Title
Role of the Contra-lesional Cortex in Recovery of Function After Traumatic Brain Injury

Author
Verley, Derek Ray

Publication Date
2017

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Role of the Contra-lesional Cortex in Recovery of Function after Traumatic Brain Injury

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Neuroscience

by

Derek Ray Verley

2017

ABSTRACT OF THE DISSERTATION

Role of the Contra-lesional Cortex

in Recovery of Function

after Traumatic Brain Injury

by

Derek Ray Verley

Doctor of Philosophy in Neuroscience

University of California, Los Angeles, 2017

Professor Neil G. Harris, Chair

After a lateralized traumatic brain injury (TBI), neural repair and regeneration in the area proximal to the injured region is known to be associated with recovery of some of the function lost to injury. However, there is a growing body of evidence, mostly from stroke research that distant, but networks which are both functionally and structurally connected to the injured cortical region are not only indirectly altered by injury, but may also be critically involved in the recovery process. One such region, called the contra-lesional cortex (CLCtx), is the uninjured, homotopic brain region mirrored on the opposite cerebral hemisphere. Here, we sought to determine how the cortical map is altered by injury and whether the CLCtx is causally involved in recovery of function after

unilateral TBI. We used functional magnetic resonance imaging (fMRI) and *in vivo* electrophysiology to chart the changes in the functional map throughout recovery from experimental TBI in the rat. To determine if the CLCtx is involved in functional recovery, we tested function with and without temporary inactivation of the CLCtx at weekly for the first month after injury. The results of these experiments indicated that the functional map shifts to the CLCtx throughout the first four weeks after injury. At 4 weeks post-injury, silencing the CLCtx re-instated the injury deficit, indicating that the CLCtx was supporting functional recovery chronically. Surprisingly, silencing activity of the CLCtx at just one week post-injury resulted in a complete recovery of function, indicating that the CLCtx is impeding function of the affected forelimb. Therefore, with its causative role in recovery of function after TBI, the CLCtx might be an important target for future interventional strategies in TBI recovery.

The dissertation of Derek Ray Verley is approved.

Chris C. Giza

David A. Hovda

Stanley Thomas Carmichael

Neil G. Harris, Committee Chair

University of California, Los Angeles

2017

TABLE OF CONTENTS

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Derek R. Verley	Graduate Student Researcher Neuroscience Interdepartmental Program
Graduate Student	

EDUCATION/ TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Santa Clara University, Santa Clara, CA	B.Sc.	06/05	Chemistry
University of California at Los Angeles, CA (UCLA)	Ph.D. Candidate	06/17	Neuroscience

A. Personal Statement. Throughout my 6 years as a research neuroscientist, I have had a record of high productivity and significant intellectual contribution, as reflected in the published manuscripts and presentations. My goal is to contribute to the growing understanding of the brain and its response to injury so that we might better understand how to we might better treat the many sequelae suffered by TBI survivors.

B. Positions and Honors

Academic Positions

2016 **Teaching Assistant**, Interdepartmental Program in Neuroscience, University of California – Los Angeles, CA

2015 **Doctoral Candidate,** Interdepartmental Program in Neuroscience, University of California – Los Angeles, CA

2011 **Teaching Assistant**, Department of Physiology, University of California – Los Angeles, CA

2009-pres. **Graduate Student Researcher**, Department of Neurosurgery, University of California – Los Angeles, CA

2006-09 **Research Scientist/Engineer1**, Department. of Neurosurgery, University of Washington, Seattle, WA

2005 **Teaching Assistant**, Department of Chemistry, Santa Clara University, Santa Clara, CA

2003-05 **Undergraduate Researcher,** Dept. of Physics, Santa Clara University, Santa Clara, CA

Other Experiences and Professional Memberships

2005 Member, Sigma Xi

Honors

- UCLA Neuroscience Travel Award/Grant for SfN –2013

C. Selected Peer-Reviews Publications

1. Harris NG, Verley DR, Gutman BA, Sutton RL. Bi-directional changes in fractional anisotropy after experiment TBI: Disorganization and reorganization? Neuroimage. Elsevier Inc.; 2016;133:129–43.

2. Harris, N.G., **Verley**, **D.R.**, Gutman B.A., Thompson, P.M., Yeh, H.J., & Brown, J.A.. (2016) Disconnection and hyper-connectivity underlie reorganization after TBI: A rodent functional connectomic analysis. *Experimental neurology*. 227, 124-138.
3. Harris NG, Nogueira MSM, **Verley DR**, Sutton RL. Chondroitinase enhances cortical map plasticity and increases functionally active sprouting axons after brain injury. [Internet]. *Journal of neurotrauma* 2013: 1–69.Available from: http://www.ncbi.nlm.nih.gov/pubmed/23517225
4. D'Ambrosio R, Eastman CL, Darvas F, Fender JS, **Verley DR**, Farin FM, et al. Mild passive focal cooling prevents epileptic seizures after head injury in rats. [Internet]. Annals of neurology 2012: 1–11.[cited 2013 Jan 11] Available from: http://www.ncbi.nlm.nih.gov/pubmed/23225633
5. Eastman, C. L., **Verley, D. R.**, Fender, J. S., Stewart, T. H., Nov, E., Curia, G., & D'Ambrosio, R. (2011). Antiepileptic and Antiepileptogenic Performance of Carisbamate after Head Injury in the Rat : Blind and Randomized Studies. *PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS*, *336*(3), 779-790. doi:10.1124/jpet.110.175133.tant
6. Eastman, C. L., **Verley, D. R.**, Fender, J. S., Temkin, N. R., & D'Ambrosio, R. (2010). ECoG studies of valproate, carbamazepine and halothane in frontal-lobe epilepsy induced by head injury in the rat. *Experimental neurology*, ***224***(2), 369-88. Elsevier Inc. doi: 10.1016/j.expneurol.2010.04.013.
7. Suljak, S. W., Rose, C. M., Sabatier, C., Le, T., Trieu, Q., **Verley, D. R.**, et al. (2010). Enhancement of muscle contraction in the stomach of the crab Cancer borealis: a possible hormonal role for GABA. *The Biological bulletin*, ***218***(3), 293-302. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20570852.
8. Stewart, T. H., Eastman, C. L., Groblewski, P. a, Fender, J. S., **Verley, D. R.**, Cook, D. G., et al. (2010). Chronic Dysfunction of Astrocytic Inwardly Rectifying K+ Channels Specific To the Neocortical Epileptic Focus After Fluid Percussion Injury in the Rat. *Journal of neurophysiology*. doi: 10.1152/jn.00398.2010.
9. D'Ambrosio, R., Hakimian, S., Stewart, T., **Verley, D. R.**, Fender, J. S., Eastman, C. L., et al. (2009). Functional definition of seizure provides new insight into post-traumatic epileptogenesis. *Brain : a journal of neurology*, ***132***(Pt 10), 2805-21. doi: 10.1093/brain/awp217.
10. **Verley, D. R.**, Doan, V., Trieu, Q., Messinger, D. I., & Birmingham, J. T. (2008). Characteristic differences in modulation of stomatogastric musculature by a neuropeptide in three species of Cancer crabs. *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology*, ***194***(10), 879-86. doi: 10.1007/s00359-008-0359-6.
11. Le, T., **Verley, D. R.**, Goaillard, J.-M., Messinger, D. I., Christie, A. E., & Birmingham, J. T. (2006). Bistable behavior originating in the axon of a crustacean motor neuron. *Journal of neurophysiology*, ***95***(3), 1356-68. doi: 10.1152/jn.00893.2005.
12. Messinger, D. I., Kutz, K. K., Le, T., **Verley, D. R.**, Hsu, Y.-W. a, Ngo, C. T., et al. (2005). Identification and characterization of a tachykinin-containing neuroendocrine organ in the commissural ganglion of the crab Cancer productus. *The Journal of experimental biology*, ***208***(Pt 17), 3303-19. doi: 10.1242/jeb.01787.

D. Research Support

UCLA Brain Injury Research Center (BIRC)

Chapter 1

Background

Epidemiology of traumatic brain injury

Traumatic brain injury (TBI) is the leading cause of death and disability worldwide (Loane and Faden 2010). TBI results from a blow to the head or a forceful acceleration/deceleration of the brain within the skull. At least 1.7 million new cases are reported annually in the United States (Loane and Faden 2010). The risk of TBI is higher for military personnel. In the wars in Iraq and Afghanistan, head trauma was diagnosed in more than 85% of all trauma admissions (Xydakis et al. 2012). Risk factors for TBI in the US civilian population include age, gender, and low socioeconomic status. Males are twice as likely of sustaining a TBI compared to females, potentially attributable to increased propensity for risk taking behaviors (Corrigan et al. 2010). The most likely ages for suffering a TBI include children aged 0-4 years, adolescents between 15-19 years, and seniors aged 75+ years and the top mechanisms of injury include vehicular accidents and falls from height (CDC 2014).

An estimated 5.3 million TBI survivors in the United States and 7.7 million in Europe require physical rehabilitation for sensorimotor deficits (Kozlowski et al. 2013). The financial burden for both acute care and rehabilitative therapy for TBI in the US has been estimated between $9-10 billion per year (Ragnarsson 2006; Bales et al. 2009). While some spontaneous recovery of function may occur after injury, it is often limited, and many TBI survivors do not recover full function. Approximately 50% of patients who survive a moderate TBI will suffer chronic disabilities (Bales et al. 2009). For example, in a follow-up study of 67 moderate-to-severely injured patients and found that 30% of patients still had upper limb impairments at 5 years post-

injury (Hillier et al. 1997). The experimental TBI model in the early adolescent male rat employed in the studies presented in this dissertation reproduces etiology of the large population of patients with upper limb impairments nicely. The use of an animal model minimizes the variability in injury location(s) observed in clinical TBI and enables control for many independent variables that might otherwise increase variability and require larger sample sizes including diet, exercise, housing environment, adherence to treatment schedules, sex and age. It is the hope that the information gleaned from these studies also address more broad concepts regarding circuit remodeling, re-organization of the cortical map, and interhemispheric interactions change throughout recovery after a lateralized brain injury.

Deciphering neural correlates of behavior: Equipotentiality vs. Cortical Localization theories

Most modern neuroscientists agree that specific regions of the mammalian brain are at least partially specialized to support for function specific behaviors. At the same time, we recognize that the brain is much more complicated than the design of an automobile, whereby each component stringently performs a single specific function to support the function of the whole machine. While specialization of function by brain structure In some cases, even single neurons have been found to be selective for single data points, such as the place cell of a rat which are preferentially activated when the rat is positioned in a specific location within a maze (Cushman et al. 2013; Ravassard et al. 2013; Acharya et al. 2016; Moore et al. 2016) or in the human, "face cells" are neurons which are activated only when a particular familiar face is recognized by human research subjects (Viskontas et al. 2009). From the signal processing of a single neuron to the ensemble of a large network of neurons firing together, it is these components of the brain which are ultimately responsible for simple-to-complex behaviors.

In the strictest view of the Cortical Localization theory, one would assert that damage to a brain region responsible for specific behavior, would result in a predictable deficit corresponding to the damaged brain region's behavioral correlate. The strict view of Equipotentiality might assert that function is distributed throughout the brain and any region of brain is capable of support most behavioral functions. These competing hypotheses were investigated and debated by philosophers beginning in the early 18th century (Karenberg 2009), was hotly debated in the 19th century by physicians and early neuroscientists (Pearce 2009), and remains a topic of interest in the literature. had been using information gleaned from post-traumatic and post-surgical case studies which depict cortical maps, the so-called homunculus. These depictions delineate specific regions of the cortex as specific centers responsible for general behavioral functions such as the vision, speech, auditory processing, memory, movement of body parts, and sensory processing for various regions of the body. Work by Lindsley seemed to show that the reticular formation of the brain is responsible for arousal (Lindsley et al. 1950), as cats with lesioned reticular formation slipped into a coma and could not be aroused back to consciousness. Then, an interesting follow-up study by John Adametz replicated these results when he lesioned all reticular nuclei at once, but found that if the lesion was produced in a stepwise manner with three week intervals between partial reticular lesioning, the cats showed normal sleep-wake cycles after the reticular formation was destroyed (Adametz 1959). This indicates that other networks in the brain can support functions for which they were not specialized for prior to injury.

However, lesioning of entire cortical regions does not always result in permanent loss of the corresponding functions. Many studies have shown that at least some spontaneous recovery of function often occurs after focal brain injury such as stroke or experimental focal brain lesion without regeneration of the most injured cortical region (Finger et al. 1971). Spontaneous partial

recovery occurs after traumatic brain injury (TBI) as well, though TBI has both focal and diffuse components. While some apparent functional improvements might attributable to behavioral compensation, whereby related networks adapt existing behavioral functions to make up for those lost by injury. When one limb is impaired, for example, the non-impaired or less impaired limb is relied upon more heavily and used more than before injury, particularly if it is the non-preferred hand. Many tests have been designed so that scientists are better able to measure true functional recovery by deciphering between compensatory behavior and true recovery of function, to measure specific deficits related to learning and memory or somatosensory and motor function (Fujimoto et al. 2004).

Diaschisis:

Regions which tend to show new activation after injury include regions that are structurally connected to and functionally related to the injured network. This reflects diaschisis early after injury, which is loosely defined as the alteration of function in an area or areas of the brain which are connected to the injured brain region. The effects of diaschisis after brain injury has been described in terms of altered metabolism, neuronal activity and excitability, and overall functionality of brain regions remote from the injury site (KEMPINSKY 1958; Von Monakow 1969; Feeney and Baron 1986; Andrews 1991; Buchkremer-Ratzmann et al. 1996; Reinecke et al. 1998; Sutton et al. 2000; Bütefisch et al. 2003; Maggiolini et al. 2008; Nishibe et al. 2010b; Holschneider et al. 2013; Carrera and Tononi 2014; Dalton 2014; Le Prieult et al. 2016). It is not clear, whether, at more chronic stages of recovery, new regions of activation indicate networks which support recovered function or if the diaschisis has a role in remodeling of compensatory networks

Clinical management of moderate-severe TBI

Clinical management of TBI begins in the intensive care unit (ICU) where the focus of interventions is aimed at minimizing secondary brain injury (Algattas and Huang 2013). All moderate-severe TBI patients receive an initial CT scan to diagnose gross pathological features such as skull fracture, midline shift, hemorrhage, sub-dural hematoma, and other features. CT images show acute blood in the brain very well, is relatively inexpensive, and the imaging process is fast (Cooper and Golfinos 2000). Intracranial pressure (ICP) and cerebral perfusion pressure (CPP) are monitored and managed using decompressive craniotomy, mannitol (hypertonic saline), hypothermia and CSF drainage (A Brain Trauma Foundation; American Association of Neurological Surgeons; Congress of Neurological Surgeons 2007). In humans, brain edema formation peaks between 2-4 days post-injury (Cooper and Golfinos 2000). Edema is swelling of a part of the brain that results in either a compensatory compression of another region or an increase in ICP. It is important to monitor and control unwanted increases in ICP because elevations in ICP are associated with poor outcome. However, CPP is the optimal parameter to use (CPP = mean arterial pressure [MAP] – ICP). In this acute phase (<24 hours), experimental treatments such as hypothermia and hyperbaric oxygen therapy have shown mixed results, but have not yet shown enough promise to take to phase IV clinical trials (Algattas and Huang 2013). There is a phase II, multi-institution stem cell therapy study ongoing at the time of the writing of this dissertation to test the potential ability of SB623 (SanBio, Inc.) cells to improve chronic motor deficits from TBI ([online access: 4/4/2017] https://www.centerwatch.com/clinical-trials/listings/). Recently, amantadine became the first pharmacotherapy that has been shown to improve the rate of functional recovery after TBI in a

Phase III randomized, placebo-controlled trial (Diaz-Arrastia et al. 2014). Other compounds including cyclosporine A (immunosuppresant), FK506 (protects mitochondria), erythropoietin, glyburide, growth hormone (steroid hormone), progesterone (steroid hormone), methylphenidate and atomoxetine (increase synaptic dopamine and norepinephrine levels), minocycline (antiinflammatory/antiapoptotic/antibiotic/antioxidant properties) and statin drugs are compounds that have shown some promise in early trials (Diaz-Arrastia et al. 2014).

Pathophysiology of TBI

The pathophysiology of moderate-severe traumatic brain injury is often described in terms of the primary and secondary injury components. The primary injury is caused directly by the initial biomechanical forces associated with injury and results in a cascade of biochemical reactions. The force of injury and displacement of affected brain regions and injury to axons, lost synaptic integrity, and produces a generally neurotoxic environment. At this acute stage, metabolic crisis is an additional cellular stressor. At 36 hours after injury there is a drop in much needed glucose fuel, as it is used up during generalized hyperactivity/hypermetabolism after injury, with an accompanying increase in metabolic fuel demand, resulting in greater cellular loss (Vespa et al. 2005). The availability and utilization of oxygen was not a limiting factor except for in the most severe traumas, from which most do not survive (Vespa et al. 2005). Differing from stroke injury in this way, these data indicated that ischemic injury is not a prominent feature of TBI. This information provides researchers a target of metabolic supplementation to protect cells from initial metabolic crisis. In the lateral CCI rat model of TBI, as used in the experiments presented herein, metabolic crisis occurs within 2-4 hours after injury (Xu et al. 2011). Further, the use of alternative fuels have shown some promise as neuroprotective treatment in the research setting (Sutton et al.

1993, 1994, 2000; Moro and Sutton 2010; Deng-Bryant et al. 2011; Moro et al. 2011; Moroa et al. 2013). Unfortunately, this approach has proved challenging, as more than 30 clinical trials in this area have failed (Loane and Faden 2010). Continued research in this line of research is clearly needed, as protecting cells from the acute insult would ultimately result in optimal recovery, as it would attenuate subsequent secondary injury.

The secondary injury features a strong immune response and neurodegeneration preceeding the initiation of programs of neuroplasticity and neural repair. Damage to the cerebral vasculature and neuronal cell bodies caused by TBI activates innate immunity and intrusion of immunocytes into the damaged brain. This initiates production of pro-inflammatory prostaglandins and cytokines with subsequent production of antibodies and proliferation of lymphocytes and activation of microglia (Gendelman 2014; Loane and Kumar 2016). Axonal transport is impaired, resulting in axonal swelling and disconnection from 1-7 days post-CCI in the rat (Chen et al. 2003). Axotomy results in Wallerian-like degeneration (Coleman and Freeman 2010). It is also important to note that some populations of axons are also more vulnerable than others. For example, the unmyelinated axons are more susceptible to secondary injury than myelinated axons (Reeves et al. 2005). Axotomized neurons showed decreased excitability while intact pyramidal neurons showed an increase in excitability within intact neurons (Greer et al. 2012). Since post-injury pathologies are specifically more damaging to certain network types or network components, they may each result in specific behavioral impairments at different times throughout recovery. In addition to the inflammatory response and axonal degeneration, epigenetic factors such as altered expression of micro RNAs (miRNAs), which are known to be involved in apoptosis (programmed cell death), inflammation, and cell proliferation (Bhalala 2015). Reduction or elimination of secondary injury would reduce the burden of recovery support in the chronic

stages after TBI, which are characterized by various mechanisms of enhanced neuroplasticity and spontaneous functional recovery.

Common models of lateralized traumatic brain injury

There are numerous experimental animal models of lateral brain injury including cryogenic brain lesions, cortical photothrombosis, medial carotid artery occlusion (MCAo), cortical undercut, blast injury, acceleration/deceleration models, weight drop models, fluid percussion injury (FPI), and controlled cortical impact (CCI). The most commonly used TBI models are FPI and CCI. For the studies presented here, the rat CCI model was utilized.

FPI is a diffuse model of TBI which exhibits damage and sheering of long-range white matter axons. Axonal damage disrupts axon transport and leads to cell death and disconnection between the affected networks in the brain. The FPI device produces injury with a brief pressure wave (usually set between 8-30ms in duration) of physiological saline in contact with the intact duramater. The pulse of saline briefly compresses the brain. Because the mechanical injury is diffuse, it causes damage not only to the underlying cortex but to subcortical structures as well. Thus, one reason that FPI is often used to study post-traumatic effects on spatial memory and fear learning is that its damage can reach deeper structures including the hippocampus and amygdala.

The CCI results in a more focal zone of gross mechanical injury compared to the FPI. Instead of using a pulse of water to briefly compress the brain, the CCI device employs the use of a pneumatic piston to drive an impactor tip into the intact dura. While white matter damage and still occurs through the stretching and sheering force of the impact, the damage is more localized. Far from being a true focal injury model like the cryogenic and ischemic stroke models, neurodegeneration occurs broadly within the peri-lesional cortex, ipsilesional thalamus,

homotopic contralesional cortex, and bilaterally across the corpus callosum beginning as early as 24 hours post-CCI and continuing through post-injury day 7 (Hall et al. 2005). The significant population of patients with upper limb impairments are most closely modeled with the CCI injury model employed herein. However, the study was designed with the goal of gaining a broader understanding of remodeling of brain circuitry after TBI. For example, gaining a better understanding of how contralesional cortical networks are affected by injury may provide insights into contralesional changes which might occur in response to a hippocampal injury, affecting memory rather than sensorimotor function. The unilateral CCI injury of the left sensorimotor cortex for the forelimb (S1FL) was used for these studies because it allowed longitudinal study of the evoked map for investigation with both fMRI BOLD and *in vivo* electrophysiological techniques.

The sensorimotor neural circuit

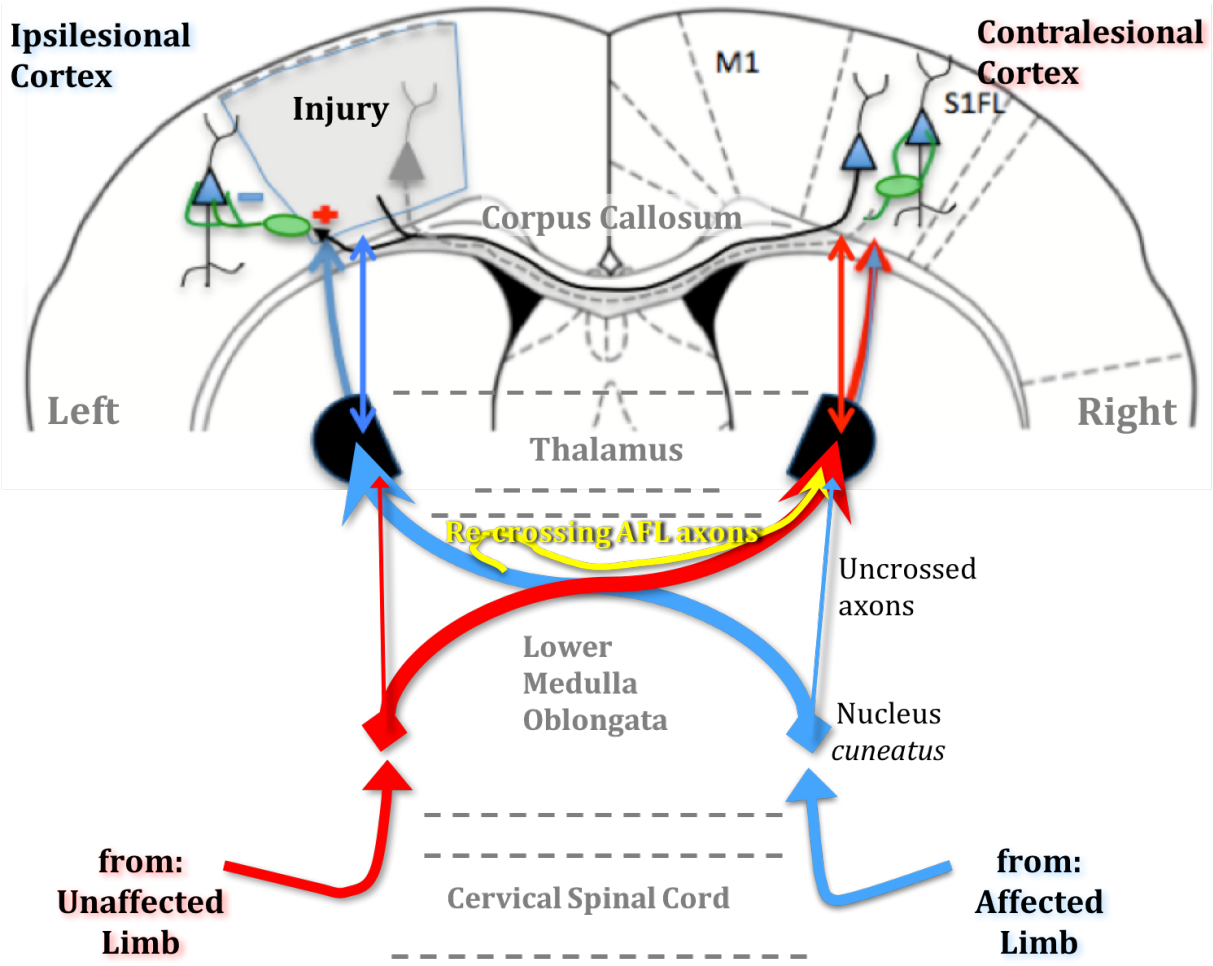


Figure 1. Ascending somatosensory pathways: Posterior columns

The somatosensory pathway from the forelimb to the S1FL cortex

The posterior columns and medial lemnisci convey impulses concerned with well-localized touch the sense of movement and position. Critical for temporal and spatial discrimination, the posterior columns make it possible for a person to hit an incoming baseball with a bat, ride a bicycle, or to type out the words of a dissertation. The posterior columns make it possible to visualize the position of any part of one's own body without looking. In the somatosensory pathway from the rat forelimb to the contralateral S1FL cortex, primary sensory neurons from the

forelimb into cervical spinal cord, rostrally to synapse on cell bodies of the secondary neurons within the *nucleus cuneatus* in the lower medulla. From the cuneate nucleus, the majority of the secondary neurons decussate in the medulla as the internal arcuate fibers and ascend to the ventral posterior-lateral nucleus of the thalamus (VPL)(Edeline 1999). However, some of the fibers do not cross, but instead ascend to the ipsilateral VPL **(Figure 1)**. After injury, fibers have been shown to sprout from the injury affected path, back across the midline to the contralesional thalamus **(Figure 1, yellow arrow)**. The tertiary VPL axons then travel through the internal capsule and radiate to the S1FL cortex via the *corona radiata*.

Learning transfers between cortical hemispheres of the brain

Bilateral brain activity occurs normally in lateralized forms of learning such as sensory motor in the uninjured brain. Some have shown that recovery of function depends upon contralesional cortex (CLCtx) activity after lateral brain injury. For example, persistent excitatory electrical stimulation of the CL motor cortex improves motor function chronically following experimental cortico-spinal tract (CST) lesion in the rat (Carmel et al. 2014). Following this gain-of-function experiment, the group went on to show that stimulated CLCtx was also the structure which became critical for supporting recovery with a direct loss-of-function-like perturbation study. They found that silencing activity of the contralesional motor cortex with $GABA_A$ agonist, muscimol reinstated the forelimb deficit only in brain stimulation-treated rats (Carmel et al. 2014).

Injury severity-dependence

Severity of unilateral injury may dictate the degree to which affected-side recovery is dependent on the CLCtx. When recovery was enhanced with four weeks of "enriched-

rehabilitation" (ER) after either large or small experimental ischemic stroke in the rat forelimb cortex, pellet reaching success was greater in rats with larger lesion volumes (Biernaskie et al. 2005). This comes as no surprise when considered within the framework of the specialization-of-function-by-cortical-location hypothesis. Both large and small lesion groups showed steady recovery of limb reaching ability throughout the 4 weeks of ER (Biernaskie et al. 2005).

If gains in functional recovery are dependent upon the peri- or ipsilesional cortex, silencing activity of the ipsilesional sensory motor cortex (iSMC) would produce an impairment to pre-ER levels. However, if gains in functional recovery are dependent upon the uninjured, contralesional cortex, silencing activity of the contralesional sensory motor cortex (cSMC) would predict return to pre-ER levels. Beirnaskie et al. found that silencing the contralesional cortex (CLCtx) significantly affected reaching performance in the small and large infarct groups as well as uninjured controls (Biernaskie et al. 2005). By week 4, ER reinstatement of limb deficit using CLCtx silencing was more devastating in the large compared to the small lesion group (Biernaskie et al. 2005). Reliance on the CLCtx for functional recovery could depend on the degree of irreversible damage to primary ipsilesional somatosensory cortex (iSMC). In the case that the TBI obliterates the entire primary SMC for the forelimb, remaining and recovered forelimb post-injury function might depend on a related intact para-lesional (S2, M2, barrel fields, or hindlimb regions). Restored function might also result from connected and related brain regions in the CLCtx. In the following studies, we investigated the spatial and electrophysiological changes in both the ipsilesional and contralesional hemispheres to better understand how lesion severity influences broad post-injury remodeling.

Brain activations correlate with functional outcomes

Functional Magnetic Resonance Imaging (fMRI) brain oxygen level-dependent (BOLD) activation maps could serve as biomarkers to improve diagnosis, prognosis, and treatment options after TBI (Lotze et al. 2006a, 2006b). The present studies seek to provide a clearer idea of how variables such as injury severity, lesion volume, and recovery time, affect how the ipsilesional and contralesional hemispheres remap during the acute and chronic recovery periods. Due to the large variability in the mechanisms of injury and resulting symptom in human patients, it is expensive and technically difficult to gather functional and structural MRI data from a sufficiently large patient sample size exhibiting very similar injuries and symptoms. To reduce such variability in these studies, the CCI was chosen because it is a commonly used rodent TBI model. The time-dependent fMRI signature provides a biomarker for progress of brain remapping over time after TBI. Functional brain activation maps might eventually help inform practitioners how to utilize modulation of brain activity, termed neuromodulation, to improve the brain's ability to optimally remodel to support the best possible functional outcomes.

MRI: Basic physics of the MR signal, functional MRI and the BOLD signal

Functional magnetic resonance imaging (fMRI) is widely used to map brain activity. Although it was developed about 29 years ago, it has become one of the most prominent brain mapping tools. With technological advancements like increasing magnetic field strength and innovations in both scan sequences and image analysis tools, fMRI continues to help researchers provide answers to critical research questions in neuroscience. FMRI works by detecting the level of blood oxygen throughout the brain over time. The signal detected by MRI is called the blood-oxygen-level-dependent (BOLD) signal. The BOLD signal comes from the magnetic properties of deoxyhemoglobin, which causes a small magnetic resonance signal. Increases in blood flow to a

particular region of brain result in a time dependent increase in signal within that region. Creation of brain activation maps relies on the fact that increased neuronal activity results in a corresponding spatially and temporally-dependent increase in blood flow. The rectangular volume of brain imaged is broken into a 3-dimensional matrix of equivalent volumes called voxels. For example, in the data presented in this dissertation, the data is composed of 14 consecutive images which are 128x128 voxels per image. Analysis of the resulting 4-dimensional data is essentially done by comparing the oxygen level at each voxel between the task "on" or task "off" condition. For example, in the present studies, forelimb stimulation is the task and images acquired during trains of forelimb stimulation compared to images acquired in the "off" or "rest" condition in which no forelimb stimulation occurs. The data sets are very large and the statistics involved are made more difficult because of the statistical necessity to make proper corrections for the many multiple comparisons that must be made for determination of statistical significance. The fMRI sequence used for the experiments presented in this dissertation acquired an image of the brain every two seconds for 360 seconds, or 180, 3-dimensional images. So, for the example above, each 3-D image collected 128x128x14 = 229,376 data points 180 times, which results in more than 41 million data points collected from each rat.

One challenge in statistical analysis of fMRI data is accounting for temporal autocorrelations, which is handled by analysis software FSL (fsl.fmrib.ox.ac.uk/fsl) by a process called "pre-whitening," whereby autocorrelations are removed by temporal filtering with known autocorrelations (Woolrich et al. 2001). Spatial autocorrelations were corrected using spatial smoothing of the autocorrelation to further improve the estimation (Woolrich et al. 2001). Recently, there is new experimental evidence that the autocorrelations used by common fMRI data analysis software could be producing inflated false-positive rates (Eklund et al. 2016). This is

because current analyses pipelines assume that the data fit a Gaussian distribution (Eklund et al. 2016). Instead, the use of nonparametric permutation test methods, which do not require that assumptions be made about the normal distribution of the data. The disadvantages of using non-parametric methods include incomplete utilization of the data, the results are more difficult to interpret, analytical methods are limited, and statistical power is lower (Nahm 2016). This issue not only applies to fMRI data, but a great deal of published biological data, which is often not normally distributed and sample sizes are often limited (Nahm 2016). It is important to note that the analysis in this study was performed with the FSL FEAT tool box. Therefore, there is a potentially increased risk of observing some regions of activation which are a result of autocorrelation and violation of assumptions of normality in the data sampled. It is possible that some time soon, some peer reviewed journals may require that published data be analyzed using nonparametric permutation tests.

