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A BEHAVIORAL AND HISTOLOGICAL COMPARISON OF FLUID PERCUSSION INJURY AND CONTROLLED CORTICAL IMPACT INJURY TO THE RAT SENSORIMOTOR CORTEX

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Abstract

Our primary goal was to evaluate the behavioral and histological outcome of fluid percussion injury (FPI) and cortical contusion injury (CCI) to the sensorimotor cortex (SMC). The SMC has been used to evaluate neuroplasticity following CCI, but has not been extensively examined with FPI. In both the CCI and FPI models, a mechanical force of 4 mm in diameter was applied over the SMC, allowing for a direct comparison to measure the relative rates of histology and recovery of function in these models. Gross behavioral deficits were found on the sensory task (tactile adhesive removal task) and multiple motor assessments (forelimb asymmetry task, forelimb placing task, and rotorod). These sensorimotor deficits occurred in the absence of cognitive deficits in the water maze. The CCI model creates focal damage with a localized injury wheras the FPI model creates a more diffuse injury causing widespread damage. Both behavioral and histological deficits ensued following both models of injury to the SMC. The neuroplastic changes and ease at which damage to this area can be measured behaviorally make this an excellent location to assess traumatic brain injury (TBI) treatments. No injury model can completely mimic

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the full spectrum of human TBI and any potential treatments should be validated across both focal and diffuse injury models. Both of these injury models to the SMC produce severe and enduring behavioral deficits, which are ideal for evaluating treatment options.

Keywords

Animal Studies; Assessment tools; Fluid Percussion Injury; Cortical Contusion Injury; Behavior; Sensorimotor Function

1. Introduction

TBI is among the leading causes of acute and chronic disability in the United States according to the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention.[1] Out of the 1.7 million Americans that endure a TBI each year, over 50,000 die.[1] Approximately 1.2 million Americans endure some sort of injury to their central nervous system, making recovery of function a major public health issue.

1.1 Primary vs. Secondary Injury

TBI occurs due to a blunt, rotational, accelerational, diffuse, focal, or concussive force to the head. Damage to the central nervous system is separated into two different classifications of injury, a primary and a secondary. Primary injuries result from the initial impact of mechanical forces. This initial disruption in tissue results in axonal shearing and cellular death of all types. This primary injury can be produced by collision forces to the skull, leading to a more focal compression of cortical tissue or by acceleration forces, inducing a more widespread injury causing brain swelling and diffuse axonal injury. Dependent upon the type of injury, a range of central nervous system (CNS) responses occur resulting in secondary damage. Secondary injury is comprised of multiple neurobiological, chemical, cytological, and physical changes that will occur for the remainder of the organism's life.[2]

Human TBI is a disorder that can cause a variety of disabilities, dependent upon several factors, including the heterogeneous nature of the type and location of the injury. Different physical forces as well as CNS locations have different pathophysiological consequences. Therefore animal models should mimic this variability so that these findings translate to clinical TBI. To approach this problem, the TBI field has developed many different ways to model human TBI in animal models.

There are many different experimental animal models of brain injury; blast injury, acceleration/deceleration models, weight drop models, cryogenic brain lesions, fluid percussion injury (FPI), and controlled cortical impact (CCI), the most common models being FPI and CCI. To date there are few direct comparisons of FPI and CCI in the rodent. Direct comparisons have examined intracranial pressure, blood brain barrier breakdown, and markers of plasticity,[3–5] but have failed to examine behavioral deficits and other common pathophysiological markers.

FPI is a diffuse model, first reported by Lindgren (1965), which induces axonal, somal, and microvascular swelling, leading to tissue distortion and axonal shearing both proximal and

distal to the injury location.[6–9] Axonal injury following this diffuse damage includes a loss of plasticity and cytoskeletal damage to axons, as well as an impairment of axonal transport. This leads to axonal swelling, rapid deformations and a loss of connectivity.[10]

The FPI device delivers a fluid pulse to the intact dural surface, creating a diffuse load to the brain.[11, 12] This model is beneficial in that different graded levels of injury can be administered, it can be used in several species of animals, and it leads to cavitation as well as axonal injury. However, there are disadvantages to this model as well. The pressure characteristics are not directly related to the mechanical impact to the brain. The direction, displacement, and velocity of the pulse are dependent upon the geometry of the brain.[11] Additionally, it has been shown that any small shift in craniotomy location alters the neurological outcome, as well as the lesion size and location of the injury.[13]

CCI's, first reported by Lighthall (1988) are focal, with localized tissue damage.[14, 15] This model displays blood brain barrier disruption and both vasogenic and cytotoxic edema similar to that seen in clinical TBI.[14, 16] The impactor device used for CCI is a pneumatic/electromagnetic cylinder, which houses a piston and impactor tip. The impactor tip is driven downwards at a specified velocity and depth, contacting the intact dural surface and creating a focal injury. The main benefit of this model is that the deformation parameters (velocity, depth, and time of dural contact) can be precisely controlled,[17] making it highly reproducible. CCI mimics the whole spectrum of focal injury, it is highly reproducible, and it translates well to human TBI.

TBI in humans can create damage to any structure causing many behavioral deficits. When creating an animal model of TBI, creating damage to a well characterized structure allows us to measure deficits in behaviors related to this structure. Recovery of function in animal models is measured through a variety of behavioral tasks, so knowledge about deficits associated with the structure being damaged is important in creating a good post-injury behavioral assessment. A large difference between the sham and injured control groups creates an injury window that is optimal to assess neuroprotective agents following injury. This makes using a model with a large injury window crucial for success. The majority of research in animal models of TBI uses either a bilateral-frontal lobe model of injury or a unilateral-parietal lobe model of injury.[18–21]

Cognitive, attentional, and spatial learning deficits associated with damage to this area are well cited in the literature[22] using the Morris water maze (MWM), which is a spatial learning task. However, the motor deficits[23] may be due to damage to motor planning areas[24] and the sensory deficits are suggested to be due to damage in areas associated with attention,[25] although this direct claim remains to be demonstrated empirically.

The unilateral-parietal lobe injury is typically centered between Lambda and Bregma and approximately 2–3 mm lateral to the midline (in rat models). This injury model creates damage to the parietal lobe as indicated by the name and creates primarily cognitive deficits. The cognitive deficits usually seen at this injury coordinate are typically measured in the MWM.[26, 27] Frequently sensorimotor deficits are seen, usually in the form of a hindlimb

motor deficit, but are only detectable in our behavioral assays for the first two weeks following injury.

We have utilized both the bilateral-frontal and unilateral-parietal lobe injury models, demonstrating substantial spatial learning deficits in the MWM.[21, 23, 27] However, the deficits seen in sensory and motor related behaviors are often present initially, but partial spontaneous recovery is seen within the first two weeks following injury[21, 28]. This spontaneous recovery makes it difficult to detect significant differences in a comparison group that received treatment. Creating an injury model with abundant and long lasting behavioral deficits is the best way to assess whether a neuroprotective drug is having beneficial effects.

Our laboratory has used the sensorimotor cortex (SMC), one of the most well characterized structures in the rodent CNS, to assess neuroprotective agents after TBI.[19, 28, 29] The forelimb SMC is well known for the plastic responses that follow both lesions and ischemic insult.[for review, see: 30, 31, 32] To summarize briefly, behavioral deficits ensue in the injured forelimb (contralateral to injury) following lesions of the forelimb SMC. However, post-injury behavioral experience (or direct cortical stimulation) alters the response to the injury. Rehabilitative training, including motor training, stimulates neural plasticity and helps compensate for loss of function. However, these post-injury experiences are time dependent.[30] More recently, it has been found that this behaviorally driven plasticity may be compromised following rodent TBI.[20] This along with the wealth of knowledge about the SMC map and the behavioral deficits associated with this area make this an exceptional target for an animal model of TBI.

