Award Number:
W81XWH-14-1-0195

TITLE:
Novel Mechanism for Reducing Acute and Chronic Neurodegeneration After Traumatic Brain Injury

PRINCIPAL INVESTIGATOR:
Bruce Lyeth, Ph.D.

CONTRACTING ORGANIZATION:
Regents of University of California. Davis, CA 95616

REPORT DATE:
July 2015

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	**2. REPORT TYPE**	**3. DATES COVERED**
July 2015	Annual	1 Jul 2014 – 30 Jun 2015

4. TITLE AND SUBTITLE
Novel Mechanism for Reducing Acute and Chronic Neurodegeneration After TBI

5a. CONTRACT NUMBER
W81XWH-14-1-0195

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Bruce Lyeth, Ph.D.

E-Mail: bglyeth@ucdavis.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

UNIVERSITY OF CALIFORNIA, DAVIS
1850 RESEARCH PARK DR, STE 300
DAVIS CA 95618-6134

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 purpose of this project is to develop a radically different strategy to reduce brain glutamate excitotoxicity and treat TBI. We will supplement a natural blood-resident enzymatic system with glutamate-oxaloacetate transaminase (rGOT) and the co-substrate oxalo-acetate (OxAc) with the objective of reducing blood levels of glutamate. This will produce a brain-to-blood gradient of glutamate which will enhance the removal of excess glutamate from the brain.
Scope: We will test this novel and powerful neuroprotective treatment in a rat model of repetitive mild (concussive) TBIs and in a model of a single moderate TBI.
Major Findings: We have:
1) Established an assay for analysis of blood serum levels of glutamate and made preliminary measurements to demonstrate sensitivity and repeatability. We have performed experiments examining the effects of administration of exogenous intravenous glutamate on serum levels of glutamate.
2) Performed experiments examining the effects of intravenous administration of rGOT on serum levels of glutamate following intravenous administration of exogenous glutamate
3) Performed experiments measuring temporal changes in serum glutamate following moderate fluid percussion TBI in rats.
4) Performed experiments examining the effects of intravenous administration of rGOT on serum levels of glutamate in naïve rats.
5) Completed behavioral experiments examining effects of a single dose of GOT on motor and cognitive outcomes.

15. SUBJECT TERMS

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
U	U	U	UU	15	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

	Page
1. Introduction……..	**3**
2. Keywords……	**3**
3. Accomplishments……...	**3**
4. Impact……..……	**12**
5. Changes/Problems..……	**12**
6. Products……	**12**
7. Participants & Other Collaborating Organizations……	**12**
8. Special Reporting Requirements……	**13**
9. Appendices……	**13**

Introduction:

Traumatic brain injury (TBI) continues to be a major problem and has affected hundreds of thousands of service personnel who have served in the Mideast war theater. Many of these personnel have sustained repeated mild or concussive brain injury and now suffer from long-lasting cognitive and physical symptoms. We have developed a radically different strategy to reduce brain glutamate excitotoxicity and treat TBI. We will supplement a natural blood-resident enzymatic system with glutamate-oxaloacetate transaminase (rGOT) and the co-substrate oxalo-acetate (OxAc) with the objective of reducing blood levels of glutamate. This will produce a brain-to-blood gradient of glutamate which will enhance the removal of excess glutamate from the brain. We will test this novel and powerful neuroprotective treatment in a rat model of repetitive mild (concussive) TBIs and in a model of a single moderate TBI. Outcome measures include blood and CSF levels of glutamate, acute neuronal degeneration, chronic neuronal cell loss, glial activation, and chronic traumatic encephalopathy (CTE) measure of β-amyloid and hyper-phosphorylated tau protein. The objective of this project is to evaluate a novel treatment strategy for reducing excessive free glutamate associated with TBI.

This project uses a highly innovative approach to address the long-recognized problem of glutamate excitotoxicity associated with TBI. This novel approach supplements a natural enzymatic system that transforms blood-borne glutamate into α-ketoglutarate. By significantly reducing blood levels of glutamate, a brain-to-blood gradient is produced that enhances the efficiency of Na^+-dependent glutamate transporters located on brain endothelial cells. Thus, excess glutamate in the brain is transported into blood. Compared to the more traditional methods of reducing glutamate excitotoxicity, treatment with rGOT and OxAc circumvents the problems of unwanted side-effect of glutamate antagonists and poor blood-brain barrier penetration associated with receptor antagonist treatments.

