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14. ABSTRACT

Closed head concussion is of significant concern to both military and civilian medicine. 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.

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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:

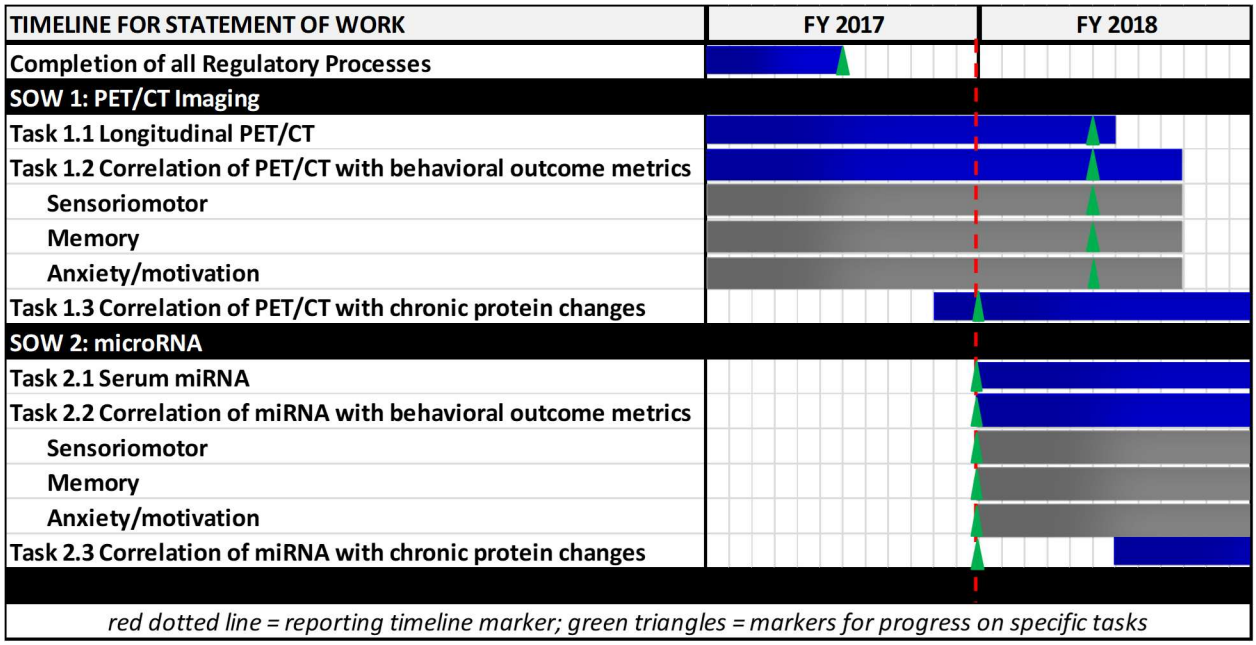
Concussion; Projectile Concussive Impact (PCI); mild TBI (mTBI); repeated mTBI; brain glucose metabolism; FDG-PET/CT imaging; microRNA; neurodegeneration; neurological deficits; behavioral impairment

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.



b. What was accomplished under these goals?

<u>SOW Major Task 1 (Months 1-24):</u> 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.

Work for SOW Major Task 1 has been completed. Four different study groups were initiated for this task: single Sham (sSham), single PCI (sPCI), repeated Sham (rSham), and repeated PCI (rPCI). Injuries were induced using the modified PCI device, which has previously been described in great detail (Leung, Larimore 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 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).

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, 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, 3m, and 6m after injury. 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 imagining was performed on the Siemens Inveon PET System. CT imagining 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 μCi/mm3 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 – 6m.