No single animal model will ever be able to replicate the complete spectrum of changes that occurs in the CNS with this disorder.[11] Translational value of these models is dependent upon how well they mimic TBI in humans, but specific models mimic selective symptoms of each type of injury and can be used as a valid way to examine mechanisms following injury or neuroprotective drugs to treat this secondary injury.[33] A characterization of FPI and CCI was completed to assess the neuroplastic and behavioral response to these two models of TBI over the SMC. Although previous work has compared these models[3–5], this is the first comparison of these commonly used models to assess behavioral and histological differences over the SMC, a structure required for performance on commonly used behavioral assessments.

2 Methods

2.1 Subjects

56 Male, Sprague Dawley (Harlan, Indianapolis, IN) rats ~3.5 months of age at the time of the injury (mean body weight = 330 g) were used in this study. 32 animals were used to assess behavioral deficits and 24 animals were used to assess histological differences after injury. All animal and surgical procedures were adhered to as described in the NIH Guide for the Care and Use of Laboratory Animals. The Southern Illinois University Institutional Animal Care and Use Committee reviewed and approved all experimental procedures. Before and after injury, animals were housed in a university-maintained, Association for

Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited vivarium, with a 12-hr light/dark schedule and a controlled environmental temperature of 22°C in standard housing cages with food and water available *ad libitum*.

2.2 Surgery

Both the CCI and FPI surgeries were performed under aseptic conditions. Animals were anesthetized using a mixture of isoflurane (2–4%) and oxygen (0.8 L/min). When the animal became unresponsive (no ocular or pedal reflexes) the head was shaved and scrubbed with 70% alcohol followed by betadine and placed into a stereotaxic device. A midline incision was made in the skin as well as through the underlying fascia. A circular craniotomy (5.0 mm) was centered 0.5 mm anterior to and 3.5 mm lateral (left) to Bregma.

The CCI model utilized in the present study was based on previous studies.[28, 34] The contusion injury was created with a sterile stainless steel impactor tip (4.0 mm in diameter) that was attached to the ImpactOne™ stereotaxic CCI instrument (http://www.leicabiosystems.com, Buffalo Grove, IL). The injury was induced with an impact speed of 3.0 m/s and an impact depth of 2.5 mm at an angle of 10° towards the midline to mimic the angle of the FPI injury. The impact tip maintained contact with the brain tissue for 0.5 sec before retraction.

The FPI model utilized in the present study was also based on previous studies from our laboratory.[26, 35, 36] Briefly stated, a midline incision was made in the skin as well as through the underlying fascia. A circular craniotomy (4.0 mm) was centered 0.5 mm anterior to and 3.5 mm lateral (left) to Bregma. A 4 mm diameter plastic female leur was secured to the skull with dental acrylic, filled with sterile saline and was fixed to the end of the FPI device. The FPI device (VCU Biomedical Engineering, Richmond, VA) consisted of a hammer on a pendulum, dropped from a 12° angle to contact a plunger at the end of a fluid filled cylinder, creating a pulse of approximately 1.5 atm. FPI injuries were delivered when animals responses indicated they were at a level III-1 anesthesia.[26, 35, 36] Additionally, the duration of ensuing apnea (latency to regain spontaneous breathing) and limb withdrawal reflex were recorded to classify injury severity. In the FPI model, the average time of apnea was 10.75 s (SEM = 1.815) and the average time for the withdrawal reflex was 138.5 s (SEM = 38.670). Animals were then placed back into the stereo-taxic frame and the incision was sutured. To maintain normal body temperature (37°C) during surgery and recovery, the rats were placed on a warm water recycling bed and pump system (EZ Anesthesia, Palmer, PA). Rats receiving sham surgeries underwent identical surgical preparation as injured animals, received craniotomies, sutured, and were then transferred to recovery.

2.3 Sensory Assessment

2.3.1 Bilateral tactile adhesive removal task—This task has been shown to be an effective assessment of somatosensory deficits following injuries to the rodent frontal lobe.[28, 37, 38] A small circular sticker approximately 113 mm^2 (Avery Dennison Corporation, Brea, CA) was applied to the radial aspect of each forelimb. The rat was returned to its home cage and the latency to initially contact and then remove the stickers was used as a dependent variables. The trial was terminated when either both the right and left sticker were

removed or two minutes had elapsed. Each animal received two trials each day with a 15 minute ITI. Two days of training occurred prior to surgery to establish a pre-injury baseline and post-injury testing occurred on days 4, 6, 8, 11, 13, and 15.

2.4 Motor Assessment

2.4.1 Forelimb assymetry task—To assess the amount of reliance on the "uninjured limb" (ipsilateral to injury), this test was administered on days 4, 6, 8, 11, 13, and 15 post-injury following methods outlined in previous studies.[28, 29] Rats were pre-tested for baseline levels for the two days prior to surgery. On each test day, the rats were placed in an empty fish tank (50 cm l × 25 cm w × 30 cm h) and were allowed to explore for 120 s. Rats were administered one trial per test day, which was recorded with a video camera. Later, the number of contacts on the vertical surface was recorded as the primary dependent variable of interest. The following equation was used to calculate the reliance on the uninjured limb: (Right forelimb contacts/ (Right forelimb contacts + Left forelimb contacts). This analysis is consistent with what has been used in the literature.[28]

2.4.2 Locomotor placing task—In order to assess recovery of coordinated, locomotor limb movement, this test was administered on days 4, 6, 8, 11, 13, and 15 post-injury following methods outlined in previous studies.[23, 29] Rats were pre-tested for baseline levels for the two days prior to surgery. On each test day, the rat was placed on an elevated grid floor (56.0 cm × 54.0 cm) with openings measuring 3.2 cm × 3.2 cm in size and allowed to freely explore for 120 s. A "foot-fault" occurred when a rat inaccurately placed a limb through one of these openings. Total movements on the grid as well as foot-faults were recorded. Rats were administered one trial per test day. The total number of foot-faults was the primary dependent variable of interest. The following equation was used to calculate the foot faults as a function of total movement on the grid: (Right forelimb faults-Left forelimb faults)/ lines crossed. This analysis is consistent with what has been used in the literature. [29]

2.4.3 Rotarod—Automated ROTOR-ROD™ (San Diego Instruments, San Diego, CA) testing was performed on post-injury days 13–15 to assess gross motor coordination.[39] Two days of training occurred prior to surgery to establish a pre-injury baseline on the rotarod. Animals were placed on the rotating cylinder against the rotation and the latency to fall was recorded. On the first day of training, the animals were placed on the cylinder, which was stationary, and replaced when they fell, until they were able to remain on the cylinder for 60 consecutive seconds so that they become acclimated to the device. Following this trial and on all other days of training, the trial was terminated and the latency was recorded when the animal fell off of the cylinder. The initial speed of 10 rpm was maintained for 15 s to allow experimenters to load the rat. Following these fifteen seconds, the speed of the rotation increased from 10 rpm to 50 rpm over the course of six minutes. An infrared beam sensor located below the cylinder recorded the latency for the animal to fall. Each animal received four trials each day with a 15 min ITI.

2.5 Cognitive Assessment

2.5.1 MWM: Reference memory acquisition—All animals were tested in the MWM using a reference memory paradigm, which has been widely utilized to assess cognitive performance following TBI.[26, 40–42] The apparatus consisted of a circular, 183 cm diameter blue plastic pool, partially filled with water (22°C) to a depth of approximately 41 cm. A clear plastic platform (10 cm × 10 cm) was submerged approximately 2 cm below the water surface. The animal's progress on the task was evaluated by a video camera affixed above the pool and this data were processed using the MWM specific computer software SMART (San Diego Instruments, San Diego, CA).

