SUPPLEMENTARY MATERIALS: Adkins et al., "*Combining Multiple Types Of Motor Rehabilitation Enhances Behavioral Recovery Following Experimental Traumatic Brain Injury In Rats*"

Supplementary Methods

Animals: Prior to behavioral manipulation, rats were pair-housed, tamed by daily handling, received housing enrichment (a PVC pipe and a cardboard roll) and were given enrichment food (nuts, seeds, grains and fruits) in addition to standard rat chow. A total of 11 animals died either at the time of surgery (n=4) or before completion of the study (n= 7). After completion of the study, three animals were excluded from analysis because they failed to reach a minimum pre-operative reaching criterion of 30% and another three animals were excluded from analysis because they failed to have impairments defined as a minimum of a 15% drop in reaching success post-CCI. Table 1 shows the number of animals in each group used for final analysis.

Controlled Cortical Impact: Rats were anesthetized with equithesin (149mg/100g chloral hydrate, 31mg/100g sodium pentobarbital i.p.), a 4mm craniotomy was created, centered over FL-SMC (0.5mm anterior and 4mm lateral to bregma), then a CCI was induced with a 3mm diameter impact tip angled 18^o away from the vertical (Benchmark Stereotaxic Impactor; Leica, Buffalo Grove, IL), depressing the brain at 1.7 D.V, at 3.0m/s for 300ms. After the impact, the wound was covered with gel foam and sutured. Topical antibiotics were applied to the incision and Buprenorphin (0.05mg/ml) was administered subcutaneously.

Single-Pellet Reaching Task: Briefly, rats were placed in a clear Plexiglas chamber (26x17x34cm). Rats were trained to reach through a window to retrieve a single palatable food piece (45 mg banana-flavored pellet, Bioserv, Inc., Frenchtown, NJ) placed on a shelf in one of two shallow wells aligned 1 cm from each edge of the window.

Prior to CCI, the preferred reaching limb was determined by placing a pellet on a shelf placed outside of a center window, permitting animals to use either limb. The number of times the animal reached for the pellets and which forelimb they used was recorded for 20 trials or fifteen minutes per day for 4-6 days until 75% of the reaches were conducted with the same forelimb for four consecutive days.

After limb preference was established, all animals were trained daily until they reached a criterion of 30% over 30 trials or after 15 minutes. A reaching trial began with the placement of a pellet in a well, which was 1 cm from the reaching window, and ended when the rat grasped the pellet and brought it to its mouth for consumption (successes), dropped the pellet before bringing it to its mouth (drops), or either knocked the pellet from the well or missed it after 5 reach attempts (misses).

Qualitative Reaching Assessment (Single-Pellet Reaching Task): For each animal on each analysis day, 5 successful reaches were scored. The scoring of a successful reach was done by breaking down the reaching movement into multiple sub-steps. The steps consisted of aim, advance, and opening of the digits, as the rat prepared to reach for the pellet. The next motion was the grasping of a pellet, which was specified by the closure of the digits around the pellet, followed by supination 1 and supination 2, which are responsible for the rotation of the paw 90^{o} to withdraw it from the slit and 45^{o} to bring the pellet to the mouth, respectively. Lastly, release of the pellet into the mouth was examined. Each step received an individual score. A score of 0 for a reach indicates normal reaching and no impairments, 0.5 indicates impairment and a 1 indicates an absent or unrecognizable aspect of a reach. A total abnormality score was obtained by adding all of the scores for all movement categories for the 5 successes. In cases where 5 successes were not obtained, the total score was divided by the ratio of the number of successes (i.e. 3/5).

Foot Fault Test: This test measures errors in coordinated forelimb placement as rats traverse a grid floor. Subjects were placed on a metal grid (test-tube rack (33.02 x 25.40 x 7.62cm with 2.54cm openings)). The number of faults (forelimb slips through the grid) were counted, both contralateral and ipsilateral to the contusion over a total of 50 steps (step=both left and right forelimb moving in the same direction).

