# Evaluating BACE-1 as a Biomarker for Experimental Traumatic Brain Injury in Rats

by

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Moderate to Severe Traumatic Brain Injuries (TBI) are known to cause synaptic damage and increase the risk of developing neurodegenerative disorders like Alzheimer's dementia, although there is no established technique to identify those at risk for this outcome. Beta-site amyloid precursor protein cleaving enzyme (BACE-1) has been studied as a promising serum biomarker to identify synaptic damage in Alzheimer's Disease (AD) due to its enzymatic role in Aβ 1-42 genesis, one of the hallmark proteins associated with AD. Since TBI produces synaptic damage, BACE-1 also has potential as a serum biomarker of posttraumatic synaptic damage. It is hypothesized that elevated BACE-1 levels lead to overproduction of toxic amyloid beta species, thereby increasing the risk of neurodegeneration after TBI. BACE-1 mRNA levels, BACE-1 protein levels and enzymatic activity of BACE-1 (Aβ 1-42 genesis) have previously been found to be elevated post experimental TBI in brain lysate samples. By detecting BACE-1 in serum samples, the identification of the extent of synaptic damage can be expedited, and the TBI can be treated appropriately. In this study, we investigated whether moderate TBI produced by controlled cortical impact (CCI) can cause detectable elevations in BACE-1 protein levels and whether BACE-1 has predictive value for cognitive or motor outcomes. We used serum collected from adult, male Sprague Dawley rats via an indwelling jugular catheter at baseline, 24, 48, and 72 hours and 2 weeks after sham or CCI injury. Serum BACE-1 was measured using a commercial ELISA kit. Additionally, the rats were tested for motor and cognitive functions using Beam Balance, Beam Walking and Morris Water Maze tests.

**This study demonstrated novel findings that there was a significant decrease in detectable serum BACE-1 concentrations post-TBI from baseline to 72 hours, and recovery trend from 72 hours to 2 weeks. There was a significant correlation between baseline BACE1 serum levels and performance on the Beam Balance task, suggesting that higher BACE-1 serum concentrations at baseline may be associated with worse motor functioning post injury.**

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## Preface

First and foremost, I would like to express my gratitude to my research mentor Dr. Edward Dixon for his continuous patience, knowledge, support, and guidance throughout my training in preparation for the completion of my Bachelor of Philosophy Degree. I would like to acknowledge and thank my lab members Sarah Svirsky, Madison Perry and Jeremy Henchir in supporting my project and training me in the laboratory throughout my undergraduate career.

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# 1.0 Literature Review

## 1.1 Traumatic Brain Injuries

Traumatic brain injuries (TBI) can range from bumps to the head to penetrative injuries that disrupt the normal functioning of the brain[19]. Each year, there are many affected by this condition, and certain specific populations are more vulnerable to having a TBI, particularly those over the age of 75, athletes and war veterans. There were 611 hospitalizations and a reported mortality rate of 176 deaths daily due to TBI-related injuries in 2020 in the US [20].

There is clear evidence that TBI leads to acute and long-term changes in behavior and brain pathology due to damage to synapses[15]. Disruptions in synaptic integrity underlie changes in motor control (or skill), learning and memory functions, which seriously affects TBI patients chronically. One of the most important regions of the brain – the hippocampus, associated with learning and memory, is especially vulnerable to damage due to TBI[17].

Controlled cortical impact (CCI) is an established animal model of TBI that uses a pneumatically or electromagnetically controlled piston to induce reproducible and well-controlled injury[13]. Previous research has shown that moderate levels of experimental TBI using the CCI injury method demonstrates synaptic changes such as decreased dendritic spines and impairments in electrophysiological activity in the hippocampus[17]. There have been studies showing synaptic changes following selective lesions, such as reactive synaptogenesis, increase in synapses and a marked 60-70% reduction in synaptic density in ipsilateral hippocampus[16,17]. TBI causes an

increase in the risk of developing neurodegenerative disorders like Alzheimer's Disease (AD) and Parkinson's disease as well as an increase in the risk of developing other forms of dementia[1].

## 1.2 Biomarkers

A condition-related biomarker, ideally, would be easy to measure and access, cost and time effective, and would be quantitatively or qualitatively reflective of a pathological process[6]. For TBI, there are several candidate proteins that show fluctuations in cerebrospinal fluid (CSF), such as PSD-95 and Neurogranin[5], however, lumbar punctures would be needed to acquire samples of CSF unless intraventricular drains were implanted for intracranial pressure management. Both methods of sampling CSF are invasive and costly to perform and should be avoided considering the discomfort that accompanies TBI in general. There are added limitations when trying to measure these protein fluctuations through peripheral biofluids like blood (plasma/serum) due to the extent of damage to the blood brain barrier varying TBI cases. Additionally, some proteins do not cross the blood brain barrier at all, so it is important to evaluate which proteins may pass and are reflective of changes in brain pathology. Current detection techniques for TBI include imaging tests like MRI and CT, or neurological assessments that involve evaluating motor skills, cognitive ability and sensorimotor function. However, none of these techniques are sufficient to quantitatively detect the extent of TBI related synaptic damage. A biomarker could also be used as an effort to prevent worsening of TBIs by having it as a quick assessment tool for mild-moderate cases of TBI where veterans or athletes are expected to return to their high injury-risk jobs after an injury event. Hence, it is important to continue evaluating candidate biomarkers that may aid in early and accurate detection of TBI.

TBI more than doubles risk of developing dementia and increases risk for AD, suggesting similar secondary mechanisms[1]. Thus, current biomarkers in AD may be useful in detecting TBI.

