Research Report

Controlled-cortical impact reduces volitional forelimb strength in rats

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Abstract

Traumatic brain injury (TBI) is one of the largest health problems in the United States and affects both cognitive and motor function. Although weakness is common in TBI patients, few studies have demonstrated a reduction in strength in models of brain injury. We have developed a behavioral method to measure volitional forelimb strength and quantify forelimb weakness following traumatic brain injury. In this paper, we report the ability of the isometric pull task to measure both acute and chronic impairments in forelimb motor function following a controlled cortical impact (CCI) in rodents. Following CCI, volitional forelimb strength is reduced by 36% and remains significantly reduced after 6 weeks of post-lesion training. We also show that CCI results in impairment of multiple additional measures of forelimb function for several weeks post-injury.

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1. Introduction

Traumatic brain injury is a serious public health problem in the United States. Annually two million people suffer a TBI, and at least 275,000 of these cases lead to prolonged stays in the hospital (Faul et al., 2010). TBI commonly results in cognitive deficiencies such as memory loss and changes in emotion or sensation. However, moderate to severe cases often result in motor dysfunction such as weakness, lack of balance, or a loss of coordination (Greiffenstein et al., 1996; Kuhtz-Buschbeck et al., 2003).

Animal models have been developed to study the effects of TBI. One common model of TBI is the controlled cortical impact (CCI) model (Edward Dixon et al., 1991). The CCI model reproduces many facets of the clinical presentation of TBI, including increased intracranial pressure (Cherian et al., 2000), brain edema (Başkaya et al., 1997), and compression of the brain. In addition, the CCI model allows for control of...
mechanical parameters such as impact velocity and the amount of brain deformation. Behavioral impairments observed in animal models of CCI resemble cognitive and motor characteristics observed in TBI patients. Both mice and rats have impaired spatial memory using the Morris water maze task extending up to 30 days post-injury (Hamm et al., 1992; Fox et al., 1998; Yu et al., 2009). Similarly, long-term impairments in motor skill-learning have been observed in rats (Ding et al., 2001).

Studies have documented motor impairments following CCI using tests such as skilled reaching, beam traversal, grid walk, and rotorod (Hamm et al., 1994; Hamm 2001; Whishaw et al., 2004). While these tests have provided much valuable data, many of them do not report chronic impairments (Edward Dixon et al., 1991; Soblosky et al., 1996), and no studies that we are aware of have reported deficits in volitional strength following CCI. Volitional forelimb strength is the voluntary application of force using the musculature of the upper limb. As weakness is believed to be the major factor contributing to disability after brain damage (Canning et al., 2004), the ability to quantitatively measure volitional forelimb strength in an animal model of TBI could provide a framework to test potential therapies to improve recovery of forelimb strength in TBI patients.

We have developed the isometric pull task, a novel method to quantitatively measure forelimb impairment after an acute brain lesion (Hays et al., 2012). In this study, we used the isometric pull task to measure multiple parameters of forelimb function following a CCI.

2. Results

2.1. Acquisition of the isometric pull task

Animals were trained on the isometric pull task as previously described (Hays et al., 2012). Animals were trained in a standard operant cage that was equipped with an aluminum handle sitting 0.75 in. beyond the inner cage wall. Animals were required to reach through a slot and pull on the handle with 120 g of force to receive a food reward. The rats took an average of 3.4 ± 0.2 days to acquire the task, as indicated by independent engagement of the pull handle. All animals became highly proficient at the task, reaching a pre-lesion performance criterion of five consecutive days averaging at least an 85% success rate in 26 ± 0.4 days. The average hit rate during the pre-lesion stage was 91.8 ± 0.7%. Average maximal pull force per trial was 165.4 ± 4.9 g, well-exceeding the 120 g threshold.

2.2. Performance after controlled cortical impact to primary motor cortex

Once animals reached the 85% pre-lesion criterion, they were given a unilateral CCI lesion to the primary motor cortex. After one week of post-surgical recovery in the home cage, animals returned to post-lesion behavioral testing for six weeks.