Cylinder Test: Forelimb use during exploratory behavior was examined by placing rats in a clear Plexiglas cylinder and videotaping them for at least two minutes or until they had ten forelimb placements on the cylinder wall. Data collected were the total number of times each limb (counted separately) was used to push off the ground to rear-before-wall, support themselves upon the wall of the cylinder, and land after a rear. Following injury to SMC, rats become hyper-reliant upon their less-impaired forelimb (ipsilateral to damage) and do not use their impaired limb as often for these exploratory behaviors. Through slow-motion playback, videotapes were analyzed and the number of times each forelimb was used for weight-bearing exploratory behaviors (rearing, landing and vertical postural support against the wall) in the cylinder.

Contusion Size: Measurement of the estimated contusion size was obtained from 8 serial coronal Nissl stained sections encompassing the full extent of the injury, from approximately 1.70 A/P through -1.06 A/P Bregma. The sections were visualized using a Leica microscope and DVC high-resolution camera using a low power objective. The area of remaining cortex of the contused hemisphere was measured using Neurolucida contour tracing (MicroBrightfield; Colchester VT). For Shams, a random hemisphere was chosen for analysis through the same sections relative to Bregma as above. Volume was calculated using the Cavalieri method [40], as the product of the summed area and the distance between section planes. Data are presented as the mean ± SEM of the volume of remaining cortex.

Supplemental Results

Performance on rehabilitation tasks: To verify that the "dose" of a particular rehabilitative treatment was similar between CCI groups and did not contribute to differences in behavioral recovery, we compared distance run between CCI groups that received exercise (CCI+RE and CCI+REC) and time in the reaching chamber (which reflects how long it took for animals to reach for all the pellets) between the CCI+R, CCI+RE, and CCI+REC. There were no significant differences in the distance run or in the time in the reaching chamber between any of our treatment groups, suggesting that a difference in exposure to the rehabilitation tasks did not influence our behavioral results. Unlike outcome measures in the main manuscript, we included only CCI + treatment animals and days 10-21 to demonstrate that constraint vests did not prevent animals from participating in reaching and aerobic exercise treatment regimens.

Distance Run: The distance run in meters was averaged in 5 or 6 day bins (see Supplemental Figure 1A). The forelimb restraint groups wore their casts during the first bin (D14-28) and the casts were removed before the 2nd bin (D21-25) was initiated. Following CCI, both groups receiving aerobic running exercise ran progressively further over testing days [effect of Days:$F(3,39) = 13.964$, $p \leq 0.0001$]. There was only a non-significant tendency of a GroupXDay interaction [$F(3,39) = 2.707$, $p = 0.058$] and no significant effect of Group [$F(1,13) = 1.350$, $p > 0.05$]. It does appear that the forelimb restraint on days 14-18 significantly reduced the distance run by the CCI+REC group ($p = 0.001$); however, these animals rebounded and in fact ran on average, although non significantly, more than the non limb-restricted animals (D35-40 bin: $p > 0.05$).

