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TITLE: Astrogliosis as the Driver for Post-Traumatic Epilepsy

PRINCIPAL INVESTIGATOR: PAMELA VANDEVORD, PH.D.

CONTRACTING ORGANIZATION: VIRGINIA TECH UNIVERSITY

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Astrogliosis as the Driver for Post-Traumatic Epilepsy

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Pamela VandeVord, PH.D.

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Nearly half of all reported cases of acquired epilepsy are caused by traumatic brain injury (TBI), which are often the result of accidental, recreational, and combat-related injuries. These acquired forms of epilepsy are predominately in young adults. Biological factors underlying post-traumatic epilepsy (PTE), as well as predictors for subgroups developing epilepsy, remain elusive, as are predictors for subgroups that may present with epilepsy. In addition, failure of the clinical trials to prevent spontaneous post-traumatic seizures using currently available anti-epileptic therapeutic approaches indicates presence of other, yet unknown mechanisms of epileptogenesis. Mounting evidence suggests mild repeated TBI pathology contributes to PTE. The influence of TBI on seizures and any cumulative TBI insults needed to cross a particular threshold to cause epilepsy have not been investigated. Further, it is uncertain if blast waves produced by explosions can cause epilepsy. Importantly, the effects of gender on PTE manifestation also require attention. Acquired epilepsy is devastating to patients, as seizures are largely unresponsive to current anti-seizure medications. Seizures significantly impair independent living and cause progressive cognitive decline. Despite our awareness of the initiating events, prevention of PTE with antiepileptic drugs has been unsuccessful. To date, nearly all research has focused on neurons and treatments exclusively addressing neuronal dysfunction. Alternatively, astrogliosis, which can decrease individual seizure thresholds, might also be implicated in TBI and is the focus of this pre-clinical study of PTE.

Despite recent recognition of mild TBI as a cause for neurological dysfunction, technical difficulties in recording EEGs from animals over long periods of time and a significantly lower PTE incidence after these types of TBI have prevented the development of effective animal models for mild or blast TBI studies with confirmed spontaneous seizures. Our group has shown spontaneous seizures occur 21 days after *repetitive mild diffuse TBI* in mice. New data using the proposed moderate *repeated blast model* have shown spontaneous, unprovoked seizures in 25% of mice beginning only three days after injury. Seizures recurred and were accompanied by additional behavioral abnormalities, including freezing, facial automatisms, tail extension, rearing and falling. The objective of this project is to capture and compare differences between blast TBI animals with seizure-confirmed PTEs and seizure-free blast TBI animals to provide a comprehensive and unbiased analysis of the molecular pathway(s) contributing to blast-related PTE. Using the blast model of repeated TBI from our studies, we will expand upon our confirmed spontaneous seizure results and take a gliocentric approach to identify biomarkers and molecular targets for the development of novel therapeutic approaches for PTE. We have readily identified cells of interest and have begun the biological assessments of the animals. We have accomplished the major first year milestones and look forward to the dissemination of our findings in the upcoming year as we continue to characterize the new PTE murine model.

15. SUBJECT TERMS:

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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Unclassified	Unclassified	Unclassified	Unclassified		

TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Our team of engineers and neuroscientists at Virginia Tech (VT) have joined to address important gaps in post-traumatic epilepsy (PTE) knowledge. We have been using a military-relevant animal model of blast neurotrauma and post-injury video-electroencephalographic (EEG) monitoring, data analytics and bioinformatics to accomplish the proposed studies. By sharing expertise across disciplines, we ensure broad hypothesis testing and robustness of research through the cross-validation of findings. Currently, the PTE research field is limited to two experimental models, with confirmed spontaneous seizures induced by moderate to severe TBIs. In these models, PTE incidence is low and heterogeneity from animal to animal has interfered with the emergence of a concise picture detailing disease mechanisms and PTE predictors. In patients, the risk for developing PTE is highest with severe injury, yet still significantly increases after mild TBI and blast exposure, which may adversely affect military personnel. Despite recent recognition of mild TBI as a cause for neurological dysfunction, technical difficulties in recording EEGs from animals over long periods of time and a significantly lower PTE incidence after these types of TBI have prevented the development of effective animal models for mild or blast TBI studies with confirmed spontaneous seizures. The use of multiple PTE models representing various forms of TBI, including blast, have the potential to inform the field if shared mechanisms underlie TBI/PTE or if unique cellular changes match to TBI/PTE in different patient subpopulations. Our group has shown spontaneous seizures occur 21 days after repetitive mild diffuse TBI in mice and data using the proposed moderate repeated blast model have shown spontaneous, unprovoked seizures in 25% of mice beginning only three days after injury. Seizures recurred and were accompanied by additional behavioral abnormalities, including freezing, facial automatisms, tail extension, rearing and falling. We **hypothesize** that blast-induced astrogliosis contributes to the development of PTE.

The **objective of this project** is to capture and compare differences between blast TBI animals with seizure-confirmed PTEs and seizure-free blast TBI animals to provide a comprehensive and unbiased analysis of the molecular pathway(s) contributing to blast-related PTE. Using the blast model of repeated TBI from our preliminary study, we have expanded upon our confirmed spontaneous seizure results and take a **gliocentric** approach to identify biomarkers and molecular targets for the development of novel therapeutic approaches for PTE. To accomplish this, we propose the following three aims:

Aim 1: Establish seizure onset, incidence, frequency and EEG properties following blast exposure. An iterative refinement process will be employed using a mouse model of repeated blast TBI with confirmed PTE to establish a reproducible injury paradigm that causes seizure patterns comparable to high PTE-prevalent male and female human injury profiles.

Aim 2: Define PTE-distinguishing molecular and physiologic features in astroglia after blast exposure. We will systematically evaluate the key PTE-relevant biological changes in astrocytes using next generation genomics, proteomics, and robust histological assessments. The PTE-confirmed subpopulation of animals will be compared to blast-treated animals without PTE.

Aim 3: Comparative assessment of blast-PTE data to database of a diffuse impact-TBI PTE model. Using data previously collected from an impact-related mTBI-PTE model that mimics sport injury, data regarding seizure onset, incidence, frequency, EEG properties and omics data will be compared to determine common elements that may exist between various TBI PTE models.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Astrogliosis, Post-Traumatic Epilepsy (PTE), Brain Injury, animal models, seizures, blast exposure, proteomics, neuropathology, genomics, electroencephalographic (EEG), astrocytes

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The goals of this project are as follows:

Aim 1: Establish seizure onset, incidence, frequency and EEG properties following blast exposure. An iterative refinement process will be employed using a mouse model of repeated blast TBI with confirmed PTE to establish a reproducible injury paradigm that causes seizure patterns comparable to high PTE-prevalent male and female human injury profiles.

Aim 2: Define PTE-distinguishing molecular and physiologic features in astroglia after blast exposure. We will systematically evaluate the key PTE-relevant biological changes in astrocytes using next generation genomics, proteomics, and robust histological assessments. The PTE-confirmed subpopulation of animals will be compared to blast-treated animals without PTE.

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SOW Goals	Proposed Timeline	% Complete
Specific Aim 1 – Blast PTE EEG - (minimum of n=16 for each group PTE+, PTE-, Sham)	Months (1-36)	
Local IACUC/ ACURO Approval	1-3	100%
Establish best practices for blast, EEG surgery, EEG monitoring and EEG data analysis, validation with existing data	1-3	100%
EEG monitoring and data collection	3-30	30%
EEG Data analysis	3-32	20%
TBI/EEG Enter data into database	12-36	0%
Milestone Achieved: ACURO Approval		01/03/2019
Milestone Achieved: Best practices for EEG monitoring and data analysis established and validated	3	03/29/2019
Milestone Achieved: Determined observation window and onset (n=30 TBI mice)	6	100%
Milestone Achieved: EEG defining profile	24	20%
Milestone Achieved: Full EEG data set acquired	30	10%
Milestone Achieved: Full EEG data set analyzed and entered into database	36	0%