Keywords:

Traumatic Brain Injury, Glutamate, GOT enzyme, Oxaloacetate, Fluid percussion, Morris water maze, Rotarod, Behavior

Accomplishments:

What were the major goals of the project?

The major goals of this project are to address the following series of related hypotheses.

- Intravenous administration of rGOT will significantly reduce the concentration of glutamate in blood and subsequently in CSF after TBI
- Treatment with rGOT will reduce functional deficits associated with TBI
- Treatment with rGOT will reduce neural and glial pathology associated with TBI

What was accomplished under these goals?

1. Established assay for analysis of blood serum levels of glutamate and made preliminary measurements to demonstrate sensitivity and repeatability.
2. Performed experiments examining the effects of administration of exogenous intravenous glutamate on serum levels of glutamate (Figure 1).
3. Performed experiments examining the effects of intravenous administration of rGOT on serum levels of glutamate following intravenous administration of exogenous glutamate (Figure 1).
4. Performed experiments measuring temporal changes in serum glutamate following moderate fluid percussion TBI in rats (Figure 2).
5. Performed experiments examining the effects of intravenous administration of rGOT on serum levels of glutamate in naïve rats (Figure 2).
6. Completed behavioral experiments examining effects of a single dose of GOT on motor (Rotarod, Figure 4) and cognitive (Morris water maze, Figures 5, 6, 7) outcomes.

Effects of rGOT administration on serum levels of glutamate:

We performed experiments to measure changes in blood serum concentrations of glutamate following tail vein administration of 3.33 ml/kg body weight of 15mM glutamate concentration (a 300 gram rat would receive 1.0 ml of the 15mM glutamate solution). A second group of rats was administered a bolus tail vein injection of rGOT enzyme (12.88 ug per 100 g rat) immediately after administration of 15 mM glutamate.

Intravenous injection of ~ 1.0 ml of 15 mM glutamate increased the blood plasma concentration of glutamate by 23 percent within 30 minutes (**Figure 1**). Co-administration of rGOT (12.88 ug/100g) along with 15 mM glutamate completely attenuated the elevation in serum glutamate. These results demonstrate that glutamate assay can reliably detect changes in blood serum concentrations of glutamate. Importantly, the dosage of rGOT tested (12.88 uG/100 g rat) was sufficient to completely reduce the elevated serum concentrations of glutamate following tail vein administration of exogenous glutamate. Ongoing experiments are examining the effects of different doses of rGOT.

