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TITLE: The Impact of PERK on Posttraumatic Tauopathy in Alzheimer's Disease

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Risk for AD is one of the most imminent threats to military personnel sustaining TBI. A major challenge in the field of TBI/AD research is elucidation of the molecular mechanisms linking TBI with tau pathogenesis that is associated with AD. This critical and urgently needed information will identify novel therapeutic targets that will benefit both military personnel and civilian population. Our recent data indicate that TBI induces sustained and dramatic endoplasmic reticulum stress, and that there is a pathological relationship between AD tau species and the ER stress sensor PERK. For the first time, we directly address the role PERK activity as a molecular mediator of TBI-induced *tau pathology* and provide unique understanding of the association between TBI and AD. Therapies aiming to interrupt the molecular events identified by this work will be monitored using our imaging technology known as MEMRI. Finally, our work will test the therapeutic value of inhibiting the PERK, which is involved in several neurodegenerative disorders.

15. SUBJECT TERMS

PERK, tauopathies, Alzheimer's disease, traumatic brain injury, controlled cortical impact

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INTRODUCTION:

Risk for AD is one of the most imminent threats to military personnel sustaining TBI. A major challenge in the field of TBI/AD research is elucidation of the molecular mechanisms linking TBI with tau pathogenesis that is associated with AD. This critical and urgently needed information will identify novel therapeutic targets that will benefit both military personnel and civilian population. Our recent data indicate that TBI induces sustained and dramatic endoplasmic reticulum stress, and that there is a pathological relationship between AD tau species and the ER stress sensor PERK. In this proposal we aimed to establish for the first time the impact of PERK on tau-mediated pathogenesis as a means by which TBI confers risk for AD. We will directly address the role PERK activity as a molecular mediator of TBI-induced *tau pathology* and provide unique understanding of the association between TBI and AD. This understanding is *critical and urgent* to develop novel therapeutic strategies. This work will further develop a novel imaging *technology* (ME-MRI) that will identify a course of functional deficits in the brain after injury and monitor the magnitude at which these phenomena persist over time. This technology will set a standard of disease course imaging from TBI to AD onset. Therapies aiming to interrupt the molecular events identified by this work will be monitored using our imaging technology. Finally, our work will test the therapeutic value of inhibiting the PERK, which is involved in several neurodegenerative disorders.

KEYWORDS:

PERK, tau, tauopathies, Alzheimer's disease, traumatic brain injury, controlled cortical impact, neurodegeneration, UPR, ER stress

ACCOMPLISHMENTS:

What were the major goals of this project?

AIM1

1. Establish mouse colony and validate anatomical and volumetric damage caused by CCI
    - *ACURO protocol approved*
    - *Wild type mouse colony established*
    - *Training to perform controlled cortical impact injury model complete*
2. Determine immunohistochemical changes in the brain (and complete all MRI analyses)
    - *Early timeline of PERK activation complete*
    - *Established which cell types show PERK activation*
3. Complete imaging analyses (this work is completed continuously as injuries are performed)

AIM2

4. Establish mouse cohorts for Aim 2
    - *rTg4510 transgenic colony established*
    - *PERK conditional knockout colony backcrossed: estimated completion date: Dec 2016*
5. Perform genetic manipulation to activate and inhibit PERK function
    - *Establishment of the PERK conditional knockout colony: estimated completion date: Dec 2016*
    - *Viral particles are continuously produced by the University of Kentucky Viral Core*
6. Perform chemical manipulations to modulate PERK
    - *Currently underway*
7. Complete data collections and analysis for all functional measurements (this work is completed continuously as injuries are performed)
    - *Cohort 1 of chemical PERK inhibition following injury data collection – MEMRI, behavioral analyses (novel object recognition, radial arm water maze)*
    - *Data collection for first four cohorts of PERK inhibition following injury (MEMRI, behavior, immunohistochemical staining) will be complete by January 1, 2017*
8. Complete data analysis (this work is completed continuously as injuries are performed)

What was accomplished under these goals?

During the past year we established the mouse colony for experiments in Aim 1 and Aim 2, we successfully performed controlled cortical impact injuries to characterize PERK activation following traumatic brain injury, we performed MEMRI and analyses following injury, we established an early timeline of PERK activation using immunohistochemical staining, and we began to investigate the impact of chemical PERK inhibition following injury on cognition and neuronal function. We are continuing to complete the goals as cohorts of mice become available.

Using immunohistochemical analyses we found that PERK is more robustly

activated in the contralateral hemisphere compared to the ipsilateral hemisphere at early time points following injury (Figure 1). We also found that PERK is active in neurons (Fig. 2) but not glia as evidenced by counterstains with GFAP (Fig. 3) or Iba1 (Fig. 4). Using the non-radioactive, puromycin based translation assay we also determined that protein synthesis is increased at early time points in neurons (Fig. 5).