CCI worsens performance of the task and alters the average force profile during trials (Fig. 1). To quantify changes in the force profile of lesioned animals, we integrated the force as a function of time for the pre-lesion stage and during the first week of post-lesion assessment. The average force profile contained two peaks, demonstrating that rats often initiated multiple pull attempts per trial. Following CCI, force peaks were dramatically reduced, consistent with a reduction in strength. A significant decrease was seen in the early post-lesion force profile resulting from weaker pull forces compared to pre-lesion. During the first second of the trial the post-lesion FTI was significantly reduced compared to pre-lesion (pre-lesion: 46.4 ± 3.6 g s; post-lesion: 29.1 ± 2.5 g s, p = 0.001). In addition, a significant increase in the force profile was seen later into the trial window for post-lesion animals compared to pre-lesion, indicating later application of force. Post-lesion FTIs calculated for the second and third seconds of the trial window were significantly greater than pre-lesion (p = 0.005 and p = 0.001 respectively). This indicates that during the first week of post-lesion assessment, lesioned animals were both pulling with weaker force at the beginning of the trial window and still attempting late into the trial window.

Volitional forelimb strength was substantially reduced following CCI (Fig. 2A). A repeated measures ANOVA on maximal force revealed a significant effect of the TBI (F(1, 44) = 10.6, p = 0.003), time point (F(1, 44) = 11.1, p < 0.001), and a significant interaction (F(1, 44) = 9.39, p < 0.001). Maximal force exerted by lesioned animals during a trial was significantly reduced when compared to pre-lesion (week 1: 105.6 ± 8.2 g; paired t-test, p < 0.001 vs. pre-lesion). This significant reduction of maximal force in TBI animals was observed during the following weeks, despite extensive training, compared to both pre-lesion (pre-lesion vs. week 2–6: all p < 0.001) and unlesioned controls (weeks 2–6: unpaired t-test, all p < 0.05 except week 5 p = 0.067), suggesting a chronic deficit in strength. Although animals failed to recover to pre-lesion levels, maximal pull force did significantly improve compared to week 1 of post-lesion for some weeks. By the third week maximal pull force was significantly increased compared to the first week of post-lesion training (week 3: 130.6 ± 7.9 g; paired t-test, p = 0.001 compared to week 1), and remained so for the duration of training (week 1 vs. weeks 4–6: all p < 0.05). Further analysis revealed that maximal force in unlesioned controls was significantly lower than pre-lesion at only one time point (week 5: 149.38 ± 5.4 g; paired t-test vs. pre-lesion, p = 0.02).

Hit rate was markedly impaired following CCI (Fig. 2B). A repeated measures ANOVA revealed significant effects of both time (F(1, 44) = 9.5, p < 0.001) and TBI (F(1, 44) = 17.9, p < 0.001), and an interaction effect (F(1, 44) = 7.32, p < 0.001). The percentage of successful trials in lesioned animals was significantly reduced compared to pre-lesion performance (week 1: 48.7 ± 5.7%; paired t-test, p < 0.001 vs. pre-lesion). Hit rate in lesioned animals demonstrates a trend toward improvement over the remainder of training, but significant improvement is only seen during weeks 3–5 (post-lesion week 1 vs. weeks 3–5; paired t-test, all p < 0.05). Hit rate of lesioned animals was significantly lower than unlesioned controls during all post-lesion epochs (post-lesion weeks 1–6; unpaired t-test, all p < 0.05). At the sixth week of training, hit rate was still significantly impaired compared to pre-lesion, indicating a chronic deficit in forelimb function (week 6: 64.1 ± 7.5%; paired
-test, $p = 0.002$ compared to pre-lesion). Unlesioned controls did not show any significant drop in hit rate at any training time point compared to pre-lesion (pre-lesion vs. weeks 1–6, all $p > 0.05$, paired t-test).

