Chronic and progressive deficits after a single closed head injury in mice

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Abstract

Background: Acute injury following brain trauma may evolve into a chronic and progressive disorder. Assessment of chronic consequences of TBI must distinguish between effects of age and injury.

Methods: C57BL/6 mice receive single closed head injury (CHI) and are analyzed at 14DPI or 180DPI for cortical atrophy and 7DPI or 180DPI for behavioral outcomes.

Results: CHI induces ipsilesional atrophy at 14DPI that increases 180 DPI due to an effect of age. On open field, injured mice develop a turn bias at 180DPI not present at 7DPI. On rotarod, injured mice have shorter latencies at 7DPI, but not at 180DPI due to worsening performance of aging uninjured mice. On beam walk, both groups at 180DPI more slowly traverse a 2cm and 1cm beam than at 7DPI. Foot-faults show no significant effects of age or injury. Limb position was assessed using Deeplabcut™ markerless tracking followed by computation of absition (integral of limb displacement over time) using custom Python scripts. On the 2cm beam, age increased absition in all limbs of uninjured mice and both forelimbs of injured mice. Injury increased left hindlimb absition at 7DPI. On the 1cm beam both forelimbs and the left hindlimb of injured mice at 180DPI have larger absition than uninjured mice at 180DPI or injured mice at 7DPI. These data suggest chronic and progressive motor deficits of injured mice at 180DPI.

Conclusions: A single impact produces ipsilesional cortical atrophy and chronic and progressive motor deficits. Quantitative behavioral analysis reveals deficits not seen using standard outcomes.

Introduction

Moderate to severe clinical TBI produces chronic and progressive cognitive and motor deficits in one-third of patients with moderate to severe TBI^[1-3]. Motor deficits are more easily detected after clinical TBI in tasks with higher motor demand ^[2, 4]. In addition, patients with motor deficits may also adopt compensatory suboptimal strategies to perform complex tasks^[4, 5]. In contrast, chronic and progressive motor deficits are less commonly seen in animal models of TBI as cognitive and motor deficits either resolve or remain unchanged over time^[6, 7]. In a rat controlled cortical impact model, severe damage to the somatosensory cortex leads to long-lasting deficits in fine, but not gross, motor tasks^[8, 9]. One challenge in studying chronic deficits is discriminating between impairments due to increased age rather than injury^[10]. A second challenge is detecting compensatory behaviors that conceal underlying deficits^[4, 9].

This study uses a mouse closed head (CHI) model of TBI in which the freely moving head of the mouse is impacted with an electromagnetically controlled piston that produces a contusion involving the motor, somatosensory, association, and visual cortices as well as producing diffuse gray and white matter injury^[11]. This model produces transient deficits in gross motor function, but it is unknown if more fine motor deficits become chronic. We therefore examine the possibility of persistence of chronic deficits in Neurological Severity Score (NSS), open field, rotarod and beam walk.

NSS qualitatively evaluates motor and neurobehavioral outcomes on ten tasks in rodent models of TBI^[12]. Open field assesses general locomotor activity, willingness to explore, basal anxiety and turning bias^[13]. The amount of time a mouse stays on an accelerating rotarod measures deficits in gait coordination, balance, and stamina^[14].

Beam walk measures motor coordination and balance^[14]. While NSS qualitatively assesses complete beam traversal during beam walk, additional quantitative beam walk assessments include time to traverse and foot-fault number^[15, 16]. None of these outcomes, however, address the presence or use of suboptimal, compensating strategies to cross the beam^[12, 17, 18]. Therefore, this study employs DeepLabCutTM, a program that utilizes deep learning to track the position of multiple sites on a mouse without physical tags^[19]. Custom python scripts are created to assess total foot-fault absition; the integral of the limb trajectory below the plane of beam. This study tests whether absition detects compensatory, suboptimal strategies on beam walk.

