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# A Study of Functional Recovery and Axonal Plasticity Following TBI and Anti-Nogo-A Immunotherapy

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LOYOLA UNIVERSITY CHICAGO

A STUDY OF FUNCTIONAL RECOVERY AND  
AXONAL PLASTICITY FOLLOWING TBI AND  
ANTI-NOGO-A IMMUNOTHERAPY

A THESIS SUBMITTED TO  
THE FACULTY OF THE GRADUATE SCHOOL  
IN CANDIDACY FOR THE DEGREE OF  
MASTER OF SCIENCE

PROGRAM IN INTEGRATIVE CELL BIOLOGY

BY

JENNIFER C. BIESIADECKI

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## LIST OF ABBREVIATIONS

aa	Amino acid
AGl	Lateral angular cortex
AGm	Medial angular cortex
AMPA	a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BBB	Blood brain barrier
Bcl-2	B-cell lymphoma 2
Bcl-XL	B-cell lymphoma extra large
BDA	Biotinylated dextran amine
BrdU	Bromodeoxyuridine
cAMP	Cyclic adenosine monophosphate
CBF	Cerebral blood flow
CCI	Controlled cortical impact
CCL2	Chemokine (C-C motif) ligand 2
CNS	Central nervous system
CP	Corticopontine

CSPG	Chondroitin-sulfate proteoglycan
CR	Corticorubral
CREB	cAMP response element-binding protein
CRMP4	Collapsin response mediator protein 4
CS	Corticostriatal
CSF	Cerebral spinal fluid
CST	Corticospinal tract
CTE	Chronic traumatic encephalopathy
DAB	Diaminobenzidine
ED	Emergency department
ER	Endoplasmic reticulum
GCS	Glasgow coma scale
GCSF	Granulocyte colony-stimulating factor
GPCR	G-protein coupled receptor
GSK3 $\beta$	Glycogen synthase kinase 3 beta
ICP	Intracranial pressure
IED	Improvised explosive device
IgG	Immunoglobulin-G
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-8	Interleukin-8



IL-10	Interleukin-10
JAM	Junction adhesion molecule
KO	Knock out
LFP	Lateral fluid percussion
Lingo	Leucine rich repeat and Ig domain containing protein
mAb 7B12	Monoclonal antibody 7B12
mAb 11C7	Monoclonal antibody 11C7
mAb IN-1	Monoclonal antibody IN-1
MAG	Myelin-associated glycoprotein
MCAO	Middle cerebral artery occlusion
MCP-1	Monocyte chemoattractant protein 1
mRNA	Messenger ribonucleic acid
MWM	Morris water maze
NgR1	Nogo-66 receptor
NMDA	N-methyl-D-aspartate
Omgp	Oligodendrocyte myelin glycoprotein
PCI	Photothrombotic cortical injury
PirB	Paired immunoglobulin-like receptor-B
PPMCC	Pearson product-moment correlation coefficient
PTSD	Post-traumatic stress disorder
PVS	Persistent vegetative state

RhoA	Ras homolog gene family, member A
RhoGEF	Rho guanine exchange factor
ROCK	Rho-associated, coiled-coil containing protein kinase 1
ROS	Reactive oxygen species
RST	Rubrospinal tract
RTN	Reticulon
S1P	Sphingosine 1-phosphate
S1PR2	Sphingosine 1-phosphate receptor 2
SEM	Standard error of the mean
SI	Primary somatosensory cortex
TBI	Traumatic brain injury
TNF- $\alpha$	Tumor necrosis factor alpha
Troy	Tumor necrosis factor receptor superfamily, member 19

## ABSTRACT

Traumatic brain injury (TBI) is a leading cause of morbidity and mortality globally and often results in long term disability. Unfortunately, the success of rehabilitation techniques as therapy for TBI is limited, which may be due to the growth restrictive environment of the adult central nervous system (CNS). This environment is thought to result from glial scars, lack of neurotrophic factors, and the presence of an array of growth-inhibitory molecules. One such growth-inhibitory molecule is the protein Nogo-A. Our laboratory has shown that neutralization of Nogo-A with anti-Nogo-A immunotherapy after ischemic stroke results in improvement in functional recovery in the skilled forelimb reaching task and is correlated with an increase in axonal plasticity. In the present study, we sought to determine whether anti-Nogo-A immunotherapy following TBI produces similar recovery as with ischemic stroke. Therefore, we hypothesized that anti-Nogo-A immunotherapy following TBI in the adult rat will result in enhanced axonal plasticity and improved recovery of motor function. Accordingly, adult rats were trained in the skilled forelimb reaching task and received either a TBI via controlled cortical impact (CCI) or no TBI. One week later, rats that received a TBI were administered anti-Nogo-A antibody (11C7), control antibody, or no treatment and tested for seven more weeks on the skilled forelimb reaching task to assess functional recovery. Once behavioral testing was completed, an anterograde

neuroanatomical tracer was injected into the sensorimotor cortex contralateral to the TBI lesion to assess axonal plasticity. Behavioral analysis did not demonstrate whether or not there was recovery of skilled forelimb function since control animals significantly recovered by week two and almost fully by the end of the study. Subsequent neuroanatomical analysis revealed no increased neuroplasticity at the level of the red nucleus in animals treated with mAB 11C7 at week eight post-TBI. To determine if anti-Nogo-A antibodies increase functional recovery and axonal plasticity following TBI, future studies should aim to produce TBI lesions that induce lasting deficits.

## CHAPTER I

### INTRODUCTION

Traumatic brain injury (TBI) has long been a leading cause of morbidity and mortality globally. It is typically caused by a blunt or penetrating trauma to the head resulting in decreased level of consciousness, amnesia, skull fracture(s), intracranial lesion(s), and/or death. It is estimated that approximately 1.7 million people in the U.S. are either hospitalized, visit the emergency department (ED), or die as a result of TBI every year (Faul et al., 2010; Coronado et al., 2012). Overall, TBI has contributed to a third of all injury-related deaths in the U.S. (Faul et al., 2010). In areas of conflict, TBI has become a signature wound resulting from a blast or blunt force trauma. From 2000 to 2012, the Department of Defense estimated that at least 250,000 service members suffered a TBI (DoD, 2012). However, these numbers do not include mild TBIs that go unreported or those that receive no medical care. Thus, it is likely these statistics are a substantial underestimate.

As for the survivors of TBI, many are plagued with disabilities. Currently, about 5.3 million Americans or 2% of the U.S. population suffer from disabilities resulting from TBI (Thurman, 2007). Annually, of the estimated 275,000 Americans hospitalized following TBI, about 80,000 of these develop longterm disability (Coronado et al., 2012). Severely injured patients may need surgery to repair or remove ruptured blood vessels

(hematomas) or bruised brain tissue (contusions). Depending upon the severity and location of the injury and the age and health of the individual, disabilities that can result include cognitive difficulties, problems with sensory processing, difficulty communicating, and behavioral or mental health problems. Even more serious TBIs can result in an unresponsive state with brief responsiveness by a strong stimulus, coma, a vegetative state, or a persistent vegetative state (PVS).

The treatment of TBI depends on the severity of the injury. While mild TBIs typically require no treatment other than rest and pain relief medication, moderate to severe TBIs are more complicated and involve increasing oxygen and blood supply to the brain and limiting further damage by relieving inflammation. Typically, however, receiving treatment is delayed and thus, neuroprotection is vastly diminished. Because of this, attention has been focused on rehabilitation as a means to enhance functional recovery. Specifically, these therapies aim to increase neuroregeneration and neuroplasticity. Neuroregeneration refers to the brain's ability to regrow or repair damaged neural tissues, cells or cell products, while neuroplasticity refers to the brain's ability to reroute neural circuitry.

Unfortunately, the success of rehabilitation techniques is limited and is thought to be due to glial scars, lack of neurotrophic factors, and the presence of an array of growth-inhibitory molecules like chondroitin-sulfate proteoglycans (CSPGs) (Davies et al., 1999) and myelin-associated proteins (Filbin, 2003). These molecules include the CSPGs Versican V2 (Schweigreiter et al., 2004) and Brevican (Schmalfeldt et al., 2000),

oligodendrocyte myelin glycoprotein (OMgp) (Wang et al., 2002), myelin-associated glycoprotein (MAG) (McKerracher et al, 1994; Mukhopadhyay et al., 1994), and Nogo-A (Schwab, 2004).

Nogo-A is an inhibitory protein initially seen as primarily expressed on the surface of oligodendrocytes and their product myelin, but has also been seen in neuronal cell bodies, neurites, and intracellularly associated with the endoplasmic reticulum (ER) of neural cells (Huber et al., 2002; Wang et al., 2002b; Josephson et al., 2001; Voeltz et al., 2006). Two regions of the Nogo-A protein have been attributed to axon-growth inhibition. The first region contains the inhibitory domain Nogo-66 and is located in the C-terminal RTN area, while the second region contains the inhibitory domain Nogo-A  $\Delta$ 20 and is located in the Nogo-A specific area towards the N-terminus (Oertle et al., 2003b). Nogo-66 is an extracellular 66-amino acid sequence common to all isoforms of Nogo, while Nogo-A  $\Delta$ 20 is a Nogo-A specific domain (Oertle et al, 2003).

Recently, neutralization of Nogo-A by treatment with monoclonal antibodies (mAb) have been of great interest in promoting axonal outgrowth for treatment of CNS injuries. The mAb IN-1 in particular has been shown to regenerate corticospinal tract fibers after adult CNS lesions (Schnell & Schwab, 1990; Schnell et al., 1994) and cause functional recovery in adult rats after spinal cord injury (Bregman et al, 1995). Although the role of Nogo-A in axonal regeneration is under active investigation, the investigation of its role in neuroplasticity has shown that treatment with anti-Nogo-A immunotherapy enhanced new axonal growth from uninjured pathways that projected to areas denervated

by the lesion (Thallmair et al., 1998; Kartje et al., 1999; Wenk et al., 1999). Some lesion models that have been used to assess neuroplasticity following treatment include unilateral pyramidotomy, cortical aspiration lesion, and ischemic stroke. In these models, administration of anti-Nogo-A antibodies have resulted in axonal plasticity of intact fiber tracts, brain reorganization (Kartje et al., 1999; Wenk et al., 1999; Emerick et al., 2003), and improved functional recovery of skilled movements in adult and aged rats (Thallmair et al., 1998; Z'Graggen et al., 1998; Emerick & Kartje, 2004; Papadopoulos et al., 2002; Tsai et al., 2007; Markus et al., 2005). However, it has also been shown in rats given a TBI that cognitive function (spatial memory) had been enhanced without an improvement in functional recovery (reaching) with anti-Nogo-A antibodies administered 24 hours after brain injury (Marklund et al., 2007; Lenzlinger et al., 2005). There are several factors that may have contributed to the observed lack in functional recovery. For example, these studies utilized the lateral fluid percussion model of TBI which, in comparison to the controlled cortical impact model, may produce more variability in lesion location and size. Also, they were only conducted up to four weeks. It is possible that more time is necessary for functional recovery to occur. Thus, the focus of this study is to examine whether anti-Nogo-A immunotherapy induces neuronal plasticity that leads to improved motor recovery eight weeks after receiving a TBI by controlled cortical impact.



## CHAPTER II

### HYPOTHESIS AND SPECIFIC AIMS

#### **HYPOTHESIS:**

**Anti-Nogo-A immunotherapy following traumatic brain injury (TBI) induced by the controlled cortical impact model (CCI) in the adult rat will result in enhanced axonal plasticity and improved recovery of sensorimotor function.**

**Specific Aim 1** will determine whether anti-Nogo-A immunotherapy given one week post-TBI results in improved motor recovery. This will be assessed using a behavioral test, the skilled forelimb reaching task to assess forelimb dexterity.

**Specific Aim 2** will determine if functional recovery is correlated with axonal plasticity. Biotinylated dextran amine (BDA), an anterograde tracer, will be used to examine whether new axonal pathways develop from the opposite, spared cortex in adult rats post-TBI to re-innervate the deafferented red nucleus.

## CHAPTER III

### REVIEW OF LITERATURE

#### **TBI INJURY PROCESS: PRIMARY AND SECONDARY INJURY**

##### **Primary Injury**

Primary injury from TBI refers to the immediate tissue damage caused by the biomechanical forces exerted on the brain at the instant of impact and can be focal, diffuse, or both (Miller et al., 2001). Often, epidural/extradural hematomas (pooling of blood between the skull and meninges), subdural hematomas (pooling of blood between the dura and arachnoid), and/or subarachnoid hemorrhages and intracerebral hematomas (pooling of blood within and around the brain) result from head injury (Moore, 2005; Morales et al., 2005).

##### **Secondary Injury**

Following and initiated by the primary injury is a complex process involving microscopic changes known as secondary injury. This leads to blood brain barrier damage, edema, increased intracranial pressure, altered cerebral blood flow, ischemia and hypoxia, increase in lactate, and energy deficits. Ultimately, brain damage characterized by cell death, axonal injury, brain atrophy, and demyelination occurs and results in functional deficits. The microscopic changes involved in secondary injury include

excitotoxicity, oxidative stress, reduction in adenosine triphosphate (ATP) levels and proinflammatory cytokine and chemokine release, as discussed in more detail below.

### ***Excitotoxicity***

Under homeostatic conditions, all cells, including neurons and glia, regulate a  $\text{Ca}^{2+}$  gradient where the concentration of  $\text{Ca}^{2+}$  extracellularly exceeds the concentration of  $\text{Ca}^{2+}$  intracellularly. Cell damage, as induced by TBI, disrupts this gradient by impairing the re-uptake of the neurotransmitter glutamate. High concentrations of glutamate in the extrasynaptic space can then overstimulate N-methyl-D-aspartate (NMDA) and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, leading to an influx of calcium ions and subsequent excitotoxicity (Szydłowska and Tymianski, 2010; Baines and Hall, 2012). When calcium ions in the cytoplasm reach non-physiological levels, mechanisms in the cell are activated to remove the excess. However,  $\text{Ca}^{2+}$  concentrations intracellularly as a result of excitotoxicity are too high to be cleared, inducing the activation of signaling pathways that lead to apoptotic or necrotic cell death. Specifically, high levels of intracellular  $\text{Ca}^{2+}$  can lead to the activation of caspases, endonucleases, and kinases, causing cell damage. In turn, this damage can lead to the overproduction of free radicals, malfunction/damage of mitochondria and endoplasmic reticula, acidosis, cell swelling, cell membrane disruption, and DNA fragmentation, causing cell death (Szydłowska and Tymianski, 2010).

### ***Oxidative Stress***

Following TBI, the oxygen loss caused by blood loss in addition to the disruption of cells' chemical gradients leads to a decrease in available ATP. Decreases in available ATP causes a disruption in protein synthesis and subsequent antioxidant enzyme synthesis. The disruption of antioxidant production leads to an increase in partially reduced oxygen and nitrogen products. It has been established that following a cortical injury, an abundant amount of hydroxyl radicals are produced (Smith et al., 1994). Since carbon is also a target for oxidation by reactive oxygen species (ROS), lipids, nucleic acids, and proteins are vulnerable to the effects of free radicals. The cells experiencing an excess of free radicals/ROS are undergoing oxidative stress. Importantly, in a homeostatic state, ROS are essential in clearing invading pathogens and are also involved in intercellular and intracellular signaling functions as mitogens. Under these conditions, potential toxicity of ROS is regulated by mechanisms that reduce oxidant levels by repairing damage and producing enzymatic antioxidants (superoxide dismutases, catalases, and glutathione peroxidases) and non-enzymatic antioxidants (vitamin E, vitamin C, and glutathione). These mechanisms after TBI, however, can become overwhelmed, subsequently causing cellular dysfunction and cell death (Bains and Hall, 2012).