Animals were assessed on the acquisition of a reference memory task beginning on post-injury day 18 and were tested for 3 subsequent days. Preliminary data (not shown) from our laboratory suggests that administering additional behavioral tasks on the same day as a MWM assessment alters animal's performance and confounds the results. Therefore, the MWM testing was administered after all of the other behavioral assessments had concluded. On every testing day, each animal received four trials during which to locate the submerged platform in the pool, starting at one of four release points in random order. In this reference memory task, the platform remained in the same place for each trial on every testing day. The trial was terminated when the rat reached the submerged platform in the northeast quadrant or when 90 secs have elapsed. If the rat did not find the platform within the 90 secs, it was gently guided to the platform and remained on it for at least 10 secs. Each rat remained on the platform for 10 secs, after which it was placed in a warm holding cage for at least 15 min before the next trial.

2.5.2 MWM: Working memory task—Working memory performance was tested on days 25–27 post-injury using established methods.[21] The procedure was the same, except the platform was submerged at the center of a new randomly chosen quadrant (south-west, north-west, and south-east) each day. Each animal was given four trials per day; starting from one of four randomly chosen release points (ITI 15 min). The first trial on each of these days was considered an information trial and was not included in subsequent analyses; the latencies from the last three trials were averaged to form a score for the day. Each trial ended when the animal located the platform, or when 90 s has elapsed. Animals not reaching the platform were guided to the platform after the trial had ended.

2.6 Histological Analysis

2.6.1 Tissue preparation—At specific time points, the rats were euthanized with Euthasol (Virbac Animal Health; 0.3 mL i.p.) and transcardially perfused with 0.9% phosphate-buffered saline (PBS), followed by 10% phosphate buffered formalin (PBF). Brains were post-fixed in PBF following removal from the cranium for 24 hrs and then transferred into a 30% sucrose solution to cryopreserve the brains four days prior to frozen sectioning. Serial, coronal sections (40 μm thickness) were sliced using a sliding microtome on a frozen stage and collected into a cryopreservative solution and stored at −20° C. Animals in the behavioral portion of the experiment underwent a cresyl violet lesion analysis 30 days after injury (both CCI and FPI). At 24 hrs post injury (both CCI and FPI),

the rats in the histological portion of this study were euthanized and underwent identical procedures and were examined for neuron degeneration as well as astrocytic activation.

2.6.2 Lesion analysis—A series of sections were brush mounted on gelatin-subbed microscope slides, stained with cresyl violet, dehydrated, and cover slipped. The extent of the lesion was analyzed with an Olympus microscope (BX-51) and an Olympus 13.5 megapixel digital camera (DP-70). Images of sections throughout the extent of the injury coordinates were captured using the digital capturing system and area measures of the lesioned tissue were determined using the ImageJ software package (1.43u, NIH). The Calvalieri method was used to calculate the volumes of the ipsilateral cortex and the contralateral cortex.[43] Four stereotaxic coordinates throughout the lesion, at approximately 1.7, 0.9, 0.1, and - 0.7 mm relative to bregma, were selected for lesion analysis. The number of sections and the section thickness (40 μm) were multiplied by the mean area of the remaining cortex. The extent of cortical injury was measured by calculating the percent reduction in the injured ipsilateral cortex compared to the contralateral cortex at each level using the formula: (1 – (ipsi/contra) × 100) and we have reliably shown that this technique is sensitive enough to detect treatment-induced reductions in injury size.[26–28, 44]

2.6.3 Neuronal degeneration—Two series of frozen sections throughout the injury cavity (+1.2 and −0.2 mm, relative to Bregma) were stained for degenerating neurons using Flouro-Jade B (Chemicon Int. Billerica, MA) stain.[45] Standard protocol was followed.[35, 46] The slides were immersed in a 95% EtOH solution for 3 min, 70% EtOH solution for 3 min, dH_20 rinse for 1 min, 0.06% potassium permanganate solution for 10 min under agitation, dH_20 rinse for 1 min, 0.004% FJ staining solution for 10 min. All the following steps including this one were performed in the dark. The slides were then rinsed with dH_20 (3 × 1 min), allowed to dry for 15 min at 50° C, xylene for 1 min and cover-slipped. Tissue sections were visualized with an Olympus fluorescent microscope (BX-51) system using a GFP filter. Each section was viewed at 4x magnification, and then increased to 40x, before the image was captured with a 13 megapixel Olympus (DP-70) digital camera. The region of interest was set to be 300 μm w × 300 μm h. The number of FJ+ cells within the captured field of view (90 mm^2) was counted using ImageTool software. There were six regions of interest examined; four in the ipsilateral cortex, surrounding the injury and two regions in the contralateral cortex, see Figure 5a.

2.6.4 Astrocyte reaction—Another series of frozen sections throughout the injury cavity (+1.2 and −0.2 mm, relative to Bregma) were examined with a protocol for GFAP immunoreactivity to determine astrocyte expression following injury. Standard protocol was followed.[35, 46] Tissue sections were mounted on subbed slides and dehydrated over several steps and placed in a citrate buffer at 90° C for 10 min, normal goat serum (Vector Laboratory Inc., Burlingame, CA, diluted 1:50) for 24 hr, incubated with a GFAP goat anti-rabbit (Dako, Carpinteria, CA, diluted 1:2000) for 48 hr, rinsed in PBS + 0.2% Triton-X (TX; 6 × 5 min),incubated using goat anti-rabbit IgG (Vector Laboratory Inc., Burlingame, CA, diluted 1:200) for 90 min, rinsed again (PBS + 0.2% TX, 6 × 5 min), avidin biotin complex (Vector Laboratory Inc., Burlingame, CA) for 90 min, rinsed with PBS + 0.2 % TX

(3 × 5 min), 0.1 PB (3 × 5 min) rinse, reacted in a nickel-intensified chromagen solution containing acetate-imidizole buffer, 2.5 % nickel ammonium sulfate, 0.05% diaminobenzidine (DAB), and 0.01% H_2O_2, rinsed with 0.1 PB (3 × 1 min), allowed to dry for 15 min at 50° C, xylene for 1 min and cover-slipped. Tissue sections were visualized, imaged, and analyzed similarly to the FJ stained tissue.

2.7 Data Analysis

2.7.1 Behavioral data analysis—Behavioral data was analyzed using a Mixed Model Factorial analysis of variance (ANOVA) or one-way between-subjects ANOVA (SPSS v. 15 for Windows). The between factor was the injury model (Sham, FPI-injured, and CCI-injured) and the within-group factor was day of testing. Both the main effects and the interaction effects were considered. Huynh-Feldt corrections and Tukey's Honestly Significant Different test (Tukey's HSD) were used to control for Type I error in the repeated measures and *post hoc* means comparison, respectively. Planned comparisons using a t-test were performed when the interaction effects were not significant to examine differences in performance on each test. A significant level of $p \leq .05$ was used for all statistical analyses.

2.7.2 Histological data analysis—A one-way ANOVA was completed where the between-subject factor was the injury model (Sham, FPI-injured, and CCI-injured) was used to analyze the lesion analysis data. Tukey's HSD were used to control for Type I error and a significance level of $p \leq .05$ was used for all statistical analyses. Inter-rater reliability measures were evaluated using the bivariate correlation of scores obtained between the two raters. *Post hoc* analyses were conducted using Tukey's Honestly Significant Difference Test (Tukey's HSD) for comparison of means whenever appropriate. A *p*-value of < 0.05 was considered significant. All data are shown as mean scores ± standard error of the mean (SEM).

Cell counts (FJ+, GFAP+) from the six selected areas of interest for each slice (at two different coordinates) in the ipsilateral and contralateral cortex were used to determine the number of cells in each area. If no population of each cell type were found in an area of interest, tissue images were still captured and used in analysis. Cell counts were performed with ImageTool software. Counts were averaged across the slices from each coordinate for each hemisphere. One-way ANOVA tests were performed on all of the data. Independent variables were treatment (Sham, FPI-injured, and CCI-injured). *Post hoc* analyses were conducted using Fischer's LSD for comparison of means whenever appropriate. A *p*-value of < 0.05 was considered significant. All data are shown as mean scores, ± SEM.