Methods

Closed Head Injury (CHI) model of TBI

Male C57/BL6 mice (16 to 18 weeks, 26-28gr, Jackson Laboratories, Bar Harbor, ME) are randomly assigned to two groups and sham-CHI or CHI administered as previously published^[11]. Baseline weights are obtained before sham-CHI or CHI. Anesthesia is induced for 2 min with isoflurane (3.5% in O₂ (1.0 L/min)) administered via a nose cone that was maintained (3% in O₂ (1.0 L/min)) until after the impact. The top of the head of the mouse is shaved and placed in a Kopf stereotaxic apparatus modified by placing a single 12.7mm sheet of polyurethane foam on the bed of the adaptor and two 12.7mm sheets of polyurethane foam wrapped around the ear bar holders. CHI is produced using a 5.0mm diameter metal impactor tip controlled by an electromagnetic impactor (Leica Microsystems, Buffalo Grove, IL). The impactor tip is placed 3mm lateral from the midline and 5mm caudal from the eyes. The impactor produces single 6.3 m/sec impact to a depth

of 3mm with a 1 sec dwell time. The impact produces a large movement of the head as described previously in Grin'kina^[11]. If breathing does not begin spontaneously within 30 sec after impact, cardiopulmonary resuscitation is initiated with chest compressions at a rate of 150–160 per min, while the mouse is ventilated with 100% O₂. Sham-injured mice receive identical treatment without the impact. CHI mice are included in both behavioral and cortical area assessments if the contusion site is centered 1.5mm lateral from the midline and -2mm posterior from Bregma. Eight CHI mice (7DPI, N=2; 180DPI, N=6) are excluded due to injury site location. Two sham-CHI mice are excluded because their weight at 180DPI was greater than 2 standard deviations above the mean weight at that age (7DPI, 30.9g ± 2.2g; 180DPI, 35.6g ± 2.1g). This study design is determined, in part, by the necessity to close authors' laboratory at the beginning of the COVID-19 epidemic. After mice received sham-CHI or CHI, the authors did not have access to these animals for 4 months post-injury until their laboratory reopened. A second cohort of mice are assessed at 7 days post-injury.

All animal experiments are carried out in accordance with the NIH guide for the care and use of Laboratory animals and approved by the Institutional Animal Care and Use Committee of the SUNY-Downstate Medical Center (protocol #18-10558).

Cortical area

Parasagittal sections are prepared as described by Sangobowale, et al.^[20] Average area between the cortical surface and the corpus callosum is measured from three 5µm thick slices 1.44-1.56mm lateral from midline, spaced 26.6µm apart within an 80µm thick section^[21, 22]. The area beginning at 0mm extending to -3mm posterior from Bregma is

traced and determined using QuPath software^[23]. Mice receiving sham-CHI or CHI are assessed for cortical area at 14 or 180DPI (Sham-CHI 14DPI, N=4; CHI 14DPI, N=4; Sham-CHI 180DPI, N=6; CHI 180DPI, N=4).

Behavioral studies

All behavioral studies are done with an average illumination of 268 lux and temperature of 24°C. Mice receiving sham-CHI or CHI begin behavioral testing at 7 or 180DPI (Sham-CHI 7DPI, N=10; CHI 7DPI, N=6; sham-CHI 180DPI, N=6; CHI 180DPI, N=4).

Neurological Severity Score

Neurological Severity Score (NSS) is performed using the method of Flierl, et al.^[12] Successful task completion is assigned one-point, unsuccessful task completion is scored zero point.

Open field

Mice are placed in a 30cm diameter open field arena for 2 minutes containing a centrally located, computer-generated 10.25cm diameter circle. AnyMaze™ (Stoelting, Wood Dale, IL) software analyzes total distance travelled, time spent in the arena central circle and absolute turn angle. Absolute turn angle sums changes in center angle as mice move around the arena. Clockwise movements are assigned positive values in degrees, anti-clockwise movements are assigned negative values in degrees.

Rotarod

Rotarod (Harvard Apparatus, Holliston, MA) testing consists of 5-minutes of training/habituation followed by 4 trials with the rod accelerating from 4–40 rpm over 10 minutes with a 15-minute intertrial interval. Total latency and trial-to-trial latency are assessed.