Neurons and glia are not the only targets of damage induced by ROS. The brain's main vascular filtering system, the blood-brain-barrier (BBB), is also affected (Smith et al., 1994). The BBB contains a network of capillaries that selectively supplies nutrients

to the brain. The difference between these capillaries and those found throughout the body lies within tight junctions that are formed between endothelial cells. These tight junctions, composed of transmembrane proteins like occludin, claudins, and junction adhesion molecules (JAMs), allow the restriction of microscopic objects (i.e. bacteria) and large or hydrophilic molecules into the cerebral spinal fluid (CSF), while allowing the diffusion of small hydrophobic molecules (i.e. O<sub>2</sub>, CO<sub>2</sub>). Along with tight junctions, pericytes, smooth muscle, and astrocytic endfeet surround these endothelial cells and strictly regulate what enters the brain from the circulatory system (Chodobski et al., 2011). Following TBI, the BBB is known to transiently break down (Chodobski et al., 2011). This result may be due to lipid peroxidation, where unsaturated fatty acids present in membrane phospholipids are targeted for peroxidation in a chain reaction, since blocking this reaction reduces BBB permeability post-TBI in a dose dependent manner (Smith et al., 1994).

### ***Reduction in ATP Levels***

Blood loss as a result of vascular damage in the primary injury reduces oxygen levels delivered to the brain and that are necessary for oxidative phosphorylation to produce energy (Hall et al., 2010). Consequently, protein functionality and production is vastly diminished. To begin, the reduction in ATP levels as a result of oxidative phosphorylation being compromised subsequently causes a reduction in Krebs cycle activity by impairing the active transport of pyruvate into mitochondria (Hall, 2012). This causes a shift in cells from consuming glucose aerobically to anaerobically, known

as the Pasteur shift, leading to lactic acidosis, a buildup of lactic acid (Hall, 2012). As the buildup of lactic acid continues, cellular pH decreases leading to protonation of amino acid side groups. This can lead to changes in protein conformation, thus affecting protein functionality. Lastly, the reduction in ATP levels causes a reduction in transcription of new proteins as ATP is necessary for this process. With transcription also being compromised, the production of proteins, like antioxidants necessary for overcoming and surviving injury, is diminished.

### ***Proinflammatory Cytokine and Chemokine Release***

Cerebral inflammation following TBI is characterized by glial activation, leukocyte recruitment, and up-regulation and secretion of mediators like cytokines and chemokines (Morganti-Kossmann et al., 2001). The disruption of the BBB allows the entry of circulating neutrophils, monocytes, and lymphocytes to the injured site (Bye et al., 2007; McIntosh et al., 1998; Clark et al., 1994; Kato & Walz, 2000; Kubes & Ward, 2000). Activated immune cells within the parenchyma release mediators including prostaglandins, free radicals, complement factors, and pro-inflammatory cytokines (Werner & Engelhard, 2007), inducing chemokine and cell adhesion molecule expression, mobilizing immune and glial cells to the injured site (Lucas et al., 2006).

Importantly, the inflammation process is complex and has both beneficial and detrimental effects. Following TBI, elevated cytokines thought to provide beneficial effects are interleukin-6 (IL-6), interleukin-10 (IL-10), and granulocyte colony-stimulating factor (GCSF). IL-6 plays an important role in host defense (Hammacher et

al., 1994) and repair as seen in a study where mice deficient in IL-6 that sustained a TBI exhibited increased oxidative stress, decreased cell survival, and delayed wound healing (Penkowa et al., 2000). IL-10 is known to have neuroprotective characteristics including suppression of microglia and astroglia activation, as well as decreased production of proinflammatory cytokines (Knobloch & Faden, 1998; Kremlev & Palmer, 2005) and ROS (Csuka et al., 1999). Lastly, GCSF has been shown to be antiapoptotic (Schneider et al., 2005) and promote neurogenesis (Sehara et al., 2007).

On the other hand, cytokines elevated following TBI that are thought to provide detrimental effects include interleukin-1 (IL-1), tumor necrosis factor alpha (TNF- $\alpha$ ), Fas ligand (FasL), interleukin-8 (IL-8), and monocyte chemoattractant protein 1 (MCP-1/CCL2). IL-1 has been seen to exacerbate neuronal injury (Rothwell, 1999), while TNF- $\alpha$  induces cerebral inflammation, BBB breakdown, and leukocyte recruitment (Ramilo et al., 1990; Kim et al., 1992). Inhibition of TNF- $\alpha$  has been shown to reduce BBB dysfunction and edema (Shohami et al., 1997). Additionally FasL has been implicated in neuronal apoptosis (Grosjean et al., 2007), IL-8 promotes neutrophil infiltration (Whalen et al., 2000) and increases BBB dysfunction (Morganti-Kossmann et al., 1997), and MCP-1/CCL2 promotes macrophage infiltration (Stirling et al., 2004; Xu et al., 2004; Maier et al., 2005).

## **INJURY SEVERITY**

TBI can be categorized as either mild, moderate, or severe based on the patient's extent of tissue injury and functional capabilities. Using the Glasgow Coma Scale

(GCS), the severity of TBI can be determined by assigning a score from 3 to 15 (from most severe to least severe) based upon functional capacity/symptom severity (O'Phelan, 2011). A score is designated as a result of a series of tests including an eye examination and verbal and motor assessments.

Patients sustaining a mild TBI may be disoriented and have difficulty localizing painful stimuli, but are conscious and responsive to verbal communication. This type of TBI may be invisible on CT scans and symptoms may evolve over several weeks, making this the most difficult to diagnose. Mild TBIs constitute a score of 13-15 on the GCS (O'Phelan, 2011).

On the other hand, patients sustaining a moderate TBI display a greater degree of disorientation than those with mild TBIs and find difficulty in communicating verbally. Much like with mild TBIs, however, these patients are also conscious. A GCS score of 9-12 is assigned to these patients (O'Phelan, 2011).

Lastly are those patients sustaining severe TBIs. These patients are typically unconscious and unable to open their eyes or communicate in any way. Severe TBI is characterized by a GCS score of 3-8 (O'Phelan, 2011).

## **MODELS OF TBI**

Modeling TBI in experimental animal models is especially difficult due to the fact that TBI severity in human patients is evaluated based upon three different criteria: eye, verbal, and motor functionality. While the GCS scores appear to be positively correlated with the extent of tissue damage, the brain's complexity makes it difficult to produce a



lesion that causes deficits in each of these three areas simultaneously in animals.

Moreover, motor/behavioral function can be assessed in animal models following TBI, but eye and verbal functions, whether there is a deficit or not after TBI, are difficult to determine. Thus, modern animal models of TBI aim to create a motor and/or cognitive deficit that can be tested with known motor and cognitive function assessments like the skilled forelimb reaching task and the Morris water maze (MWM).

Currently, there exists three commonly used animal models of TBI: the weight drop model (WD), the lateral fluid percussion model (LFP), and the controlled cortical impact model (CCI) (Figure 1a-d). Each model aims to emulate the primary and secondary injury processes as well as the motor and sensory functional deficits seen in humans.

As the name suggests, the WD model involves a weight of a chosen mass and size being dropped from a defined height either onto the skull or directly onto the brain. Unlike other models, this model is non-penetrating, and depending on kinetic variables, leads to a mild to moderate TBI. TBI can even be severe and lead to spacial memory deficits if the weight of the chosen mass is great enough. The WD model is not typically useful for evaluating persistent behavioral deficits because deficits that arise do not usually last more than a week (Morales et al., 2005). Thus, this model is not very useful in evaluating the effects of a given treatment since the animal recovers whether a treatment is given or not.

Another TBI model, the LFP model, is produced by rapidly injecting saline into the brain. In this model, a pendulum is swung into a syringe-like device around a craniotomy, hitting the end of the syringe and forcing saline into the brain. As a result, the mortality rate seen in this model is the greatest due to brainstem herniation, subarachnoid hemorrhage, and intrapetechial hemorrhage (Morales et al., 2005). This model has been used frequently to evaluate treatments of TBI, including anti-Nogo-A immunotherapy (Lenzlinger et al., 2005; Marklund et al., 2007).

Lastly, the CCI model is obtained by rapidly extending a piston at a defined velocity and duration directly into the brain. To produce this, an incision is made over the skull, a craniotomy is performed, and the pneumatic or magnetic piston is angled over the cortex and extended into the brain. This injury model appears to most closely mirror human TBI, leading to both focal and diffuse injury as well as decreasing variability between animals (Hall et al., 2008; Morales et al., 2005). Along with the LFP model, the CCI model has also been used frequently to evaluate treatments of TBI. However, this model has not yet been used in conjunction with anti-Nogo-A immunotherapy until now.

### **Other Models of TBI**

Recently, blast injuries as a result of the current war conflicts and repetitive sports-related head injuries have suggested that current models of TBI either need to be modified or new models of TBI need to be generated to replicate these types of injuries for further study. In the current conflict, TBI as a result of blast injury produced by improvised explosive devices (IEDs) has become quite common. The damage caused by

a blast injury is produced by a rapid pressure wave moving through the brain tissue (Risling and Davidsson, 2012). As a result of a blast, however, additional blunt force trauma may arise from shrapnel or environmental structures (Sundaramurthy et al., 2012). Currently, rodent models of blast injury do exist and consist of a shock-blast wave generator that sends a pressurized wave through a metallic tube where an anesthetized animal receives the blast (See Figure 2). This model leads to diffuse brain damage, intracranial hematoma, and brain edema (Svetlov et al., 2010). Animal behavior in these models, however, has not been as extensively studied as in other models, thus it is likely modifications will need to be made to make this model an accurate reflection of human blast injury.

As of yet, repetitive sports-related TBI models in animals are infrequent but are being developed. Recently, concussions sustained from sports have been of increasing interest and thought to be more serious than previously believed. Although concussions of this kind are considered to be mild TBIs, they often occur multiple times and can lead to changes in personality, sleep problems, cognitive impairments, increased risk for suicide, post-traumatic stress disorder (PTSD), depression, and anxiety. In more serious cases, these injuries can result in chronic traumatic encephalopathy (CTE). Because sports are a large part of many childrens' lives and the lives of professional athletes, it is important to develop a model for this type of TBI to fully understand its effects and how to effectively treat it (Peskind et al., 2013).

## **ANATOMY OF CORTICO-RUBRAL-SPINAL MOTOR SYSTEM**

The projection of neurons in different motor tracts originate and end in specific areas and follow distinct pathways. Corticospinal tract (CST) neurons originate in lamina V in two distinct cortical areas as small to large pyramidal neurons and end in the spinal cord contralaterally. The origin of neurons for the corticopontine (CP) and corticorubral (CR) tracts are also localized in cortical lamina V. However, these fibers end ipsilaterally in the pontine nuclei and magnocellular red nucleus, respectively. As for the corticostriatal (CS) tract, neurons originate again in lamina V, but also in laminae III and VI, ending ipsilaterally in the striatum. It has been shown that CP, CR, and CS cortical neurons occupy one large cortical area, including parts of the medial (AGm) and lateral (AGl) agranular cortices and the primary (SI) somatosensory cortex. Interestingly, it appears that there is considerable overlapping of the cortical neurons of origin for the four different motor fiber systems. However, each individual neuron projects axons to only one of the four motor associated nuclei and rarely to more than one (Akintunde and Buxton, 1992).

Like the CST, the rat rubrospinal tract (RST) is a contralateral pathway. It primarily originates in the caudal 2/3 of the magnocellular portion of the red nucleus, follows through the central tegmental decussation and dorsolateral funiculus of the spinal cord, terminating in laminae V, VI, and VII of the spinal cord gray matter. It has been shown that RST fibers directly project to specific populations of motoneurons in forelimb areas of the cervical spinal cord (Kuchler et al., 2002). In this study, RST axons were

seen in close apposition to distal (paw) and intermediate (forearm) muscle motoneurons, but absent with regard to motoneurons projecting to proximal (upper arm) muscles (Kuchler et al., 2002). Thus, there appears to be a specific role of the RST in distal forelimb muscle control. It has also been seen that the CST is involved in precise rat limb movements (Hyland, 1998) much like the RST. However, the difference between these two fiber tracts may lie in the context of the movement, where the CST is primarily involved when a new movement is being learned and the RST is active when automated movements are being executed (Kennedy, 1990).

### **CURRENT TBI TREATMENTS**

The severity of TBI dictates treatment. Mild TBIs typically require no treatment other than rest and pain relief medication. Moderate to severe TBIs are more complicated and involve stabilizing the individual to prevent further injury, increasing oxygen and blood supply to the brain, controlling blood pressure, limiting further damage by relieving inflammation, and allowing the body to rest (Traumatic Brain Injury, 2013; NINDS Traumatic Brain Injury Information Page, 2013). In many incidents, however, there is delay in receiving treatment and thus, neuroprotection is vastly diminished. Because of this, attention has been focused on rehabilitation as a means to enhance functional recovery. Rehabilitation techniques are tailored to the individual's needs and may include physical therapy, occupational therapy, speech/language therapy, physiatry, psychology/psychiatry, and social support (NINDS Traumatic Brain Injury Information Page, 2013). Specifically, these therapies aim to increase neuroregeneration and

neuroplasticity. Neuroregeneration refers to the brain's ability to regrow or repair damaged neural tissues, cells or cell products. This includes, but is not limited to, the generation of new glia, myelin, and dendritic and axonal tracts. Neuroplasticity refers to the brain's ability to reroute neural circuitry, as can be done around damaged tissue resulting from TBI.

## **ANTI-NOGO-A IMMUNOTHERAPY**

### **Introduction**

Unfortunately, the success of rehabilitation techniques after TBI is limited. This is thought to be due to the environment in which adult neurons reside--limiting the capacity for neuronal plasticity in the adult CNS. Glial scars, lack of neurotrophic factors, and the presence of an array of growth-inhibitory molecules like chondroitin-sulfate proteoglycans (CSPGs) (Davies et al., 1999) and myelin-associated proteins (Filbin, 2003) are part of this growth-limiting environment. These molecules include the CSPGs Versican V2 (Schweigreiter et al., 2004) and Brevican (Schmalfeldt et al., 2000), oligodendrocyte myelin glycoprotein (OMgp) (Wang et al., 2002), myelin-associated glycoprotein (MAG) (McKerracher et al, 1994; Mukhopadhyay et al., 1994), and Nogo-A (Schwab, 2004). Of these, Nogo-A is the most studied inhibitory molecule.

