

Award Number:

W81XWH-08-1-0624

TITLE:

Neural Plasticity and Neurorehabilitation Following Traumatic Brain Injury

PRINCIPAL INVESTIGATOR:

Dorothy Kozlowski, Ph.D.

CONTRACTING ORGANIZATION:

DePaul University

Chicago, IL 60604

REPORT DATE:

April 2011

TYPE OF REPORT:

Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. <b>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</b>					
1. REPORT DATE (DD-MM-YYYY) 1 April 2011		2. REPORT TYPE Final		3. DATES COVERED (From - To) 15 Sep 2008 – 14 Mar 2011	
4. TITLE AND SUBTITLE  Neural Plasticity and Neurorehabilitation Following Traumatic Brain Injury				5a. CONTRACT NUMBER W81XWH-08-1-0624	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)  Dorothy Kozlowski Ph.D. & Theresa Jones, Ph.D.				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  DePaul University  Chicago, IL 60604				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT  Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT  Rehabilitation following traumatic brain injury is not well understood and has relied primarily on findings from studies conducted in stroke. We have demonstrated that following CCI (a rodent model of contusion TBI), behavioral function was most enhanced by combining 3 types of forelimb rehabilitation: tray reaching, exercise, and forelimb constraint. This enhancement was seen in tests of Forelimb Reaching, Coordination, and Forelimb Use. Combining all three rehabilitation therapies also enhanced performance on rehabilitation tasks. CCI results in a drastic loss of movement representations in the motor cortex, an effect far more severe than expected based on stroke models. Despite this, the motor cortex near the contusion maintains the capacity for motor map plasticity.					
15. SUBJECT TERMS  Controlled Cortical Impact, Reach Training, Exercise, Constraint Induced Therapy, Motor Mapping,					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT  UU	18. NUMBER OF PAGES  19	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

## Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	12
Reportable Outcomes.....	12
Conclusion.....	13
References.....	13
Appendices.....	14

# **Research Technical Report - Neural Plasticity and Neurorehabilitation Following Traumatic Brain Injury**

**Dorothy Kozlowski Ph.D. & Theresa Jones, Ph.D.**

**Final Report: 9/2008 – 4/2011**

**INTRODUCTION:** Individuals with traumatic brain injury (TBI) rarely have the benefit of pharmacological treatments that provide neuroprotection. Instead, they may receive multiple treatments aimed at managing brain injury followed by rehabilitation. While much TBI research is focused on neuroprotection strategies, there remain many individuals who can be expected to either miss the window of opportunity or to benefit incompletely from early neuroprotective treatment. The focus of our research projects is on treatment strategies for individuals who have passed the acute stage of TBI and rely on rehabilitation. The effects of rehabilitation on the brain have been extensively studied in animal models of stroke and have significantly influenced rehabilitation of stroke patients (for review see (T. A. Jones et al., 2009). These findings and practices are being applied to TBI; however, animal studies examining the effectiveness of rehabilitation and its effect on TBI-induced cellular sequelae and neuroplasticity are lacking. The innovation of the proposed research is that it is the first to examine different types of rehabilitation strategies focused on upper limb function following an animal model of TBI: the controlled cortical impact (CCI). The studies assess the therapeutic utility of these rehabilitative strategies, and examine their impact on the injured brain and on compensatory neuroplasticity.

## **BODY:**

### **Experiment 1 – Does motor rehabilitative training improve function and promote restorative neural plasticity in remaining brain tissue? Is it improved by adjunctive behavioral therapies?**

Conducted at DePaul University, Lab of Dorothy Kozlowski Ph.D.

**Rationale:** Our labs have demonstrated that compensatory neural plasticity may not occur following TBI. Given this, we believe that forelimb rehabilitation will need to be more rigorous following TBI than what has been shown to be effective following stroke. Therefore, we employed combinations of rehabilitative training and examined their effects on behavioral outcome and neural plasticity.

**Animals:** Adult male Hooded Long Evans rats were placed in the groups listed below. All animals have been run in the study. Injuries and behavioral analysis are complete.

<b>Group:</b>	<b>n=</b>
CCI + reach training	9
CCI + reach training + exercise	13
CCI + reach training + exercise + forelimb constraint	11
CCI + forelimb constraint only	9
CCI + yoked control	11
Sham + reach training	6
Sham + reach training + exercise	7
Sham + reach training + exercise + forelimb constraint	8
Sham + forelimb constraint only	6
Sham + yoked control	6

**Surgery:** Rats received a unilateral CCI over the forelimb representation in the sensorimotor cortex (FL-SMC or Sham surgery (J. E. Minnich et al., 2010, in press))

**Rehabilitation training regime:** *Reach training* - animals were trained on a forelimb reaching task prior to the CCI to determine baseline performance and preferred forelimb. The rehabilitative reach training of the impaired forelimb (on a tray reaching task) started on day 3 and was administered once a day until the end of the study (J. E. Hsu and T. A. Jones, 2006). *Reach training + exercise* – reach training occurred as above. In addition, starting at 14 days post-CCI, rats had voluntary access to a running wheel for 6 h/day, 3h each in the light and dark cycle and running distance was measured daily. *Reach training + exercise + forelimb constraint therapy*– reach training and exercise occurred as above. *Forelimb constraint therapy*- on post-CCI Day 10 rats were placed into unilateral unimpaired-limb (one-holed) restricting vests. They were briefly anesthetized with isoflurane so that vests can be customized. The vests were worn continuously for 10 days. Animals in the “CCI+forelimb constraint only” group wore the vests but did not receive reach training or exercise. As a yoked control, non-rehabilitated animals were placed in the reaching chamber and received pellets on the floor at the same rate that a reaching rat retrieves them in the tray. Non-exercised rats received access to a locked running wheel during exercise periods. Furthermore, animals not receiving forelimb constraint were anesthetized and fitted with control (2-holed) vests during the forelimb constraint period. All vests were removed on Day 20 prior to final outcome measures.

**Behavioral analysis:** Forelimb function (of both forelimbs) was analyzed pre-CCI and on days 3, 7, 14, 21, & 28, 35 & 42 post-CCI using , footfault, vermicelli and limb use tests and impaired limb function was assessed on the single pellet reaching task (J. E. Hsu and T. A. Jones, 2006; J. E. Minnich et al., 2010)

**Histology:** Animals were sacrificed on day 42 post-CCI and intracardiacally perfused. Brains were harvested and sent to Dr. Theresa Jones. Initially we proposed sacrificing on day 30, however preliminary analysis indicated that a slight behavioral effect was starting to be seen on day 30 and that extending the testing period out to day 42 would be beneficial. This change was submitted to ACURO and approved. Therefore, the amount of time spent working with the animals increased from 30 to 42 days.

**Results:** Unlike what is seen following stroke (T. A. Jones et al., 1999; J. E. Hsu and T. A. Jones, 2006; M. A. Maldonado et al., 2008), rehabilitation reach training alone was not sufficient to enhance behavioral function following CCI. In a sensitive motor assessment task, the single pellet reaching test, the group that benefited the most was the one that received all three rehabilitative training paradigms (See Figure 1). In this task, animals that received all 3 rehabilitation tasks not only had a greater success rate at retrieving pellets, almost to pre-injury levels. Slow motion analysis of successful reaches revealed that injured animals receiving the combination of 3 therapies used more normalized reaching strategies and movements compared to the other injury groups and more similar to sham animals. Interestingly, the other rehabilitation tasks, also diminished abnormal behaviors (although the reaching success was not affected).

