

AWARD NUMBER: W81XWH-16-2-0044

TITLE: “Evaluation of Clinically Relevant Prognostic Indicators in a Model of Mild TBI/Concussion”

PRINCIPAL INVESTIGATOR: Dr. Deborah Shear

CONTRACTING ORGANIZATION: The Geneva Foundation
Silver Spring, MD 20910

REPORT DATE: October 2017

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 is 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 Oct 2017		2. REPORT TYPE Annual		3. DATES COVERED 20Sep2016 - 29Sep2017	
4. TITLE AND SUBTITLE Evaluation of Clinically Relevant Prognostic Indicators in a Model of Mild TBI/Concussion				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-16-2-0044	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Dr. Deborah A Shear (PI), Dr. Kristen DeDominicis, Dr. Lai Yee Leung Email: deborah.a.shear.civ@mail.mil				5d. PROJECT NUMBER 10497	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Geneva Foundation 917 Pacific Ave, Suite 600 Tacoma, WA 98402				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 Closed head concussions, also known as mild traumatic brain injuries (mTBIs), are of great concern to both military and civilian populations alike. While acute concussion symptoms resolve for most patients, a subset will experience effects that persist chronically. Emphasis has been placed upon identifying prognostic indicators to distinguish these vulnerable patient populations for the purpose of providing enhanced care. Two potential clinically-relevant prognostic indicators include altered brain glucose metabolism as detected by FDG-PET imaging and changes in serum microRNA levels. This aim of this work is to comprehensively characterize longitudinal profiles of these two potential prognostic indicators following single and repeated injuries in a rodent model of closed head concussion. These studies utilize the WRAIR Projectile Concussive Impact (PCI) model, which is a military relevant model of closed head concussion developed under the directive of the Combat Care Casualty Research Program (CCCRP). In this Year 1 Report, we provide results to characterize longitudinal alterations in brain glucose uptake and associated neurobehavioral changes following single or repeated closed head concussions obtained in our studies thus far. In addition, plans for the assessment of serum miRNA changes following single or repeated closed head concussions are discussed.					
15. SUBJECT TERMS Nothing listed					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

TABLE OF CONTENTS

<u>No.</u>	<u>Page</u>
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	20
5. Changes/Problems	20
6. Products	21
7. Participants & Other Collaborating Organizations	22
8. Special Reporting Requirements	23
9. Appendices	23

1. INTRODUCTION:

The WRAIR Projectile Concussive Impact (PCI) model of closed-head mTBI was previously established under the directive of the Combat Casualty Care Research Program (CCCRP). The histopathological, molecular, and acute neurobehavioral profiles of this military-relevant mTBI model, which includes a custom designed helmet and sensor film system provided by the Army Research Laboratory, have been well characterized by previous studies. The primary goals of the current proposal are to a) characterize clinically relevant acute metrics of brain trauma following PCI and b) determine their prognostic value for chronic neurological and cognitive deficits and/or neurodegeneration. The two clinically relevant mTBI metrics assessed here will be brain glucose metabolic dysfunction and alterations in serum microRNA levels.

Following either single or repeated PCI injuries, studies in SOW Major Task 1 will assess brain glucose uptake by [18F] FDG-PET/CT imaging while studies in SOW Major Task 2 will evaluate serum microRNA profiles. This proposal expands upon our ongoing collaboration with the Uniformed Services University Health Science (USUHS) Translational Imaging Facility, which is highly experienced with the study of brain glucose metabolism in brain trauma models. The long-term objective of this proposal is to determine a clinically relevant mechanism for discerning mTBI patients whose symptoms will persist chronically, thereby identifying which patients may need increased care and treatment to mitigate chronic deficits and neuropathology. The findings from this study will be the basis for future preclinical studies following single or repeat PCI and will inform future clinical studies of mTBI.

2. KEYWORDS:

Concussion; Projectile Concussive Impact (PCI); mild TBI (mTBI); repeated mTBI; brain glucose metabolism; FDG-PET/CT imaging; microRNA; neurodegeneration; neurological deficits; behavioral impairment

3. ACCOMPLISHMENTS:

a. What were the major goals of the project?

SOW Major Task 1: Determine if acute brain glucose metabolism dysfunction following single or repeat PCI correlates with longitudinal behavioral outcome measures and chronic protein changes relating to CTE or neurodegenerative pathology.

SOW Major Task 2: Determine if acute changes in serum miRNA biomarkers have prognostic value for deficits in longitudinal behavioral outcome measures and CTE related neuropathology following single or repeat PCI.

TIMELINE FOR STATEMENT OF WORK	FY 2017	FY 2018
Completion of all Regulatory Processes		
SOW 1: PET/CT Imaging		
Task 1.1 Longitudinal PET/CT		
Task 1.2 Correlation of PET/CT with behavioral outcome metrics		
Sensoriomotor		
Memory		
Anxiety/motivation		
Task 1.3 Correlation of PET/CT with chronic protein changes		
SOW 2: microRNA		
Task 2.1 Serum miRNA		
Task 2.2 Correlation of miRNA with behavioral outcome metrics		
Sensoriomotor		
Memory		
Anxiety/motivation		
Task 2.3 Correlation of miRNA with chronic protein changes		
<i>red dotted line = reporting timeline marker; green triangles = markers for progress on specific tasks</i>		

b. What was accomplished under these goals?

SOW Major Task 1 (Months 1-24): Determine if acute brain glucose metabolism dysfunction following single or repeat PCI correlates with longitudinal behavioral outcome measures and chronic protein changes relating to CTE or neurodegenerative pathology.

SOW Major Task 1 is progressing ahead of schedule and includes four different study groups: single Sham (sSham; N=24), single PCI (sPCI; N=24), repeated Sham (rSham; N=22), and repeated PCI (rPCI; N=22). Injuries were induced using the modified PCI device, which has previously been described in great detail (Leung et al., 2014). In the repeated sham and injury groups, a total of 4 hits or sham control manipulations were performed for each rat with a one hour interval between procedures. All experimental tasks for this SOW Major Task 1 occurred at Site 1 (WRAIR; PI: Dr. Deborah Shear), with the exception of the PET/CT imaging experiments described in Subtask 1.1, which occurred at Site 2 (USUHS; PI: Dr. Bernard Dardzinski). All data for tasks occurring at or before 3 months following PCI has been collected. Procedures for tasks which include 6 month time points are currently underway.

Subtask 1.1: Determine the acute alterations in brain glucose metabolism in specific regions of interest (ROI) following single and repeated PCI by combined [18F] FDG-PET and CT.