We quantified the average time to reach the 120 g threshold in trials resulting in a hit throughout post-lesion training (Fig. 2C). Prior to the lesion, animals typically achieved threshold in successful trials within $370 \pm 30.4$ ms. Repeated measures ANOVA revealed a significant effect of time post-lesion on the time within a trial to reach threshold ($F[84,6] = 8.57$, $p < 0.001$). After CCI, the time to threshold significantly increased in the first week (week 1: $649 \pm 39.4$ ms; paired t-test, $p < 0.001$ compared to pre-lesion). This time to threshold remained significantly elongated throughout post-lesion assessment in lesioned animals (pre-lesion vs. weeks 2–6; paired t-test, all $p < 0.05$), and compared to unlesioned controls a transient

Fig. 1 – CCI worsens performance and causes a significant change in the trial force profile. (A) Representative force plots of trials performed by a rat in the pre-lesion stage. (B) Representative force plots of trials in the first week of training following CCI. Note the reduced force peaks compared to pre-lesion. (C) A picture showing a rat performing the isometric pull task. The rats were trained to reach out a small slot in the booth and pull on an aluminum handle. (D) The data depicts average force generated during a trial. The blue line indicates average force during pre-lesion training, and the red line indicates average force during the first week of post-lesion assessment for lesioned animals. Multiple peaks indicate repeated pull attempts. Note the post-lesion reduction in force early in the trial and the increase in force during the later phases of the trial. Shaded areas around each line denote 95% confidence intervals to clarify significant differences.
increase was also observed (CCI vs. unlesioned animals, unpaired t-test; week 1 \( p=0.001 \); week 2 \( p=0.014 \)). A repeated measures ANOVA revealed that there was no effect of time on the within-trial time to reach threshold in unlesioned controls (\( F[60,6]=0.89, p=0.51 \)). These findings indicate that CCI results in a chronic increase in the amount of time necessary for the animals to successfully pull 120 g.

To investigate chronic deficits in motor function following CCI, we quantified the speed of force generation during individual pull attempts in lesioned animals. The time to reach a given force was measured from a baseline of 10 g up to 120 g, in 10 g increments (Fig. 3). We investigated the effect of CCI at different training time-points using a two-way factorial ANOVA. We found that there was a significant difference in speed of force generation between the CCI and control groups during the pre-lesion assessment phase (\( F[263,1]=11.1, p<0.001 \)), but a post-hoc analysis failed to reveal any significant differences between the two groups at individual force levels (unpaired t-tests, all \( p>0.05 \)). We then investigated the effect of the CCI on speed of force generation in the first week of training following the lesion. A two-way ANOVA revealed a significant effect of the lesion (\( F[274,1]=72.3, p<0.001 \)), consistent with a slowing of force generation. Additionally, post-hoc analysis also revealed that at each individual force level the lesioned animals were significantly slower to reach all levels of force than unlesioned controls (unpaired t-tests, all \( p<0.05 \)). Finally, we investigated the chronic effects of CCI on speed of force generation by analyzing the sixth week of training following the lesion. A two-way ANOVA revealed that also after six weeks of training there was an effect of CCI on the speed of force generation (\( F[263,1]=98.04, p<0.001 \)). Post-hoc analyses indicated that in the sixth week, as was seen in the first week post-lesion, the CCI animals had significantly greater times to reach all levels of force (all \( p<0.05 \)). These results reveal that a CCI results in a decrease of the speed of force generation.