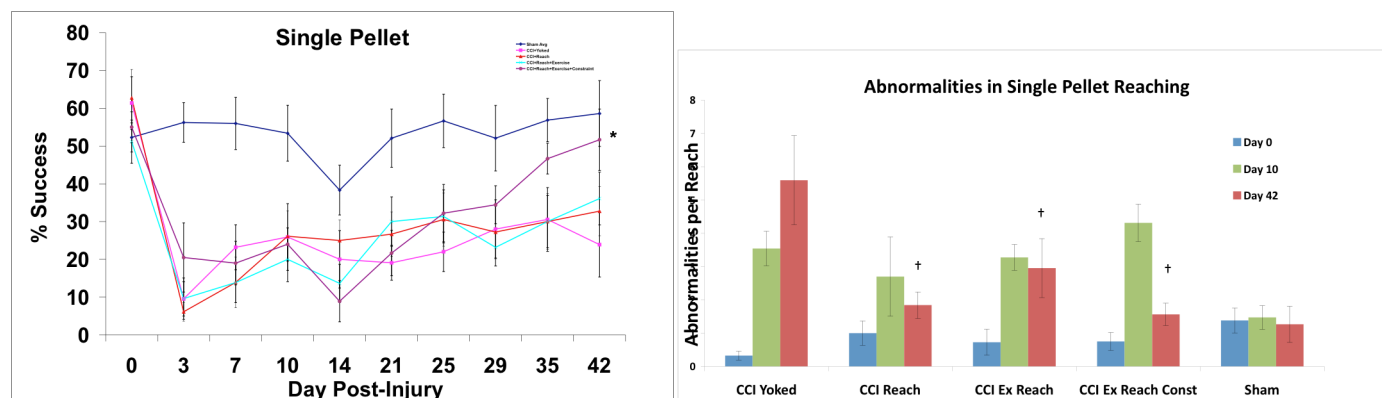


Figure 1. – Combining all 3 rehabilitation tasks enhances success rate on the single pellet test (left) and significantly reduces abnormal reaching behaviors (right).

Tests which examine forelimb coordination and asymmetries in forelimb use also demonstrate that combining all 3 rehabilitation tasks (but not each individually) promote enhanced coordination and lessen asymmetrical forelimb use (Figure 2).

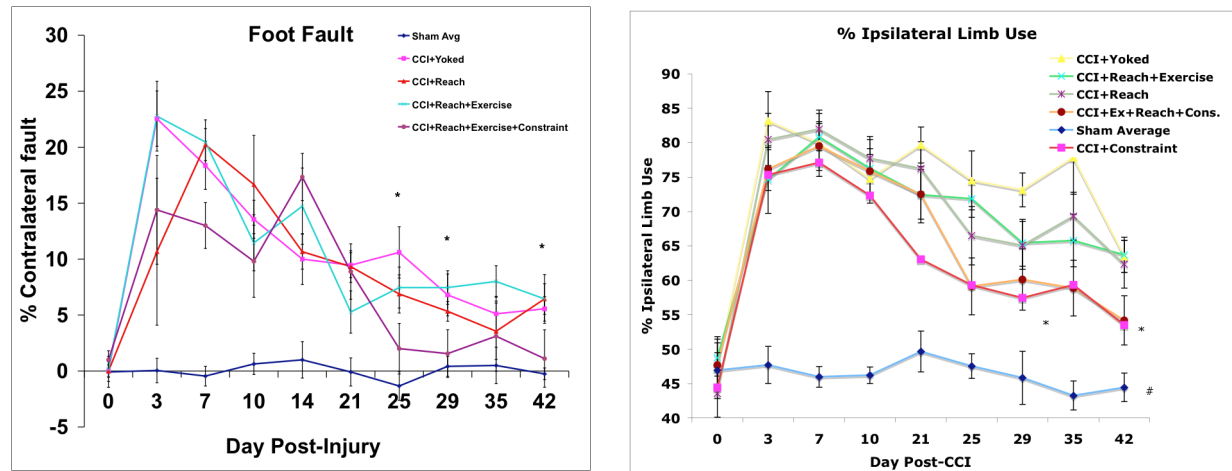


Figure 2. Combining all three rehabilitation tasks promotes the recovery of forelimb coordination (left) and the return to more symmetrical limb use (right) following injury.

The Vermicelli Test is a task of manual dexterity that has shown to be sensitive to deficits following stroke, but has not been used to assess manual dexterity following TBI (CCI). On the vermicelli task, we have found that CCI produces a decrease in the number of adjustments of the impaired forelimb and unimpaired limb and an increase in abnormal behaviors during pasta eating. There were trends for rehabilitation enhancing performance on this test, however due to increased variability there were no significant differences between rehabilitation groups and CCI-Yoked. See Figure 3 – next page.

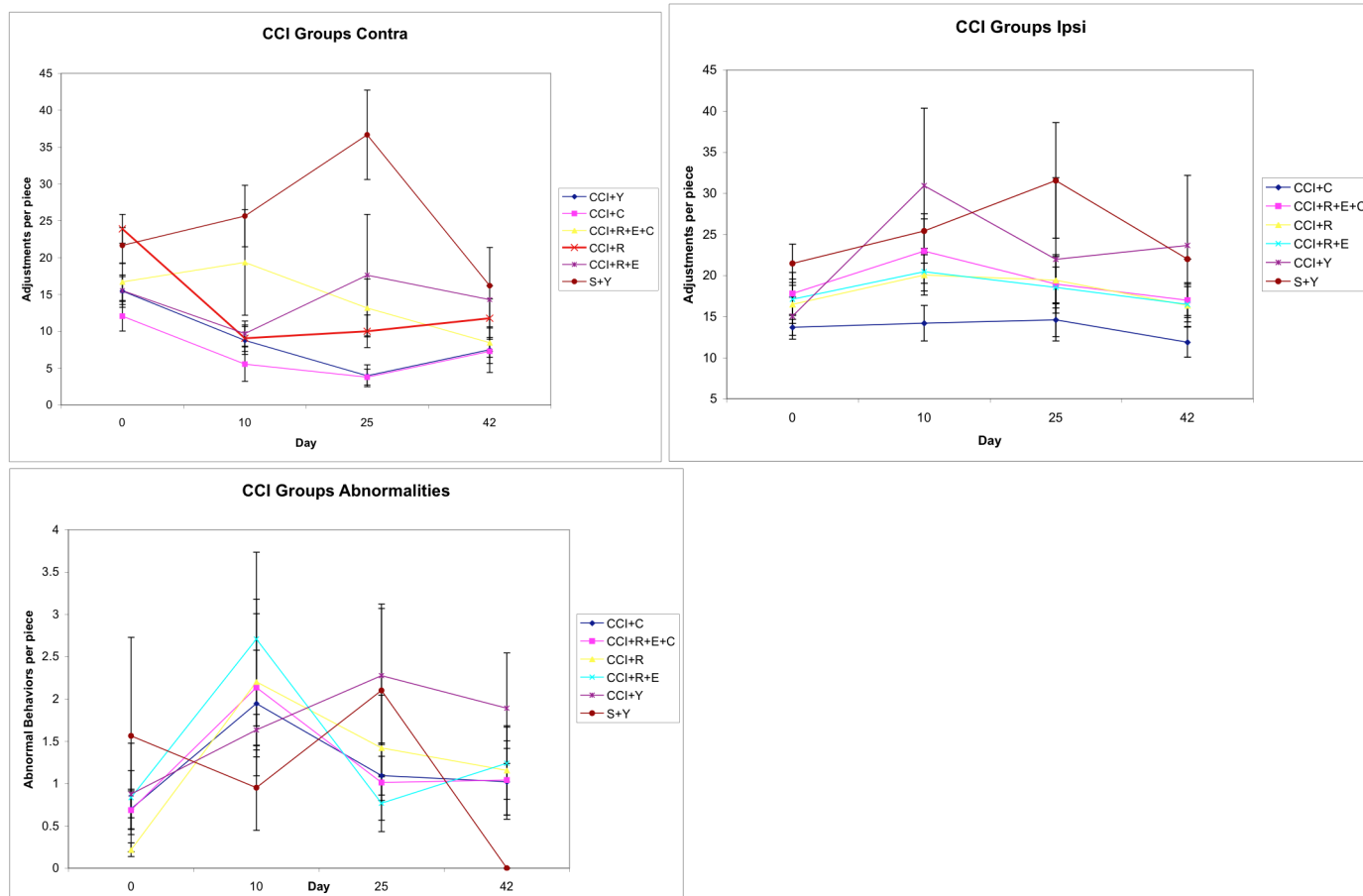


Figure 3. Analysis of Vermicelli Test. Rats that receive a TBI have deficits in manual dexterity (compared to sham) that are not significantly affected by rehabilitation.