Beam walk

Beams are 35cm long, 3cm, 2cm, or 1cm wide and elevated 10cm. Three different beam widths are used to modulate task difficulty and increase sensitivity in detecting motor deficits. Mice are video recorded traversing the beam from each direction using an iPhone11Pro placed 18 inches lateral from the beam midpoint. Videos are recorded at 60 frames per second with 4K resolution. Video recording begins when the mouse is placed on one beam end and ends after the mouse traverses the beam with all four feet planted on the opposite end. Mice traverse each beam twice, once in each direction. Time to traverse is measured as the average video time of two trials per beam width. A foot-fault is recorded if the innermost fore or hindlimb digit cannot grip the top of the beam edge as described in Fox et al^[18]. Each forelimb and hindlimb are assayed separately.

DeepLabCut™ analysis of beam walk

Beam walk videos are further analyzed using DeepLabCut™ v.2.1.9^[19] for *post-hoc* pose tracking and estimation. Briefly, 8 anatomical points, including both fore- and hindlimbs, ears, nose, and base of tail, are marked manually in 146 frames from 4 different videos. Dataset creation and labeling are created with DeepLabCut™ installed under an Anaconda 3 environment. DeepLabCut™ Google Colaboratory is used for network

creation, training, evaluation, and analysis of novel videos. Initial network is trained for 330,400 iterations reaching a train error of 1.54 pixels and test error of 12.09 pixels. Outlying labels are extracted, manually corrected, and merged into a new dataset and trained for 263,000 iterations with a train error of 11.37 pixels and test error of 6.25 pixels. One hundred forty-four new videos of beam walking are analyzed, and the horizontal (X) and vertical (Y) coordinates for each body part for each frame recorded. Custom Python scripts are written for extracted limb position data analysis. DeepLabCut™ labeled frames are utilized to extract the vertical position of the top of the beam. The vertical position for the top of the beam is defined as the average vertical position of a fore or hindlimb resting on the beam surface. Total foot-fault absition for each limb is defined as the integral of vertical limb displacement (pixels*seconds) below the beam surface over time during all foot-faults. The python analysis code used to determine absition is publicly available at <https://github.com/suny-downstate-medical-center/CHIanalysis>.

Statistics

Mann-Whitney U test analyzes Neurological Severity Score. Kruskal-Wallis analyzes foot-faults with Dunn's post-hoc test. Repeated measures ANOVA analyzes treatment and time effects on rotarod. Two-way ANOVA analyzes weight at testing, cortical area, open field, beam walk, and absition. Statistically significant differences on two-way ANOVA are further analyzed by one-way ANOVA followed by Tukey's post hoc test for pairwise comparisons. Pairwise comparisons between the Sham-CHI 7DPI and CHI 180DPI groups are not assessed since both age and injury status differ. In all tests, statistical significance is set at 0.05.

Results

Mouse weight significantly increases between 7DPI and 180DPI ($F_{1,22} = 26.7$, $p < 0.0001$). Mice at 180DPI ($35.6\text{g} \pm 2.1\text{g}$) are heavier than at 7DPI ($30.9\text{g} \pm 2.2\text{g}$) in both the sham-CHI and CHI groups ($F_{3,22} = 10.8$, $p = 0.0001$, sham-CHI 7 vs 180DPI, $p = 0.0005$; CHI 7 vs 180DPI, $p = 0.05$). Mouse weight is not significantly affected by injury ($F_{1,22} = 1.0$, $p = 0.3$) nor interaction between injury and age ($F_{1,22} = 0.6$, $p = 0.4$).

Cortical atrophy is a common feature of TBI and rodent TBI models^[24]. Cortical area is reduced significantly in the ipsilesional hemisphere as mice age ($F_{1,14} = 48.882$, $p = 0.0005$). A significant ipsilesional cortical atrophy is seen after CHI ($F_{1,14} = 48.882$, $p = 0.00001$) that does not change significantly beyond an effect of age (Age*Injury, $F_{1,14} = 2.38$, $p = 0.15$). The lesion area encompasses portions of the sensory, motor, association, and visual cortices^[25]. These data suggest a chronic and ongoing ipsilesional cortical degeneration initially due to injury later due to age.

NSS does not differ in CHI and sham-CHI mice at 7DPI or at 180DPI suggesting neither an effect of injury ($U = 78.0$, $p = 0.4$) nor of age ($U = 66.5$, $p = 0.9$). These data suggest NSS score between 7 and 180DPI is unaffected by injury or age.