A time window of enhanced neuroplasticity may occur after TBI

Reorganization of neural networks within the injured hemisphere is suggested by altered cortical maps (Lotze et al. 2006b; Kasahara et al. 2009) and altered functional connectivity (Harris et al. 2016b) after TBI. There is strong evidence that neuroplasticity is enhanced after TBI and, perhaps more importantly, neuroplasticity is more easily enhanced during a period of time after injury which can take the form of axonal sprouting, dendritic remodeling, altered neuromodulatory systems such as changes in receptor populations (Jørgensen et al. 1997; Biernaskie and Corbett 2001; MacDonald et al. 2007; Nishibe et al. 2010a; Harris et al. 2010a, 2013a, 2013c; Chen et al. 2011; Greer et al. 2011a; Lee et al. 2011; Machado et al. 2013; Addington et al. 2015; Song et al. 2016). These studies also showed that there are several approaches to enhance plasticity after

injury, including pharmacological agents, exercise, training, enriched environment, or brain stimulation, to name a few. Enhanced neuroplasticity within the cerebral cortex have been found to occur during times of altered balance between inhibition and excitation within the cortex (Fujioka et al. 2004; Benali et al. 2008). However, not all increases in post-injury plasticity will result in improvements in functional outcome (Harris et al. 2010a; Wang et al. 2011). Indeed, some forms of post-injury plasticity are maladaptive and result in post-traumatic epilepsy, characterized by sponteneous chronic recurrent seizures (D'Ambrosio et al. 2004, 2005, 2009b; Hunt et al. 2009; Yang et al. 2010; Kharlamov et al. 2011). Therefore, more research must be done in this area, as it may be one key to solving both post-traumatic epilepsy and plasticity which supports functional recovery after TBI.

The balance of trans-callosal inhibition is disrupted in lateralized TBI

Basic mechanisms of trans-callosal cortical inhibition have been characterized in the normal, uninjured vertebrate brain (Ferbert et al. 1992; Meyer et al. 1995; Gerloff et al. 1998; Li et al. 2011). Silencing of activity in the rodent forelimb motor cortex with the sodium channel blocker lidocaine caused a 42.2% expansion of the forelimb representation in the opposite cortex (Maggiolini et al. 2008). This data agrees with the widely-supported notion that each hemisphere works constantly to maintain the forelimb representation of its contralateral, homotopic counterpart, and that this maintenance is achieved through mostly inhibitory influences (Ferbert et al. 1992; Meyer et al. 1995; Gerloff et al. 1998; Li et al. 2011). According to this basic mechanism, injury to one cortical hemisphere would result in dysfunction of that injured area imparting disinhibition of its contralesional, uninjured counterpart. Conversely, if the uninjured, CLCtx is

disinhibited it could become hyper-excitable, as observed in the PP-SEP experiments for **Aim 1** (upcoming **Figure 6**). Indeed, after a focal, cortical ischemic lesion, use of the unaffected forelimb results in reduced ILCtx-to-CLCtx inhibition and increased CLCtx-to-ILCtx inhibition (Rehme et al. 2011) but it is not known whether the brain's response to TBI is like that of stroke in this regard. Any differences might provide insights into the source of the difference in terms of differences between the disease pathophysiologies.

Overview and Specific Aims:

Traumatic brain injury (TBI) is a leading cause of death and disability throughout the world, with at least 1.7 million new cases reported annually in the United States alone (Loane and Faden 2010). Recovery after TBI remains of foremost concern, as incomplete or prolonged recovery is common. Recent estimates indicated that 5 million TBI survivors in the United States require physical rehabilitation for sensorimotor (SM) deficits (Kozlowski et al. 2013). Although some deficits resolve spontaneously over time after brain injury, recovery is often limited. In a follow-up study of 67 moderate-severely injured TBI patients, for instance, 30% still had upper limb impairments at five years (Hillier et al. 1997). After SM cortex injury, various brain regions may exhibit new "abnormal" functional activation throughout recovery, including regions around the injured cortex, and surprisingly within the opposite, uninjured cortical hemisphere (Lotze et al. 2006a). It is unclear whether newly activated brain regions are causally involved in facilitating spontaneous recovery after TBI. Non-invasive brain imaging and electrophysiological techniques may serve as important biomarkers of the brain reorganization associated with the most optimal functional recovery. If distant networks are best suited to support optimal functional recovery for permanently damaged brain regions

Clear understanding of both the time-dependent brain activation map changes and mechanisms that enable the brain to dynamically reorganize to support recovery of function after TBI remain elusive. While some studies have been performed in rat models of lateralized focal ischemia, ablation, and electrolytic lesion, little is known about TBI-associated functional remodeling within the opposite, contralesional hemisphere. Clearly, more work in this area of TBI research because trauma exhibits unique etiological features. The overall goal of this thesis project was to determine whether the uninjured, contralesional cortex (CLCtx)--located in the opposite, or

"homotopic" cerebral hemisphere—mirroring the site of brain trauma—plays a role in recovery of function following lateral controlled cortical impact injury (CCI) in the rat. In **Aim 1** of the present study, the temporal involvement of the injured and contralesional cortical hemisphere was studied throughout the first 4 weeks of recovery in terms of changes in both the cortical activation maps **(Aim 1A)** and the underlying neurological activity **(Aim 1B)**. **Aim 2** sought to determine the causal role of the non-injured, CLCtx in spontaneous functional recovery of the injury-impaired forelimb.

The CLCtx may be an important target for therapeutic intervention after TBI

A time window of enhanced brain plasticity following TBI has been documented (Griesbach et al. 2007, 2009, Harris et al. 2010a, 2013b, 2013c; Nishibe et al. 2010b; Greer et al. 2011b; Campbell et al. 2012). Potential for the brain to make more substantial anatomical and physiological changes after TBI may be a substrate for recovery from functional deficits. Within the injured cortex, surviving neurons have been shown to sprout axons in order to remodel connections lost to injury throughout the acute period (Harris et al. 2010b). Enhancement of axonal sprouting in the ILCtx did not enable the brain to regenerate neural networks lost to injury to support recovery of function (Harris et al. 2010a). However, after CCI in the adult rat, an initial up-regulation of plasticity-associated markers after injury subsides in the injured, ipsilesional (ILCtx) and hippocampus 21 days after injury (Griesbach et al. 2009). ILCtx may be most amenable to reorganization within three weeks post-injury in the CCI injury model of TBI in the rat. Correspondingly, much of the spontaneous recovery occurs within the first several weeks after injury in the rat. As with life span, the human the time scale of functional recovery is longer. It is difficult to make some comparisons between humans and rats (Xiong et al. 2014; Failla and Wagner 2015), as their life spans are much shorter (~3 years). Interestingly, at 21 days after CCI,

synapsin and BDNF are increased in the CLCtx, implying that the potential for contralesional plasticity is enhanced throughout the early recovery period after TBI (Griesbach et al. 2009). However, during more chronic recovery periods, the potential for return of function diminishes as the time window of injury-induced plasticity closes (Griesbach et al. 2007, 2009). Interventions that enhance or extend the period of post-traumatic plasticity may provide potential for better recovery (Harris et al. 2010a, 2010b), but no such treatment has been approved for TBI patients yet.

The temporal component of the CLCtx had not yet been investigated longitudinally in the rat. To determine how the role of the CLCtx might change over time, function was tested with and without silencing activity of the CLCtx both acutely and chronically after injury. The results of such studies would help inform the future directions for altering activity and plasticity in specific brain regions at various time points throughout recovery. If the uninjured, CLCtx is found to support re-learned function for the injured, ILCtx therapeutic intervention to enhance plasticity in this region might be a beneficial approach. At this point, most experimental interventions aimed at enhancing plasticity have focused their attention mainly within the peri-lesional cortex or more distant sites within the injured cerebral hemisphere. In the case our studies indicated that activation from this region impedes functional recovery, the goals of therapeutic intervention might be best directed toward decreasing activity and plasticity in this region throughout recovery, while up-regulating plastic potential in the perilesional or other cortical regions. The overall goal is to determine if the CLCtx is supportive of renewed function in early and late recovery. In pursuit of this overall goal, the spatio-temporal alterations in the cortical map were first characterized. The role of the CLCtx regions identified by fMRI in spontaneous recovery of affected forelimb dexterity was then determined by CLCtx inactivation, and sought to determine which brain regions

mediate altered function by transiently silencing CLCtx activity at both the acute and chronic time points.

Could the use of neuromodulation improve therapeutic gains?

We know that injury to one part of the brain affects activity of not only those regions directly affected by the initial mechanical injury, but of more distant brain regions functionally connected to the injury cite. One such site is the uninjured, CLCtx. Functional MRI data have shown that interaction between the injured ILCtx and the CLCtx is altered throughout recovery from cortical stroke(Rehme et al. 2011, 2012; Volz et al. 2015). Stroke lesioning studies have reported increased excitability in the CLCtx (Buchkremer-Ratzmann et al. 1996; Reinecke et al. 1999; Mohajerani et al. 2011). Hyperexcitability after stroke is thought to be due, at least in part, to a disruption of the inhibitory, GABAergic networks. In support of this stroke studies have reported reduced $GABA_A$ receptor binding after experimental (Qü et al. 1998) and clinical stroke (Bütefisch et al. 2003). Modulation of medial prefrontal cortical GABAergic (inhibitory) systems have been found to improve working memory deficits within the first month following lateral CCI in the adult rat (Kobori and Dash 2006). In the first several days following injury in the rat, however, constraint-induced movement therapy (forced use of the affected limb often coupled with restraint of the non-affected limb) results in greater cell death and increase in lesion volume (Kozlowski et al. 1996). To enhance the effectiveness of rehabilitative therapy one strategy is to use the brain's innate ability to modify remaining networks to support recovery. In more severe injury, such as stroke or contusive TBI, adjacent regions of surviving perilesional cortex and other more distantly connected cortical regions within the injured hemisphere are thought to remodel in order to compensate for lost function of injured tissue after either experimental stroke (Carmichael

et al. 2001; McNeal et al. 2010) or TBI (Harris et al. 2010a). However, some have speculated that CLCtx activation is maladaptive or an indicator of poor outcome, since better recovery of limb function is achieved in stroke patients in which activation has returned to the ILCtx (Dijkhuizen et al. 2003). Functional magnetic resonance imaging (fMRI) data acquired from stroke patients shows that the CLCtx is more functionally active during motor activity of the impaired hand, even after full clinical recovery (Riecker et al. 2010), suggesting that a more permanent reorganization of networks inluding the CLCtx contributes to recovery. Compared with stroke, far less is known about the role of the CLCtx in TBI recovery. Functional recovery after unilateral TBI, where white matter injury is a signature feature, is accompanied by distinct cortical remapping and physiological adaptations in both the injured and uninjured cortical hemispheres. Determination of how CLCtx networks are involved in functional recovery might aid in future studies.

These studies were designed to address the following specific aims:

- **Specific Aim 1: To investigate bilateral, longitudinal changes in the limb-evoked sensorimotor maps and underlying neurological activity after unilateral TBI in the rat**
- **Specific Aim 2. To determine if the CLCtx is causally involved in recovery of limb function after TBI and whether this is temporally limited.**
- **Specific Aim 3: To identify brain regions involved in affected forelimb activity during silencing of the CLCtx at the acute or chronic recovery periods.**

Chapter 2

Aim 1: To investigate bilateral, longitudinal, **[A]** changes in the cortical map and **[B]** underlying neurophysiological activity in the contra-lesional cortex after unilateral TBI in the rat

Introduction

In the normal mammalian brain, most axons in both the sensory and motor pathways cross the midline sagittal plane as they ascend or descend respectively, between the brain's cerebral hemispheres and their associated parts of the body. Due to the crossing of fibers, cortical representations for the body reside in the opposite cortical hemisphere from the body region to which it connects. However, approximately 5% of primary motor neurons of the cortico-spinal tract remain uncrossed, but they normally do not contribute significantly to skilled reaching in the uninjured rat (Whishaw and Metz 2002). For the spino-thalamic sensory pathways approximately 8% of fibers in the rat, between 5-10% in the cat, and 17% of fibers in the monkey make up the uncrossed (ipsilateral) pathway (Granum 1986; Jones et al. 1987; Apkarian and Hodge 1989). Typically, damage to regions associated with sensorimotor cortex (SMC) in one cerebral hemisphere causes functional impairments on the opposite side of the body (Willis et al. 1979; Soblosky et al. 1996; Nathan et al. 2001; Alwis et al. 2012).

Some bilateral cortical activation is a normal feature of the uninjured brain, shown to occur during tactile learning (Harris and Diamond 2000). A large portion of brain activation ipsilateral to the stimulated limb is due to activity of inhibitory interneurons responding to input from the opposite hemisphere through the corpus callosum (Kobayashi et al. 2003a). This normal trans-hemispheric inhibition dampens mirror movements and enables functional independence between

the right and left cortical hemispheres allowing lateralization of function. It is possible that a basic sensorimotor imprint exists on the homotopic contra-lesional cortical hemisphere, enabeling the intact contralesional cortex to take on sensory processing and motor control for the affected limb. Local cerebral blood flow (LCBF) autoradiographic studies in a controlled cortical impact (CCI) rat model of unilateral TBI revealed a significant increase in limb-stimulation-evoked contra-lesional cortex (CLCtx) activation at 4, 7, and 30 days post-injury (Harris et al. 2013a). The CLCtx is the uninjured cortical region in the same location as the injury, in the opposite cortical hemisphere. Early recovery of the affected forelimb was temporally correlated with new areas of activation spread diffusely across CLCtx, the cortical hemisphere contralateral (on the opposite cortical hemisphere) to the injury site, while a return of ipsi-lesional (on the same hemisphere as the injury) activation was observed at 30 days (Harris et al. 2013a). CLCtx activation after brain injury evoked by the injury-affected limb has been reported in both clinical (Riecker et al. 2010) and experimental stroke studies (Dijkhuizen et al. 2001; Ward et al. 2003), but far fewer studies have investigated this phenomenon after TBI. At least with stroke injury, greater prevalence of contralesional activation has been reported in patients with more extensive lesions during both the acute (Biernaskie et al. 2005; Hsu and Jones 2006) and chronic (Carey et al. 2002; Feydy et al. 2002; Biernaskie et al. 2005; Calautti et al. 2007; Dong et al. 2007; Riecker et al. 2010; Jang 2011) phases of stroke recovery. However, even less is known about alterations after TBI, and due to the paucity of fMRI studies in TBI, the forelimb sensory map changes. The biomechanical forces associated with TBI cause stretching and shearing of both myelinated and unmyelinated axons in white matter, likely contributing to a global injury, and heterogeneous outcomes associated with TBI. The larger, unmyelinated axons of the white matter are particularly susceptible to irreversible damage from diffuse axonal injury (DAI)(Reeves et al. 2005). While stroke injury features a major

ischemic component, except in the most severe, and often fatal cases, TBI does not (Vespa et al. 2005). Also, despite growing evidence of a trans-hemispheric shift in activation both in unilateral stroke and TBI, the CLCtx shows significant time-dependent changes in dendritic plasticity after experimental stroke (Jones 1999), but not after unilateral CCI, when researchers used the MAP2 staining for histological quantification of dendritic spines (Jones et al. 2012). To my knowledge, careful longitudinal assessment of somatosensory map changes throughout recovery from unilateral CCI injury has not yet been performed. Based upon pilot data and that published in the literature, it was anticipated that CCI injury in the rat would initiate new affected forelimb-evoked CLCtx fMRI activation patterns that evolved throughout recovery.

Neurovascular coupling: BOLD signal reflects activation of neurons

The neurophysiological signal underlying altered CLCtx activation after TBI is also not well understood. Both LCBF autoradiography and fMRI measure temporal changes in blood flow, which increases by dilation of small, localized arterial blood vessels, in response to the increased metabolic and oxygen demand from increased neuronal activation. Despite the vastly different technologies employed in these techniques, given the similarity of the essential measures, similar results from fMRI studies were expected. The blood oxygen level-dependent (BOLD) signal measured by fMRI provides a measure of neuronal activity over both space and time (Logothetis et al. 2001). Changes in blood flow have been shown to correlate positively with excitatory neuronal activity in the normal human and rodent cortices (Ureshi et al. 2004; Mishra et al. 2011; Devonshire et al. 2012). With the exception of only a few non-cortical regions including the caudate and putamen, the assumption of linearity apply when interpreting BOLD activation data (Mishra et al. 2011). In the primary structures of interest, in particular, the sensorinotor cortex and

thalamus, the linear relationship between forelimb stimulus frequency and both neuronal activity and blood flow have proven reliable (Kida and Yamamoto 2010; Mishra et al. 2011; Nasrallah et al. 2011; Devonshire et al. 2012).

While both fMRI and EEG can give us some information regarding brain activity, each technique features a different set of strengths and weaknesses. Overall, in regard to event-related (i.e. somatosensory stimulation-evoked) brain activation, fMRI data provide much better spatial resolution while EEG provides superior temporal resolution. Together, these two techniques complement one another to provide a more comprehensive look at brain activity. For this dissertation, electrophysiology was performed in two parallel series of rats to investigate the neuronal activity underlying the dynamic BOLD activation maps throughout the first four weeks after injury.

Materials and Methods:

Experimental Protocol:

Two separate cohorts of male, adult rats were used to acquire either somatosensory evoked potential (SEP) electrophysiology recordings (n=8 rats) or somatosensory evoked functional magnetic resonance imaging (fMRI) data (n=11 rats) before and at 7, 14, 21 and 28 days after controlled cortical impact (CCI) injury. All experimental procedures were approved by the UCLA Institutional Animal Care and Use Committee.

Brain Injury:

Adult male Sprague-Dawley rats (320-350g) were acquired from Charles River Breeding Labs, (Hollister, CA), housed two per cage, and acclimated to standard housing conditions for one week prior to their use as experimental subjects. Throughout the study, rats were allowed access to rat chow (LabDiet Inc., St. Louis, MO, USA) and water *ad libitum.* Animals were anesthetized with isoflurane (3% induction, 1-1.5% for maintenance) vaporized in oxygen flowing at 0.8 L/min. The scalp was shaved after which it was placed in a stereotaxic framed (Stoelting Co., Wood Dale, IL, USA). Body temperature was maintained at 37±0.5°C throughout the surgery using a thermostatically-controlled heating pad. After cleaning the scalp with alternating betadine and ethanol scrubs, 0.1ml of the local anesthetic bupivicaine was injected subcutaneously at the midline of the scalp before the skull was exposed via a midline incision. A 5mm diameter craniotomy was made using a dental drill with intermittent perfusion of sterile saline to prevent heating of the skull caused by drilling. A surgical microscope was used during exposure of the left hemispheric sensorimotor cortex centered at 0mm from Bregma in the anterior-posterior (AP) plane and -3mm lateral (ML) from the midline (over the left cerebral hemisphere. Injury was induced by controlled cortical impact (CCI) whereby a 4mm diameter (Ø), flat, circular impactor tip was advanced through the craniotomy tangentially onto the dural surface at 20psi (2.2m/s) or 30psi (2.8m/s) to a depth 2mm below the dural surface with a 250ms dwell time, resulting in a left-side injury of the primary sensorimotor cortex for the right forelimb (S1FL Ctx). Animals were randomized to mild-moderate (20psi or ~2.2m/sec) or moderate (30psi or ~2.8m/sec) groups prior to CCI surgery. Images were acquired through the microscope for all injuries for later verification of the presence of a sub-dural hematoma. The craniotomy was then sealed with a layer of non-bioreactive, Kwik-Cast silicon elastomer (Sarasota, FL, USA) and the scalp was re-sutured and covered with a layer of bupivicaine local anesthetic solution and triple antibiotic ointment.

Animals were placed in a recovery chamber with ambient temperature maintained at ~28°C until they awoke from anesthesia after which they were returned to their home cages. No mortalities occurred due to acute post-surgical complications.

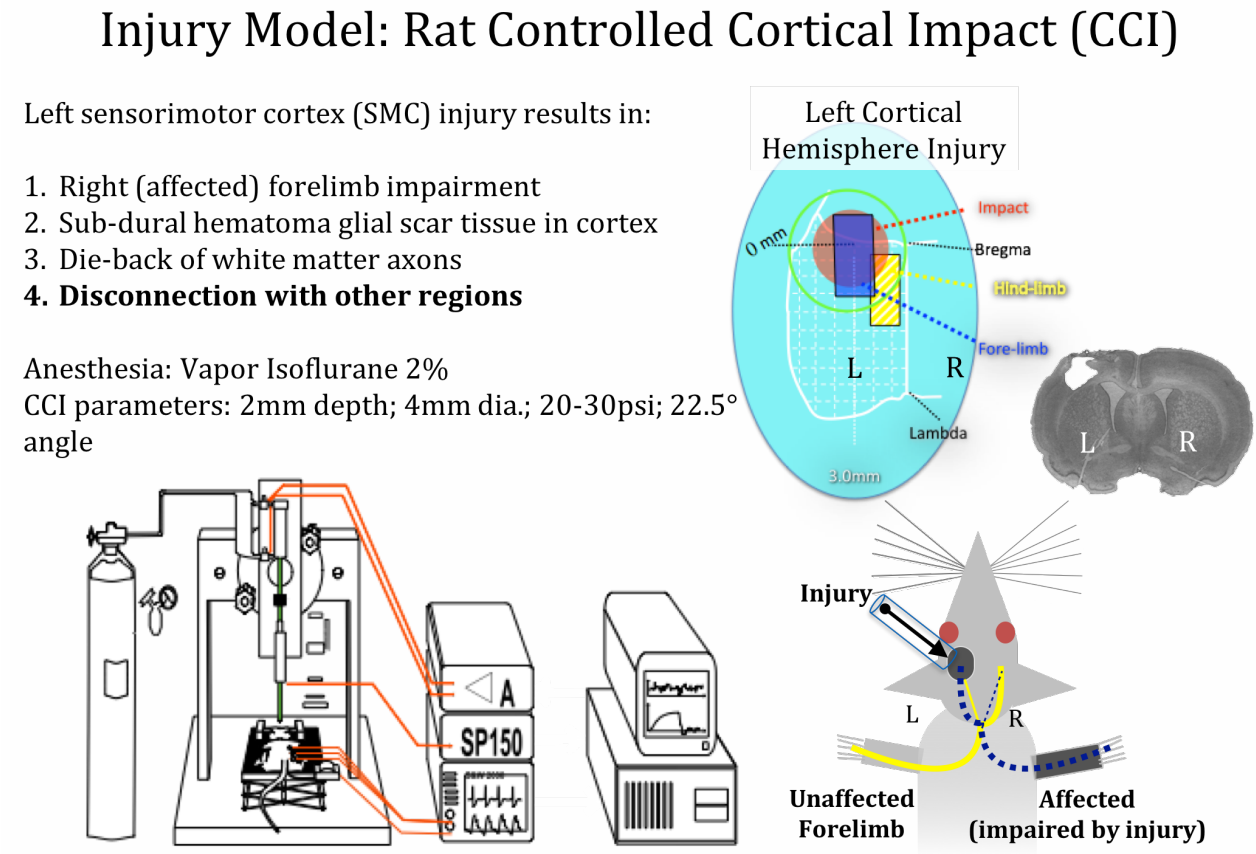


Figure 2. The controlled cortical impact (CCI) injury model in the rat used for the experiments presented here utilizes a pneumatic piston to produce a contusion injury directly to the left forelimb sensorimotor cortex, which controls the right forelimb (including forepaw). The piston—set at either 20psi (moderate) or 30psi (moderate-severe injury) impacts the intact duramater at a 22.5° lateral angle, 2mm depth, with a flat 4mm diameter impact tip.

Injury severity assessment in vivo & ex vivo

Differences in cortical lesion size were compared across our subjects using Tensor-Based Morphometry (TBM), a technique developed by Paul Thompson and colleagues (Hua et al. 2011,

2013). TBM measures local volumetric differences between two images by analyzing the Jacobian matrices of displacement fields resulting from registering the images. In our case, a non-linear atlas was constructed from the data determinant of each subject over this region to compute a univariate measure of injury severity. As the Jacobian determinant represents the fractional volume change required to match the average image to each subject, this was found to be a good measure of relative injury severity for our dataset, producing results similar to those measured by microscope (Zeiss, Switzerland) from post-mortem tissue sections.

Magnetic Resonance Imaging:

A 7 Tesla Bruker MR spectrometer running Paravision 5.1 (Bruker, Billerica, MA, USA) was used to acquire structural and functional data before, and at weekly intervals after brain injury. Rats were briefly anesthetized with 4% isoflurane in oxygen flowing at 0.6l/min in order to administer dexmedetomidine sedation (0.05mg/kg in sterile saline (Williams et al. 2010) via the penile vein. Following placement of a subcutaneous cannula 3cm above the base of the tail dorsally. Continuous rate infusion (Hamilton syringe pump) of dexmedetomidine (0.1mg/kg/hr) commenced as the rat was transferred to a purpose-built cradle and secured using three-point immobilization of the head with two ear bars and a tooth bar. Respiration was monitored remotely and temperature was thermostatically-controlled by forced air (SA11 Instruments, Inc., USA) through the bore of the MRI scanner. The S116 Bruker gradients (400mT/m) were used in combination with a birdcage transmit and an actively decoupled receive-only surface coil to acquire the data. Following a multi-slice, gradient echo pilot scan to optimize positioning within the magnet, localized shimming was performed on the head to improve B0 homogeneity. fMRI: a multi-slice, gradient-echo, single shot, echo-planar imaging sequence (repetition time (TR)=2s, echo time (TE)=30ms, no averages and using 14×0.75mm thick slices with a 30mm2 field-of-view

(FOV) and a data matrix of 128 read by 128 phase-encoding steps) was used to acquire brain oxygen level-dependent (BOLD) signal data during repeated electrical stimulations (10 Hz, 3.5 mA, Grass Instruments, Warwick, RI, USA) of affected and unaffected forelimbs in separate experimental trials, each with an "off-on-off" block design repeated thrice with each block consisting of 60s-off, 40s-on, and 60s off.

Limb stimulation was elicited through a pair of steel electrodes positioned subcutaneously at the 1st and 4th pad of each forepaw. Electrodes were placed while the rat was under the initial period of isoflurane anesthesia and tested by verifying hand movement upon stimulation. FMRI data acquisition began at 20-30mins after the termination of the initial 10min period of isoflurane anesthesia. After two runs of stimulus-evoked fMRI acquisition, isoflurane anesthesia was reinstated and dexmedetomidine infusion was discontinued. *Structural images:* A 2-dimensional rapid acquisition with relaxation enhancement (RARE) sequence was used to collect T2-weighted images with the following parameters: TR = 5000 ms, TE = 60 ms, RARE factor = 8, 2 averages and using 14x0.75mm thick slices with a 30mm2 FOV and a data matrix of 128 read by 64 phase-encoding steps in a total time of 8 mins. At the end of the imaging session sedation was reversed using antisedan (1.0mg/kg, intraperitoneally). Rats recovered to the awake state and resumed exploratory behavior in their home cages within two minutes following this reversal injection.

Image Analysis:

fMRI: Data were weighted Fourier transformed to 128x128 and converted to compressed NIFTI format for entrance into the FEAT analysis pipeline (FSLtools, (Worsley 2001). First-level analysis was run after brain extraction, motion and slice timing correction, 5mm spatial smoothing and a high-pass filter. Data were analyzed using the general linear model by fitting a hemodynamic

response function composed of a square wave according to the stimulus protocol timing, convolved with a single gamma variate function to derive the temporal derivative waveform. Analysis was performed using temporal filtering and using FMRIB's improved linear modeling with pre-whitening while including motioning correction parameters in the model. Data were co-registered to a study-specific FMRI template using FLIRT (Jenkinson et al. 2002) with 12 degrees of freedom and a co-ratio cost function. Statistical maps were cluster thresholded at $z=1.7$ and $p<0.05$ based upon pre-injury data showing robust activation within the sensory-motor cortex. Higher level, group analyses were run between baseline and post-injury data using FMRIB's' local analysis of mixed effects (Woolrich et al. 2004a, 2004b) with automatic outlier de-weighting and with contrasts cluster thresholded at $z=1.7$ and $p<0.05$. Data were displayed on a rat brain template.

Tensor-based morphometry:

Tensor-based morphometry (TBM) was used to measure local volumetric differences at 28 days post-injury as an indicator of injury severity. This was achieved by co-registration of the structural data from all pre-injury and 28d post-injury data to a non-linear atlas constructed from this data. The Jacobian matrices were derived from the displacement field calculated from each image-to-template co-registration. The Jacobian determinant of the matrix is a univariate measure that represents the fractional volume change required to match the template image to each subject.

Sensory-Evoked Cortical Recordings

A parallel series of rats was used to investigate alteration of the cortical sensory evoked potential (SEP) signal before and at various time points after injury. Two electrode montages were used to cover the extent of the CLCtx. Montage A (n=4 rats) consisted of three stainless steel

epidural screw electrodes (Ø=1.19mm including threading) over the anterior aspect of the forelimb sensorimotor cortex (SMC) at stereotaxic (x, y) coordinates relative to the skull-based landmark, Bregma: (2mm,2mm); (1.5mm,-1.mm); and (4mm, -1.0mm. Montage B (n=4) consisted of five screw electrodes placed at: (3mm, 2.0mm), (2, 0mm, (4.5mm, 0mm), (2.0mm, -2.0mm), and (4.5mm, -2.0mm). For both montages, a reference electrode was placed midline in the frontal bone. We began these experiments with Montage A and added two additional electrodes to Montage B and combined the groups for analysis. A hand drill (model DH-1 holder, D#60 bit; Plastics1.com) set to 0.8mm depth and diameter of 1.00mm was used to fine tune the depth and width of the smaller pilot hole so that recording electrode screws could be set into the skull with 1/2 turn or less without pushing the remaining bone into the brain space. These precautions ensured that the dura was not deformed at any time throughout the drilling or electrode placement procedure (D'Ambrosio et al. 2009a). Insulated electrode leads were connected to gold-plated sockets within a plastic pedestal and dental acrylic was used to anchor it to the skull over the electrodes. The location of the skull that would later be removed for CCI injury was covered with silicon elastomer (WP Instruments, Sarasota, FL) and a thin layer of dental acrylic for easy removal. Each montage was implanted one week prior to control recordings and two weeks in advance of CCI injury.

Sensory-evoked potentials (SEPs) were recorded using the MP150 Biopac acquisition system and Acknowledge Software v4.1.1 (Biopac Systems, Inc., Goleta, CA). Paired-pulse SEPs (PP-SEPs) were recorded over the contra-lesional cortex and evoked by electrical stimulation of the unaffected forelimb and while under the same dexmedetomidine sedation as detailed before MRI study. The inter-stimulus interval (ISI) between pulses was varied between 30, 35, 40, 50, 100, and 200ms.

Changes in synchronous neural activity are inferred from changes in local blood flow in the BOLD fMRI signal. Several studies have shown that the hemodynamic response is linearly coupled to the neuronal activity using simultaneous fMRI-EEG techniques in the rat sensorimotor cortex before and as early as 3 days after injury (Ureshi et al. 2004; Mishra et al. 2011; Devonshire et al. 2012). In order to determine if activation of the CLCtx observed in the present fMRI studies were associated with neurophysiological signal, SEPs were measured from electrocorticography (ECoG) electrodes. The decision to use skull-based electrophysiological recordings of local field potential (LFP) without depth electrodes, which would record multi-unit activity (MUA) was based on several advantages of the skull-based epi-dural electrode headset paradigm compared to depth electrode: Spatial consistency, reliability, good signal-to-noise ratio and minimal brain trauma. The electrode headsets enable reliable data acquisition over the exact same locations throughout the longitudinal study duration. Epidermal electrodes, while simple and convenient, struggle with low signal due to their distance from the brain and greater noise artifact from underlying skin, muscle, and bone. Epidermal electrodes provide poor spatial resolution due to their large size compared to the relatively small rat brain, while epidural electrodes are small and could be placed in a tightly spaced grid. Finally, the non-invasive skull-based electrode implantation does not cause cortical tissue damage and inflammation (D'Ambrosio et al. 2009a), whereas micro wire depth electrodes initiate strong astrocyte and microglial activation caused by cortical damage (Paralikar et al. 2009). Electrode damage itself can lead to altered excitability of neurons therein, so the electrodes and implantation procedures were designed to avoid potentially confounding damage in the CLCtx. Still, addition of a subgroup of rats with both depth and ECoG electrodes would have help to determine the relative contributions of both inhibitory post-synaptic potentials (IPSPs) and excitatory post-synaptic potentials (EPSPs) composing the SEP signal data

reported here. This would provide additional information about the local changes in the balance between cortical excitation/inhibition thought to occur after brain injury. This may not be necessary in the future, however, as model-based analysis of LFPs are showing promise for bettering our understanding of the inhibitory/excitatory balance in cellular activity by decomposing the signal into its component inhibitory and excitatory conductances (Zheng et al. 2012).