Injuries were performed as previously described in the proposal; briefly, mice were anesthetized using isoflurane and a midline incision was made. A 3mm in diameter craniotomy was performed and mice were injured using the electromagnetic CCI machine at 1.5m/s with 500msec dwell time. A cranioplasty was then placed over the injury site and the incision was sutured. Mouse brains were collected following cardiac perfusion using 0.9% saline. Tissue was sectioned using a freezing microtome and stained using immunofluorescence.

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

This project provided the opportunity to become proficient in the CCI models of injury. One graduate student successfully completed training from Dr. Kathryn Saatman, and she is working to train other students in our lab.

This project has also allowed for professional development in allowing us to expand our knowledge and relationships with TBI experts at the National Neurotrauma Society annual meeting and the Alzheimer's Association International Conference. One graduate student presented her work at both meetings and received feedback to aid in data interpretation. These meetings greatly expanded our knowledge on the most recent findings in the TBI field.

How were the results disseminated to communities of interest?

Data collected from this project was presented at two separate meetings and has been presented at multiple department level seminars. We anticipate submitting a manuscript within the next few months.

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

During the next reporting period, we aim to complete a full timeline of early PERK activation up through 7 days post-injury, complete data collection and analyses from experiments on chemical PERK inhibition following injury, and publish our findings.

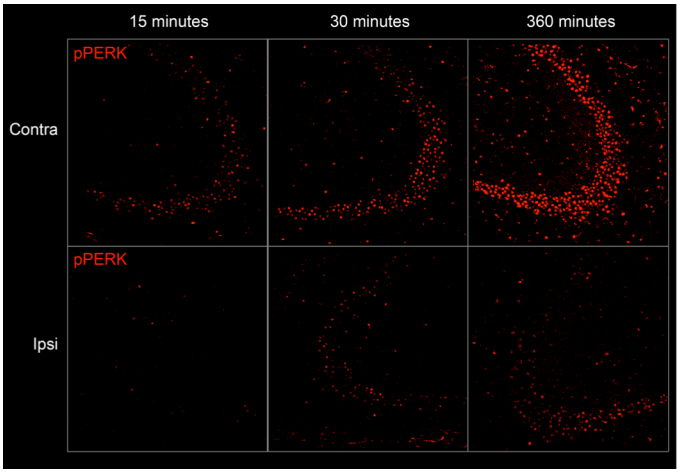

Fig. 1: PERK is more active in the contralateral hemisphere compared to the ipsilateral hemisphere, and PERK activation increases with time.

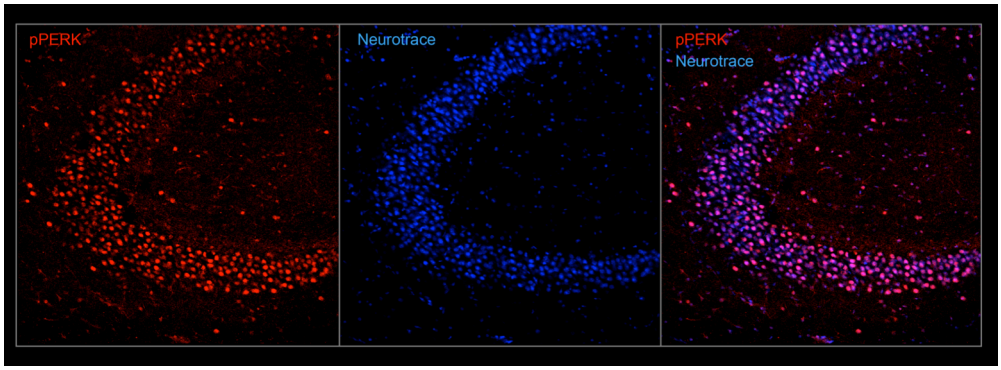

Fig. 2: PERK is active in neurons.

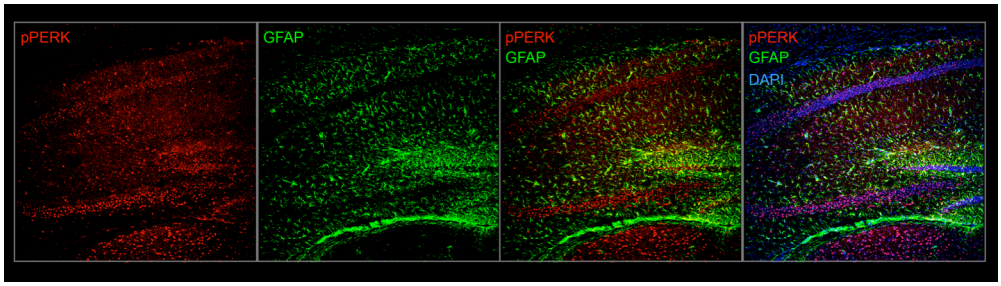

Fig. 3: PERK is not active in GFAP positive cells.