Combining all three rehabilitation tasks also enhanced performance on the rehabilitation tasks themselves. Analysis of the time it takes to complete the tray reaching rehabilitation task demonstrated that animals that received all 3 rehab tasks completed the tray reaching faster, and also seemed to run further in the exercise wheel. See Figure 4.

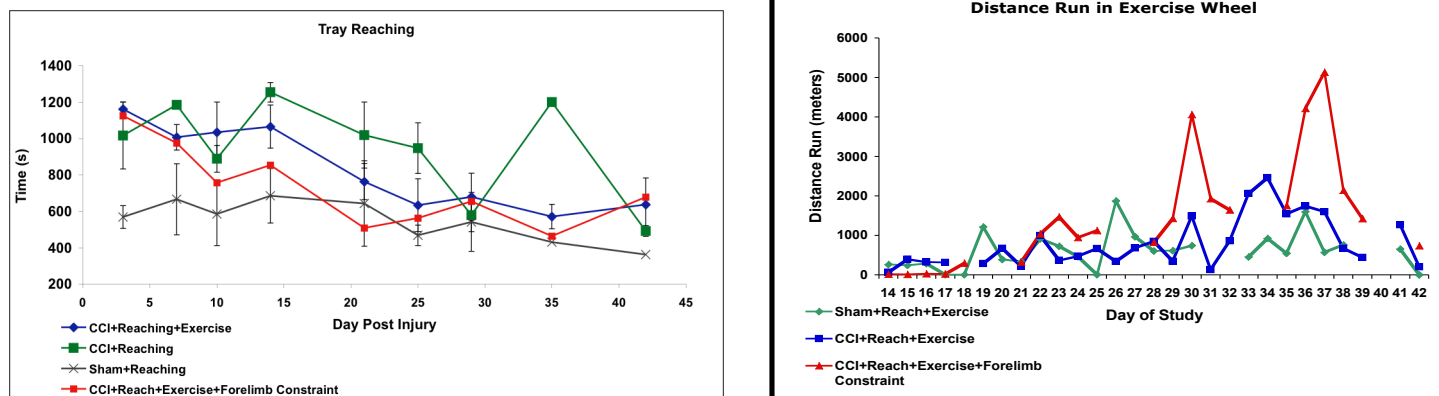


Figure 4. Combining all 3 rehabilitation tasks enhances the performance of the injured animals on the tray reaching tasks (left) and increases the distance they run in the exercise wheel (right).

**Histology Results:** The animals listed above have been sacrificed, brains harvested and sent to the lab of Theresa Jones for sectioning and staining. To date, the brains have been sectioned and one set stained for Nissl. Using the Nissl stained sections, Dorothy Kozlowski's lab has analyzed the size of the contusions. Previous studies have shown that if animals receive too strenuous of a rehabilitation regime too early, it may result in an expansion of the size of the injury (D. A. Kozlowski et al., 1996). We have demonstrated that rehabilitation does not increase the size of the contusion. Combining the three rehabilitation regimes seemed to slightly decrease contusion size (increased cortical volume compared to CCI yoked). See Figure 5 below.

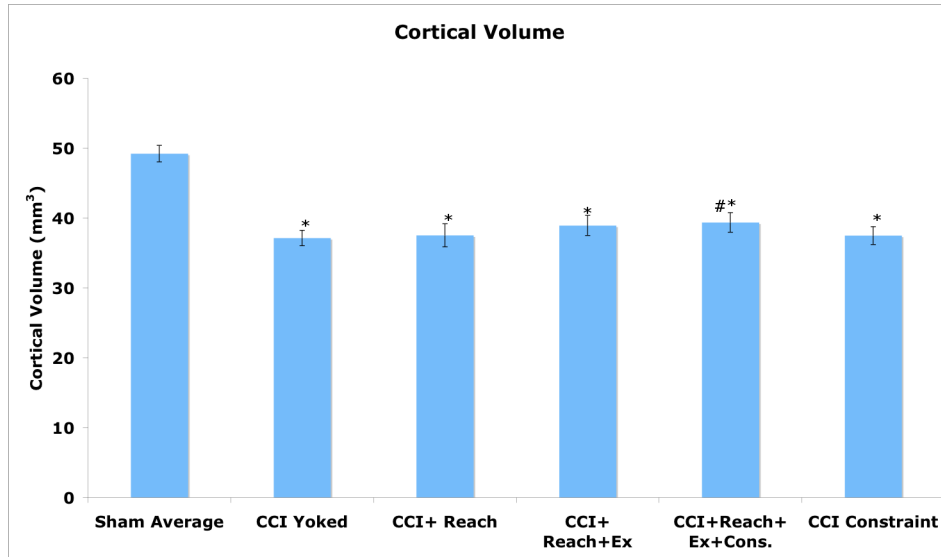


Figure 5. Volume of remaining cortex is significantly decreased in all CCI groups compared to Sham(\* $p < 0.05$ ). However the animals receiving rehabilitation do not have a larger contusion size than CCI+yoked animals. Combining all three rehabilitations decreases contusion size compared to CCI-Yoked (# $p = 0.051$ ).

The remaining sets of brain sections have been stained with microtubule-associated protein 2 (MAP2) for analysis of dendritic arborization, BrdU for analysis of cellular proliferation, Nogo for analysis of proteins that inhibit plasticity, and phosphorylated axonal neurofilament subunit H (pNfH). Quantitative analysis will rely on stereological measures or optical densitometry, as appropriate, given staining patterns. Sample locations will be the remaining sensorimotor cortex around the injury, in the contralateral homotopic cortex and subcortical structures, including the striatum and thalamus (Hsu & Jones, 2006). This quantification is still underway and will be completed by the end of 2011.

## **Experiment 2 – Does motor rehabilitation promote synaptic structural and functional plasticity in remaining motor cortex following TBI?**

**Rationale:** After stroke, functional improvements resulting from motor rehabilitation focused on an impaired forelimb has been found to induce functional reorganization and synaptic plasticity in remaining motor cortex. This experiment determined whether a rehabilitation regime shown to be most effective in improving function in Exp. 1 also results in parallel plasticity of motor cortical movement representations (motor maps) which reflect motor cortex neural integrity and organization. In addition to validating and extending the results of Exp.1, this study is important for revealing potential mechanisms of functional recovery that might be manipulated or facilitated to further improve function.



Animals: Adult male hooded Long Evans rats were placed in the following groups. Power Analysis was conducted as in Exp. 1. An “n” of 13 animals per group was conservatively estimated (a larger sample size than that used in Experiment 1 was needed based on variability in the measure of motor map plasticity evident in preliminary results from a non-DOD funded experiment that were summarized in the last project report.)