CCI surgery procedure with electrode headsets

One week after acquiring the baseline, pre-injury PP-SEP recording, rats were anesthetized with isoflurane. The left skull over the S1FL cortex was exposed through the electrode headset using a dental drill to remove the overlying acrylic layer and surgical forceps to extract the underlying silicone elastomer (WP Instruments, Inc.) plug. CCI was then performed through the left side of the open headset as described above. The contused cortex and craniotomy were sealed over with a thin protective layer of silicon elastomer (WP Instruments, Inc.) and the headset was re-closed with pre-mixed dental acrylic. PP-SEP data was acquired prior to injury and at 3, 7, 14, 21, and 28 days after injury.

SEP Analysis:

All 35 acquired time-locked PP SEP epochs of 350ms duration were averaged and subsequently analyzed off-line with Acqknowledge Software v4.1.1 (BioPac Systems, Inc., Goleta, CA). For the more standard paradigm of left (unaffected by injury, non-impaired) forelimb stimulation through the principal pathway to the right (uninjured, contra-lesional) peak amplitudes of the evoked response (N1) were measured from the baseline immediately following the first

(stimulus A) and second (B) stimulus artifacts for N1A and N1B for each ISI at each time point. The paired-pulse ratio (PPR) was calculated as the ratio of the second response amplitude relative to the first (N1B/N1A) to assess modulations in the time-locked evoked amplitude throughout the first month of recovery. This was repeated for each ISI. When PPR=1, the second response is of the same magnitude as the first, indicating that the cortex is neither inhibited nor hyper-excitable at the tested ISI after the stimulus. In the case where the PPR < 1, the second response to the equivalent stimulus input is unable to produce the same response because the cortical neurons are being inhibited by local inhibitory interneurons. When the PPR > 1, the second response is greater than the first, the cortex is thought to be hyper-excitable at the ISI period after the first stimulation. An increase in the PPR over time indicates decreased inhibition (disinhibition) over time.

For SEP waveforms featuring peaks which are broader and less clearly defined, the peak integrals were used as the primary measure of cortical response rather than peak heights and latencies. The post-injury PP SEP recorings in the ipsi-lesional cortex around the injury or in the contra-lesional cortex during affected forelimb stimulation are not well characterized in the literature.

Results

Trans-hemispheric shift of the cortical representation for the injury-affected forelimb occurs over four weeks

To characterize how the cortical map changes throughout recovery after TBI (**Aim 1**), affected forelimb-evoked fMRI data was acquired from 11 adult rats before and at weekly intervals following either moderate or moderate-severe CCI injury of the left primary sensorimotor forelimb (S1-FL) cortex. These data showed that CCI injury initiated a trans-hemispheric shift in the group-

averaged affected forelimb-evoked BOLD activation from the injured cortex to the uninjured, CLCtx over the first four weeks of recovery **(Figure 3A-C)**. The activation patterns show a new, wide-spread bilateral pattern of activation during the first two weeks after injury, covering a large region of the uninjured cortical hemisphere and broad ipsilesional cortical activation surrounding the injury site. Over the next two weeks of recovery, the mean activation map shifted completely to the opposite and homotopic contralesional S1-FL cortex. Functional MR images of average BOLD brain activations in response to affected-limb stimulation at pre-injury baseline, 7, 14, 21, 28 and days post-CCI injury (corrected for multiple comparisons, $z=1.7$, $p=0.05$) show widespread ipsi- and contra-lesional cortical activation after stimulation of the right (injury-affected) fore-paw **(Figure 3A)**. This evolved to a more focused volume of S1-FL CLCtx activation at 4 weeks post-injury. The number of activated voxels in the injured cortex elicited by the right, affected limb stimulation prior to and following injury shows significantly decreased ipsilesional activation throughout the first four weeks following CCI injury to the left sensorimotor cortex (Kruskal-Wallace test, $p=0.0018$) **(Figure 3B)**. For these box-and-whisker plots, the top of the box marks the upper quartile, the bottom of the box marks the lower quartile range, the horizontal line in each box denotes the median number of activated voxels, and finally, the "whiskers" mark the maximum and minimum number of activated voxels. Compared to pre-injury the number of significantly activated voxels in the ipsilesional cortex are decreased significantly at 21 and 28 days (Dunn's Multiple Comparison test, $p<0.05$). The volume of activated cortex within the injured hemisphere decreased to approximately half of pre-injury values over the first two weeks following injury, then again at both 21 and 28 days to approximately 10% of the pre-injury volume. The average activation volume within the ILCtx shrank significantly throughout 4 weeks of post-injury recovery. The average activation volume elicited by affected limb stimulation prior to and

following injury in the uninjured, contralesional cortex did vary significantly over time (Kruskal-Wallace test, p=0.0231) **(Figure 3C)**. However, post-hoc rank sum tests showed no significant differences by post-injury time point compared to the activation volumes at the pre-injury baseline.

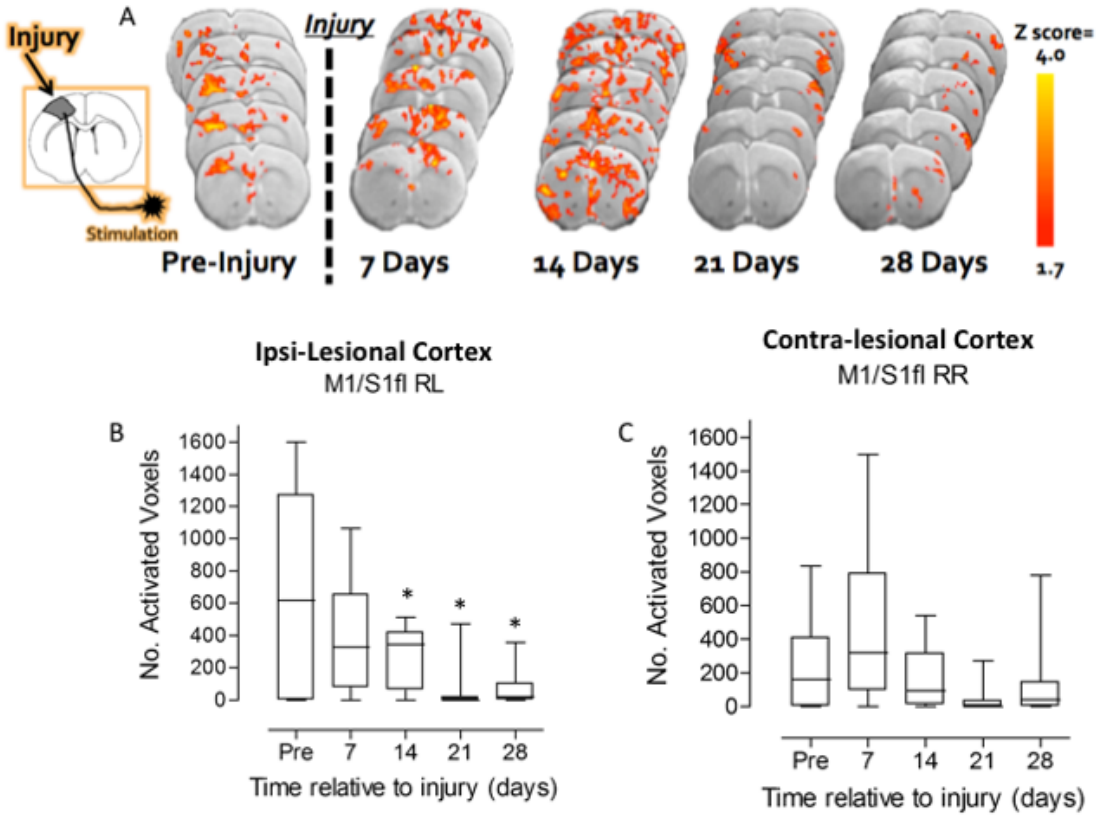


Figure 3**.** The cortical BOLD activation map for the affected forelimb shifts to the uninjured, contra-lesional cortex throughout the first four weeks after unilateral CCI injury in the rat. [**A]** Functional MR images of average BOLD brain activations in response to affected-limb stimulation at pre-injury baseline, 7, 14, 21, 28 and days post-CCI injury (corrected for multiple comparisons, z=1.7, p=0.05). Stimulation of the right (injury-affected) fore-paw initiates widespread ipsi- and contra-lesional wrong-side activation, which leads to focused wrong-side S1 activation at 4 weeks post-injury. [**B]** Box-and-whisker plot showing the number of activated voxels in the injured cortex elicited by affected limb stimulation prior to and following injury. How to read these box-and-whisker plots: The top of the box marks the upper quartile, the bottom of the box marks the lower quartile range, the horizontal line in each box denotes the median number of activated voxels, and

finally, the "whiskers" represent the maximum and minimum number of activated voxels. Ipsilesional activation is significantly decreased throughout the first four weeks following CCI injury to the left sensorimotor cortex (Kruskal-Wallace test, $p=0.0018$). Compared to pre-injury the number of significantly activated voxels in the ipsilesional cortex are decreased significantly at 21 and 28 days (Dunn's Multiple Comparison test, $p<0.05$). **[C]** Box-and-whisker plot showing the number of activated voxels in the uninjured, contralesional cortex elicited by affected limb stimulation prior to and following injury. Activation volumes in the uninjured, contralesional cortex vary significantly over time (Kruskal Wallis test, $p=0.0231$). Post-hoc rank sum tests showed no significant differences by post-injury time point.

Affected forelimb-evoked contralesional activation is correlated with injury severity only acutely after injury

To determine if more severely injured rats exhibit more pronounced CLCtx activation, the relative cortical lesion size was used to divide rats into either "mild" (TBM < 15) or "moderate-severe" (TBM > 15) injury groups (**Figure 4A-D**). Relative lesion size was measured using the TBM analysis comparing anatomical images acquired before and at four weeks post-injury. The resulting within-ROI-averaged Jacobian determinate calculated for each animal provided a good index of relative injury severity in this dataset, as lesion size measured by TBM significantly correlates with lesion volumes measured by histological tissue post mortem from the same rats (Spearman $r = 0.782$, p-value (two tailed) = 0.0064, see **Supplementary Figure 1** in the Appendix).

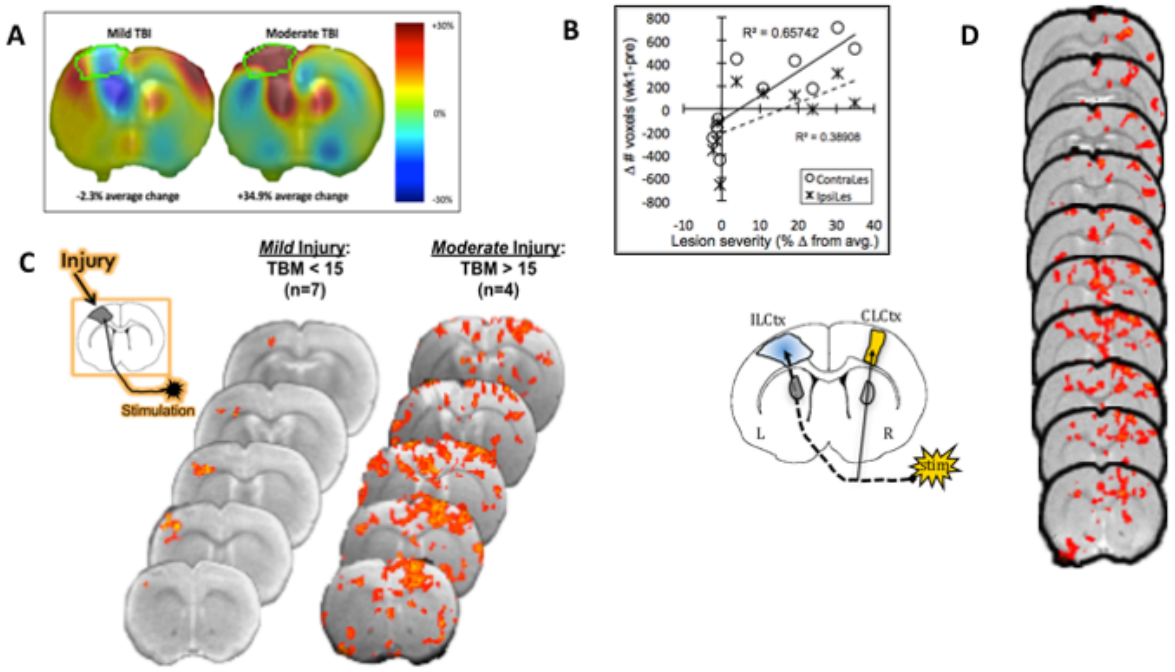


Figure 4. More extensive damage to the sensorimotor cortex leads to increased contra-lesional "wrong-side" activation. **[A]** Example images of the mildest and most severe cortical lesions within the region of interest (ROI, green trace) Jacobian maps overlaid on the mean (n=11) RARE structural image for mild or moderate-severe TBI. The color scale (right) indicates the relative % change required to warp the image to the group average image at 28 days post-injury. Tensor-based morphometry (TBM) analysis of t2-weighted MR images was used to compare relative, within-group cortical lesion size as a measure of injury severity. **[B]** The degree of wrong-side activation at 7 days post-injury is significantly correlated with injury severity in the rat (Spearman r = 0.782, p-value (two tailed) = 0.0064) but not at subsequent time points ($p<0.05$). **[C]** Greater damage to the sensorimotor cortex leads to increased contra-lesional "wrong-side" activation. Comparison between average activation maps of mild-moderate (n=7) to moderate (n=4) injured rats. **[D]** Affected limb stimulation-evoked BOLD activations correlate positively with lesion volume at one week post-injury, but not at later time points. At PID7 activated white and grey matter volumes positively correlate with lesion volumes (corrected for multiple comparisons, $p<0.05$, $z>1.0$, n=11). Lower right: Z-value color scale for significant regions of affected limb-evoked BOLD activation.

The volume of CLCtx BOLD activation one week post-injury was significantly correlated to the degree of cortical damage (Spearman r=0.782, p-value(two-tailed) =0.0064) **(Figure 4B)**.

There is a severity-dependent increase in both peri-lesional and contralesional BOLD activation in rats with moderate-severe compared to mild cortical lesions at 7 days post-injury **(Figure 4C)**. On average, the mild injury group displayed ipsilesional activation without activation of the CLCtx. However, rats with moderate-severe injury showed broad bilateral activation, indicating that more severe damage to the cortex initiates a broad contralesional activation acutely. Surprisingly, there was no correlation between injury severity and increased CLCtx activation at two, three, or four weeks post-injury ($p>0.05$) **(Figure 4D)**. Therefore, the acute activation pattern at one week post-injury could serve as a biomarker for either injury severity and prospective recovery outcomes.

Unaffected forelimb-evoked CLCtx BOLD signal increases at day 28 is associated with increased excitability

The location of CLCtx activation did not change over time **(Figure 5A)**. However, the BOLD signal magnitude within the CLCtx (S1-FL area) during stimulation of the unaffected forelimb was significantly enhanced four weeks after injury relative to pre-injury, but not at early time points **(Figure 5B)**. Statistical contrasts between pre-injury activation showed no difference in contralesional activation at 7, 14, or 21 days following injury. However, at 28 days following injury, activation was significantly enhanced within the contralesional S1-FL somatosensory cortex **(Figure 5B-D)**.

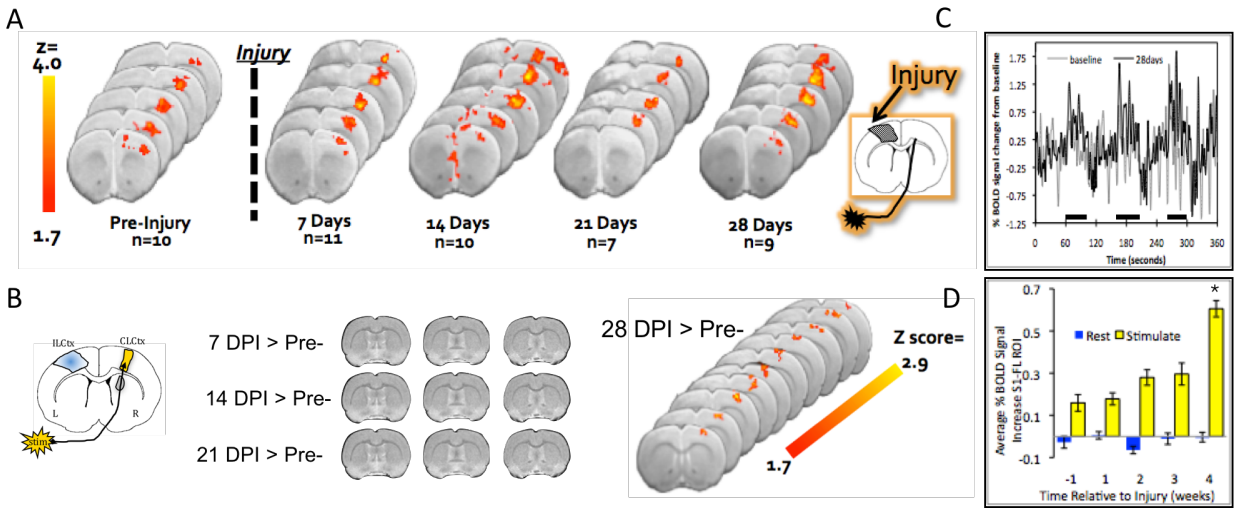


Figure 5. Unaffected forelimb-evoked BOLD signal increases in signal intensity at 4wks post-injury but the cortical map location does not change over time. **[A]** Average BOLD unaffected forelimb-evoked activation maps overlaid onto the mean group T2-weighted structural image in rats before and at 1, 2, 3, and 4 weeks following CCI injury. **[B]** Interestingly, stimulation of the unaffected limb resulted in significantly enhanced activation of the contralesional cortex at 28 days (Paired contrast, $z>1.7$, $p>0.05$, corrected for multiple comparisons). Earlier post-injury time points did not show significant differences in activation intensity (Anatomical MRI brain sections with no significant activations). **[C]** Average raw BOLD activation signal (y-axis) from the contralesional S1FL cortex for all rats during pre-injury baseline (grey) or 28DPI (black) over 360 seconds. The black horizontal lines above the x-axis indicate periods of unaffected forelimb stimulation. **[D]** Average % BOLD signal increase from un-thresholded 4-D data within a region of interest comprising the S1-FL registered from a standard brain atlas to functional space. Average BOLD activation increased significantly over time ($p<0.0001$, linear mixed models ANOVA). Post-hoc comparison showed significant increase in bold signal at 4wks compared to pre-injury (Dunn's Multiple Comparison test, $p<0.05$). *=$p<0.05$.

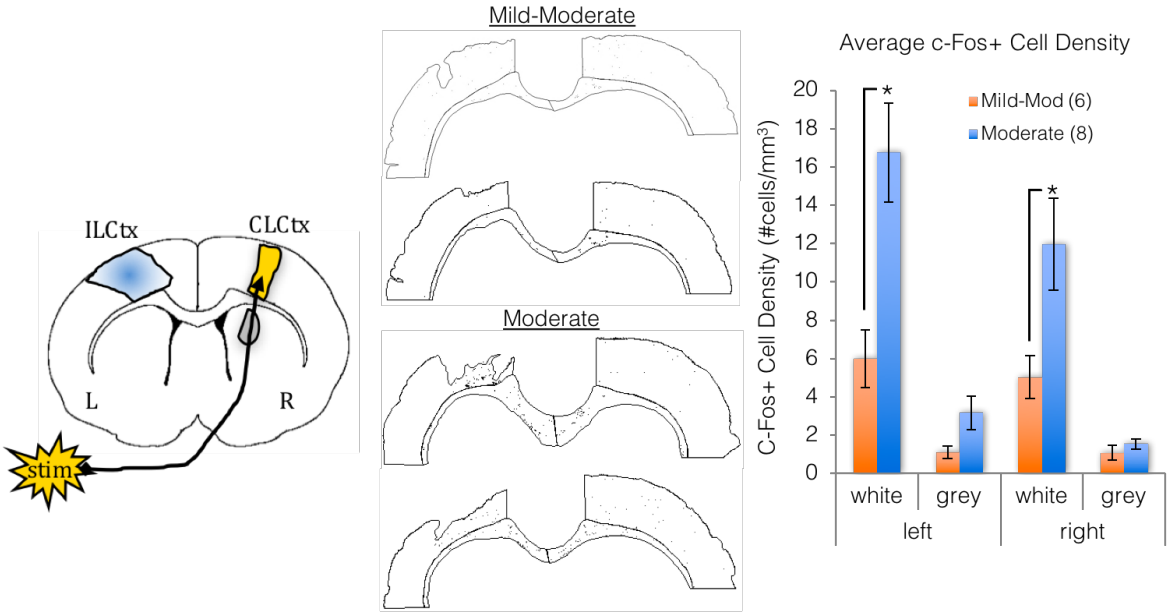


Figure 6. Rats with larger lesions show increased bilateral c-Fos expression across the corpus callosum white matter tract, which connects the injured and contra-lesional cortices. **[A]** Column graph comparing c-Fos early immediate gene protein expression between moderately (n=6) or moderately-severly (n=8) injured rats after stimulation of the unaffected forelimb at 5 wks. post-injury. Rats with moderate injury severities expressed significantly increased c-Fos activation in both the left and right corpus callosum compared to rats with less severe, mild-moderate injuries ($p<0.05$). In either right or left cortical grey matter, however, the density of c-Fos+ activated neurons was not significantly different between injury severity (Mild-Mod vs. Moderate, t-test, $p>0.05$).

To investigate the neurophysiological signal underlying the unaffected forelimb-evoked activation of the CLCtx, paired-pulse sensory-evoked potentials (PP SEPs) were recorded from skull-based electrodes over the primary somatosensory forelimb (S1FL) cortex. The unaffected forelimb was stimulated using inter-stimulus intervals (ISIs) of 35, 50, 100, and 200 ms, whereby the time between the first, A, and the second, B stimuli is varied **(Figure 7A)**. At longer ISI, the B SEP waveform is similar to the A waveform. At shorter ISI, active intercortical inhibition in the CLCtx during the response to stimulus A blunts the response to stimulus B. This paired-pulse

suppression (PPS) results from inhibition of the excitatory networks by other, integrated networks of inhibitory GABAergic interneurons. The $N1B_{35ms}$ becomes broader than $N1A_{35ms}$ because the neuronal ensemble is desynchronized by the pulse of inhibition 35ms into the first response, resulting in a slower/longer latency time-to-peak. Latencies of the $N1B_{35ms}$ peaks decreased significantly at both 14 and 21 days **(Figure 7B)**. Residual inhibition 100ms after the onset of the first "A" stimulus increased significantly at 7 days ($p=0.030$) and again at 28 days ($p=0.016$) post-injury paired t-tests compared to pre-injury latencies. The N1A peak latencies decreased significantly at both 21 and 28 days. Naïve rats did not show any significant change in the latencies of the N1A nor N1B peaks over time (one-way ANOVA, $p>0.05$) **(Figure 7C)**.

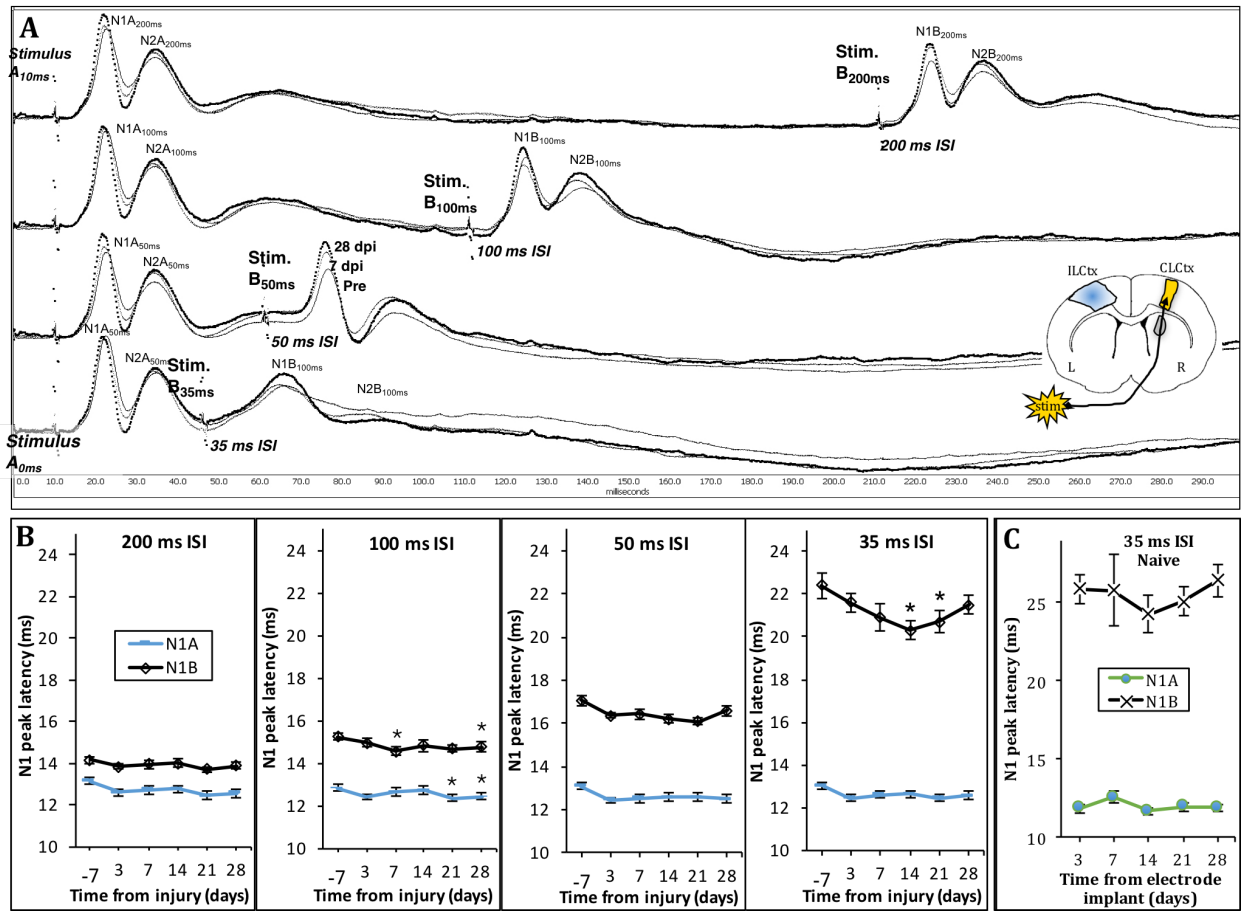


Figure 7. **[A]** Representative paired-pulse sensory-evoked potentials (PP SEPs) averaged traces organized by decreasing inter-stimulus interval (ISI) from 200 ms (top traces) to 35 ms (bottom traces). For each ISI, traces from pre-injury (solid line), seven days post-injury (dotted line), and 28 days post-injury (bold dotted line) are overlaid. **[B]** PP SEP Latency plots of the N1A and N1B peaks recorded from the right cortical hemisphere during stimulation of the left (non-affected) forelimb. The latencies of the N1B Compared to pre-injury, latency of the N1B peak at 100ms ISI was significantly shorter at both 7 ($p=0.030$) and 28 days ($p=0.016$, paired t-test). At 35ms ISI latency of N1B peak was significantly shorter at both 14 ($p=0.025$) and 21 days ($p=0.048$) compared to pre-injury. **[C]** Plot of N1A (green dot) and N1B (black X) peak latencies from a group of uninjured control rats (n=5). Naïve, age-matched controls received electrode headset implantation surgery but not the CCI injury surgery. Latencies were measured 3, 7, 14, 21, and 28 days after implantation of their electrode headsets. Peak latencies did not change significantly over time ($p>0.05$). ISI=Inter-stimulus interval. *=$p<0.05$.

Bi-phasic excitability: An early and late wave of hyper-excitability?

The paired-pulse ratio (PPR) of the amplitude of the second, N1B to the first, N1A is a standard measure of inhibition, with decreasing values indicating increased inhibition and increasing PPR indicating increasing excitability. The PPR did not vary significantly with PID for the 35ms, 50ms or 200ms ISI **(Figure 8B)**. However, the PPR of the N1 peak at 100ms ISI did vary significantly over time (Friedman statistic=15.79, p=0.0075) **(Figure 8A-B)**, but *post-hoc* testing showed no significant differences between pre-injury and each of the post-injury time points ($p>0.01$, Dunn's Multiple Comparison Test). The PPR first increased at PID3 then decreased to below pre-injury levels, then increasing again to above pre-injury levels at PID28. The amplitude of the $N1A_{100ms}$ peak increased significantly at PID21 **(Figure 8C),** while the amplitude of the $N1B_{100ms}$ peak increased significantly at both the early-acute PID3 and the more chronic PID28 ($p<0.01$, Dunn's Multiple Comparison Test) **(Figure 8D)**, indicating an increase in the size and/or synchronicity of the receptive field and/or amplification of the signal along the sensory pathway.

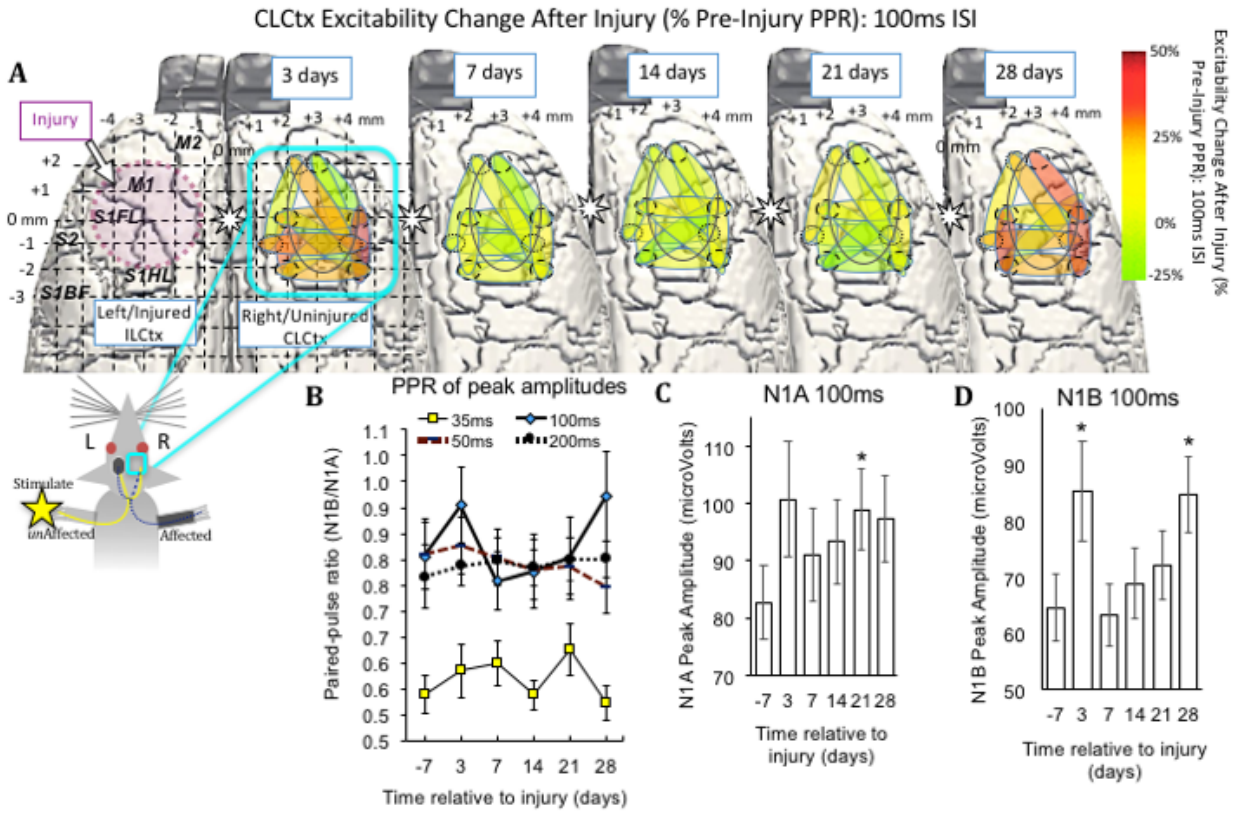


Figure 8. PP SEP#1: **[A]** Visualization of the average change in CLCtx excitability by electrode pair over all time points. compared to pre-injury paired-pulse sensory-evoked potential (PP SEP) paired-pulse ratio at 100ms inter-pulse interval (PPR_{100ms}) spatially overlaying a cortical map template. Key: Oval marks the region of increased BOLD response at 28 DPI in a parallel group of rats stimulated at 10Hz (100ms ISI). Violate-filled broken circle over the left S1FL and M1 cortical regions is the CCI injury site. **[B]** PPR plotted over time for each inter-stimulus interval (ISI) from CLCtx PP SEPs during stimulation of the left (non-affected) forelimb. PPR of the N1 peak at 100ms ISI changed significantly over time (Friedman statistic=15.79, p=0.0075). Post-hoc testing using Dunn's Multiple Comparison test showed only non-significant differences between pre-injury and each of the post-injury time points. The PPR did not vary significantly with time for the 35ms, 50ms or 200ms ISI. **[C]** The amplitude of the $N1A_{100ms}$ peak increased significantly at PID21 ($p<0.01$, Dunn's Multiple Comparison Test). **[D]** The amplitude of the $N1B_{100ms}$ peak increased significantly at PID3 and PID28 ($p<0.01$, Dunn's Multiple Comparison Test).