In these experiments, brain region specific uptake of [18F]FDG was measured by PET with corresponding CT as a surrogate for assessing brain glucose

metabolism. FDG-PET/CT imaging experiments were conducted at 24h, 3d, 7d, 1m, and 3m after injury. These imaging experiments are also underway at 6m post injury; however, they have not yet concluded for all cohorts and are therefore not being presented here. All imaging was performed at the USUHS Center for Neuroscience and Regenerative Medicine (CNRM) Translational Imaging Facility (TIF). The morning of the scan, animals were transferred from WRAIR to USUHS/CNRM TIF. All transportation of animals to and from WRAIR and USUHS/TIF was performed by the WRAIR Veterinary Services Program (VSP). PET imaging was performed on the Siemens Inveon PET System. CT imaging was performed on the Siemens Multimodality System during the same acquisition session as the PET Imaging. For analysis, FDG uptake in μCi was determined in both the right (ipsilateral) and left (contralateral) hemispheres in the following broad area regions of interest (ROIs) using the invicroRatAtlas54 on the VivoQuant software: basal ganglia, thalamus, amygdala, cerebellum, cortex, hypothalamus, midbrain, corpus callosum, olfactory bulb, hippocampus, septal area, ventricles, and white matter. FDG concentrations in each right and left ROI were calculated in $\mu\text{Ci}/\text{mm}^3$ and were normalized to the concentration of FDG in the whole brain. These normalized values were used for subsequent data analysis.

Altered FDG uptake between PCI injured rats and their corresponding shams (ie, sSham vs sPCI; rSham vs rPCI) were analyzed in both ipsilateral and contralateral ROIs listed above. No comparisons were made between sPCI and rPCI rats due to the effects of multiple anesthesia administrations, which results in significant alterations in the absence of injury. Statistically significant injury effects are described below. Figures for these brain regions were included if a brain region (either ipsilateral or contralateral) demonstrated altered FDG uptake as a consequence of injury at any time point between 24h – 3m.

Both increased and decreased FDG uptake were observed acutely after PCI. Following sPCI, uptake in both the ipsilateral and contralateral olfactory bulbs increased by 2.63% and 1.89%, respectively, at 24h ($p < 0.05$, Fig. 1A). After rPCI, uptake increased by 2.76% in the ipsilateral olfactory bulb at 24h ($p < 0.05$, Fig. 1A) but decreased in the ipsilateral thalamus at 3d by 1.32% ($p < 0.05$, Fig. 1B).

While no significant injury effects were present at 7d or 1m following injury, altered FDG uptake re-emerged chronically in a novel regional distribution. At 3m following rPCI, uptake increased by 2.95% in the contralateral cortex ($p < 0.01$, Fig. 1D) and decreased by 1.68% and 3.14% in the ipsilateral thalamus ($p < 0.01$, Fig. 1B) and white matter ($p < 0.01$, Fig. 1C), respectively. No changes in FDG uptake were observed in the ipsilateral or contralateral basal ganglia, amygdala, cerebellum, hypothalamus, midbrain, corpus callosum, hippocampus, septal area, and ventricles at any time point assessed thus far.

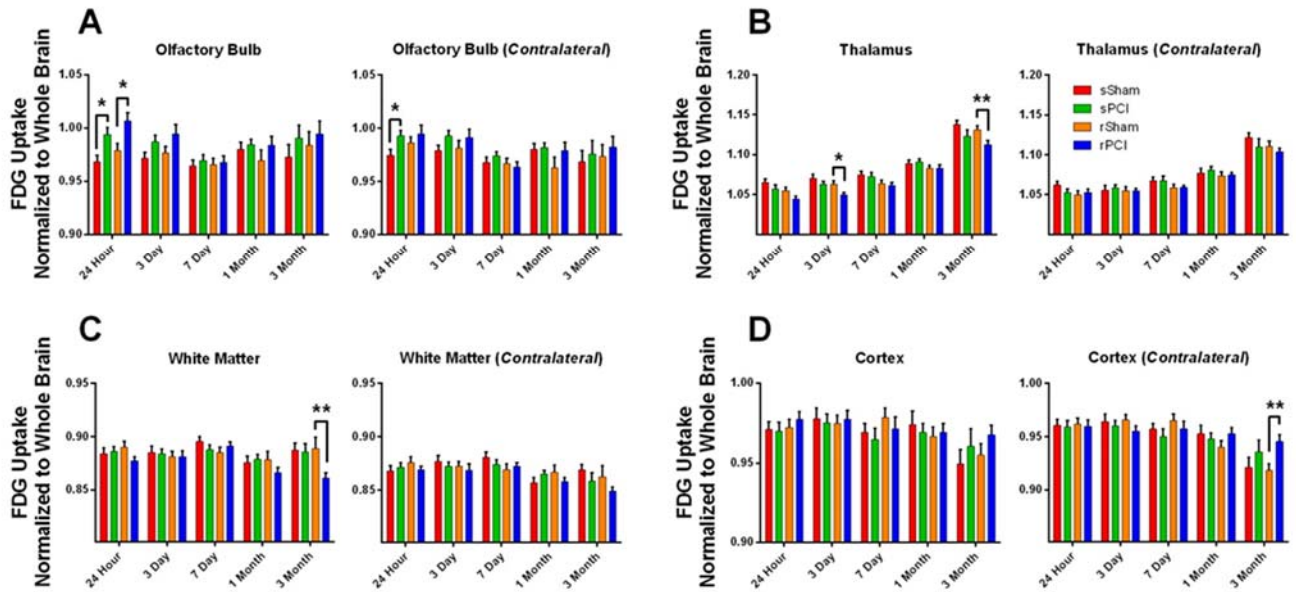


Figure 1: PCI alters FDG uptake. Longitudinal changes in FDG uptake were evaluated following PCI (A-D). Regions are presented here if at least one significant alteration was observed in either PCI group at any time point (* $p < 0.05$, ** $p < 0.01$ against respective sham control; Two-way ANOVA with Fisher's LSD post test). Ns for sSham,sPCI,rSham,rPCI at each time point are as follows: 24h - 22,22,22,22; 3d - 22,22,22,22; 7d - 21,22,22,22; 1m - 24,24,22,22; 3m - 18,16,18,17.

Subtask 1.2: Determine if brain glucose metabolism correlates with changes in established acute, subacute, and chronic behavioral outcomes following single or repeat PCI.