### **Genetic and Protein Characterization**

By differential splicing or alternative promotor usage, the nogo gene can give rise to three isoforms: Nogo-A, -B, and -C (Chen et al., 2000; Dodd et al., 2005; Huber et al., 2002; Oertle et al., 2003b; Prinjha et al., 2000). The C-terminus of all three isoforms

shares a 180 amino acid (aa) sequence with two highly conserved hydrophobic stretches (Dodd et al., 2005). In particular, Nogo-A is a transmembrane protein of about 1200-aa and contains two inhibitory domains: Nogo-66 and Nogo-A  $\Delta$ 20 (Oertle et al., 2003b). Nogo-66 is located within the C-terminal 200-aa reticulon (RTN) region while the 160-aa Nogo-A  $\Delta$ 20 domain is located within the Nogo-A specific region closer to the N-terminus (Oertle et al., 2003b; Chen et al., 2000; GrandPre et al., 2000; Schwab et al., 2006) (See Figure 3).

### **Expression of Nogo-A**

Nogo-A was initially seen to be primarily expressed by oligodendrocytes in the adult CNS, with the highest levels present in the innermost adaxonal and outermost myelin membranes (Huber et al., 2002; Wang et al., 2002b). This localization allows direct interaction between axons and oligodendrocytes, further substantiating its role as a myelin-associated growth inhibitor (Filbin, 2003).

Nogo-A has also been found to be expressed in neuronal cell bodies and neurites (Huber et al., 2002; Josephson et al., 2001). Additionally, it has been seen to be strongly expressed by developing and immature neurons and, counterintuitively, persists at a high level in plastic (constantly changing and reshaping) regions of the CNS like the cortex, hippocampus, and dorsal root ganglia (Huber et al., 2002; Peng et al., 2011). Because of this observation, it is likely that Nogo-A has additional functions in plastic areas of the CNS unrelated to axonal plasticity. It has also been seen that, although Nogo-A is expressed in nearly all neurons (Hasegawa et al, 2005; Huber et al., 2002), the Nogo-66

receptor NgR1 is not (Hasegawa et al., 2005). In areas where neurons illustrate a high capacity for regeneration, no NgR1 mRNA is found (Hasegawa et al., 2005), suggesting that the lack of this receptor may be a reason why these neurons are so highly regenerative.

Lastly, Nogo-A is found intracellularly, associated with the endoplasmic reticulum (ER) of neural cells. Because of Nogo-A's 200-aa C-terminus, it belongs to the reticulon (RTN) protein family, which is composed of four members (RTN1, RTN2, RTN3, RTN4) present in eukaryotes (Oertle et al., 2003a). These proteins have been found to influence the curvature of the ER and appear as structural regulators for the subcompartment "tubular ER" (Voeltz et al., 2006).

### **Nogo-A Expression after CNS Injury**

Following CNS injury, Nogo-A expression has been found to be increased directly within or proximal to the site of injury. This has been observed in spinal cord injury (Hunt et al., 2003), ischemia (Zhou et al., 2003), and optic nerve damage (Hunt et al., 2003). More recently, an up-regulation in Nogo-A levels in a subpopulation of retinal ganglion cells with larger soma sizes was observed following axonal severing in the optic nerve (Pernet et al., 2011). Furthermore, following ischemic stroke, an increase in Nogo-A expression was seen even 28 days post-MCAO in the pyramidal neurons and interneurons of the perilesional cortex (Cheatwood et al., 2008). To our knowledge, Nogo-A expression following TBI has not been examined. These results suggest



additional functions of Nogo-A unrelated to axonal plasticity, however these functions are currently unknown.

Along with observing an increase in Nogo-A expression after CNS injury, it is important to note that this increase has not always been observed. It has also been seen that the high endogenous expression of Nogo-A by oligodendrocytes shows little increase around a spinal cord injury site (Huber et al., 2002). Whatever the case, it appears that following injury in the CNS, Nogo-A expression does not decrease, but rather either increases or stays the same.

## **Molecular Mechanisms of Nogo-A**

### ***Cell Surface Nogo-A***

To understand how Nogo-A mediates axonal growth inhibition, it is important to first understand the structure and organization of the growth cone--an extension of a developing axon that dynamically seeks its synaptic target. The growth cone contains two domains: a microtubule-containing central domain and an actin-containing peripheral domain. It is within the peripheral domain that actin assembles and disassembles to dynamically extend the axon's filopodia in search for its synaptic target (See Figure 4a).

As mentioned, there are two domains that have been shown to exert inhibitory effects on neurite outgrowth in the Nogo-A protein: the Nogo-66 sequence in the C-terminal RTN region and Nogo-A  $\Delta$ 20 in the Nogo-A-specific region (Oertle et al., 2003b). Nogo-66 in particular has a clear receptor complex and mechanism of action. Along with other myelin inhibitors exhibited on the oligodendrocyte cell surface (MAG,

OMgp), Nogo-66 binds to and stimulates the NgR1 receptor complex. This complex includes a glycosyl-phosphatidylinositol-linked leucine-rich repeat protein (Fournier et al., 2001) and the NgR1-associated proteins Lingo (Mi et al., 2004) and p75 (Wang et al., 2002a) or Troy (Shao et al., 2005). The interaction of Nogo-66 with this complex passes through the neurite and activates the RhoA/ROCK pathway. Downstream in this pathway, cofilin is inactivated by LIMK/Slingshot, stabilizing actin, subsequently preventing actin cytoskeleton depolymerization in the growth cone and thus blocking dynamic extension and retraction of the neurite (Nash et al., 2009) (See Figure 4c). It appears that RhoA's activation by myelin-associated inhibitors may occur by the phosphorylation and inactivation of GSK3 $\beta$ , subsequently allowing CRMP4 and RhoA interaction (Alabed et al., 2010).

More recently, an additional receptor has been identified for Nogo-66, MAG, and OMgp known as paired immunoglobulin-like receptor-B (PirB) (Atwal et al., 2008). Blocking of PirB in primary neuron cultures partially relieved myelin-protein-induced inhibition of axonal outgrowth (Atwal et al., 2008). However, genetic deletion of PirB in vivo after corticospinal tract transection (Nakamura et al., 2011), TBI (Omoto et al., 2010), or optic nerve crush (Fujita et al., 2011) failed to increase axonal growth. Thus, how PirB contributes to neurite outgrowth inhibition is not yet well understood.

As for the inhibitory domain in Nogo-A of particular interest in this study, Nogo-A  $\Delta$ 20, the mechanisms it triggers in neurons is less well-known. Unlike with Nogo-66, Nogo-A  $\Delta$ 20's receptor(s) has(have) not been identified until very recently and has been

shown to be the G-protein coupled receptor (GPCR) sphingosine 1-phosphate receptor 2 (S1PR2) (Kempf et al., 2014; Arzt et al., 2013; Theide-Stan et al., 2013). S1PR2 belongs to a subfamily of five S1PRs, which are known to be activated by sphingosine 1-phosphate (S1P) (Kempf et al., 2014). Nogo-A  $\Delta$ 20 has been shown to also bind S1PR2 via extracellular receptor loops 2 and 3, which are distinct from S1P's binding site (Kempf et al., 2014). Once bound, Nogo-A  $\Delta$ 20 signals through the G protein G<sub>13</sub>, leukemia-associated Rho guanine exchange factor (RhoGEF), LARG, and RhoA (Kempf et al., 2014). In addition to Nogo-A  $\Delta$ 20's interaction with S1PR2, it appears that it also interacts with Tetraspanin-3, a 4-transmembrane-spanning protein that organizes a functional membrane scaffold for the ligand-receptor interaction between Nogo-A  $\Delta$ 20 and the GPCR (Theide-Stan et al., 2013). It is now believed that the rapid endocytosis of Nogo-A  $\Delta$ 20, subsequently leading to RhoA activation, cytoskeleton destabilization, and neurite retraction seen in *in vitro* experiments (Joset et al., 2010) is dependent upon this specific, spatiotemporally coordinated Tetraspanin protein scaffold (Theide-Stan et al., 2013). Upon endocytosis, it has been shown that the Nogo-A  $\Delta$ 20/receptor complex, via a pincher-dependent mechanism, is retrogradely transported in signalosomes to the neuronal cell body where it activates RhoA and negatively regulates phospho-CREB (cAMP response element-binding protein), subsequently attenuating neuronal growth (Joset et al., 2010) (See Figure 4c).

Along with Nogo-A, other signaling molecules, including neurotrophic factors, Wnt (a group of signal transduction pathways made of proteins that pass signals from

outside of a cell through cell surface receptors to the inside of the cell), semaphorins, and netrin, contain more than one active site that interacts with more than one subunit of a multi-subunit receptor complex (Schwab, 2010). It is possible the two inhibitory domains of Nogo-A may contribute to growth repression and plasticity differently. Where Nogo-66/NgR1 may be able to cause acute/short-term growth cone collapse and neurite growth inhibition, Nogo-A  $\Delta$ 20 may induce short-term along with chronic/long-term inhibitory mechanisms at the cytoskeleton and gene expression level (Chivatakarn et al., 2007). Thus, it is possible that these two domains, upon receptor complex activation, together contribute to both acute and chronic impairment of axonal plasticity.

### ***Intracellular Nogo-A***

Nogo-A belongs to the RTN protein family, which is composed of four members in eukaryotes (Oertle et al., 2003a). Several functions have been described for RTNs, including influencing ER curvature, structurally regulating the subcompartment “tubular ER” (Voeltz et al., 2006), and interacting with the anti-apoptotic intracellular proteins Bcl-2 or Bcl-XL (Tagami et al., 2000; Wan et al., 2007). The latter has eluded to RTNs’ role in cell death regulation (Tagami et al., 2000; Wan et al., 2007).

Although Nogo-A and its inhibitory domains are known to induce growth cone collapse and subsequent neurite outgrowth inhibition, Nogo-A may also act as a neuroprotective molecule, possibly explaining its upregulation of expression in areas of CNS injury. One more recent report has shown that Nogo-A knockout (KO) or the acute blockade of Nogo-A with antibodies 24 hours before focal cerebral ischemia decreased

neuronal survival after middle cerebral artery occlusion (MCAO) (Kilic et al., 2010). It has also been seen that transfection of Nogo-A  $\Delta 20$  in cortical neurons is protective against hydrogen-peroxide-induced cell death *in vitro* by possibly interacting with peroxiredoxin 2, which scavenges ROS (Mi et al., 2012). Therefore, Nogo-A and timing of its blockade may have multiple downstream effects on neurons that are as of yet not well understood.

### **Anti-Nogo-A Immunotherapy after CNS Injury**

To date, several antibodies targeted against Nogo-A exist including IN-1, 7B12, and of particular interest in this study, 11C7. The reactivity of IN-1 and 11C7 to Nogo-A has been well-characterized both *in vitro* and *in vivo* (Chen et al., 2000; Buffo et al., 2000; Schnell et al., 2003; Wiessner et al., 2003; Oertle et al., 2003b; Baumann et al., 2008).

Original studies of Nogo-A blockade by *in vivo* administration of anti-Nogo-A antibodies focused on the use of mAb IN-1 after spinal cord injury. This resulted in an enhancement of CST regeneration in adult rats (Schnell and Schwab, 1990; Bregman et al., 1995). Additionally, significant functional improvements in locomotor function were observed in adult spinal cord injured rats following treatment with mAb IN-1 (Merkler et al., 2001).

Aside from spinal cord regeneration studies with anti-Nogo-A immunotherapy, anti-Nogo-A antibodies have been implicated in neuroanatomical plasticity. Work in this area has shown that after unilateral pyramidotomy (severing of the corticospinal tract

immediately rostral to the pyramidal decussation) in rats treated with mAb IN-1, a significant increase in axonal sprouting was observed from the non-lesioned side into the deafferented red nucleus, basilar pontine nuclei, and spinal cord (Thallmair et al., 1998; Z'Graggen et al., 1998; Bareyre et al., 2002). Moreover, this increase in neuroplasticity correlated to a significant improvement in functional recovery as observed through qualitative analyses of skilled forelimb reaching in lesioned rats treated with mAb IN-1 compared to control rats (Thallmair et al., 1998; Z'Graggen et al., 1998).

Work examining the increase in neuroplasticity in the context of anti-Nogo-A immunotherapy extended further to adult cortical lesions. Following unilateral aspiration lesion in the sensorimotor cortex, it was observed that projections were sent from the opposite intact motor cortex into the deafferented dorsolateral striatum (Kartje et al., 1999), red nucleus, basilar pontine nuclei (Wenk et al., 1999), and spinal cord (Emerick and Kartje, 2004). Again, behavioral recovery was observed with a full recovery in forelimb reaching and a significant improvement in the skilled ladder rung walking test in adult rats treated with mAb IN-1 following unilateral sensorimotor cortex aspiration lesion (Emerick and Kartje, 2004).

Studies continued further in the area of cortical lesions treated with anti-Nogo-A immunotherapy to experimental stroke. In adult rats treated with mAb IN-1 following middle cerebral artery occlusion (MCAO), significant functional recovery was observed in the skilled forelimb reaching task along with new cortico-efferent projections from the opposite, unlesioned hemisphere (Papadopoulos et al., 2002). In one particular study, a

different monoclonal anti-Nogo-A antibody, 7B12, was administered 24 hours following two different models of stroke--photothrombotic cortical injury (PCI) and MCAO. In both experimental stroke models, significant improvement in behavioral outcomes was observed along with a significant increase in midline crossing corticospinal fibers originating in the unlesioned sensorimotor cortex in animals treated with mAb 7B12 following stroke (Wiessner et al., 2003). Even when adult rats had obtained a MCAO and were treated with anti-Nogo-A antibody 11C7 nine weeks after stroke, significant functional improvement in the skilled forelimb reaching task was observed along with significant enhancement in corticorubral axonal sprouting from the contralesional forelimb motor cortex to the deafferented red nucleus in comparison to control rats (Tsai et al., 2011).

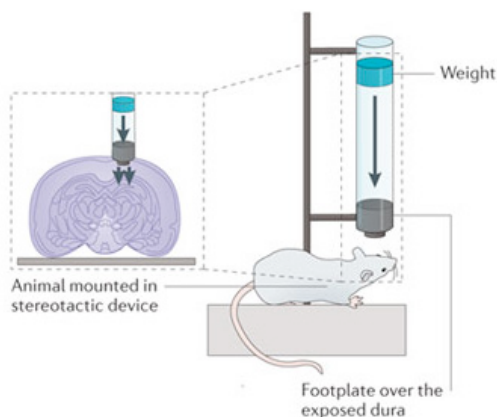
In yet another cortical lesion model, unilateral medial agranular cortex lesion, rats were treated with anti-Nogo-A antibodies IN-1, 7B12, or 11C7 immediately following injury. This injury was used to model neglect--a human cognitive spatial disorder typically induced by damage to prefrontal or posterior parietal association cortices. Each group of adult rats treated with either IN-1, 7B12, or 11C7 showed significant behavioral recovery from neglect compared to control rats (Brenneman et al., 2008).