### 1.3 BACE-1

A well-known characteristic of AD is the formation of β-amyloid (Aβ) plaques, which are also known markers of CNS injury[2]. Aβ (1-42) is produced from amyloid precursor protein (APP) by proteases such as β-secretase and γ-secretases[2]. β-secretase 1 is also known as β-site amyloid cleaving enzyme (BACE-1). BACE-1 is primarily a presynaptic protein that has been identified as a transmembrane aspartic protease[10] is 70kDa in weight. BACE-1 knockout mice display complex neurological phenotypes, including growth retardation, memory deficits, axon guidance defects, and schizophrenia-like behaviors[3] possibly due to the enzyme's many substrates other than APP. BACE-1 is found to be expressed most in neurons and astrocytes but is also found in peripheral tissues such as pancreas and liver. This is expected, as most cells produce Aβ from APP[4]. In a clinical study, BACE-1 in CSF and peripheral blood samples was observed to be elevated in patients with mild cognitive impairment (MCI) who subsequently developed Alzheimer's disease when compared to healthy controls[14,6]. However, recording baseline BACE-1 concentrations from healthy subjects would be important for establishing an acceptable "normal" range, and further research must be conducted in order to explore the fluctuations in BACE-1 in organs outside the central nervous system post injury, in order to determine if BACE-1 can selectively reflect CNS damage.

## 1.4 Hypotheses

This study is investigating BACE-1 as a biomarker for TBI by evaluating changes in BACE-1 serum concentrations as well as evaluating its predictive value in behavioral outcomes post CCI. Based on previous studies that showed elevated levels of BACE-1 in ipsilateral hippocampal sections at 24 hours post-injury (PI), we hypothesize that BACE-1 levels will be elevated, suggesting an overproduction of this enzyme in an animal model of TBI. Additionally, we assessed synaptic integrity after TBI at 2-weeks PI by analyzing concentrations of the pre-synaptic protein synaptophysin and the post-synaptic marker postsynaptic density protein 95 (PSD-95), using Western blots. Based on previous literature, we hypothesized that synaptophysin will increase post injury and PSD-95 will decrease post injury.

## 2.0 Methods

### 2.1 Animals and CCI Injury

All experimental procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee in accordance with the guidelines established by the National Institutes of Health in the Guide for the Care and Use of Laboratory Animals. Animals were housed up to two rats per cage in the University of Pittsburgh vivarium with a 12:12 light/dark photoperiod (lights on at 7:00 a.m.) and provided food and water *ad libitum*. Daily monitoring of animals was done by veterinary technicians. A total of 29 adult male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing 285-385 g were used in this study and were randomly assigned to receive either a sham procedure (n=11) or CCI injury (n=9). Twelve animals (n=6 each) were processed for immunoblotting, and all rats were used for biofluid collection. Rats were anesthetized using 4% isoflurane with a 2:1 $N_2O/O_2$ mixture in a ventilated anesthesia chamber. Following endotracheal intubation, the rats were mechanically ventilated with a 2% isoflurane mixture. An intrajugular catheter was surgically implanted prior to sham or CCI injury. Animals were then placed in a stereotaxic frame, and body temperature was monitored by rectal thermistor probe and maintained at 37°C with a heating pad. Following a midline incision, the soft tissues of the scalp were reflected and a 7 mm craniectomy was performed over the right parietal cortex, between bregma and lambda, and centered 5 mm lateral of the sagittal suture to expose the dura mater. Control sham injury animals were subjected to anesthesia and surgical procedures but did not receive a TBI. The CCI injury device was a small bore (1.975 cm) double-acting stroked-constrained pneumatic cylinder with a 5.0 cm stroke. An impactor tip (6 mm in diameter, flat tip)

was set to produce a tissue deformation of 2.8 mm at a velocity of 4 m/sec with a dwell time of 150 msec. After each sham or CCI injury, the scalp was sutured closed, gas anesthetics were turned off, and righting time was monitored. Once ambulatory, the animals were returned to their home cages.

### 2.2 Biofluid and Serum Collection

Serum was collected via the indwelling jugular catheter pre-injury (baseline), 24, 48 and 72 hours post injury. At the 2-week timepoint, blood was collected via a cardiac puncture at sacrifice. Upon collection, each sample was centrifuged, aliquoted in polypropylene cryovials, and stored at −80°C until the time of assay. For sham control subjects, blood was drawn, processed and stored for later batch analysis. BACE-1 levels post-TBI were measured using ELISA in a total of 20 samples. 7 animals were excluded because serum collection was unsuccessful at all necessary timepoints due to catheter damage. Two additional animals (one from each group) were excluded due to space restrictions on the ELISA 96-well plate.