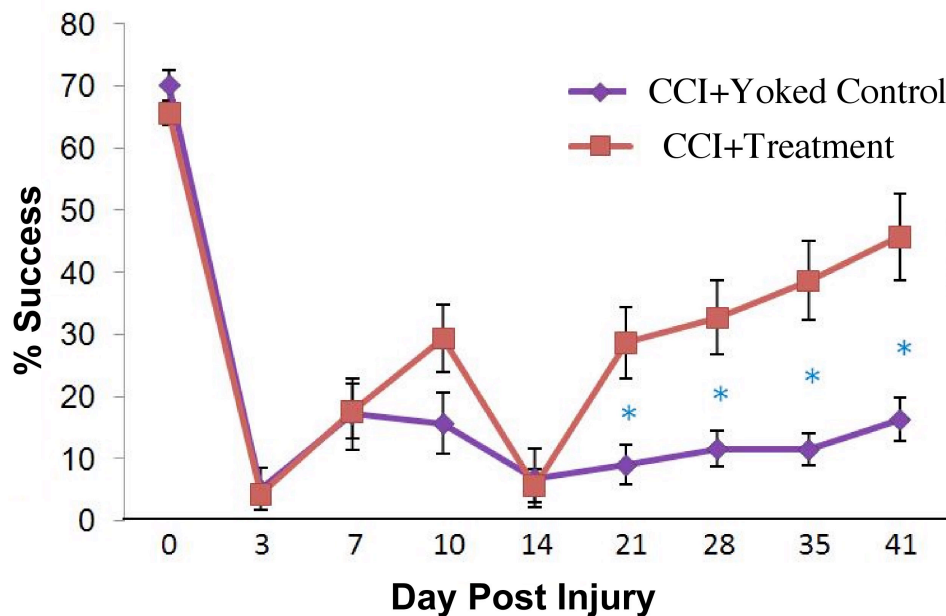
Group:	n=
CCI+Treatment = Reach Training+Exercise+Constraint	13
CCI+Yoked Control	13
TOTAL =26	

Motor Mapping: The effects of the most efficacious rehabilitative training regime in Exp 1, the combination of 3 treatments, on functional plasticity and motor cortical integrity was examined using intracortical microstimulation (ICMS) mapping. After pre-CCI training on the skilled reaching task, as described in Exp. 1, all animals received a CCI over the motor cortex. Animals were randomly placed into either the combination treatment or yoke control groups (as described in Exp. 1), with the exception that animals were matched for pre-CCI and post-CCI (Day 3) performance on the single pellet-reaching task. On day 42-45 post CCI, the caudal and rostral forelimb areas and surrounding regions of the motor cortex of both hemispheres were mapped using standard rodent ICMS techniques under ketamine-xylazine anesthesia, a procedure established in collaboration with Jeffrey Kleim (unpaid consultant). In this method, the organization of motor cortical movement representations is revealed in detail by stimulating primary motor cortex in a systematic (grid like) manner. In this initial experiment, mapping was not conducted multiple times (because repetitive ICMS may impact the size or severity of CCI), but it is expected that this study will set the stage for future within-animal analyses of the time course of motor map changes. \*Immediately after mapping, animals were transcardially perfused and their brains were stored for later immunohistochemistry processing.

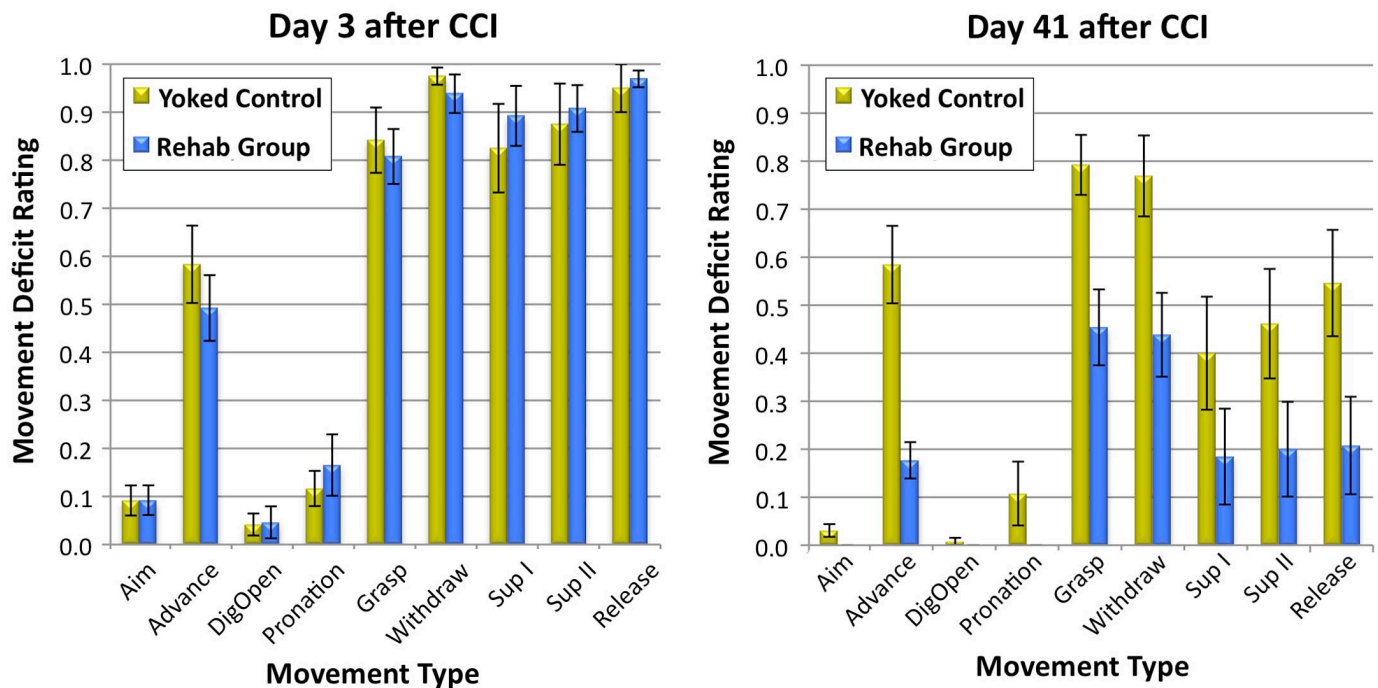
Anatomical Analyses: In the initial grant proposal, we planned to examine synaptic structural plasticity of remaining motor cortex using transmission electron microscopy. However, the equipment, a high quality vibratome, used to section brain tissue for EM malfunctioned at the time the animals needed to be sacrificed. EM tissue processing is extremely sensitive to the timing and quality of sectioning. There were no other appropriate vibratomes available and the time-sensitive nature of EM tissue processing and experimental procedures dictated that we could not wait until our equipment was fixed or replaced. Thus, to avoid losing the opportunity to examine anatomical effects of the training and correlate this with the motor mapping results, we processed the tissue after ICMS for immunohistochemistry analysis to enable the same anatomical measurements as used in Experiment 1.

## Results:

Experiment 2 relied on knowing the results of Exp1 to reveal the optimal rehabilitation regime, which was found to be Reach Training+ Exercise+ Forelimb Constraint. (Figure 7). In Exp 2 we replicated the findings of Experiment 1 in Dr. Theresa Jones’ laboratory, further demonstrating that the combination of 3 treatments significantly improved reaching success (Figure 6) and normalized reaching movements and strategies (Figure 7). Further, the rehabilitation increased wrist representations in the motor cortex, as established using intracortical microstimulation mapping of the injured hemisphere, indicating an expansion or reinstatement of this area compared with controls. These findings indicate that sufficient rehabilitative training can greatly improve motor function and improve the functional integrity of remaining motor cortex. When compared with findings from stroke models, they also suggest that more intense rehabilitation may be needed to improve motor function and remodel the injured cortex after TBI.