Results regarding FDG uptake following single and repeat PCI from 24h – 3m after injury were reported in the Year 1 Annual Report. At 6m following sPCI, uptake decreased in the ipsilateral ventricles by 2.05% ($p < 0.05$, Fig. 1C) and the ipsilateral and contralateral thalamus by 1.65% and 1.83%, respectively ($p < 0.05$, Fig. 1B). After rPCI, FDG uptake decreased in both the ipsilateral and contralateral thalamus by 1.56% and 1.51%, respectively, at 6m after injury ($p < 0.05$, Fig. 1B). In addition, uptake decreased in the ipsilateral hemisphere by 0.59% but increased in the contralateral hemisphere by 0.64% at 6m after rPCI ($p < 0.01$, Fig. 1F).

No changes in FDG uptake were observed in the ipsilateral or contralateral basal ganglia, amygdala, cerebellum, hypothalamus, midbrain, corpus callosum, hippocampus, and septal area at any time point assessed.

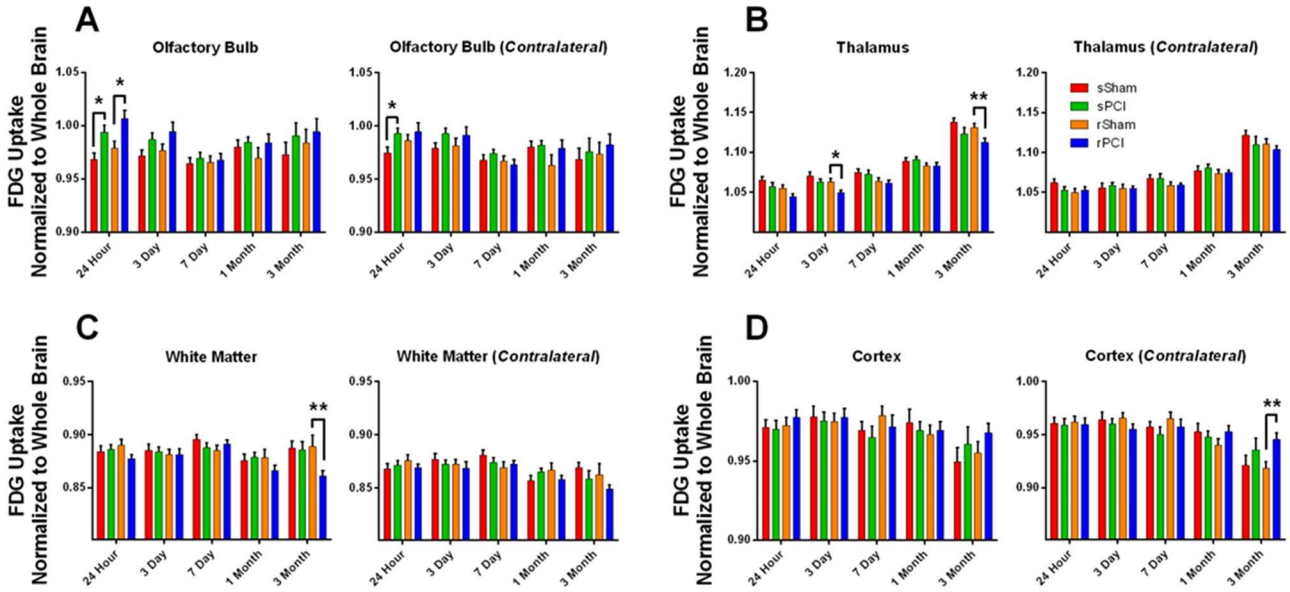


Figure 1: PCI alters FDG uptake. Longitudinal changes in FDG uptake were evaluated following PCI (A-D). Regions are presented here if at least one significant alteration was observed in either PCI group at any time point (* $p < 0.05$, ** $p < 0.01$ against respective sham control; Two-way ANOVA with 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. 3A). 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. 3B). 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. 3B).

