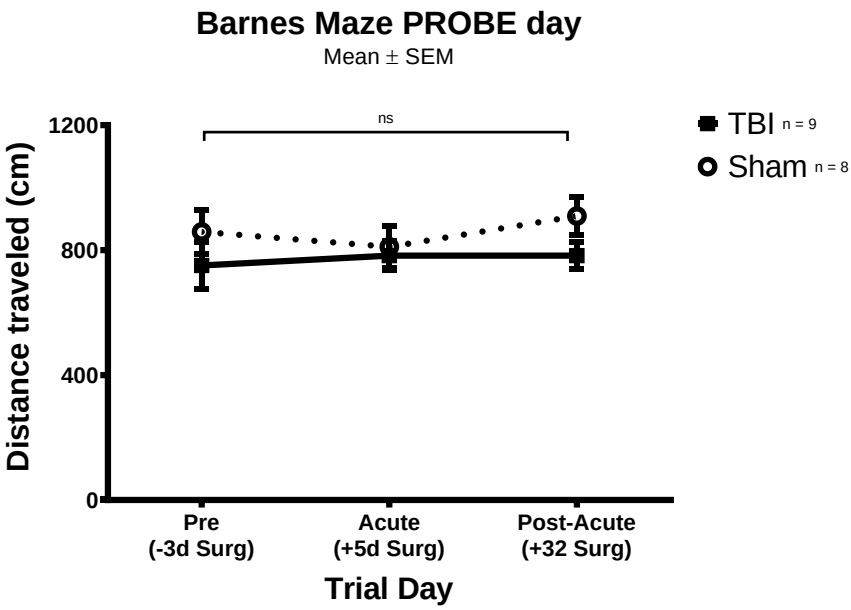


Figure 6. Preliminary results on the Barnes maze, a test of hippocampal-dependent memory, show no significant difference between TBI and sham control mice at the acute (5 days) or pre-aging (1 month) time points. We will continue to monitor the performance of mice on our battery of behavioral tests as they age following TBI/sham surgery.

4) Other achievements:
- Monthly meetings between the Co-PIs for discussion, updates, strategic planning
- Frequent (at least monthly) meetings with key hands-on personnel involved in animal studies.
- Quarterly meeting with the entire study team to develop and optimize study protocols, share updates, and discuss questions/concerns.

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?
During the next reporting period, we will complete all TBI/sham surgeries, perform imaging and behavioral studies at the 1 month time point, euthanize and collect brain tissue from 1-month survival mice, and begin to age the 15-month survival cohort. We will prepare collected tissues for mtDNA sequencing and mitochondrial enzyme activity assays.

Once data collection from the 1-month time point has been completed for all study subjects, we will begin to process and analyze the data to prepare our findings for dissemination.

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
Once our study was initiated, we found that completion of the four mouse behavioral tests that we proposed was very time-consuming. This was limiting the number of mice that could be assessed per day which was slowing the progress of our study. To prevent further delays, we

chose to eliminate one test from our protocol, the Novel Object recognition. Our battery of behavioral assessments still includes the Barnes maze, Grid walk, and Rotorod.

Actual or anticipated problems or delays and actions or plans to resolve them
As noted above, the start of our *in vivo* animal work was delayed by a parvovirus outbreak in our University's animal facility. Our animals were not directly affected by the virus, but the decontamination of shared animal use areas, and the ensuing scheduling backup of multiple investigators using the shared resources, delayed the start of our experiments.
However, at the end of year 1, animal experiments are now actively underway. The overall timeline of our studies has enough flexibility that these delays in year 1 should not ultimately affect the timely completion of the overall project.

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
N/A

Significant changes in use or care of vertebrate animals.
Nothing to Report.