Given the vast amount of work that has been done on anti-Nogo-A immunotherapy following CNS injury and the overwhelming evidence that this therapy induces behavioral recovery as a result of neuroplasticity, research in this area is now expanding to TBI. To date, there are two major studies that have looked at the results of

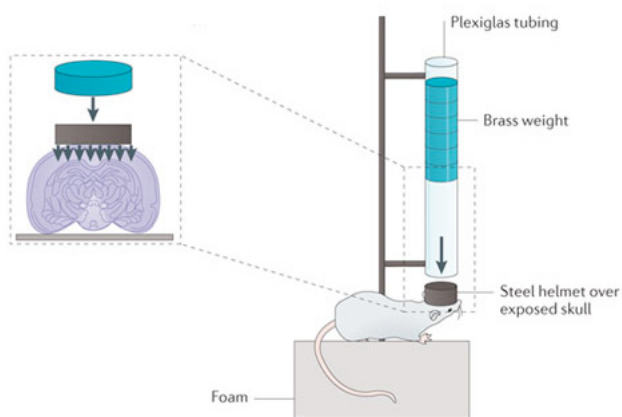
anti-Nogo-A immunotherapy following TBI. Interestingly, these studies have not seen any differences in neurological motor function between rats treated with an anti-Nogo-A antibodies and control rats (Lenzlinger et al., 2005; Marklund et al., 2007). Furthermore, rats treated with mAb 11C7 did not show an increase in the extent of axonal sprouting from the uninjured corticospinal tract related to control rats (Lenzlinger et al., 2005). Both studies, however, used the lateral fluid percussion model (LFP) of TBI and tested neurological motor function for only four weeks post-TBI. Therefore, in this study, we chose the CCI model of TBI (produces less variability in lesion size and location than the LFP model) and tested functional recovery for much longer (eight weeks post-TBI) since all studies showing functional improvement post-ischemic lesions saw significant recovery at five and six weeks after treatment with anti-Nogo-A antibodies.



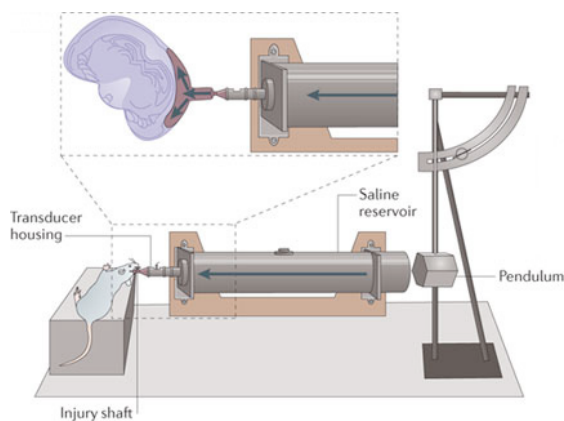
**A. Feeney's Weight Drop Model  
(directly onto brain)**



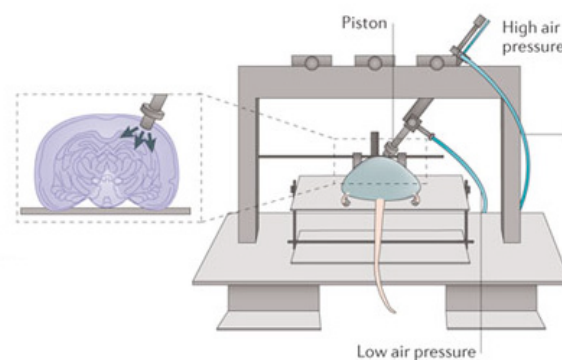
**B. Marmarou's Weight Drop Model  
(directly onto skull)**



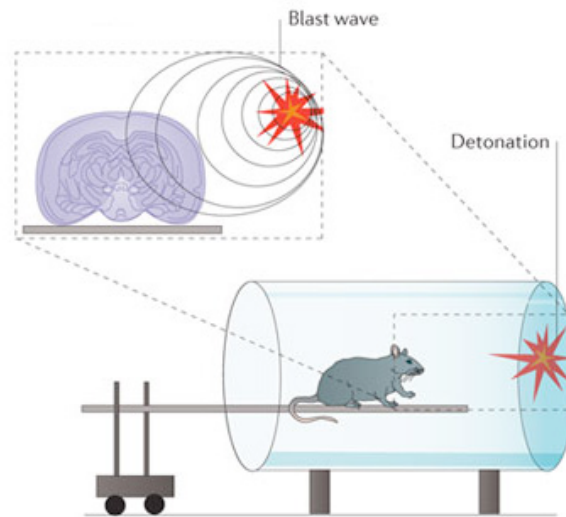
**C. Lateral Fluid Percussion Model**



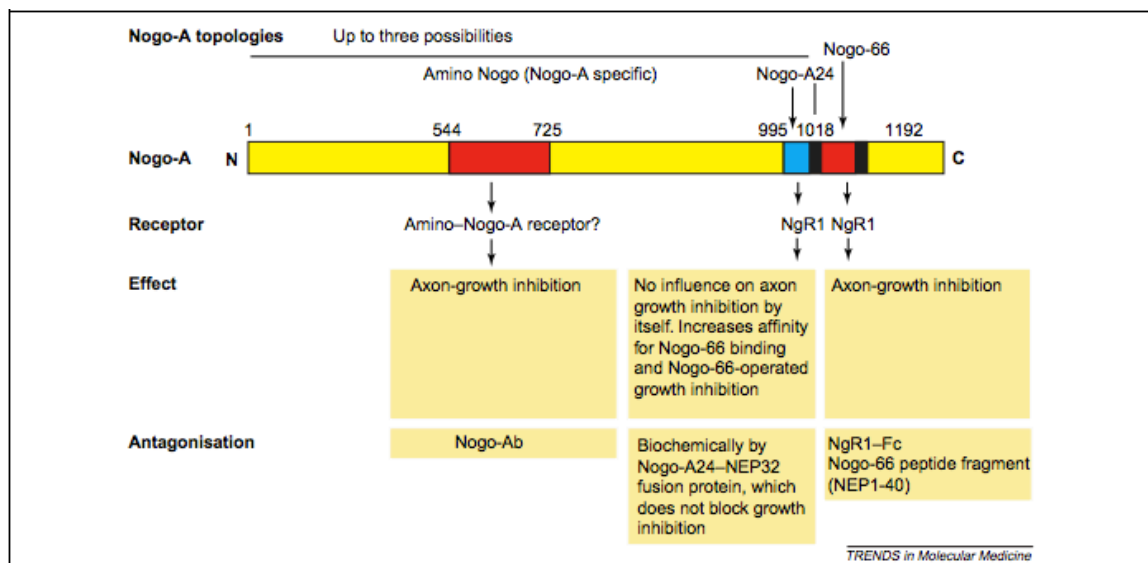
**D. Controlled Cortical Impact Model**



**Figure 1. Commonly used models of TBI.** **A.** Feeney's weight drop model; a weight of a given mass is dropped directly onto the exposed dura. **B.** Marmarou's weight drop model; a weight of a given mass is dropped directly onto the exposed skull. **C.** Lateral fluid percussion model; saline is pushed through a reservoir at a given velocity by a pendulum into the brain. **D.** Controlled cortical impact model; a piston at a given velocity is forced by air pressure into the exposed brain to a given distance. Adapted from Xiong et al., 2013.

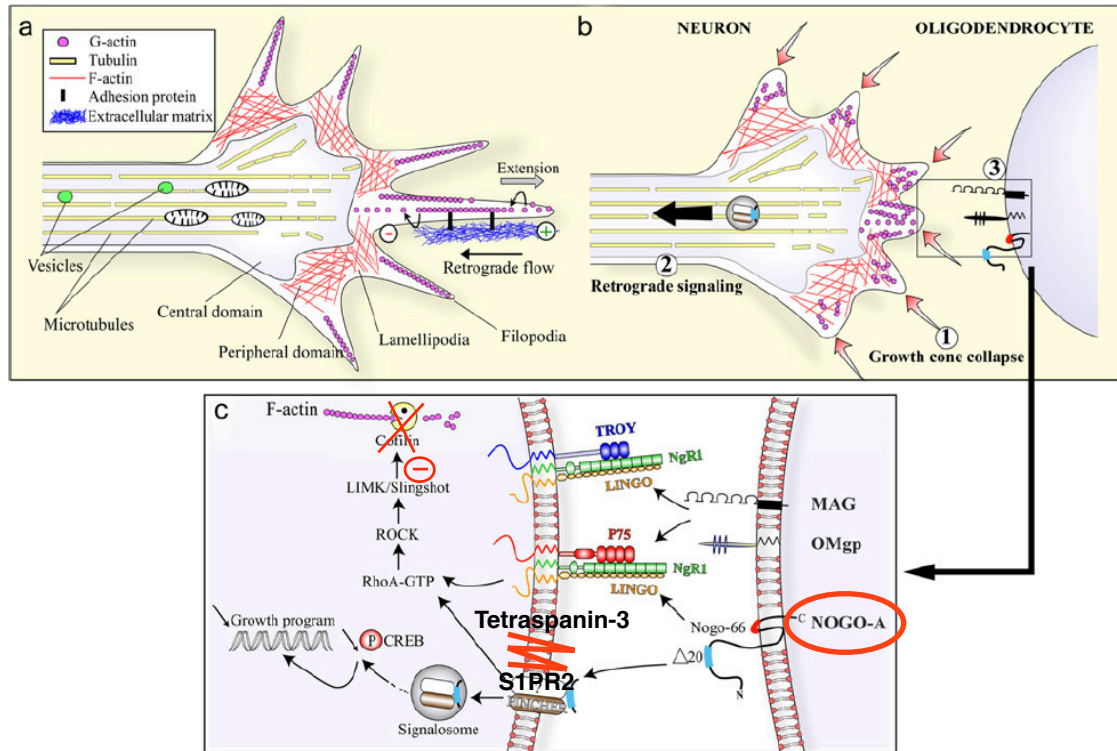


**Figure 2. Blast injury model of TBI.** An anesthetized rodent is placed inside a metallic tube where a pressurized wave is sent through by a shock-blast wave generator. Adapted from Xiong et al., 2013.



**Figure 3. Schematic of human Nogo-A.** Nogo-A contains two inhibitory domains (red): Nogo-A<sup>544-725</sup> (Nogo-A  $\Delta$ 20) and Nogo-66 which result in axon-growth inhibition.

Adapted from Schwab et al., 2006.



**Figure 4. Molecular mechanisms of Nogo-A-mediated axonal growth inhibition. A.** Organization of the growth cone. **B.** Growth cone inhibition. **C.** Nogo-66 and other myelin inhibitors on the cell surface of oligodendrocytes binds to and stimulates a common neuronal receptor complex on neurons while Nogo-A  $\Delta 20$  binds to and stimulates a GPCR via Tetrspanin-3 (transmembrane protein shown in red). Image adapted from Pernet & Schwab, 2012.

## CHAPTER IV

### MATERIALS AND METHODS

**Animals.** Adult male Sprague Dawley rats (40, 200-300g, ages 8-10 weeks) (Harlan, Indianapolis, IN) were divided into four experimental groups: (1) TBI only (n=12); (2) TBI plus control antibody (n=8); (3) TBI plus mAb 11C7 (n=10); (4) no TBI or treatment (n=10) (See Table 1). To ensure investigators were blinded to treatment groups, the animals were number- and color-coded. Experiments were approved by the Institutional Animal Care and Use Committee of Hines Veterans Affairs Hospital.

**Skilled Forelimb Reaching Task.** Animals were placed in a transparent Plexiglas chamber (30 x 36 x 30 cm) with a rectangular opening (1.5 x 3 cm) in one wall containing a Plexiglas shelf attached and underneath the opening. Animals were food restricted to 95% of their normal body weight to encourage reaching; this diet was maintained throughout the testing period. This task was used according to previous work to assess skilled motor functional recovery via forelimb dexterity following ischemic stroke (Papadopoulos et al., 2002) and other injuries to motor pathways governing forelimb function (Z'Graggen et al., 1998). Small round sucrose pellets (45 mg; Research Diets, Frenchtown, NJ) were placed one at a time onto the shelf about 1.5 cm from the opening. Animals reached through the opening for the pellet until a total of 20 attempts and/or 5 minutes had passed. During the training period it typically takes

anywhere from 2-5 weeks for each rat to successfully obtain 16 or greater pellets out of 20 attempts per session. A successful attempt was characterized by the animal locating the pellet, reaching with its preferred forelimb through the opening, grasping the pellet, and bringing to pellet to its mouth (Figure 5). The preferred forelimb was determined once the animal had been consistently reaching with only one forelimb. Once the animal was successful 16 or more times for three consecutive sessions, the animal had reached baseline. The baseline value was an average of the last three sessions. After baseline was established, each animal received a TBI as described below and was then tested the next day and daily 3-5 times a week for eight weeks. The timeline of this experimental design is shown in Figure 6. Following TBI, reaching success in the first seven days after TBI was examined for considerable variability. To minimize variability between the groups, a set of exclusion criteria was incorporated and any animal that met one of the two criteria was excluded from statistical analyses. These animals were part of a group called “included only”. Animals were excluded based on the following criteria: (1) does not successfully obtain a pellet within seven days following TBI and (2) successfully obtains a pellet eight or more times during one or more sessions within seven days following TBI. For completeness and comparison, all animals were also analyzed. These animals were part of a group called “all animals”.

**Traumatic Brain Injury via Controlled Cortical Impact (CCI).** TBIs were induced via controlled cortical impact as previously described (Dixon et al., 1991). Rats were anesthetized with 2% isoflurane with oxygen. Directly above the forelimb sensorimotor

cortex opposite of the preferred forelimb, a 4 mm craniotomy was performed at a position of 0.5 mm anterior and 4 mm lateral to bregma. The cortical impact was delivered by a controlled cortical impact injury device consisting of a small bore and double acting, pneumatic piston cylinder with a 40 mm stroke mounted on a stereotaxic micromanipulator. Accurate determination of the piston coordinates was performed by placing a sharp concentric tip on bregma and moving it to the above coordinates. The concentric tip was then replaced by the impactor tip (3 mm diameter). The pneumatic piston cylinder was angled 22.0 degrees away from vertical so that the flat impactor tip was perpendicular to the surface of the brain. The impactor tip penetrated the brain at 2.5 m/sec at a depth of 2.0 mm below the cortical surface for 250 msec. After impact, the bone fragment removed from the craniotomy was replaced, and the scalp was sutured closed. During surgery, the animal's body temperature was maintained at 37 degrees celsius. The animal was then returned to its home cage. (See Figure 7)

**Antibodies.** The anti-Nogo-A antibody used for this study, 11C7, is an IgG<sub>1</sub>-isotype monoclonal antibody that recognizes an epitope of rat Nogo-A corresponding to amino acids 763-820. The control antibody, mouse anti-bromodeoxyuridine (BrdU), is also an IgG<sub>1</sub>-isotype monoclonal antibody.

**Osmotic Pump and Cannula Placement and Removal.** One week following TBI, animals were anesthetized with 2% isoflurane with oxygen and pumps containing either 11C7 or control antibody were inserted into a subcutaneous pocket surgically created in the midscapular area. The Alzet osmotic pump (model 2ML) is manufactured for

delivery of factors to the brain ventricles of rats that are between 250 g and 300 g in weight. The pumps are 5.1 cm in length and 1.4 cm in diameter and are regulated by a flow moderator with a pumping rate of 5.0 uL/hr ( $\pm 0.75$  uL/hr). Once the pump had been placed, a catheter connected to the pump was led subcutaneously to the cranial site for cannula placement. A burr hole was made contralateral to CCI injury to receive the cannula. The cannula was placed 1.3 mm lateral, 0.8 posterior, and 3.8 dorsoventral, relative to bregma into the lateral cerebral ventricle. Both incisions, for pump insertion and cannula insertion, were sutured, and the animal was placed in a recovery cage and monitored until awake.