Neurophysiological signal underlying affected forelimb-evoked CLCtx BOLD activation maps

To determine if cortical pyramidal neurons are the source of the affected forelimb stimulation-evoked ILCtx and CLCtx BOLD activation maps after injury, a second PP SEP study was conducted, this time with the post-injury addition of ipsi-lesional electrodes for bilateral recording and unaffected or affected forelimb stimulation. During affected forelimb stimulation, both the average Response A_{100ms} and Response B_{100ms}, measured as the amplitude of the first dominant peak within the first 30ms following each stimulus.

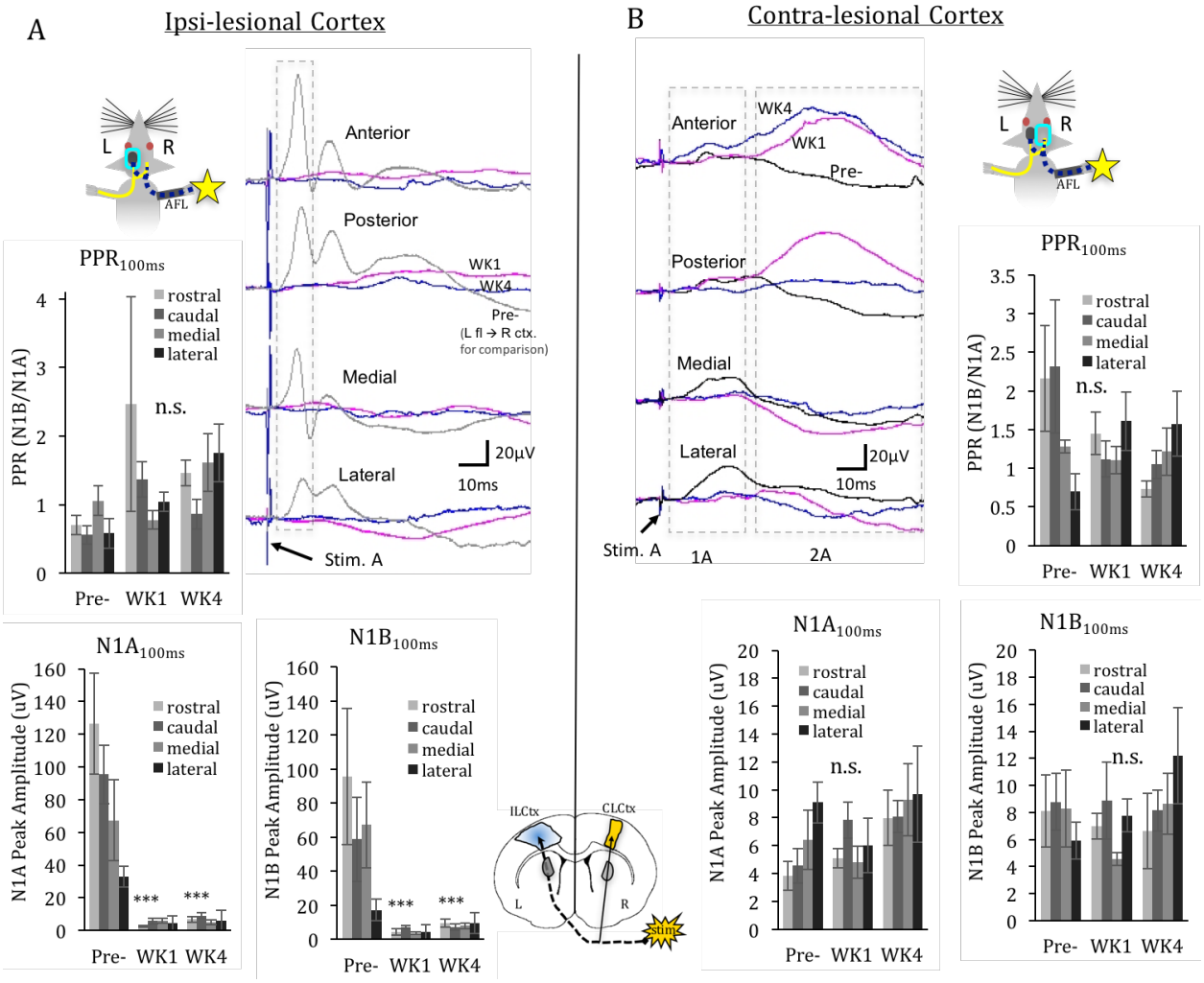


Figure 9. PP SEP waveforms and analysis from affected forelimb stimulation with paired-pulse stimuli at 100ms ISI shown according to stereotaxic location within the ipsi-lesional **[A]** or contra-

lesional cortex **[B]**. **Upper** column graphs in **[A]** and **[B]** show the PPR_{100ms} plotted by electrode location over time. **Lower column graphs** plot the $N1A_{100ms}$ and $N1B_{100ms}$ peak amplitudes by electrode location over time. **[A]** Ipsi-lesional $N1A_{100ms}$ and $N1A_{100ms}$ signal amplitudes diminished significantly in rostral, caudal, and medial ILCtx regions at both 1wk and 4wks post-injury (two-way ANOVA, $p<0.05$; Bonferonni-corrected post-hoc comparison, $p<0.05$). The PPR_{100ms} in the ILCtx did not change significantly after injury. **[B]** The $N1A_{100ms}$, $N1B_{100ms}$, and PPR_{100ms} did not change significantly in the CLCtx (**bar graphs**) (two-way ANOVA, $p>0.05$).

Key: Waveforms represent group averaged PP SEP recordings from pre-injury (**black trace**), one week (**magenta trace**) and four weeks (**blue trace**) post-injury, and prior to injury from the right cortex during left forelimb stimulation (**gray trace** in **[A]**) for comparison. ILCtx=ipsi-lesional cortex; CLCtx=contra-lesional cortex; *=significantly different from pre-injury, $p<0.05$, two-way ANOVA; */ =significant variation by cortical location; n.s.=no significant differences compared to pre-injury.

In the CLCtx, analysis $N1_{100ms}$ peak amplitude measures did not show a significant change in the peak height or PPR (**Figure 9B**). Further investigation into the excitability of the CLCtx using the shorter 35ms ISI indicated region-specific increases in excitability. The $N1A_{35ms}$, like the $N1A_{100ms}$, did not change significantly over time **(Figure 10B, lower left plot)**. However, the $N1B_{35ms}$ peak amplitude increased significantly in the anterior CLCtx at 7DPI. Likewise, the medial and anterior CLCtx PPR_{35ms} increased significantly at 7DPI but had returned to pre-injury levels by 28DPI **(Figure 10B, upper and lower right plots)**.

In addition to measuring peak amplitude, because of the wide, prominent peak between 56.4-103.8 ms of the recording made amplitude measurement less sensitive, (upper traces **Figure 9B**, gray broken boxed region 2A), the area under the peak was also measured for the affected forelimb-evoked, anterior, CLCtx SEP data (see **Supplementary Figure 2C** in Appendix). These data showed that the cortical response magnitude increased significantly in the CLCtx at PID28 (**Figure 9C top**) in agreement with the fMRI BOLD map (**Figure 3**). Similarly, response B_{35ms} (**Figure 9B, bottom graph**) peak integral (area under the peak) measured at the dominant peak

between 48-135ms increased significantly over the CLCtx on PID7 (post-hoc paired t-tests: Pre-Wk1 p=0.003 and Pre-Wk4 p=0.006 (two-tail)). Unlike the amplitude data, the peak integral analysis showed that affected limb CLCtx activation increases according to the PP SEP, and this is concordant with the results of the fMRI data (**Figure 3**).

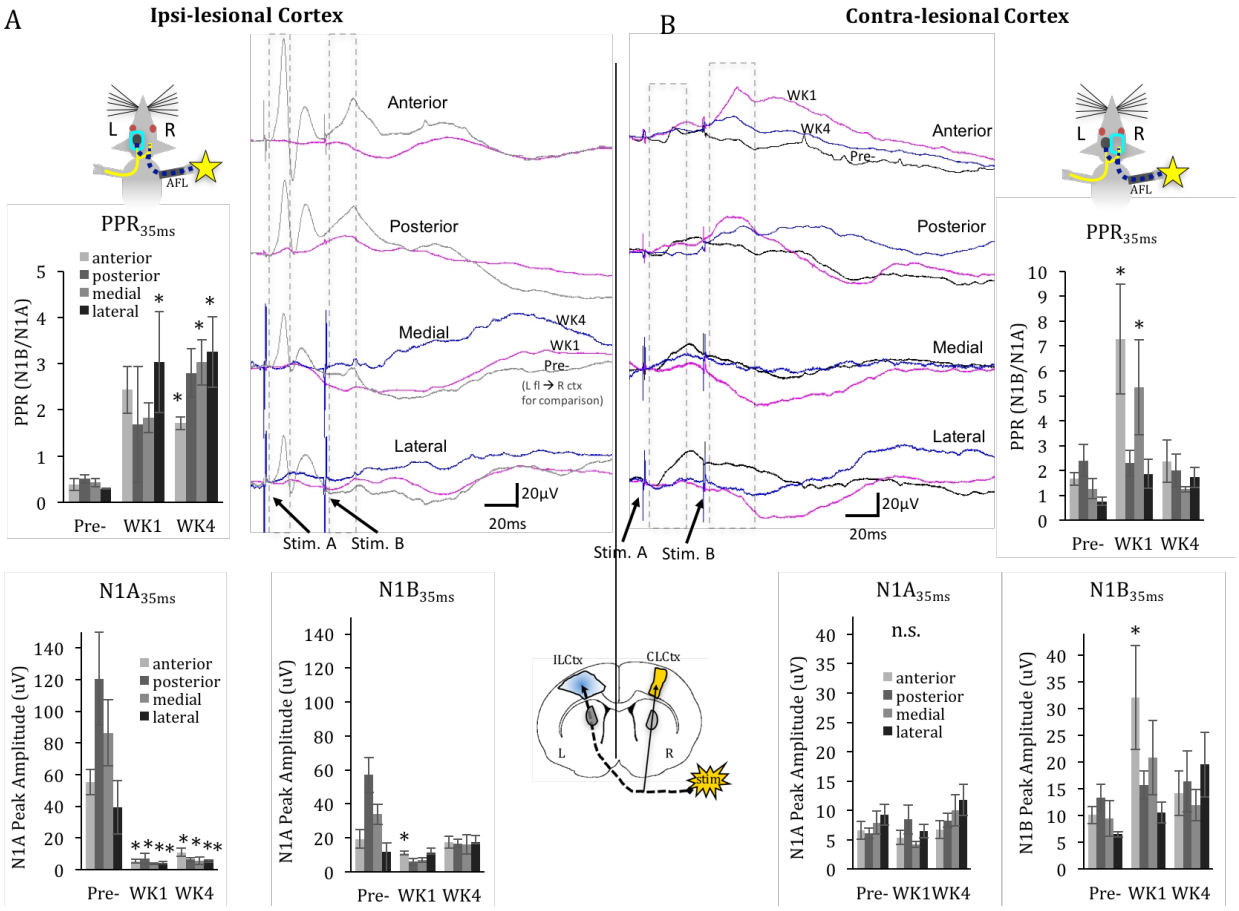


Figure 10. Affected forelimb stimulation at 35ms ISI and resulting average PP SEP waveforms according to location within the ipsi-lesional **[A]** or contra-lesional cortex **[B]**. **Upper** column graphs in **[A]** and **[B]** show the PPR_{35ms} of the N1B/N1A peak amplitudes plotted by electrode location over time. **Lower column graphs** plot the $N1A_{35ms}$ and $N1B_{35ms}$ peak amplitudes by electrode location over time. **[A]** Ipsi-lesional $N1A_{35ms}$ signal amplitudes decreased significantly in all ILCtx regions at both 1wk. and 4wks. post-injury (two-way ANOVA, $p<0.05$; Bonferonni-corrected post-hoc comparison, $p<0.05$). The PPR_{35ms} in the ILCtx increased significantly in the lateral region at 1wk and in the anterior, medial and lateral regions of the ILCtx at 4wks post-

injury. **[B]** The PPR_{35ms} increased significantly in the anterior and medial regions of the CLCtx (**upper right graph**) (two-way ANOVA, $p<0.05$; Bonferroni-corrected post-hoc comparison, $p<0.05$) with greatest hyperexcitability originating from the anterior and posterior regions of the contra-lesional cortex at both 1wk and 4wks post-injury (two-way ANOVA, $p<0.05$; Bonferonni-corrected post-hoc comparison, $p<0.05$). The $N1A_{35ms}$ amplitude did not change significantly over time (**[B] lower graphs**) with the $N1B_{35ms}$ amplitude increasing significantly in the anterior CLCtx 1wk. post-injury.

Key: Waveforms represent group averaged PP SEP recordings from pre-injury (**black trace**), one week (**magenta trace**) and four weeks (**blue trace**) post-injury, and prior to injury from the right cortex during left forelimb stimulation (**gray trace** in **[A]**) for comparison. ILCtx=ipsi-lesional cortex; CLCtx=contra-lesional cortex; *=significantly different from pre-injury, $p<0.05$, two-way ANOVA; */ =significant variation by cortical location; n.s.=no significant differences compared to pre-injury.

In the ILCtx, the average affected forelimb stimulation-evoked N1A and N1B SEP signal amplitude decreased significantly at both 1wk and 4wks post-CCI **(Figure 9A, bottom)** and **(Figure 10A, bottom graphs)** ISI trials ($p<0.05$, two-way ANOVA, Bonferroni post-hoc comparisons). The loss of ILCtx SEP at 4wks **(lower graphs in Figures 9A and 10A)** agrees with the absence of ILCtx fMRI BOLD signal at 4wks post-injury. At 1wk post-injury, the electrophysiological signal was significantly diminished, while the fMRI data indicated clear BOLD activation surrounding the injury site **(Figure 3A)**. This mismatch could be indicative of an inhibitory interneuronal signal in the ILCtx at 1wk post-injury during affected forelimb stimulation, since the SEP primarily reflects the activity of cortical pyramidal neurons near the surface of the epidural electrodes.

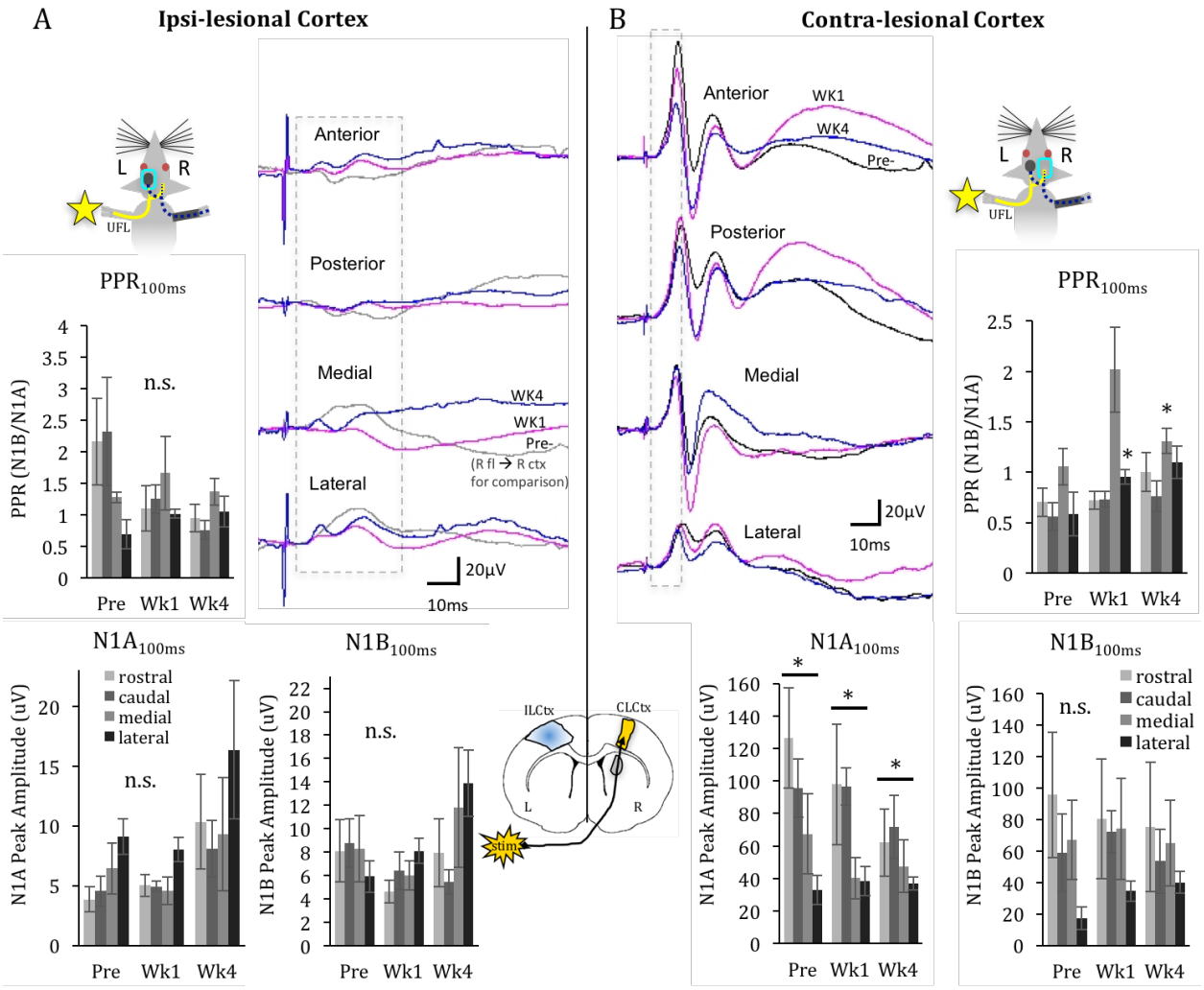


Figure 11. Affected forelimb stimulation at 100ms ISI and resulting average PP SEP waveforms according to location within either the ipsi-lesional cortex **[A]** or contra-lesional cortex **[B]**. **Upper** column graphs in **[A]** and **[B]** show the PPR_{100ms} of the N1B/N1A peak amplitudes plotted by electrode location over time. **Lower column graphs** plot the $N1A_{100ms}$ and $N1B_{100ms}$ peak amplitudes by electrode location over time. **[A]** Ipsi-lesional PPR_{100ms} and peak amplitudes did not change significantly by electrode location or time relative to injury (two-way ANOVA, $p>0.05$). The $N1A_{100ms}$ and $N1B_{100ms}$ peak amplitudes did not change significantly after injury. **[B]** CLCtx $N1A_{100ms}$ and $N1B_{100ms}$ peak amplitudes did not vary significantly over time, but did vary significantly by electrode location (two-way ANOVA, $p<0.05$) with greatest signal originating from the anterior and posterior regions of the CLCtx at all time points tested (two-way ANOVA, $p<0.05$; Bonferonni-corrected post-hoc comparison, $p<0.05$). CLCtx PPR_{100ms} increased significantly in the lateral region at 1wk and in the medial CLCtx at 4wks post-CCI.

Key: Waveforms represent group averaged PP SEP recordings from pre-injury (**black trace**), one week (**magenta trace**) and four weeks (**blue trace**) post-injury, and prior to injury from the right

cortex during right forelimb stimulation (**gray trace** in **[A]**) for comparison. ILCtx=ipsi-lesional cortex; CLCtx=contra-lesional cortex; *=significantly different from pre-injury, $p<0.05$, two-way ANOVA; */ =significant variation by cortical location; n.s.=no significant differences compared to pre-injury.

Surprisingly, the ILCtx SEP showed no significant change in $N1A_{100ms}$, $N1B_{100ms}$, or PPR_{100ms} after injury during UFL stimulation (**Figure 11A**). It was expected that, since injury resulted in a significant and dramatic decrease in AFL-ILCtx SEP, injury would similarly diminish the magnitude of the UFL-ILCtx SEP. However, this lack of significant change might be due to the much lower signal magnitude at pre-injury baseline (**Figures 9A** compared to **Figures 11A and 12A**), thus resulting in a floor effect. It seems less likely that the UFL-ILCtx SEP signal generator either lies outside of the affective injury zone or that it moves to a new location after injury, as the signal does not appear to shift from one location to another over time, but the waveforms at all time points look similar for each location, even though the pre-injury control was recorded in the opposite, right cortex. Similarly, the only significant change in amplitude was a decrease in $N1B_{35ms}$ at 1wk post-injury (**Figure 12A**, lower right graph) and excitability measured with PPR_{35ms} did not change significantly (**Figure 12A**, upper column plot).

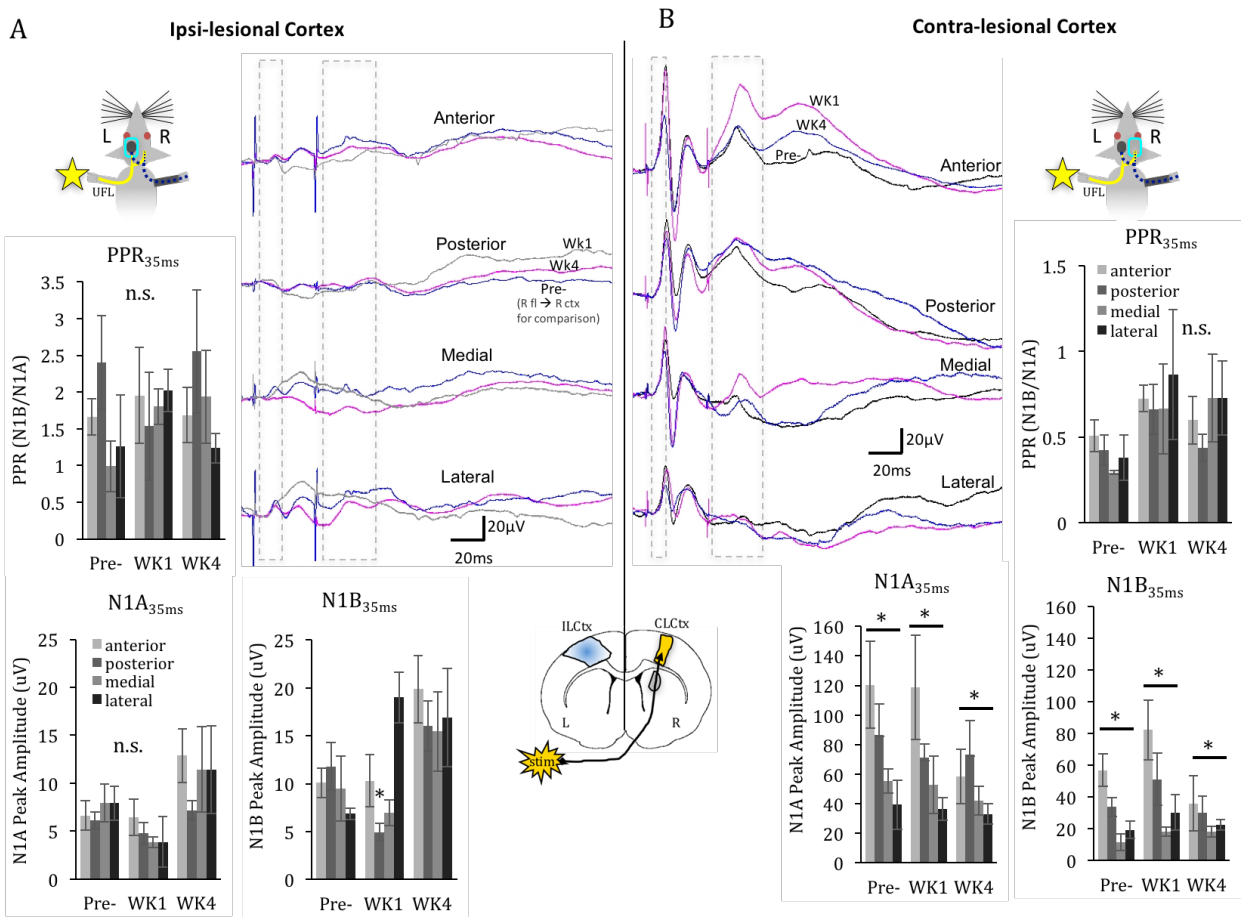


Figure 12. Unaffected forelimb 35ms ISI paired-pulse stimulation at and resulting average PP SEP waveforms according to location within the ipsi-lesional **[A]** or contra-lesional cortex **[B]**. **Upper** column graphs in **[A]** and **[B]** show the PPR_{35ms} of the N1B/N1A peak amplitudes plotted by electrode location over time. **Lower column graphs** plot the $N1A_{35ms}$ and $N1B_{35ms}$ peak amplitudes by electrode location over time. **[A]** Ipsi-lesional $N1A_{35ms}$ signal amplitudes decreased significantly in all ILCtx regions at both 1wk. and 4wks. post-injury (two-way ANOVA, $p<0.05$; Bonferonni-corrected post-hoc comparison, $p<0.05$). The PPR_{35ms} in the ILCtx increased significantly in the lateral region at 1wk and in the anterior, medial and lateral regions of the ILCtx at 4wks post-injury. **[B]** The PPR_{35ms} increased significantly in the anterior and medial regions of the CLCtx (**upper right graph**) (two-way ANOVA, $p<0.05$; Bonferonni-corrected post-hoc comparison, $p<0.05$) with greatest hyperexcitability originating from the anterior and posterior regions of the contra-lesional cortex at both 1wk and 4wks post-injury (two-way ANOVA, $p<0.05$; Bonferonni-corrected post-hoc comparison, $p<0.05$). The $N1A_{35ms}$ amplitude did not change significantly over time (**[B] lower graphs**) with the $N1B_{35ms}$ amplitude increasing significantly in the anterior CLCtx 1wk. post-injury.

Key: Waveforms represent group averaged PP SEP recordings from pre-injury (**black trace**), one week (**magenta trace**) and four weeks (**blue trace**) post-injury, and prior to injury from the right cortex during right forelimb stimulation (**grey trace** in **[A]**) for comparison. ILCtx=ipsi-lesional cortex; CLCtx=contra-lesional cortex; *=significantly different from pre-injury, $p<0.05$, two-way ANOVA; n.s.=not significant. */=significantly different between cortical positions across the contra-lesional cortex, two-way ANOVA with Bonferroni post-hoc comparison.

Both the $N1A_{35ms}$ and $N1A_{100ms}$ data showed significant spatial differences in signal magnitude across the UFL-CLCtx PP SEP (**Figures 11B and 12B,** two-way ANOVA, $p<0.05$). The strongest signal tended to come from the more anterior (rostral) portion of the S1FL cortex, and the weakest, but least variable signal, was detected in the lateral region of the CLCtx (**Figures 11B and 12B**). The spatial distribution pattern of SEP signal amplitude remained consistent across time points in agreement with the UFL stimulation-evoked fMRI map (**Figure 5A**), does not shift after injury. UFC-CLCtx PPR_{35ms} did not reveal a change in excitability (**Figure 12B**, upper graph). However, the PPR_{100ms} did show that the lateral CLCtx was hyperexcitable at 1wk and the medial region was significantly more excitable at 4wks post-CCI (**Figure 11B**, upper bar graph).

Discussion

Lesion size-dependent increase in CLCtx activation is transitory

Throughout the first four weeks of recovery after CCI, the steady resumption of more normal limb-reaching is a stereotypical feature in this lateral TBI model (Harris et al., 2010; Nobuhiro et al., 2011). If the ILCtx were responsible for spontaneous recovery of limb function, a concomitant return of ipsilesional activation would be expected. However, the regions of activation appear to shift to other, more distant regions of the brain. These new regions of activation point to brain regions which are part of a network responsible for improving limb

function over the first month of recovery. The CLCtx appears to be an interesting candidate for supporting functional recovery because it consistently exhibited new activations from the impaired limb throughout recovery **(Figure 3A).** The affected limb-evoked fMRI data show a progressive trans-hemispheric shift from the injured to the CLCtx after unilateral CCI injury until, at four weeks post-injury, affected limb stimulation elicits activation in the CLCtx only **(Figure 3A)**. Increase in CLCtx activation from pre-injury to PID7 may serve as a bio-marker indicating more severe injury **(Figure 4)** and requirement of a particular treatment such as early neuromodulation combined with focused therapies to improve outcome in the human patient. This result differs from most in the stroke literature, where CLCtx activation has been shown to increase with lesion volume (Kobayashi et al. 2003b). The index of injury severity was essentially the chronic injury lesion volume. The ability of the ILCtx to activate dissipated over the first four weeks post-injury **(Figure 3A, B)**.

While mechanisms leading to ipsilateral activations after brain injury are currently under investigation, it has not been determined whether CLCtx activations play any causal role in recovery from TBI-induced deficits. Improved functional recovery after CCI injury in the rat is associated with sprouting of axons within the corticospinal tract from the CLCtx to the affected limb (Smith et al. 2007; Carmel et al. 2010; Zhang et al. 2010; Meng et al. 2014). This increased input to the injury-impaired forelimb through the uncrossed (ipsilateral) fibers and the re-crossing of sprouting axons could be responsible for sustained ipsilateral activation in the uninjured homotopic CLCtx observed in the present fMRI studies (see **Figure 3A**). Axonal sprouting has been shown to occur as early as one day post-injury (Greer et al. 2011b) and appears to peak around PID7-PID14 (Harris et al. 2010b). It is possible that sprouting could strengthen the uncrossed (or rather recrossed) pathway could enhance injury-affected forelimb (AFL)-stimulation-evoked

CLCtx activation by transmitting more signal to the contra-lesional thalamus and from there to the CLCtx. Enhanced AFL-evoked CLCtx activation, particularly when it is widespread in the first two weeks after injury, can also arise by amplification of the signal from the uncrossed pathway to the CLCtx in the thalamus. Another potential mechanism of CLCtx activation from sensory inputs from the AFL activation is through the corpus callosum from injured to CLCtx.

Unaffected-forelimb-stimulus-evoked CLCtx map: Evidence of an affected "unaffected" pathway

If the CLCtx takes over function of the affected forelimb, it must adapt, restructure, and remodel throughout recovery to enable some existing or new networks to take on function for the affected limb. These newly purposed or re-purposed networks could be integrated into the same cortical space as networks still responsible for maintaining function of the unaffected limb. Enhanced neuroplasticity within the cerebral cortex have been found to occur during times of altered balance between inhibition and excitation within the cortex (Fujioka et al. 2004; Benali et al. 2008). The unaffected forelimb stimulus-evoked $N1B_{100ms}$ peak latencies were significantly reduced at both PID7 and PID28, indicating two temporally distinct periods of CLCtx disinhibition (increased excitability) and enhanced cortical map plasticity in the CLCtx over the first month of recovery in the rat **(Figure 7B)**.

In the first PP SEP study (PPSEP#1), in which electrodes were placed only over the CLCtx and the paired-pulse stimulation was performed at ISI of 35ms, 50ms, 100ms, and 200ms. The goal of PPSEP#1 was to test the hypothesis that the increased unaffected limb-evoked BOLD signal observed in the CLCtx at PID28 **(Figure 5)** is due to disinhibition of the CLCtx. Results of pilot data indicated that this range would be sensitive to a change in contra-lesional excitability if it were to occur within the first four weeks after injury. It was expected that the 35ms ISI would

show the greatest increase in CLCtx excitability, because the second response is strongly attenuated (lower PPR) and would be less likely to be hindered by a ceiling effect. The CLCtx was most strongly influenced by changes in inhibition over time at the 100ms ISI, since the 100ms ISI was also used for forelimb stimulation in the parallel fMRI experiment and thus is best suited to show the electrophysiological signal that underlies the BOLD activation maps. Also, co-incidental, was the significantly increased CLCtx signal magnitude, in both the BOLD fMRI signal and the SEP electrophysiological signal during 100ms ISI unaffected forelimb stimulation at PID28.