Significant changes in use of biohazards and/or select agents
N/A

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.**
  "Brain Injury and Aging: A Now and Later Story" seminar presentation by Dr. Harris at the University of Kentucky Department of Neuroscience and Spinal Cord and Brain Injury Research Center. May 2019.
- **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:	Janna Harris
Project Role:	Multiple PI (Contact)
Research Identifier:	0000-0002-4829-6360
Nearest person month worked:	4.7 Calendar Months
Contribution to Project:	Dr. Harris obtained the Animal Protocol approvals from IACUC and ACURO. She has supervised work by study team members to optimize experimental protocols, and to source materials that will be needed, and to schedule and carry out animal experiments. She has coordinated meetings with the co-PI Dr. Swerdlow, as well as meetings with other individuals on the project.
Funding Support:	No change

Name:	Russell Swerdlow
Project Role:	Multiple PI
Research Identifier:	0000-0003-2948-7230
Nearest person month worked:	1.35 Calendar Months
Contribution to Project:	Dr. Swerdlow has worked collaboratively with co-PI Dr. Harris and the study team to supervise development of the study protocols and schedules, to troubleshoot when any issues arise, and to review the progress of animal experiments.
Funding Support:	No change

Name:	Xiaowan Wang
Project Role:	Postdoctoral Fellow
Research Identifier:	Not applicable
Nearest person month worked:	2.8 Calendar Months
Contribution to Project:	Dr. Wang has carried out the mouse behavioral studies, and worked to develop protocols for the mitochondrial enzyme assays, brain tissue dissection and isolation of total DNA, and tau tangle stereology.
Funding Support:	No change

Name:	Judit Perez-Ortiz
Project Role:	Postdoctoral Fellow
Research Identifier:	0000-0002-8953-4281
Nearest person month worked:	2.8 Calendar Months
Contribution to Project:	Dr. Perez-Ortiz has carried out the mouse behavioral studies, and worked to develop protocols for behavioral testing, brain tissue dissections, DNA isolation, and stereology analysis.
Funding Support:	No change

Name:	(Harry) Scott Barbay
Project Role:	Senior Scientist
Research Identifier:	0000-0003-2470-7775
Nearest person month worked:	3.0 Calendar Months

Contribution to Project:	Dr. Barbay assisted with preparation of the animal protocols and sourcing of research supplies. During the reporting period he completed 32 TBI/sham surgeries and assisted with animal health monitoring and medical records.
Funding Support:	No change

Name:	Sarah Christian
Project Role:	Research Assistant
Research Identifier:	Not applicable
Nearest person month worked:	10.2 Calendar Months
Contribution to Project:	Ms. Christian assisted with preparation of the animal protocols and development of experimental protocols for animal behavior assessments. She has also been responsible for sourcing and ordering research supplies, animal health monitoring, maintenance of study records and laboratory compliance.
Funding Support:	No change

Name:	DongWei Hui
Project Role:	Senior Research Associate
Research Identifier:	0000-0003-1020-8940
Nearest person month worked:	2.45 CYM
Contribution to Project:	Dr. Hui developed and optimized methodologies for mitochondrial DNA isolation from mouse brain and preparation for deep sequencing without contamination from nuclear DNA.
Funding Support:	No change

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

Brooks, William Miles:

AWARDED
R01AG060733-01A1 (Swerdlow, Russell) 02/01/2019 - 11/30/2023 0.9 CYM
NIH $471,784
Validation and Mechanistic Interrogation of Metabolism Targeting for AD
Role: Co-Investigator
Major Goals: Aim 1: Determine the effect of a KD on cognition and function in AD. Aim 2: Use biomarkers to define KD physiological effects and mechanisms of action.

Nudo, Randolph J:

AWARDED
R03HD094608-01A1 (Guggenmos, David J) 09/20/2018 - 08/31/2020 0.6 CYM
NIH $100,000
Shaping Motor Recovery After Stroke Using Activity-Dependent Stimulation
Role: Co-Investigator
Major Goals: The proposed study addresses questions regarding the role of cortical stimulation in promoting reorganization and recovery of spared brain areas after ischemic injury within neocortex. The answers will help shape strategies for treating the underlying disruption in communication that results from these types of injuries. Aim 1 will determine the role of somatosensory cortical stimulation on the

behavioral recovery profiles of M1 lesioned rats.Aim 2 will determine how cortical stimulation impacts functional connectivity between PM and S1 after a lesion to M1.Aim 3 will determine if S1 stimulation alters the expression of neuroplastic markers within the spared motor and sensory areas.

Swerdlow, Russell:

AWARDED
R01 AG061194 (Swerdlow, Russell) 02/01/2019 - 11/30/2023 2.4 CYM
NIH $250,000
Mechanistic Basis of the mtDNA Haplogroup J-Alzheimer's Disease Association
Major Goals: In Aim 1, we use cytoplasmic hybrid (cybrid) cell lines to define how the haplogroup J variants affect mitochondrial function and integrity. In Aim 2, we use clinical biospecimen and biomarker data to further explore the haplogroup J, AD, and aging nexus.