Two weeks following pump insertion, the animals were anesthetized with 2% isoflurane with oxygen, and the pump and cannula were removed. After removal, the amount of antibody left in the pump was assessed to determine adequate antibody distribution. Once again, the incisions were sutured and the animal was placed in a recovery cage and monitored until awake.

**Biotinylated Dextran Amine (BDA) Tracing.** Following behavioral testing and data collection (8 weeks post-TBI), all rats were anesthetized with 2% isoflurane with oxygen and placed in a stereotaxic instrument for the anterograde tracer/BDA injection. Contralateral to the TBI lesion, a craniotomy was made exposing the forelimb sensorimotor cortex. Using a 5  $\mu$ L Hamilton microsyringe, 1  $\mu$ L of a 10% solution of BDA (Molecular Probes, Eugene, OR) in 0.01 M phosphate buffer (pH 7.4) was injected at two sites (1-3 mm lateral to bregma at a depth of 1.5 mm from the cortical surface) in



the unlesioned forelimb motor cortex as previously described (Neafsey et al., 1986) (Figure 8).

After a survival period of two weeks after BDA injection to allow for adequate transport of BDA, animals were deeply anesthetized with sodium pentobarbital (150 mg/kg; i.p.) and perfused transcardially with Ringer's solution followed by fixative (4% paraformaldehyde in 0.1M phosphate buffer, pH=7.4). The brains were removed, post-fixed overnight, and then transferred to a 30% sucrose solution in 0.1 M phosphate buffer (pH=7.4) for 3 days. The tissue was blocked and then embedded in optimal cutting temperature (OCT) compound and immediately frozen by immersion in cold isopentane and preserved in a -80°C freezer. 50 micron sections were cut coronally on a cryostat and sections were collected in a solution of 50 mM Tris-buffered 0.9% saline (pH=8.0) with 0.5% Triton X-100 (TBST-X). Sections were washed in TBST-X and incubated overnight with an avidin-biotin-peroxidase complex (ABC complex) diluted in TBST-X (4 drops avidin:4 drop biotin:10 mL TBST-X). The sections were washed again and preincubated for 10 min with 0.4% ammonium nickel sulfate (0.4 g/100 mL TB, pH=8.0) followed by a second preincubation with 0.4% ammonium nickel sulfate and 0.015% 3,3-diaminobenzidine (DAB). The tissue was then reacted in 0.4% ammonium nickel sulfate, 0.015% DAB, and 0.004% hydrogen peroxide in 50 mM Tris buffer (pH=8) for 1-10 min. until staining is visible. Once reacted, sections were mounted on slides. Additional alternate sections were taken and processed for Nissl stain used to analyze lesion volume.

**Crossed Projections Quantification.** All BDA-positive fibers at the level of the parvocellular red nucleus crossing the midline on seven consecutive sections were counted. A 2500  $\mu\text{m}^2$  grid was placed in an eyepiece to ensure that fibers counted actually did cross the midline (Figure 9). To correct for the inter-animal tracing differences, the values were divided by the number of labeled CST fibers in the cerebral peduncle and expressed as fibers crossing the midline per ten thousand labeled CST axons.

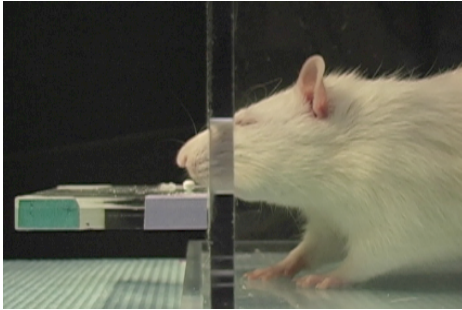
**TBI Lesion Analysis.** The lesion volume of each animal was quantitatively analyzed on Nissl stained sections +4.7 to -5.2 mm from bregma (Paxinos & Watson, 1998). All slides containing Nissl-stained sections were scanned at high resolution (1200 dpi) to a computer in TIFF file format. Once scanned, each hemisphere was cut and pasted (using Adobe Photoshop CS5) onto a high resolution template. Each section was corrected for artifacts and colored black (Figure 10). Using a script in Photoshop, all black pixels were counted for each section, allowing lesion volume and location to be easily calculated. This novel method adapted the method described previously that the area of the intact contralateral hemisphere minus the area of the lesioned hemisphere is multiplied by the total distance between sections to obtain lesion volume (Kawamata et al., 1997). Lesion volume was expressed as a percentage of the intact contralateral hemispheric volume.

**Statistics.** Analysis of all data was performed with SPSS version 20.0 (SPSS, Inc.). For behavioral data, a repeated measures analysis of variance (ANOVA) with a Tukey HSD *post hoc* test was used to test for overall significance, differences in initial deficits, and to

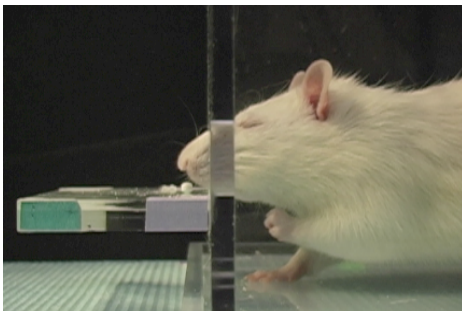
determine the times points at which the predicted recovery differed among the groups. Additionally, a repeated measures *t*-test was used to determine the significance of differences between time points for each group, and a paired samples *t*-test was used to determine the significance between baseline reaching success scores and the success scores at each week post-TBI. For anatomical data, a oneway ANOVA was used to compare the mean lesion volume percents along with the mean number of axonal fibers crossing the midline per 10,000 cerebral peduncle fibers among the different groups along with *post hoc* Tukey HSD tests. For all analyses,  $p < 0.05$  was considered significant. All data are presented as mean values  $\pm$  standard error of the mean (SEM). Correlation between lesion volume and reaching success was evaluated using the Pearson product-moment correlation coefficient (PPMCC).

**Table 1: Experimental Groups**

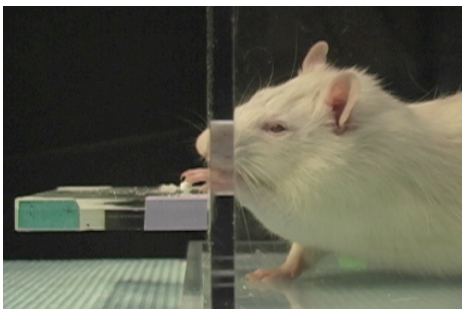
Groups		
Treatment	Histology	n
TBI Only	BDA/Nissl	12
TBI/Control Antibody	BDA/Nissl	8
TBI/11C7 Antibody	BDA/Nissl	10
No TBI	BDA	10
Total		40



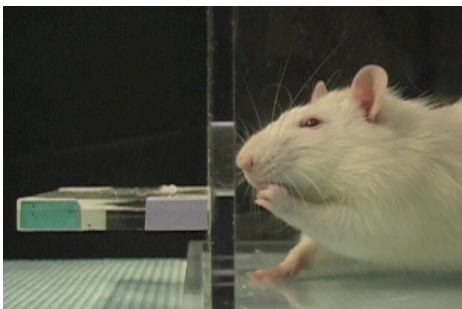
**Identifies sucrose pellet with olfaction cues.**



**Reaches with preferred forelimb (left in this case) for pellet through the window.**



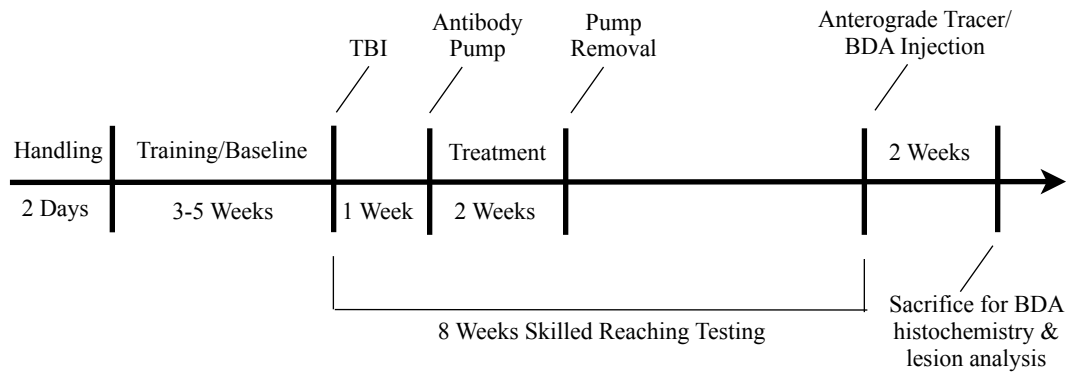
**Grasps pellet with digits.**



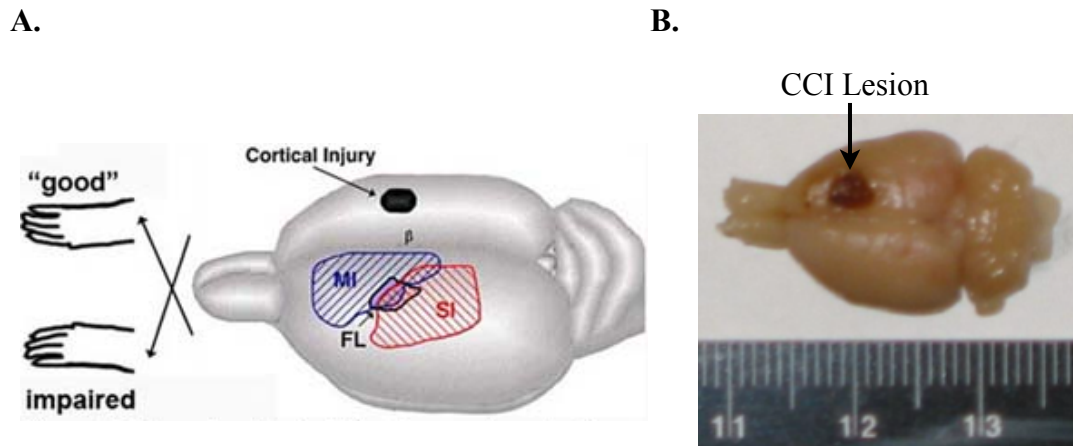
**Brings pellet back through the window to mouth without dropping.**

**Figure 5. Skilled forelimb reaching task.** A rat is shown successfully obtaining a sucrose pellet.

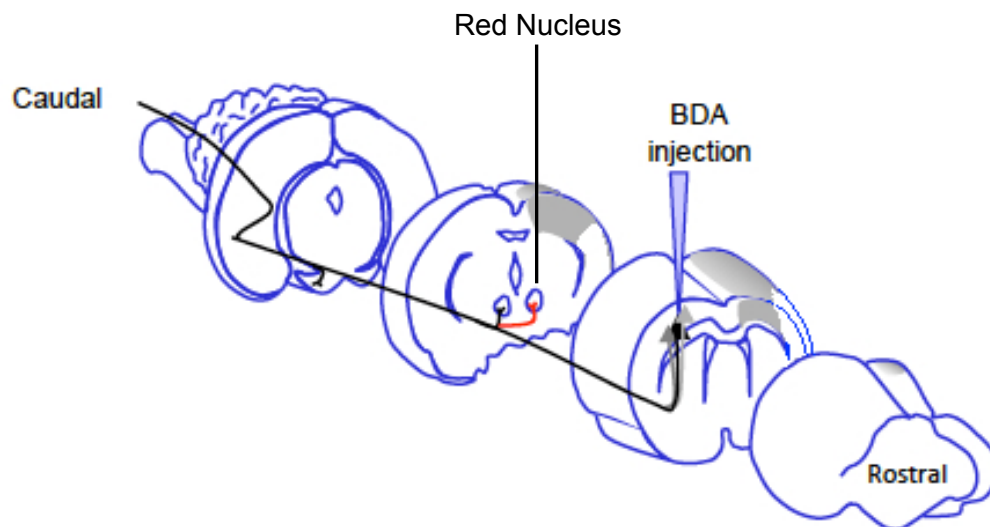
### Timecourse of Experimental Design for Aims 1 and 2



**Figure 6. Timecourse of experimental design for Specific Aims 1 and 2.**

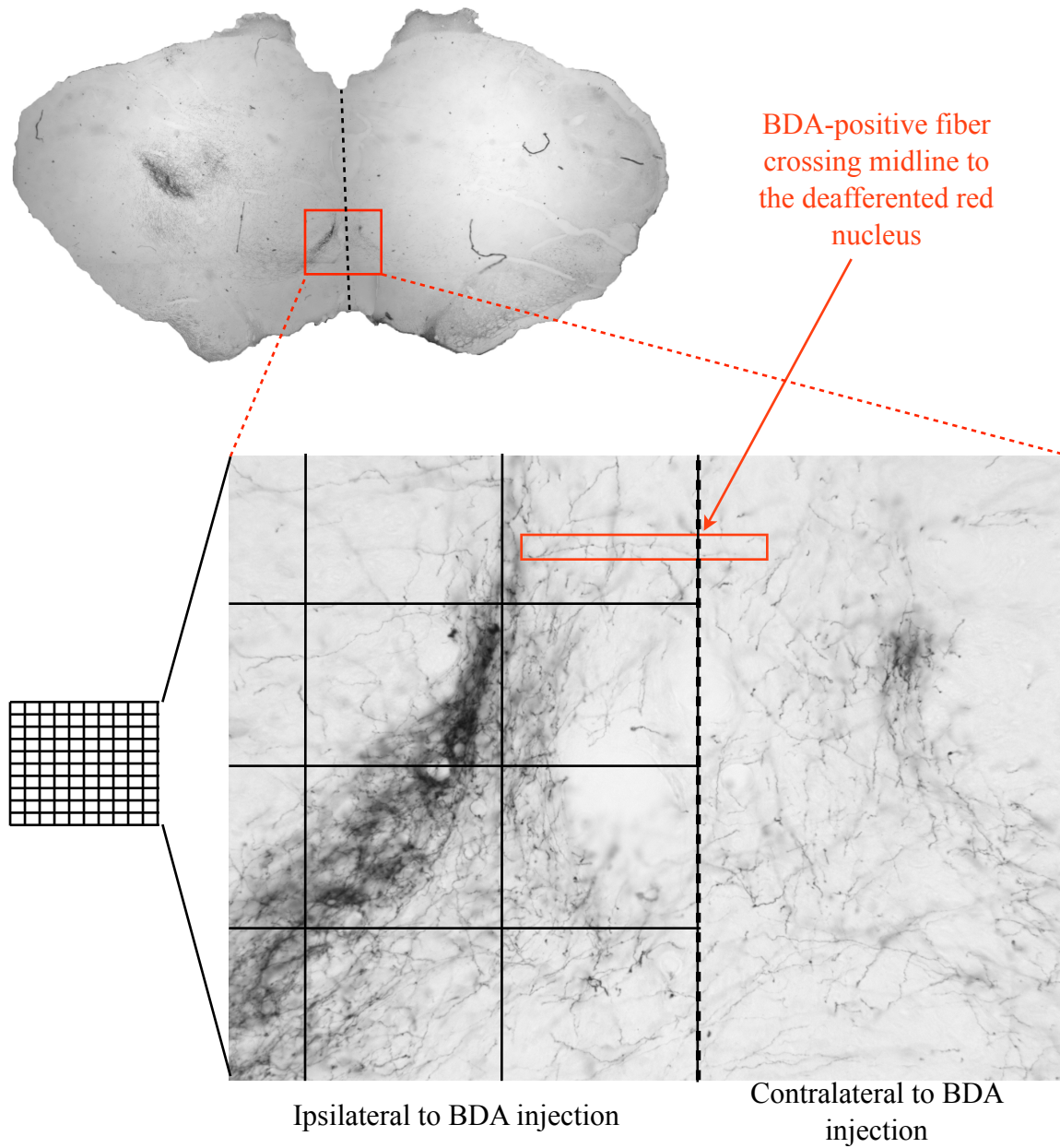


**Figure 7. The controlled cortical impact procedure produces a lesion in the forelimb sensorimotor cortex.** **A.** Dorsal view of a rat brain animation depicting approximate location of the forelimb sensorimotor cortex (FL in black circle) with cortical injury (shown in black) and corresponding impaired forelimb. MI = primary motor cortex, SI = primary somatosensory cortex. Image adapted from <http://homepage.psy.utexas.edu/HomePage/Faculty/Jones/JonesBio/Joneslab/ressum.html>. **B.** Photograph of a rat brain that sustained a controlled cortical impact (CCI) lesion (arrow).

