CORTICAL STIMULATION TO ENHANCE MOTOR IMPROVEMENTS AFTER STROKE

By

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This work is dedicated to my dad for his seemingly limitless support

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AIF	Apoptosis-inducing factor
ApoE	Apolipoprotein E
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
CFA	Caudal forelimb area
CNS	Central nervous system
CS	Cortical stimulation (motor cortex)
CS/RT	Cortical stimulation combined with rehabilitative training/therapy
CST	Cortical spinal tract
DNA	Deoxyribonucleic acid
E1-CS/RT	E1 configuration cortical stimulation with rehabilitative training condition
E2-CS/RT	E2 configuration cortical stimulation with rehabilitative training condition
E3-CS/RT	E3 configuration cortical stimulation with rehabilitative training condition
ET-1	Endothelin-1
FASr	Apoptosis-stimulating factor receptor
GABA	Gamma-Aminobutyric acid
GDNF	Glial cell line-derived neurotrophic factor
HC	Healthy control condition
IC	Internal capsule
ICMS	Intracortical microstimulation
LTD	Long-term depression
LTP	Long-term potentiation

M1	Primary motor cortex
MCA	Middle cerebral artery
MCAo	Middle cerebral artery occlusion
MCA-CS/RT	MCAo plus cortical stimulation combined with rehabilitative training condition
MCAo	Middle cerebral artery occlusion
MCA-RT	MCAo plus rehabilitative training condition
MEP	Motor evoked potential
MT	Movement/motor threshold
NMDA	N-methyl D-aspartate
NO	Nitric oxide
NT	Non-trained condition
O_2	Oxygen
PARP1	Poly-(ADP ribose) polymerase 1
PMd	Dorsal premotor cortex
PMv	Ventral premotor cortex
RFA	Rostral forelimb area
RT	Rehabilitative training/therapy condition
S1	Primary somatosensory cortex
SMA	Supplementary motor area
Sub-CS/RT	Subcortical ischemia plus cortical stimulation combined with rehabilitative training condition
Sub-RT	Subcortical ischemia plus rehabilitative training condition
tDCS	Transcranial direct cortical stimulation
TIA	Transient ischemic attack
TMS	Transcranial magnetic stimulation

VEGF	Vascular-endothelial growth factor

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Motor rehabilitation after cerebral ischemia can enhance motor performance and induce cortical plasticity. Electrical stimulation of the motor cortex (CS) during rehabilitative training (CS/RT) augments motor map plasticity and enhances motor improvements. CS/RT's ability to enhance motor improvements after ischemic insult has only been tested in a limited number of situations that do not completely represent stroke conditions in the human population. Indeed, the lack of treatment effect in a recent phase III clinical of CS/RT reflects knowledge gaps in how to fully translate basic science findings of CS/RT into the clinical setting. The goal of this dissertation is to use rodent models of stroke to examine several parameters of CS/RT therapy that may account for its lack of benefit in human stroke patients.

Study #1: It is unclear how the distribution of electrical stimulation across the cortex influences both motor map reorganization and improvements in motor performance. Here, we examined the behavioral and neurophysiological effects of delivering CS/RT through a distributed versus focal arrangement of electrical contacts. In this experiment, stroke was modeled by focal ischemic damage with motor cortex.

Results showed that animals given CS/RT with a distributed contact configuration condition exhibited greater motor improvements than animals given CS/RT with a focused contact arrangement or given rehabilitative training alone (RT). All groups that received rehabilitation exhibited greater increases in motor map area and reaching accuracy than animals that received no rehabilitative training (NT). However, both CS/RT groups exhibited larger motor maps than the RT animals. The results indicate that although both focal and distributed forms of CS/RT promote motor map reorganization, only the distributed form of CS/RT enhances motor performance with rehabilitation.

Study #2: While distributed CS/RT proved more effective than focal CS/RT in the first experiment, the number of distributed stimulation contacts sites that will induce the greatest motor improvements with CS/RT is not known. Here, we examined the behavioral effects of delivering CS/RT with either four or nine independent contact sites that were distributed across motor cortex following ischemic insult. In this experiment, stroke was modeled by temporary occlusion of the middle cerebral artery. The results showed that both types of distributed CS/RT enhanced motor improvements regardless of the number of independent sites. Our results indicate that the number of independent stimulation sites does not affect CS/RT with distributed stimulation.

Study #3: Human stroke patients who are candidates for CS/RT will likely have received standard forms of rehabilitation prior to the onset of CS/RT. It is not known whether CS/RT can induce motor improvements in individuals who have received rehabilitative experience following ischemic insult. Further, the phase III clinical trial that failed to find an effect of CS/RT had recruited stroke patients who had already received

standard rehabilitative therapy. Here, the behavioral effects of administering CS/RT after early application of RT alone were measured. In this experiment, stroke was modeled by temporary occlusion of the middle cerebral artery. The results showed that CS/RT magnifies behavioral improvements above and beyond those occurring from rehabilitative training alone. Our results suggest that CS/RT and RT have a complimentary rather than antagonizing relationship.