In the second PP SEP study (PPSEP#2), PPR_{100ms} also showed significant disinhibition at both PID7 and PID28 (**Figure 11B**, upper column graph). These data agree with the PPSEP#1 results and also support the idea that the CLCtx becomes hyperexcitable through the mechanism proposed in **Figure 13** below:

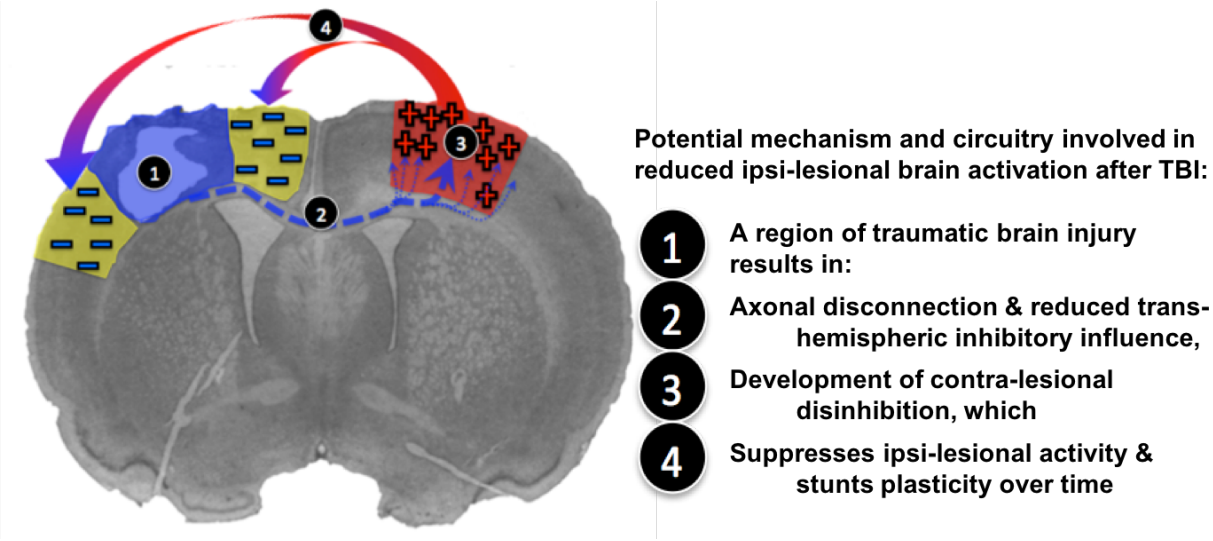


Figure 13. Proposed mechanism leading to long-term map changes after lateral CCI in the rat

Imbalance in inhibitory control between the hemispheres could lead to increased excitability within the contralesional S1-FL cortex **(Figure 11)**. Reduced ipsilesional-to-contralesional transcortical inhibition through trans-colossal fibers may reduce the threshold of

contralesional circuitry to activate. Disinhibition of the CLCtx occurs, at least in part, through a down-regulation of the inhibitory neurotransmitter gamma-aminobutyric acid type-A receptors containing the α1 subunit ($GABA_AR$-α1) (Lee et al. 2011). The α1 subunit-containing $GABA_A$ receptors are predominantly located post-synaptic side of the synaptic cleft between the pre-synaptic GABAergic inhibitory interneuron and the post-synaptic excitatory neuron. These receptors are responsible for phasic inhibition of excitatory pyramidal cells in the cerebral cortex. Phasic inhibition from interneurons in the cerebral cortex, for example, is responsible for modulating various EEG-detectable frequency bands such as the theta and gamma network ocillations (Farrant and Nusser 2005). Recently, phasic GABAergic transmission was shown to be impaired as early as in the CLCtx 24-48 hours after unilateral CCI in the mouse (Le Prieult et al. 2016). So, in addition to injury-induced changes in excitatory networks, changes in phasic inhibition is also likely to influence cortical map plasticity and changes in excitability observed in the fMRI and PP SEP data presented here.

At the same time, the number of extrasynaptic $GABA_ARs$ associated with tonic inhibition, such as the α4/δ and α5 subunit-containing $GABA_A$ receptors, increased significantly in the CLCtx, yet the GABAR-mediated currents showed slower kinetics to result in an overall increase in the excitation/inhibition ratio (Le Prieult et al. 2016).

On the other side of the brain, numbers and tonic inhibitory activity of GABAARs containing the α5 subunit increased in the peri-lesional cortex after experimental stroke injury (Clarkson et al. 2010). When the superfluous receptors were treated with an inverse agonist tonic inhibition was normalized and functional impairment caused by the SMC lesion was improved significantly (Clarkson et al. 2010). Together, the data from this study and those mentioned above in this section indicate that the deficits affecting behavior and function after TBI are associated

with an imbalance of transhemispheric excitation/inhibition between the injured and contra-lesional cortices.

The current working model **(Figure 11)** depends on the well-supported idea that each cortex inhibits activity of its homotopic counterpart in the opposite, contra-lateral cortex through trans-callosal white matter fibers that connect the lateral hemispheres. Initially, injury stuns the ipsi-lesional cortex, which attenuates ipsi-to-contra-lesional inhibition, resulting in hyperexcitability in the contra-lesional cortex. In the same way as silencing one cortical region with $GABA_AR$ agonist results in immediate expansion of the contra-lateral receptive field. This trans-hemispheric disinhibition thus causes increased activity within the CLCtx, which, in turn, has the recipricol effect of increased trans-callosal inhibition of the already stunned ILCtx. This basic working model for the effects of lateral brain injury on circuitry remodeling has been supported by data from studies in stroke patients (Calautti and Baron 2003; Calautti et al. 2007; Rehme et al. 2011, 2012; Volz et al. 2015). The model is also supported by the data presented herein, and this is not surprising because it is based on brain circuitry which has been well studied and validated.

Ipsi-lesional BOLD activation may reflect activation of networks of inhibitory interneurons

The presence of the BOLD and SEP signals coincided in the CLCtx as explained by the excitatory activity of cortical pyramidal neurons. In the ILCtx, the presence of an AFL-evoked BOLD signal at PID7 (**Figure 3B**) coincided with a conspicuous loss of SEP signal (**Figure 10A**). However, the average cortical SEPs recorded from electrodes around the edge of the contusion site were not statistically different from control recordings of background brain activity in the absence of stimuli **(Supplementary Figure 2A)**. There are several plausible explanations for the

discrepancy in ILCtx activation signal between the two techniques. First, the signal could be blunted by their proximity to the blood and scar tissue from the contusion from the CCI. Because the electrodes were implanted after injury, there are no pre-injury baseline PP SEP recordings from the left S1FLCtx to compare to and the space between the IL electrodes is greater than that between the electrodes over the CLCtx. Or potentially, because the electrodes must be screwed into pilot holes in the skull at the edge of the craniotomy, they may be farther from the signal generators in the underlying brain due to shifting of the map or death of tissue forming a cavity below the electrodes and insulating them from the cortical signal. The most compelling explanation is that the signal observed by BOLD results from activation of the networks of inhibitory interneurons in the injured cortex through trans-callosal fibers from the CLCtx. Since the local field potentials generated by synchronous firing of excitatory pyramidal cells are detected by the skull electrodes but the signal from networks of inhibitory interneurons cannot be measured by the induction of magnetic fields because their heterogeneous orientations cancel each other out resulting in a net B field vector equal to zero. This latter explanation does also fit the current working model for the mechanisms leading to disruption of the normal interhemispheric balance between excitation and inhibition toward suppressed ILCtx activity and hyperexcitation of the CLCtx. The c-Fos study results are also consistent with the idea that increased contra-to-ipsi-lesional trans-callosal inhibition occurs after lateralized TBI in the rat. Larger lesions showed greater activation across the corpus callosum interconnecting the ILCtx and CLCtx **(Figure 6)**. Recently published histological and diffusion tensor MR imaging data show that unilateral CCI initiates a significant loss of transcallosal structural connectivity by PID35 (Harris et al. 2016a).

The electrophysiological underpinnings of affected limb-stimulation-evoked contra-lesional cortex BOLD activation maps

At the same ISI as the fMRI stimulus, the magnitude of the 100ms ISI cortical response increased significantly at both 7 and 28 days after injury **(Response A in upper Figure 9B and upper graphs in Figure 9C)**. The peak with the most profound post-injury change in Response A for 100ms ISI is a broad N2-like peak. Since the ipsi-lateral SEP has not been studied and characterized in the published literature, the pathway of the evoked signal from the hand to the ipsi-lateral (same side as the stimulated hand) CLCtx is uncertain, particularly after brain injury and subsequent post-injury remodeling has altered the circuitry. Regardless of the pathway, the significant increase in the magnitude of the AFL-CLCtx SEP response agrees with the BOLD data.

Biomarkers from neurophysiology and fMRI BOLD cortical mapping

Poor motor recovery has been shown to be associated with increased CLCtx-to-ILCtx interhemispheric inhibition in chronic stroke patients (Murase et al. 2004; Duque et al. 2005; Rehme et al. 2011, 2012; Volz et al. 2015) Paired-pulse SEP techniques provide markers of altered excitability. At the shorter 35ms ISI and longer 100ms ISI we detected an overall change in inhibition by measuring the latency of the first synchronous excitatory response of pyramidal neurons in the contralesional cortex when the stimulus travels through the in-tact sensory pathway from the unaffected limb. At 4 weeks post-injury, the increase in the AFL-CLCtx BOLD signal corresponded with increases in the $N1_{100ms\ ISI}$ PPR **(Figure 12D)** and $N1B_{100ms\ ISI}$ peak amplitude **(Figure 10D)**, indicating that these measures might serve as a useful biomarker of remodeling within the CLCtx. The 100ms inter-stimulus interval (ISI) is most comparable to the 10Hz forelimb stimulation frequency in the parallel fMRI experiment. Also, co-incidental was

the significantly increased CLCtx signal magnitude, in both the BOLD fMRI signal and the SEP electrophysiological signal during 100ms ISI unaffected forelimb stimulation at PID28. If the CLCtx takes over function of the affected forelimb, it must adapt, restructure, and remodel throughout recovery to enable some existing or new networks to take on function for the affected limb, to work in the same cortical space than the networks still responsible for maintaining function of the normal limb. The neuroplastic changes within the cortex have been found to occur during times of altered balance between inhibition and excitation within the cortex (Benali et al. 2008). The $N1B_{100ms}$ peak latency data show distinct increases in excitation at PID7 and PID28, with the $N1B_{35ms}$ latency data showing hyperexcitability in the two weeks between **(Figure 9)**, possibly indicative of temporally distinct periods of functional modifications in the CLCtx over the first month of recovery in the rat. These results highlight the importance of timing for any interventions targeting the dynamic changes in excitation/inhibition within the injured brain. It is unclear from these data, however, whether CLCtx hyper-responsiveness is maladaptive to recovery after experimental TBI.

Chapter 3

Specific Aim 2. To determine if the contralateral cortex (CLCtx) is causally involved in recovery of limb function after TBI and whether its influence is temporally limited.

Background

Much of the evidence showing involvement of the CLCtx after lateralized brain injury has been gleaned from stroke and other focalized cortical lesion studies, while there is a relative dearth of research in TBI. Thus, while the results of stroke and lesion studies are very helpful, the published data remains limited and, as noted previously, the results may not be translatable to TBI (Voorhies and Jones 2002; Vespa et al. 2005; Jones et al. 2012; Kozlowski et al. 2013; Lu et al. 2013). Even after stroke, it remains unclear whether new CLCtx activity signifies that the homotopic cortex acts to support or impede affected limb recovery. While CLCtx activation is present with recovering limb function, a clear role has not yet been determined. The controversy regarding the apparent influence of the uninjured cortical hemisphere in recovery after stroke has led some to suggest that each hemisphere should be treated differently from a therapeutic standpoint (Schallert et al. 2003). The cortical representation of the affected forelimb remains dynamic into the more chronic recovery period. For this reason, brain-targeted treatments must consider timing and location for treatments such as neuromodulation to force trans-hemispheric balance of inhibition and excitation. This study sought to provide a step toward a clearer understanding of the role of the uninjured hemisphere at multiple time points in recovery after TBI.

Although not yet investigated after TBI, studies of stroke patients indicate that up-regulation of contralesional motor cortex (M1) may play an important adaptive role in regaining

function (Ward et al. 2003; Belanger et al. 2007). In the early post-stroke stages, fMRI studies have shown enhanced CLCtx activation during various motor tasks in early recovery (Johansen-Berg et al. 2002; Ward et al. 2003; Tombari et al. 2004). During later periods of chronic recovery, however, the role of the undamaged cortex appears to diminish as activation patterns decline in patients with good recovery. These studies indicate that in chronic recovery, outcome would improve if activation were shifted back from the CLCtx to the ipsilateral, injured cortex (ILCtx) if possible. The ability of the ILCtx to support functional recovery may, however, depend upon how extensively the injury has destroyed the critical brain structures. A better understanding of any causal role that the CLCtx plays in TBI-associated impairment and spontaneous recovery is necessary to develop a direct, neuromodulatory treatment paradigm to maximize the effects of therapeutic interventions. To help fill this critical knowledge gap in the TBI field, the following studies aimed to determine whether the CLCtx is causally involved in behavioral impairment and recovery time course in the acute and chronic phases after unilateral, cortical TBI.

Materials and Methods

Experimental Groups and Procedure:

Adult, male, naïve rats (16-week-old adult male Sprague-Dawley rats) were food-restricted to 15-20g/day, received forelimb reach training and subsequent testing on a staircase reaching task (Montoya et al. 1991). Subsequently, a left controlled cortical impact (CCI) injury was performed. Forelimb reaching success was measured using a modified version of the staircase test prior to, and after pharmacologically silencing the CLCtx by infusion of intracortical muscimol or artificial cerebrospinal fluid (aCSF) vehicle control. Post-injury testing for both aCSF and muscimol-treated rats was performed at 6d and at 27d. At

7d and 28d rats were re-tested 30 minutes after the right CLCtx was silenced by intraparenchymal muscimol (n=11) or aCSF (n=7) infusion. A subset of rats (n=7/group) were tested at 35d post-injury to determine if temporary silencing produced any longer-term carry-over effects on limb function. Experimental design is shown in **Figure 13**.

Brain Injury

All study protocols were approved by the University of California, Los Angeles (Los Angeles, CA) Chancellor's Animal Research Committee and adhered to the Public Health Service Policy on Humane Care and Use of Laboratory Animals. The method for induction of moderate CCI injury was performed as previously described. Briefly, rats (220–250 g in body weight) were anesthetized with 2% isoflurane vaporized in O2 flowing at 0.8 L/min and placed on a homeostatic temperature-controlled blanket while being maintained in a stereotactic frame. CCI was produced using a 4mm diameter impactor tip that was advanced through a 6mm craniotomy (centered at 0mm Bregma and 3mm left lateral to the sagittal suture) onto the brain using a 20-psi pressure pulse and to a deformation depth of 2 mm below the dural surface. Following injury, a cannula was placed into the right, contralesional sensorimotor cortex (SMC) through a 0.8mm diameter burr hole and secured to the skull with super glue gel and dental cement. Care was taken to prevent heat damage to the brain during drilling by cooling the skull with sterile saline (0.9%) during drilling.

Cortical Silencing

A permanent in-dwelling cannula (Plastics1 inc., Roanoke, Virginia) was placed into the contralesional homotopic primary sensory forelimb (S1-FL) cortex immediately following

CCI injury. Using stereotaxic coordinates from Paxinos and Watson Rat Atlas, cannulas were centered within the S1-FL area using the following coordinates, based on our previous fMRI data: +3.5mm lateral, +0.5mm rostral relative to Bregma, and 2.5mm below the outer skull surface (~1.5mm below the cortical surface) and into the middle of the S1-FL cortex according to both The Rat Brain in Stereotaxic Coordinates brain atlas (Paxinos and Watson 1998) and forelimb stimulus-evoked fMRI activation maps presented in Chapter 1.

Immediately after surgery, injured animals were randomized (randomizer.org) into two groups, Muscimol or aCSF. On days 7 and 28, S1-FL cortex activity was temporarily silenced by infusion of 1ul of the $GABA_A$ channel receptor agonist muscimol (1ug/ul in aCSF vehicle) through the cannula over a period of 10 mins. Vehicle-treated control rats received equivalent volume of aCSF (Harvard Apparatus, Holliston, MA) by the same injection procedure.

Assessment of forelimb function

Rats were habituated and trained in a staircase forelimb reaching/ pellet retrieval apparatus (Lafayette Instruments, Lafayett, IN) for 1 and 2 weeks respectively until they reached 50% reaching success rate on both limbs, as with prior studies (Harris et al. 2010). The number of steps cleared of sugar pellets within a 10-min period (pellet retrieved and eaten) was used as an objective measure of forelimb skilled-reaching ability. The number of pellets dropped was used as a measure of unskilled limb performance. Two trials were run for each rat per testing day and the results were averaged. Each trial was video-recorded for offline analysis. Reaching dexterity was assessed offline on a frame-by-frame basis.

The reviewer was blinded to the treatment group and asked to count the number of pellets eaten, dropped, and attempted with each forelimb. An unsuccessful attempt was counted when a rat reached for a pellet inside a stairwell and then closed its digits in a grasping effort, but ultimately failed to hold and eat the pellet. To calculate reaching success for each forelimb, the number of pellets eaten or dropped was divided by the total number of reaching attempts and expressed as a percentage. The rate of success provides a more precise measure of dexterity and sensory function compared to the number of total pellets eaten as it considers how much difficulty the rat had in feeling and grasping each pellet.

Functional MRI of CLCtx silencing

To investigate the effects of silencing the CLCtx on the cortical map at five weeks after CCI injury (**Aim 3**), forelimb-evoked BOLD MRI was acquired (as described in **Aim 1 Methods**) prior to and 1 hour after CLCtx silencing at 5 weeks post-injury from 11 rats from the forelimb reaching studies in **Aim 2**.

Results

To determine whether CLCtx activation is involved in behavioral outcome after TBI, forelimb reaching ability was assessed in the staircase reaching apparatus before and immediately after pharmacological silencing of the CLCtx by infusion of intracortical muscimol or aCSF vehicle control at one and four weeks post-injury (see **Figure 12** for overview).

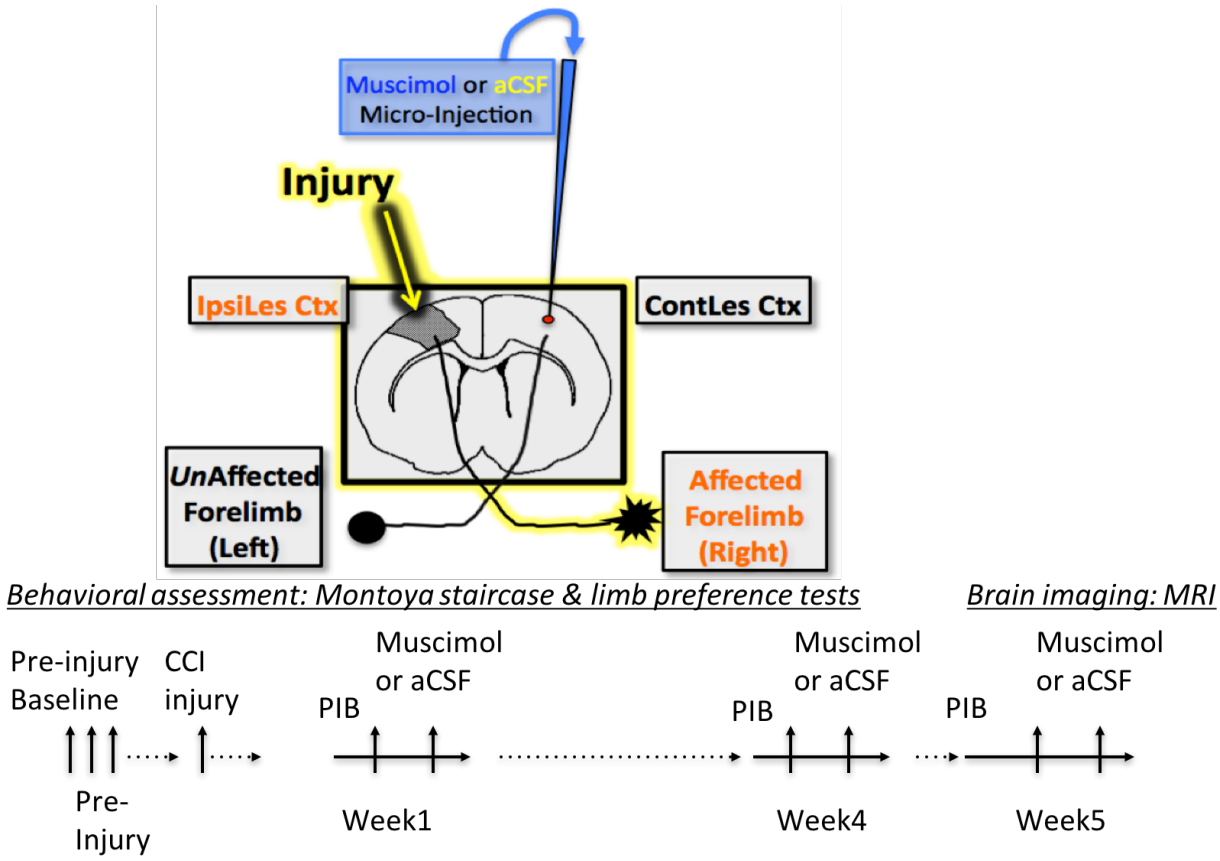


Figure 14. To determine the functional role of the contra-lesional cortex after TBI, rats were trained to reach in the staircase-reaching task to establish pre-injury baseline reaching accuracy for each rat. Controlled cortical impact (CCI) injury was delivered to the left, sensorimotor cortex, impairing the right forelimb. Pre-injection baseline reach performance was assessed on days 6 and 27 post-injury. On days 7 and 28 post-injury, a small portion of the contra-lesional S1-FL cortex was injected with either 1uL of Muscimol to silence part of this cortex or aCSF to control for any potential effects of vehicle injection. Functional MRI data was acquired during week 5 post-injury to test the UnAffected or Affected forelimb-evoked response before and during silencing of the contra-lesional S1-FL cortex.

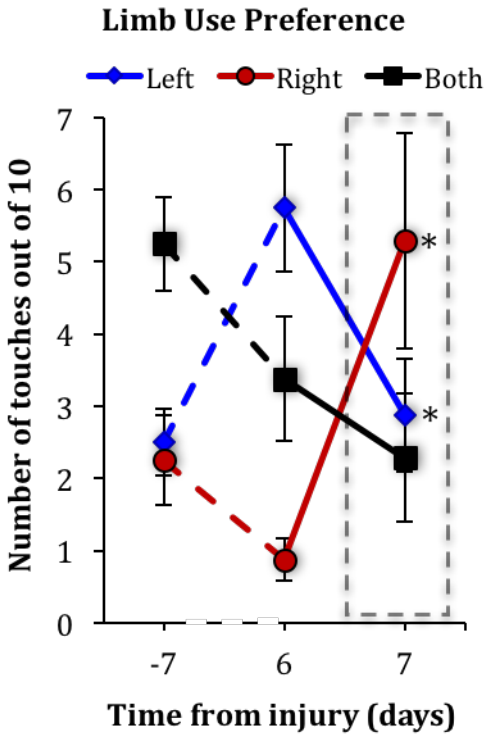


Figure 15. The limb use preference changed significantly over time after injury for use of the right, left, and both limbs (simultaneously) (one-way ANOVA, $p<0.05$). Testing limb preference before silencing on PID6 and after silencing the CLCtx on PID7 revealed a significant decrease in left, unaffected forelimb use preference (Blue diamonds) (paired t-test, $p<0.05$) and a significant increase in preference for use of the right, affected forelimb (red circles) (paired t-test, $p<0.05$), but no significant change in simultaneous use of both limbs (black squares) (paired t-test, $p>0.05$). Broken grey box highlights data acquired during silencing of the contralesional S1FL cortex. Broken line between -7d and 6d post-injury indicates non-linear time scale. *$p<0.05$, paired t-test between pre-silenced and silenced conditions.

CLCtx silencing reversed the injury-induced limb use preference favor affected forelimb at one week post injury

Prior to injury, rats did not show a preference for either the right or left limb in the Schallert Cylinder Test **(Figure 13).** Injury caused an increase in unaffected forelimb use and a decrease in affected forelimb use, as expected **(Figure 13)**. Also anticipated was the silencing-induced

decrease in use of the left forelimb (paired t-test, $p<0.05$). Surprisingly, however, silencing the CLCtx by muscimol injection on PID7 resulted in a significant increase in the proportion of affected forelimb use.

Silencing of the uninjured cortex impairs the UFL

To assess the efficacy of the silencing procedure, reaching success of the unaffected (by injury) forelimb was assessed before (6 or 27 DPI) and after (7 or 28 DPI) injection of either muscimol or the aCSF vehicle control **(Figure 14)**. Prior to injury, the future unaffected forelimb reaching accuracy was at or above 50% for both the muscimol (54%) and aCSF (61%) groups, and did not differ between the two groups **(Figure 14A)**. Injury did not impair forelimb reaching for the unaffected limb at 1 week post-CCI, as both groups were reaching at 55% success. At 7 days after TBI, infusion of muscimol impaired forelimb reaching ability of the unaffected forelimb by reducing it to an average of 17%. Reaching success for the aCSF group was not impaired, as these animals reached a 60% success rate after infusion compared to the 58% success rate on day six, prior to infusion.

At 27 days post-injury, unaffected forelimb reaching success was not significantly different from pre-injury reaching levels for either group **(Figure 14B)**. The day 28 injections initiated temporary unaffected forelimb reaching impairments in muscimol-treated rats (accuracy declining from 55% pre-drug to 17.5% post-infusion) while aCSF control animals maintained a reaching accuracy post-infusion (59%) that was not significantly different from the prior day's performance (61%). These combined results indicate that temporary CLCtx silencing was equally effective at both one and four weeks post-injury. To verify that the second injection also had no effect on later unaffected forelimb reaching, forelimb reaching success was measured a final time at 35 days post-

CCI. At that timepoint, the muscimol-treated group was reaching with 62% success rate while the control group was reaching with 54% accuracy, a difference that was not statistically significant (**Figure 15C**, students t-test, $p>0.05$). These data show that transient muscimol-induced cortical silencing did not have lasting effects on cortical function as measured by normal reaching success of the injury-unaffected forelimb at 35 days post-injury and after multiple injections of either muscimol or aCSF.

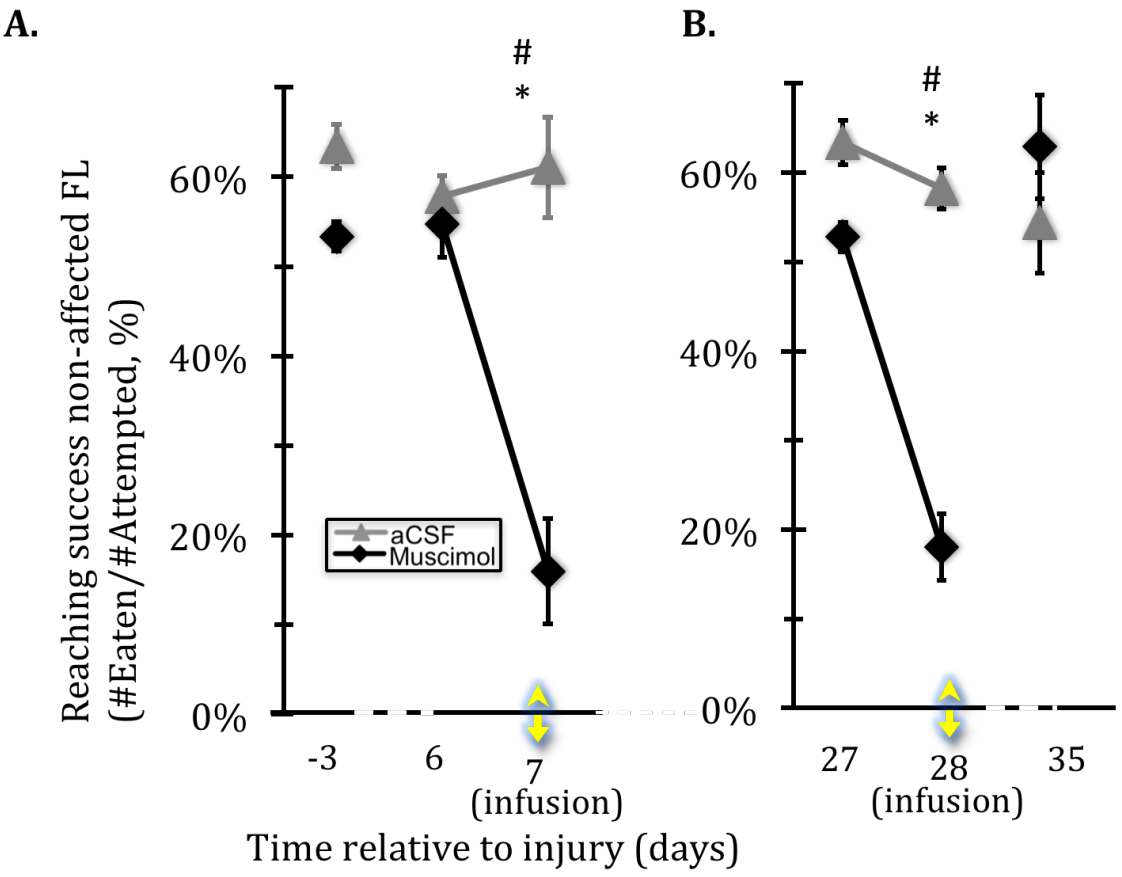


Figure 16. Muscimol injection-induced changes on PID7 in unaffected forelimb reaching success verified silencing of the contra-lesional cortical function resulted from muscimol but not aCSF (vehicle control). These results also suggest that silencing was equally affective at both 1 and 4 weeks post-injury, and that transient silencing did not have lasting effects on cortical function. tx=injection (Muscimol or aCSF), # $p<0.05$ between group, *$p<0.05$ pre-tx vs. tx for muscimol.

CLCtx silencing improved AFL function in acute recovery

At six days, post-CCI rats assigned to muscimol and aCSF groups had equivalent and significant deficits in forelimb reaching accuracy compared to pre-injury baselines **(Figure 15A).** Contralesional silencing resulted in a significant improvement in reaching accuracy of the affected forelimb compared to vehicle infused rats one week post-injury (muscimol vs aCSF at day 7, linear mixed effects modeling, corrected for multiple comparisons, $p=0.001$). Silencing improved reaching success to pre-injury levels (paired t-test, $p<0.045$). This contrasts with results seen for the unaffected forelimb, where no carryover effects of the first muscimol infusion were observed **(Figure 15A)**.