On open field testing, sham-CHI and CHI groups travel equal distances suggesting similar motivation to ambulate and explore (Table 1). Time spent in the center circle shows no effect of injury ($F_{1,22} = 0.56$, $p = 0.5$) but does show a significant effect of age ($F_{1,22} = 8.53$, $p = 0.01$) and significant interaction of injury and age ($F_{1,22} = 5.639$, $p = 0.03$) (Figure 2, Panel A). Injured mice at 180DPI spend significantly more time in the center circle than at 7DPI ($F_{3,22} = 3.92$, $p = 0.02$, CHI180 vs CHI7 $p = 0.013$). These data suggest

lowered basal anxiety in injured mice at 180DPI. Absolute turn angle assessed over the entire arena shows no significant effect of injury ($F_{1,22}=3.3$, $p=0.1$), age ($F_{1,22}=0.7$, $p=0.4$), or interaction between injury and age ($F_{1,22}=2.2$, $p=0.2$). Within the center circle, however, absolute turn angle significantly increases due to injury ($F_{1,22}=5.98$, $p=0.02$), age ($F_{1,22}=8.009$, $p=0.010$), and a significant interaction between injury and age ($F_{1,22}=6.01$, $p=0.02$) (Figure 2, Panel B). CHI mice at 180 DPI turn more towards the ipsilesional hemisphere than injured mice at 7DPI or sham-injured mice at 180DPI ($F_{3,22}=5.2$, $p=0.007$; CHI180 vs CHI7, $p=0.013$; CHI180 vs Sham-CHI180, $p=0.02$). These data suggest that a turning bias towards the ipsilesional hemisphere developing in injured mice after 7DPI.

Total latency on the rotarod is significantly reduced in injured mice at 7DPI ($F_{1,14}=11.57$, $p=0.004$) (Figure 3). This injury effect at 7DPI is no longer present at 180DPI ($F_{1,7}=2.56$, $p=0.2$) due to a significant effect of age in Sham-CHI mice but not in injured mice (Sham-CHI, 7DPI vs. 180DPI, $F_{1,13}=5.96$, $p=0.03$; CHI, 7DPI vs. 180DPI, $F_{1,8}=0.49$, $p=0.51$). These data suggest that CHI acutely produces rotarod deficits while age decreases latency in the sham-injured mice. Trial-to-trial latency differs significantly in Sham-CHI ($F_{3,11}=5.71$, $p=0.01$) but not CHI mice ($F_{3,6}=1.1$, $p=0.4$) (Figure 3). Trial-to-trial latency does not differ between 7DPI and 180DPI among any group suggesting no age effect. These data suggest that sham-CHI mice improve their performance on rotarod, but CHI mice do not. These data also suggest that latency of sham-CHI mice on rotarod worsens as the mice age.

On beam walk, time to traverse the 3cm beam shows no significant effect of injury ($F_{1,22}=2.7$, $p=0.1$), age ($F_{1,22}=0.001$, $p=1.0$), and no interaction between injury and age

($F_{1,22}=1.8$, $p=0.2$) (Table 1). Time to traverse the 2cm beam shows significant effects of injury ($F_{1,22}=4.4$, $p=0.05$) and age ($F_{1,22}=12.7$, $p=0.002$) but no interaction between injury and age ($F_{1,22}=0.7$, $p=0.4$). Time to traverse the 1cm beam also shows significant effects of injury ($F_{1,22}=6.1$, $p=0.02$) and age ($F_{1,22}=8.5$, $p=0.01$) with no interaction of injury and age ($F_{1,22}=2.1$, $p=0.2$). The injury effect on the 2cm and 1cm beams is driven by CHI mice at 180DPI spending significantly more time traversing than Sham-CHI mice at 7DPI (2cm, $F_{3,22}=7.1$, $p=0.002$, CHI180 vs Sham-CHI7, $p=0.004$; 1cm, $F_{3,22}=4.8$, $p=0.01$, CHI180 vs Sham-CHI7, $p=0.005$). Thus, neither injury nor time effect time to traverse beams of different widths. Sham-CHI mice have an age effect on the 2cm beam (Sham-CHI180 vs Sham-CHI7, $p=0.01$) while CHI mice display an age effect on the 1cm beam (CHI180 vs Sham-CHI7, $p=0.05$). These data suggest disparate effects of age depending upon the width of the beam.