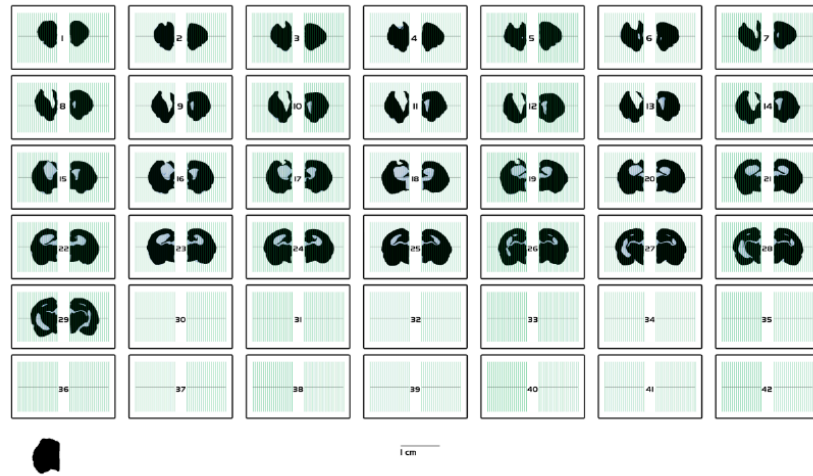


**Figure 8. Schematic illustration of the BDA procedure.** Corticofugal fibers from the contralesional forelimb motor cortex to the termination areas in the red nucleus are represented. The BDA tracer injection site is contralateral to the TBI lesion (gray shaded area). Red lines indicate possible sprouting fibers. Image adapted from Papadopoulos et al., *Annals of Neurology*, 2002.





**Figure 9. Schematic for counting BDA-positive fibers crossing the midline.** A circular glass grid was placed inside the left eyepiece of the microscope. At 20X magnification, the edge of the grid was placed along the midline of the section, and any BDA-positive fibers crossing that edge were counted.



**Figure 10. Traumatic brain injury lesion analysis.** Brain sections were stained with Nissl, scanned, and placed digitally using Photoshop CS5 onto a high resolution template. Sections were then colored black and the pixels were used to obtain lesion volume data.

## CHAPTER V

### RESULTS

**Animal body weight post-TBI.** All animals (Figure 11a) and “included only” animals (Figure 11b) gained weight consistently post-TBI. One TBI/11C7 animal lost weight for two weeks after TBI, but then regained that weight and continued gaining weight to the end of the study (Figure 11a,b). Another animal in the TBI/Control Ab group gained weight consistently through week three, but then passed away due to an infection as a result of cannula insertion during antibody pump placement (Figure 11a).

**Quantification of TBI Lesions in the Forelimb Sensorimotor Cortex.** All TBI lesions were localized in the forelimb sensorimotor cortex in the hemisphere opposite of the preferred forelimb (Figure 12a-c). Analysis of TBI lesion size showed no lesion volume differences between groups for all animals in the study (TBI Only =  $13\% \pm 6\%$ ,  $n = 10$ ; TBI/Control Ab =  $17\% \pm 9\%$ ,  $n = 7$ ; TBI/11C7 =  $12\% \pm 5\%$ ,  $n = 9$ ;  $p > 0.05$ ; Figure 13a) and “included only” animals (TBI Only =  $9\% \pm 5\%$ ,  $n = 3$ ; TBI/Control Ab =  $19\% \pm 6\%$ ,  $n = 4$ ; TBI/11C7 =  $13\% \pm 5\%$ ,  $n = 8$ ;  $p > 0.05$ ; Figure 13b). However, there is a trend that the control antibody TBI lesion volume was greater than TBI only lesion volume in “included only” animals.

**Functional Recovery in Animals Treated with mAb 11C7 One Week after TBI.** To determine whether blocking the neurite inhibitory protein Nogo-A one week after TBI

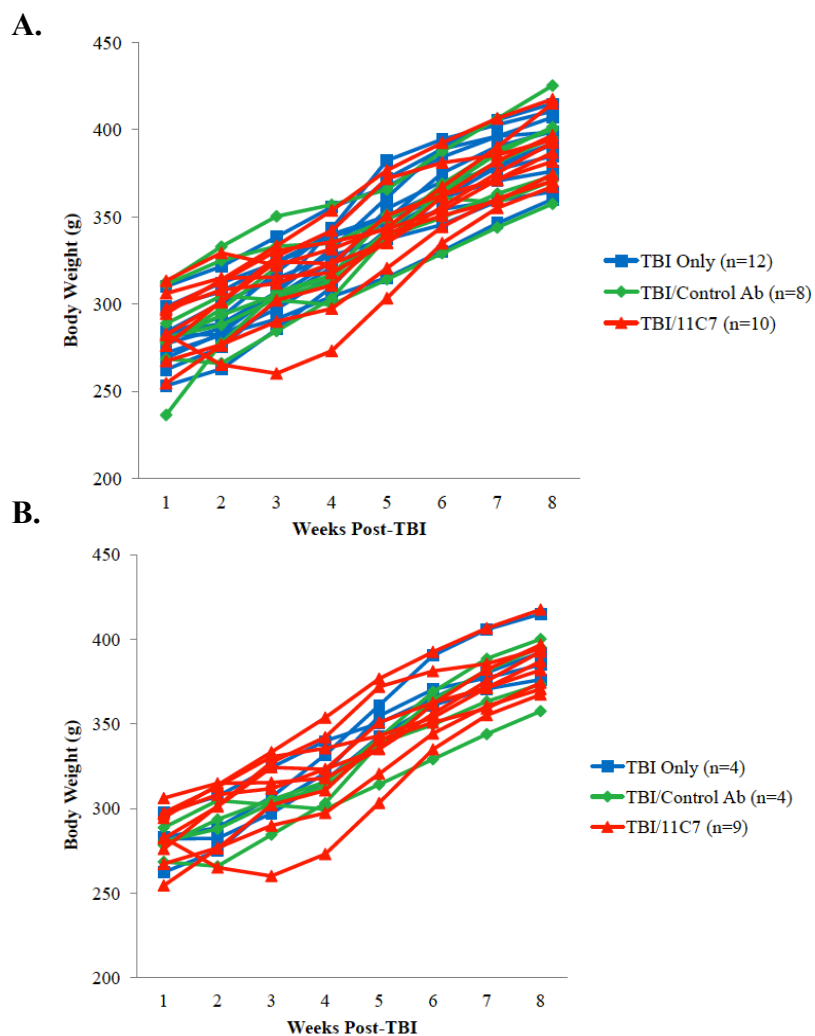
resulted in functional recovery, rats were tested on the skilled forelimb reaching task. By design, all animals achieved the same success score in grasping pellets at baseline testing prior to TBI with no significant differences between groups ( $p > 0.05$ ). Within the first week after TBI, all three groups showed a severe deficit in pellet grasping (mean of 3 pellets, or 17.5% of baseline), with no significant differences between groups ( $p > 0.05$ ). At weeks three and six, TBI/11C7 animals had significantly less success in pellet grasping than TBI only animals ( $p < 0.005$  and  $p < 0.05$ , respectively). However, at every other time point, no significant differences in pellet grasping were seen between the groups ( $p > 0.05$ ). In comparison to week one post-TBI, all groups achieved significant recovery by week two (TBI Only,  $p < 0.0005$ ; TBI/Control Ab,  $p < 0.005$ ; TBI/11C7,  $p < 0.05$ ). All groups showed even more recovery at week eight with TBI only animals successfully grasping a mean of 13 pellets (76% of baseline), TBI/Control Ab animals successfully grasping a mean of 11 pellets (67% of baseline), and TBI/11C7 animals successfully grasping a mean of 11 pellets (65% of baseline). (See Figure 14a)

“Included only” animals achieved the same success score in grasping pellets at baseline testing prior to TBI with no significant differences between groups ( $p > 0.05$ ). Within the first week after TBI, all three groups showed a severe deficit in pellet grasping (mean of 2.25 pellets, or 13.5% of baseline), with no significant differences between groups ( $p > 0.05$ ). Throughout the completion of testing, no significant differences in pellet grasping were seen between the groups ( $p > 0.05$ ). In comparison to week one

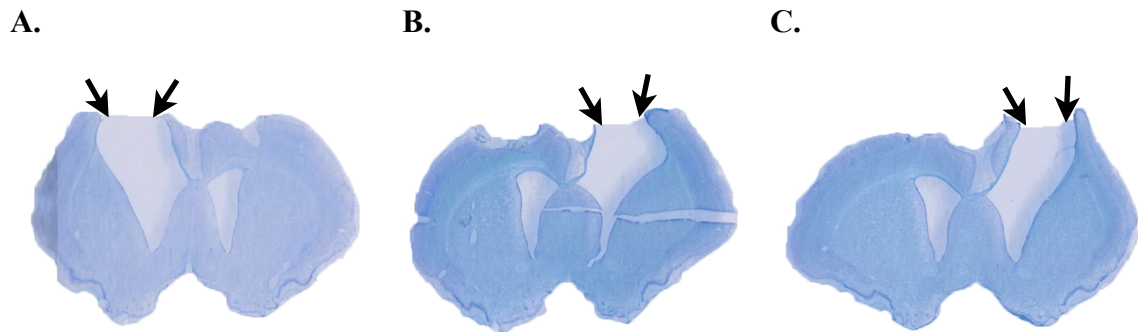
post-TBI, all groups achieved significant recovery by week two ( $p < 0.05$ ). All groups showed marked recovery at week eight with TBI only animals successfully grasping a mean of 11.6 pellets (70% of baseline), TBI/Control Ab animals successfully grasping a mean of 12.5 pellets (74% of baseline), and TBI/11C7 animals successfully grasping a mean of 10.6 pellets (64% of baseline). (See Figure 14b)

**Correlation between Lesion Volume and Reaching Success.** No correlation between lesion volume and reaching success was seen in any treatment group for all animals (Figure 15a; TBI Only,  $r = 0.47$ ,  $p > 0.05$ ; TBI/Control Ab,  $r = 0.36$ ,  $p > 0.05$ ; TBI/11C7,  $r = -0.05$ ,  $p > 0.05$ ) and “included only” animals (Figure 15b; TBI Only,  $r = 0.90$ ,  $p > 0.05$ ; TBI/Control Ab,  $r = 0.17$ ,  $p > 0.05$ ; TBI/11C7,  $r = -0.44$ ,  $p > 0.05$ ).

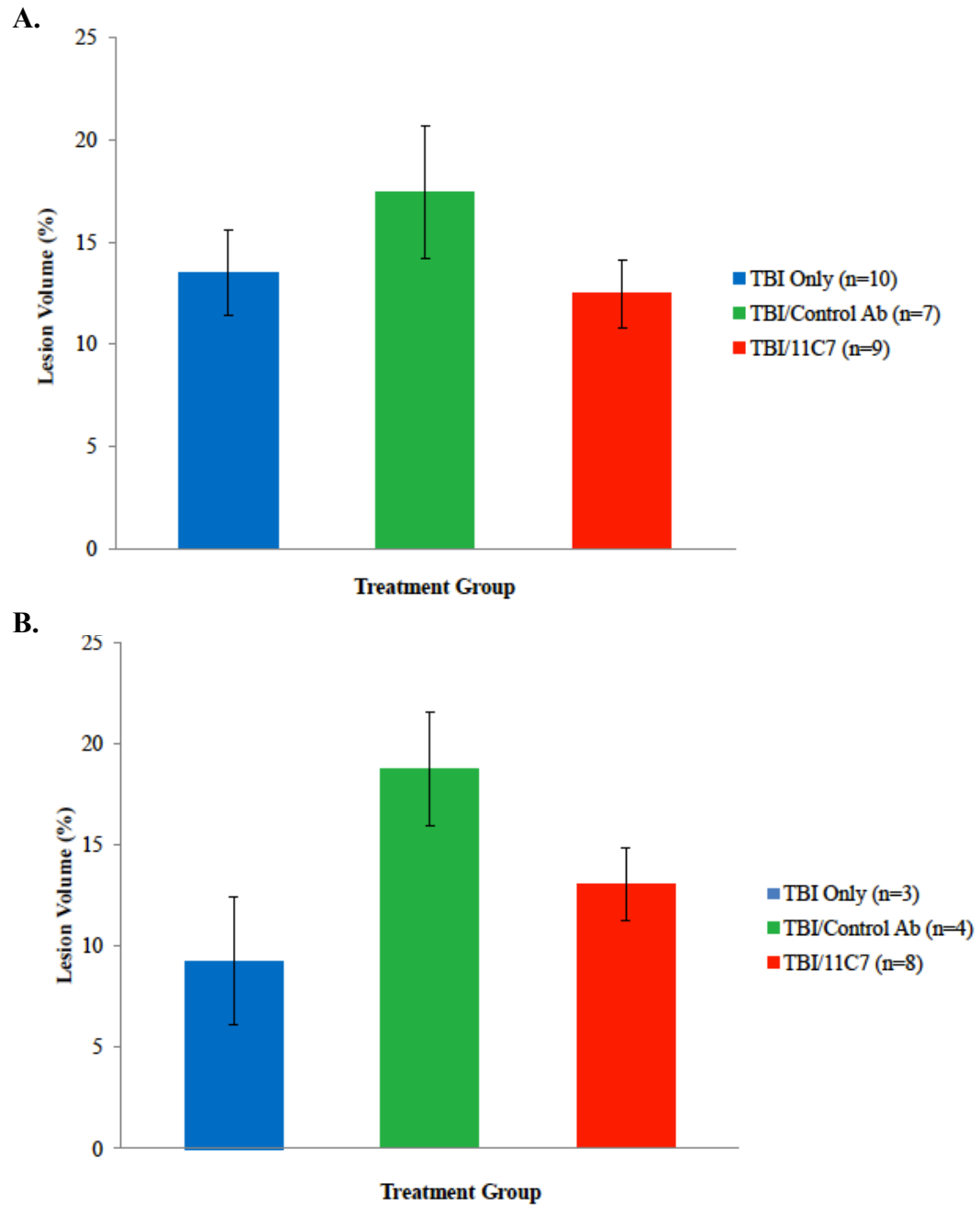
**Corticorubral Axonal Plasticity in Animals Treated with mAb 11C7 One Week after TBI.** The cortico-rubral projections crossing the midline from the unlesioned hemisphere were examined with the anterograde tracer BDA in order to relate behavioral changes to the development of new cortico-efferent pathways. In the undamaged CNS, the cortico-rubral pathway primarily involves ipsilateral projections with minor contralateral projections. Thus, axonal fibers crossing the midline of an intact brain is sparse. The parvocellular region of the red nucleus on the BDA-injection side showed dense BDA-positive fibers ipsilateral to the injection site (Figure 16a-d). No significant differences in the amount of fibers crossing the midline between treatment and control groups for all animals and “included only” animals were seen ( $p > 0.05$ ) (Figure 17a,b).