Although some of the tasks required subjective scoring, each scoring procedure was very well defined with operational definitions. Additionally, 25% of the data were scored by a second, blind experimenter. These data were analyzed with a Pearson's correlation to assess the covariance between the two independent datasets.

3 Results

3.1 Sensory Assessment

3.1.1 Tactile Adhesive Removal Task—The animals' latencies to remove the adhesive stimuli were evaluated using repeated measures ANOVA, with the within-subjects factor of Day (4, 6, 8, 11, 13, and 15) and the between-subjects factor of Group (Sham, FPI-injured, and CCI-injured). Both the main effect of Day [F (4.452, 129.102) = 3.84, $p < 0.01$] and Group [F (2, 29) = 4.079, $p < 0.05$] were significant, see Figure 1.

The test for the interaction of Day × Group was significant [F (8.904, 129.102) = 2.070, $p < 0.05$]. Simple main effects of Group resulted in significant changes across the 6 days. The FPI-injured [F (3.415, 37.569) = 3.218, $p < 0.05$] and the CCI-injured [F (5, 45) = 2.415, $p < 0.05$] changed across the days, whereas the Sham [F (1.537, 13.834) = 1.517, $p = 0.250$] animals had low latencies throughout.

Post hoc analysis for the main effect of Group was completed to determine specific injury model effects. The CCI-injured animals performed significantly [HSD (20) = 23.126, $p < 0.001$] worse than the FPI-injured animals. Additionally, simple main effects revealed significant differences between the CCI-injured and Sham groups on all days of testing ($p < 0.05$), whereas the FPI-injured group was only significantly different than the Sham group on days 4 [F (2, 29) = 7.445, $p < 0.01$], 6 [F (2, 29) = 17.001, $p < 0.001$], and 8 [F (2, 29) = 13.278, $p < 0.001$].

3.2 Motor Assessments

3.2.1 Forelimb asymmetry task—A bias score was used to determine the amount of reliance on the "uninjured limb" (ipsilateral to injury). To examine Group differences, performance was evaluated using repeated measures ANOVA, with the within-subjects factor of Day (4, 6, 8, 11, 13, and 15) and the between-subjects factor of Group (Sham, FPI-injured, and CCI-injured). Both the main effect of Day [F (4.287, 124.323) = 7.728, $p < 0.001$] and Group [F (2, 29) = 18.943, $p < 0.001$] were significant, see Figure 2a.

The test for the interaction of Day × Group was significant [F (8.904, 129.102) = 2.070, $p < 0.05$]. Simple main effects of Group resulted in significant changes across the 6 days. The FPI-injured [F (5, 55) = 6.135, $p < 0.001$] and the CCI-injured [F (5, 45) = 6.618, $p < 0.001$] changed across the days, whereas the Sham group [F (5, 45) = 0.879, $p = 0.503$] did not have a bias throughout testing.

Post hoc analysis for the main effect of Group was done to determine specific injury model effects. The FPI-injured [HSD (20) = 25.051, $p < 0.001$] and the CCI-injured [HSD (18) = 27.151, $p < 0.001$] animals performed significantly worse than the Sham animals. There were no significant differences between the CCI-injured and FPI-injured group on any day of testing ($p < 0.05$)

3.2.2 Locomotor placing task—The animal's right and left forelimb faults and total steps were counted and evaluated as a percent of total steps that were faults on the grid surface. To examine Group differences, performance was evaluated using repeated measures

ANOVA, with the within-subjects factor of Day (4, 6, 8, 11, 13, and 15) and the between-subjects factor of Group (Sham, FPI-injured, and CCI-injured). Both the main effect of Day [F (5, 145) = 3.001, $p < 0.05$] and Group [F (2, 29) = 39.380, $p < 0.001$] were significant. The interaction of Day × Group was not significant [F (10, 145) = 1.293, $p = 0.239$].

Post hoc analysis for the main effect of Group was done to determine specific injury model effects. The FPI-injured [*HSD* (20) = 0.409, $p < 0.001$] and the CCI-injured [*HSD* (18) = 0.715, $p < 0.001$] animals performed significantly worse than the Sham animals. There were no significant differences between the FPI-injured and the CCI-injured animals [*HSD* (20) = 0.113, $p = 0.07$]. Performance on this task is shown in Figure 2b.

3.2.3 Rotarod—The animal's latencies to fall were evaluated using repeated measures ANOVA, with the within-subjects factor of Day (13, 14, and 15) and the between-subjects factor of Group (Sham, FPI-injured, and CCI-injured). The main effect of Day [F (2, 58) = 24.630, $p < 0.001$] and the main effect of Group [F (2, 29) = 15.215, $p < 0.001$] were significant. The interaction of Day × Group was not significant [F (4, 58) = 1.922, $p = 0.119$]. Performance on this task is shown in Figure 3.

Post hoc analysis for the main effect of Group was done to determine specific injury model effects. The FPI-injured [*HSD* (20) = 55.386, $p < 0.001$] and the CCI-injured [*HSD* (18) = 45.156, $p < 0.001$] animals performed significantly worse than the Sham animals. There were no significant differences between the FPI-injured and the CCI-injured animals [*HSD* (20) = 10.230, $p = 0.598$].

3.3 Cognitive Assessments

3.3.1 Reference memory acquisition—In the reference memory task of the MWM, the latency to reach the platform was averaged over the four trials for each testing day during reference memory acquisition. The animal's latencies to reach the platform were evaluated using repeated measures ANOVA, with the within-subjects factor of Day (18, 19, 20, and 21) and the between-subjects factor of Group (Sham, FPI-injured, and CCI-injured). The main effect of Day [F (2.720, 78.868) = 70.854, $p < 0.001$] was significant, but the main effect of Group [F (2, 29) = 2.879, $p = 0.072$] was not significant. The interaction of Day × Group was also not significant [F (5.439, 78.868) = 0.768, $p = 0.586$], data not shown.

3.3.2 Working memory task—In the working memory task, the latency to reach the platform was averaged over the last three trials for each testing day to assess working memory. The animal's latencies to reach the platform were evaluated using repeated measures ANOVA, with the within-subjects factor of Day (25, 26, and 27) and the between-subjects factor of Group (Sham, FPI-injured, and CCI-injured). Both the main effect of Day [F (2, 58) = 1.512, $p = 0.229$] and Group [F (2, 29) = 2.145, $p = 0.135$] were not significant. The interaction of Day × Group was also not significant [F (4, 58) = 0.147, $p = 0.963$], data not shown.

3.4 Histological Analyses

3.4.1 Lesion analysis—A lesion analysis was completed with the histological portion of the experiment, 24 hrs post-injury, as well as following the behavioral assessment, 30 days post-injury. Representative images of cresyl stained coronal slices demonstrating damage 24 hrs post-injury are displayed in Figure 4a. The ratio of lesion volume in the cortices 24 hrs post-injury was compared in a one-way ANOVA [Group (Sham, FPI-injured, and CCI-injured)]. There was a significant difference between the Groups [F (2, 21) = 4.802, p < 0.05]. More specifically, the CCI-injured animals were significantly different than the FPI-injured group [*HSD* (14) = 12.203, p < 0.05] and Sham group [*HSD* (14) = 10.252, p < 0.05]. The FPI-injured [*HSD* (14) = 0.048, p = 0.992] group was not significantly different than the Sham group, see Figure 4c.

Representative images of cresyl stained coronal slices demonstrating damage 30 days post-injury are displayed in Figure 4b. The ratio of lesion volume in the cortices 30 days post-injury was also compared in a one-way ANOVA [Group (Sham, FPI, and CCI)]. There was a significant difference between the Groups [F (2, 29) = 61.512, p < 0.001].