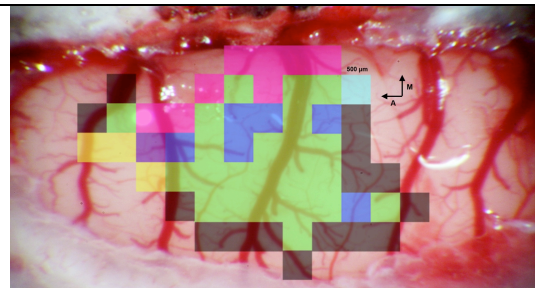


**Figure 6.** The impaired forelimb was assessed using the single-pellet reaching task. As previously shown, the combination of reach training, exercise and constraint therapy significantly improved reaching performance compared to CCI+Yoked control group. \* $p < .05$



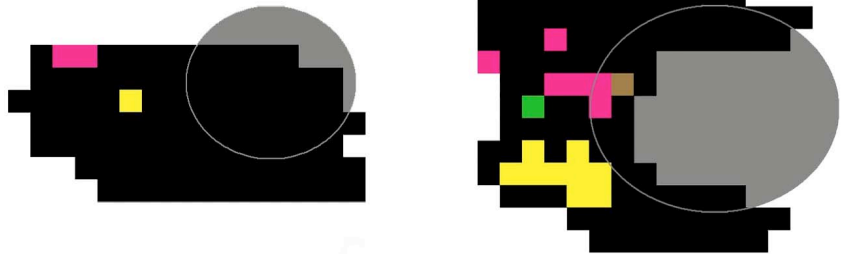
**Figure 7 -** Frame-by-frame movement analysis revealed a reduction in movement abnormalities in performance of the skilled reaching task as a result of rehabilitative training. The impaired limb was assessed at the beginning (Day 3) and end (Day 41) of the rehabilitation period to determine the level of impairment. On day 41, the Rehab Group demonstrated a significant reduction in several movement abnormalities compared to the Control group ( $p$ 's < 0.05). Movements types are shown left to right in the sequence in which they are performed during reaching: aiming and advancing the limb through the chamber window towards the pellet, then opening the digits, pronating the paw and grasping the pellet, followed by

withdrawing the limb back through the window, supinating at the wrist (which has 2 phases) and releasing the pellet into the mouth). Larger scores indicate greater abnormality.

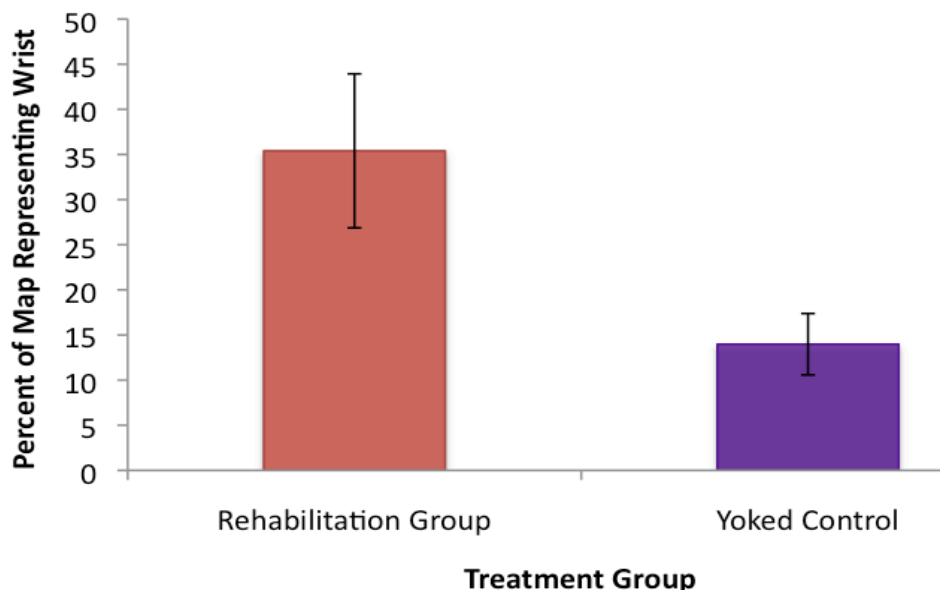
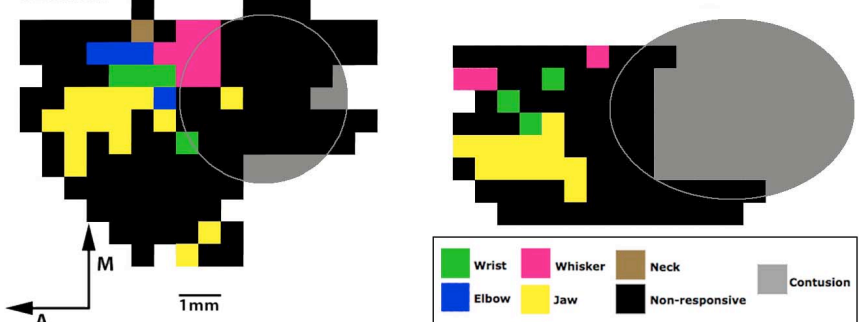


**Figure 7 - Left**, Example of ICMS derived movement representation maps from an intact motor cortex (top) and following CCI (right). Colors correspond to movements elicited by stimulation. **Right** Examples of ICMS derived motor maps from a control animal and treatment group representative of the increase in forelimb movement representations (green and blue in the maps) in animals receiving rehabilitative training

### Yoked Control



### Rehab



**Figure 8. ICMS motor mapping results.** Previous research in injured and intact animals has revealed greater wrist representation after training or rehabilitation. Following 42 days of the combination of treatments, animals had a greater proportion of remaining motor cortex from which wrist movements could be evoked ( $p < 0.05$ ). These data indicate that improvements in reaching success may be linked either to the expansion or reinstatement of wrist representation in CCI injured motor cortex.

## **KEY RESEARCH ACCOMPLISHMENTS:**

- **Following CCI, behavioral function was most enhanced by combining tray reaching, exercise, and forelimb constraint on all behaviors measured.**
- **Combining all three rehabilitation therapies enhanced performance on rehabilitation tasks.**
- **Rehabilitation does not increase the size of the contusion.**
- **CCI results in a drastic loss of movement representations in the motor cortex, an effect far more severe than expected based on stroke models.**
- **Despite this, the motor cortex near the contusion maintains the capacity for motor map plasticity, and this can be driven by intense rehabilitative training**

**This study demonstrates that neural plasticity may be limited following TBI and that the rehabilitation protocol required to produce behavioral enhancements is more extensive than that seen in a similarly sized lesion due to stroke. However, it also indicates that the capacity for functionally beneficial reorganization of motor cortex is possible.**

## **REPORTABLE OUTCOMES:**

### **Poster/Oral Presentations:**

Combs, H., Jones, T.A., Kozlowski, D.A., & Adkins, D.L. Skill training, exercise and constraint-like therapy together promote major functional reorganization of remaining motor cortex after controlled cortical impact injury in rats. *2011 National Neurotrauma Society Meeting.*

K. McDonough, R. De La Torre, L. Ferguson, J. Stamschror, M. Colella, S. Lance, A. Pevstov, E. Ramos, D. Adkins, T. Jones, D. A. Kozlowski. "The vermicelli handling test: A new behavioral test to examine manual dexterity following traumatic brain injury (TBI) in the rat" . *Presented at the National Neurotrauma Symposium, 6/10.* Received Student Travel Award.