The total number of trials per day was transiently reduced following CCI (Fig. 2D). Prior to lesion, animals performed 350±14 trials/day. Following CCI, lesioned animals performed significantly fewer trials during week 1 (week 1: 225±27 trials/day; paired t-test, \( p<0.001 \) compared to pre-lesion). Repeated measures ANOVA revealed a significant effect of time in the lesioned group (\( F[84,6]=4.76, p<0.001 \)), but not in the unlesioned controls (\( F[60,6]=1.3, p=0.27 \)). By the second week of post-lesion testing, the number of trials per day in lesioned animals was not significantly reduced compared to pre-lesion (week 2: 279±37 trials/day; paired t-test, \( p=0.06 \) compared to pre-lesion), and remained so for the duration of testing. In addition, the number of trials in lesioned animals compared to unlesioned controls was significantly different at only the first week post-lesion (week 1 lesioned vs. unlesioned, unpaired t-test, \( p=0.03 \)), but not at any other time points. This indicates that animals are still engaged in the task following CCI.

Fig. 2 – Controlled-cortical impact impairs multiple measures of forelimb function. (A) Maximal volitional pull force is significantly reduced following CCI. (B) The percentage of successful trials by animals was also significantly reduced following CCI. (C) After trial initiation, the latency to reach the hit criterion of 120 g was increased following CCI. (D) The total number of trials attempted by animals during each day of training was transiently reduced following CCI. * Indicates \( p<0.05 \) compared to unlesioned controls. Error bars indicate mean ± SEM.
2.3. Histology

CCI resulted in a large lesion to the motor cortex (Fig. 4A). Lesion volume was not significantly correlated with the percent reduction in maximal force following CCI ($r = 0.07$, $p = 0.83$). In addition, lesion volume was not significantly correlated with maximal force at the end of post-lesion assessment ($r = -0.56$, $p = 0.06$). The ventricle size in the lesioned hemisphere was reliably found to be larger than that of the intact hemisphere (mean ventricle size ratio = 4.52).

We measured the percent reduction in white matter as the ratio of intact corpus callosum and external capsule in the lesioned hemisphere to the intact hemisphere. Percent reduction in white matter was significantly negatively correlated with maximal force both at the beginning ($r = -0.652$, $p = 0.02$) and the end of post-lesion assessment ($r = -0.651$, $p = 0.02$; Fig. 4B). This indicates that animals with more damage to white matter typically had larger deficits in strength, consistent with findings from patients of brain damage (Benson et al., 2007). Additionally, we measured the percent reduction...
in gray matter as a ratio of the lesioned hemisphere to the unlesioned hemisphere. We found that percent reduction in gray matter was significantly correlated with force used in the first week of post-lesion assessment ($r = 0.6$, $p = 0.03$), but not in the sixth week ($r = 0.47$, $p = 0.12$).

### 3. Discussion

In this study, we show that CCI results in a significant impairment in multiple parameters of forelimb function as measured by the isometric pull task, including maximal pull force, time to reach threshold, hit rate, speed of force generation, and number of pull attempts per trial. The isometric pull task is sensitive enough to measure sustained deficits following CCI that persist despite extensive training.

The isometric force task provides a rich dataset and high statistical power, as an average of 13,750 trials were performed by each subject over the course of the experiment. While the number of trials is significantly reduced for the first week after lesion, subjects still perform more than 200 trials per day. This reduction in the number of trials during the first week of post-lesion assessment could be attributed to less motivation to pull after CCI. Despite this initial reduction in trials, the effect is transient and by the second week of post-lesion assessment there were no significant differences in the number of trials initiated by lesioned and unlesioned animals. The large number of trials on a daily basis allows for an accurate assessment of strength and exceeds the number of repetitions typically used in other forelimb tasks. Along with previous studies in a model of stroke, the results from this study demonstrate the flexibility and sensitivity of the isometric force task in monitoring various models of motor impairment (Hays et al., 2012; Khodaparast et al., 2013). Here we chose to use the CCI model of TBI, but this task could likely be applied to fluid percussion or blast models of TBI with minor or no modifications. In addition, since the isometric force task is automated, it is suitable for screening potential molecular, cellular, or behavioral therapies.