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, 3m, and 6m post injury. At baseline, the composite NSS-R scores from all groups

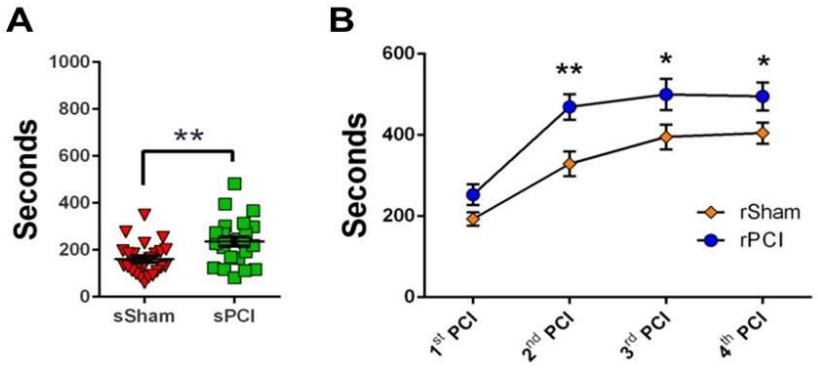


Figure 3: PCI increases righting reflex Time to regain righting reflex was recorded following each PCI injury or sham control manipulation for both the single injury group (A) and repeat injury group (B). Values for each individual rPCI impact were compared against the matched rSham impact (* $p < 0.05$, ** $p < 0.01$). 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.

were comparable. NSS-R results for 24h – 3m following rPCI were presented in the Year 1 Annual Report. At 6m, no chronic deficits or sPCI or rPCI were observed as detected by the NSS-R (Fig. 3).

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. 4A-C, non-significant parameters not shown).

Overall, dynamic gait parameters revealed that PCI animals moved more slowly than their corresponding sham controls (Fig. 4A). Temporal parameters indicated that injured

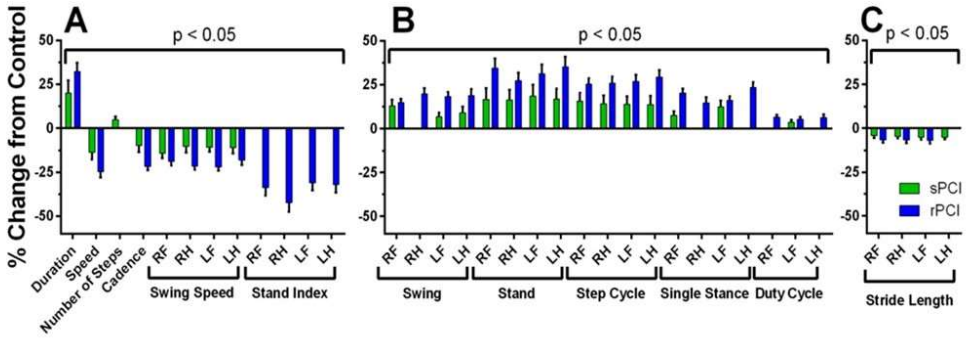


Figure 4: PCI induces acute gait dysfunction Analysis of dynamic (A), temporal (B), and static (C) gait analysis parameters 2 hours following injury reveals significant injury effects in both the single and repeat PCI groups. Values are presented as a percent change in each injury group from the appropriately 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.

rats spent more time moving through individual gait components compared to matched sham controls (Fig. 4B). Static paw positioning parameters revealed few differences between injured and uninjured rats (Fig. 4C). No injury effects were seen in parameters that examine inter-limb coordination.

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, 3m, and 6m post injury. At baseline, the composite NSS-R scores from all groups were comparable. NSS-R results for 24h – 3m following rPCI were presented. At 6m, no chronic deficits or sPCI or rPCI were observed as detected by the NSS-R. All NSS-R results were provided in the Year 1 & 2 Annual Reports.

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.

Experiment 1.2.2 Memory Assessments:
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sec, it was manually guided there. Once on the platform, 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. Results from 24h – 3m after injury were reported in the Year 1 Annual Report and results from the 6m post-injury time point were included in the Year 2 Annual Report. No significant effects of sPCI or rPCI were observed at 6m following injury.