Post-hoc analysis indicated that the FPI-injured group [*HSD* (20) = 27.029, p < 0.001] and the Sham group [*HSD* (18) = 39.038, p < 0.001] had a significantly smaller mean percent reduction when compared to the CCI-injured group. Additionally, the Sham group had a significantly smaller mean percent reduction than the FPI-injured group [*HSD* (20) = 12.009, p < 0.01], see Figure 4c.

3.4.2 Neuronal degeneration—Degenerating neurons in the cortices both ipsilateral and contralateral to the injury were measured by counting the number of FJ+ cells within specific regions of the cortices. Representative images of FJ+ cells in the cortices 24 hrs post-injury for each group are displayed in Figure 5b. This FJ+ cell quantification was analyzed by a oneway ANOVA with Group (Sham, FPI-injured, and CCI-injured) as the between factors. Analysis of the ipsilateral cortices showed that there were significant differences in the number of FJ+ cells between the groups [F (2, 21) = 43.768, p < 0.001].

Post-hoc analysis indicated that the FPI-injured group [*HSD* (14) = 35.766, p < 0.001] and the CCI-injured group [*HSD* (14) = 53.938, p < 0.001] had significantly more FJ+ cells when compared to the Sham group. Further analysis indicated that the FPI-injured group had significantly fewer FJ+ cells than the CCI-injured group [*HSD* (14) = 18.172, p < 0.01], see Figure 6a.

Analysis of the contralateral cortex showed that there were significant differences in the number of FJ+ cells between groups [F (2, 21) = 38.081, p < 0.001]. Post-hoc analysis indicated that the FPI-injured group had significantly more FJ+ cells when compared to the CCI-injured group [*HSD* (14) = 5.563, p < 0.001] and the Sham group [*HSD* (14) = 6.625, p < 0.001]. There was not a significant difference of FJ+ cells between the CCI-injured group and the Sham group [*HSD* (14) = 1.063, p = 0.207], Figure 6a.

3.4.3 Astrocyte reaction—Reactive astrocytes in the cortices both ipsilateral and contralateral to the injury were measured by counting the number of GFAP+ cells within

specific regions of the cortices. Representative images of GFAP+ cells in the cortices 24 hrs post-injury for each group are displayed in Figure 5b. This GFAP+ cell quantification was analyzed by a one-way ANOVA with Group (Sham, FPI-injured, and CCI-injured) as the between factors. Analysis of the ipsilateral cortices showed that there were significant differences in the number of GFAP+ cells between the groups [F (2, 21) = 35.545, p < 0.001].

Post-hoc analysis indicated that the FPI-injured group [*HSD* (14) = 17.234, p < 0.001] and the CCI-injured group [*HSD* (14) = 29.547, p < 0.001] had significantly more GFAP+ cells when compared to the Sham group. Further analysis indicated that the FPI-injured group had significantly fewer GFAP+ cells than the CCI-injured group [*HSD* (14) = 12.313, p < 0.01], see Figure 6b.

Analysis of the contralateral cortex showed that there were significant differences in the number of GFAP+ cells between groups [F (2, 21) = 21.502, p < 0.001]. Post-hoc analysis indicated that the FPI-injured group had significantly more GFAP+ cells when compared to the CCI-injured group [*HSD* (14) = 14.781, p < 0.001] and the Sham group [*HSD* (14) = 13.531, p < 0.001]. There was not a significant difference of GFAP+ cells between the CCI-injured group and the Sham group [*HSD* (14) = 1.250, p = 0.622], see Figure 6b.

4 Discussion

Millions of dollars each year are invested into animal research designed to discover and refine new treatments and rehabilitative strategies to treat TBI. The animal model is the tool that neurotrauma scientists use to mimic human TBI and to assess these different treatments. The primary goal of the current research was to assess, characterize, and compare the two most common animal models of TBI: CCI and FPI over the SMC.

These models create damage by different physical forces and create a different physiological response. The injury location, over the SMC, created substantial and enduring behavioral deficits, as well as histological damage in both of these models of TBI. Additionally, our data suggest that the CCI model creates focal damage with a localized injury wheras the FPI model creates a more diffuse injury causing widespread damage. However both injury model types to the SMC created behavioral deficits in both sensory and motor behavioral measures.

Injury severity was assessed with a battery of behavioral tasks to measure functional recovery following injury. Animals were assessed in both fine and gross motor and sensory tasks, as well as a spatial learning task. Both the CCI and FPI to the sensorimotor cortex created substantial and enduring deficits in the fine and gross motor assessments. Deficits with both of these models were severe in the Locomotor placing task and the Forelimb asymmetry task and endured longer than two weeks post-injury. Creating this longer lasting deficit is helpful when identifying potential treatments and may correspond to more chronic deficits seen in the human population. The severity of deficit demonstrated in the Rotorod was similar in both of these injury models.

In the Tactile Adhesive Removal Task, both the injury models created deficits when compared to the Sham animals. However, the FPI animals demonstrated spontaneous recovery after eight days post-injury. The CCI deficits were significantly more severe and recovered slightly, but were still significantly different than Sham animals, even after two weeks of assessment.

The lasting behavioral deficits seen in both the CCI and FPI models over the SMC are beneficial to researchers that are interested in finding potential treatments for TBI through the use of animal models. Other models, including the bilateral frontal and the unilateral parietal typically demonstrate spontaneous recovery in sensorimotor related tasks within the first two weeks of injury[21, 28]. Extending these deficits with the SMC model allows for a more long-term evaluation of any potential strategies of improving recovery of function. This benefit of using the SMC as a target structure for TBI to measure combination therapy treatment has been demonstrated in the literature[47]. However, one benefit to the use of both the bilateral frontal model and the unilateral parietal model is the appearance of MWM deficits several weeks after injury[21, 23, 27].

Neither the CCI nor FPI injury models over the SMC demonstrated spatial learning deficits in the MWM. However, this finding was hypothesized due to the injury location and was used as an internal behavioral standard. Damage to the sensorimotor cortex was hypothesized to create sensory and motor deficits in the absence of cognitive deficits. Damage from these models did not create substantial damage to the prefrontal cortex or the hippocampus, two areas thought to be important for spatial learning.

A cortical volumetric lesion analysis was completed at both 24 h and 30 days post-injury to determine the extent of the injury cavity. At 24 h post-injury, the CCI animals had a significant mean percent reduction compared to the Sham animals, whereas the FPI animals did not. One plausible explanation for this is the nature of the progression of damage following traumatic brain injury. The initial, primary injury causes a secondary injury cascade including cerebral edema, excitotoxicity, oxidative stress, inflammation, and a glial response all of which leads to increased scarring and cell death as time progresses. Over the course of several weeks, the secondary injury mechanisms accrue additional damage and increase the amount of total tissue loss. Recent studies demonstrated that at 24 hrs post-injury, injury models have significantly more cortical cavitation than sham animals, but it is significantly less than the total cavity that develops 30 days after injury. This increased cavity is initiated by the primary injury but is likely perpetuated by increases in edema, excitotoxicity, oxidative stress, inflammation, glial responses, and reduced cellular energy metabolism.

At 30 days post-injury both the FPI and the CCI animals had a significant increase in injury cavity compared to the Sham animals. Additionally, the CCI animals had an increased cortical mean percent reduction when compared to the FPI animals. This is likely due to the level of severity of the injury as well as the physical forces of each of the models. The physical nature of a stainless steel impactor tip contacting the dural surface creates a more focal, localized injury to the cortices. The FPI on the other hand creates a diffuse, widespread injury, throughout the brain. Using a mean percent reduction score of tissue loss

is a valid initial assessment, but does not evaluate underlying tissue damage. At this time point there is tissue that is intact, but is inflamed and undergoing degeneration.