CCI results in persistent forelimb deficits despite intensive training of the impaired limb after lesion. The impairments measured include elements of both a loss of strength and of speed. The chronic loss of strength bears similarity to long-term weakness observed in some patients who suffer a moderate or severe TBI (Jang, 2009). The persistent weakness observed in this study is significant because several rat models of forelimb impairment recover over time with training (Bierenaskie and Corbett, 2001; Krakauer et al., 2012; Whishaw et al., 2008; Alavardashvili et al., 2008). This lasting loss of function measured by the isometric force task is advantageous because it allows testing of therapeutic interventions at both acute and chronic time points following injury. Minimizing training-dependent recovery over time could improve the power to observe recovery that is due to potential interventions.

In our histological analysis, we found a significant relationship between the amount of force used by animals at both the beginning and end of post-lesion behavioral assessment and the percent reduction in white matter. Furthermore, we found a significant correlation between the percent reduction in gray matter and the amount of force used in the first week of post-lesion assessment, but not in the final week of post-lesion assessment. This finding indicates that a loss of white matter may be more detrimental to motor function in the chronic time span. While a loss of gray matter may initially predict behavioral deficits, plasticity in cortical regions may eliminate any significant correlation chronically.

Based on the findings from this study, we report that the CCI model of TBI results in a persistent loss of volitional forelimb strength after lesion. Several parameters related to forelimb function remain impaired for at least six weeks after lesion despite extensive task-specific training. The chronic loss of volitional forelimb strength provides a novel context in which to test potential therapeutic strategies to restore forelimb function after TBI.

### 4. Experimental procedures

#### 4.1. Subjects

Twenty-nine adult female Sprague-Dawley rats purchased from Charles River, weighing between 250 g and 300 g at the beginning of behavioral training, were used in this experiment. The rats were housed in a 12:12 h reversed light cycle and were food deprived to no less than 85% of their normal body weight. All handling, housing, surgical procedures, and behavioral training of the rats were approved by the University of Texas Institutional Animal Care and Use Committee.

#### 4.2. Behavioral apparatus and software

The behavioral apparatus and software were used as previously described (Hays et al., 2012). The apparatus consisted of an acrylic box ($10 \times 12 \times 4.75$ in.$^3$). The box contained a slot in the front right corner which rats could reach through to access the aluminum pull handle. The slot was sized and positioned such that rats could only reach the pull handle using their right forelimb. The pull handle was centered in the slot at a height of 2.5 in. from the cage floor, and the distance of the pull handle to the cage was varied from 0.75 in. inside the cage (relative to the inner cage wall) to 0.75 in. outside the cage. The aluminum pull handle was connected to a force transducer (Vulintus, LLC) which could measure the force with which the rat pulled to an accuracy of a few grams in force. The force transducers were inspected daily and were recalibrated when necessary. Custom-built software was used to control the behavioral apparatus and collect data. A microprocessor controller (Vulintus, LLC) sampled the force transducer at frequencies of either 20 Hz or 100 Hz and sent the information to MATLAB software which displayed the data on the screen, controlled the behavioral session, and saved the data to permanent files.

#### 4.3. Behavioral training

Animals underwent training for two 30-min sessions per day, with at least 2 h between sessions. Behavioral sessions were conducted five days per week. Shaping procedures were similar to those previously described (Hays et al., 2012). When animals
were initially being trained, an experimenter would manually dispense pellets when the rat contacted the pull handle to promote association between the pull handle and reward delivery. After the food reward association was learned, animals were trained to pull on the handle with greater amounts of force, and single pellets were dispensed following successful trials during training (45 mg dustless precision pellet, BioServ, Frenchtown, NJ). If rats did not receive 50 pellets per day, they were given 10 g of additional pellets after daily training sessions were completed.

During the early stages of training the pull handle was placed 0.75 in. inside the cage. As the animal learned the association between pulling the handle and receiving a reward, the pull handle was gradually retracted at increments of 0.25 in. until it was at a maximum distance of 0.75 in. from the inner cage wall.