Foot-faults on the 3cm, 2cm, or 1cm beams show no effects of injury or age (Table 1). A simple foot-fault count, however, does not assess differences in time or distance the limb is off the beam. Therefore, total foot-fault absition of each limb is also assayed. On the 3cm beam, forelimb absition shows no effect of injury (Left, $F_{1,22}=1.7$, $p=0.2$; Right, $F_{1,22}=1.1$, $p=0.3$) (Figure 4, Panel A). The left forelimb has a significant age effect (Left, $F_{1,22}=5.0$, $p=0.04$; Right, $F_{1,22}=0.4$, $p=0.5$) that is due to insignificant group differences ($F_{3,22}=3.1$, $p=0.05$, Sham-CHI180 vs Sham-CHI7, $p=0.1$, CHI180 vs Sham-CHI7, $p=0.1$). Forelimb absition shows no interaction of injury and age (Left, $F_{1,22}=0.7$, $p=0.4$; Right, $F_{1,22}=1.9$, $p=0.2$). Hhindlimb absition shows no effect of injury (Left, $F_{1,22}=1.8$, $p=0.2$; Right, $F_{1,22}=2.3$, $p=0.1$), age (Left, $F_{1,22}=2.9$, $p=0.1$; Right, $F_{1,22}=0.2$, $p=0.7$) or an interaction of injury and age (3cm, Left, $F_{1,22}=0.6$, $p=0.5$, Right, $F_{1,22}=2.5$, $p=0.1$) (Figure

4, Panel A). These data suggest neither forelimb nor hindlimb absition are effected by injury or age on the 3cm beam.

On the 2cm beam, forelimb absition shows no effect of injury (Left, $F_{1,22}=0.3$, $p=0.2$; right, $F_{1,22}=0.1$, $p=0.7$), a significant effect of age (Left, $F_{1,22}=32.8$, $p=0.00001$, right, $F_{1,22}=20.42$, $p=0.0002$), and no significant interaction between injury and age (Left, $F_{1,22}=0.5$, $p=0.5$, right, $F_{1,22}=0.05$, $p=0.8$) (Figure 4, Panel B). Age effects on the 2cm beam, however, are driven by both Sham-CHI and CHI mice between 7DPI and 180DPI (Left, $F_{3,22}=12.6$, $p<0.0001$, Sham-CHI180 vs Sham-CHI7, $p<0.0001$, CHI180 vs CHI7, $p=0.02$, Right, $F_{3,22}=7.3$, $p=0.001$, Sham-CHI180 vs Sham-CHI7, $p=0.005$, CHI180 vs CHI7, $p=0.05$). These data suggest that age affects absition of both forelimbs on the 2cm beam. Hindlimb absition shows a significant injury effect in the left hindlimb (Left, $F_{1,22}=9.8$, $p=0.005$; Right, $F_{1,22}=0.9$, $p=0.3$), significant age effects of both hindlimbs (Left, $F_{1,22}=11.6$, $p=0.003$, Right, $F_{1,22}=12.7$, $p=0.002$) and no significant interaction between injury and age (Left, $F_{1,22}=0.01$, $p=0.9$, Right, $F_{1,22}=0.1$, $p=0.7$) (Figure 4, Panel B). Increased hindlimb absition is driven by injury in CHI mice at 7DPI (Left, $F_{3,22}=7.8$, $p=0.001$, CHI7 vs Sham-CHI7, $p=0.07$) and age in Sham-CHI mice (Left, Sham-CHI180 vs Sham-CHI7, $p=0.05$; Right, $F_{3,22}=5.1$, $p=0.008$, Sham-CHI180 vs Sham-CHI7, $p=0.005$). These data suggest injury effects absition of the left hindlimb at 7DPI and age effects absition of both hindlimbs in uninjured mice.