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.

Experiment 1.2.3 Anxiety and Motivation:
Anxiety behavior was assessed prior to injury and at 1, 3, and 6 months after injury with the elevated plus maze (EPM). 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.

Results from 1m and 3m following injury were previously reported in the Year 1 Annual Report. No significant alterations were observed between injured rats and matched sham controls at 6m for arm durations, arm entries, distance travelled, or velocity.

<u>Experiment 1.2.4 Correlation Analysis</u>:

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*	*Pressure (PSI)*		
	4th Hit	0.5598	0.0067 **
	3rd Hit	0.5515	0.0078 **
	SUM	0.4702	0.0315 *
Thalamus (Ipsilateral) *3 Day*	*Pressure (PSI)*		
	2nd Hit	-0.5499	0.008 **
	SUM	-0.5291	0.0137 *
	Force (lbs)		
	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*	*Righting Reflex (s)*		
	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*	*Righting Reflex (s)*		
	SUM	0.4514	0.0065 **
Thalamus (Ipsilateral) *3 Month*	*Righting Reflex (s)*		
	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
Olfactory Bulb (Ipsilateral) *24 Hour*	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 *
	Step Cycle (RH)	0.3781	0.0162 *
	Step Cycle (LH)	0.3513	0.0262 *
	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 *
Thalamus (Ipsilateral) *3 Day*	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 *
	Step Cycle (RH)	-0.3807	0.0154 *
	Step Cycle (LH)	-0.3785	0.016 *
	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) *3 Month*	Swing Speed (LH)	-0.4058	0.0156 *
	Swing (LH)	0.3878	0.0213 *
	Single Stance (RH)	0.3625	0.0324 *
Thalamus (Ipsilateral) *3 Month*	Swing Speed (LH)	0.4927	0.0026 **
	Swing Speed (RH)	0.3658	0.0307 *
	Stand Index (RF)	-0.4404	0.0081 **
	Swing (LH)	-0.4302	0.0099 **
	Stand (RF)	-0.3558	0.0359 *
White Matter (Ipsilateral) *3 Month*	Swing (RF)	-0.3596	0.0398 *
	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: The effect of PCI on the neurodegenerative markers amyloid beta and phosphorylated tau was evaluated. At 6 months following PCI, rats were perfused with 4% paraformaldehyde and brains were removed for evaluation by immunohistochemistry. Paraffin embedded coronal brain sections were stained with 6E10 (amyloid beta) and AT8 (phosphorylated tau) antibodies. Positive staining was quantified in both the ipsilateral and contralateral hemispheres.

Results were summed across six slices per rat and analyzed as a percent of positive staining relative to total slice area.

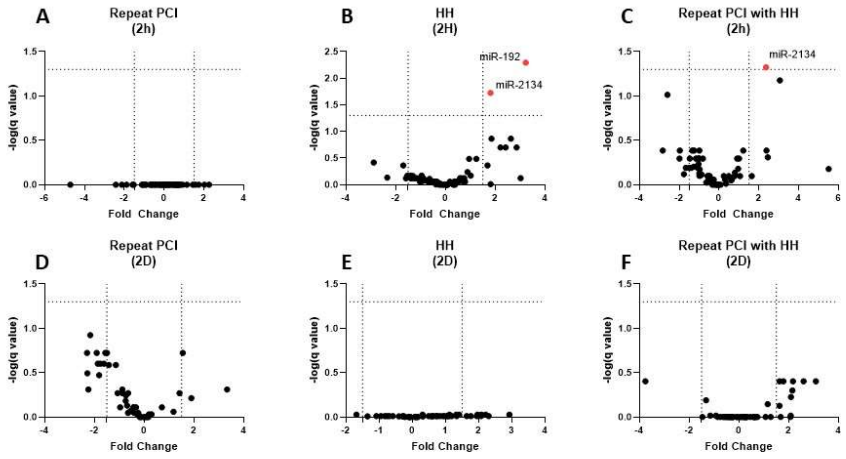
At 6 months following PCI, preliminary analysis of the rSham and rPCI groups demonstrated no change in chronic neurodegeneration as detected by staining for amyloid beta and phosphorylated tau (Fig. 8). As a result, this experiment was not explored further with additional animals or in the sPCI group.