Specific Aim 2 – Blast PTE Biological Parameters	Months (2-36)	
Establish best work practices and consistent protocols for omics and histology studies using appropriately aged animals	1-3	100%
Omics (minimum of n=6 for each group PTE+, PTE-, Sham)		
Blast Sham, PTE+/- tissue harvest, astrocyte isolation and processing for omics	3-32	20%
Blast Sham, PTE+/- data analysis	12-36	10%
Enter data into database	24-36	0%
Milestone Achieved: Preliminary data for hippocampus samples that were obtained until this point	12	50%
Milestone Achieved: Entire data set and analysis completed for BLAST	32	0%
Astrogliosis (minimum of n=10 for each group PTE+, PTE-, Sham)		
Blast PTE+/- tissue harvest and processing	3-24	25%
Blast PTE+/- tissue analysis	6-30	10%
Enter data into database	24-36	0%
Milestone Achieved: **Preliminary Analysis** of first and second cohort blast tissues **(minimum of n=3 for each group PTE+, PTE-, Sham)**	9- 12	80%
Milestone Achieved: Stereology and analysis completed for **Blast (minimum of n=10 for each group PTE+, PTE-, Sham)**	12-24	20%
Milestone Achieved: Data analysis completed a **(minimum of n=10 for each group PTE+, PTE-, Sham)**	24-36	0%
Specific Aim 3 – Compare Blast and Impact PTE Data	Months (24-36)	
Complete database entry	12-28	0%
Cross-comparison of Blast with Diffuse TBI data sets and **analysis against EEG data** set	24-36	10%
Milestone Achieved: Cross comparison of data of Blast and Impact models **(minimum of n=6 for each group PTE+, PTE-, Sham)**	36	5%

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) MAJOR ACTIVITIES FOR THIS REPORTING PERIOD

During this past year, we have accomplished the goals we have set forth for the current project. Specifically, all university and government approvals were granted and the experimentation efforts of the proposed work has begun. We have completed several rounds of animal blasting, EEG recording and analysis. Several challenges were found along the way, however, with the expertise within the team, we have been able to overcome some of the technical issues and still be on track to complete the project in the approved period.

Group 1 (n=6) - Blast testing and EEG completed

1. EEG recordings of Group 1 mice has been completed. With Group 1, 5 animals completed the 6 weeks of recordings. This is a total: 5472 hours of data**.**
2. Brains were collected from Group 1 mice for omics biological assessments. Magnetic bead separation of astrocytes from the cortex of the brain was conducted and quality control measurements depicted the mRNA to be highly purified mRNA samples. GFAP PCR was conducted on the isolated astrocyte mRNA samples and demonstrated that blast animals have elevated levels of GFAP as comparted to sham (figure 1).
   Sample are stored and waiting for increase numbers before further processing.
3. Total animals per category; 1 PTE+Blast, 3 Blast only, 1 Sham

Group 2 (n=7) - Blast testing and EEG completed

1. EEG recordings of Group 2 mice has been completed. With Group 2, 7 animals completed the 6 weeks of recordings. A total of 8192 hours of recordings was collected**.**
2. Brains were collected from Group 2 mice for omics biological assessments. Magnetic bead separation of astrocytes from the cortex of the brain was conducted and quality control measurements depicted the mRNA to be highly purified mRNA samples. Sample are stored and waiting for increase numbers before further processing.
3. Total animals per category; 1 PTE+Blast, 3 Blast only, 3 Sham.

Group 3 (n=20) – Blast testing completed; In the process of EEG analysis

1. EEG recordings of Group 3 mice has been completed. With Group 3, 20 animals completed the 6 weeks of recordings. A total of 24192 hours of recordings was collected**.**
2. Brains were collected from Group 3 mice and split between the omics and histological biological assessments. Magnetic bead separation of astrocytes from the cortex of the brain was conducted and quality control measurements depicted the mRNA to be highly purified mRNA samples. For immunohistological assessments, animals were perfused with 4% PFA and brains were collected for processing. Sample are stored and waiting for increase numbers before further processing.
3. Total animals per category; Pending analysis.