Experiment 1.2.1 Sensorimotor Assessments:

Righting Reflex: Immediately following each PCI impact, rats were returned to their home cage in the supine position and the time to return to an upright position, or righting reflex, was recorded. Rats in the sPCI group had significantly greater righting reflex times than those in the sSham group ($p < 0.01$, Fig. 2A). For repeat injury groups, righting reflex times were assessed after each sequential 1h impact. While the mean time to right was increased following the first PCI impact over sham control, this did not reach statistical significance ($p = 0.056$, Fig. 2B). Following the 2nd, 3rd, and 4th sequential PCI impacts, the mean time to right was significantly increased over the corresponding sham control anesthesia administration (2nd impact: $p < 0.01$, 3rd and 4th impacts: $p < 0.05$; Fig. 2B).

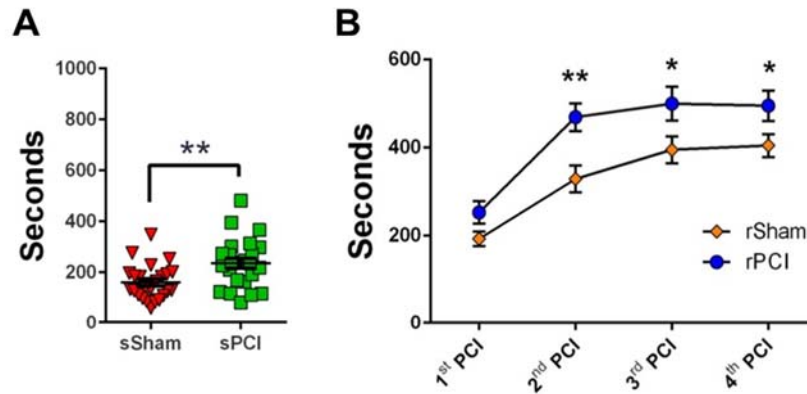


Figure 2: PCI increases righting reflex. Time to regain righting reflex was recorded following each PCI injury or sham control manipulation for both the single injury group (A) and repeat injury group (B). Values for each individual rPCI impact were compared against the matched rSham impact (* $p < 0.05$, ** $p < 0.01$). Statistical significance evaluated against the respective sham control by an unpaired t-test (A) or two-way ANOVA with Fisher's LSD post test (B). $N = 24, 24, 22, 22$ for sSham, sPCI, rSham, rPCI, respectively.

Gait Analysis: Rats were subjected to the automated gait analysis task at baseline, 2h, 2d, and 1m after injury using The CatWalk Automated Gait Analysis System (Noldus Information Technology, Leesburg, VA) as previously described (Mountney et al., 2013). Briefly, following acclimation to a darkened goal box (5 min), rats completed trial runs across a glass walkway towards the goal box. A camera positioned underneath the walkway recorded illuminated pawprints resulting from direct contact between the paws and glass surface, which were digitized for processing and analysed using the CatWalk XT 9. 55 different gait parameters were assessed. Limited gait alterations were observed at baseline, 2d, and 1m after injury while robust injury effects were seen at 2h. As such, only the 2h data is presented here.

At 2h, gait dysfunction compared to matched sham controls was detected in all four paws (RF, RH, LF, LH) at both injury severities. The data indicate a greater number of significantly altered parameters and larger percent changes compared to sham controls in the rPCI group than the sPCI group. 26/55 analyzed gait parameters were significantly altered following sPCI compared to sSham ($p < 0.05$) while after rPCI, 33/55 parameters were significantly altered from rSham ($p < 0.05$). The significant differences are presented as percent change from the appropriate sham control for both the sPCI and rPCI injury groups (Fig. 3A-C, non-significant parameters not shown).

Overall, dynamic gait parameters revealed that PCI animals moved more slowly than their corresponding sham controls (Fig. 3A). Temporal parameters indicated that injured rats spent more time moving through individual gait components compared to matched sham controls (Fig. 3B). Static paw positioning parameters revealed few differences between injured and uninjured rats (Fig. 3C). No injury effects were seen in parameters that examine inter-limb coordination.

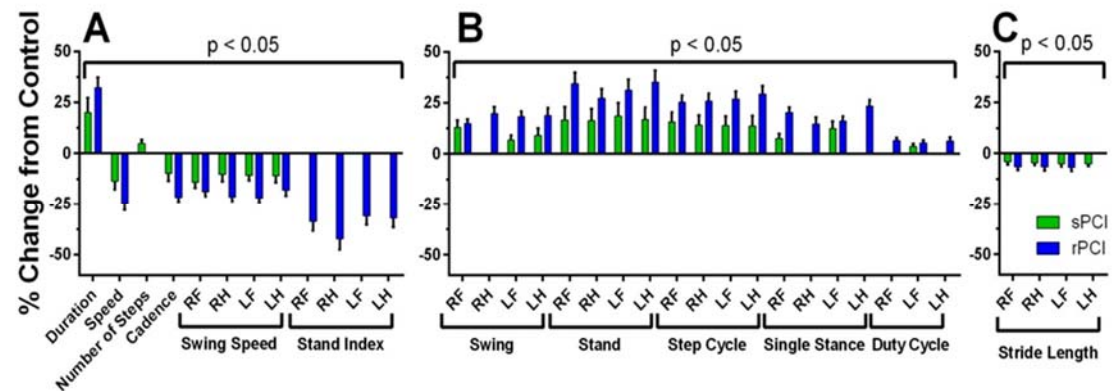


Figure 3: PCI induces acute gait dysfunction. Analysis of dynamic (A), temporal (B), and static (C) gait analysis parameters 2 hours following injury reveals significant injury effects in both the single and repeat PCI groups. Values are presented as a percent change in each injury group from the appropriately matched sham control. Statistical significance was determined using raw data values; only parameters which were significant in either PCI group are presented here ($p < 0.05$ from respective sham, unpaired t-test). $N = 24, 24, 20, 20$ for sSham, sPCI, rSham, rPCI, respectively.

NSS-R: The Revised Neurological Severity Scale (NSS-R) includes 10 separate neurological tests to evaluate motor, sensory, and reflex skills. These individual tests include a balance beam test, a landing test, a tail raise test, a drag test, righting reflex, ear reflex, eye blink response, sound reflex, tail reflex, and paw flexion reflex. Performance on each test is scored using the following system: 0 for no impairment, 1 for partial impairment, or 2 for severe impairment. Composite scores for each animal were tabulated at baseline, 4h, 2d, 1m, and 3m post injury. At baseline, the composite NSS-R scores from all groups were comparable. rPCI rats had significantly higher scores compared to repeat sham controls 4h after injury ($p < 0.001$, Fig. 4), indicating worse neurological deficits in this group acutely. Differences between rPCI and rSham groups were not apparent at 2d after injury or at any subacute or chronic time points assessed. No differences were observed between sPCI and sSham groups. Performance on the

NSS-R at 6 months is also currently being assessed but has not yet been completed in all experimental cohorts.