SOW Major Task 1 Summary and Conclusions:
Work for SOW Major Task 1 has been completed. 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. Work through 3 month endpoints was previously reported in the Year 1 Annual Report. Assessment at 6 months has now been completed and demonstrated chronic alterations in FDG uptake which occurred in both ipsilateral and contralateral hemispheres. The data at 6 months post injury provide additional support to the previously reported conclusion that brain glucose uptake following PCI follows a pattern of acute hypermetabolism with hypometabolism prevailing chronically. This was especially apparent in the thalamus, where decreased uptake was observed in both the sPCI and rPCI groups both ipsilateral and contralateral to the injury impact location. 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 SOW Major Task 1 included the characterization of neurobehavioral deficits and chronic neurodegenerative pathology after single and repeat PCI. Behavioral deficits through 3 months post injury were previously reported in the Year 1 Annual Report. At 6 months post injury, no alterations were observed in the spatial learning and probe trials of the MWM or in behavior in the EPM. In the working memory MWM, while both PCI groups took longer to find the hidden platform in individual trials, no changes were seen in working memory when assessing the difference between trial 1 and trial 2. In considering all behavioral deficits from 24h – 6m, PCI resulted in acute neurobehavioral deficits but minimal changes in anxiety and cognitive performance at 1m and 3m following injury. By 6m, all behavioral deficits had resolved. Additionally, at 6m post injury, no evidence for chronic neurodegenerative pathology was observed in rPCI rats compared to rSham rats.

<u>SOW Major Task 2:</u> 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.

All live animal work for SOW Major Task 2 is 100% completed. In the original experimental design, four different study groups were included in this Task: sSham, sPCI, rSham, and rPCI. Injuries were induced using the modified PCI device, which has previously been described in great detail (Leung, Larimore et al. 2014). All experimental tasks for SOW Major Task 2 occurred at Site 1 (WRAIR; PI: Dr. Deborah Shear). miRNA characterization of sSham and sPCI, and of rSham and rPCI has been completed. In addition, because initial results showed only modest trends, additional

animals (per group) and additional groups (polytrauma) and (PCI+polytrauma) were added as part of a Year 3 no-cost-extension to determine whether exposure to polytrauma would exacerbate the effects of concussion on FDG-PET imaging data (Site 2) and serum miRNA profiles. Results on serum miRNA profiles were provided in Annual Report 3 (see Figure 5 below). Overall, results showed that miRNAs were more significantly altered following rPCI vs. sPCI (Fig. 5 A, D). Conversely, following polytrauma alone, miRNA signaling was altered at the 2h post-injury time point but not at 2 days post-injury. However, in animals that received both PCI and polytrauma, miRNA changes were detected at both post-injury time points. Notably, miR-2134 is a pre-synaptic/astrocytic microRNA that is involved in neural plasticity. The finding that we see an elevation of this microRNA level at 2h post injury is an injury specific observation, rather than an actual gene expression modulation. It may be that the synaptic shear forces exerted by concussion is causing the release of this microRNA to the blood. miR-192 on the other hand is in involved in blood coagulation and venous thrombosis (PMID: 32160777).
Further work is ongoing to validate (i.e. replicate) these results and to analyze for potential correlations between imaging, behavioral and histopathological data.



Target	Fold Change	P value	t ratio	df	q value
mmu-miR-2134	2.359	0.000587	4.337	15.00	0.047451

Figure 5. Volcano plots showing alterations in miRNA at 2 hours (top row) and 48 hours post-injury. Inserts shows fold changes for combined PCI+HH (polytrauma) injury effects.