Figure 1

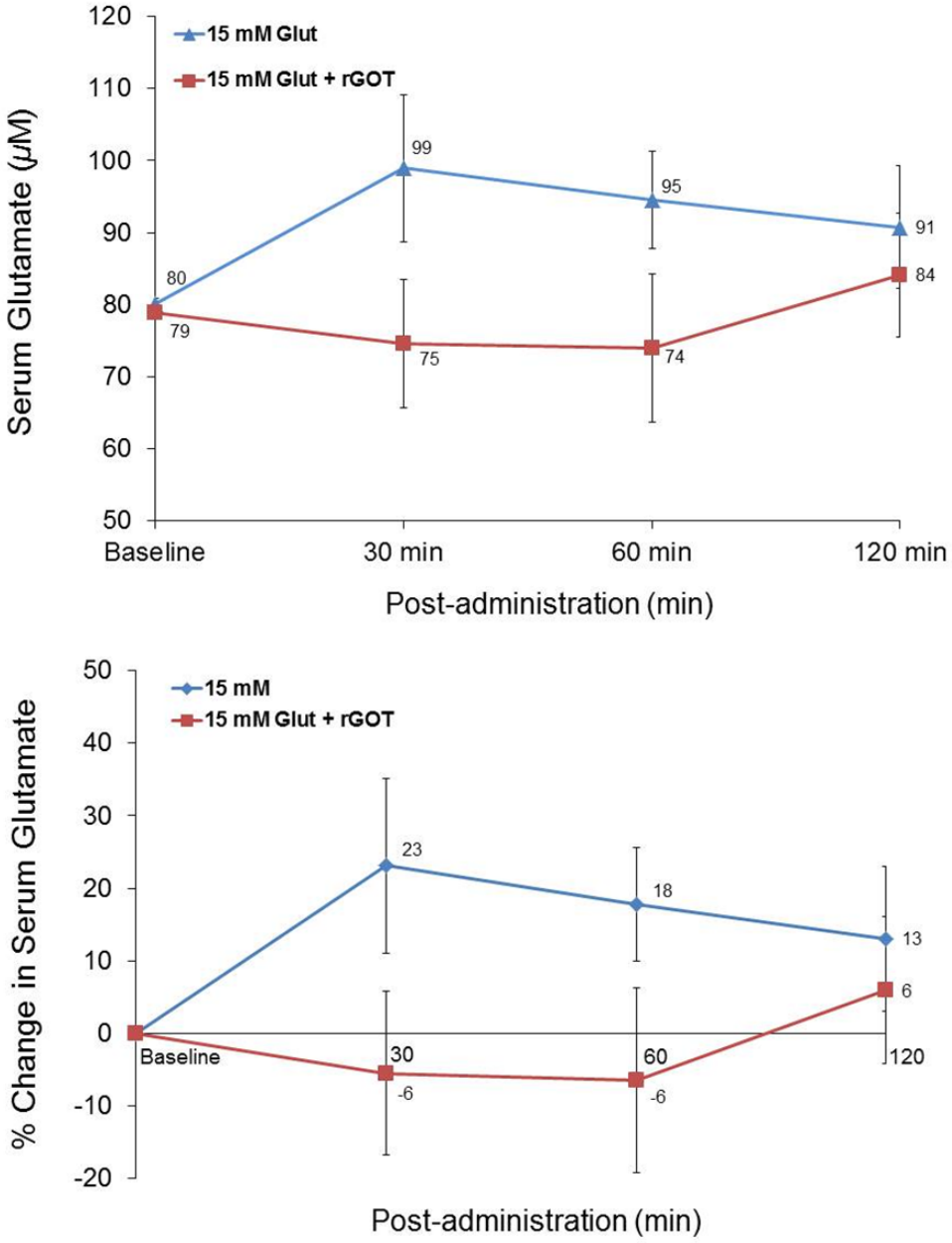


Figure 1. Serum glutamate concentrations in adult rats treated with 15 mM exogenous glutamate (n=6) and in combination with rGOT (n=6) (A). Tail vein blood samples were taken at different time points (pre-injection or baseline, 30, 60, 120 min). Animals that received 15 mM Glut showed an increase in serum glutamate concentration at 30 min and it remained elevated at 60 min and 120 min post-administration. Treatment with rGOT (12.88 µg per 100 g) immediately after administration of 15 mM Glutamate abolished the increase in serum glutamate level at similar time points. Data from the same experiment expressed as percent change from baseline in the lower graph. All data are mean ± SEM.

Effects of moderate TBI on serum levels of glutamate:

We have increased the number of animals in this experiment (see **Figure 2**). A baseline blood sample was drawn from the tail vein of male Sprague Dawley rats 24 hours prior to moderate fluid percussion TBI. Tail vein blood samples were drawn at 10, 20, 30 and 60 minutes after TBI. Blood was processed for serum and immediately frozen on dry ice for later analysis of glutamate concentration using Amplex Red glutamic acid assay kit (Molecular Probes). Preliminary results indicate that moderate TBI (2.14 ATMs) produced no change in serum glutamate after TBI Figure 3-A,B). This indicates that any movement of glutamate from brain interstitial fluid to blood is likely diluted in the large volume of circulating blood (~21 ml). Importantly, rGOT treatment significantly reduced the blood serum concentration of glutamate. This should increase the concentration gradient between brain and blood thereby enhancing the driving force of glutamate from the brain into the blood circulation.