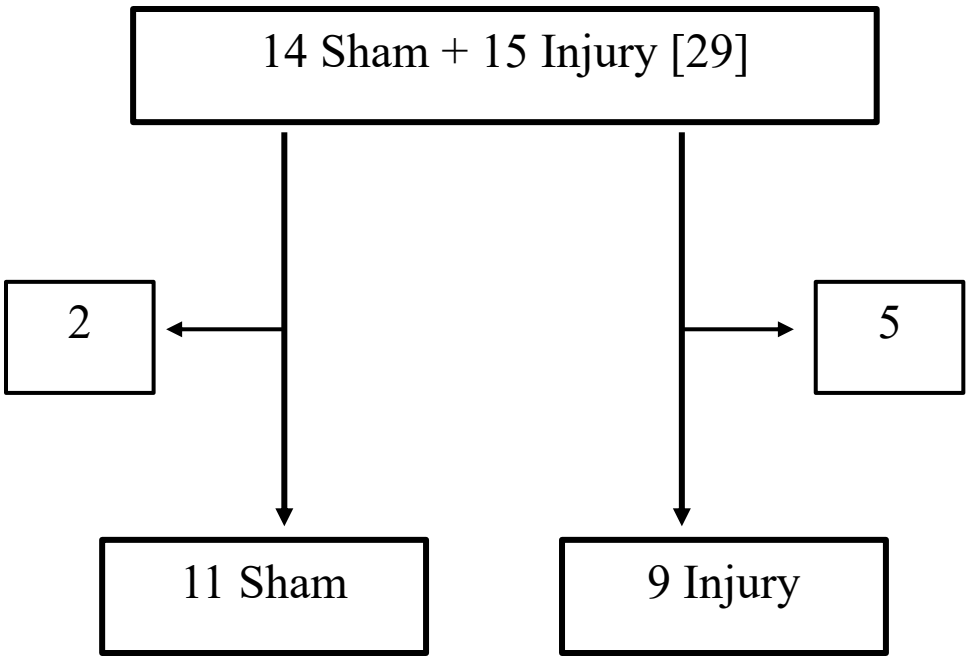



***Figure 1: Animal information for serum assessment***

*The project started with 29 animals total (n=14 sham; n=15 injury). 7 animals were excluded (n=2 sham; n=5 injured) since serum collection was unsuccessful for all required timepoints and 2 (n=1 sham and injury) more animals were excluded due to ELISA plate space restriction.*

### 2.3 Tissue Processing and Sample Preparation

At 2 weeks post-injury, animals received an overdose of sodium pentobarbital (intraperitoneally, 100 mg/kg Fatal-plus, Vortech Pharmaceuticals, Dearborn, MI) and were rapidly decapitated. Ipsilateral hippocampus was dissected on a chilled ice plate and immediately frozen in liquid nitrogen and stored at -80°C. Samples were homogenized in SynPer lysis buffer (0.1 M NaCl, 0.01 M Tris-Cl, 0.001 M ethylenediaminetetraacetic acid [EDTA], pH 7.6) with protease and phosphatase inhibitors (Sigma-Aldrich, St. Louis, MO). To acquire synaptosome

samples, the homogenized whole cell samples were centrifuged at 13,000g at 4 °C for 30 min, and the supernatants collected. The supernatants were centrifuged at 15,000g for 20 min at 4°C to be collected for the synaptosome samples.

### 2.4 Motor Testing (Post Injury Days 1-5)

Gross vestibulomotor function was assessed by the beam-balance task in which the total time the animal stayed balanced on the elevated, 1.5-cm-wide wooden beam was recorded. Three trials were performed per animal per day, with a maximum of 30 sec/trial. Training and pre-assessment was completed on the day prior to sham/CCI injury. Spinning on the beam was counted as a fall. The animals fell onto a cushioned pad placed below the beam to prevent additional injury.

Vestibulomotor function and coordination were further assessed using a beam-walking task. Rats were trained to escape a bright light and loud white noise (model #15800C; Lafayette Instruments, Inc., Lafayette, IN), by traversing a narrow wooden beam (2.5 × 100 cm) and entering the darkened goal box at the opposite end. The noise and light were terminated when the rat entered the goal box. Four pegs (3 mm diameter and 4 cm high) were equally spaced along the center of the beam to increase the difficulty of the task and mark progression of distance. Performance was assessed by measuring the latency to traverse the beam. The rats remained in the goal box for 30 sec between trials. If the rats did cross the beam in more than 60 sec or fell off, the light and noise were stopped, and the rat was placed in the goal box. The mean of three trials was used as the data for each day.

### *2.5* Cognitive Assessment – Morris Water Maze (Post Injury Day 9-14)

The Morris Water Maze[18] (MWM) has been shown to be sensitive to cognitive function/dysfunction following TBI[18]. This water maze task was used to compare rates of acquisition between injured and sham groups. The Morris water maze consisted of a dark blue plastic pool 180 cm in diameter and 60 cm in depth. The pool was filled with water to a depth of 28 cm and a clear acrylic glass platform 10 cm in diameter and 26 cm high (i.e., 2 cm below the surface) was positioned 26 cm from the maze wall in the southwest quadrant and held constant for each trial. Testing for spatial learning began on postoperative day 9 and consisted of providing a block of four daily trials (4-min inter-trial interval) for 5 consecutive days (days 9–13) to locate the platform when it was submerged (i.e., invisible to the rat). An additional day (14) was provided to locate the platform when it was raised 2 cm above the water's surface (i.e., visible to the rat) and when it was removed from the pool (Probe trial). The visible platform task was used as a control procedure to determine the contributions of non-spatial factors (e.g., sensorimotor performance, motivation, and visual acuity) on MWM performance. The Probe trial was used for testing memory recall and learning the task. For each daily block of trials, the rats were randomly placed in the pool facing the wall at each of the four possible start locations (north, east, south, and west). Each trial lasted until the rat climbed onto the platform or until 120 sec had elapsed, whichever occurred first. Rats that failed to locate the goal within the allotted time were manually guided to it and placed for 30 seconds. All rats remained on the platform for 30 sec before being placed in a heated incubator between trials. The mean latency scores of the four trials for each rat were used in the statistical analyses. Swim speed and distance were recorded with a video analysis system. The pool was located in a 2.5 × 2.5-meter room with numerous extra-maze visual cues that remained constant throughout the experiment.

## 2.6 BACE-1 Serum Assessment

We used the BACE-1 ELISA (Euroimmun AG (Lübeck, Germany) which was engineered for CSF and plasma samples. This is a novel study that utilizes serum samples on this kit. The pilot study data showed that several samples had BACE-1 levels which were lower than standards. In order to overcome this, the preset Euroimmun protocol was optimized to use 30uL of undiluted peripheral serum samples, instead of 15uL as suggested by the protocol, and the standards stayed as 15uL in order to use the same reference scale of concentration.

Briefly, the plate wells were coated with 30uL of sample and 100uL of biotin conjugate for 3 hours on a plate shaker at room temperature. The samples were undiluted and vortexed before pipetting into the wells. Washing wells with 1x wash buffer was repeated for five times between each incubation step. 100 uL of the secondary antibody, horseradish peroxidase (HRP)-conjugated, was added and incubated at room temperature for 30 minutes. After washing, 100 uL of tetramethylbenzidine (TMB) solution was added and incubated for 30 minutes. Lastly, 100uL of Stop buffer was added. All samples were performed in duplicates for detection. SoftPro Max plate reader was used to detect BACE-1 concentrations at 450nm wavelength.