Study #4: While animal studies of CS/RT have observed enhanced motor improvements in models of cortical stroke, they have not tested its effects in subcortical stroke models. Subcortical damage is relatively common in stroke patients. Further, the phase III clinical trial that found no benefit with CS/RT had enrolled patients with subcortical stroke damage. In this experiment, the behavioral effects of CS/RT in a rodent model of subcortical ischemia involving damage within the internal capsule were compared to model of cortical ischemia. The results showed that CS/RT confers no additional motor improvements compared to RT following subcortical white matter ischemia. These data suggest that CS/RT may not be as effective in treating human stroke patients with subcortical damage.

CHAPTER 1
NEURAL PLASTICITY, STROKE AND CORTICAL STIMULATION

General Introduction

In the United States, approximately 795 000 new or recurrent strokes occur each year with an estimated 6.5 million individuals currently living with the effects of this disease (American Heart Association, 2009). The functional deficits following stroke involve a number of systems ranging from cognitive, affective, communicative, motor and sensory domains. Motor impairments are found in more than 75% of stroke victims (Lawrence et al., 2001) and are the primary factor contributing to serious long-term adult disability (McNeil and Binette, 2001) and decreased quality of life (Hacket et al., 2000; Sturm et al., 2002). For 2009, the projected financial burden of stroke is an estimated 68.9 billion dollars when estimates of the direct costs involving the treatment of the injury and the indirect costs involving loss of productivity are combined. More effectives treatments for motor impairments after stroke will alleviate some of the financial burden and improve the lives of both stroke survivors and care givers.

Given the incidence of motor deficits in stroke patients, motor rehabilitation is the primary treatment. Unlike many other medical conditions, there is currently no single treatment intervention that is recognized by therapists for enhancing motor function. After stroke, some motor improvements occur within the first three months while substantially fewer improvements are observed at later time points (Duncan et al., 2000). Further, the degree of motor improvement after stroke is typically incomplete and highly variable across individuals (Gresham et al., 1995). The extent of motor improvement after stroke depends on many factors such as the initial impairments, age, sex, the nature of the training experience and patient genotype (Duncan et al., 2000).

After stroke, the severity of motor impairments during the initial hospital admission predicts the severity of motor impairments and the ability to perform activities of daily living at the time of discharge (Shelton et al., 2001). Moderate to severe hemiparesis within the first month post stroke strongly predicts poor functional outcomes at least up to three years following the injury (Samuelsson et al., 1996). The location of injury can also predict functional outcomes in stroke patients. Subcortical stroke is typically associated with more severe impairments and fewer behavioral gains when there is damage to white matter connections (Norrving, 2003; Arakawa et al., 2006). In particular, ischemic damage in the posterior region of the internal capsule has been associated with relatively severe impairments and little restoration of motor function (Morecraft et al., 2002; Lie et al., 2004; Wenzelburger et al., 2005). Reduced axonal integrity as assessed by lower fractional anisotropy with MR imaging correlates with further loss of fractional anisotropy and motor function in patients with poor motor outcome (Møller et al., 2007). More symmetrical bilateral fractional anisotropy after stroke correlates with improved motor function at a three-month assessment (Jang et al., 2005). Functionally, the absence of TMS responses within the first forty-eight hours is associated with complete hand palsy (Pennisi et al., 1999). The nature of the training experience also affects motor improvements after stroke. In animal studies, skilled motor training is associated with improvements in skilled motor function that are absent during endurance or strength motor training or training on simple motor tasks (Adkins et al., 2006; Kleim and Jones, 2008). Human stroke patients given one of two types of rehabilitative therapy in a single study can exhibit different degrees of motor improvements (Langhammer and Stanghelle, 2000). In a meta-analysis, Cifu and

Steward (1999) observed early initiation of rehabilitative therapy had a strong relationship with improved functional outcomes. However, immediate therapy may be harmful as animal data have observed exacerbated tissue damage with extreme use of an impaired limb in the acute hours after injury (Kozlowski et al., 1996; Risedal et al., 1999). Many patient characteristics that are independent of the stroke can influence the level of impairments as well as the degree of motor improvements over time. Analysis of motor improvements on the Barthel Index have been used in statistical modeling to demonstrate that older age, prestroke disability, and the female sex are associated with fewer behavioral gains at least up to 1 year following injury (Tilling et al., 2001). Increasing age at stroke onset also correlates with poorer performance on activities of daily living (Nakayama et al, 1994). Functional status assessed by having fewer disabilities in activities of daily living before stroke is also strongly associated with fewer difficulties in activities of daily living after stroke (Colantonio et al., 1996). There is emerging evidence that the genotype of stroke patients affects their recovery. Given the complexity of the human genome there are likely numerous genes that affect recovery from stroke. In the context of stroke recovery, two of the more characterized genes include brain-derived neurotrophic factor (BDNF) and apolipoprotein E (ApoE). It is estimated that 30-40% of the human population carries at least one copy of the BDNF gene with a val66met polymorphism that is associated with diminished release of the active protein (Egan et al., 2003; Chen et al., 2004). BDNF is a highly expressed growth factor in the brain that affects the survival, structure and function of neurons (Desai et al., 1999; Lu and Chow, 1999). Levels of BDNF are altered in response to variety of manipulations making it a putative signal for encoding experience in the brain (Pearson-