Group 4 (n=6) – Blast testing completed; In the process of EEG analysis

1. EEG recordings of Group 6 mice has been completed. With Group 4, 6 animals completed the 6 weeks of recordings. A total of 6192 hours of recordings was collected**.**
2. Brains were collected from Group 4 mice for histological biological assessments. For immunohistological assessments, animals were perfused with 4% PFA and brains were collected for processing. Sample are stored and waiting for increase numbers before further processing.
3. Total animals per category; Pending analysis.

2) SPECIFIC OBJECTIVES:

The objective of this project is to capture and compare differences between blast TBI animals with seizure-confirmed PTEs and seizure-free blast TBI animals to provide a comprehensive and unbiased analysis of the molecular pathway(s) contributing to blast-related PTE. Using the blast model of repeated TBI from our preliminary study, we will expand upon our confirmed spontaneous seizure results and take a gliocentric approach to identify biomarkers and molecular targets for the development of novel therapeutic approaches for PTE. To accomplish this, we propose the following three aims:

Aim 1: Establish seizure onset, incidence, frequency and EEG properties following blast exposure. An iterative refinement process will be employed using a mouse model of repeated blast TBI with confirmed PTE to establish a reproducible injury paradigm that causes seizure patterns comparable to high PTE-prevalent male and female human injury profiles.

Aim 2: Define PTE-distinguishing molecular and physiologic features in astroglia after blast exposure. We will systematically evaluate the key PTE-relevant biological changes in astrocytes using next generation genomics, proteomics, and robust histological assessments. The PTE-confirmed subpopulation of animals will be compared to blast-treated animals without PTE.

Aim 3: Comparative assessment of blast-PTE data to database of a diffuse impact-TBI PTE model. Using data previously collected from an impact-related mTBI-PTE model that mimics sport injury, data regarding seizure onset, incidence, frequency, EEG properties and omics data will be compared to determine common elements that may exist between various TBI PTE models.

3) SIGNIFICANT RESULTS:

Our proposed animal model uses adult C57Bl/6 mice (8-9 weeks) that were initially acclimated for two weeks prior to blast. Animals were maintained on a 12-12-hour light-dark cycle at 70°F and 70% humidity with ad libitum access to food and water. Blast testing was conducted using the Advanced Blast Simulator (ABS) shown in Figure 1. Prior to each blast, animals were administered general anesthesia (4% isoflurane in infusion chamber) for five minutes. Animals were then placed inside the test chamber of the ABS within a mesh sling that was designed to minimize wave hindrance, suspends and immobilizes the anesthetized animals within the test section. Blast exposures consisted of a series of three (one-hour intervals) anterior blasts. The three pressure measurements were collected at 250 kHz using a Dash 8HF data acquisition system (Astro-Med, Inc., West Warwick, RI) for each resulting blast wave. Peak static overpressures for the wave's positive phase were calculated by determining the measured wave velocity (m/s) at the specimen's position. The resulting pressure profiles resemble Friedländer-like waveforms. For the positive phase, the average peak static overpressure was 97 kPa (14 psi) with a positive phase duration of 2 ms. A high-speed camera records each event and videos confirm there is no impact of the animals with the walls of the chamber during blast. Recovery from anesthesia was monitored on a warming pad until the rat is mobile and animals awoke within one minute following blast. Anesthetized shams endured the aversive auditory stimulus produced in close proximity to the ABS. Upon completion, animals were returned to their respective rooms at the animal care facility. The following day, animals undergo electrode placement surgery and EEG recordings and videos began within 48 hours post blast series.