## 2.7 Immunoblotting

Total protein concentration was determined by a bicinchoninic acid (BCA) protein assay kit (Thermo Scientific, Pittsburgh, PA) using a 96-well microplate reader (Biotek, Winooski, VT). To assess BACE-1, synaptophysin and PSD-95 expression, synaptosome lysates were boiled for 10 min prior to blotting. The blots were blocked in 5% nonfat dry milk in tris-buffered saline (TBS)

for 1 h. The primary antibodies rabbit anti-PSD-95 (diluted, 1:2500, Abcam, Cambridge, MA), rabbit anti-BACE-1 (diluted, 1:1000, Abcam, Cambridge, MA) and mouse anti-synaptophysin (diluted, 1:10,000, Sigma-Aldrich) were mixed in blocking solution and incubated with the blots overnight at 4$^{o}$C. The following day, the membranes were washed with TBS buffer, incubated in blocking solution containing HRP-conjugated secondary antibodies (goat anti-mouse HRP 1:5000 or goat anti-rabbit HRP 1:2500) for 1 h. Proteins were visualized using a chemiluminescence detection system (Supersignal, Pierce). Blots were imaged with the Chemidoc Imager (BioRad). Optical density of BACE-1, synaptophysin and PSD-95 were measured using ImageJ (National Institutes of Health) and normalized to actin levels. Ipsilateral hippocampal cortices for both sham and CCI-injured animals were loaded onto one gel to directly compare injury effect. Values were presented as the ratio of optical densities that represented the percentage of sham levels (100%) for each time point. Data were expressed as the group means +/– standard error of the mean (SEM).

### 2.8 Statistical Analysis

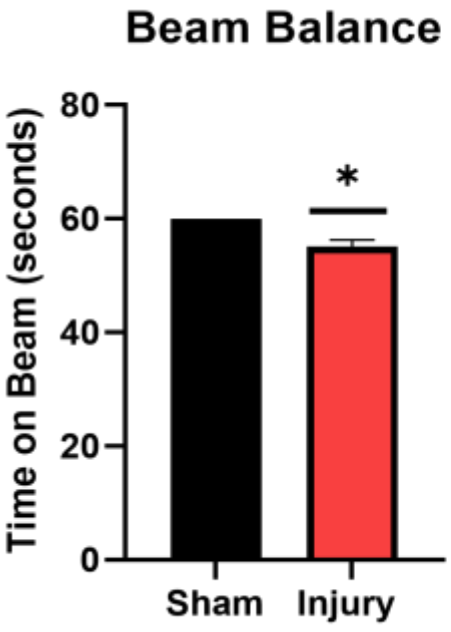
ELISA data was analyzed using Arigo Biolaboratories Corp.'s ELISA calculator. Immunoblot data were compared using a non-parametric, Mann–Whitney, t-test for each time point and a p value $<0.05$ was considered statistically significant for all tests. A Mixed-Effects analysis was used to assess the time course data with post hoc tests utilized to analyze differences from baseline. Statistical tests on ELISA and Behavioral data were completed using GraphPad Prism (GraphPad, La Jolla, CA).

## 3.0 Results

### 3.1 Motor Deficits Following CCI Injury

Time spent by rats successfully balanced on the Beam Balance task was significantly lower for the CCI animals when compared to sham groups (*p= 0.0005, Fig.2a). Time taken to traverse the beam, or the beam walking latencies were higher for CCI injured animals (*p<0.001, Fig.2b).

A

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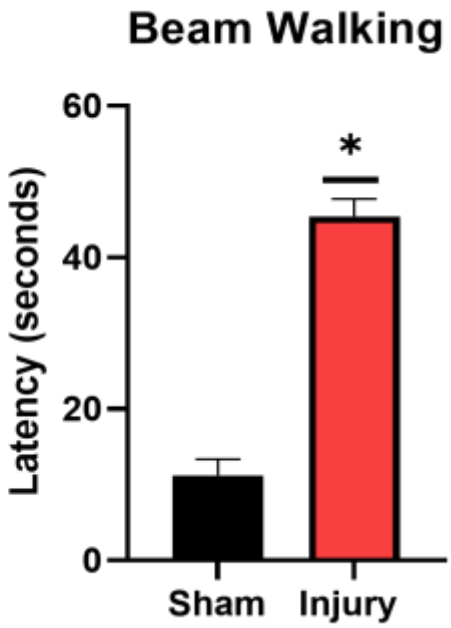


***Figure 2: Motor deficits after CCI injury***

***(A):*** *Injured animals spent significantly lower time on the beam as compared to sham counterparts (*p=0.0005)* ***(B):*** *Injured animals took significantly longer to find the safe box as compared to sham counterparts (*p < 0.0001).*

### 3.2 Morris Water Maze

Post Injury MWM testing showed that the CCI group had significantly longer swim latencies (*p=0.0012, Fig.3a) to reach the submerged target platform as compared to the sham group. The probe trial performed on day 14 showed no significant difference (p=0.6072, Fig.3b) when comparing CCI and sham groups. By breaking up the data to separate latencies to various zones around the platform, however, significant differences were observed. The CCI group had longer average latencies to entering the inner platform zone (*p=0.0491, Fig.3c) when compared to the sham group during the visible trials. The CCI animals did not take significantly longer to enter the outer platform zone, although there was an increasing trend (p=0.1307, Fig.3d) when compared to the sham group. Lastly, the visible platform trial performed on day 14 showed the CCI group spent no significant differences in % time spent in target quadrant compared to sham group (p=0.9871, Fig.3e).