Subtask 2.1: Determine the acute serum miRNA biomarker change profiles following single or repeat PCI occurring alone or in conjunction with induced hypoxemia and hemorrhagic shock (HH = polytrauma).

Serum miRNA profiles was assessed at 30 min, 24 hours, and 3 days post injury using serial blood draws. Terminal serum miRNA analysis were also 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 assessed 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 data collected in Exp. 1.2.2 was used to evaluate potential correlations between memory dysfunction and miRNA profiles at 1 and 3 months following injury. No significant correlations were detected.

Experiment 2.2.3 Anxiety and Motivation: The elevated plus maze task results from Exp 1.2.3 were used to determine potential correlations between anxiety and motivation at 1, 3, and 6 months post injury. No significant correlations were detected.

Experiment 2.2.4 Correlation Analysis: Correlation analyses between acute serum miRNA and behavioral outcome metrics following PCI were evaluated. No significant correlations were detected between these outcome metrics.

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. Histopathological processing was delayed due to shutdowns resulting from the pandemic. Brain specimens are currently being processed by FuDu technologies and we anticipate the analysis of those sections will be completed by the end of the current NCE

Experiment 2.3.2 Correlation Analysis: Correlation analyses between acute serum miRNA profiles and protein hallmarks of neurodegeneration following PCI will be evaluated. Animals were divided into 6 groups (n=6/group): Sham, Hemorrhage and Hypoxia (HH60), single projectile concussive injury (sPCI), sPCI+HH60, repeated PCI (rPCI), and rPCI+HH60. Serum was collected from each animal at 2 hours and 24 hours after injury. A biomarker panel (GFAP, UCHL1, NF-L, tau) was conducted on serum samples using the SIMOA HD-1 Neurology 4-plex assay.

Our results (Figure 6 below) suggest that hemorrhage and hypoxia play an important role in the biomarker levels. In the absence of TBI, hemorrhage and hypoxia increase GFAP and UCHL1 at 24 hours after injury. sPCI and rPCI increased NF-L at 2 hours but we did not observe any changes to GFAP, UCHL1, or tau. PCI also caused no changes

at the 24 hour time point. When hemorrhage and hypoxia occur as a comorbidity to TBI, the biomarker levels increased significantly. GFAP, NF-L, and UCHL-1 increased significantly at 2 hours with GFAP and UCHL1 levels remaining increased at 24 hours. These results demonstrate that extracranial injuries exacerbate biomarker levels and may augment neuropathology.