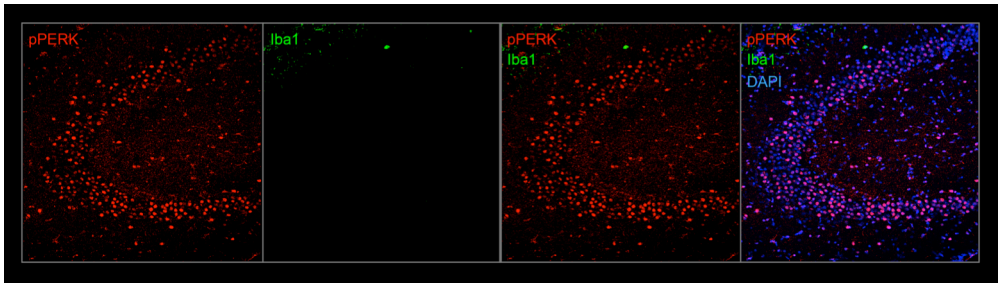

Fig. 4: PERK is not active in Iba1 positive cells.

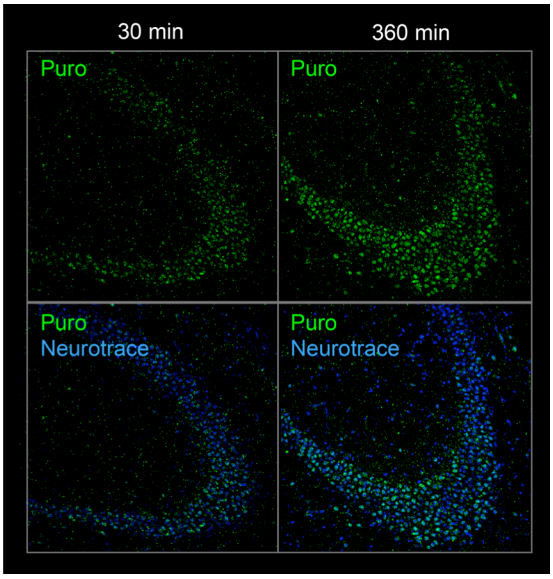

Fig. 5: Puromycin uptake increases with time following injury.

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
Although we are continuously working on increasing our sample size for each experimental group, our preliminary data suggest that CCI impacts neurons before glia. This is a surprising finding considering that previous data in related fields of neurodegeneration suggest that inflammatory responses would be more prevalent. In addition, it would explain why cognition is the first faculty to be impacted immediately after injury. Another surprising finding is that injured neurons are the primary cell type that increases protein synthesis. The identity of these proteins is unknown, and we hypothesize that they correspond to stress proteins, such as PERK, and not synaptic proteins.

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

CHANGES/PROBLEMS:

Changes in approach and reasons for change

We injured mice and analyzed PERK activation at earlier time points than originally suggested to optimize the timing of the drug delivery for chemical PERK inhibition. We wanted to make sure we were targeting an appropriate window. Now that we have a clear therapeutic window, we will continue our time points as established in the proposal.

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

Currently, our major delay comes from establishing the PERK conditional knockout colony. The animals have been backcrossed, but now we must have the appropriate parental cross to ensure maximal usable offspring for future experiments. This requires the animals to age to appropriate breeding age before we can perform any experiments.

Changes that had a significant impact on expenditures

No changes that have had significant impact on expenditures

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

Not applicable

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

PRODUCTS:

Publications, conference papers, and presentations *Report only the major publication(s) resulting from the work under this award.*

Journal publications.

Nothing to report

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

Nothing to report

Other publications, conference papers, and presentations.

1. Alzheimer's Association International Conference 2016 – "Activation of PERK in controlled cortical impact model of traumatic brain injury", poster presentation
2. National Neurotrauma Society Annual Meeting 2016 – "PERK activation in controlled cortical impact model of TBI"

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

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project? *Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."* ***Example****:*

Name:	Joe Abisambra
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
Funding Support:	No change

Name:	Kathryn Saatman, PhD
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Saatman trained Shelby Meier in the CCI model of injury
Funding Support:	No change

Name:	Shelby Meier
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	0000-0003-1946-9004
Nearest person month worked:	6
Contribution to Project:	Shelby has performed all CCI injuries, the majority of the MEMRI scans and analyses, all immunohistochemical staining and analyses, and all behavioral studies.
Funding Support:	University of Kentucky IBS program

Name:	Grant Nation
Project Role:	Colony manager

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	10
Contribution to Project:	Grant takes care of all the animals for this project and ensures proper genotype.
Funding Support:	R01 NS091329-01A1

Name:	Bret Smith
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Smith offered feedback and expertise in interpreting results.
Funding Support:	Collaborator

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?

Organization Name: GlaxoSmithKline
Location of Organization: Collegeville, PA
Partner's contribution to the project: GSK developed the chemical PERK inhibitor used for this project, and supplies it for us.