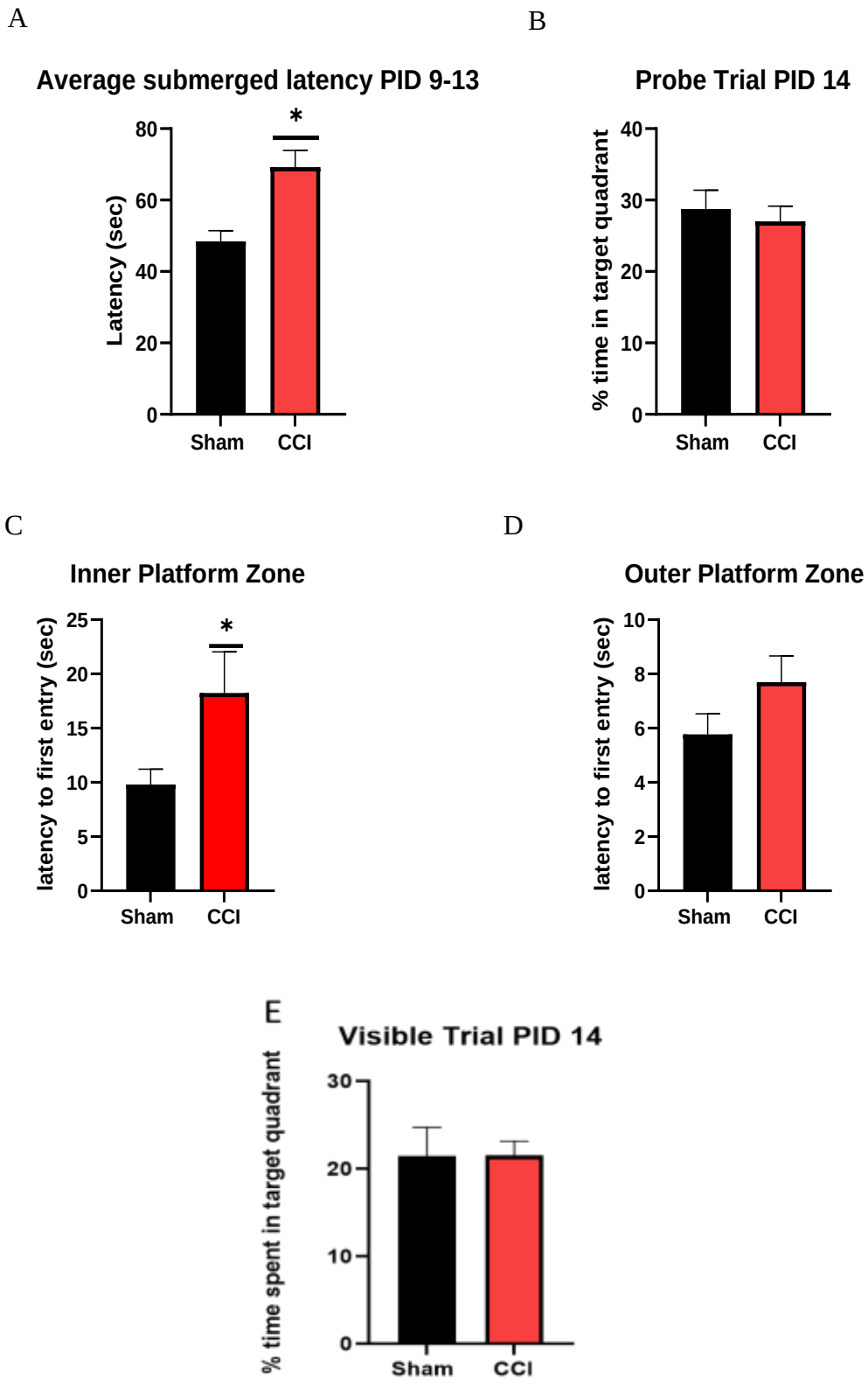


***Figure 3: Cognitive deficits after injury (MWM)***

***(A)*** *Average Submerged Swim Latencies (9-13 days post injury) - Student's t-test shows a significant difference in CCI group compared to sham group (*p=0.0241);* ***(B):*** *Probe Trial (14 days post injury) - Student's t-test does not show significant differences in CCI group compared to sham group (p=0.6072);* ***(C):*** *Average Swim Latencies of First Entry to the Inner Platform Zone (IPZ) (Days 14 post injury) - Student's t-test shows significant differences in CCI group compared to sham group (*p=0.0491);* ***(D):*** *Average Swim Latencies of First Entry to Outer Platform Zone (OPZ) (Days 14 post injury) - Student's t-test shows no significant difference in CCI group compared to sham group (p=0.1307);* ***(E):*** *Visible platform Trial (Day 14 post injury) - Student's t-test shows no significant difference in CCI group compared to sham group (p=0.9871).*

### 3.3 Serum BACE-1 Concentrations Showed Reduction in Expression After Injury

Serum BACE-1 expression after CCI was assessed by ELISA. As shown in Figure 4, there was a significant reduction in BACE-1 expression in both post CCI and sham groups when comparing to baseline levels. Tukey's multiple comparisons tests revealed that in the sham group, BACE1 levels were significantly lower ($p<0.05$) from baseline at 48hrs and 72hrs after TBI. This analysis was utilized since no injury effect was noticed with ANOVA repeated measures analysis and post-hoc analysis could not be performed. In the CCI group, BACE-1 levels were significantly lower ($p<0.05$) from baseline on 48hrs, 72hrs, and 2-weeks after TBI. This reduction in BACE-1 levels after both sham and CCI treatment, indicates a possible effect of surgery or anesthetic on peripheral BACE-1. Baseline serum concentrations were taken pre-surgery and therefore may not

be the best correlation to observe injury effect. There were no significant differences between sham and injury groups, calculated by an unpaired t-test excluding timepoints (p=0.4074, Fig. 4).