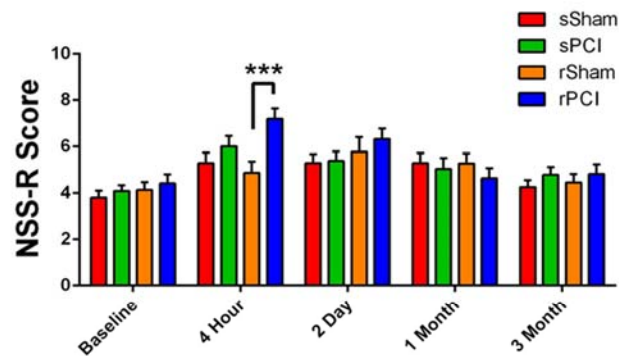


Figure 4: PCI results in acute neurological deficits. Composite NSS-R scores were elevated in the rPCI group at 4h after injury. Statistical significance of the injury groups was evaluated against their respective sham controls at each time point (***) $p < 0.001$, two-way ANOVA with Fisher's LSD post test). Ns for sSham,sPCI,rSham,rPCI at each time point are as follows: baseline - 24,24,22,22; 4h - 24,24,22,22; 2d - 24,24,22,22; 1m - 24,24,22,22; 3m - 22,23,19,21.

Experiment 1.2.2 Memory Assessments:

Memory assessments were performed at 1 and 3 months after injury using the Morris water maze (MWM) task (Noldus EthoVision XT) with a video-tracking system. Performance on these tasks is also being evaluated at 6 months but is not yet complete in all cohorts. The water maze apparatus consisted of a circular pool (75 cm deep; 175 cm diameter) filled with clear water (22 C, room temperature) to a depth of 60 cm. A clear, Plexiglas platform was submerged to a depth of 1 cm from the water surface and placed approximately 35 cm from the wall of the pool. Trials were performed in a darkened room with visual light cues.

Spatial Learning: In the spatial learning task, the rat was placed in the pool (snout facing the pool-wall) at one of four equally spaced starting positions: north (N), south (S), east (E), and west (W). Each rat was allowed to swim freely in the pool until finding the submerged platform or until 60 sec had elapsed. If the rat did not find the platform in 60 sec, it was manually guided there. Once on the platform, rats were allowed to rest for 10 sec prior to removal and return to their home cage. Rats were given 2 trials per day (5 min. ITI) for 4 consecutive days followed by a missing platform (probe) trial on the 5th day to assess memory retention. The platform location varied for each time point tested. The primary outcome measures were: (1) latency (sec) to find the hidden platform; (2) percent time spent swimming in outer

annulus (thigmotaxic behavior); and (3) percent time searching in the target (missing platform) zone during the probe trial.

The acquisition trials of the spatial learning MWM task revealed no significant injury effect in the latency to find the hidden platform compared to matched sham controls at any time point (Fig. 5). Thigmotaxic behavior (perimeter swimming) significantly increased in the sPCI compared to sSham group on the first acquisition trial one month following injury ($p < 0.05$, Fig. 6A) but significantly decreased in the rPCI group compared to rSham in the second acquisition trial at 3 months post-injury ($p < 0.05$, Fig. 6B). Both sPCI and rPCI groups had significantly lower mean thigmotaxic scores during the acquisition trials at 3 months after injury ($p < 0.001$, Fig. 6E) compared to their respective sham controls. No other differences in thigmotaxic swimming behavior were observed. Surprisingly, at 1m, sPCI rats spent significantly more time in the probe trial platform quadrant than sSham animals ($p < 0.001$, Fig. 7A). No other differences in memory retention were observed between injury groups.

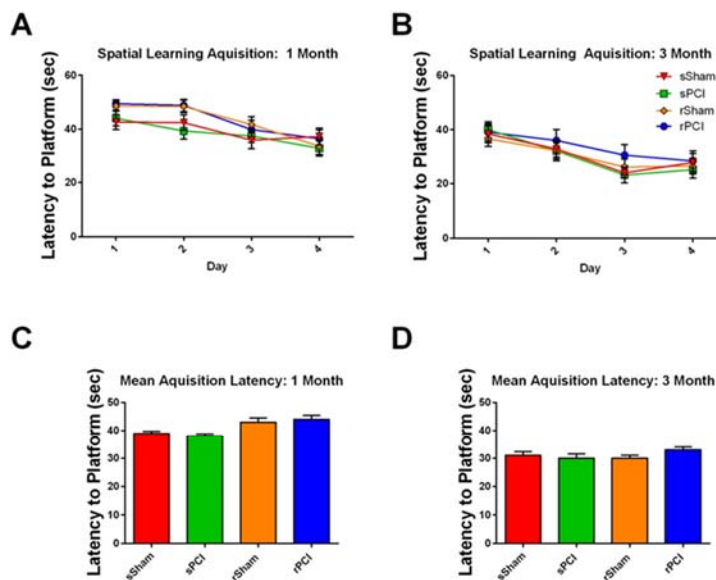


Figure 5: PCI does not alter spatial learning acquisition. Spatial learning was assessed after PCI in the Morris water maze task. No injury effects were observed compared to matched sham controls in individual daily trials (A and B, Two-way repeated measures ANOVA with Fisher's LSD post test) or mean acquisition latencies (C and D, unpaired t-test). Ns for sSham,sPCI,rSham,rPCI at each time point are as follows: 1m - 24,24,22,22; 3m - 24,23,22,20.