A trial was initiated when at least 10 g of force were applied to the aluminum pull handle. After initiation the force on the pull handle was sampled for 4 s. At the beginning of training, the force threshold for a successful attempt was 10 g (the same as the force required to initiate a trial), and this force threshold was increased to 35, 65, and 120 g as each animal progressed in training. If the force threshold required for a hit was met within a 2 s window after force initiation, the trial was recorded as a success and a reward pellet was delivered. If the force threshold was not reached within 2 s, the trial was recorded as a failure and no reward was delivered.

Rats were considered fully trained after they maintained a performance of 85% success at the 120 g threshold for five consecutive days. None of the animals in this study failed to meet this criterion. After the animals successfully reached this stage, they were placed into either the lesion group or the unlesioned control group. Rats in the lesioned group received a CCI and were given seven days to recover in their home cage following the injury before behavioral testing resumed. Animals then returned to behavioral testing for six weeks (30 days) with the same parameters as the pre-lesion stage to allow for a direct comparison of performance. Unlesioned controls continued in behavioral training for six weeks after reaching the lesion criteria required for the lesioned animals.

4.4. Controlled-cortical impact

Rats were anesthetized with a cocktail of ketamine hydrochloride (50 mg/kg), xylazine (20 mg/kg), and acepromazine (5 mg/kg) injected intramuscularly, and given supplemental doses as needed. Throughout the surgery, the rat’s body temperature was maintained using a T-Pump Localized Temperature Therapy System (Gaymar) set to 38°C. Ventricle size was maintained using a T-Pump Localized Temperature Therapy System (Gaymar) set to 38°C. A trial was initiated when at least 10 g of force were applied to the aluminum pull handle. After initiation the force on the pull handle was sampled for 4 s. At the beginning of training, the force threshold for a successful attempt was 10 g (the same as the force required to initiate a trial), and this force threshold was increased to 35, 65, and 120 g as each animal progressed in training. If the force threshold required for a hit was met within a 2 s window after force initiation, the trial was recorded as a success and a reward pellet was delivered. If the force threshold was not reached within 2 s, the trial was recorded as a failure and no reward was delivered.

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4.5. Histology

At the completion of the post-lesion assessment period, animals were transcardially perfused with 4% paraformaldehyde, and the brain was removed. Coronal sections 40 μm thick extending through the lesioned area were cut using a cryostat and stained with cresyl violet. Each brain was analyzed with regard to the lesion volume, intact white matter, ventricle size, and the caudal and rostral extents of the lesion. White matter was measured as the area of the corpus callosum and external capsule in each hemisphere of the brain. A ratio of the total area of white matter in the lesioned hemisphere compared to the total area of white matter in the unlesioned hemisphere was calculated for each brain section, and this ratio was averaged across all sections for each brain. Ventricle size was similarly calculated, and the average ventricle size ratio was then calculated for each brain. Histological analysis was completed using ImageJ and was done blind to the performance of the animals on the isometric pull task. Of animals that completed the behavioral testing, histological analysis could not be performed on three animals due to technical reasons, and their brains were excluded.

4.6. Statistics

Fifteen lesioned animals (n=15) and eleven unlesioned controls (n=11) were included in behavioral data analysis. Data from each subject was grouped by combining each 5 consecutive days into epochs. Each day consisted of two sessions of data. Post-lesion performance at each epoch was compared with the last five days of pre-lesion performance, and significant differences were determined using repeated measures ANOVA, paired t-tests, and unpaired t-tests. All data are reported as the mean±SEM. Significant differences are noted in the figures as * p<0.05. Error bars indicate mean±SEM, except in Fig. 1D where shaded areas denote 95% confidence intervals to clarify significant differences. Matlab was used for all analyses.

Disclosures

Robert L. Rennaker II is the owner of Vulintus, LLC. Other authors declare no competing financial interests.
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