Award Number: W81XWH-10-2-0092

TITLE: "Neurocognitive and Biomarker Evaluation of Combination mTBI from Blast Overpressure and Traumatic Stress”

PRINCIPAL INVESTIGATOR: Stephen Ahlers

CONTRACTING ORGANIZATION: The Geneva Foundation
Tacoma, WA 98402

REPORT DATE: September 2012

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
September 2012	Annual	1September2011-31August2012

Neurocognitive and Biomarker Evaluation of Combination mTBI from Blast Overpressure and Traumatic Stress

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-2-0092

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Rayond Genovese, Ph.D., Jitandra Dave, Ph.D., and Stephen Ahlers Ph.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

The Geneva Foundation
Tacoma, WA 98402

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
US Army MRMC
Ft Detrick, MD 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
There is a high co-morbidity of mild traumatic brain injury (mTBI) and post traumatic stress disorder (PTSD) in Warfighters. Co-morbid mTBI and PTSD appears to be more prevalent than mTBI cases in isolation. Mild TBI and PTSD are statistically ranked the highest of battlefield injuries in OIF and OEF. It is generally assumed that the manifestation of mTBI symptoms result from one or more exposures to improvised explosive devices (IEDs) and that PTSD symptoms result from exposure to prolonged battlefield stress. The high incidence and comorbidity of PTSD and mTBI underscore an imperative for the DoD research community to gain an understanding of the underlying mechanisms that precipitate these conditions together with the often associated post-concussive syndrome (PCS) which appears to share many of the same cognitive and emotive symptoms associated with TBI and PTSD. The purpose of the proposed experiments is to determine the relative contributions of repeated exposure to blast overpressure (BOP) and exposure to stressful (predatory) events, when presented alone and in combination, in a rodent model. The level of BOP used in the proposed experiments has been demonstrated by the PI (Ahlers) to be associated with mild outcomes where there is evidence of cognitive impairment in the absence of demonstrable pathology. The proposed experiments take advantage of years of extensive experience from the primary investigators (Ahlers & Genovese) in studies of the effects of BOP and stressful events and their effects on behavior. The assessment behavioral outcomes resulting from exposure to BOP and stress will be complemented by the assessment of the potential protein biomarkers by Dr. Dave and his group who have considerable experience identifying protein biomarkers for brain injury.

15. SUBJECT TERMS none provided

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**		7	**19b. TELEPHONE NUMBER** *(include area code)*

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

Table of Contents

INTRODUCTION:

There is a high co-morbidity of mild traumatic brain injury (mTBI) and post traumatic stress disorder (PTSD) in Warfighters. Co-morbid mTBI and PTSD appears to be more prevalent than mTBI cases in isolation. Mild TBI and PTSD are statistically ranked the highest of battlefield injuries in OIF and OEF. It is generally assumed that the manifestation of mTBI symptoms result from one or more exposures to improvised explosive devices (IEDs) and that PTSD symptoms result from exposure to prolonged battlefield stress. The high incidence and comorbidity of PTSD and mTBI underscore an imperative for the DoD research community to gain an understanding of the underlying mechanisms that precipitate these conditions together with the often associated post-concussive syndrome (PCS) which appears to share many of the same cognitive and emotive symptoms associated with TBI and PTSD. The purpose of the proposed experiments is to determine the relative contributions of repeated exposure to blast overpressure (BOP) and exposure to stressful (predatory) events, when presented alone and in combination, in a rodent model. The level of BOP used in the proposed experiments has been demonstrated by the PI (Ahlers) to be associated with mild outcomes where there is evidence of cognitive impairment in the absence of demonstrable pathology. The proposed experiments take advantage of years of extensive experience from the primary investigators (Ahlers & Genovese) in studies of the effects of BOP and stressful events and their effects on behavior. The assessment behavioral outcomes resulting from exposure to BOP and stress will be complemented by the assessment of the potential protein biomarkers by Dr. Dave and his group who have considerable experience identifying protein biomarkers for brain injury.

BODY:

The objective of this research proposal is to systematically assess the combined effects of BOP and exposure to traumatic stress in rodents with the aim of understanding how these forces may interact with the manifestation of cognitive and emotive dysfunction, as well as evaluating their outcome on known biomarkers involved in TBI and stress response system activation.

Specific Aims

- Specific Aim 1: Assess the effects of repeated exposure to BOP and stress on cognitive and emotional performance. The primary investigator for this aim is Dr. Ahlers with support (predator exposures and performing the elevated plus maze) from Dr. Genovese.

- Specific Aim 2: To characterize the extent to which BOP will specifically modify the process of conditioned fear in rats. The primary investigator for this aim is Dr. Genovese with support (blast exposures) from Dr. Ahlers.

- Specific Aim 3: Evaluate the combined effects of repeated exposure to BOP and stress on established biomarkers of traumatic brain injury (TBI). Primary

investigator is Dr. Dave working in tandem with Drs. Genovese and Ahlers. Dr. Dave's effort is structurally aligned with Dr. Genovese's effort, as they are both WRAIR performers. Because of the technical sophistication of the experiments the overall plan emphasized the efforts of specific aim 2, which, as stated previously, has been completed. Note that the analysis of brain biomarkers relating to the exposure conditions from specific aim 2 are underway but not yet complete. Aim 1 to assess the acute effects of blast on cognition and anxiety is ongoing.