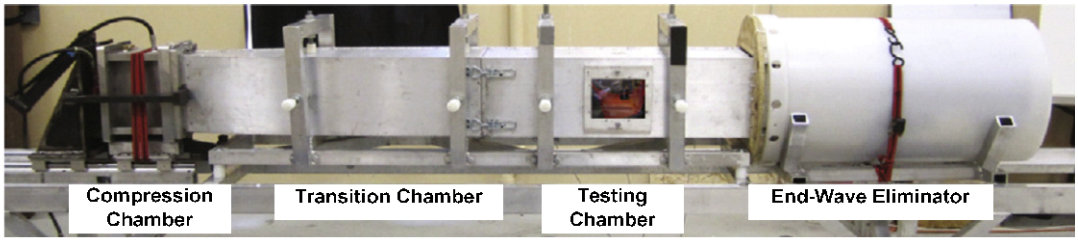

Figure 1 The ABS at Virginia Tech. The gas compression chamber (driver) is shown on left. This chamber is separated from the larger test section by a calibrated plastic membrane. The end–wave eliminator (shown on the right) diffuses the shock wave. Animals are loaded inside the chamber where open window is located. Blast pressures are measured and depicted with pressure magnitudes (kPa) expressed over specific times (msec) at specimen location.

Animals underwent video EEG recordings for 8 weeks to determine PTE status. Analysis of this data has been the most challenging aspect of the research thus far. The team meets frequently to discuss ways of distinguishing non-convulsive seizure from convulsive by frequency of EEG as videos are not 100% conclusive during dark hours if animal appears from its backside.

Once animals were determined to be either blast PTE+, blast PTE- or sham, animals were identified for either the histological or the omics outcomes. This past year, we have optimized all our biological methodologies. Figure 2 depicts the histological staining that will be conducted to identify astrogliosis. Figure 3 depicts the omics isolations of astrocytes from the fresh brain tissue and levels of GFAP within the tissues.

To accomplish the comparative assessment of Aim 3, our group has been collecting data from an impact-TBI model of PTE. We have structured the proposed research design to allow for a direct comparison of blast PTE with the ongoing impact-TBI PTE data. This unique dataset will be used to establish PTE predictors and therapeutic targets unique and similar to each of the TBI models. This established expertise in multiple models of TBI and recent advances across our laboratories can generate vital data that will inform the TBI/PTE fields, and importantly reduce the utilization of resources for duplicative research. The impact-TBI work has continued and will provide much data for comparison.