Figure 2

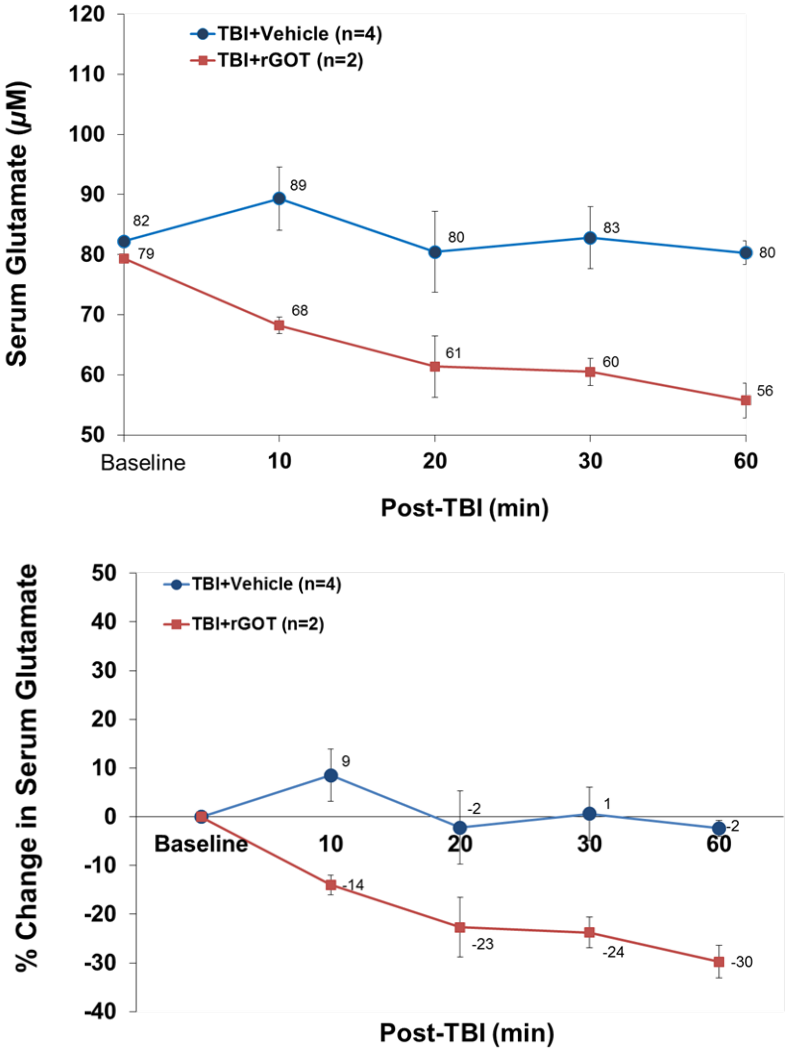


Figure 2. Serum glutamate concentrations in adult rats following moderate traumatic brain injury treated with rGOT (125ug/kg) or saline immediately following TBI. **(A)** Tail vein blood samples were taken at different time points (basal, 10, 20, 30, and 60 min). Serum glutamate levels were essentially unchanged by TBI, but rGOT treatment significantly reduced serum glutamate

concentrations. Data from the same experiment expressed as percent change from baseline are shown in the lower graph. Treatment with rGOT reduced serum glutamate concentrations by as much as 30 percent at one hour after injection. All data are mean ± SEM.

Behavioral experiments:

**Note that the group code identity for this blinded experiment was just broken at the time of writing this report. We are in the process of checking and verifying accuracy of data entries. Thus, the graphs below should be considered as preliminary and statistical analyses will not be performed until all data entries are verified. Final data graphs and statistical analyses will be completed during the next quarter (Year 2, quarter 1).*

Group parameters of body weight, injury magnitude, and temperature are provided in **Table 1.** The sham-operated rats were not subjected to TBI. Values of the experimental parameters were similar for all treatment groups. Importantly, the magnitude of the injury (ATM) was nearly identical for the three TBI treatment groups.

Groups	Weight (g)	ATM	Temporalis Temp.		Rectal Temp.	
			Pre	Post	Pre	Post
Sham (n=6)	333 ± 17	n/a	35.4 ± 0.06	35.3 ± 0.10	36.8 ± 0.41	36.8 ± 0.39
TBI + Vehicle (n=10)	340 ± 21	2.13 ± 0.01	35.8 ± 0.31	35.4 ± 0.31	37.1 ± 0.29	37.1 ± 0.38
TBI + rGOT (n=9)	339 ± 18	2.15 ± 0.01	35.9 ± 0.36	35.5 ± 0.50	37.1 ± 0.39	37.3 ± 0.46
TBI + rGOT + Oxal (n=9)	326 ± 21	2.15 ± 0.02	36.0 ± 0.33	35.5 ± 0.92	37.3 ± 0.35	37.2 ± 0.54