**Figure 11. Animal body weight consistently increased over time post-TBI.** Animals were food-deprived to encourage reaching. All animals (A) and “included only” animals (B) increased body weight consistently post-TBI over time.

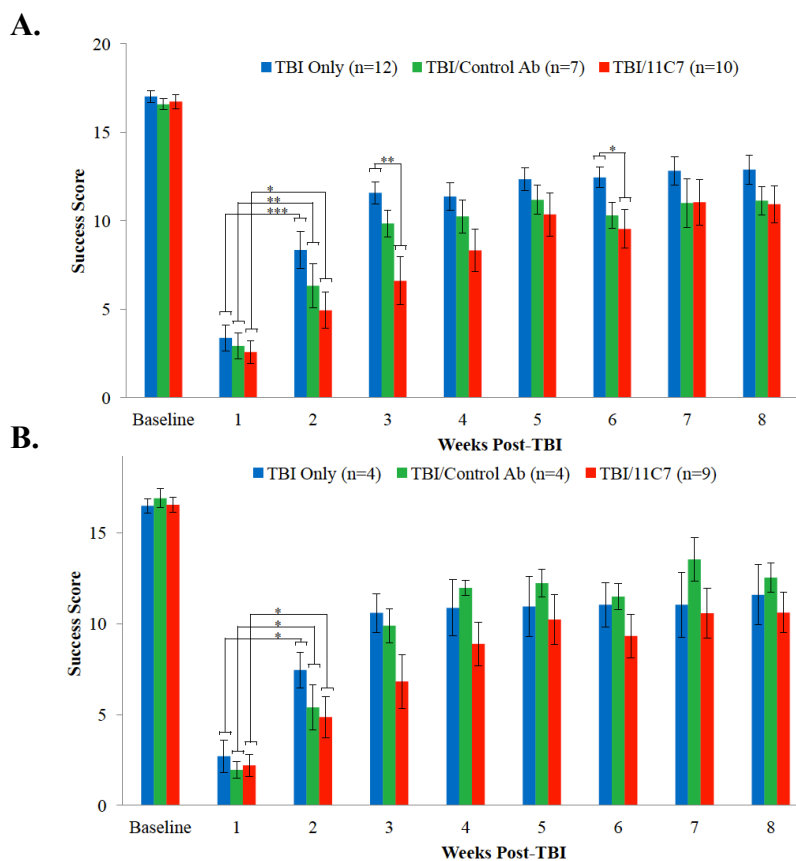


**Figure 12. Representative photomicrographs of TBI lesions in the sensorimotor cortex.** (A) TBI Only brain. (B) TBI/Control Ab brain. (C) TBI/11C7 brain. Location of the TBI lesion is indicated by arrows, Nissl stain.

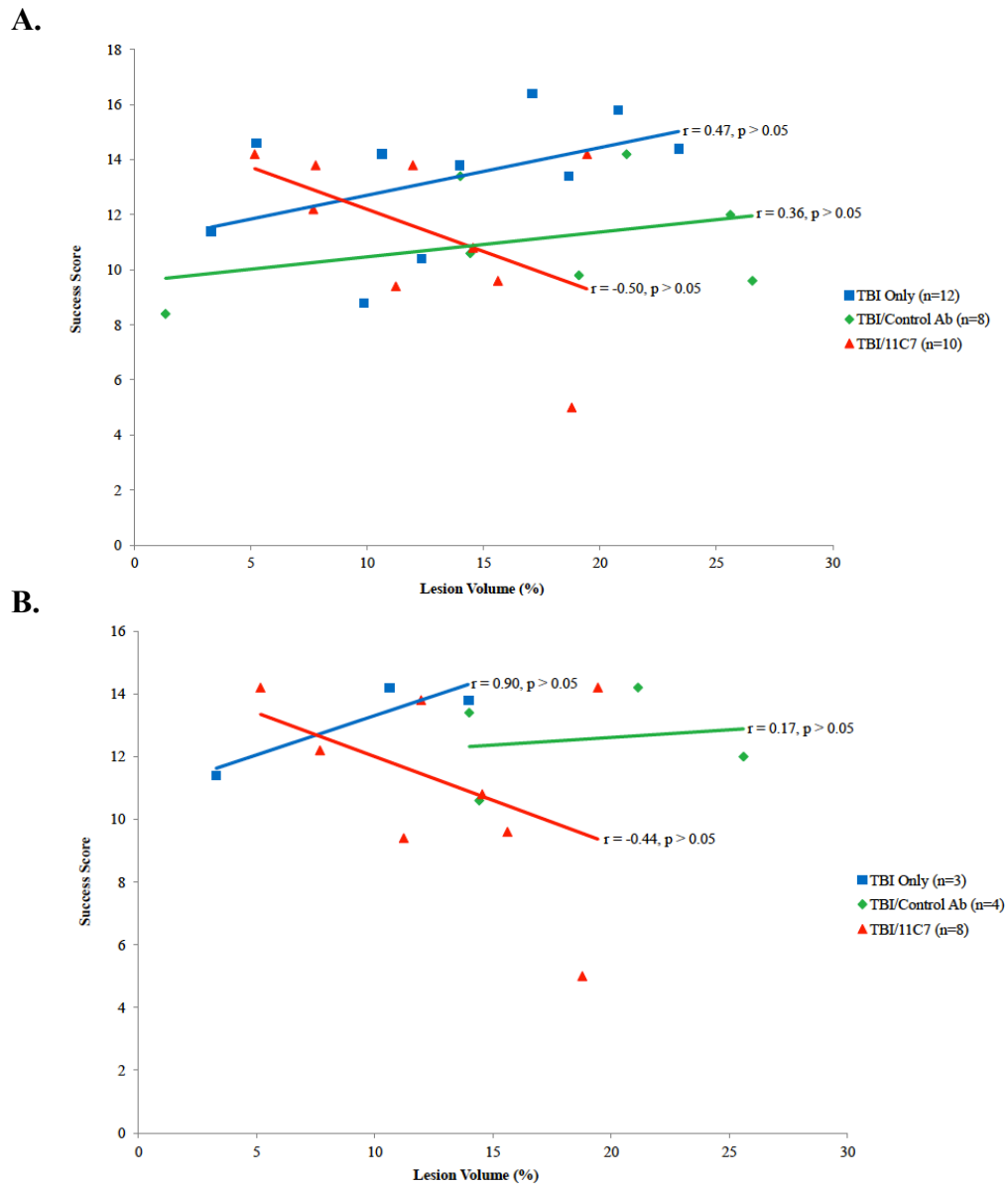


**Figure 13. Average lesion volume per treatment group.** No significant differences in lesion volume were seen between groups for all animals (A) or “included only” animals (B) (Oneway ANOVA;  $p > 0.05$ ; error bars indicate SEM).

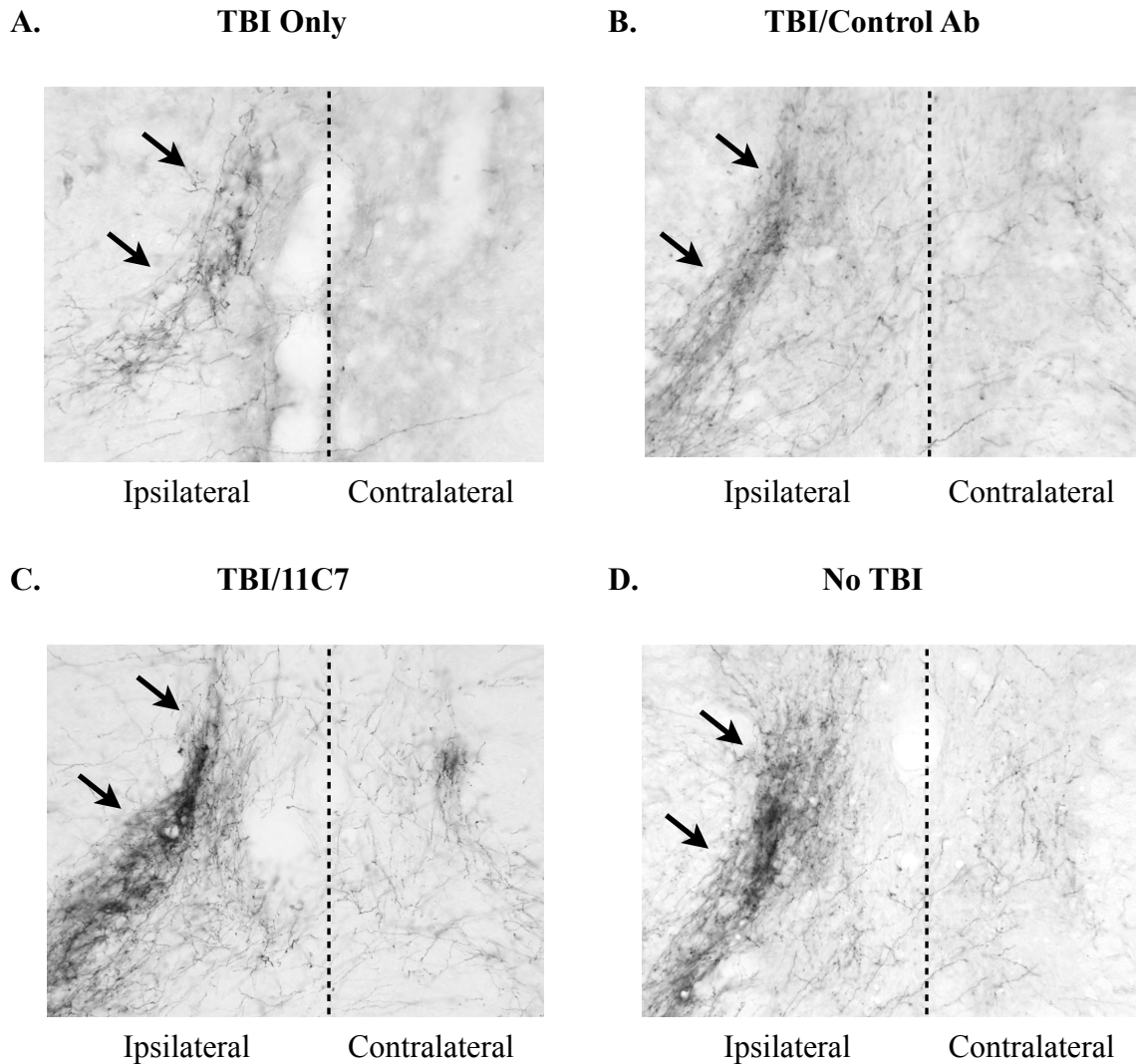




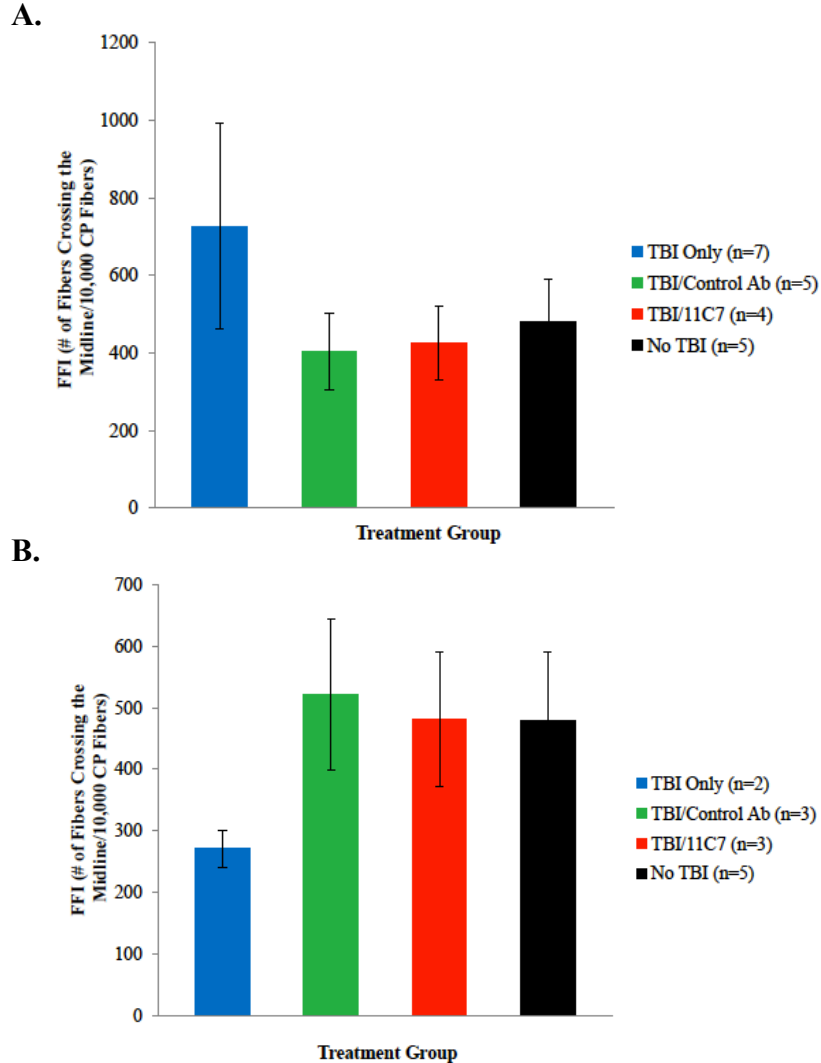
**Figure 14. The skilled forelimb reaching task.** By design, all groups had a success score around 16 pellets at baseline; one week post-TBI, all groups had a significant deficit with no difference between groups; and all groups significantly improved by week two post-TBI and continued improving through week 8 post-TBI for all animals (A) and “included only” animals (B) (Repeated measures *t*-test; \* =  $p < 0.05$ , \*\* =  $p < 0.005$ , \*\*\* =  $p < 0.0005$ ). At weeks three and six, TBI/11C7 animals were significantly less successful than TBI only animals for all animals (A), and no significant differences were seen between the groups at any time point for “included only” animals (B) (Repeated measures ANOVA, *post hoc* Tukey HSD Test; \* =  $p < 0.05$ , \*\* =  $p < 0.005$  and  $p > 0.05$ , respectively; error bars indicate SEM).