On the 1cm beam, forelimb absition shows significant effects of injury (Left, $F_{1,22}=6.2$, $p=0.02$; Right, $F_{1,22}=5.1$, $p=0.03$), and age (Left, $F_{1,22}=7.1$, $p=0.01$, Right, $F_{1,22}=11.8$, $p=0.002$) (Figure 4, Panel C) with the left forelimb having a significant interaction of injury and age (Left, $F_{1,22}=4.6$, $p=0.04$, Right, $F_{1,22}=3.2$, $p=0.09$). CHI mice at 180DPI have

greater forelimb absition than CHI mice at 7DPI or Sham-CHI mice at 180DPI (Left, $F_{3,22}=4.8$, $p=0.01$, CHI180 vs CHI7, $p=0.03$, CHI180 vs Sham-CHI180, $p=0.03$, Right, $F_{3,22}=5.6$, $p=0.005$, CHI180 vs CHI7, $p=0.01$, CHI180 vs Sham-CHI180, $p=0.07$). These data suggest that CHI produces a chronic and progressive increase in forelimb absition. Hindlimb absition shows significant effects of injury (Left, $F_{1,22}=10.6$, $p=0.004$; Right, $F_{1,22}=5.1$, $p=0.04$), age (Left, $F_{1,22}=20.2$, $p<0.0001$, Right, $F_{1,22}=5.9$, $p=0.02$), with a significant interaction between injury and age of the left hindlimb (Left, $F_{1,22}=4.5$, $p=0.05$, Right, $F_{1,22}=2.1$, $p=0.2$) (Figure 4, Panel C). CHI mice at 180DPI have significantly greater left hindlimb absition than CHI mice at 7DPI or Sham-CHI mice at 180DPI (Left, $F_{3,22}=10.1$, $p<0.0001$, CHI180 vs CHI7, $p=0.002$, CHI180 vs Sham-CHI180, $p=0.01$, Right, $F_{3,22}=3.7$, $p=0.03$, CHI180 vs CHI7, $p=0.09$, CHI180 vs Sham-CHI180, $p=0.1$). These data suggest that injury produces a chronic and progressive increase in absition deficits in both forelimbs and the left hindlimb on the 1cm beam.

Discussion

Comparison of histological and motor outcomes at 7, 14 and 180 DPI reveal both age and injury effects in the CHI mouse model of TBI. Ipsilateral cortical area shows an effect of injury that does not change beyond the normal effects of age (Figure 1, Panel B). In contrast, latency on rotarod shows a significant age effect and acute injury effect only at 7DPI (Figure 3). Open field and beam walk outcomes are affected by significant interactions between injury and age suggesting that injury produces chronic and progressive deficits (Figures 2 and 4). Thus, a study of chronic deficits in preclinical TBI models must assess how much of a long-term deficit is due to aging of the mice.

Injured mice at 180DPI showed a significant increase in time spent in the open field arena center suggesting lowered basal anxiety (Figure 2, Panel A). A similar reduction in basal anxiety was reported in adult mice 7 weeks following a single controlled cortical impact^[26]. In the center of the arena, injured mice showed a strong turning bias toward the injured cortex (Figure 2, Panel B). Turning bias is thought to be a consequence of unilateral brain damage^[27]. A similar turning developed bias is reported in rats 2 to 6 weeks after a single controlled cortical impact^[8]. Additional examples of turning bias have been reported in models of cerebral ischemia^[27-30].

Rotarod latency shows large age effects at 180DPI that are larger than the acute effects of injury (Figure 3). Mice are significantly heavier at 180DPI than at 7DPI. Heavier mice perform worse on rotarod suggesting that the increased weight of mice at 180DPI underlies the large age effect on rotarod latency^[13]. Thus, a large age effect lowers the utility of using rotarod to examine chronic motor effects.

Age effects seen on time to traverse the 2cm and 1cm, but not the 3cm beams suggest that chronic injury effects may be more readily detected in tasks with higher motor demand (Table 1). Assessing foot-fault number does not reveal significant pairwise effects (Table 1), yet absition analysis of the same mice reveals both age and injury effects (Figure 4). An increase in absition without changing foot-fault number suggests the use of alternative compensatory motor strategies. The assay of forelimb and hindlimb absition using DeepLabCutTM tracking reveals chronic and progressive motor deficits not seen by assessing time to traverse the beam or number of foot-faults (Figure 4).