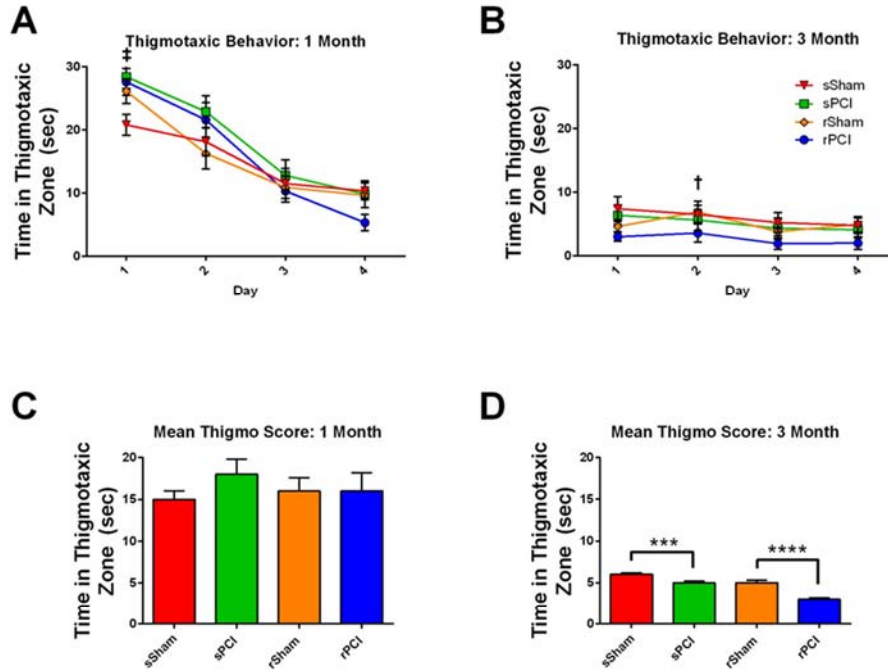


Figure 6: PCI affects thigmotaxic swimming behavior. Differences in thigmotaxic swimming in the spatial learning acquisition trials of the Morris water maze task were assessed by individual acquisition days (A and B, † $p < 0.05$ sSham vs sPCI, † $p < 0.05$ rSham vs rPCI, Two-way repeated measures ANOVA with Fisher's LSD post test) and mean duration in zone (C and D, *** $p = 0.001$, **** $p < 0.0001$ against respective sham control, unpaired t-test). Ns for sSham,sPCI,rSham,rPCI at each time point are as follows: 1m - 24,24,22,22; 3m - 24,23,22,20.

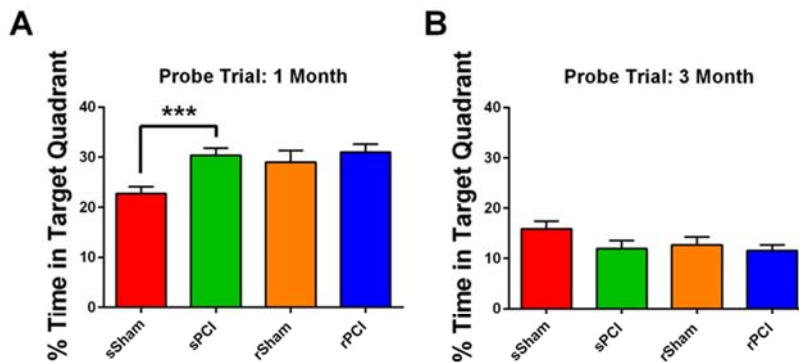


Figure 7: PCI has mild effects on memory retention. Percent of time spent searching the target quadrant for the missing platform was evaluated during the probe trial. Significant injury effects were assessed against the corresponding sham control group (A and B, *** $p < 0.001$, unpaired t-test). Ns for sSham,sPCI,rSham,rPCI at each time point are as follows: 1m - 24,24,22,22; 3m - 24,23,22,20. Ns for sSham,sPCI,rSham,rPCI at each time point are as follows: 1m - 24,24,22,22; 3m - 24,23,22,20.

Working Memory: The working memory testing was a delayed matching-to-place task that consisted of two sets of two trials each with a 5 minute inter-set interval. Within a single set, the second trial occurred immediately following the first. The starting position and platform location remained consistent for both trials within a set but was moved to a new starting position and platform location between trial sets. The difference in latency to locate the platform between trials in each trial set was determined and analyzed for statistical significance. No differences as a consequence of injury were observed on the working memory task at either 1 month or 3 months following PCI (Fig. 8).

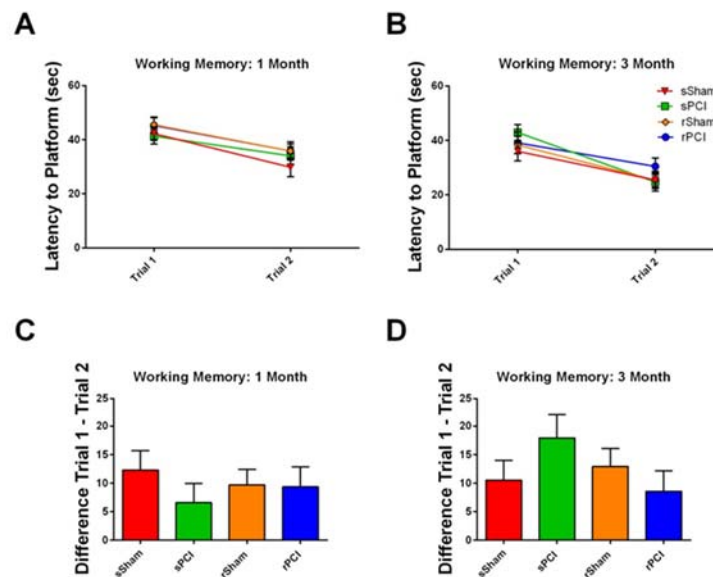


Figure 8: PCI does not result in working memory deficits. At both 1 and 3 months post injury, no significant working memory deficits were observed in latency to find the hidden platform in either the first or second trial (A and B, Two-way repeated measures ANOVA with Fisher's LSD post test) or in the difference between trials 1 and 2 (C and D, unpaired t-test). Ns for sSham,sPCI,rSham,rPCI at each time point are as follows: 1m - 24,24,22,22; 3m - 20,19,22,20.

Experiment 1.2.3 Anxiety and Motivation:

Anxiety behavior was assessed prior to injury and 1 and 3 months after injury with the elevated plus maze (EPM). Trials on the EPM are also underway at 6 months post injury, but these experiments have not yet concluded. The EPM (Noldus Technologies) consisted of two perpendicular intersecting walkways elevated 1 meter above the floor. One walkway (2 arms) had no wall while the other walkway (2 arms) had high walls. Rats were placed in an open arm facing the center of the maze and

were allowed to explore for 5 minutes. Animal movements were recorded and analyzed using Ethovision software (Noldus Technologies). All trials were performed in a darkened room without the experimenter present. The primary outcome measures were duration in open or closed arms, frequency of entering open or closed arms, distance travelled, and velocity.

Trials in the EPM revealed that sPCI rats entered the open arm significantly fewer times than sSham rats at 1m after injury ($p < 0.05$, Fig. 9C). No other significant alterations were observed between injured rats and matched sham controls at any time point for arm durations, arm entries, distance travelled, or velocity.