L. Ferguson, J. Stamschror, S. Lance, A. Pevtsov, M. Colella, E. Ramos, K McDonough, R. De La Torre, D. Adkins, T. Jones, D. A. Kozlowski. "Motor rehabilitative training, exercise, and forelimb constraint after a controlled cortical impact (CCI) to the forelimb sensorimotor cortex: Differential effects on skilled motor function and recovery". *Presented at the National Neurotrauma Symposium, 6/10.*

E. Ramos, L. Ferguson, J. Stamschror, S. Lance, A. Pevtsov, M. Colella, K. McDonough, R. De La Torre, D. Adkins, T. Jones, D. A. Kozlowski. "Forelimb constraint therapy following a controlled cortical impact (CCI) to the forelimb sensorimotor cortex (FL-SMC) in rats hastens recovery of skilled motor function." *Presented at the National Neurotrauma Symposium, 6/10.* – Travel award.

Ferguson, L., Adkins, D.L., McDonough, K., Stamschror, J., Colella, M., Jones, T.A., & Kozlowski, D.A. Cortical electrical stimulation of the motor cortex, exercise, and motor rehabilitative training after a controlled cortical impact to the forelimb sensorimotor cortex: Differential effects on skilled motor function. *Society for Neuroscience Meeting*, 2009.

Kozlowski D.A., & Jones, T.A. Neurorehabilitation and Neuroplasticity Following Traumatic Brain Injury. *Military Health Research Forum 2009. (poster and oral presentation)*

Adkins DL, Tennant KA, Donlan N, Jones TJ, Kozlowski D (2009) Cortical electrical stimulation of the motor cortex after a controlled cortical impact to the forelimb sensorimotor cortex improves skilled motor function. *National Neurotrauma Society Abstract #226. Peer Reviewed*

### **Invited Lectures and Presentations:**

Kozlowski, D.A. Neurorehabilitation and Neuroplasticity in a Rodent Model of Traumatic Brain Injury. *Purdue University – Calumet*. March, 2011.

Kozlowski, D.A. Searching for Treatment Strategies for Traumatic Brain Injury. *DePaul University Grants Luncheon. DePaul University, Chicago, IL* March, 2011.

Jones, T. A. Learning to remodel the injured brain. *Neuroscience Lecture Series*, Southern Illinois University, March, 2011

Kozlowski, D.A. Traumatic Brain Injury – Rodent Models, Rehabilitation, and Regenerative Therapy. *University of Auckland, Center for Brain Research, New Zealand*. November, 2010.

Kozlowski, D.A. Neurorehabilitation and Neuroplasticity in a Rodent Model of Traumatic Brain Injury. *University of California-Los Angeles, Brain Repair Institute Seminar*, October, 2010.

Kozlowski, D.A. Neurorehabilitation and Neuroplasticity in a Rodent Model of Traumatic Brain Injury. *University of Wisconsin-Milwaukee Neuroscience Seminar*. September, 2010.

### **CONCLUSIONS:**

**This study demonstrates that neural plasticity may be limited following TBI and that the rehabilitation protocol required to produce behavioral enhancements is more extensive than that seen in a similarly sized lesion due to stroke. However, it also indicates that the capacity for functionally beneficial reorganization of motor cortex is possible.**

### **RESEARCH PERSONNEL SUPPORTED**

#### **DEPAUL UNIVERSITY:**

Dorothy Kozlowski  
Lindsay Ferguson  
Juston Stamschror  
Kevin McDonough

## UNIVERSITY OF TEXAS-AUSTIN:

Theresa Jones  
Nicole Donlan

### REFERENCES:

- Hsu JE, Jones TA (2006) Contralesional neural plasticity and functional changes in the less-affected forelimb after large and small cortical infarcts in rats. *Exp Neurol* 201:479-494.
- Jones TA, Chu CJ, Grande LA, Gregory AD (1999) Motor skills training enhances lesion-induced structural plasticity in the motor cortex of adult rats. *J Neurosci* 19:10153-10163.
- Jones TA, Allred RP, Adkins DL, Hsu JE, O'Bryant A, Maldonado MA (2009) Remodeling the brain with behavioral experience after stroke. *Stroke* 40:S136-138.
- Kozlowski DA, James DC, Schallert T (1996) Use-dependent exaggeration of neuronal injury after unilateral sensorimotor cortex lesions. *J Neurosci* 16:4776-4786.
- Maldonado MA, Allred RP, Felthausen EL, Jones TA (2008) Motor skill training, but not voluntary exercise, improves skilled reaching after unilateral ischemic lesions of the sensorimotor cortex in rats. *Neurorehabil Neural Repair* 22:250-261.
- Minnich JE, Mann SL, Stock M, Stolzenbach KA, Mortell BM, Soderstrom KE, Bohn MC, Kozlowski DA (2010) Glial cell line-derived neurotrophic factor (GDNF) gene delivery protects cortical neurons from dying following a traumatic brain injury. *Restor Neurol Neurosci* 28:293-309.
- Minnich JE, Mann SL, Stock M, Stolzenbach KA, Mortell BM, Soderstrom KE, Bohn MC, Kozlowski DA (in press) Glial cell line-derived neurotrophic factor (GDNF) gene delivery protects cortical neurons from dying following a traumatic brain injury. *Restor Neurol Neurosci*.

### APPENDIX:

#### Abstracts from Meetings

National Neurotrauma Society, 2009 Abstract

#### CORTICAL ELECTRICAL STIMULATION OF THE MOTOR CORTEX AFTER A CONTROLLED CORTICAL IMPACT TO THE FORELIMB SENSORIMOTOR CORTEX IMPROVES SKILLED MOTOR FUNCTION

Adkins, D.L.<sup>1,2</sup>, Tennant, K.A.<sup>2</sup>, Donlan, N.<sup>1</sup>, Jones, T.J.<sup>1,2</sup>, Kozlowski, D.<sup>3</sup>

1. Department of Psychology, University of Texas at Austin, 2. The Institute for Neuroscience, University of Texas at Austin, 3. Department of Anthropology, University of Texas at Austin, 4. DePaul University, Department of Biology, Chicago, IL.

In animal models, we have consistently found that motor cortical electrical stimulation (CS) coupled with motor rehabilitative training (RT) improves motor function following ischemic stroke. However, we have also found that if the initial forelimb impairments are extensive or if CS+RT treatment is delayed for 30 days, that CS is less effective. Together, these studies have suggested that CS+RT is linked to plasticity of neural structures and a reorganization of motor cortex after ischemic damage. It is unknown whether CS+RT will also be an effective treatment for motor impairments following traumatic brain injury (TBI). Recently, it was reported that although a unilateral controlled cortical impact (CCI) injury to the sensorimotor cortex induces similar behavioral impairments compared to comparably placed electrolytic or ischemic lesion models, there is a difference in pathological cellular sequelae between the stroke and TBI models (Liput et al., 2008) abstract). Liput et al. reported that unlike in the stroke models, the CCI injury did not induce behaviorally linked neural plasticity in the contra-injury motor cortex. Thus, it is not clear if CS+RT will be as effective following TBI or what the most effective parameters may be to enhance functional recovery. In this initial investigation, we found that CCI to the sensorimotor cortex produces profound deficits in reaching ability. After 6 weeks of RT, 100HZ stimulation at 50% movement threshold during reaching training improves reaching

performance compared to the no stimulation training group. However, animals are still highly impaired compared to pre-injury levels of performance and thus likely require longer or more intense CS+RT intervention for greater motor recovery.