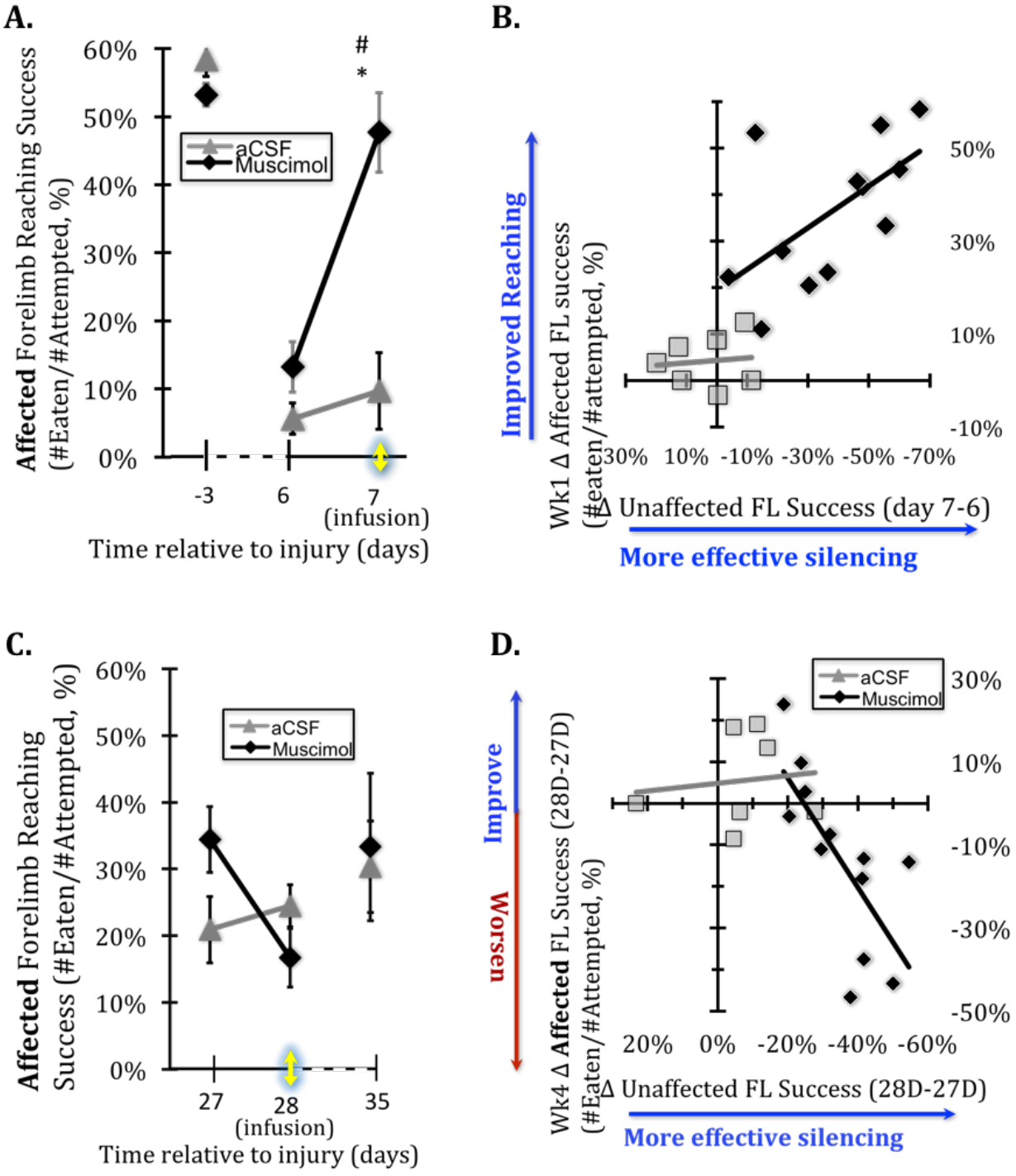


Figure 17. Silencing the contralesional cortex by muscimol injection ameliorated injury-induced impairment one week after injury. **[A]** Temporary silencing the contra-lesional S1-FL cortex by injection of muscimol one week after unilateral CCI injury resulted in significant improvements in affected forelimb reaching success compared to aCSF vehicle (linear mixed-effects model,

p=0.0011). **[B]** Week one improvement in affected forelimb reaching accuracy (y-axis) was dependent on the degree of impairment in the unaffected forelimb (x-axis) caused by muscimol (linear regression: F=19.43, P=0.0029 (two-tailed), and r2=0.7083), but not by vehicle injection (grey squares; p>0.05). **[C]** Silencing did not result in a significant difference between groups four weeks after injury. [**D]** At 4 weeks post-injury reinstatement of deficits in affected forelimb function (y-axis) was dependent on the degree of impairment in the unaffected forelimb (x-axis) produced by cortical silencing by muscimol (linear regression: F=10.43, P=0.0090, and r^2=0.5106), but not by vehicle injection (grey squares; F=0.07487, P=0.7953, r≤0.1475).

There was also some evidence of spontaneous recovery in the aCSF group from day 6 (~6-8 reaching success) to day 27 (20% success), concomitant with the improvements seen in the muscimol group from day 6 (~12% success) to day 27 (~35% success) **(Figures 13A and 13C)**. At five weeks post-injury, the muscimol-treated group was reaching with ~33% success rate while the control group was reaching with ~28% accuracy (**Figure 13A**; not significantly different between groups). This level of performance with the affected limb was not significantly worse than that of the unaffected limb at 5 weeks post-CCI.

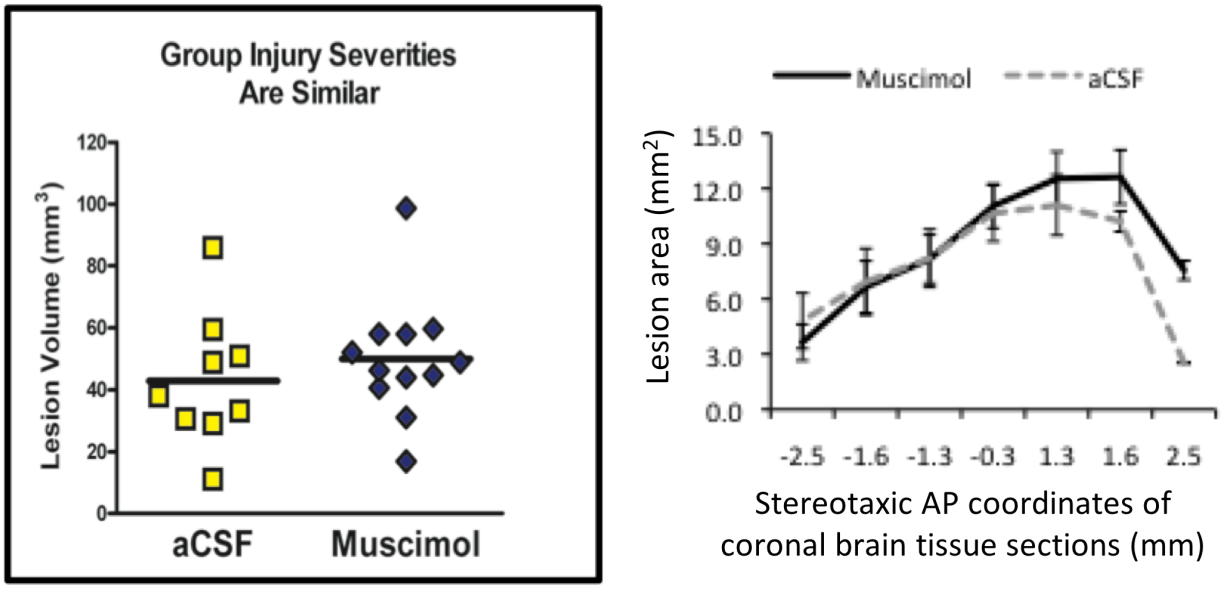


Figure 18. Lesion volumes were similar between aCSF and Muscimol groups. **[A]** scatter plot of lesion volume for each rat by treatment group. Horizontal line denotes the group average. Lesion volume did not differ between aCSF and Muscimol rats by volume analysis (Signed-rank test,

p>0.05). **[B]** Average lesion area (mm^2) for each coronal brain section mapped by the A-P distance from anatomical landmark, bregma.

Dose-response evaluation

The silencing technique employed in these experiments produced greater than expected variability in forelimb reaching impairments, suggesting that the cortex was not silenced to the same degree among all rats **(Figures 13B and 13D)**. However, this variability in the effectiveness of the muscimol-silencing technique provided an opportunity to determine if there is a dose-response relationship between the amount of silencing (change in reaching success for the unaffected forelimb) and the influence of the CLCtx on affected limb reaching ability at one **(Figure 15B)** and four **(Figure 15D)** weeks post-injury. Interestingly, reaching improvement at one week post-injury showed a significant positive correlation, with greater resumption of reaching ability resulting from a greater effectiveness of cortical silencing indicated by loss of reaching function in the unaffected limb **(Figure 15B)**. Crucially, in vehicle control rats, there was no association between silencing of unaffected limb and affected forelimb after aCSF infusion. The situation at four weeks post-injury was the opposite effect, as muscimol worsened outcome, and this was dependent on the degree of silencing. A reinstatement of deficits in the affected forelimb was significantly correlated with the degree of impairment produced by muscimol in the unaffected forelimb, an effect not found in the aCSF-treated rats **(Figure 15D)**.

Muscimol effectively silences unaffected forelimb-evoked BOLD activation of S1FL CLCtx.

Preliminary fMRI results from 5 weeks post-injury indicate that the contralesional silencing procedure effectively dampens activity of the sensorimotor cortex surrounding the injection site. **Figure 17A** shows the contrasts between unaffected forelimb stimulation-evoked activations

before and during active silencing of the CLCtx with muscimol. The blue activation maps **(Figure 17A, left)** indicate regions of the brain that are more strongly activated prior to silencing. Put another way, the blue brain regions exhibit reduced brain activation due to muscimol injection. On the contralesional hemisphere, there is significantly greater involvement of the sensorimotor cortex, caudate/putamen, compared to the same rats one hour after contralesional muscimol injection ($P<0.05$, $z>1.0$, corrected for multiple comparisons). Conversely, the red-yellow activation maps **(Figure 17A, right)** indicate new regions of brain activation that occur due to affected forelimb stimulation during silencing, compared to the pre-silencing condition. Several small brain regions show increases in unaffected forelimb-evoked activation **(red-yellow activations; Figure 17A)**. The cortical regions of silencing-induced increases include the CLCtx secondary somatosensory cortex (S2) and part of the ipsilesional S1-FL cortex. This data verifies that overall muscimol injection successfully inhibited the contralesional sensorimotor forelimb cortex.

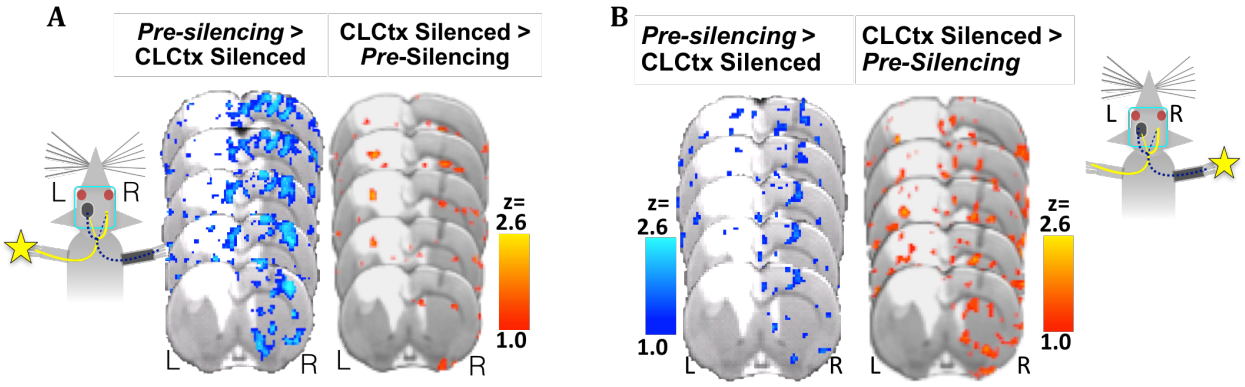


Figure 19. **A.** Comparisons of unaffected forelimb stimulation-evoked BOLD activation contrast maps compared between the same rats (N=8) prior to and during contralesional silencing by muscimol infusion into the contralesional S1-FL cortex through a cannula (imaging artifact from cannula visible in right cortical hemisphere). Silencing resulted in an increase in activation in the contra-lesional thalamus but the S1FL region of the CLCtx was not responsive to affected forelimb

stimulation. **B.** Comparisons of affected forelimb-evoked activation areas are compared between the same rats (N=8) prior to and during contralesional silencing by muscimol infusion into the contralesional S1-FL cortex through a cannula (imaging artifact from cannula visible in right cortical hemisphere). No new areas of activation are present following stimulation of the affected, with exception of the thalamus, which is more greatly activated while the contra-lesional cortex is activated.

Contralesional silencing of affected forelimb-evoked activation at 5 weeks unmasks very few new regions of brain activation

Stimulation of the injury-affected forelimb of this group **(Figure 17B)** prior to contralesional cortical silencing showed the contralesional S1-FL cortex activation, as observed in the first MRI study, and showed some activation lateral to the injury site and within the injured cortex. The lesion observed in the averaged RARE images are dominated by the largest lesions, and are representative of only the most severely injured rats. Prior to silencing, the contralesional S1-FL cortex is activated in response to stimulation of the injury-affected forelimb (**Figure 17B**, blue activations).

Discussion

In the present chapter, functional MRI was used to image nearly the whole cerebrum to identify regions responsible for time-dependent effects of silencing the CLCtx in acute and more chronic post-injury recovery periods. Experiments for **Aim 3** were designed to identify new areas of activation which might be responsible for the surprising result of silencing-induced improvements in forelimb reaching at one week post-injury and the worsening of affected forelimb impairment when the CLCtx is silenced with muscimol at four weeks post-injury. Forelimb-evoked fMRI data was collected from 11 rats in the

chronic post-injury recovery period. By measuring brain activation prior to injection of muscimol, and then a second session 1 hour after the injection of muscimol into the CLCtx, this experimental design provided the opportunity to compare the effect of silencing of the CLCtx by muscimol to the non-silenced condition within the same animals. The idea was that the within animal comparison would be more powerful than having two groups, because it would minimize the variability across animals, particularly regarding lesion size and location. In addition, to control for any potential effect of the vehicle injection, five animals were scanned prior to and one hour following injection of artificial cerebrospinal fluid vehicle (aCSF). BOLD activation data acquired before injection could again be compared, within-subject, to that acquired one hour after injection to determine if there are any differences which may be caused by the injection itself. We expected that there would be no difference in average group activations caused by injection of aCSF vehicle control since there was no effect on forelimb reaching ability (see **Figures 11, 12, and 13**). At the level of a cortical network, such as the S1 forelimb region of the CLCtx, application of muscimol reversibly dampens the cortical response in a concentration-dependent manner.

Silencing effectiveness by muscimol injection was verified by the disappearance of CLCtx activation during unaffected forelimb stimulation at the chronic time point. Stimulation of the affected forelimb further verified the efficacy of contralesional silencing because the contralesional activation was eliminated in cortex surrounding the cannulas **(Figure 16)**. At 4 weeks post-injury, silencing re-enacted the deficits in affected limb function, so the silenced CLCtx was at least part of a network critical for supporting spontaneously recovered function. Unsurprisingly, no new areas of activation were observed upon silencing the CLCtx at PID35.

Balance of trans-callosal inhibition is disrupted in TBI

Basic mechanisms of trans-callosal cortical inhibition have been characterized in the normal, uninjured vertebrate brain (Ferbert et al. 1992; Meyer et al. 1995; Gerloff et al. 1998; Li et al. 2011). Silencing of activity in the rodent forelimb motor cortex with the sodium channel blocker lidocaine caused a 42.2% expansion of the forelimb representation in the opposite cortex (Maggiolini et al. 2008). This data agrees with the widely-supported notion that each hemisphere works constantly to maintain the forelimb representation of its contralateral, homotopic counterpart, and that this maintenance is achieved through mostly inhibitory influences (Ferbert et al. 1992; Meyer et al. 1995; Gerloff et al. 1998; Li et al. 2011). According to this basic mechanism, injury to one cortical hemisphere would result in dysfunction of that injured area imparting disinhibition of its contralesional, uninjured counterpart. Conversely, if the uninjured, CLCtx is disinhibited it could become hyper-excitable, as observed in the PP-SEP experiments from **Aim 1** (**Figure 6**). Indeed, after a focal, cortical ischemic lesion, use of the unaffected forelimb results in reduced ILCtx-to-CLCtx inhibition and increased CLCtx-to-ILCtx inhibition (Rehme et al. 2011) but it is not known whether the brain's response to TBI is like that of stroke in this regard. Any differences might provide insights into the source of the difference in terms of differences between the disease pathophysiologies.

Inactivation of the CLCtx one week after injury resulted in a dramatic recovery of function in the injury-affected limb (**Figure 15**; 6 vs. 7 days, $p=0.0156$, Wilcoxon two-tailed signed rank test). Therefore, activity of the uninjured homotopic S1-FL region could impair limb function by relieving CLCtx-to-ILCtx inhibition. It may be that silencing prevents the uninjured cortex from inhibiting an otherwise still-functional ILCtx through inhibitory trans-callosal activity. While it

seems that the injured cortex is "stunned" with metabolic crisis early after injury once that has resolved, the volume of activated ILCtx continues to decline due to ramping up of CLCtx-to-ILCtx inhibition over the first four weeks post-injury. Another possibility is that the injured cortex is truly dysfunctional in moderate-severe injury so that it is less capable of supporting function one week after injury. Thus, inactivation of contralesional S1-FL cortex may rescue limb function through disinhibition of other regions of brain that might be able to support function early after injury. These could be enhanced by functional alterations in connected subcortical regions such as the thalamus and caudate, or in associated cortical regions such as the prefrontal cortex or supplementary sensory and motor areas. For example, in human stroke patients, fMRI studies of hand movement found connectivity changes between injured primary motor cortex (M1) for the hand and contralesional M1, premotor, and supplementary motor cortices (Rehme et al. 2011). Increased peri- and contralesional activations in the first two weeks after injury by affected forelimb stimulation were observed in the fMRI data presented here (Aim1/chapter 1, **Figure 3** above). Corresponding electrophysiological data **(Figure 7A)** suggest that the underlying activity is associated with post-synaptic activity of pyramidal cells, because sensory-evoked cortical signal was also detected over this cortical region. It is possible that the increased activation of the CLCtx elicited by the affected limb arises from reduction of trans-callosal inhibition from the contused or perilesional cortex. By silencing this CLCtx at seven days post-injury contralesional to ipsilesional inhibition is released, allowing the contused cortex to resume control of the affected forelimb. This hypothesis presumes that the injured and or ILCtx remains functional at seven days post-injury but it is merely inhibited by the increased activation and inhibition from the CLCtx. Additional fMRI studies are required to determine which region of the brain is responsible for the reinstatement of limb function that occurs with silencing of the CLCtx at seven days post-injury.

The site of direct unilateral focal injury does not activate in more severely injured brains (see **Figures 3A** and **4B**). Over time, however, it is possible that the brain makes adaptive changes to the hemispheric imbalance in inhibition and excitation. Biernaskie et al. (2005) used lidocaine to temporarily inactivate the contralesional motor cortex after rats had recovered from unilateral stroke (during exposure to an enriched environment). In their stroke model, silencing strongly and significantly reinstated a functional deficit in a skilled forelimb-reaching task following either three or four weeks of post-injury enriched-rehabilitation, indicating that the CLCtx is critically involved in functional recovery after physical rehabilitation (Biernaskie et al. 2005). Similarly, in the present study, muscimol silencing showed a trend toward reinstatement of the injury-induced deficits at week four—worsening reaching performance in eight of the 11 animals tested—the within-group effect was not significant in the post-hoc comparison. The between-animal variability of reaching success is probably one contributing factor. However, we did not observe clear evidence that lesion volume in the chronic period, indicating that lesion volume may not be a sensitive predictor of functional outcome. A second possibility is that the cannula placement did not completely target the cortical region which aids in support of function for the recovering forelimb by four weeks post-injury, as we have observed the CLCtx map shifts laterally throughout this period. Deficits were not significantly reinstated in our model of TBI at four weeks post-injury in either aCSF or muscimol treated rats from 27 to 28 days post-injury. This discrepancy may arise from differences in etiology between stroke and TBI. The homotopic contralesional (S1-FL) cortex may be among several different networks that support limb function for injured cortex after TBI.

Taken together, these data support the hypothesis that CLCtx-to-ILCtx trans-callosal inhibition prevents normal forelimb function and ipsilesional map plasticity. I suspect that the mechanism by which the CLCtx aids in latent recovered function of the affected forelimb involves

the chronic inhibition of activity in the injured cortex throughout recovery and a reciprocal loss of ILCtx-to-CLCtx inhibition. Thus, the CLCtx is an important target for intervention and a biomarker signaling the need to re-establish balance of inhibition/excitation between the hemispheres and other interconnected regions (McNeal et al. 2010; Rehme et al. 2012) to potentiate recovery of lost function resulting from TBI. The altered functional map may help provide information about the spatial and temporal status of the recovering brain (Hoskison et al. 2009; Rehme et al. 2011, 2012; Algattas and Huang 2013) so that rehabilitative and interventional strategies (Mansoori et al. 2014) are tailored to each individual case throughout recovery.

Excitability is also attenuated in aged rats (David-Jürgens and Dinse 2010) and humans with diminished tactile acuity (Takeuchi et al. 2010). Increased inhibition around the injury site may be a maladaptive phenomenon when lesions are small and some of the network is spared. Research on stroke patients show reduced inhibitory drive from the ILCtx-to-CLCtx during early recovery (Rehme et al. 2011). At chronic stages patients showed stronger CLCtx-to-ILCtx inhibitory drive which was also correlated with poor functional outcome(Rehme et al. 2011). In a well-characterized cortical focal stroke model, there is convincing evidence that increased intra-cortical tonic inhibition within the ILCtx prevents recovery, and that general pharmacologic attenuation of tonic inhibition with $GABA_A$ receptor inverse agonist significantly improves functional outcome (Clarkson et al. 2010; Clarkson 2012).

The current model-based data point towards remote regions of changes in excitation/inhibition. The BOLD data from the first MRI study indicated that the contra-lesoinal cortex was the only area that showed persistent new activation every week for at least four weeks and electrophysiological studies verified the hyperexcitability. Imbalance between inhibition and excitation initiated by injury should be addressed to recover the balance between the injured cortex

and other remotely connected networks. It may be possible to drive improved recovery of the ILCtx, re-enable its activation over time, and provide an opportunity for a better overall improvement in functional outcome.

Early time point MRI

The finding that limb use preference (Schallert cylinder test) and dexterous function of the injury-affected forelimb had essentially normalized by silencing the CLCtx at one week post-injury was surprising and perhaps the most interesting finding for further study by investigators in the future. These findings imply that CLCtx activity interferes with function of the injury-affected forelimb acutely, but not chronically after injury. The prolonged contra-to-ipsi-lesional inhibition, which begins within the first week after injury, could act to divert somatosensory signals from the affected limb to the CLCtx rather than ILCtx, thereby "learning" to process information from the affected forelimb through mechanisms of activity-dependent plasticity. If this winner-take-all inter-hemispheric competition type model is at work after CCI, then rebalancing the interhemispheric excitability might promote survival and sustained use of the ipsi-lesional cortical map. Thus, for future investigators, it would region of the brain is responsible for the silencing-induced return of forelimb function at one week post-injury. My hypothesis is that the CLCtx is responsible for inhibiting this region, either directly or indirectly. The contusional or peri-contusional cortex is the most obvious region of potential reactivation since there is direct inhibition of this region through the trans-callosal fibers from the CLCtx. Given the various other cortical and subcortical regions both structurally and functionally connected to the S1FL CLCtx, it is possible that silencing CLCtx produces a second

diaschisis resulting in inhibition or disinhibition of another network which is then able to support full limb function just one week after injury. Regions connected to the silenced S1FL CLCtx which could also support forelimb function might include the prefrontal cortex, higher-level somatosensory processing cortices like the secondary somatosensory (S2) cortex, the thalamus, primary (M1) and secondary motor (M2) corticies, and the cerebellum (Pawela et al. 2010). Reestablishment of balanced excitatation/inhibition between, not only the injured and contra-lesional cortices, but between all brain regions that undergo excitability-related diaschisis, may be useful in directing neural repair and remodeling. Ideally, the use of a targeted approach guided by the fMRI and electrophysiological results may help facilitate a more complete recovery following a traumatic brain injury.

Chapter 4

General Discussion

There is currently no cure for traumatic brain injury and all clinical trials to date have failed. Fortunately, some spontaneous recovery can occur after injury and rehabilitative therapies have proven to be effective in enhancing spontaneous recovery. Even with current rehabilitative therapeutic approaches, recovery is often incomplete, so many TBI survivors are left to struggle with long-term disabilities. The data in these studies indicate that network-specific, targeted approaches to modulate the excitability of specific nodes of injury-affected networks, such as the CLCtx, might improve the efficacy of rehabilitation by unlocking the ability of a patient to temporarily practice physical therapy exercises with the injury-impaired limb. In the clinic, this could translate to the use of repeat transcranial magnetic stimulation (rTMS) during physical therapy sessions. The author proposes that future treatment might implement a wearable headset cap device with multiple stimulators to balance excitability across the brain to optimize recovery. Such an idealized theoretical device would be a closed-loop system implementing sensors to use brain function biomarkers to dynamically modulate neural excitability throughout the brain during affected and unaffected forelimb use. This sort therapeutic strategy hinges upon the assumption that the effects of stimulation will have carry-over effects between treatment sessions so that there is a synergy between the stimulation and rehabilitation to maximize recovery. Since map changes have been shown to occur in response to rehabilitation, rehabilitative therapies could improve outcome by encouraging the cortical map to shift back to the ipsi-lesional cortex or to other regions which might better support function (Hamzei et al. 2006; Richards et al. 2008; Donahoo and Richards 2009). With the addition of brain stimulation strategies, rehabilitation

might be more effective in shifting the map to the most optimal region(s) of brain to support functional recovery.

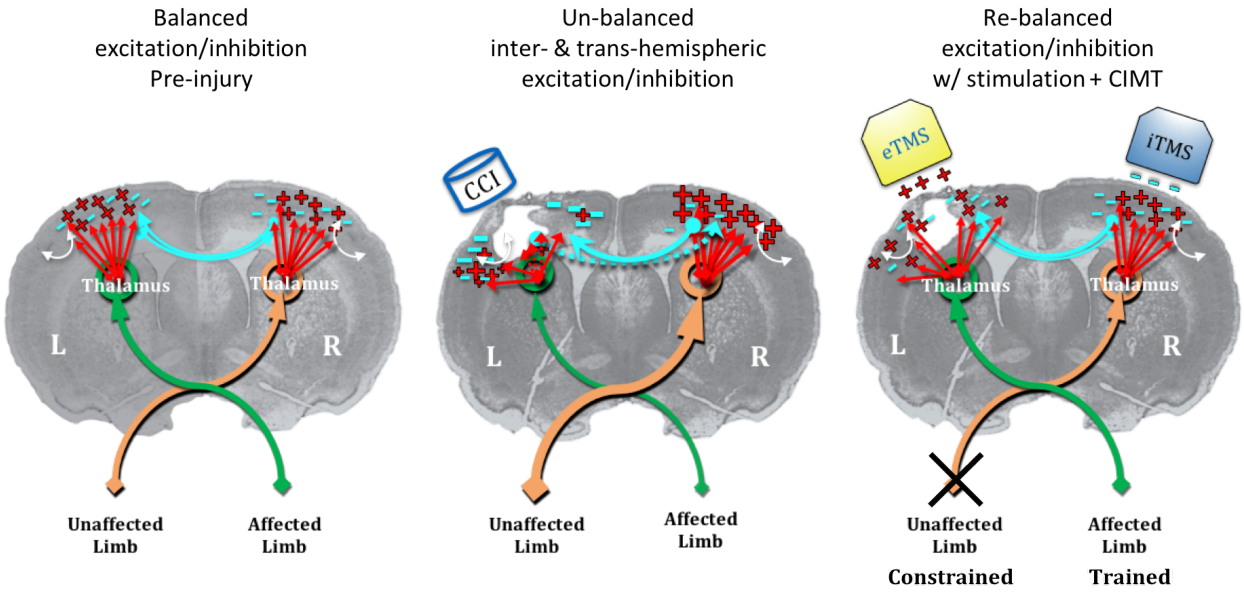


Figure 20. Schematic diagram of the hypothesized mechanism of trans-hemispheric excitability disruption by lateral injury and re-balancing of excitation/inhibition by combined neuro-stimulation and CIMT treatments. **Left:** Prior to injury, interhemispheric inhibitory fibers are functional, and excitation (red "+" signs) and inhibition (blue "-" signs) is balanced in homeostasis. Excitatory sensory input frome each limb to the corresponding thalamus then to the S1FL cortex, each being excitatory inputs. **Middle:** Injury results in loss of ipsi-to-contra-lesional inhibition and mostly intact contra-to-ipsi-lesional inhibition when the contused cortex is "stunned" and further when axons die due to Wallerian degeneration and secondary axonal injury. **Right:** Excitatory stimulation of the ILCtx and inhibitory stimulation of the CLCtx rebalances excitation/inhibition across the injured brain and re-enable affected limb function, which is then re-enforced using CIMT and AFL training.

CCI Injury: Disinhibition, Expansion of AFL-CLCtx receptive field, Activity-dependent plasticity

Decreased AFL Input: Impairment leads to learned non-use

Increased UFL Input: Compensatory over use, Activity-dependent plasticity, Expansion of AFL-CLCtx receptive field;

eTMS = excitatory transcranial magnetic stimulation, iTMS = inhibitory transcranial magnetic stimulation, L = Left, R = Right, CCI = Controlled cortical impact injury, CIMT = constraint-induced movement therapy.

Current neurorehabilitation strategies and map plasticity

Post-injury rehabilitative therapies have been developed to target various types of impairments including speech, memory, cognition, or movement. Here, the focus will remain on therapies geared toward sensorimotor recovery, since the injury model used for these experiments feature TBI affecting the sensorimotor networks of the central nervous system. Impairment of forelimb/upper limb after brain injury naturally results in increased use of the UFL to compensate for lost function of the AFL (Taub et al. 2006).

Constraint-induced movement therapy (CIMT) is one of the most proven and common interventions for limb impairments due to stroke. CIMT encourages use of the AFL and decreases voluntary activity of the UFL by constraining the UFL in a sling CIMT has also been shown to alter excitability (Tarkka et al. 2008). While more studies of CIMT's effectiveness have been performed in stroke research, some have been emerging in TBI research. A study of CIMT in adolescent TBI showed significant improvements in a variety of outcome measures (Cimolin et al. 2012; CDC 2014). This is particularly important because of the bimodal increased incidence of TBI among the young and the old (Testa et al. 2005). In a more elderly population, a study of 29 chronic stroke patients with hand impairment, used fMRI and TMS to investigate cortical reorganization after CIMT (Hamzei et al. 2006). These investigators found that AFL training with CIMT was associated with a change in the sensorimotor map and intracortical excitability for the affected hand (Hamzei et al. 2006). Others have found CIMT to both change the cortical map and improve function (Schaechter et al. 2002). The Extremity Constraint

Induced Therapy Evaluation (EXCITE) trial, a prospective, single-blind, randomized, multicenter clinical trial conducted at 7 US academic institutions from 2001-2003 found that when a 2-week CIMT program was implemented between 3-9 months post-stroke, patients showed significant and lasting improvements in arm function (Wolf et al. 2012). Sensorimotor rehabilitation significantly improves function of the affected forelimb after CCI in the rodent, but rehabilitation therapies must be more intense and more varied to show improvements, whereas stroke injury responds more readily to individual therapies.

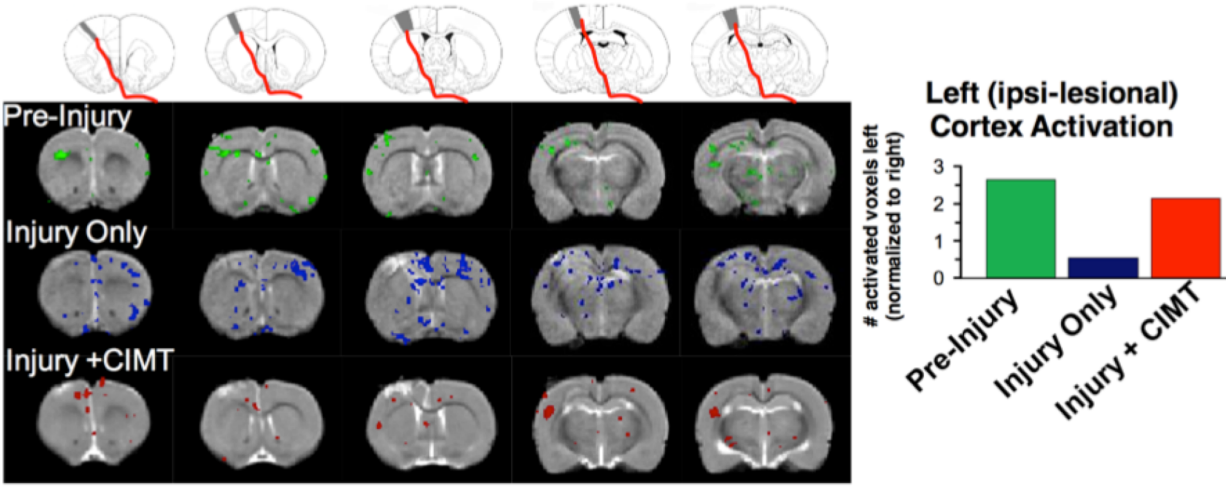


Figure 21. Images: Affected forelimb-evoked fMRI data acquired pre-injury (green mean activation area) and at 28d after injury with CIMT at 4-14d post-injury (red mean activation area) or injury only (blue mean activation area; n=3/group) revealed an effect of CIMT increasing more ipsilesional activation (left side of images). Activation data are over-plotted onto mean anatomical data showing no effect of early CIMT on contusion volume. Graph: Number of voxels significantly activated in ipsilesional (left) cortex normalized by the number of voxels activated in the contralesional cortex. Figure adapted with permission from Professor Neil G. Harris, Ph.D.

In the rat, preliminary studies (3 rats/group) showed that compared to no treatment controls, CIMT from days 4-14 post-injury resulted in a shift in the average activation to the

ipsilesional cortex around the injury site at 28 days post-injury **(Figure 21)**. Though this is a small sample of 3 rats per group, the activation maps clearly show that CIMT rats had greater ipsilesional activation and less CLCtx activation. These preliminary data still support the hypothesized mechanism of action **(Figure 20)**. These results should encourage future use of fMRI to analyze the neurophysiological alterations which result in optimal recovery, particularly in an animal model in which injury location can be controlled. While effectively preventing UFL input activity, CIMT also results in an obligatory increase in AFL use. For quadrupedal animals like rats, walking with the UFL constrained in a vest necessitated consistent use of the impaired, AFL. In humans, who are bipedal and use their upper limbs for daily tasks, CIMT is employed during daily tasks to "re-train" the impaired limb.