In the brain of animals sacrificed 24 hrs post-injury, there is tissue that appears damaged, but not completely lost yet. This type of tissue is still stained using a nissl stain (cresyl violet), but the stain is hypointensive, indicating that some of the cells in this tissue may be impaired.[46] The nissl body stained with cresyl violet is composed of rough endoplasmic reticulum, which can dissolve and disappear (chromatolysis) under pathological conditions like injury.[48, 49] Many of these damaged neurons are in the process of degenerating and the surrounding tissue is inflamed. It is likely that this is the tissue that is dying over the course of the next several weeks, becoming a significantly larger lesion cavity.

To assess this damaged tissue, we conducted cell counts of reactive astrocytes (GFAP+) and degenerating neurons (FJ+) throughout the cortices. Several regions of interest from both the cortices ipsilateral and contralateral to the injury were analyzed. Both of the injury models showed a significant increase in these two markers when compared to Sham animals in the ipsilateral cortices. In addition the CCI animals had significantly more GFAP+ and FJ+ cells in the ipsilateral cortices than the FPI animals. This is in agreement with the lesion analysis data suggesting the CCI creates more localized damage than the FPI.

In the contralateral cortices there were no significant differences in the number of reactive astrocytes or degenerating neurons between the CCI and the Sham group. However, the FPI animals had a significant increase in the number of GFAP+ and FJ+ cells in the cortex contralateral to the injury. These data substantiate the claim that the FPI injury model is diffuse and widespread, creating damage to areas distal to the injury location, including the contralateral cortices.

5 Conclusions

Damage to the SMC in both of these injury models creates abundant, long lasting sensorimotor behavioral deficits in the absence of cognitive deficits, ideal for multiple group comparisons. FPI is a diffuse injury that leads to widespread histological damage, including the contralateral cortices, in the absence of excessive cortical damage. The occurrence, size, and location of the lesion cavities were variable, without behavioral variability. Cortical contusion injury is primarily focal, with the majority of the histological damage forming proximal to the site of injury. These models mimic the variability that occurs in human traumatic brain injury and can be used in preclinical animal models to screen multiple neuroprotective compounds simultaneously.

Our results indicate that even a moderate FPI injury creates long term functional behavioral deficits, which are ideal for multiple drug comparisons. It has been agreed upon that administration of a drug that targets only one of several complex components of secondary injury will not be a sufficient neuroprotective treatment. A combination of drug treatments as well as behavioral rehabilitation that target multiple components of secondary injury is necessary. Behavioral characterization of these models as well as studies examining

neuroplastic changes and rehabilitative strategies following TBI allow for the evaluation of the effectiveness of these multicomponent polytreatments.

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Highlights

- We characterized two animal models of traumatic brain injury (TBI): Cortical Contusion Injury (CCI) and Fluid Percussion Injury (FPI)
- We evaluated behavioral and histological deficits in these models over the sensorimotor cortex (SMC)
- Gross behavioral sensory and motor deficits were found in the absence of cognitive deficits
- Histological data verifying a more focal CCI injury and more diffuse FPI injury were found
- Both of these injury models over the SMC produce severe and enduring behavioral deficits, ideal for evaluating treatment options

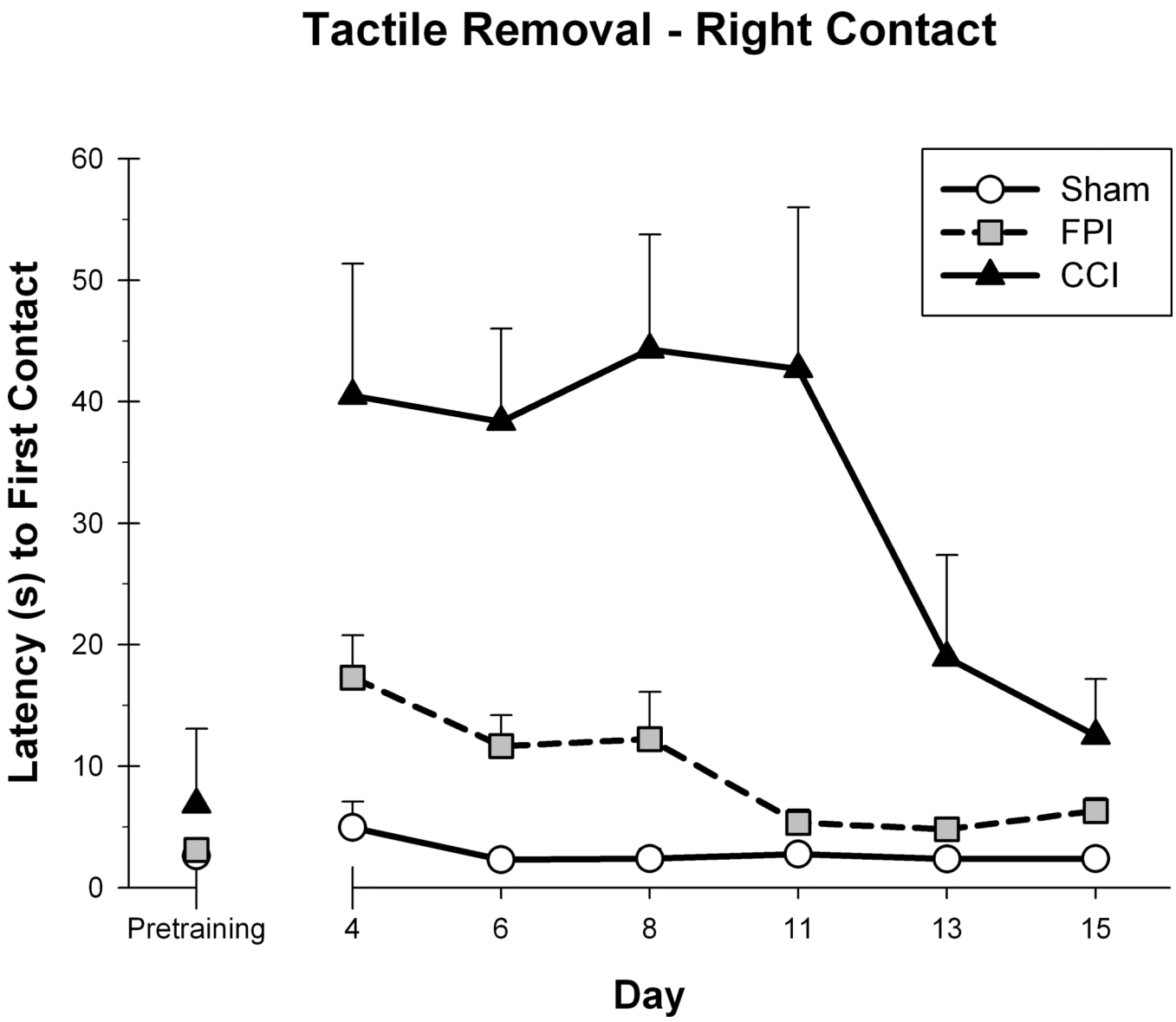

Figure 1.

Tactile Adhesive Removal Task. The average latencies (+ SEM) to remove the adhesive from the impaired forelimb, both during pretraining (2 and 1 days prior to being injured), and following brain injury (days 4, 6, 8, 11, 13, 15).