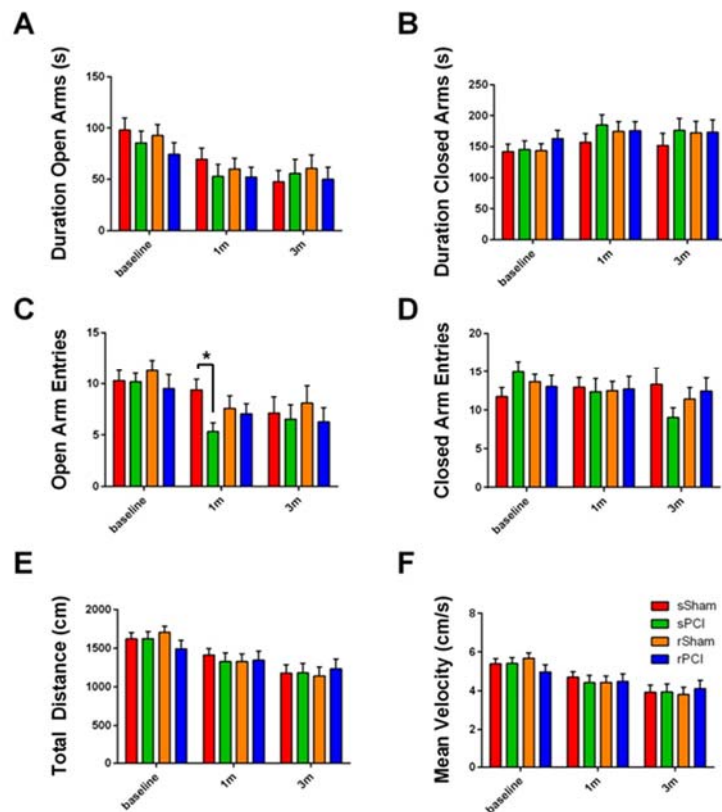


Figure 9: PCI results in limited anxiety-like behavior. The longitudinal effects of PCI on anxiety-like behavior were assessed using the elevated plus maze task. Significant alterations in open arm parameters (A&C), closed arm parameters (B&D), distance (E), and velocity (F) were evaluated for each PCI group against the respective sham (* $p < 0.05$, Two-way ANOVA with Fisher's LSD post test). Ns for sSham,sPCI,rSham,rPCI at each time point are as follows: baseline - 24,24,22,22; 1m - 24,24,22,22; 3m - 24,23,22,21.

Experiment 1.2.4 Correlation Analysis:

To assess if clinically relevant metrics of concussion may have prognostic value for acute - chronic alterations in brain glucose metabolism, correlational analyses between significantly altered brain regions of FDG uptake and injury impact factors, righting reflex times, and significantly altered gait parameters were performed. Correlational analyses between regions of altered brain glucose metabolism and chronic behavioral deficits will be performed following completion of the 6 month behavioral experiments.

No significant correlational relationships were obtained for single injury groups with any parameter assessed. For repeat injury groups, however, many significant correlations between acute concussion metrics and longitudinal FDG uptake alterations were obtained. For clarity and ease of interpretation, weak correlations ($-0.35 < r < 0.35$) have been omitted. Metrics which quantify the strength of the injury impact (Table 1) correlated significantly with acutely altered FDG-PET ROIs but did not correlate with chronic alterations in glucose uptake. Conversely, righting reflex (Table 2), which acts as a measure of loss of consciousness in the rat, correlated with acute through chronic changes in FDG uptake. Numerous significantly altered gait parameters detected at 2h post injury correlated with acutely (Table 3) and chronically (Table 4) altered FDG-PET ROIs.

FDG-PET ROI	Outcome Measure	Pearson r	p value
Olfactory Bulb (Ipsilateral) 24 Hour	<i>Pressure (PSI)</i>		
	4th Hit	0.5598	0.0067 **
	3rd Hit	0.5515	0.0078 **
	SUM	0.4702	0.0315 *
Thalamus (Ipsilateral) 3 Day	<i>Pressure (PSI)</i>		
	2nd Hit	-0.5499	0.008 **
	SUM	-0.5291	0.0137 *
	<i>Force (lbs)</i>		
	2nd Hit	-0.5385	0.0143 *
	3rd Hit	-0.4793	0.0325 *

Table 1: Significant results from two-tailed Pearson correlation analyses of injury impact factors with significantly altered FDG-PET ROIs. All data is from the repeat injury groups.

FDG-PET ROI	Outcome Measure	Pearson r	p value
Olfactory Bulb (Ipsilateral) 24 Hour	<i>Righting Reflex (s)</i>		
	SUM	0.5822	<0.0001 ****
	3rd Hit	0.5146	0.0004 ***
	4th Hit	0.5017	0.0005 ***
	2nd Hit	0.3719	0.0129 *
Cortex (Contralateral) 3 Month	<i>Righting Reflex (s)</i>		
	SUM	0.4514	0.0065 **
Thalamus (Ipsilateral) 3 Month	<i>Righting Reflex (s)</i>		
	4th Hit	-0.4742	0.004 **
	SUM	-0.4552	0.006 **

Table 2: Significant results from two-tailed Pearson correlation analyses of righting reflex with significantly altered FDG-PET ROIs. All data is from the repeat injury groups.

FDG-PET ROI	Outcome Measure	Pearson r	p value
	Average Speed	-0.4762	0.0022 **
	Duration	0.4216	0.0067 **
	Stand (RF)	0.421	0.0068 **
	Stand (RH)	0.4053	0.0095 **
	Stand (LH)	0.3805	0.0154 *
	Step Cycle (RF)	0.42	0.007 **
	Step Cycle (LF)	0.3799	0.0156 *
Olfactory Bulb (Ipsilateral)	Step Cycle (RH)	0.3781	0.0162 *
	Step Cycle (LH)	0.3513	0.0262 *
24 Hour	Swing Speed (LF)	-0.4138	0.0079 **
	Duty Cycle (RF)	0.411	0.0084 **
	Stand Index (LH)	0.4076	0.009 **
	Stand Index (RH)	0.3668	0.0216 *
	Single Stance (RF)	0.3692	0.0191 *
	Stride Length (RH)	-0.3679	0.0195 *
	Stride Length (LH)	-0.3544	0.0249 *
	Cadence	-0.3538	0.0251 *
	Swing Speed (RH)	0.4586	0.0029 **
	Swing Speed (RF)	0.3837	0.0145 *
	Swing Speed (LF)	0.3829	0.0148 *
	Step Cycle (LF)	-0.402	0.0101 *
Thalamus (Ipsilateral)	Step Cycle (RH)	-0.3807	0.0154 *
	Step Cycle (LH)	-0.3785	0.016 *
3 Day	Step Cycle (RF)	-0.3724	0.0179 *
	Cadence	0.3911	0.0126 *
	Stand (RF)	-0.3752	0.017 *
	Stand (LH)	-0.3673	0.0197 *
	Stand (LF)	-0.3635	0.0211 *
	Swing (RF)	-0.3609	0.0221 *

Table 3: Significant results from two-tailed Pearson correlation analyses of gait parameters with acute FDG-PET ROIs. Only gait parameters and ROIs which were significantly different from sham controls were assessed for a correlational relationship. All data is from the repeat injury groups.