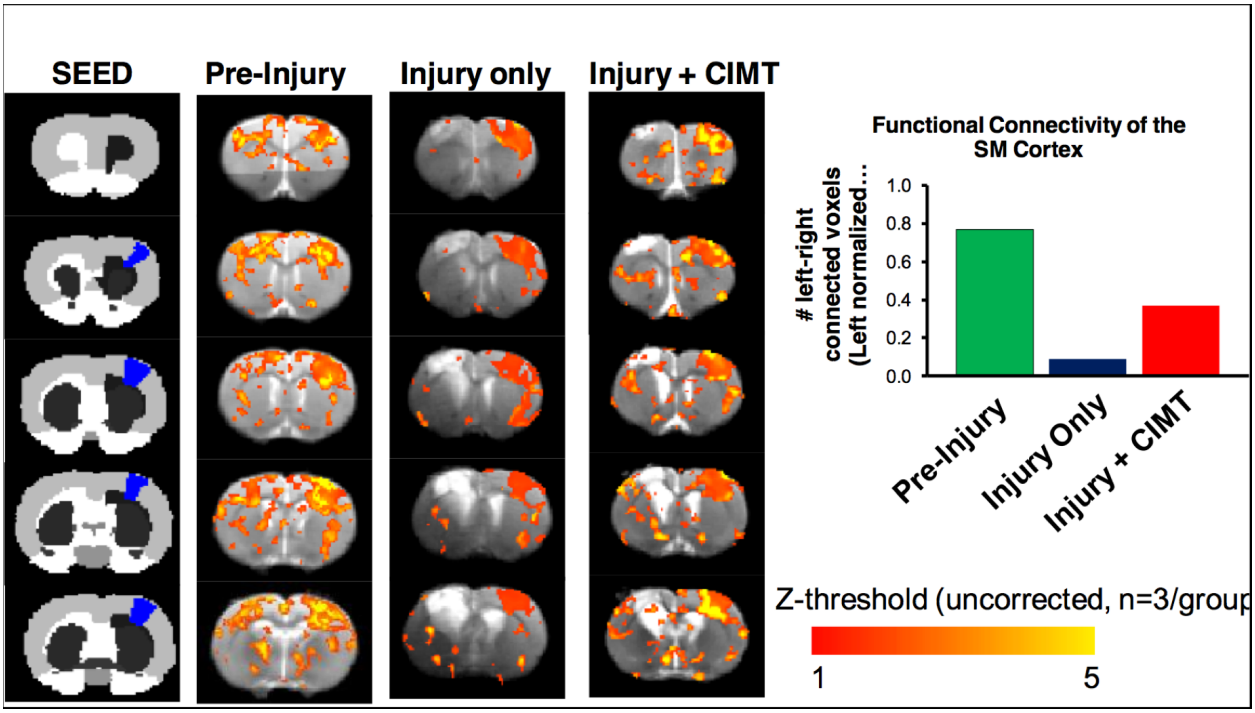


Figure 22. Images: Resting- state fMRI data were acquired from rats before injury and then again at 4 wks post-CCI (CIMT+injury, injury only, n=3/group). After co- registration to a rat atlas, data were processed for seed- based functional connectivity (fc) analysis of the contralesional SM cortex (blue area). CIMT increased fc of the homotopic, ipsilesional SMC compared to injury alone as shown by mixed effects analysis Z score (see Figure legend). Graph: Number of

functionally connected voxels in ipsilesional (right) SM cortex normalized to contralesional (left) obtained from group mean image data shown.

In the same groups of CIMT and control rats, resting-state fMRI was also acquired before and then 4 weeks post-injury and analyzed for functional connectivity to the S1FL CLCtx **(Figure 22)**. These data indicate that inter-hemispheric functional connectivity was greatest in rats rehabilitated using CIMT. Thus, CIMT may improve AFL function by changing the laterality of input toward more ILCtx activation from AFL activity. In this regard, inhibitory stimulation of the CLCtx and excitatory stimulation of the ILCtx are the afferent compliment to CIMT and therefore has potential to enhance current rehabilitative treatment paradigms in TBI survivors **(see Figure 20)**. After injury cortical reorganization is dependent upon time-dependent imbalance between glutamatergic excitation and GABAergic inhibition (Benali et al. 2008).

Does non-invasive region-specific brain stimulation hold the potential to be an adjunctive approach to enhancing the effectiveness of current rehabilitative therapies?

The Food and Drug Administration (FDA) has approved use of vagus nerve stimulation (VNS) for epilepsy and depression, deep brain stimulation (DBS) for Parkinson's disease and dystonia, and repetitive transcranial magnetic stimulation (rTMS) for the treatment of depression. Another stimulation method, transcranial direct current stimulation (tDCS) has recently been tested as experimental treatment of stroke and neuropsychiatric patients (Aparicio et al. 2016). Network-specific neuromodulation might also show promise in a potential ability to enhance rehabilitative therapies after TBI. The proposed mechanism of action for CLCtx inhibition (silencing) to enhance the effectiveness of rehabilitative therapies to improve recovery can be summarized as the following: CLCtx silencing results in disinhibition of the ILCtx and

other connected regions, making these networks newly receptive fields. Therapy then uses these new networks, promoting cortical re-specialization by mechanisms of activity-dependent-plasticity (Martin et al. 1999; Overman et al. 2012). Advanced analysis of longitudinal fMRI data from stroke patients at the acute and chronic phases of recovery shows altered balance in inhibitory or excitatory tone between interconnected network nodes after injury (Rehme et al. 2011, 2012; Volz et al. 2015). The work of Rehme and colleagues provides strong incentive for the investigator to be mindful that there are likely to be multiple close or distant networks which will be affected by injury and probably important for recovery (Rehme et al. 2011, 2012; Volz et al. 2015). These studies serve as a powerful reminder that the most affective stimulation paradigm treatments will probably require stimulation of multiple regions simultaneously, each with custom stimulation parameters, depending on whether the network excitability was to be increased or decreased. With this approach and results of the current literature in mind that spatially balanced activity between regions is required to re-establish their appropriate connectivity.

There is still much pre-clinical work to be done to understand the significance of the many variables which are inherent after TBI but much more difficult to control and measure. Some important variables which need to be better understood include timing of treatment, method of stimulation or other task relevance, location and distribution of primary and secondary injury/injuries, and mechanism of injury. Although there are limitations to pre-clinical animal models, the ability to control the above variables, they provide the most promising opportunity to understand not only the function of each variable, but also the interaction between each variable. Analysis of large public data sets from many clinical and pre-clinical studies might ultimately provide the best understanding of the mechanisms and the potential points of intervention.

Neuroimaging still holds promise as a TBI biomarker but is limited by a lack of clear relationship between the neuropathology of injury and recovery and the quantitative image-based data obtained. Specifically lacking is the data on electrophysiological changes that lead to alterations in cortical map changes after TBI.

When inhibitory rTMS (iTMS, ≤1 Hz) is applied to the motor cortex in one hemisphere it works to diminish excitability there, while simultaneously increasing the excitability of the non-stimulated hemisphere through a reduction of interhemispheric inhibition from the stimulated (inhibited) to the non-stimulated motor cortex. When rTMS is applied with higher frequency stimulation (≥3Hz), rTMS can be used to increase cortical excitability (Kubis 2016). Regarding the model **(Figure 20)** of re-balancing interhemispheric excitation/inhibition this could be applied to the injured cortex or other brain regions affected by excitability-related diaschisis after TBI.

Importance of timing

The results of the current studies document several main findings regarding post-injury-time-dependent changes in cortical map excitability, plasticity, and function in brain regions remote from the site of injury following controlled cortical impact (CCI) injury in a rat model of unilateral TBI. First, the group averaged cortical representation for the affected forelimb (AFL) stimulation-evoked BOLD activation map shifted from its usual unilateral location in the left M1/S1FL sensorimotor cortex before injury, to a broad bilateral/bi-hemispheric coverage in the first two weeks after injury. Then, the bilateral activation patterns focused down to more compact regions at 3 weeks post-injury, and finally by 4 weeks post-injury, the average AFL

cortical map had made a complete trans-hemispheric shift to a the CLCtx. The appearance of new AFL BOLD activations in the CLCtx are both spatially and temporally associated with periods of increased SEP signal and altered excitability. Additionally, the role of the CLCtx in affected forelimb function at one week post-injury is the exact opposite as its role at four weeks post-injury. These data show the importance of timing of treatment, as the brain dynamically remodels throughout recovery.

Is the uncrossed ipsilateral pathway to the CLCtx the most optimal or just the new path of least resistance?

It may seem unlikely that the small proportion of axonal fibers that make up the uncrossed (ipsilateral) sensory and motor pathways could support the same load of information that the primary, crossed pathway does prior to injury. There is a plethera of evidence that fibers from the damaged pathway sprout axons back across to the contralesional side at the level of the spinal cord, structurally bolstering the ipsilateral pathway (Martin et al. 1999; Dancause et al. 2005; Lee et al. 2011; Lindau et al. 2014). Damage to the dorsal columns, spontaneous recovery is dependent on the sprouting of preserved afferent fibers and their enhanced role in activating neurons throughout the somatosensory system in rats, primates, and humans, alike (Kaas et al. 2008). Another potential neuroplastic mechanism that the brain may use to compensate for the smaller ipsilateral tract is amplification of the signal from the AFL at the synaptic junctions within the contralesional thalamus or the CLCtx. While the present data supports the idea that the CLCtx plays a role in supporting recovered limb function in chronic recovery, it may not be capable taking on 100% functional support. If the entire network is destroyed, then for complete functional recovery to occur, other brain regions must be tasked with supporting the lost function

beyond what the CLCtx is capable. It may be that the imbalance in excitability leading to increased CLCtx excitability and functional take over can be corrected to enable other nodes of the sensorimotor network to support some of the function lost to injury.

Early map changes are reliably reported by fMRI BOLD

The experiments for the first study were designed to investigate cortical map changes, which have been shown to occur and are thought to be associated with spontaneous functional recovery of the injury-affected forelimb. It is unlikely that abnormal CLCtx activations pathophysiological events associated with TBI that might alter the hemodynamic response include vasogenic edema and blood-brain barrier (BBB) permeability. Holmin and Mathiesen described a biphasic temporal profile of edema occurring over the first week after injury in the damaged cortical hemisphere (1995). The first phase of edema occurs from 1 hour to 2 days post-CCI before subsiding from days 3-5 post-CCI (Holmin and Mathiesen 1995). The secondary phase of edema occurred at PID6 within the injured, but not the CLCtx (Holmin and Mathiesen 1995). These pathophysiologies were restricted to the injured cerebral hemisphere and thus are by themselves not sufficient to explain the increased CLCtx activation and altered excitability. Thus, the data presented here indicates that the neurovascular coupling required for reliable fMRI brain mapping is intact within the CLCtx. Thus, fMRI may be a very useful biomarker of altered excitability and might help inform a patient-specific treatment approach for rehabilitation and stimulation protocol. Further studies using DCM analysis methods of fMRI data, similar to that done in stroke (Rehme et al. 2011, 2012; Volz et al. 2015), could provide valuable information about how the excitability of each network node has been altered by injury and thus, how stimulation can be used to rebalance these network interactions during rehabilitative therapy.

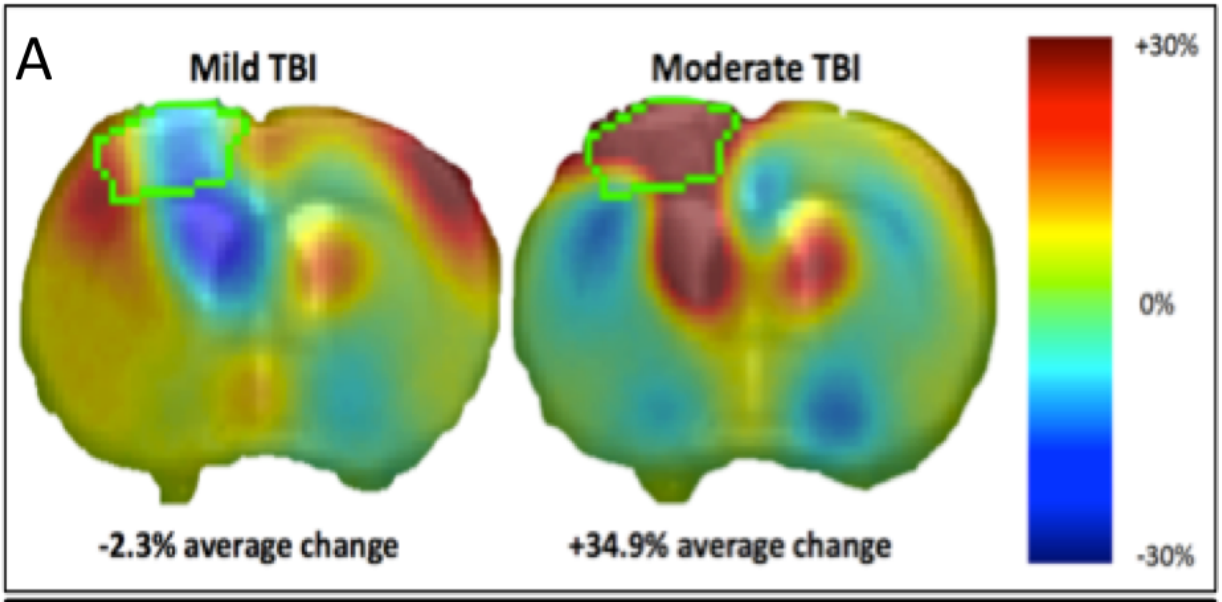
Concluding remarks:

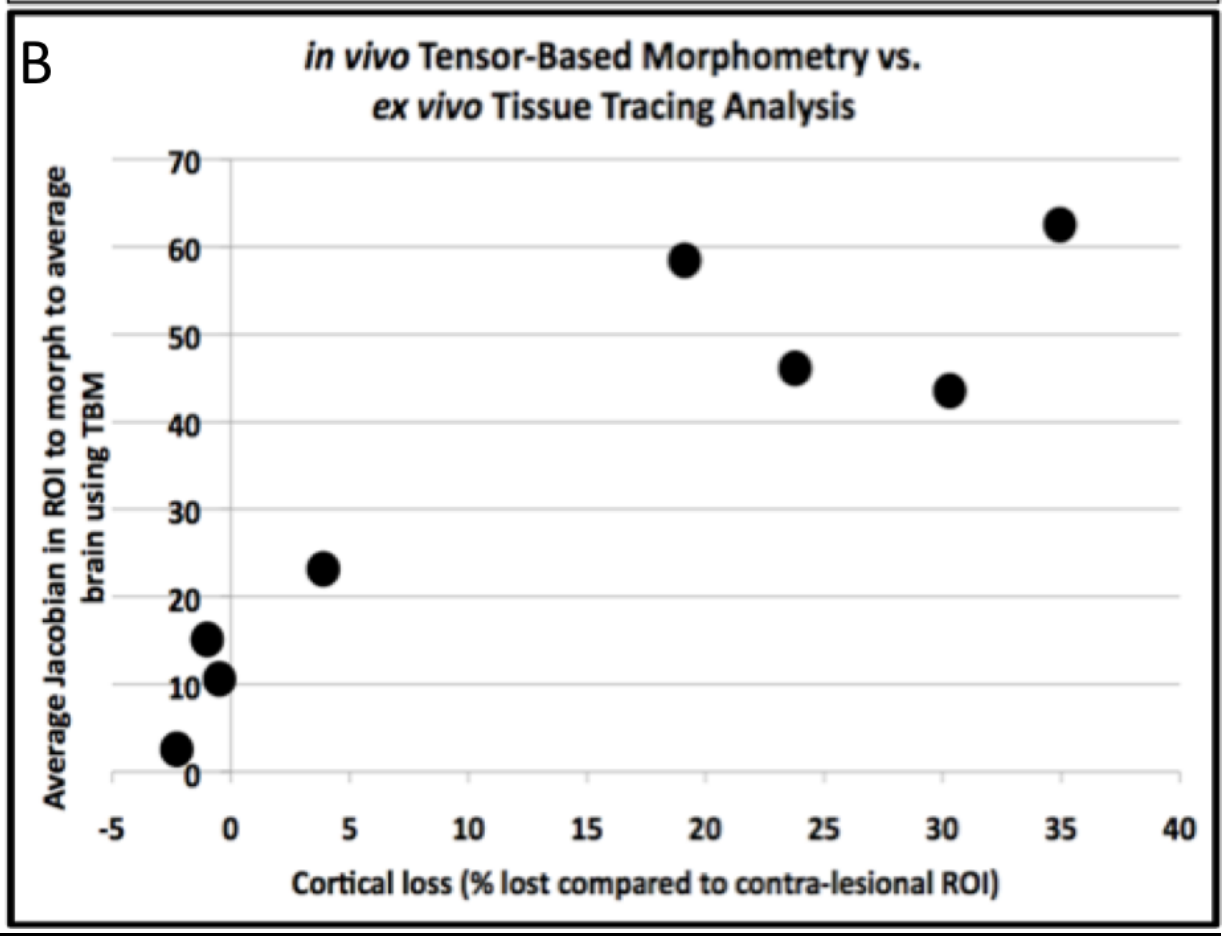
Neuroscientific discovery has always been limited by the technologies available to study the brain. With the advent of immunocyto- and immunohistochemistry and microscopy, patch clamp electrophysiology we could study the brain at a cellular level. With the electroencephalogram (EEG) neuroscientists could get a gross view of the brain's activity. More recently, with the advent of non-invasive functional brain imaging and brain stimulation techniques we are entering an era whereby activity and excitability might soon be measured and modulated in near real time with noninvasive technologies to treat TBI. One similar concept, deep brain stimulation (DBS) devices now used to treat certain forms of pharmaco-resistant epilepsy. There is some precedent for the use of non-invasive brain stimulation after chronic stroke. Some investigators have used an excitatory stimulus paradigm to increase excitability in ILCtx and an inhibitory stimulus to reduce excitability in the uninjured cortex and found that patients have shown significant improvements (Hummel and Cohen 2006). While these treatments remain experimental and their effectiveness has been somewhat controversial (Hummel et al. 2008), it appears with further research, this approach holds some promise as a future adjuvant treatment with physical therapy (Takeuchi et al. 2012).

In the studies presented here we have made some progress toward a better understanding about the role of the CLCtx in recovery of function after TBI. While single treatment paradigms have been shown to improve functional recovery, to maximize recovery, a multimodal approach at once addressing neuroprotection, neuroplasticity, and multi-focal neuromodulation is required. The findings of this study add to the growing body of data indicating that spatially balanced

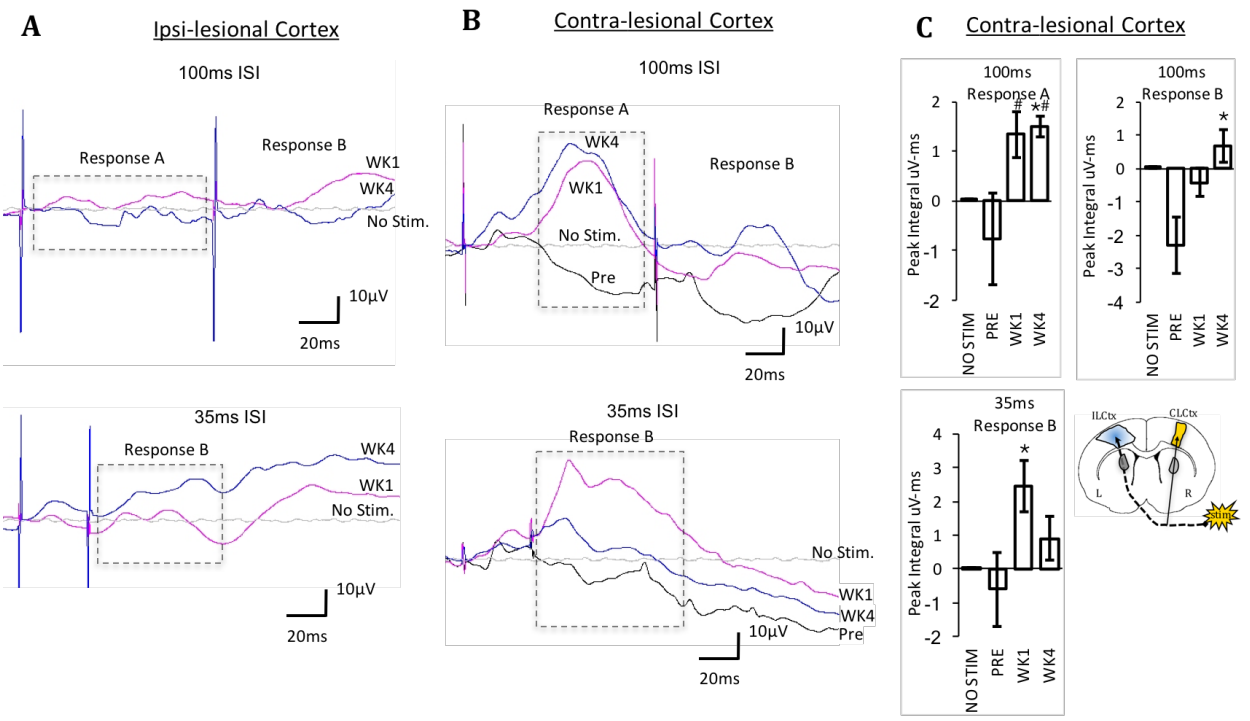
activity between regions is required to re-establish their appropriate connectivity after TBI specifically, and, perhaps, lateralized brain injury in general.

Appendix


A
Mild TBI
Moderate TBI
+30%
0%
-30%
-2.3% average change
+34.9% average change


B
in vivo Tensor-Based Morphometry vs.
ex vivo Tissue Tracing Analysis
Average Jacobian in ROI to morph to average brain using TBM
70
60
50
40
30
20
10
0
-5
0
5
10
15
20
25
30
35
40
Cortical loss (% lost compared to contra-lesional ROI)

Supplementary Figure 1**.** A. Example images of the mildest and most severe cortical lesions within the region of interest (ROI, green trace) Jacobian maps overlaid on the mean (n=11) RARE structural image for mild or moderate-severe TBI. The color scale (right) indicates the relative % change required to warp the image to the group average image at 28 days post-injury. B. Validation of TBM by comparison to tissue tracing volume analysis. Linear regression analysis indicates a significant deviation from zero (p=0.0013). Thus, our lesion analysis method using TBM will serve as a suitable non-invasive indicator of injury severity for subsequent analyses.



Supplementary Figure 2. Right affected forelimb stimulation, bilateral recording electrodes:

[A] Upper: ILCtx @ ISI 100ms: Average PPSEP responses were not significantly different from control traces without limb stimulation. **Lower:** ILCtx @ ISI 35ms: PPSEP responses were not significantly different from control traces without limb stimulation. **[B] Upper** CLCtx @ ISI100ms: One-way ANOVA of peak integral (area under the peak) measured across the dominant peak between 56.4-103.8 ms in the first response after the first of the 100ms ISI paired-pulse stimuli showed a significant increase in the neuronal response magnitude over the homotopic contralesional S1FL cortex. **[C] Lower:** CLCtx @ ISI 35ms: One-way ANOVA of peak integral (area under the peak) measured at the dominant peak between 48-135 ms (boxed region) in $ResponseB_{35ms}$ showed a significant increase in the neuronal response magnitude over

the homotopic contralesional S1FL cortex. Post-hoc paired t-tests: Pre-Wk1 p=0.001535 and Pre-Wk4 p=0.002972 (one-tail).

Key: Waveforms represent group averaged PP SEP recordings from pre-injury (**black trace**), one week (**magenta trace**) and four weeks (**blue trace**) post-injury, and in the absence of stimuli (**grey trace**) for comparison. ILCtx=ipsi-lesional cortex; CLCtx=contra-lesional cortex; *=significantly different from pre-injury, $p<0.05$; #=significantly different from non-stimulated control

References

A Brain Trauma Foundation; American Association of Neurological Surgeons; Congress of Neurological Surgeons. Guidelines for the management of severe traumatic brain injury (3rd Edition). J Neurotrauma. 2007;24(Suppl 1(suppl 1):S1-106.

Acharya L, Aghajan ZM, Vuong C, Moore JJ, Mehta MR. Causal Influence of Visual Cues on Hippocampal Directional Selectivity. Cell. Elsevier Inc.; 2016;164(1–2):197–207.

Adametz JHJH. Rate of recovery of functioning in cats with rostral reticular lesions; an experimental study. J Neurosurg. 1959;16(1):85.

Addington C, Roussas A, Dutta D, Stabenfeldt S. Endogenous Repair Signaling after Brain Injury and Complementary Bioengineering Approaches to Enhance Neural Regeneration. Biomark Insights. 2015;10:43.

Algattas H, Huang J. Traumatic Brain Injury Pathophysiology and Treatments: Early, Intermediate, and Late Phases Post-Injury. Int J Mol Sci. 2013;15(1):309–41.

Alwis DS, Yan EB, Morganti-Kossmann MC, Rajan R. Sensory Cortex Underpinnings of Traumatic Brain Injury Deficits. PLoS One. 2012;7(12).

Andrews RJ. Transhemispheric diaschisis. A review and comment. Stroke. 1991;943–9.

Aparicio LVM, Guarienti F, Razza LB, Carvalho AF, Fregni F, Brunoni AR. A Systematic Review on the Acceptability and Tolerability of Transcranial Direct Current Stimulation Treatment in Neuropsychiatry Trials. Brain Stimul. Elsevier Inc.; 2016;9(5):671–81.

Apkarian AV, Hodge CJ. Primate Sphothalamic Pathways: I. A Quantitative Study of the Cells of Origin of the Spinothalamic Pathway. J Comp Neurol. 1989;288:447–73.

Bales JW, Wagner AK, Kline AE, Dixon CE. Persistent cognitive dysfunction after traumatic brain injury: A dopamine hypothesis. Neurosci Biobehav Rev. 2009;33(7):981–1003.

Belanger HG, Vanderploeg RD, Curtiss G, Warden DL. Recent neuroimaging techniques in mild traumatic brain injury. J Neuropsychiatry Clin Neurosci. 2007 Jan;19(1):5–20.

Benali A, Weiler E, Benali Y, Dinse HR, Eysel UT. Excitation and inhibition jointly regulate cortical reorganization in adult rats. J Neurosci. 2008 Nov;28(47):12284–93.

Bhalala OG. Chapter 26 The Emerging Impact of microRNAs in Neurotrauma Pathophysiology and Therapy. Brain Neurotrauma Mol Neuropsychol Rehabil Asp. 2015.

Biernaskie J, Corbett D. Enriched rehabilitative training promotes improved forelimb motor function and enhanced dendritic growth after focal ischemic injury. J Neurosci. 2001 Jul;21(14):5272–80.

Biernaskie J, Szymanska A, Windle V, Corbett D. Bi-hemispheric contribution to functional motor recovery of the affected forelimb following focal ischemic brain injury in rats. Eur J Neurosci. 2005 Feb;21(4):989–99.

Buchkremer-Ratzmann I, August M, Hagemann G, Witte OW. Electrophysiological Transcortical Diaschisis After Cortical Photothrombosis in Rat Brain. Stroke. 1996 Jun 1;27(6):1105–11.

Bütefisch CM, Netz J, Weßling M, Seitz RJ, Hömberg V. Remote changes in cortical excitability after stroke. Brain. 2003 Feb 1;126(2):470–81.

Calautti C, Baron J-C. Functional neuroimaging studies of motor recovery after stroke in adults: a review. Stroke. 2003 Jun;34(6):1553–66.

Calautti C, Naccarato M, Jones PS, Sharma N, Day DD, Carpenter AT, et al. The relationship between motor deficit and hemisphere activation balance after stroke: A 3T fMRI study. Neuroimage. 2007 Jan 1;34(1):322–31.

Campbell JN, Register D, Churn SB. Traumatic Brain Injury Causes an FK506-Sensitive Loss and an Overgrowth of Dendritic Spines in Rat Forebrain. J Neurotrauma. 2012;29:201–17.

Carey JR, Kimberley TJ, Lewis SM, Auerbach EJ, Dorsey L, Rundquist P, et al. Analysis of fMRI and finger tracking training in subjects with chronic stroke. Brain. 2002 Apr;125(Pt 4):773–88.

Carmel JB, Berrol LJ, Brus-Ramer M, Martin JH. Chronic electrical stimulation of the intact corticospinal system after unilateral injury restores skilled locomotor control and promotes spinal axon outgrowth. J Neurosci. 2010 Aug 11;30(32):10918–26.

Carmel JB, Kimura H, Martin JH. Electrical stimulation of motor cortex in the uninjured hemisphere after chronic unilateral injury promotes recovery of skilled locomotion through ipsilateral control. J Neurosci. 2014 Jan 8;34(2):462–6.

Carmichael ST, Wei L, Rovainen CM, Woolsey T a. New patterns of intracortical projections after focal cortical stroke. Neurobiol Dis. 2001 Oct;8(5):910–22.

Carrera E, Tononi G. Diaschisis: Past, present, future. Brain. 2014;137(9):2408–22.

CDC. Traumatic Brain Injury in the United States. 2014.

Chen JL, Flanders GH, Lee W-C a. W-CA, Lin WC, Nedivi E. Inhibitory dendrite dynamics as a general feature of the adult cortical microcircuit. J Neurosci. 2011 Aug 31;31(35):12437–43.

Chen S, Pickard JD, Harris NG. Time course of cellular pathology after controlled cortical impact injury. Exp Neurol. 2003;182(1):87–102.

Cimolin V, Beretta E, Piccinini L, Turconi AC, Locatelli F, Galli M, et al. Constraint-Induced Movement Therapy for Children With Hemiplegia After Traumatic Brain Injury. J Head

Trauma Rehabil. 2012;27(3):177–87.

Clarkson AN. Perisynaptic GABA Receptors The Overzealous Protector. Adv Pharmacol Sci. 2012 Jan;2012:708428.

Clarkson AN, Huang BS, Macisaac SE, Mody I, Carmichael ST. Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. Nature. Nature Publishing Group; 2010 Nov 11;468(7321):305–9.

Coleman MP, Freeman MR. Wallerian Degeneration, Wld(S), and Nmnat. Annu Rev Neurosci. 2010 Mar;(March):245–67.

Cooper PR, Golfinos JG. Head injury. Appleton & Lange; 2000.

Corrigan JD, Selassie AW, Orman JAL. The epidemiology of traumatic brain injury. J Head Trauma Rehabil. 2010 Jan;25(2):72–80.

Cushman JD, Aharoni DB, Willers B, Ravassard P, Kees A, Vuong C, et al. Multisensory control of multimodal behavior: Do the legs know what the tongue is doing? PLoS One. 2013;8(11).

D'Ambrosio R, Fairbanks JP, Fender JS, Born DE, Doyle DL, Miller JW. Posttraumatic Epilepsy Following Fluid Percussion Injury in the Rat. Brain. 2004;127(Pt 2):304–14.

D'Ambrosio R, Fender JS, Fairbanks JP, Simon EA, Born DE, Doyle DL, et al. Progression from frontal-parietal to mesial-temporal epilepsy after fluid percussion injury in the rat. Brain. 2005 Jan;128(Pt 1):174–88.

D'Ambrosio R, Hakimian S, Stewart T, Verley DR, Fender JS, Eastman CL, et al. Functional definition of seizure provides new insight into post-traumatic epileptogenesis. Brain. 2009a;132(10):2805–21.

D'Ambrosio R, Hakimian S, Stewart T, Verley DR, Fender JS, Eastman CL, et al. Functional definition of seizure provides new insight into post-traumatic epileptogenesis. Brain. 2009b Oct;132(Pt 10):2805–21.

Dalton JC. The Monakow Concept of Diaschisis. 2014;61:283–8.

Dancause N, Barbay S, Frost SB, Plautz EJ, Chen D, Zoubina E V, et al. Extensive cortical rewiring after brain injury. J Neurosci. 2005 Nov;25(44):10167–79.

David-Jürgens M, Dinse HR. Effects of aging on paired-pulse behavior of rat somatosensory cortical neurons. Cereb Cortex. 2010 May;20(5):1208–16.

Deng-Bryant Y, Prins ML, Hovda D a, Harris NG. Ketogenic diet prevents alterations in brain metabolism in young but not adult rats after traumatic brain injury. J Neurotrauma. 2011 Sep;28(9):1813–25.

Devonshire IM, Papadakis NG, Port M, Berwick J, Kennerley AJ, Mayhew JEWW, et al. Neurovascular coupling is brain region-dependent. Neuroimage. Elsevier B.V.; 2012 Sep;59(3):1997–2006.