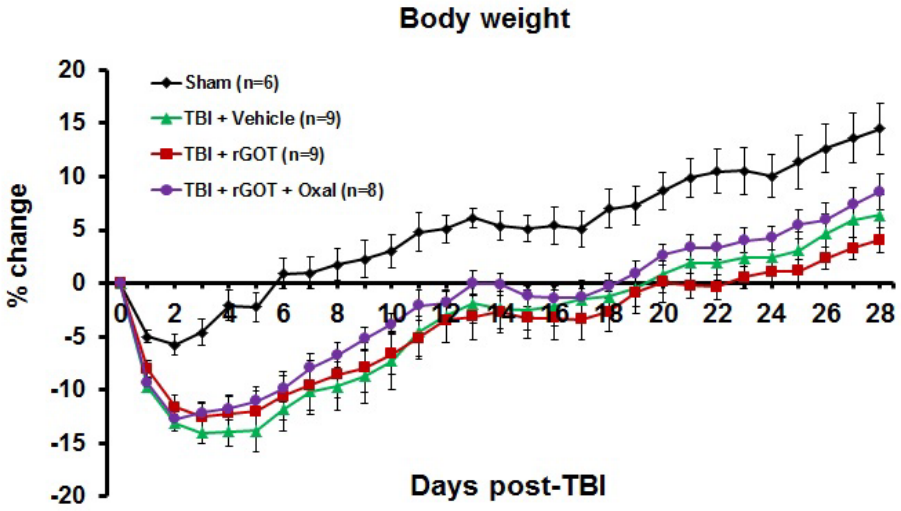
Table 1. Groups, Sample size, Body weight, ATM, Temporalis and Rectal temperatures (means ± SD)

Body Weight Post-TBI

The average animal body weight (% change) was normalized to the pre-surgery body weight and was calculated as the ratio of the difference between the post-TBI day and pre-TBI body weight over the pre-TBI body weight. Over the course of 28 days following TBI, the mean body weight of the three TBI groups decreased compared to the sham-TBI group (**Figure 3**). However, there appears to be no difference in the body weight of the animals in the TBI groups for any of the days following TBI. The mean maximum body weight loss for the sham and TBI groups were 6% (day 2) and 10-14% (days 3-5), respectively. The mean percent of body weight increased steadily with the sham and TBI groups regaining their pre-surgery body weight at

approximately 6 days and 18-20 days post-TBI, respectively.

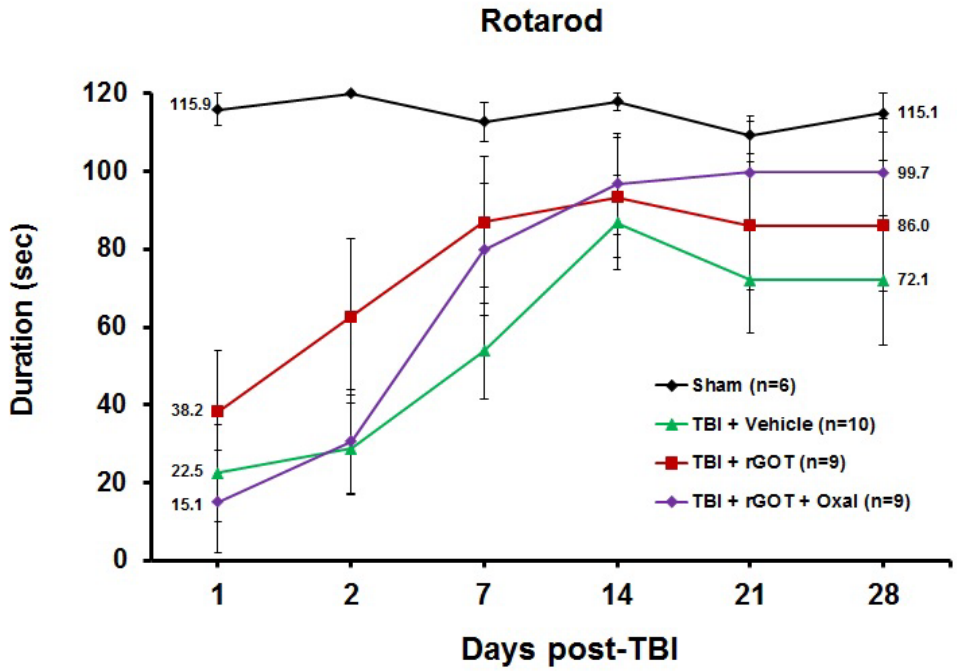
Figure 3



Motor deficits following TBI

The Sham-TBI group performed consistently near the maximum 120 seconds per trial over the 28 day testing period. TBI caused impairment of performance in sensorimotor functions assessed with the Rotarod test as evident by reduced durations (**Figure 4**). The animals in the three TBI groups showed progressive improvements in motor functions over the 28 day testing period. The TBI + GOT + Oxal animals nearly matched the performance of the sham –TBI animals at days 21 and 28. The vehicle TBI group had overall shorter durations in the Rotarod test.