CHI produces atrophy of the ipsilesional hindlimb/torso motor and sensory, retrosplenial, and association cortices^[25] (Figure 1). Increases in absition in the absence

of increases in foot-faults may result from a decreased ability for the mouse to recover from a foot-fault. Increasing absition during recovery from foot-faults may suggest impairments arising from the injured sensory and motor cortices during tasks of high motor demand^[31, 32].

Two caveats of this study are that separate cohorts of mice are assessed at 7 and 180DPI and that the 180 DPI study may be underpowered. Studies are ongoing assaying a larger cohort at acute and chronic timepoints. Multiple structures of the mouse were marked using DeepLabCut™ yet only forelimb and hindlimb absition are assessed. We are further testing the ability of DeepLabCut™ to assess additional body parts of the mouse in the hope to provide an even more detailed assessment of mouse motor function. Ongoing studies are incorporating mirrors placed above the mouse during motor tasks to allow simultaneous video recording of mouse movement from additional angles. These additions have the potential to increase the number of motor parameter assessments and further improve our analysis of mouse motor deficits in the acute and chronic phases of experimental TBI.

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Author Contributions S.L., E.N. and P.J.B. designed the experiments in this study. S.L., D.H. and E.N. performed these studies. S.L., D.H., C.K. and P.J.B. analyzed the results. S.L., E.N., C.K., D.H. and P.J.B. wrote the manuscript.

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Behavioral test	Parameter	Sham-CHI7		CHI7		Sham-CHI180		CHI180	
Open Field	Distance traveled (meters)	3.5 ± 0.5		4.6 ± 0.6		2.7 ± 0.3		3.8 ± 0.5	
	Absolute Turn Angle in the arena (degrees)	9047.2 ± 761.3		9381.7 ± 886.9		6722.2 ± 798.2		10037.3 ± 1713.8	
3cm Beam Walk	Time to traverse beam (seconds)	7.9 ± 0.8		16.3 ± 5.2		11.6 ± 1.4		12.5 ± 2.9	
	Forelimb foot-faults	Left	Right	Left	Right	Left	Right	Left	Right
		0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Hindlimb foot-faults	0.5 ± 0.3	0.5 ± 0.3	0.3 ± 0.2	1.0 ± 0.7	0.3 ± 0.3	0.3 ± 0.3	1.0 ± 0.7	0.3 ± 0.3
2cm beam walk	Time to traverse beam (seconds)	7.8 ± 0.5		11.0 ± 1.4		12.6 ± 1.1*		14.0 ± 1.6	
	Forelimb foot-faults	Left	Right	Left	Right	Left	Right	Left	Right
		0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.5 ± 0.5	0.3 ± 0.3
	Hindlimb foot-faults	0.1 ± 0.1	0.6 ± 0.3	1.2 ± 0.4	1.0 ± 0.45	0.3 ± 0.2	0.5 ± 0.2	1.8 ± 0.9	1.0 ± 0.4
1cm Beam walk	Time to traverse beam (seconds)	9.0 ± 0.7		12.1 ± 1.6		13.4 ± 2.4		25.1 ± 8.4*	
	Forelimb foot-faults	Left	Right	Left	Right	Left	Right	Left	Right
		0.3 ± 0.2	0.1 ± 0.1	0.8 ± 0.5	0.3 ± 0.3	0.3 ± 0.2	1.0 ± 0.3	1.0 ± 0.8	1.0 ± 0.5
	Hindlimb foot-faults	0.6 ± 0.3	0.9 ± 0.3	2.3 ± 0.6	2.5 ± 0.5	3.2 ± 0.7	1.5 ± 0.7	6.0 ± 0.7	3.3 ± 1.1

Table 1 Behavioral parameters Distance traveled shows no significant difference due to injury ($F_{1,22}=4.0$, $p=0.06$), age ($F_{1,22}=2.4$, $p=0.14$), or interaction between injury and age ($F_{1,22}=0.001$, $p=0.9$). Absolute turn angle assessed in the entire arena shows no significant effect of injury ($F_{1,22}=3.3$, $p=0.1$), age ($F_{1,22}=0.7$, $p=0.4$), or interaction between injury and age ($F_{1,22}=2.2$, $p=0.2$). Forelimb foot-faults do not significantly differ on 3cm or 2cm, but was significant on the 1cm beam (3cm Left, $H(3)=2.7$, $p=0.5$, Right, $H(3)=3.3$, $p=0.3$; 2cm Left, $H(3)=2.3$, $p=0.5$, Right, $H(3)=5.5$, $p=0.1$; 1cm Left, $H(3)=1.5$, $p=0.7$,