Skill training, exercise and constraint-like therapy together promote major functional reorganization of remaining motor cortex after controlled cortical impact injury in rats.

Combs, H. L., Jones, T.A., Kozlowski, D.A., & Adkins, D.L.

There is a great deal of research on the neural mechanisms underlying motor rehabilitation after stroke, but not after traumatic brain injury (TBI). This is problematic given growing evidence that rehabilitative training regimes effective after stroke may be ineffective after TBI, even when injuries are similar. Unlike similarly placed stroke-like lesions, rehabilitative reach training alone was recently found to be insufficient to improve motor behavior in rats with controlled cortical impact (CCI) injury to the sensorimotor cortex. However, the combination of reach training, exercise, and forced forelimb use effectively improved motor function (Ferguston et al., 2010, J. Neurotrauma, 26: A-83). We examined the effects of this behavioral treatment combination after CCI on the functional reorganization of forelimb representations in motor cortex, and its the relationship with behavioral function. Adult male rats that were proficient in a skilled reaching, received a CCI and three days later began rehabilitation (n = 13) or yoked control procedures (n = 13). The rehabilitation group participated in daily skilled reach training and exercise over 42 days. For the first 10 days, rats wore vests that constrained the intact forelimb to force reliance on the impaired limb. Yoked controls were exposed to reaching chambers, locked running wheels, and were placed in non-limb restricting vests. The combination treatment significantly improved reaching success and normalized reaching strategies. Further, the rehabilitation increased wrist representations in the motor cortex, as established using intracortical microstimulation mapping of the injured hemisphere, indicating an expansion or reinstatement of this area compared with controls. These findings indicate that sufficient rehabilitative training can greatly improve motor function and improve the functional integrity of remaining motor cortex. When compared with findings from stroke models, they also suggest that more intense rehabilitation may be needed to improve motor function and remodel the injured cortex after TBI.<sup>1</sup>

1.Combs HL,Jones TA,Kozlowski DA,Adkins DL.Skill training, exercise and constraint-like therapy together promote major functional reorganization of remaining motor cortex after controlled cortical impact injury in rats.National Neurotrauma Society.2011;28:A-1–A-134

## **THE VERMICELLI HANDLING TEST: A NEW BEHAVIORAL TEST TO EXAMINE MANUAL DEXTERITY FOLLOWING TRAUMATIC BRAIN INJURY (TBI) IN THE RAT**

Kevin E. McDonough<sup>1</sup>, Roxanna De La Torre<sup>1</sup>, Lindsay M. Ferguson<sup>1</sup>, Justin D. Stamschror<sup>1</sup>, Michael N. Colella<sup>1</sup>, Steven Lance<sup>1</sup>, Alexandr Pevstov<sup>1</sup>, Elena M. Ramos<sup>1</sup>, DeAnna L. Adkins<sup>2</sup>, Theresa A. Jones<sup>2</sup>, Dorothy A. Kozlowski<sup>1</sup>

<sup>1</sup>DePaul University, Department of Biology, Chicago, IL 60614

<sup>2</sup>University of Texas-Austin, Institute for Neuroscience, Department of Psychology, Austin, TX 78712

The Vermicelli Handling Test is a useful test of manual dexterity that has been used to evaluate fine motor function in animal models of stroke and Parkinson's disease (Allred et al. 2008). The test involves examining the use of forelimbs while rodents eat uncooked vermicelli pasta. In unilateral stroke and Parkinson's models, animals show deficits in the way they use their paws to manipulate the pasta as it is eaten. This test has never been used to examine forelimb function in animal models of traumatic brain injury (TBI). The current study compared the handling behaviors of sham (uninjured) rats with rats that received a unilateral controlled cortical impact (CCI) to the forelimb sensory motor cortex (FL-SMC). At different time points throughout a 42-day testing period, animals were video-recorded eating five pieces of vermicelli

pasta. These video tapes were analyzed for time to eat the pasta, paw adjustments while eating (both contralateral and ipsilateral to the injury), and atypical behaviors, such as failing to contact the piece with both paws during eating. The results show that a CCI to the FL-SMC results in lateralized deficits in the Vermicelli Handling Test when compared to Shams. These deficits include an increase in the number of adjustments of the ipsilateral paw, a decrease in the number of adjustments of the contralateral paw, an increase in atypical behaviors while eating the pasta, and an increased time to eat the pasta. These findings suggest that this test could be a useful tool to examine forelimb function following TBI. In addition, it could be very useful in examining therapeutic potential following TBI and is currently being used to examine the effects of rehabilitation following CCI in this laboratory. This project was supported by the Department of Defense TBI Concept Award and the DePaul University Research Council.

## **FORELIMB CONSTRAINT THERAPY FOLLOWING A CONTROLLED CORTICAL IMPACT (CCI) TO THE FORELIMB SENSORIMOTOR CORTEX (FL-SMC) IN RATS HASTENS RECOVERY OF SKILLED MOTOR FUNCTION.**

Elena Ramos<sup>1</sup>, Lindsay Ferguson<sup>1</sup>, Justin Stamschror<sup>1</sup>, Steven Lance<sup>1</sup>, Alexandr Pevtsov<sup>1</sup>, Michael Colella<sup>1</sup>, Kevin McDonough<sup>1</sup>, Roxanna De La Torre<sup>1</sup>, DeAnna L. Adkins<sup>2</sup>, Theresa A. Jones<sup>2</sup>, Dorothy A. Kozlowski<sup>1</sup>

<sup>1</sup>DePaul University, Department of Biology, Chicago, IL 60614

<sup>2</sup>University of Texas-Austin, Institute for Neuroscience, Department of Psychology, Austin, TX 78712

It has been demonstrated that animals receiving a unilateral controlled cortical impact (CCI) of the forelimb sensorimotor cortex (FL-SMC) have long-lasting impairments in forelimb sensory and motor function. It has been shown in animal models of ischemic stroke and in human patients with stroke, that ipsilateral forelimb constraint (FC) in animals and constraint-induced movement therapy (CIMT) in humans aids in the recovery of motor function of the upper extremity. CIMT is being tried in patients with traumatic brain injury, yet the effects of FC alone as a form of rehabilitation in an animal model of TBI have never been examined. Therefore, this study examined the effects of FC on motor function following CCI. Animals received FC alone following a unilateral CCI over the FL-SMC. FC was performed using a two-armed vest followed by taping of the ipsilateral or non-injured forelimb so that it could move, but not be used for locomotion, grooming, or eating. FC was applied from days 10-20 following CCI. This forced the animals to rely solely on the use of their impaired limb for 24 hours a day for ten days. Control animals received the two-armed vest alone. Throughout the course of this study, the animals' behavioral deficits were assessed using a test of forelimb reaching (single pellet test (SP)) and a test for motor coordination (foot fault (FF)). The SP test consisted of reaching for a single banana-flavored pellet for 20 trials and a percent success was obtained. The FF test consisted of the animals walking on a grid and recording the number of times that the animals' forelimbs fell through the grid over a period of 50 steps. Both tests were performed on the animal prior to the injury and throughout the study until day 42 following CCI. It was determined that there were significant deficits in reaching ability and motor coordination following CCI. Animals that had FC alone showed a faster recovery in both of these measures compared to injured animals not receiving any motor rehabilitation. These results suggest that the use of FC alone following CCI has the ability to accelerate the recovery of both reaching ability as well as motor coordination following TBI. Other studies in our lab are showing that adding FC to other modes of motor rehabilitation further



enhances behavioral recovery. This work was funded by the Department of Defense TBI Concept Award and the DePaul University Research Council.