FDG-PET ROI	Outcome Measure	Pearson r	p value
Cortex (Contralateral)	Swing Speed (LH)	-0.4058	0.0156 *
	Swing (LH)	0.3878	0.0213 *
3 Month	Single Stance (RH)	0.3625	0.0324 *
	Swing Speed (LH)	0.4927	0.0026 **
Thalamus (Ipsilateral)	Swing Speed (RH)	0.3658	0.0307 *
	Stand Index (RF)	-0.4404	0.0081 **
3 Month	Swing (LH)	-0.4302	0.0099 **
	Stand (RF)	-0.3558	0.0359 *
White Matter (Ipsilateral)	Swing (RF)	-0.3596	0.0398 *
3 Month	Swing Speed (LH)	0.3509	0.0452 *

Table 4: Significant results from two-tailed Pearson correlation analyses of gait parameters with chronic FDG-PET ROIs. Only gait parameters and ROIs which were significantly different from sham controls were assessed for a correlational relationship. All data is from the repeat injury groups.

Subtask 1.3: Determine if acute brain glucose metabolism dysfunction following a single or repeat PCI correlates with chronic protein changes relating to CTE or neurodegenerative pathology (tau, tau phosphorylation, and amyloid precursor protein) using end-term protein analysis.

Experiment 1.3.1 Neurodegenerative Pathology:

Following PCI, this experiment will evaluate chronic alterations in proteins related to neurodegenerative disease pathology such as tau, phosphorylated tau, and amyloid precursor protein. At sacrifice 6 months

post injury, rats are being perfused with 4% paraformaldehyde and the brains are being removed for evaluation by immunohistochemistry. Paraffin embedded coronal brain sections will be stained with an antibody against phosphorylated tau (AT8) and an antibody against amyloid beta (6E10). Sacrifice of all injury cohorts at the 6 month time point has not yet been completed. These experiments are currently underway.

Experiment 1.3.2 Correlation Analysis:

Correlation analyses between acute alterations in brain glucose metabolism as detected by PET/CT and protein hallmarks of neurodegeneration following PCI will be evaluated. Currently, experiments to evaluate neurodegeneration following PCI are ongoing. There are no correlational results to report at this time.

SOW Major Task 1 Summary and Conclusions:

The primary goal of SOW Major Task 1 was to characterize longitudinal alterations in brain glucose metabolism with FDG-PET imaging following single or repeated concussions induced with the WRAIR PCI model. This work has been completed through the 3 month endpoints. Assessment at 6 months post-injury, is ongoing. Thus far, we have found that PCI resulted in longitudinal alterations in FDG uptake that occurred in specific regions of interest (ROI) both ipsilateral and contralateral to the injury impact site. In the ROI analysis, altered uptake was present in the acute post-injury window. It resolved entirely by 7d, but re-emerged in a novel pattern at 3m post injury, which may be reflective of primary and secondary injury effects. While not true in every instance, the general pattern of these changes revealed an acute hypermetabolic profile with instances of hypometabolism prevailing chronically. The pattern of dysregulation in the olfactory bulb and thalamus are noteworthy. In the olfactory bulb, PCI injury resulted in increased uptake at 24h following injury in both the contralateral and ipsilateral hemispheres. One potential explanation for this result arises from the anatomy of the rat olfactory bulbs within the skull, with their protruding nature likely making them particularly vulnerable to the coup countercoup effects of the concussion injury. Also of note is the frequency and distribution of dysregulation observed in the thalamus. Here, decreased FDG uptake was observed acutely but re-emerged chronically. Some studies have examined the relevance of the thalamus in mTBI symptomology [for review, see (Grossman and Inglese, 2016)]. Given the importance of this brain region in relaying sensory and motor signals to the cortex, future research into thalamic dysfunction may provide insight into the etiology of chronic concussion symptomology.

Secondary goals of this study include the characterization of neurobehavioral deficits after single and repeat PCI. In accordance with previous studies, PCI resulted in robust acute neurofunctional deficits including increased righting reflex time, impaired performance detected by NSS-R, and numerous gait

alterations at 2h after injury. Chronic behavioral deficits in anxiety and cognition were also assessed. A reduced frequency of entry into the open arms of the EPM was observed in the sPCI group at 1m, which may be indicative of increased anxiety and decreased exploratory behavior following injury. However, this reduced frequency of entry into the open arms did not result in a reduction of time spent in the open arms of the maze. Cognitive deficits on the MWM were also observed following PCI. At 1m, the sPCI group displayed increased thigmotaxic swimming behavior on the first acquisition day, indicative of impaired focus on the task. In contrast, significant decreases in thigmotaxic behavior in both PCI groups were observed at 3m. This may reflect an injury-induced biphasic pattern. Surprisingly and contrary to expectations, the sPCI group at 1m spent increased time in the target quadrant of the probe trial, suggesting improved memory retention after injury compared to sham. However, upon closer examination, it appears likely that this sSham cohort spent an atypically lower amount of time in the target quadrant compared to all other groups rather than the sPCI group displaying improved performance on the task. Overall, the chronic behavioral deficits at 1m and 3m indicate mildly increased anxiety and decreased cognitive performance. The ongoing behavioral assessments at 6 months may strengthen the demonstrated chronic functional impairment following PCI.

SOW Major Task 2: Determine if acute changes in serum miRNA biomarkers have prognostic value for deficits in longitudinal behavioral outcome measures and CTE related neuropathology following single or repeat PCI.

Experiments for SOW Major Task 2 have not yet been initiated. Upon project commencement, the workload for Major Task 1 was scheduled ahead of Major Task 2. Since work for Task 1 occurs at two different sites and requires transportation of rats between facilities, it is more challenging to schedule and thus was prioritized to offset potential delays due to scheduling conflicts between sites. There are no results to report for Major Task 2 at this time.

Subtask 2.1: Determine the acute serum miRNA biomarker change profiles following single or repeat PCI.