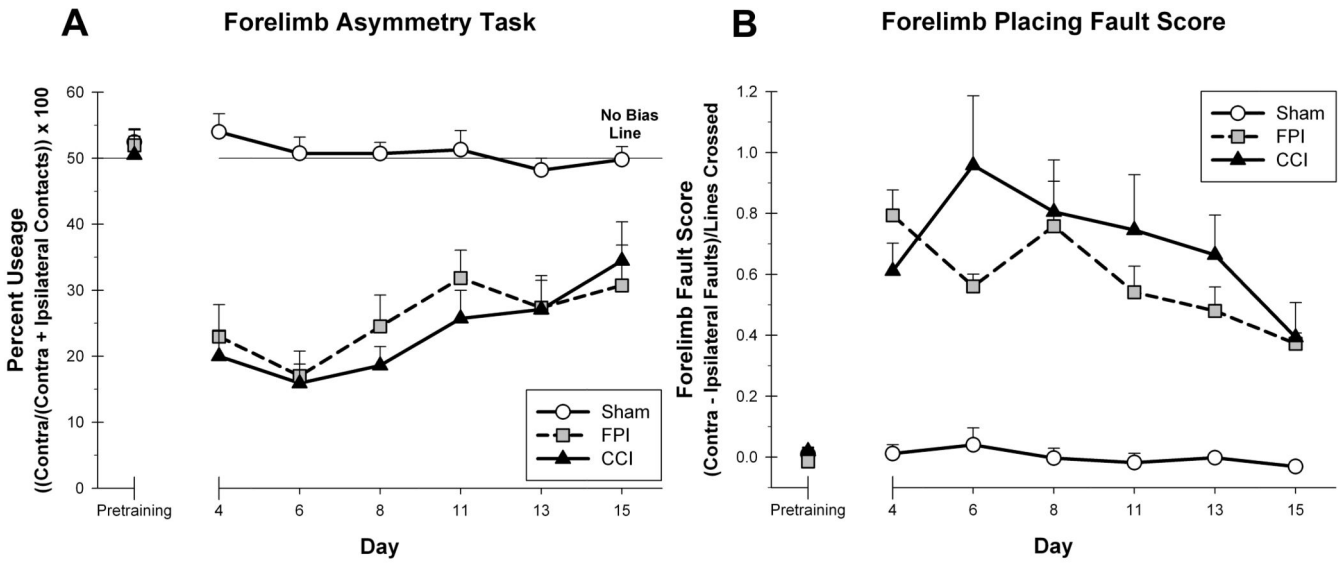

Figure 2.

Fine Motor Assessment. A). Forelimb Assymetry Task. The average percent useage bias (contralateral contacts/(contralateral +ipsilateral contacts) *100) for the unimpaired forelimb, both during pretraining (2 and 1 days prior to being injured), and following brain injury (days 4, 6, 8, 11, 13, 15). B). Locomotor Placing Task. The average fault scores ((contralateral faults – ipsilateral faults)/lines crossed) for the impaired forelimb, both during pretraining (2 and 1 days prior to being injured), and following brain injury (days 4, 6, 8, 11, 13, 15).

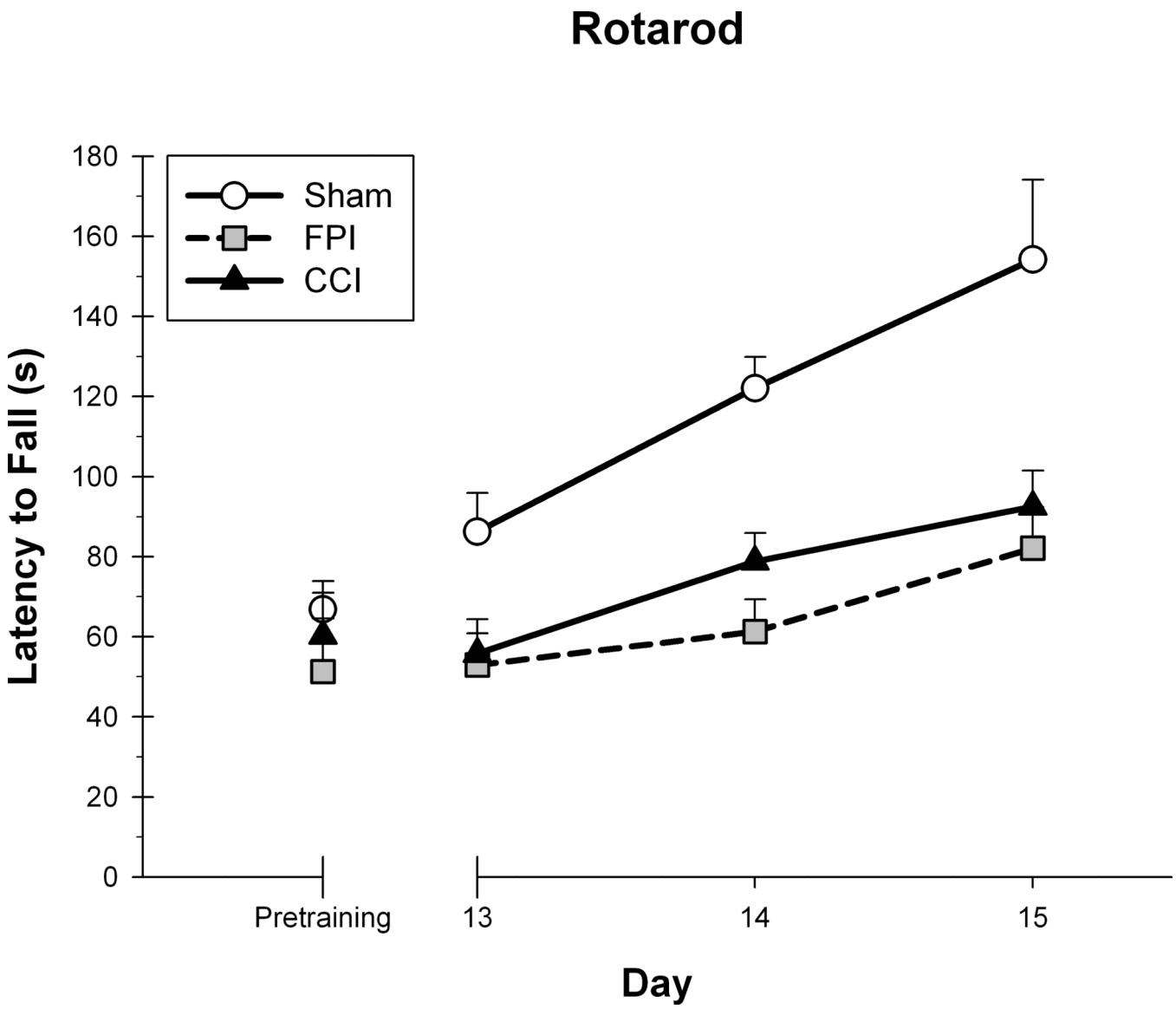

Figure 3.
Rotarod. The average latencies to fall from the rotating cylinder, both during pretraining (2 and 1 days prior to being injured), and following brain injury (days 13, 14, and 15).

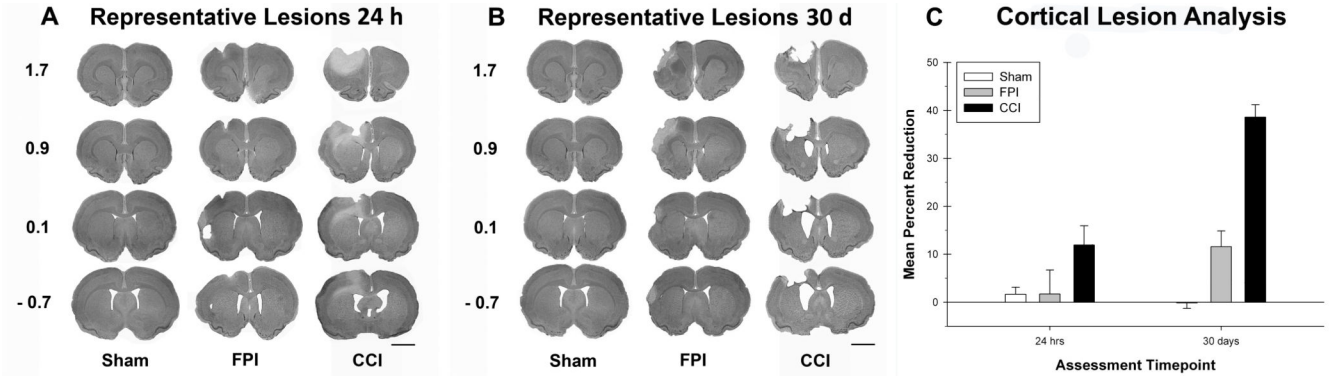

Figure 4.