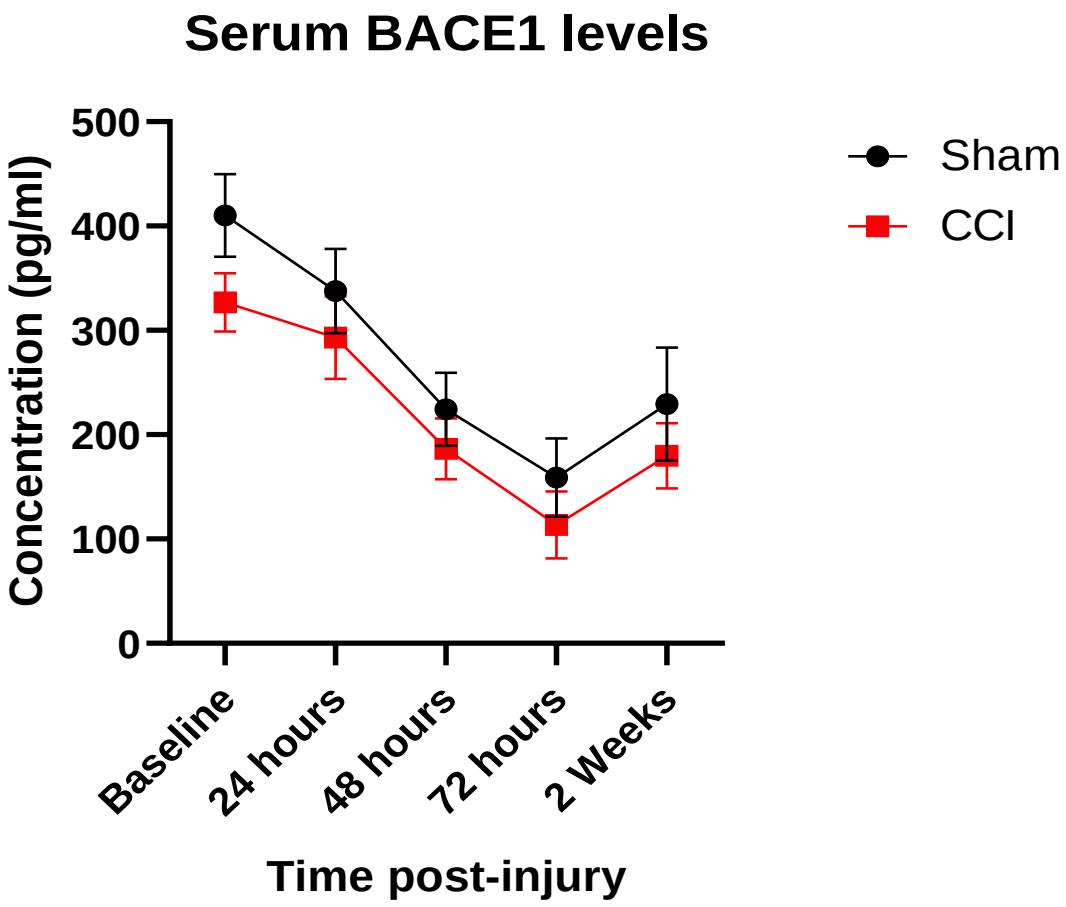


***Figure 4: BACE-1 serum concentration after surgery***

*There were no significant changes between BACE-1 levels between sham and CCI, calculated by a Mixed Effects analysis an unpaired student t-test (p=0.3084). Baseline serum was collected before any surgical interventions. Decline in serum BACE-1 concentrations is evident after 24 hours post-injury, whereas a slow recovery began after 72 hours.*

### 3.4 No Significant Change in BACE-1 Concentration in Ipsilateral Hippocampal Samples.

Figure 5a shows ipsilateral hippocampal BACE-1 protein expression in synaptosomes after CCI, assessed by Western blot at a 2-week timepoint (n=6 each). Figure 5b shows representative Western blots for BACE-1 and actin, a protein chosen as a control (n=2). A Student's t-test was performed to determine differences in protein expression between sham and CCI animals (n=6 per group). BACE-1 expression in synaptosomes of ipsilateral hippocampus in the CCI group was not significantly different 2 weeks post injury when compared to sham controls (p=0.7890).

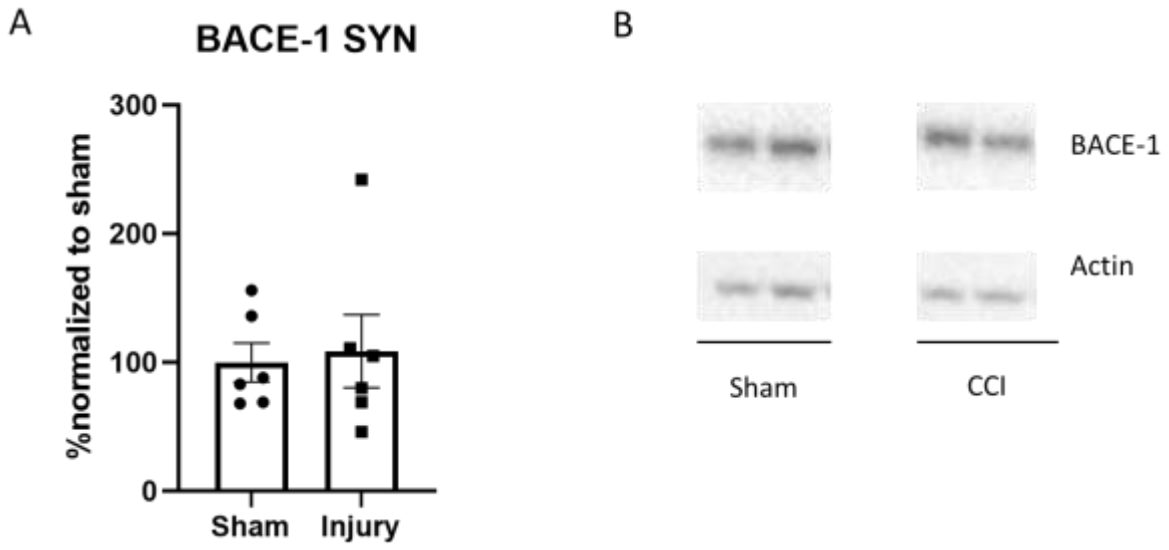


***Figure 5: BACE-1 expression quantitatively calculated using Western blot***

***(A):*** *No statistically significant difference is seen in BACE-1 concentrations in ipsilateral hippocampal synaptosome samples comparing sham and CCI groups (p= 0.7890; n=6 per group).* ***(B)*** *Representative actin and BACE-1 Western blots (n=2 per group).*