Diaz-Arrastia R, Kochanek PM, Bergold P, Kenney K, Marx CE, Grimes CJB, et al. Pharmacotherapy of traumatic brain injury: state of the science and the road forward: report of the Department of Defense Neurotrauma Pharmacology Workgroup. J Neurotrauma. 2014;31(2):135–58.

Dijkhuizen RM, Ren J, Mandeville JB, Wu O, Ozdag FM, Moskowitz M a, et al. Functional magnetic resonance imaging of reorganization in rat brain after stroke. Proc Natl Acad Sci U S A. 2001 Oct;98(22):12766–71.

Dijkhuizen RM, Singhal AB, Mandeville JB, Wu O, Halpern EF, Finklestein SP, et al. Correlation between brain reorganization, ischemic damage, and neurologic status after transient focal cerebral ischemia in rats: a functional magnetic resonance imaging study. J Neurosci. 2003 Jan;23(2):510–7.

Donahoo A-LS, Richards LJ. Understanding the Mechanisms of Callosal Development Through the Use of Transgenic Mouse Models. Semin Pediatr Neurol. Elsevier Inc.; 2009;16(3):127–42.

Dong Y, Winstein CJ, Albistegui-DuBois R, Dobkin BH. Evolution of FMRI activation in the perilesional primary motor cortex and cerebellum with rehabilitation training-related motor gains after stroke: a pilot study. Neurorehabil Neural Repair. 2007;21(5):412–28.

Duque J, Hummel F, Celnik P, Murase N, Mazzocchio R, Cohen LG. Transcallosal inhibition in chronic subcortical stroke. Neuroimage. 2005 Dec;28(4):940–6.

Edeline J. Learning-Induced Physiological Plasticity in the Thalamo-Cortical Sensory Systems : a Critical Evaluation of Receptive Field Plasticity , Map Changes and Their Potential Mechanisms. 1999;57(98).

Eklund A, Nichols TE, Knutsson H. Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. Proc. Natl. Acad. Sci. 2016.

Failla MD, Wagner AK. Models of Posttraumatic Brain Injury Neurorehabilitation. CRC Press; 2015.

Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci. 2005 Mar;6(3):215–29.

Feeney DM, Baron JC. Diaschisis. Stroke. 1986 Sep 1;17(5):817–30.

Ferbert a, Rothwell JC, Day BL, Colebatch JG, Marsden CD. Interhemispheric Inhibition of the Human Motor Cortex. J Physiol. 1992;453(453):525–46.

Feydy A, Carlier R, Roby-Brami A, B. Bussel FC, Pierot L, Burnod Y, et al. Longitudinal Study of Motor Recovery After Stroke: Recruitment and Focusing of Brain Activation. Stroke. 2002 Jun 1;33(6):1610–7.

Finger S, Marshak RA, Cohen M, Scheff S, Trace R, Niemand D. Effects of successive and simultaneous lesions of somatosensory cortex on tactile discrimination in the rat. J Comp Physiol Psychol. 1971;77(2):221–7.

Fujimoto ST, Longhi L, Saatman KE, McIntosh TK. Motor and cognitive function evaluation following experimental traumatic brain injury. Neurosci Biobehav Rev. 2004;28(4):365–78.

Fujioka H, Kaneko H, Suzuki SS, Mabuchi K. Hyperexcitability-associated rapid plasticity after a focal cerebral ischemia. Stroke. 2004 Jul;35(7):e346-8.

Gendelman MLK and HE. Bridge Between Neuroimmunity and Traumatic Brain Injury. Curr Pharm Des. 2014;20(26):4284–4298.

Gerloff C, Cohen LG, Floeter MK, Chen R, Corwell B, Hallett M. Inhibitory influence of the ipsilateral motor cortex on responses to stimulation of the human cortex and pyramidal tract. J Physiol. 1998 Jul 1;510 (Pt 1(1998):249–59.

Granum SL. The spinothalamic system of the rat. I. Locations of cells of origin. J Comp Neurol. 1986;247(2):159–80.

Greer JE, Mcginn MJ, Povlishock JT. Diffuse Traumatic Axonal Injury in the Mouse Induces Atrophy, c-Jun Activation, and Axonal Outgrowth in the Axotomized Neuronal Population. Neurobiol Dis. 2011a;31(13):5089–105.

Greer JE, McGinn MJ, Povlishock JT. Diffuse traumatic axonal injury in the mouse induces atrophy, c- Jun activation and axonal outgrowth in the axotomized neuronal population. 2011b.

Greer JE, Povlishock JT, Jacobs KM. Electrophysiological Abnormalities in Both Axotomized and Nonaxotomized Pyramidal Neurons following Mild Traumatic Brain Injury. J Neurosci. 2012 May 9;32(19):6682–7.

Griesbach GS, Gómez-Pinilla F, Hovda D a. Time window for voluntary exercise-induced increases in hippocampal neuroplasticity molecules after traumatic brain injury is severity dependent. J Neurotrauma. 2007;24(7):1161–71.

Griesbach GS, Sutton RL, Hovda D a, Ying Z, Gomez-Pinilla F. Controlled contusion injury alters molecular systems associated with cognitive performance. J Neurosci Res. 2009 Feb 15;87(3):795–805.

Hall ED, Sullivan PG, Gibson TR, Pavel KM, Thompson BM, Scheff SW. Spatial and temporal characteristics of neurodegeneration after controlled cortical impact in mice: more than a focal brain injury. J Neurotrauma. 2005;22(2):252–65.

Hamzei F, Liepert J, Dettmers C, Weiller C, Rijntjes M. Two different reorganization patterns after rehabilitative therapy: an exploratory study with fMRI and TMS. Neuroimage. 2006 Jun;31(2):710–20.

Harris J a, Diamond ME. Ipsilateral and contralateral transfer of tactile learning. Neuroreport. 2000 Feb 7;11(2):263–6.

Harris NG, Chen S, Pickard JD. Cortical Reorganization after Experimental Traumatic Brain Injury: A Functional Autoradiography Study. J Neurotrauma. 2013a;30(13):1137–46.

Harris NG, Chen S, Pickard JD. Cortical Reorganization after Experimental Traumatic Brain Injury: A Functional Autoradiography Study. J Neurotrauma. 2013b;30:1137–46.

Harris NG, Mironova Y a, Hovda D a, Sutton RL. Chondroitinase ABC enhances pericontusion axonal sprouting but does not confer robust improvements in behavioral recovery. J Neurotrauma. 2010a Sep;12(November):1–12.

Harris NG, Mironova Y a, Hovda D a, Sutton RL. Pericontusion axon sprouting is spatially and temporally consistent with a growth-permissive environment after traumatic brain injury. J Neuropathol Exp Neurol. 2010b Feb;69(2):139–54.

Harris NG, Nogueira MSM, Verley DR, Sutton RL. Chondroitinase Enhances Cortical Map Plasticity and Increases Functionally Active Sprouting Axons after Brain Injury. J Neurotrauma. 2013c;30(14):1257–69.

Harris NG, Verley DR, Gutman BA, Sutton RL. Bi-directional changes in fractional anisotropy after experiment TBI: Disorganization and reorganization? Neuroimage. Elsevier Inc.; 2016a;133:129–43.

Harris NG, Verley DR, Gutman BA, Thompson PM, Yeh HJ, Brown JA. Disconnection and hyper-connectivity underlie reorganization after TBI: A rodent functional connectomic analysis. Exp Neurol. Elsevier Inc.; 2016b;277:124–38.

Hillier SL, Sharpe MH, Metzer J. Outcomes 5 years post-traumatic brain injury (with further reference to neurophysical impairment and disability). Brain Inj. 1997;11:661–75.

Holmin S, Mathiesen T. Biphasic edema development after experimental brain contusion in rat. Neurosci Lett. 1995 Jul 14;194(1–2):97–100.

Holschneider DP, Guo Y, Wang Z, Roch M, Scremin OU. Remote Brain Network Changes after Unilateral Cortical Impact Injury and Their Modulation by Acetylcholinesterase Inhibition. J Neurotrauma. 2013 Jan 23;30(June 1, 2013):907–19.

Hoskison MM, Moore a. N, Hu B, Orsi S, Kobori N, Dash PK. Persistent working memory dysfunction following traumatic brain injury: evidence for a time-dependent mechanism. Neuroscience. IBRO; 2009 Mar 17;159(2):483–91.

Hsu JE, Jones T a. Contralesional neural plasticity and functional changes in the less-affected forelimb after large and small cortical infarcts in rats. Exp Neurol. 2006 Oct;201(2):479–94.

Hua X, Gutman B, Boyle CP, Rajagopalan P, Leow AD, Yanovsky I, et al. Accurate measurement of brain changes in longitudinal MRI scans using tensor-based morphometry. Neuroimage. Elsevier Inc.; 2011 Jul 1;57(1):5–14.

Hua X, Hibar DP, Ching CRK, Boyle CP, Rajagopalan P, Gutman B a, et al. Unbiased tensor-based morphometry: improved robustness and sample size estimates for Alzheimer's disease clinical trials. Neuroimage. Elsevier Inc.; 2013 Feb 15;66:648–61.

Hummel FC, Celnik P, Pascual-Leone A, Fregni F, Byblow WD, Buetefisch CM, et al. Controversy: Noninvasive and invasive cortical stimulation show efficacy in treating stroke patients. Brain Stimul. Elsevier Inc; 2008 Oct;1(4):370–82.

Hummel FC, Cohen LG. Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? Lancet Neurol. 2006;5(8):708–12.

Hunt RF, Scheff SW, Smith BN. Posttraumatic epilepsy after controlled cortical impact injury in mice. Exp Neurol. Elsevier Inc.; 2009 Feb;215(2):243–52.

Jang SH. Contra-lesional somatosensory cortex activity and somatosensory recovery in two stroke patients. J Rehabil Med. 2011 Feb;43(3):268–70.

Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage. 2002;17(2):825–41.

Johansen-Berg H, Rushworth MFS, Bogdanovic MD, Kischka U, Wimalaratna S, Matthews PM. The role of ipsilateral premotor cortex in hand movement after stroke. Proc Natl Acad Sci U S A. 2002 Oct 29;99(22):14518–23.

Jones MW, Apkarian A V, Stevens RT, Hodge Jr. CJ. The spinothalamic tract: an examination of the cells of origin of the dorsolateral and ventral spinothalamic pathways in cats 93. J Comp Neurol. 1987;260(0021–9967 (Print)):349–61.

Jones T a. Multiple synapse formation in the motor cortex opposite unilateral sensorimotor cortex lesions in adult rats. J Comp Neurol. 1999 Nov;414(1):57–66.

Jones TA, Liput DJ, Maresh EL, Donlan N, Parikh TJ, Marlowe D, et al. USE-DEPENDENT DENDRITIC REGROWTH IS LIMITED FOLLOWING UNILATERAL CONTROLLED CORTICAL IMPACT TO THE. J Neurotrauma. 2012;1–47.

Jørgensen OS, Hansen LI, Hoffman SW, Fülöp Z, Stein DG. Synaptic remodeling and free radical formation after brain contusion injury in the rat. Exp Neurol. 1997;144(2):326–38.

Kaas JH, Qi H, Burish MJ, Gharbawie OA, Onifer SM, Massey JM. Cortical and subcortical plasticity in the brains of humans, primates, and rats after damage to sensory afferents in the

dorsal columns of the spinal cord. Exp Neurol. 2008;209(2):407–16.

Karenberg A. Cerebral localization in the eighteenth century--an overview. J Hist Neurosci. 2009;18(December):248–53.

Kasahara M, Menon DK, Salmond CH, Outtrim JG, Tavares JVT, Carpenter T a, et al. Restructuring of functional connectivity in motor network after traumatic brain injury (TBI). 2009;114(2002).

KEMPINSKY WH. Experimental study of distant effects of acute focal brain injury; a study of diaschisis. A M A Arch Neurol psychiatry. 1958;79(4):376–89.

Kharlamov E a, Lepsveridze E, Meparishvili M, Solomonia RO, Lu B, Miller ER, et al. Alterations of GABA(A) and glutamate receptor subunits and heat shock protein in rat hippocampus following traumatic brain injury and in posttraumatic epilepsy. Epilepsy Res. 2011 Jun;95(1–2):20–34.

Kida I, Yamamoto T. Comprehensive correlation between neuronal activity and spin-echo blood oxygenation level-dependent signals in the rat somatosensory cortex evoked by short electrical stimulations at various frequencies and currents. Brain Res. 2010 Mar;1317:116–23.

Kobayashi M, Hutchinson S, Schlaug G, Pascual-Leone A. Ipsilateral motor cortex activation on functional magnetic resonance imaging during unilateral hand movements is related to interhemispheric interactions. Neuroimage. 2003a;20(4):2259–70.

Kobayashi M, Hutchinson S, Schlaug G, Pascual-Leone A. Ipsilateral motor cortex activation on functional magnetic resonance imaging during unilateral hand movements is related to interhemispheric interactions. Neuroimage. 2003b Dec;20(4):2259–70.

Kobori N, Dash PK. Reversal of brain injury-induced prefrontal glutamic acid decarboxylase expression and working memory deficits by D1 receptor antagonism. J Neurosci. 2006;26(16):4236–46.

Kozlowski D a., Leasure JL, Schallert T. The control of movement following traumatic brain injury. Compr Physiol. 2013 Jan 1;3(1):121–39.

Kozlowski D a, James DC, Schallert T. Use-dependent exaggeration of neuronal injury after unilateral sensorimotor cortex lesions. J Neurosci. 1996 Aug;16(15):4776–86.

Kubis N. Non-Invasive Brain Stimulation to Enhance Post-Stroke Recovery. Front Neural Circuits. 2016;10(July):56.

Lee S, Ueno M, Yamashita T. Axonal remodeling for motor recovery after traumatic brain injury requires downregulation of γ-aminobutyric acid signaling. Cell Death Dis. Nature Publishing Group; 2011 Jan;2(3):e133.

Li N, Downey JE, Bar-Shir A, Gilad AA, Walczak P, Kim H, et al. Optogenetic-guided cortical plasticity after nerve injury. PNAS. 2011;108(21):8838–43.

Lindau NT, Bänninger BJ, Gullo M, Good NA, Bachmann LC, Starkey ML, et al. Rewiring of the corticospinal tract in the adult rat after unilateral stroke and anti-Nogo-A therapy. Brain. 2014;137(3):739–56.

Lindsley DB, Schreiner LH, Knowles WB, Magoun HW. Behavioral and EEG changes following chronic brain stem lesions in the cat. Electroencephalogr Clin Neurophysiol. 1950;2(4):483–98.

Loane DJ, Faden AI. Neuroprotection for traumatic brain injury: Translational challenges and emerging therapeutic strategies. Trends Pharmacol Sci. 2010;31(12):596–604.

Loane DJ, Kumar A. Microglia in the TBI brain: The good, the bad, and the dysregulated. Exp Neurol. Elsevier Inc.; 2016;275:316–27.

Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A. Neurophysiological investigation of the basis of the fMRI signal. Nature. 2001 Jul 12;412(6843):150–7.

Lotze M, Grodd W, Rodden F a, Gut E, Schönle PW, Kardatzki B, et al. Neuroimaging patterns associated with motor control in traumatic brain injury. Neurorehabil Neural Repair. 2006a;20(1):14–23.

Lotze M, Markert J, Sauseng P, Hoppe J, Plewnia C, Gerloff C. The role of multiple contralesional motor areas for complex hand movements after internal capsular lesion. J Neurosci. 2006b May;26(22):6096–102.

Lu X-CM, Mountney A, Chen Z, Wei G, Cao Y, Leung LY, et al. Similarities and differences of acute nonconvulsive seizures and other epileptic activities following penetrating and ischemic brain injuries in rats. J Neurotrauma. 2013 Apr 1;30(7):580–90.

MacDonald E, Van der Lee H, Pocock D, Cole C, Thomas N, Vandenberg PM, et al. A Novel Phosphodiesterase Type 4 Inhibitor, HT-0712, Enhances Rehabilitation-Dependent Motor Recovery and Cortical Reorganization After Focal Cortical Ischemia. Neurorehabil Neural Repair. 2007;21(6):486–96.

Machado AG, Cooperrider J, Furmaga HT, Baker KB, Park H-J, Chen Z, et al. Chronic 30-Hz deep cerebellar stimulation coupled with training enhances post-ischemia motor recovery and peri-infarct synaptophysin expression in rodents. Neurosurgery. 2013 Aug;73(2):344–53; discussion 353.

Maggiolini E, Viaro R, Franchi G. Suppression of activity in the forelimb motor cortex temporarily enlarges forelimb representation in the homotopic cortex in adult rats. Eur J Neurosci. 2008 May;27(10):2733–46.

Mansoori BK, Jean-Charles L, Touvykine B, Liu A, Quessy S, Dancause N. Acute inactivation of

the contralesional hemisphere for longer durations improves recovery after cortical injury. Exp Neurol. The Authors; 2014 Apr;254:18–28.

Martin JH, Kably B, Hacking a. Activity-dependent development of cortical axon terminations in the spinal cord and brain stem. Exp Brain Res. 1999 Mar;125(2):184–99.

McNeal DW, Darling WG, Ge J, Stilwell-Morecraft KS, Solon KM, Hynes SM, et al. Selective long-term reorganization of the corticospinal projection from the supplementary motor cortex following recovery from lateral motor cortex injury. J Comp Neurol. 2010 Mar;518(5):586–621.

Meng Y, Chopp M, Zhang Y, Liu Z, An A, Mahmood A, et al. Subacute Intranasal Administration of Tissue Plasminogen Activator Promotes Neuroplasticity and Improves Functional Recovery following Traumatic Brain Injury in Rats. PLoS One. 2014;9(9):e106238.

Meyer BU, Röricht S, Gräfin von Einsiedel H, Kruggel F, Weindl A. Inhibitory and excitatory interhemispheric transfers between motor cortical areas in normal humans and patients with abnormalities of the corpus callosum. Brain. 1995 Apr;118 (Pt 2:429–40.

Mishra a. M, Ellens DJ, Schridde U, Motelow JE, Purcaro MJ, DeSalvo MN, et al. Where fMRI and Electrophysiology Agree to Disagree: Corticothalamic and Striatal Activity Patterns in the WAG/Rij Rat. J Neurosci. 2011 Oct 19;31(42):15053–64.

Mohajerani MH, Aminoltejari K, Murphy TH. Targeted mini-strokes produce changes in interhemispheric sensory signal processing that are indicative of disinhibition within minutes. Proc Natl Acad Sci U S A. 2011 May 31;108(22):E183-91.

Von Monakow C. Diaschisis [1914 article translated by G. Harris]. In: Brain and Behavior I: Mood States and Mind. Balt Penguin. 1969;27–36.

Montoya CP, Campbell-Hope LJ, Pemberton KD, Dunnett SB. The “staircase test”: a measure of independent forelimb reaching and grasping abilities in rats. J Neurosci Methods. 1991 Feb;36(2–3):219–28.

Moore JJ, Ravassard PM, Ho D, Acharya L, Kees AL, Vuong C, et al. Dynamics of Cortical Dendritic Membrane Potential and Spikes in Freely Behaving Rats. 2016;1497:1–38.

Moro N, Ghavim SS, Hovda D a., Sutton RL. Delayed sodium pyruvate treatment improves working memory following experimental traumatic brain injury. Neurosci Lett. Elsevier Ireland Ltd; 2011 Mar 17;491(2):158–62.

Moro N, Sutton RL. Beneficial effects of sodium or ethyl pyruvate after traumatic brain injury in the rat. Exp Neurol. Elsevier Inc.; 2010 Oct;225(2):391–401.

Moroa N, Ghavima S, Harris NG, Hovda DA, Sutton RL, Moro N, et al. Glucose administration after traumatic brain injury improves cerebral metabolism and reduces secondary neuronal injury. Brain Res. Elsevier; 2013 Aug 29;18(9):1–13.

Murase N, Duque J, Mazzocchio R, Cohen LG. Influence of interhemispheric interactions on motor function in chronic stroke. Ann Neurol. 2004 Mar;55(3):400–9.

Nahm FS. Nonparametric statistical tests for the continuous data: The basic concept and the practical use. Korean J Anesthesiol. 2016;69(1):8–14.

Nasrallah F a, Tan J, Chuang K-H. Pharmacological modulation of functional connectivity: α(2)-adrenergic receptor agonist alters synchrony but not neural activation. Neuroimage. Elsevier B.V.; 2011 Dec 22;

Nathan PW, Smith M, Deacon P. The crossing of the spinothalamic tract. Brain. 2001;124(Pt 4):793–803.

Nishibe M, Barbay S, Guggenmos D, Nudo RJ. Reorganization of motor cortex after controlled cortical impact in rats and implications for functional recovery. J Neurotrauma. 2010a;27(12):2221–32.

Nishibe M, Barbay S, Guggenmos D, Nudo RJ. Reorganization of Motor Cortex after Controlled Cortical Impact in Rats and Implications for Functional Recovery. J Neurotrauma. 2010b Sep;27(12):2221–32.

Overman JJ, Clarkson a. N, Wanner IB, Overman WT, Eckstein I, Maguire JL, et al. PNAS Plus: A role for ephrin-A5 in axonal sprouting, recovery, and activity-dependent plasticity after stroke. Proc Natl Acad Sci. 2012;109(33):E2230–9.

Paralikar K, Clement R, Webb A, Barber A, Smith NB, Herbert H. Lipowsky. NOVEL APPROACHES TO QUANTIFYING, TRACKING AND ENHANCING THE PERFORMANCE OF THE ELECTRODE-TISSUE INTERFACE IN MICROWIRE BRAIN IMPLANTS. The Pennsylvania State University; 2009.

Pawela CP, Biswal BB, Hudetz AG, Li R, Jones SR, Cho YR, et al. Interhemispheric neuroplasticity following limb deafferentation detected by resting-state functional connectivity magnetic resonance imaging (fcMRI) and functional magnetic resonance imaging (fMRI). Neuroimage. Elsevier Inc.; 2010 Feb;49(3):2467–78.

Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. Acad Press. 1998;(1):1–474.

Pearce JMS. Marie-Jean-Pierre Flourens (1794-1867) and cortical localization. Eur Neurol. 2009;61:311–4.

Le Prieult F, Thal SC, Engelhard K, Imbrosci B, Mittmann T. Acute Cortical Transhemispheric Diaschisis after Unilateral Traumatic Brain Injury. J Neurotrauma. 2016;33:[Epub ahead of print].

Qü M, Buchkremer-Ratzmann I, Schiene K, Schroeter M, Witte OW, Zilles K. Bihemispheric reduction of GABAA receptor binding following focal cortical photothrombotic lesions in the rat brain. Brain Res. 1998 Dec;813(2):374–80.

Ragnarsson KT. Traumatic brain injury research since the 1998 NIH Consensus Conference: accomplishments and unmet goals. J Head Trauma Rehabil. 2006;21(5):379–87.

Ravassard P, Kees A, Willers B, Ho D, Aharoni D, Cushman J, et al. Multisensory control of hippocampal spatiotemporal selectivity. Science (80-). 2013;340(6138):1342–6.

Reeves TM, Phillips LL, Povlishock JT. Myelinated and unmyelinated axons of the corpus callosum differ in vulnerability and functional recovery following traumatic brain injury. Exp Neurol. 2005 Nov;196(1):126–37.

Rehme AK, Eickhoff SB, Rottschy C, Fink GR, Grefkes C. Activation likelihood estimation meta-analysis of motor-related neural activity after stroke. Neuroimage. Elsevier Inc.; 2012;59(3):2771–82.

Rehme AK, Eickhoff SB, Wang LE, Fink GR, Grefkes C. Dynamic causal modeling of cortical activity from the acute to the chronic stage after stroke.Rehme AK, Eickhoff SB, Wang LE, Fink GR, Grefkes C. Dynamic causal modeling of cortical activity from the acute to the chronic stage after stroke. Neuroimage. El. Neuroimage. Elsevier Inc.; 2011 Apr 1;55(3):1147–58.

Reinecke S, Lutzenburg M, Hagemann G, Bruehl C, Neumann-Haefelin T, Witte OW. Electrophysiological transcortical diaschisis after middle cerebral artery occlusion (MCAO) in rats. Neurosci Lett. 1999 Feb;261(1–2):85–8.

Reinecke S, Lutzenburg M, Hagemann G, Witte OW. Electrophysiological transcortical diaschisis after middle cerebral artery occlusion in rat. Eur J Neurosci. 1998;10:225.

Richards LG, Stewart KC, Woodbury ML, Senesac C, Cauraugh JH. Movement-dependent stroke recovery: a systematic review and meta-analysis of TMS and fMRI evidence. Neuropsychologia. 2008 Jan 15;46(1):3–11.

Riecker A, Gröschel K, Ackermann H, Schnaudigel S, Kassubek JR, Kastrup A. The role of the unaffected hemisphere in motor recovery after stroke. Hum Brain Mapp. 2010 Jan;1029(June 2009):1017–29.

Schaechter JD, Kraft E, Hilliard TS, Dijkhuizen RM, Benner T, Finklestein SP, et al. Motor Recovery and Cortical Reorganization after Constraint-Induced Movement Therapy in Stroke Patients: A Preliminary Study. Neurorehabil Neural Repair. 2002 Dec 1;16(4):326–38.

Schallert T, Fleming SM, Woodlee MT. Should the injured and intact hemispheres be treated differently during the early phases of physical restorative therapy in experimental stroke or parkinsonism? Phys Med Rehabil Clin N Am. 2003 Feb;14(1 Suppl):S27-46.

Smith JM, Lunga P, Story D, Harris N, Le Belle J, James MF, et al. Inosine promotes recovery of skilled motor function in a model of focal brain injury. Brain. 2007;130:915–25.

Soblosky JS, Matthews M a., Davidson JF, Tabor SL, Carey ME. Traumatic brain injury of the forelimb and hindlimb sensorimotor areas in the rat: Physiological, histological and behavioral correlates. Behav Brain Res. 1996 Sep;79(1–2):79–92.

Song W, Amer A, Ryan D, Martin JH. Combined motor cortex and spinal cord neuromodulation promotes corticospinal system functional and structural plasticity and motor function after injury. Exp Neurol. Elsevier Inc.; 2016;277:46–57.

Sutton RL, Hovda DA, Adelson PD, Benzel EC, Becker DP. Metabolic changes following cortical contusion: relationships to edema and morphological changes. Acta Neurochir Suppl (Wien). 1994;60:446–8.

Sutton RL, Hovda DA, Chen MJ, Feeney DM. Alleviation of Brain Injury-Induced Cerebral Metabolic Depression by Amphetamine: A Cytochrome Oxidase Histochemistry Study. Neural Plast. 2000;7(C):109–26.

Sutton RL, Lescaudron L, Stein DG. Unilateral Cortical Contusion Injury in the Rat : Vascular Disruption and Temporal Development of Cortical Necrosis. J Neurotrauma. 1993;10(2):135–55.

Takeuchi N, Oouchida Y, Izumi S-I. Motor control and neural plasticity through interhemispheric interactions. Neural Plast. 2012;2012:823285.

Takeuchi N, Tada T, Toshima M, Ikoma K. Correlation of motor function with transcallosal and intracortical inhibition after stroke. J Rehabil Med. 2010 Nov;42(10):962–6.

Tarkka IM, Könonen M, Pitkänen K, Sivenius J, Mervaalat E. Alterations in cortical excitability in chronic stroke after constraint-induced movement therapy. Neurol Res. 2008 Jun;30(5):504–10.

Taub E, Uswatte G, Mark VW, Morris DM. The learned nonuse phenomenon: implications for rehabilitation. Eura Medicophys. 2006;42(3):241–55.

Testa JA, Malec JF, Moessner AM, Brown AW. Outcome after traumatic brain injury: Effects of aging on recovery. Arch Phys Med Rehabil. 2005;86(9):1815–23.

Tombari D, Loubinoux I, Pariente J, Gerdelat A, Albucher J-F, Tardy J, et al. A longitudinal fMRI study: in recovering and then in clinically stable sub-cortical stroke patients. Neuroimage. 2004 Nov;23(3):827–39.

Ureshi M, Matsuura T, Kanno I. Stimulus frequency dependence of the linear relationship between local cerebral blood flow and field potential evoked by activation of rat somatosensory cortex. Neurosci Res. 2004 Feb;48(2):147–53.

Vespa P, Bergsneider M, Hattori N, Wu H-M, Huang S-C, Martin N a, et al. Metabolic crisis without brain ischemia is common after traumatic brain injury: a combined microdialysis and positron emission tomography study. J Cereb Blood Flow Metab. 2005 Jun;25(6):763–

74.

Viskontas I V, Quian R, Fried I. Human medial temporal lobe neurons respond preferentially to personally relevant images. Proc Natl Acad Sci. 2009;106(50):21329–21334.

Volz LJ, Sarfeld A-S, Diekhoff S, Rehme AK, Pool E-M, Eickhoff SB, et al. Motor cortex excitability and connectivity in chronic stroke: a multimodal model of functional reorganization. Brain Struct Funct. 2015;220(2):1093–107.

Voorhies AC, Jones T a. The behavioral and dendritic growth effects of focal sensorimotor cortical damage depend on the method of lesion induction. Behav Brain Res. 2002;133:237–46.

Wang Y, Neumann M, Hansen K, Hong SM, Kim S, Noble-Haeusslein LJ, et al. Fluoxetine increases hippocampal neurogenesis and induces epigenetic factors but does not improve functional recovery after traumatic brain injury. J Neurotrauma. 2011;28(2):259–68.

Ward NS, Brown MM, Thompson a J, Frackowiak RSJ. Neural correlates of motor recovery after stroke: a longitudinal fMRI study. Brain. 2003 Nov;126(Pt 11):2476–96.

Whishaw IQ, Metz G a. Absence of impairments or recovery mediated by the uncrossed pyramidal tract in the rat versus enduring deficits produced by the crossed pyramidal tract. Behav Brain Res. 2002 Aug 21;134(1–2):323–36.

Williams K a., Magnuson M, Majeed W, LaConte SM, Peltier SJ, Hu X, et al. Comparison of alpha-chloralose, medetomidine and isoflurane anesthesia for functional connectivity mapping in the rat. Magn Reson Imaging. Elsevier Inc.; 2010 Apr;28(7):995–1003.

Willis WD, Kenshalo DR, Leonard RB. The cells of origin of the primate spinothalamic tract. J Comp Neurol. 1979;188(4):543–73.

Wolf SL, Winstein CJ, Miller JP, Taub E, Uswatte G, Morris D, et al. Interpreting the Randomized Clinical Trial of Constraint-Induced Movement Therapy. JAMA. 2012;64:336–8.

Woolrich MW, Behrens TEJ, Beckmann CF, Jenkinson M, Smith SM. Multilevel linear modelling for FMRI group analysis using Bayesian inference. Neuroimage. 2004a;21(4):1732–47.

Woolrich MW, Behrens TEJ, Smith SM. Constrained linear basis sets for HRF modelling using Variational Bayes. Neuroimage. 2004b;21(4):1748–61.

Woolrich MW, Ripley BD, Brady M, Smith SM. Temporal Autocorrelation in Univariate Linear Modeling of FMRI Data. Neuroimage. 2001;14(6):1370–86.

Worsley KJ. Statistical analysis of activation images. Ch 14, in Functional MRI: An Introduction to Methods. Stat Anal Act images. 2001.

Xiong Y, Mahmood A, Chopp M. Animal models of traumatic brain injury. Nat Rev Neurosci. 2014;14(2):128–42.

Xu S, Zhuo J, Racz J, Shi D, Roys S, Fiskum G, et al. Early microstructural and metabolic changes following controlled cortical impact injury in rat: a magnetic resonance imaging and spectroscopy study. J Neurotrauma. 2011 Oct;28(10):2091–102.

Xydakis MS, Ling GSF, Mulligan LP, Olsen CH, Dorlac WC. Epidemiologic aspects of traumatic brain injury in acute combat casualties at a major military medical center: A cohort study. Ann Neurol. 2012 Oct 11;1–9.

Yang L, Afroz S, Michelson HB, Goodman JH, Valsamis H a, Ling DSF. Spontaneous epileptiform activity in rat neocortex after controlled cortical impact injury. J Neurotrauma. 2010 Aug;27(8):1541–8.

Zhang Y, Xiong Y, Mahmood A, Meng Y, Liu Z, Qu C, et al. Sprouting of corticospinal tract axons from the contralateral hemisphere into the denervated side of the spinal cord is associated with functional recovery in adult rat after traumatic brain injury and erythropoietin treatment. Brain Res. Elsevier B.V.; 2010;1353:249–57.

Zheng Y, Luo JJ, Harris S, Kennerley A, Berwick J, Billings S a., et al. Balanced excitation and inhibition: Model based analysis of local field potentials. Neuroimage. Elsevier Inc.; 2012;63(1):81–94.