Lesion Analysis. A). Representative Representative images of cresyl violet stained (24 hr) tissue throughout the injury coordinates; 1.7 mm, 0.9 mm, 0.1 mm, and −0.7 mm, relative to Bregma. Scale bar = 2.0 mm. B.) Representative Representative images of cresyl violet stained (30 days) tissue throughout the injury coordinates; 1.7 mm, 0.9 mm, 0.1 mm, and −0.7 mm, relative to Bregma. Scale bar = 2.0 mm. C). The average (+SEM) percent reduction (1 – (ipsi/contra) × 100) of cortical volume between the ipsilateral and contralateral sides to the injury following 24 hrs and 30 days.

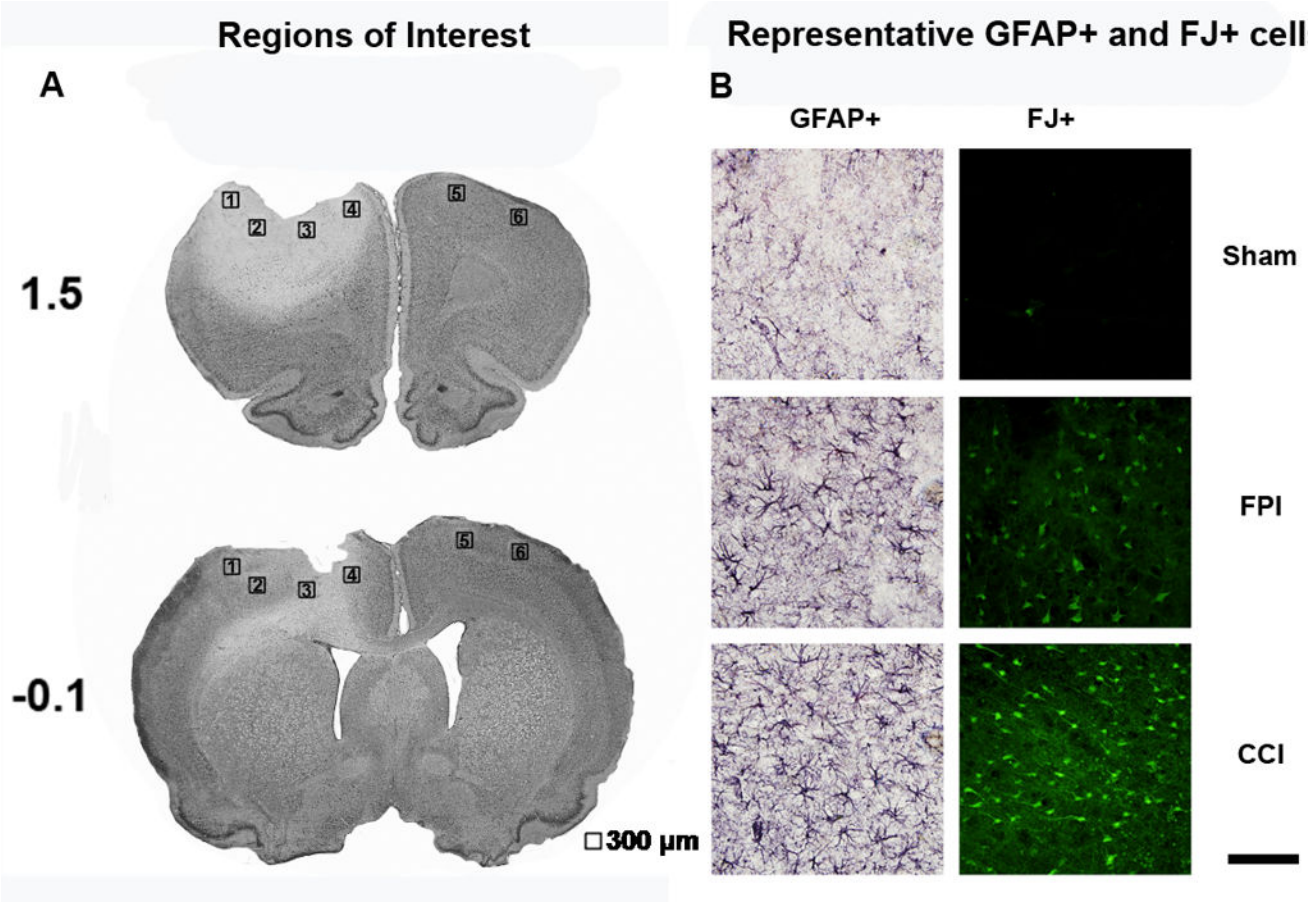

Figure 5.

Regions of Interest. A). Depiction of the six areas used for cell counts (GFAP+ and FJ+ cells), analyzed across two sections spaced equally throughout the injury. B). Photomicrograph representing FJ+ and GFAP+ stained cell bodies.

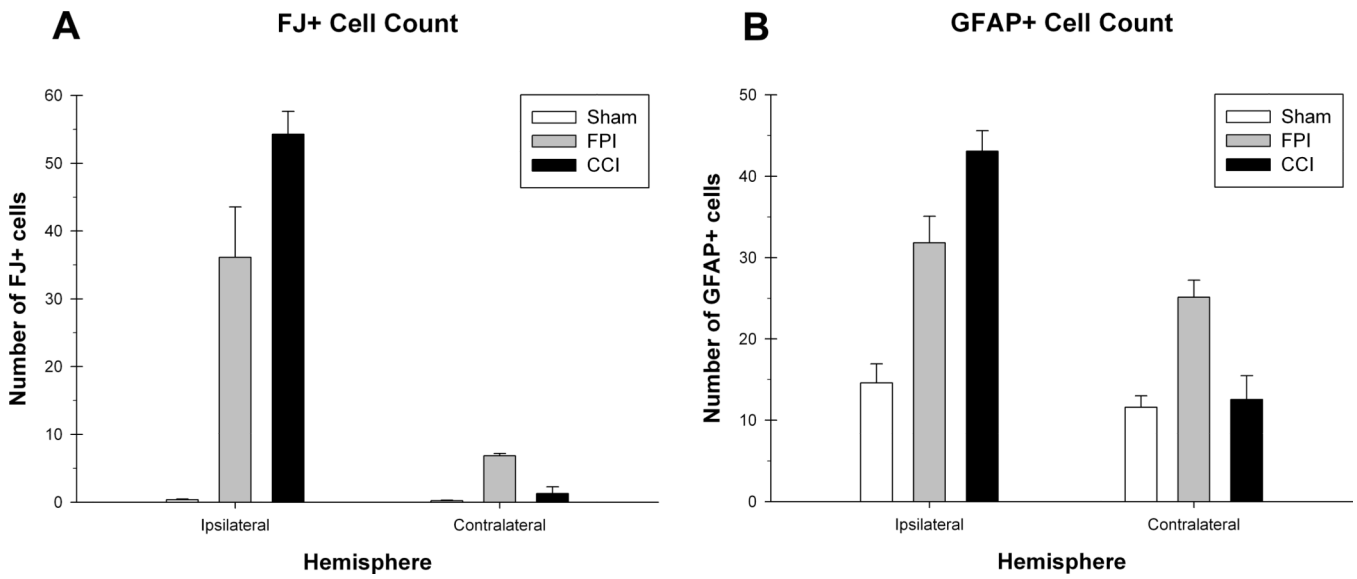

Figure 6.

Cell Counts. A). FJ Cell Counts. Quantification of FJ+ degenerating neurons in the cortices both ipsilateral and contralateral to the injury. B). GFAP Cell Counts. Quantification of GFAP+ reactive astrocytes in the cortices both ipsilateral and contralateral to the injury.