### *3.5* Ipsilateral Hippocampal Samples Showed a Trend Toward an Increase in Synaptophysin After Injury

Hippocampal synaptophysin protein expression after CCI was assessed by Western Blot at a 2-week timepoint using synaptosome samples. Figure 6b shows representative Western blots for synaptophysin and actin (n=2). A Student's t-test was performed to determine differences in protein expression comparing sham and CCI tissues. Synaptophysin expression in ipsilateral hippocampus synaptosomes of the CCI group showed a trend toward an increase as compared to the sham group 2-weeks post injury (p=0.1905).


***Figure 6: Synaptophysin expression quantitatively calculated using Western blot***

***(A):*** *A trend toward an increase is seen with CCI animals having higher synaptophysin concentrations compared to sham samples (p=0.1905; n=6 per group).* ***(B):*** *Representative actin and synaptophysin Western blots (n=2 per group).*

### 3.6 Ipsilateral Hippocampal Samples Showed a Trend Toward a Reduction in PSD-95 Expression After Injury

Hippocampal PSD-95 protein expression after CCI was assessed by Western blot at a 2-weeks timepoint post injury using synaptosome samples. A Student's t-test was performed to determine differences in protein expression comparing sham and CCI tissues. PSD-95 expression in synaptosome samples of ipsilateral hippocampus in CCI animals had a trend toward a decrease when compared to sham (p=0.0613) (n=6 per group).

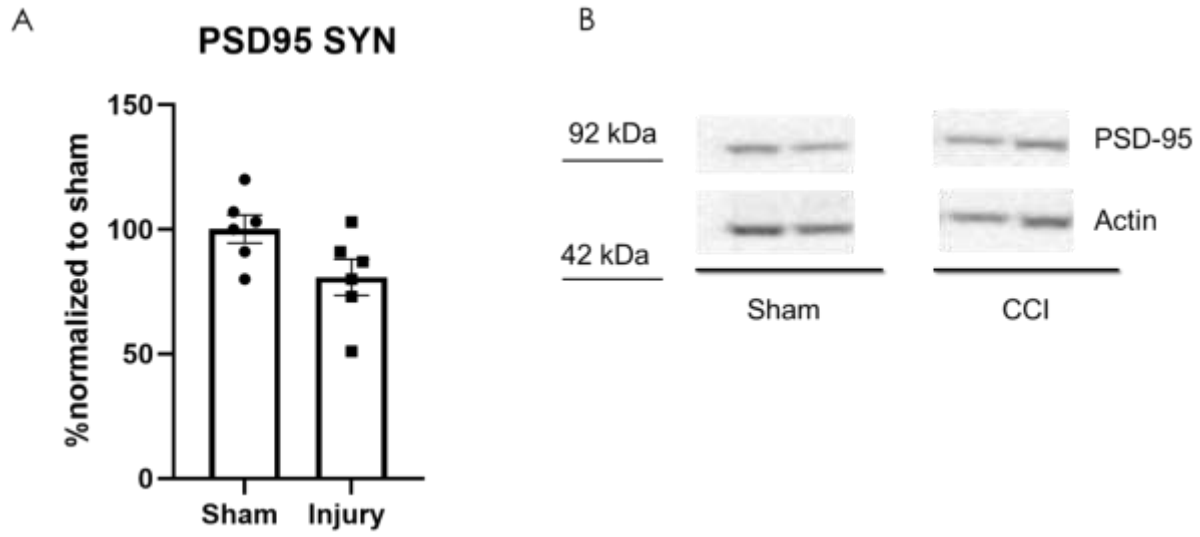


***Figure 7: PSD-95 expression quantitatively calculated using Western blot***

***(A):*** *A trend toward a decrease is seen with CCI animals having lower PSD-95 concentrations compared to sham samples (p=0.0613; n=6 per group).* ***(B):*** *Representative actin and PSD-95 Western blots (n=2 per group).*

### 3.7 BACE-1 Expression Predictive of Motor Performance

When comparing baseline BACE-1 (BL BACE-1) serum levels with performance on the Beam Balance task, we found a significant positive correlation within animals (*p=0.0165, Fig. 8). The data suggests that higher BACE-1 baseline concentrations in rat serum predict higher latencies on the Beam Balance task. There were no other correlations with other behavioral assessments.