Serum miRNA profiles will be assessed at 30 min, 24 hours, and 3 days post injury using serial blood draws. Terminal serum miRNA analysis will also be conducted

Subtask 2.2: Determine if acute miRNA biomarker profiles correlate with changes in established acute, subacute and chronic behavioral outcomes following single or repeat PCI.

Experiment 2.2.1 Sensorimotor Assessments: This experiment will assess sensorimotor function following PCI with the following tasks: Righting Reflex

immediately after injury; CatWalk gait analysis at 2h post injury, and the Neurological Severity Scale – Revised (NSS-R) at 48 hours, 3 months, and 6 months post injury.

Experiment 2.2.2 Memory Assessments: The Morris water maze task will be used to assess memory dysfunction at 1 and 3 months following injury.

Experiment 2.2.3 Anxiety and Motivation: The elevated plus maze task will be utilized to evaluate anxiety and motivation at 1, 3, and 6 months post injury.

Experiment 2.2.4 Correlation Analysis: Correlation analyses between acute serum miRNA and behavioral outcome metrics following PCI will be evaluated.

Subtask 2.3: Determine if acute miRNA biomarker profiles following a single or repeat PCI correlates with chronic protein changes relating to CTE or neurodegenerative pathology (tau, tau phosphorylation, and amyloid precursor protein) using end-term protein analysis.

Experiment 2.3.1 Neurodegenerative Pathology: Chronic alterations in proteins related to neurodegenerative disease pathology, such as tau, phosphorylated tau, and amyloid precursor protein, will be evaluated following PCI.

Experiment 2.3.2 Correlation Analysis: Correlation analyses between acute serum miRNA profiles and protein hallmarks of neurodegeneration following PCI will be evaluated.

SOW Major Task 2 Summary and Conclusions: There are no results or conclusions to present at this time for SOW Major Task 2.

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

Nothing to report.

d. How were the results disseminated to communities of interest?

Selected results from SOW Major Task 1 were presented in poster format at the 2016 National Neurotrauma Symposium. Details of this presentation may be found in Section 6 (Products) of this report. Additionally, a manuscript to disseminate the results of SOW Major Task 1 is currently in preparation for submission and publication in a peer reviewed journal.

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

To accomplish the remaining goals and objectives from SOW Major Task 1, work will continue as planned. This includes completing experiments at the 6 month time point (FDG-PET imaging, NSS-R, memory assessments, and anxiety assessments) for all remaining injury cohorts. Histopathology to assess protein accumulation related to neurodegeneration and its potential correlation between brain glucose metabolic changes will also proceed as planned. Work will also commence on SOW Major Task 2.

4. IMPACT:

a. What was the impact on the development of the principal discipline(s) of the project?

The brain regions identified in this project as being sensitive to glucose metabolic dysregulation following concussive injury will inform future pre-clinical and clinical studies that examine metabolic disturbances following mTBI. The data generated from this study thus far also highlight the potential importance of acquiring a baseline PET imaging scan to assess changes after injury on an individual basis. This is an important consideration in the design of future preclinical imaging studies.

b. What was the impact on other disciplines?

Nothing to report.

c. What was the impact on technology transfer?

Results from SOW Major Task 1 demonstrating chronic disruptions in brain glucose metabolic activity, in conjunction with findings future preclinical and clinical studies to better define these changes, may impact the usage and duration of use of FDG-PET imaging clinically following concussion.

d. What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

a. Changes in approach and reasons for change

Nothing to report.

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

Nothing to report.

c. Changes that had a significant impact on expenditures

Nothing to report.

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

Nothing to report.

e. Significant changes in use or care of human subjects

Nothing to report.

f. Significant changes in use or care of vertebrate animals.

Nothing to report.

g. Significant changes in use of biohazards and/or select agents

Nothing to report.

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.

DeDominicis KE, Mountney A, Wilson C, Jones S, Jaiswal S, Deng-Bryant Y, Braverman S, Hwang H, Hahn J, Hoy A, Dardzinski B, Shear D, Selwyn R, Cartagena C. (2016) Longitudinal FDG uptake changes following mild concussive brain injuries and correlation to clinical mTBI assessors in rats. Poster presentation. 34th National Neurotrauma Society Symposium in Lexington, KY, USA.

- **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**

This project has demonstrated that the previously established WRAIR PCI model of mild head trauma captures the chronic metabolic depression which has previously been described in clinical patients following brain injury, thus supporting its use as an effective animal model in which to study this phenomenon.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. What individuals have worked on the project?

Name: Deborah Shear
Project Role: Principal Investigator (Site 1)
Research Identifier:
Nearest person month worked: 2
Contribution to Project: Data Analysis; Reporting
Funding Support: CCCRP

Name: Kristen DeDominicis
Project Role: Associate Investigator
Research Identifier:
Nearest person month worked: 6
Contribution to Project: PCI Injuries; Behavioral Assessments; Data Analysis; Reporting
Funding Support: CCCRP

Name: Lai Yee Leung
Project Role: Associate Investigator
Research Identifier:
Nearest person month worked: 2
Contribution to Project: Data Analysis; Reporting
Funding Support: The Geneva Foundation

Name: Katherine Cardiff

Project Role: Research Associate
Research Identifier:
Nearest person month worked: 2
Contribution to Project: PCI Injuries; Behavioral Assessments
Funding Support: CCCRP

Name: Shalini Jaiswal
Project Role: Research Associate
Research Identifier:
Nearest person month worked: 2
Contribution to Project: PET/CT Imaging; Data Analysis
Funding Support:

Name: Bernard Dardzinski
Project Role: Principal Investigator (Site 2)
Research Identifier:
Nearest person month worked:
Contribution to Project: PET/CT Imaging; Data Analysis; Reporting
Funding Support:

b. 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.

c. What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Not applicable.

9. APPENDICES

References

Grossman EJ, Inglese M (2016) The Role of Thalamic Damage in Mild Traumatic Brain Injury. J Neurotrauma 33:163-167.

- Leung LY, Larimore Z, Holmes L, Cartagena C, Mountney A, Deng-Bryant Y, Schmid K, Shear D, Tortella F (2014) The WRAIR projectile concussive impact model of mild traumatic brain injury: re-design, testing and preclinical validation. *Annals of biomedical engineering* 42:1618-1630.
- Mountney A, Leung LY, Pedersen R, Shear D, Tortella F (2013) Longitudinal assessment of gait abnormalities following penetrating ballistic-like brain injury in rats. *J Neurosci Methods* 212:1-16.