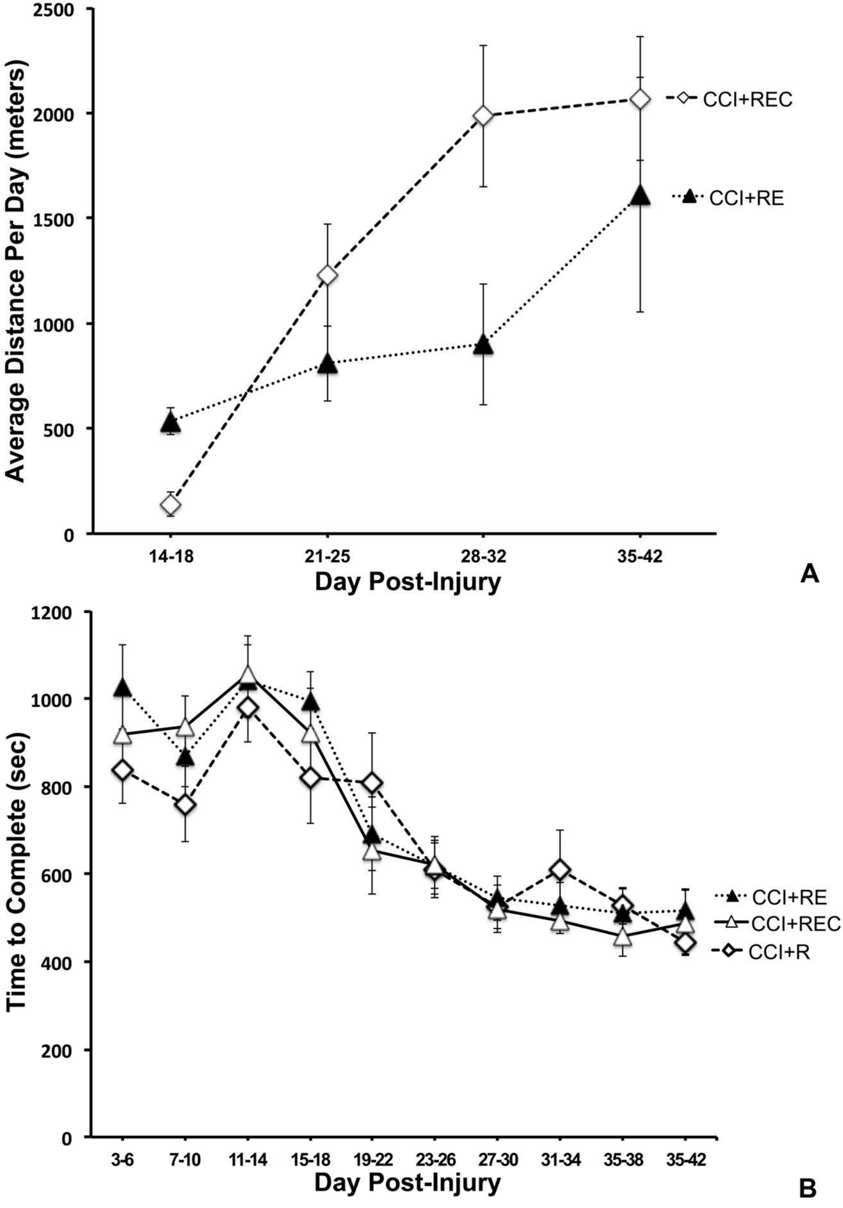
Time to complete Tray Reach Training: There were no significant differences between treatment groups in the time taken to grasp 100 pellets from the tray following CCI (See Supplemental Figure 1B). All groups significantly decreased the time to complete the tray reaching rehabilitation treatment [Day:$F(9,198) = 44.354$, $p \leq 0.001$]; however, there were no significant differences between groups [$F(2,22)=0.164$, $p > 0.05$] nor a GroupXDay interaction[$F(18,198)=1.407$, $p > 0.05$].

Single Pellet Reaching Task: There were no statistical differences between Sham groups in reaching percent success [Day x Group: $F(32,208) = 1.307$, $p > 0.05$; Group: $F(4, 26) = 1.71$, $p > 0.05$]. Additionally, there were no significant effects of testing across days, suggesting that animals had reached a stable performance prior to sham surgery [Days: $(8,208) = 0.35$, $p > 0.05$]. In all further analyses, sham groups were combined for comparison with the CCI groups.

Cylinder Test: There was no statistical differences observed between the Sham groups in rANOVA (p's > 0.05) and they were thus combined for all further analysis.

Foot-Fault Test: There were no significant differences between sham groups [Days:[$F(8,208) = 0.99 > 0.05$; DayXGroup: $F(32, 208) = 1.22$, $p > 0.05$; Group: $F(4,26) = 1.91$, $p > 0.05$] and thus they were combined to compare against the CCI treatment groups.

Contusion Size: No significant difference was observed between Sham groups ($p = 0.38$) and therefore they were pooled.



2500
2000
1500
1000
500
0
Average Distance Per Day (meters)
14-18
21-25
28-32
35-42
Day Post-Injury
CCI+REC
CCI+RE
A
1200
1000
800
600
400
200
0
Time to Complete (sec)
3-6
7-10
11-14
15-18
19-22
23-26
27-30
31-34
35-38
35-42
Day Post-Injury
CCI+RE
CCI+REC
CCI+R
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