## **MOTOR REHABILITATIVE TRAINING, EXERCISE, AND FORELIMB CONSTRAINT AFTER A CONTROLLED CORTICAL IMPACT (CCI) TO THE FORELIMB SENSORIMOTOR CORTEX: DIFFERENTIAL EFFECTS ON SKILLED MOTOR FUNCTION AND RECOVERY**

Lindsay Ferguson<sup>1</sup>, Justin Stamschror<sup>1</sup>, Steven Lance<sup>1</sup>, Alexandr Pevtsov<sup>1</sup>, Michael Colella<sup>1</sup>, Elena Ramos<sup>1</sup>, Kevin McDonough<sup>1</sup>, Roxanna De La Torre, DeAnna L. Adkins<sup>2</sup>, Theresa A. Jones<sup>2</sup>, Dorothy A. Kozlowski<sup>1</sup>

<sup>1</sup>DePaul University, Department of Biology, Chicago, IL 60614

<sup>2</sup>University of Texas-Austin, Institute for Neuroscience, Department of Psychology, Austin, TX 78712

In animal models of ischemic stroke, motor rehabilitative training improves motor function, while exercise (EX) alone does not. Recently, it was reported that although a unilateral controlled cortical impact (CCI) to the sensorimotor cortex (FL-SMC) induces similar behavioral impairments compared to comparably placed ischemic lesion models, the CCI did not induce behaviorally linked neural plasticity in the contra-injury motor cortex, as is evidenced following stroke. Therefore, It is unknown which types of rehabilitation alone or in combination will be an effective treatment for motor impairments following traumatic brain injury (TBI). Animal models of TBI have examined the effects of exercise and enriched environments and found various results on cognitive and motor function, but motor rehabilitative training focused on the forelimb has not been examined. Animals received reach training (RT), exercise (EX) or forelimb constraint (FC) alone or in combination at different times following unilateral CCI of the FL-SMC. RT consisted of reaching for a tray of banana pellets daily (day 3-42), EX was voluntary access to a running wheel for 6 hours (days 14-42) and FC consisted of a vest which immobilized the animals non-impaired forelimb for 24 hours (days 10—20) forcing the use of the impaired forelimb. Animals were tested on reaching performance and motor coordination tests until day 42 post-CCI. Tests used were the Single Pellet Reaching Test, Foot Fault, and Forelimb Use. We found that CCI to the FL-SMC produces profound deficits in reaching ability, motor coordination and an over-reliance on the ipsilateral forelimb for postural support. Unlike what is seen following stroke, these deficits are not significantly improved following RT alone or RT+EX. Animals that receive RT+EX+FC increase reaching ability to pre-injury levels, as well as recover faster on tests of motor coordination and forelimb use. Additionally, the recovery of reaching ability measured qualitatively by analyzing abnormalities in reaching indicates that CCI+RT+EX+FC animals reach as they did post-injury, not using abnormal compensatory reaching strategies. These rats also perform better during rehabilitation tasks, i.e. run farther on the exercise wheel than injured rats receiving RT+EX. Examination of remaining cortical volume in these animals indicates that these rehabilitation strategies do not increase nor decrease cortical volume, therefore the behavioral effects are not linked directly to contusion size. These results suggest that rehabilitative motor training following CCI is most effective when coupled with multiple types of motor rehabilitation tasks. Funded by: Department of Defense TBI Concept Award and the DePaul University Research Council.

Cortical electrical stimulation of the motor cortex, exercise, and motor rehabilitative training after a controlled cortical impact (CCI) to the forelimb sensorimotor cortex: Differential effects on skilled motor function.

Lindsay Ferguson<sup>2</sup>, DeAnna L. Adkins<sup>1</sup>, Kevin McDonough<sup>2</sup>, Justin Stamschror<sup>2</sup>, Michael Colella<sup>2</sup>, Theresa A. Jones<sup>1</sup>, Dorothy A. Kozlowski<sup>2</sup>

<sup>1</sup>University of Texas-Austin, Institute for Neuroscience, Department of Psychology, Austin, TX 78712

<sup>2</sup>DePaul University, Department of Biology, Chicago, IL 60614

In animal models of ischemic stroke, motor rehabilitative training (RT) alone or in combination with cortical electrical stimulation (CS) improves motor function, while exercise (EX) alone does not. However, if the initial impairments are extensive or if CS+RT treatment is delayed for 30 days, CS is less effective. CS and RT, but not EX, are also associated with greater cortical reorganization of distal forelimb representation in the lesioned motor cortex following ischemia. It is unknown whether EX, RT (alone or in combination), or CS+RT will also be an effective treatment for motor impairments following traumatic brain injury (TBI). Recently, it was reported that although a unilateral controlled cortical impact (CCI) to the sensorimotor cortex (FL-SMC) induces similar behavioral impairments compared to comparably placed electrolytic or ischemic lesion models, there is a difference in pathological cellular sequelae between the stroke and TBI models (Liput et al., 2008) abstract). Unlike in the stroke models, the CCI injury did not induce behaviorally linked neural plasticity in the contra-injury motor cortex. Thus, it is not clear if these rehabilitative strategies will be as effective following TBI. Animals received RT, RT+EX or CS+RT following unilateral CCI of the FL-SMC. RT consisted of reach training, exercise was voluntary access to a running wheel and the CS consisted of a 100 HZ stimulation of the injured FL-SMC. Animals were tested on reaching performance and motor coordination tests. In this initial investigation, we found that CCI to the sensorimotor cortex produces profound deficits in reaching ability. These deficits do not seem to be improved following RT alone, however following both RT+EX and CS+RT, animals increase their reaching ability significantly, but not to pre-injury levels. These results suggest that rehabilitative motor training following CCI may be most effective when coupled with adjunctive treatments

## **PT073978 – Kozlowski - Neural Plasticity and Neurorehabilitation Following Traumatic Brain Injury**

### **Non-Technical Abstract**

Individuals with traumatic brain injury (TBI) can't take a drug to prevent brain cell death and enhance recovery. Instead, they receive multiple treatments aimed at managing the initial brain trauma followed by months of rehabilitation. While much TBI research is focused on finding a miracle drug, many patients may either miss the drug's window of opportunity, or may benefit incompletely from early drug treatment. The focus of this proposal is on treatment strategies for individuals who rely on rehabilitation. The effects of rehabilitation on the brain have been extensively studied in animal models of stroke and have significantly influenced rehabilitation of stroke patients. These findings and practices are being applied to TBI, however, animal studies examining rehabilitation and its effect on the brain following TBI are lacking. This study will be the first to examine motor rehabilitation strategies following a rodent model of TBI. We will produce a controlled cortical impact (CCI) over the area of the brain responsible for the use of the animals' front paws. At specific time points post-injury, we will utilize three different animal models of physical therapy, ie. Forelimb reaching, exercise, and constraint induced therapy, alone and in combinations. This would mimic physical rehabilitation strategies employed in humans with limited arm use. We will examine the therapeutic utility of motor rehabilitation by measuring behavioral indices of forelimb use and examine its impact on the

injured brain by looking at neural plasticity markers and extent of injury. Once we know which rehabilitation strategy is most beneficial we will see exactly how that strategy affects the reorganization of the injured brain using motor mapping. Answering these questions using animal models of TBI will provide significant direction for the development of new approaches for TBI rehabilitation. Furthermore, it will provide the groundwork necessary for investigating therapies that combine rehabilitation with non-behavioral therapies, including pharmacotherapy aimed at neuroprotection or decreasing inhibitory molecules to induce regeneration.