Figure 4



Cognitive deficits following TBI

Moderate TBI produced a significant spatial memory deficit (MWM) in the TBI + vehicle treated animals when compared to the sham –TBI animals at days 13-16 post-TBI (**Figure 5**). The TBI + rGOT group did not show a significant difference in the cognitive performance from the vehicle-treated animals until day 5 of testing. Our data suggested that the cognitive performance improved from days 13-16 when the animals were treated with a combination of rGOT + Oxal. The average swim speed did not differ significantly for all the groups (Figure 5).

The probe trials (60 sec) of the Morris water maze were performed seven days following the final test trial on day 5 of the spatial learning task. The data showed no cognitive deficits existed in any of the groups in regards to the time spent in the target quadrant (**Figure 6**).

Swim speeds were nearly identical for all groups (**Figure 7**).

Figure 5

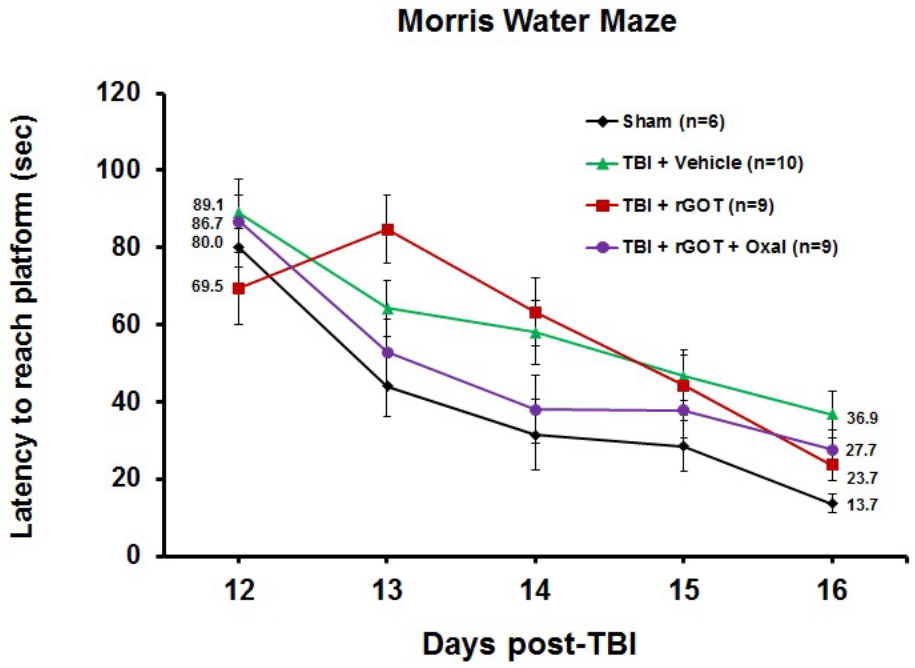


Figure 6

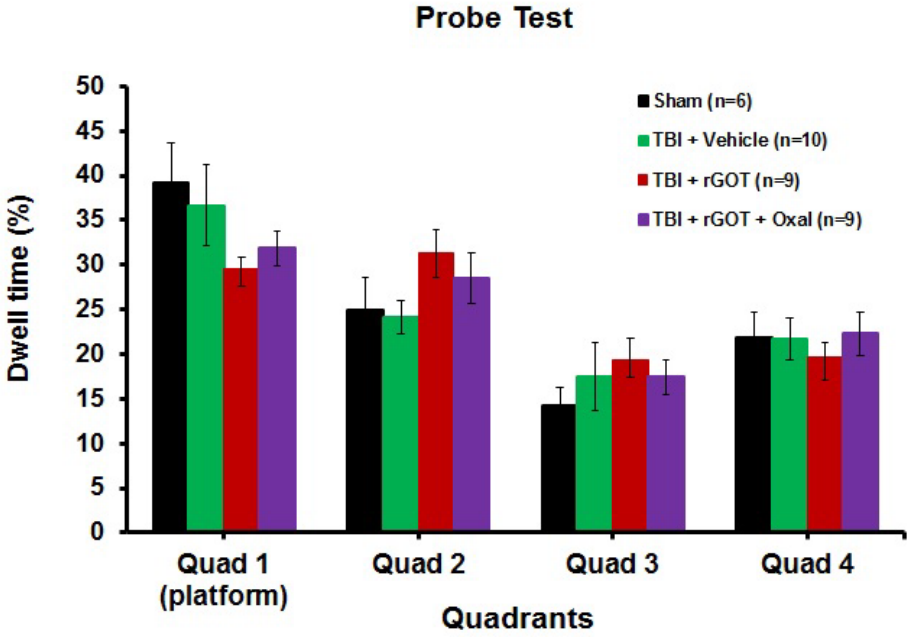
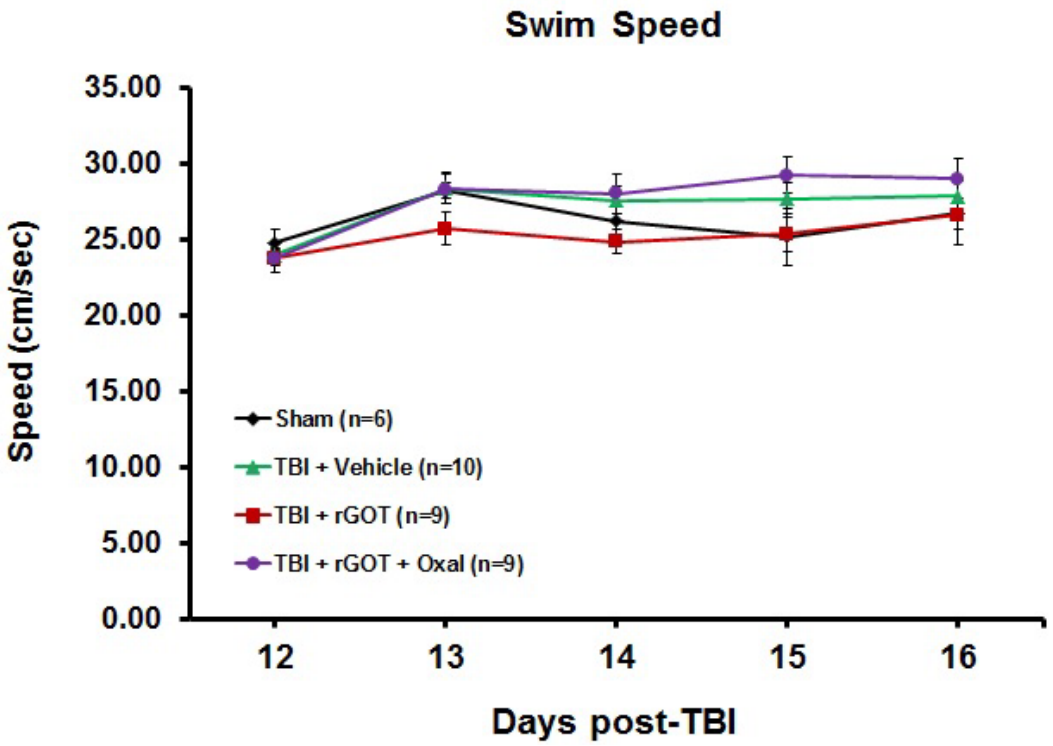


Figure 7



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 quarter one of year two, we will proceed with complete statistical analysis of the behavioral results reported herein. We will also begin processing the brains for histological evaluation of injury and treatment effects on long-term neuronal survival.

Reportable Outcomes:

Nothing to Report