What other organizations were involved as partners?
Nothing to Report.

8. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:**
     Not applicable.
   - **QUAD CHARTS:**
     An updated quad chart is provided as a separate attachment.

9. **APPENDICES:**
   None.

Does TBI Affect mtDNA Heteroplasmy?

AZ170111
W81XWH-18-1-0497

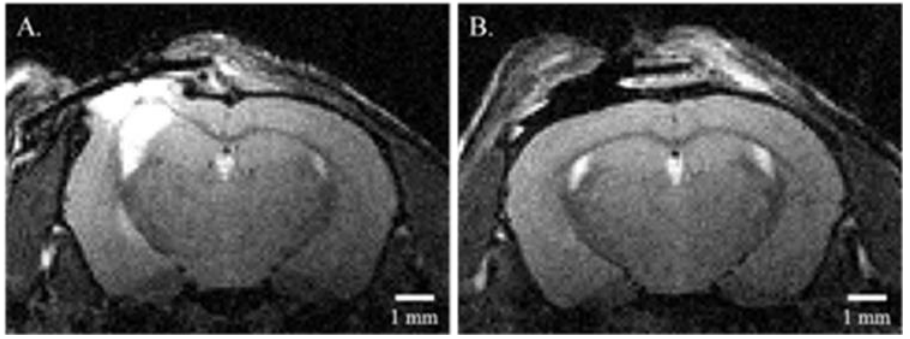
PIs: Janna Harris PhD & Russell Swerdlow MD **Org:** University of Kansas Medical Center **Award Amount:** $1,300,000

Study Aims

- **Aim 1**: Test whether a young adulthood TBI accelerates the age-dependent accumulation of mtDNA heteroplasmy.
- **Aim 2**: Test whether brain mtDNA heteroplasmy in aged mice subjected to young adulthood TBI relates to behavioral function, brain metabolism, and brain structure.
- **Aim 3**: Test whether neurofibrillary tangle burden in aged rTg4510 mice subjected to young adulthood TBI relates to mtDNA heteroplasmy.

Approach

We will expose young adult mice to a TBI or sham injury, and let the mice age. We will measure the rate of accumulation of mtDNA heteroplasmy and explore connections with neurocognitive decline, MRI measures, and AD-associated neuropathology.



In our experimental model of traumatic brain injury, MRI at 1 month post-surgery shows the brain lesion in a controlled cortical impact mouse (A) and the lack of damage in a sham control mouse (B).

Accomplishments in Year 1: Regulatory approvals obtained for animal work, experimental protocols developed and optimized, C57Bl/6 mice acquired, surgical/behavioral/imaging studies initiated.

Timeline and Cost

Activities CY	'18	'19	'20	'21
Aim 1		Year 1	Year 2	Year 3
Aim 2		Year 1	Year 2	Year 3
Aim 3		Year 1	Year 2	Year 3
Estimated Budget ($K)		**$387**	**$494**	**$418**

Updated: September 2019 for Year 1

Goals/Milestones

Year 1 Goals:

- ✓ Obtain regulatory approvals for animal studies (Months 1-6)
- ✓ Acquire C57Bl/6 mice (Months 6-10)
- ☐ Pre-surgery clinical evaluations (Months 6-10) *In progress*
- ☐ Acquire rTg4510 mice and start on doxycycline (Months 6-10) *In progress*
- ☐ Administer CCI surgeries to rTg4510 mice (Months 7-11) *In progress*
- ☐ Administer CCI and Sham surgeries to C57Bl/6 mice (Months 7-11) *In progress*
- ☐ Acute clinical evaluations (Months 8-12) *In progress*
- ☐ Obtain post-injury/pre-aging MRIs (Months 8-12) *In progress*
- ☐ Obtain mtDNA from post-injury/pre-aged mice (Months 8-12) *In progress*

Comments/Challenges/Issues/Concerns

- In May 2019 mouse parvovirus was detected in our institution's animal facility. Although our mice were not directly affected, this outbreak delayed our experiments due to the decontamination of the shared animal surgery, behavior, and imaging facilities.

Budget Expenditure to Date

- Projected Expenditure: $387,078
- Actual Expenditure: $266,040