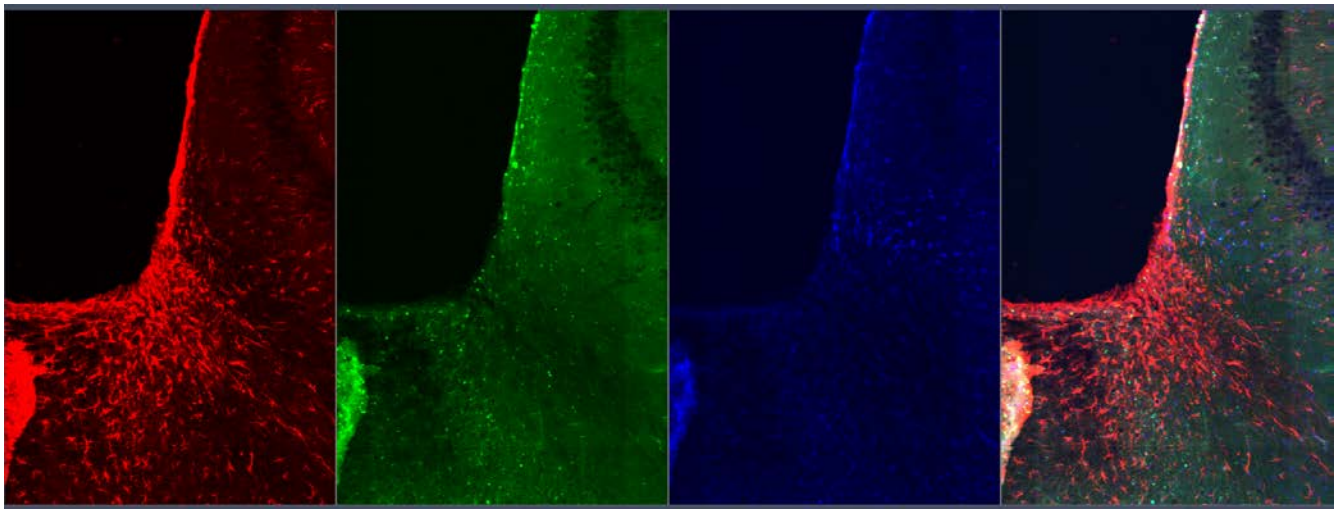

Figure 3. Images represent the histological assessments that have been optimized for the PTE study. Brain tissue from the brain of repeated TBI animals were collected and several iterations of the immunohistochemistry were conducted to optimize the protocols for triple labeling of gliosis markers. Astrocytic markers GFAP, S-100 and Dapi are shown individually from left to right. The far right depicts the combined images giving a comprehensive analysis of the pathology.

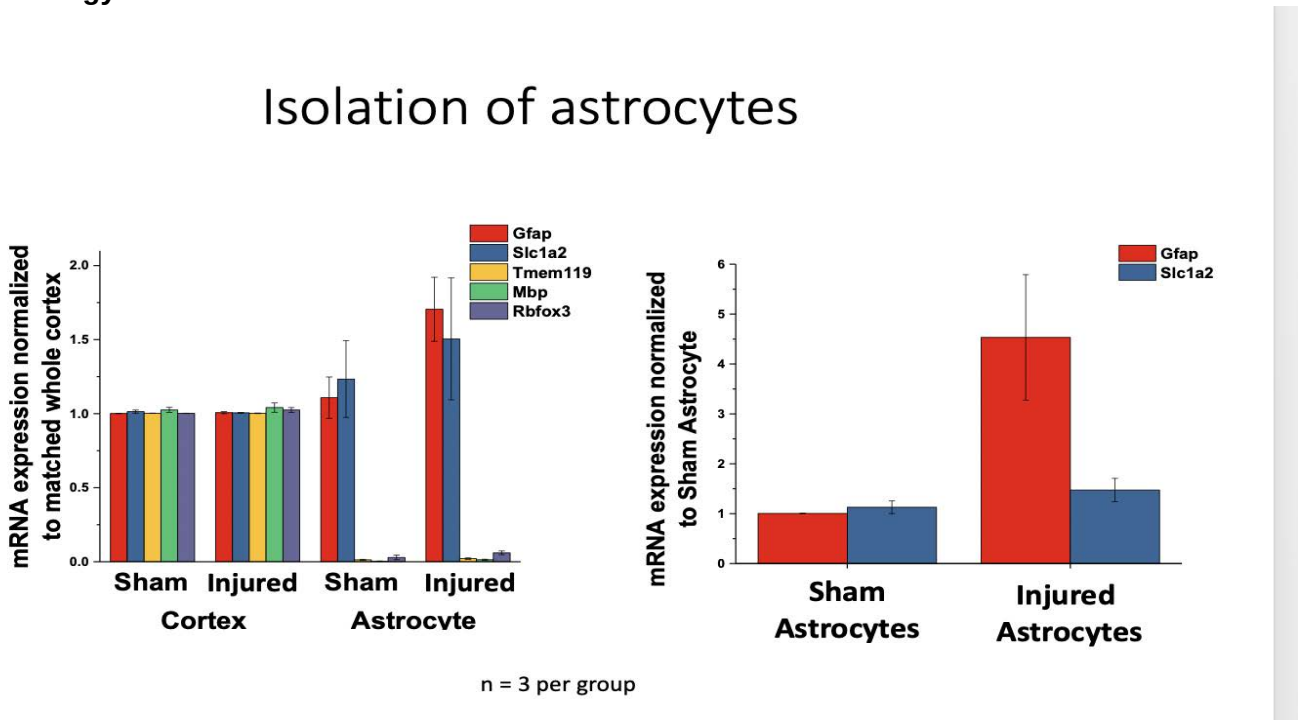

Figure 3. Cortex tissue from the brain of blast and sham animals were collected at 6 weeks post blast and continuous EEG recordings. On left, whole cortex features mRNA from various neural cells markers. Following magnetic bead isolation of astrocytes, only astrocytic mRNA was present indicating highly purified samples. On right, GFAP was elevated in blast animals compared to sham.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We expect this second year to be very productive as our momentum is high. Our staff has been trained and all methodology has been optimized. We expect to accomplish the majority of the experimental testing in Year 2 so that Year 3 will be focused on analysis and the comparative study.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research