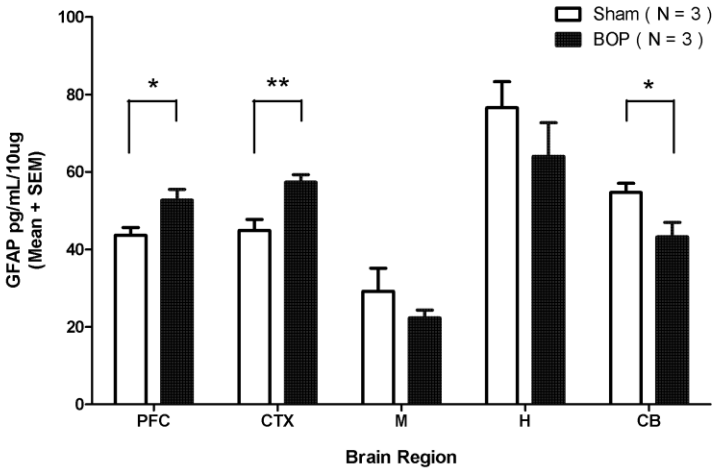
There are no changes to the original Statement of Work.

KEY RESEARCH ACCOMPLISHMENTS: (Ahlers portion) None significant, owing to the fact that the effort is in the early stages. We have exposed rats to BOP as prescribed in the proposal.

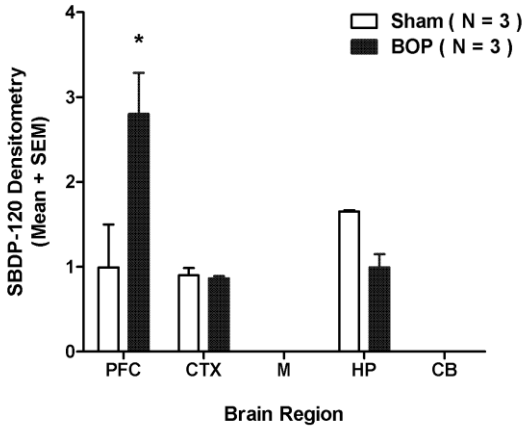
REPORTABLE OUTCOMES:

For the first phase of the study the co-PI role was to provide animals exposed to blast overpressure and to assist in the preparation of the animals and tissue for the biomarker portion of the experiment. The major portion of the work beyond the above was the conditioned fear experiments performed by Gr. Genovese's laboratory. These data were reported in the report submitted by Dr. Genovese and will not be repeated here.

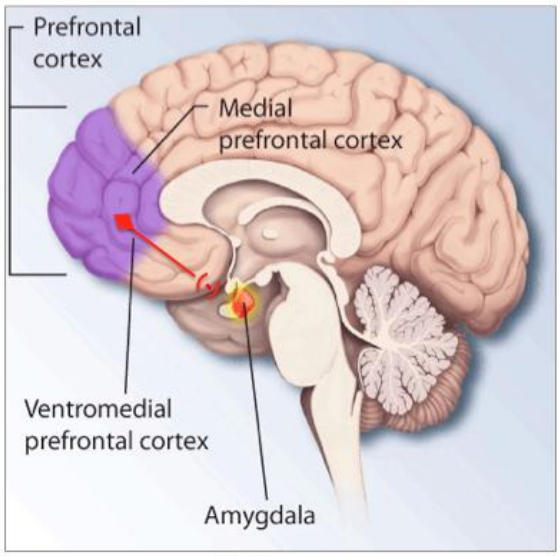
The preliminary biomarker analysis has revealed the possibility of significant outcomes. Exposure to blast overpressure appears to cause damage to brain regions that modulate the outward expression of anxiety. Specifically, in the figures below there are significant increases in glial fibrillary acidic protein (GFAP) in the prefrontal cortex (PFC) and other cortical regions in rats exposed to blast (vs. controls). Evidence of apoptotic changes in the PFC are shown in the next slide where there were significant increases in spectrin breakdown product 120 (SBDP 120), again potentially reflecting damage to the PFC.



* p<0.05, ** p<0.01, 1-tailed t-Test, CI = 95%



* p < 0.0001, 2-way ANOVA with Bonferroni Correction



CONCLUSION: The project is on pace to complete the milestones provided in the proposal. The next portion of the study will focus on exposure of rats to BOP and to the stressful stimuli with the focus on the assessment of anxiety and cognitive behavior.

Preliminary data from the biomarker portion of the study suggests that blast exposure may preferentially damage brain regions such as the PFC. The PFC is thought to exert inhibitory influences on limbic regions (amygdala) that have been implicated in the manifestation of anxiety (see diagram above).

APPENDICES: None.

SUPPORTING DATA:

As part of another collaboration we (Ahlers) recently published a paper examining the long-term effects of exposure to blast overpressure on the manifestation of anxiety behaviors several months after the blast exposure. The blast exposure parameters are similar to those employed in this study, however the studies are distinct in several ways. The primary emphasis of this effort is the near simultaneous exposure to blast and stress whereas the Elder et al. paper examined the effects of blast on anxiety behaviors 4 months or longer after the exposure to blast only (no stress exposures). The reference to the Elder paper is provided below.

Elder, G.A., Dorr, N. P., De Gasperi, R., Gama Sosa, M. A., Shaughness, M. C., Maudlin-Jeronimo, E., Hall, A. A., McCarron, R. M., Ahlers, S. T. Blast Exposure Induces Post Traumatic Stress Disorder-Related Traits in a Rat Model of Mild Traumatic Brain Injury. *Journal of Neurotrauma*, 29:1-12, 2012