Right, $H(3)=9.4$, $p=0.02$). Post-hoc tests reveal no significant difference between groups. Hindlimb foot-faults did not significantly differ on the 3cm beam, but did differ significantly on the 2cm and 1cm beams (3cm Left, $H(3)=1.6$, $p=0.7$, Right, $H(3)=6.5$, $p=0.09$; 2cm Left, $H(3)=8.9$, $p=0.03$, Right, $H(3)=1.2$, $p=0.7$; 1cm Left, $H(3)=17.1$, $p=0.001$, Right, $H(3)=8.3$, $p=0.04$). Post hoc tests reveal only a significant difference between Sham-CHI7 and CHI180 mice. Thus, forelimb or hindlimb foot-faults have no significant effects of side, age, or treatment.

A) Cortical Loss in Injured Mice

B) Summary of Cortical Loss

A) Time in the center

B) Absolute turn angle in the center

A) 3cm

B) 2cm

C) 1cm

Figure 1, Cortical Area, Panel A, Cortical loss. Representative parasagittal sections of ipsilesional mouse cortex at 14 or 180 days after sham-CHI or CHI. **Panel B,** Summary of Cortical Loss. Cortical area is significantly lower in injured mice at 14 and 180DPI compared to sham-CHI mice (14DPI, ** $p=0.01$; 180DPI, *** $p=0.0001$). Cortical area is significantly decreased between 14 and 180DPI (CHI, $p=0.005$, Sham-CHI, $p=0.2$). Scale bar, 0.4mm.

Figure 2 Chronic deficits in open field testing. Panel A, Time in the arena center. CHI mice spend significantly more time in the arena center at 180DPI than at 7DPI (** $p=0.01$). **Panel B,** Absolute turn angle in the center. CHI mice develop a significant turning bias towards the ipsilesional hemisphere at 180DPI compared to both CHI mice at 7DPI (** $p=0.01$) and Sham-CHI mice at 180DPI (* $p=0.02$).

Figure 3. Rotarod Latency, Injury significantly affects total rotarod latency at 7DPI (sham-CHI vs CHI, $F_{1,14}=11.57$, ** $p=0.004$) but not 180DPI (sham-CHI vs CHI, $F_{1,7}=2.56$, $p=0.2$). Total rotarod latency of sham-CHI but not CHI mice show significant worsening between 7DPI and 180DPI (Sham-CHI, $F_{1,13}=5.96$, * $p=0.03$; CHI, $F_{1,8}=0.49$, $p=0.51$). The trial-to-trial latency differs significantly at 7 and 180DPI for sham-CHI mice, but not CHI mice (sham-CHI, $F_{3,11}=5.71$, ** $p=0.01$; CHI, $F_{3,6}=1.1$, $p=0.4$).

Figure 4 Beam Walk Forelimb and Hindlimb Absition. Panel A, 3cm beam, No significant differences are seen on the 3cm beam. **Panel B, 2cm beam.** Forelimb absition

of sham-CHI and CHI mice increases significantly between 7 and 180DPI (Sham-CHI, Left, **** $p < 0.0001$; Right, *** $p = 0.005$; CHI, Left, ** $p = 0.02$, Right, * $p = 0.05$). Hindlimb absition of sham-CHI mice increases significantly between 7 and 180 DPI (Left, # $p = 0.05$; Right, ## $p = 0.005$). **Panel C, 1cm beam**, Forelimb absition of CHI mice increases significantly between 7 and 180 DPI (Left, + $p = 0.03$, Right, ++ $p = 0.01$). Left forelimb absition differs significantly between sham-CHI and CHI mice at 180DPI (+ $p = 0.03$). Left hindlimb of CHI mice differs significantly between 7 and 180 DPI ($\forall p = 0.002$). The left hindlimb of sham-CHI and CHI mice differs significantly at 180DPI ($\forall\forall p = 0.01$).