**Figure 15. Correlation between lesion volume and reaching success score per treatment group.** No significant correlation between lesion volume and reaching success score was seen per treatment group for all animals (A) and “included only” animals (B). The Pearson correlation coefficients (r values) are shown with corresponding significance (PPMCC;  $p > 0.05$ ).



**Figure 16. Representative histological images of BDA-positive fibers at the level of the red nucleus.** BDA-positive fibers in TBI only rats (A), TBI/control Ab rats (B), TBI/11C7 rats (C), and No TBI or treatment rats (D). Dense BDA-positive fibers are seen ipsilateral to the injection site for all animals (indicated by arrows). The number of fibers crossing the midline (indicated by dotted lines), however, did not differ significantly between groups ( $p > 0.05$ ).



**Figure 17. Rats treated with mAb 11C7 one week after TBI demonstrate no change in cortico-rubral plasticity, compared to controls.** The final fiber index (FFI), or the number of axonal fibers crossing the midline per 10,000 cerebral peduncle fibers, did not significantly differ between treatment groups for all animals (A) and “included only” animals (B) (Oneway ANOVA;  $p > 0.05$ ; error bars indicate SEM).

## CHAPTER VI

### DISCUSSION

Our results do not conclusively demonstrate whether intracerebral treatment with the mAb 11C7 to neutralize Nogo-A following TBI in adult rats promotes functional recovery or not as measured by the skilled forelimb reaching task requiring fine digital dexterity. Furthermore, cortico-efferent plasticity was not observed. Specifically, our results showed a consistent TBI lesion in the forelimb motor cortex that led to a significant decrease in skilled reaching success one week post-TBI with all groups showing significant recovery by week two. For the remainder of the study, all groups increased in skilled reaching recovery with no significant differences in reaching between groups. Furthermore, no correlation between lesion volume and reaching success was seen along with no significant differences seen in the number of axonal fibers crossing the midline at the level of the red nucleus between the treatment groups. Although it may appear that anti-Nogo-A immunotherapy following TBI may not be effective in promoting functional recovery through axonal plasticity, several factors, including low number of animals per group and significant recovery in control animals, may have contributed to these findings, making it impossible to conclude this at this time.

Previous studies that utilized anti-Nogo-A immunotherapy following ischemic stroke have shown significant recovery in the skilled forelimb reaching task

(Papadopoulos et al., 2002; Seymour et al., 2005). Specifically, animals that received anti-Nogo-A mAb IN-1 following stroke first showed significant recovery at week 6 with a success of approximately 80%, while controls remained around 45-50% for the remainder of the study (Papadopoulos et al., 2002). When animals received delayed treatment with mAb IN-1 following stroke, similar results were seen. At week 5, animals that received mAb IN-1 following stroke were significantly more successful in the skilled forelimb reaching task than controls, with a success of approximately 47% as compared to 23% for controls (with respect to baseline success score) (Seymour et al., 2005). At the end of this study (week nine), stroke animals that received anti-Nogo-A immunotherapy had a success of about 76% while controls had a success of about 30% (Seymour et al., 2005). Furthermore, these studies have shown a significant increase in cortico-efferent plasticity in stroke animals that received mAb IN-1 as compared with stroke controls (Papadopoulos et al., 2002; Seymour et al., 2005). Because of the success of anti-Nogo-A immunotherapy shown in these and other studies, a substantial precedent had been set that this therapy may be beneficial in other CNS injury models like TBI.

However, an increase in functional recovery in the skilled forelimb reaching task in adult rats that received anti-Nogo-A immunotherapy following TBI was not seen. Interestingly, by week eight all animals, including control animals, recovered to approximately 70% of baseline. This is unlike the stroke studies that showed that control animals had significantly lower recovery than stroke animals treated with anti-Nogo-A immunotherapy from weeks five and six through the end of the studies (Papadopoulos et

al., 2002; Seymour et al., 2005). In fact, marked recovery of all animals in the present study was seen from week two through the end. Therefore, it is impossible to say conclusively that anti-Nogo-A immunotherapy following TBI did or did not cause an increase in functional recovery since all animals recovered quickly and almost fully. Additionally, no increase in axonal plasticity was seen in anti-Nogo-A antibody treated animals. Interestingly, animals that received a stroke and IN-1 treatment had a final fiber index (FFI) around 500, which is what “included only” animals in all groups except TBI only in this study had (Papadopoulos et al., 2002). However, it is again difficult to say these results illustrate what really occurred due to the low number of animals in the TBI only, TBI/Control Ab, and TBI/11C7 groups for these animals. This low number of animals can be attributed to improper injection of BDA by the investigator, thereby not allowing the anterograde tracer to travel through the red nucleus for subsequent visualization of fibers crossing the midline. When we looked at the FFI for all animals, no significant differences were seen between the groups. Although, it does appear an opposite trend from the “included only” animals occurred. It could be possible that if even more animals are studied, this trend may become statistically significant, indicating that TBI with addition of antibodies increases axonal plasticity to normal levels (as seen by the No TBI group).

Other studies conducted utilizing anti-Nogo-A immunotherapy as a means to improve functional recovery as related to an increase in axonal plasticity following TBI had also failed to produce similar results as seen with stroke and anti-Nogo-A

immunotherapy. To date, two studies have been conducted to assess functional recovery, cognitive recovery, and axonal plasticity following TBI and anti-Nogo-A immunotherapy (Lenzinger et al., 2005; Marklund et al., 2007). Although both studies had shown that anti-Nogo-A immunotherapy does not alter functional recovery as measured by composite neuroscore and an increase in axonal plasticity between TBI/anti-Nogo-A Ab and TBI controls was not seen, several factors may have played a role in the discrepancies seen between these studies and those conducted with stroke and other CNS injury models (Lenzinger et al., 2005; Marklund et al., 2007). First, these studies used the lateral fluid percussion (LFP) model of TBI, possibly producing more variability in lesion size and location than the more direct controlled cortical impact (CCI) model of TBI. Second, and most importantly, these studies were conducted only up to four weeks post-TBI. As seen in the stroke studies above, significant functional recovery was not observed until five and six weeks post-injury. Thus, these studies may have concluded before recovery could occur. In the present study, we addressed these issues by using a different model of TBI (CCI) and extending the study to eight weeks post-injury.

Although we strongly believe the CCI model coupled with the skilled forelimb reaching task is one the best ways to reproduce TBI and effectively test functional recovery, it is impossible to make thoughtful conclusions in any study utilizing this procedure if the deficit produced by the CCI model is not a lasting one. All animals in this study recovered to about 70% of baseline--around the same amount stroke animals that had received anti-Nogo-A antibodies had recovered. The difference is that stroke



controls consistently had significantly less success (30-45% of baseline) than stroke/IN-1 animals. In order to say conclusively that anti-Nogo-A immunotherapy is not effective in enhancing functional recovery in animals that sustained a TBI, success in the skilled forelimb reaching task for the control animals would have to start and remain low (30-45% of baseline) for the whole study. Because all animals recovered so quickly (by week two), the TBI lesion produced by the CCI model is more than likely not large enough. To produce a lasting deficit, the parameters of this model need to be reworked, i.e. the depth at which the piston penetrates the brain and/or velocity of penetration need to be increased.

Once the parameters of the CCI model have been improved and a lasting deficit is produced, it is still possible that anti-Nogo-A immunotherapy may not enhance functional recovery. Thus, it is important to understand the cellular differences between stroke and TBI that may lead to conflicting conclusions. Similar to ischemic stroke, focal or global cerebral ischemia occurs frequently following TBI (Bouma et al., 1992; Coles et al., 2004; Inoue et al., 2005; Overgaard & Tweed, 1983) leading to the ischemic cascade involving excitotoxicity, oxidative stress, and apoptosis. Cerebral hypoperfusion and subsequent poor neurological outcome suggest that ischemic stroke and TBI share the same fundamental mechanisms. While this may be accurate to an extent, substantial differences lie in the primary injury between ischemic stroke and TBI. For example, irreversible tissue damage occurs when the cerebral blood flow (CBF) in patients sustaining a TBI is 15mL/100g/min while in patients sustaining an ischemic stroke, it is

5-8.5mL/100g/min (Cunningham et al., 2005). Ischemic stroke leads primarily to metabolic stress and ionic perturbations. However, in TBI the ischemic cascade is a result of the structural damage produced by the shear forces of the injury to neuronal cell bodies, astrocytes, microglia, and cerebral microvascular and endothelial cells (Bramlett & Dietrich, 2004; DeWitt & Prough, 2003; Rodriguez-Baeza et al., 2003).

Mechanistically, post-traumatic ischemia occurs because of morphological injury, i.e. vessel distortion as a result of mechanical displacement, hypotension in the presence of autoregulatory failure (McIntosh et al., 1996; Rodriguez-Baeza et al., 2003), inadequate availability of nitric oxide or cholinergic neurotransmitters (DeWitt & Prough, 2003; Scremin & Jenden, 1997), and potentiation of prostoglandin-induced vasoconstriction (Armstead, 2006). Additionally, in the early stages of injury, TBI patients may develop cerebral hyperperfusion (CBF > 55mL/100g/min) and subsequent hyperaemia immediately following post-traumatic ischemia (Kelly et al., 1996; Martin et al., 1997; Sakas et al., 1995). Increases in CBF beyond matching metabolic demand (hyperaemia) can cause even more damage by leading to vasoparalysis with subsequent increases in cerebral blood volume and intracranial pressure (ICP) (Kelly et al., 1997). Therefore, while cerebral ischemia and its cellular cascade is involved in both ischemic stroke and TBI, additional injury as a result of the mechanical forces that create TBI causes an increased risk of poor neurological outcome.

Another important difference between stroke and TBI involves the timing of injury. TBI occurs very rapidly in comparison to stroke, involving a quick mechanical

force causing immediate trauma to the brain. Ischemic stroke, on the other hand, is an evolving brain injury that occurs over a period of hours to days with an infarct core (the area exposed to the most dramatic blood flow reduction) and a surrounding zone (ischemic penumbra) of less affected metabolically active tissue that is functionally silent (Majno & Joris, 1995; Broughton et al., 2009). It is in this ischemic penumbra where injury evolves slowly and therapeutic intervention is focused. Because injury evolves over a greater amount of time in ischemic stroke than in TBI, it is possible that anti-Nogo-A immunotherapy may be working through growth factors or cytokines that are released over a prolonged period of time in ischemic stroke that are not present for as long or at all in TBI. Consequently, while anti-Nogo-A immunotherapy is effective in restoring functional activity and increasing neuroplasticity in ischemic stroke, this therapy may not have the opportunity to provide the same effectiveness in TBI.

Additionally, the animal models of TBI and ischemic stroke produce CNS lesions in two very different ways. TBI is produced by a mechanical force penetrating the brain in three commonly used models: a weight of a given mass and velocity is dropped on the exposed skull or brain tissue (weight drop model), a saline solution is forcefully injected into the brain (lateral fluid percussion model), or a piston penetrates the brain tissue at a given velocity and depth (controlled cortical impact model). On the other hand, ischemic stroke is commonly produced by middle cerebral artery occlusion in animals. This model involves occlusion of one or more branches of the middle cerebral artery for a period of minutes to hours. Therefore, even if both injuries are considered CNS lesions, the way

these two injuries are produced may shed light on the true differences between them and why anti-Nogo-A immunotherapy may be beneficial for one and ineffective in the other.

Overall, even though this study did not produce significant results that anti-Nogo-A immunotherapy improves functional recovery and increases axonal plasticity, it also did not disprove the idea that anti-Nogo-A antibodies could play a positive role in functional recovery and axonal plasticity. In particular, this study pointed out areas in procedures that need to be fine-tuned to produce a model which can effectively be tested and produce reliable results from. In the future, the CCI model parameters needs to be refined to produce TBI lesions that cause lasting deficits in reaching in order to effectively determine whether anti-Nogo-A immunotherapy aids in improving functional recovery and increasing axonal plasticity. Also, special care needs to be taken throughout the histological processes in order to keep the number of animals that can be used to a maximum. Furthermore, it is possible that the skilled forelimb reaching task is not the best assessment of functional recovery following TBI. Thus, it may be beneficial to use a battery of additional sensorimotor assessments in conjunction with the skilled forelimb reaching task. Once the CCI model parameters have been refined, the number of animals for each procedure is large enough to obtain meaningful statistical conclusions, and additional sensorimotor assessments are used, it is still possible that anti-Nogo-A immunotherapy will not increase functional recovery in comparison to control animals as related to an increase in axonal plasticity. This may be due to the idea that ischemic stroke and TBI are much more different types of CNS injury than originally thought.

Thus, while anti-Nogo-A immunotherapy appears to be effective in increasing functional recovery as related to an increase in axonal plasticity in ischemic stroke, specific differences between TBI and ischemic stroke that are as of yet unknown may be playing a pivotal role in anti-Nogo-A antibodies' effectiveness. Although no conclusive results were obtained, this study has brought us one step closer to understanding the pathology of TBI and how to produce an effective model to test potential treatments of TBI.

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## VITA

The author, Jennifer Biesiadecki, was born on October 27, 1988 in Harvey, Illinois, to Bogumila and Jan Biesiadecki. She received her secondary education at Sycamore High School in Sycamore, Illinois, graduating in 2007. Jennifer attended the University of Illinois in Urbana-Champaign, Illinois. While at UIUC, she co-founded two chapters of two separate global organizations known as Global Business Brigades and Global Medical Brigades. Both organizations are part of Global Brigades with two different and distinct missions. Global Business Brigades aims to empower rural communities in Panama to become more economically stable by providing financial consulting and planning to those where business guidance and financial assistance are not available. Global Medical Brigades aims to provide medical care in the form of mobile medical units consisting of students volunteering in triage and pharmacy, doctors, and nurses to those in need where medical attention is inaccessible. Currently, Global Medical Brigades focuses their attention in Ghana, Nicaragua, Panama, and Honduras. While at UIUC, Jennifer's work with Global Medical Brigades focused on providing medical attention to those in need in all parts of Honduras. As a junior and senior at UIUC, Jennifer worked with a post-doc in a lab that focused on the evolution and ecology of the live-bearing fish, *Heterandria formosa*, specifically looking at maternal and fetal interactions and how nutrients are allocated from mother to offspring. In May 2011,

Jennifer graduated from UIUC with a Bachelor of Science degree in Integrative Biology (pre-med) and a minor in Chemistry.

In August 2011, Jennifer entered the Master's Program in the Department of Integrative Cell Biology at Loyola University Chicago Stritch School of Medicine. She joined the laboratory of Dr. Gwendolyn L. Kartje, M.D., Ph.D to pursue a potential treatment for traumatic brain injury. Two years later, Jennifer is applying to medical schools in hopes to attend a medical school in the fall of 2014 and continue pursuing her dream to become a doctor.