in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The risk of blast-induced PTE is a rising concern for current and retired military personnel. Currently, there is no blast-related preclinical model for PTE. Through use of a military-relevant murine model of blast-induced neurotrauma, the proposed study aims to identify key biomarkers and pursuable therapeutic targets to help effectively monitor and treat Veterans affected by PTE. By the end of the proposal period, we expect to have successfully developed a reliable and reproducible experimental animal model of blast PTE is crucial to advance the field. This includes time to onset of first seizure and each subsequent seizure, the percentage of animals that develop epilepsy in the model, and weather a predicative, EEG characteristic signature occurs during the latent period and prior to spontaneous seizures. Furthermore, using a multi-omics approach, we aim to identify a list of molecular targets that are predictive for PTE. We will use the NIH NINDS TBI CDEs and the FITBIR data repository, which is a publicly available resource space to ensure that all data generated, is available to inform the TBI/PTE field, and reduce the utilization of resources for duplicative research.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

The successful development of the blast PTE preclinical model will help establish correlations between the physical components of blast and the associated PTE pathophysiology. Our intent in identifying PTE-pertinent biomarkers is to one day improve PTE diagnostics both on and off the battlefield, perhaps enabling intervention in the early stages of PTE pathologic progression. This multidisciplinary approach will further aid the framework needed to compare molecular and physiologic changes across different types of military-relevant PTE. In defining a causative link between chronic astrogliosis and PTE, the proposed study would expand basic insight into PTE pathologic sequelae may cause a shift in pharmacotherapeutic research strategies.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

There is a significant gap in understanding the mechanism of PTE. Until recently, there has been very limited discussion on the possibility that blast exposure can result in PTE. This body of research will help inform the public on this subject and open communication lines between patients and physicians to help identify those affected by the condition.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

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

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Although we had some challenges with the initial EEG recordings, we were able to eliminate the issues early on within the reporting period, so we are confident that we will be able to accomplish the tasks in the proposed time frame,

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

There were some initial delays in recruiting personal for the project, but the team has been established and is running smoothly.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

None to report

Significant changes in use or care of vertebrate animals

None to report

Significant changes in use of biohazards and/or select agents

None to report

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

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

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  None to report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication*

(published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

None to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

None to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

None to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

None to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

None to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or

research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*

None to report

7. 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".

Name: Pamela VandeVord, PhD
Project Role: PI
Researcher Identifier (e.g., ORCID ID): 0000-0003-3422-2704
Nearest person month worked: 0.6
Contribution: Oversees overall project. Conducted blast testing and histological assessment of brain tissues. Writes technical reports.

Name: Stefanie Robel, PhD
Project Role: Co-PI
Researcher Identifier (e.g., ORCID ID): 0000-0001-6716-3670
Nearest person month worked: 0.6
Contribution: Constructed 3 EEG analysis work stations for undergraduate and graduate students. EEG monitoring related to blast TBI, EEG analysis training and electrode implantation.

Name: Michelle Olsen, PhD
Project Role: Co-I
Researcher Identifier (e.g., ORCID ID): 0000-0003-1394-664X
Nearest person month worked: 0.36
Contribution: Oversees OMICs and development of SOPs related to OMICs. Oversees molecular experiments and analysis.

Name: Harald Sontheimer, PhD
Project Role: Co-I
Researcher Identifier (e.g., ORCID ID): 0000-0002-5843-9871
Nearest person month worked: 0.36
Contribution: Oversees EEG analysis and advises on technical protocols and writing.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

-
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*