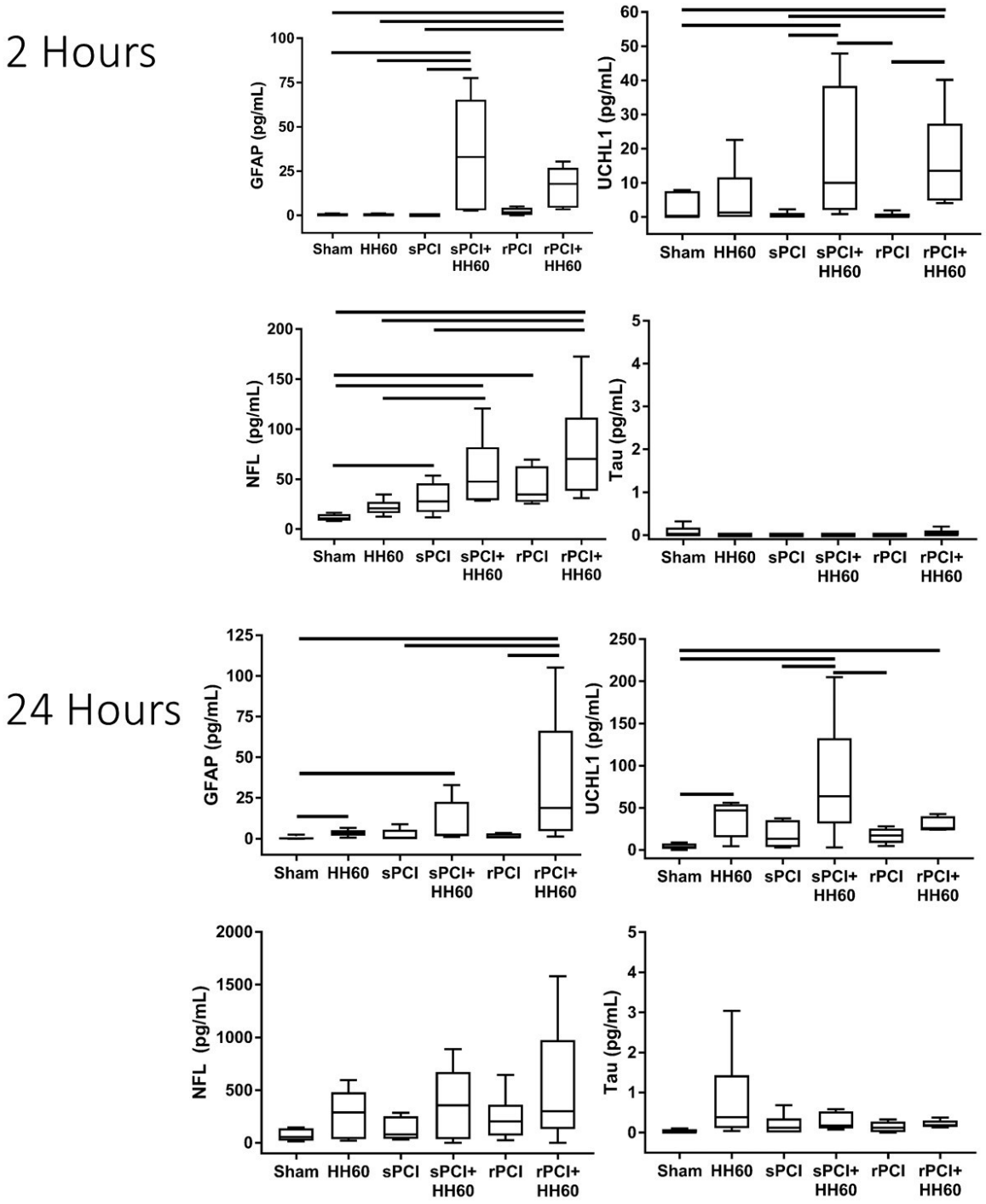


Figure 6. Box plots showing serum levels for GFAP, UCHL1, NFL, and Tau at 2 hours and 24 hours post-injury.

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

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 and 2 were presented in poster format at the 2017 – 2019 National Neurotrauma Symposiums. 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 has been submitted for 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 2, histopathological analysis will be completed. Histopathology to assess protein accumulation related to neurodegeneration and its potential correlation between brain glucose metabolic changes will also proceed as planned.

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, Dardzinksi 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:	Dr. 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:	Dr. Bernard Wilfred

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

Name:	Dr. Angela Boutte
Project Role:	Associate Investigator
Research Identifier:	
Nearest person month worked:	2
Contribution to Project:	Data Analysis; Reporting
Funding Support:	The Geneva Foundation

Name:	Dr. Zachary Bailey
Project Role:	Research Associate
Research Identifier:	
Nearest person month worked:	4
Contribution to Project:	PCI – Polytrauma Injuries; Serum biomarkers; Behavioral Assessments
Funding Support:	CCCRP

Name:	Katherine Cardiff
Project Role:	Research Associate
Research Identifier:	
Nearest person month worked:	2
Contribution to Project:	PCI – Polytrauma Injuries; Serum biomarkers; Behavioral Assessments
Funding Support:	The Geneva Foundation

Name:	Dr. 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.