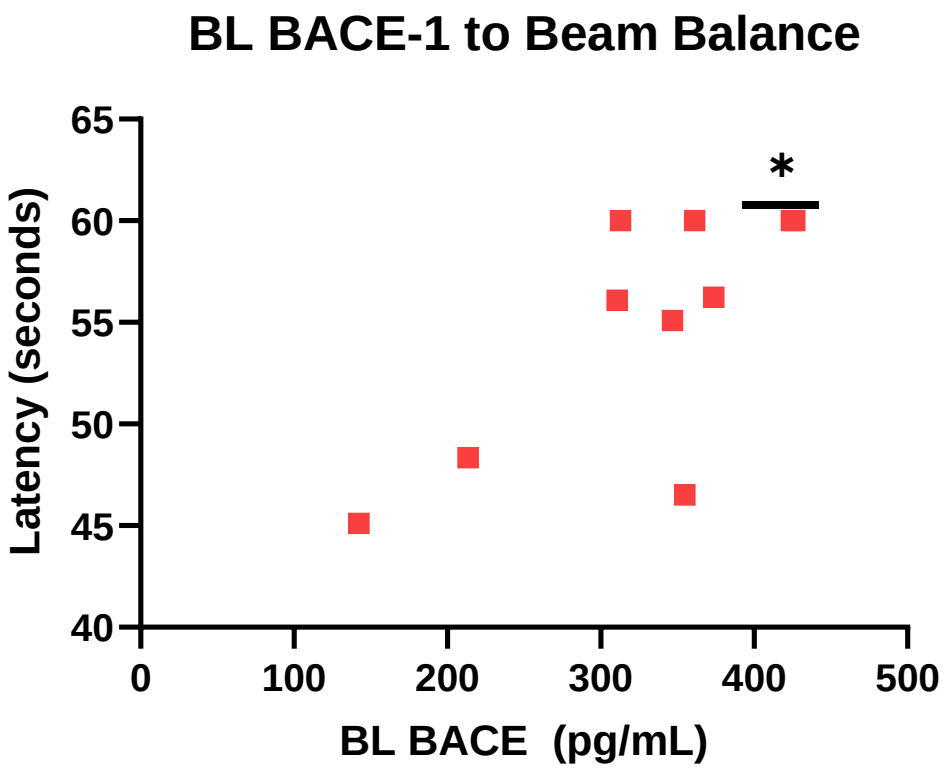


***Figure 8: BACE-1 concentrations predictive of gross motor functioning post injury***

*There is a correlation between baseline BACE-1 serum concentrations (BL BACE pg/ml) and the 5-day average performance on the Beam Balance task for the injured animals, suggesting higher baseline BACE-1 levels predict better function on Beam Balance task (r= 0.7303, *p=0.0165).*

## 4.0 Discussion

The study shows novel evidence that BACE-1 levels in serum decrease from baseline to 72 hours in both sham and CCI groups. This is not evidence that correlates CCI injury with changes in serum BACE-1, but rather suggests that the general surgical procedures such as anesthesia or the craniectomy are likely altering serum BACE-1 levels. Optimizing the ELISA for serum by increasing sample load to 30uL led to smaller number of wells showing undetectable samples, suggesting that it worked (6 samples had to be repeated in subsequent ELISA plates). In future studies, increasing the sample load by another 10uL as well as incubating for a longer period of time, such as overnight, may be beneficial if suspecting lower concentrations. As for peripheral BACE-1 levels being affected by surgical procedures, future studies are needed to assess serum of naïve rat models at different time points, as well as using alternative anesthetics than isoflurane. While past studies have shown that BACE-1 expression in brain lysates stays unaffected with exposure to isoflurane[11], our study raises the question whether isoflurane may alter BACE-1 expression in the periphery. It may be possible that metabolizing isoflurane in the liver may affect the BACE-1 concentrations and it is important to record fluctuations in BACE-1 to determine how organs outside the CNS may contribute to peripheral BACE-1 levels.

Blasko et al. discovered that concentrations of BACE-1 in brain lysates returned to baseline levels 1-week post injury, and that sham and CCI groups had similar concentrations of the protein[2]. Our findings showed that BACE-1 levels in synaptosome samples of the ipsilateral hippocampal tissue remained comparable to sham levels after the CCI injury in rats. To our knowledge, this is a novel finding showing BACE-1 concentrations in brain lysates post CCI. A future direction of

this could be replicate the study with a larger sample size to ensure that the results are not underpowered and any possible changes in BACE-1 post CCI at a longer timepoint can be identified.

A past study showed that CCI causes phosphorylation of PSD-95, which in turn causes it to downregulate, which led us to expect PSD-95 to decrease post CCI[22]. Thus, our results that show a trend in reduction of PSD-95 further support this data. Synaptophysin has varying evidence in past literature regarding expression post TBI. Our results showed a trend to increase post CCI, however the study was underpowered and must be replicated with a larger sample size.

Results confirmed a TBI effect by showing motor deficits assessed by the Beam Balance and Beam Walking tasks as expected and in agreement with past studies investigating performance post CCI. Morris Water Maze was used to assess cognitive function post injury and showed significant deficits in the CCI group compared to sham while analyzing the following parameters: Average submerged platform latency, Latency to Inner platform zone, latency to outer platform zone. An interesting interpretation of the probe trial data revealed that segregating data into duration to varying zones of entry show significant differences for sham and CCI groups.

This study showed novel evidence that demonstrates BACE-1 having predictive value in motor functioning post injury. An interesting positive correlation between serum BACE-1 baseline concentrations and functioning on beam balance assessment post CCI was observed. This provides an additional tool in determining patient groups at risk for greater motor problems post CNS injury, by suggesting that there may be a biological basis of vulnerability.

Varying proteins in the synapse give us insights on various mechanisms and functions being disrupted due to CCI injury. Using BACE-1 in conjunction to other candidate biomarkers

can be useful in obtaining a more accurate picture of synaptic disruptions in the brain. Future studies may aim to explore this correlation more, utilizing various motor functioning assessments to better understand the role of BACE-1 with motor function. BACE-1 being predictive in motor functioning was a surprising finding since BACE-1 was primarily was correlated in AD literature with cognitive impairment, however, there was a study that demonstrated the role of BACE-1 in forming and maintaining muscle spindles in the body that may contribute to its role in motor functioning, however it is still unclear how organs outside the CNS are affecting peripheral BACE-1 levels[21,23].

The clinical implications of this study may apply to groups particularly vulnerable to TBI such as war veterans, athletes and senior populations who are at fall risk, but also suggest a biological basis for identifying groups vulnerable to motor deficits due to TBI. The possible predictive capacity of BACE-1 may help tailor rehabilitation plans and better prepare patients to take extra precautions in an event of injury.

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