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

Changes/Problems

Nothing to Report

Products:

Nothing to Report

Participants & Other Collaborating Organizations:

What individuals have worked on the project?

Name:	Bruce Lyeth, PhD
Project Role:	Principle Investigator
Researcher Identifier:	252972781 (UC Davis ID)
Nearest person month:	5
Contribution to the project:	Dr. Lyeth performed the fluid percussion TBIs and supervised the conduct of the project.
Funding Support:	No other source

Name:	Ken Van, MS
Project Role:	Staff Research Associate
Researcher Identifier:	613144013 (UC Davis ID)
Nearest person month:	10
Contribution to the project:	Mr. Van performed the surgeries, the blood draws, glutamate assays, and behavioral testing.
Funding Support:	No other source

Name:	Gene Gurkoff, PhD
Project Role:	Assistant Researcher
Researcher Identifier:	727993875 (UC Davis ID)
Nearest person month:	1
Contribution to the project:	Dr. Gurkoff assisted with the serum glutamate assays.
Funding Support:	No other source

Name:	Emily Doisy, BS
Project Role:	Staff Research Associate
Researcher Identifier:	897554960 (UC Davis ID)
Nearest person month:	1
Contribution to the project:	Ms. Doisy is the Laboratory Manager & Safety Officer. She managed ordering of supplies and managed safety training and concerns in the laboratory. She also assisted with the serum glutamate assays.
Funding Support:	No other source

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?

None

Special Reporting Requirements:

Collaborative Awards:

Not applicable

Quad Chart:

Updated quad chart is attached.

Appendices:

None

Novel Mechanism for Reducing Acute and Chronic Neurodegeneration after TBI

Log number: PT120075

Award Number: W81XWH-14-1-0195

PI: Bruce Lyeth, Ph.D. **Org: University of California, Davis** **Award Amount : $763,916**

DMRDP

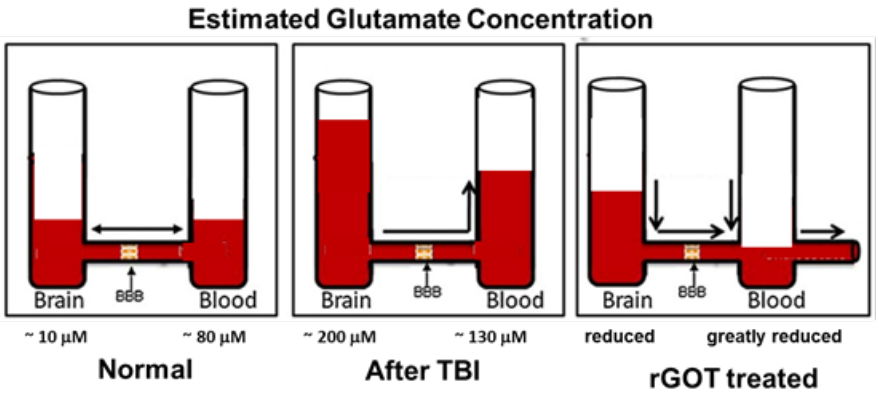
Study Aims

- **Specific Aim 1:** Determine the effects of TBI on glutamate levels in serum and CSF after TBI and determine the effects of rGOT on glutamate after multiple mild or a single moderate TBI in rats.
- **Specific Aim 2:** Determine the effects of the optimal doses of rGOT on acute and chronic brain pathology and behavioral outcome after TBI in rats.

Approach

An intravenous administration of the enzyme, glutamate oxaloacetate transaminase (GOT) that converts glutamate into α-ketoglutarate will be evaluated for reducing excessive free glutamate associated with TBI. We will examine the mechanism of action of GOT on blood and CSF levels of glutamate and the therapeutic potential of GOT to reduce cellular and behavioral pathology associated with TBI. These objectives are addressed using two clinically relevant models of experimental TBI in the rat.

Mechanism of glutamate diffusion between blood and brain: rGOT treatment reduces blood glutamate, creating a diffusion gradient that scavenges glutamate from the brain.



Timeline and Cost

Activities FY	14	15	16
Aim 1a: Determine optimal rGOT dosing for reducing blood and brain levels of glutamate.	■		
Aim 1b: Determine effects of rGOT on chronic pathology and behavior after repeated mild TBI		■	
Aim 2: Determine effects of rGOT on pathology and behavior after moderate TBI with & without secondary insults.			■
Estimated Budget ($K in total costs)	254	254	254

Updated: 27 JUL 2015

Goals/Milestones

CY14 Goal – Experiment ramp-up & determine optimal dosing

☑ Optimize experimental methods and glutamate assay

☑ Determine optimal rGOT dosing for reducing glutamate in blood

CY15 Goals – Evaluate treatment effects on repeated mild TBI

☐ Determine rGOT effects on brain pathology after mild TBI

☐ Determine rGOT effects on behavior after mild TBI

CY16 Goal – Evaluate treatment effects on moderate TBI

☑ rGOT effects on pathology/behavior after moderate TBI

☐ rGOT effects on pathology/behavior after moderate TBI + hypoxia

Comments/Challenges/Issues/Concerns

- No changes in timeline.
- spending off by 1 quarter due to early start in spending.

Budget Expenditure to Date

Projected Expenditure: $254,000

Actual Expenditure: $359,967