Fuhrhop et al., 2009). Disrupting BDNF levels in the brain limits synaptic plasticity (Ma et al., 1998; Gorski et al., 2003; Genoud et al., 2004) and impairs many types of learning (Linnarsson et al., 1997; Minichiello et al., 1999; Mizuno et al., 2000). In the context of stroke rehabilitation, animals given infusions of antisense BDNF oligonucleotide that diminish BDNF signaling do not exhibit motor improvements during motor training after experimental stroke (Ploughman et al., 2009). Humans that carry the val66met polymorphism exhibit attenuated responses of cortical plasticity as assessed by TMS motor mapping (Kleim et al., 2006) or fMRI (McHughen et al., 2009). These findings suggest the Met allele may limit neuroplastic changes following injury that support motor improvements (Pearson-Fuhrhop et al., 2009). Indeed, the val66met polymorphism has been associated with poorer outcome after subarachnoid hemorrhage (Siironen et al., 2007). The ApoE gene has two common single nucleotide polymorphisms resulting in three distinct alleles termed ApoE2-4 or E2-4 (Mahley et al., 2000). E3/E3 is the most common genotype with estimated frequencies of 43%-74% in human populations depending on ethnicity (Eichner et al., 2002). Approximate frequencies of the others include: 22% E3/E4, 12% E2/E3, 3% E4/E4, 2% E2E4, 1% E2/E2 (Eichner et al., 2002; Bersano et al., 2008). ApoE mediates lipid transport from one cell type to another as well as serves in neuronal repair, remodeling and protection (Mahley et al., 2000; Cedazo-Minguez, 2007). In a meta-analysis, ApoE genotype was associated with functional outcome following subarachnoid hemorrhage (Martínez-González and Sudlow, 2006). In another study, ApoE genotype related to behavioral outcome measures at 1 and 3 months post stroke and ApoE4 was associated with poorer outcome (Pearson-Fuhrhop et al., 2009). Motor improvements after stroke must

therefore be thought of as the product of numerous behavioral and neurobiological factors. The growing identification of stroke recovery factors and their interactions indicate our present lack of understanding for how to facilitate brain repair and drive behavioral improvements.

Neurobiology of Stroke

Stroke is a heterogeneous injury involving disruption of blood flow to the brain. The resulting dysfunction or death of neural tissue creates neurological deficits that reflect loss of function by the compromised areas. The two general ways that blood flow is disrupted during stroke are through ischemic and hemorrhagic processes. Hemorrhagic stroke occurs when a blood leaks from the cardiovascular system resulting in reduced blood flow as well as toxic effects of the misplaced blood. Hemorrhagic stroke has an intracerebral origin in 10% and a subarachnoid origin in 3% of all stroke cases (American Heart Association, 2009). Ischemic stroke accounts for approximately 87% of all stroke types and is characterized by compromised blood flow due to a blood vessel obstruction or an inability for the cardiovascular system to maintain adequate supply such as cardiac arrest (American Heart Association, 2009). The overall diminished ability of the cardiovascular system to supply blood to the brain is termed global ischemia. If reduced blood flow is due to obstruction of a specific vessel or set of vessels it is termed focal ischemia. Thrombotic focal ischemia occurs when the blockage is formed at the site where it disrupts blood flow whereas embolic focal ischemia occurs when the blockage is formed distant to the site where it disrupts blood flow and is then carried in the blood stream to the place of the blockage. An additional type of ischemic injury is a transient ischemic attack (TIA). TIA's are not considered strokes but rather temporary reductions in the blood flow where neurological symptoms

last less than 24 hours. The onset of a TIA is usually sudden and the duration brief, generally lasting between 2 and 30 minutes (Hankey, 1996). While TIA's are highly transient they tend to foreshadow upcoming ischemic embolism strokes that are typically more severe (Hankey, 1996).

Cell Death During Stroke

The pathophysiology of focal ischemia stroke is reviewed here because it is most relevant to the stroke models used in this dissertation (for a review of hemorrhagic stroke see Carmichael et al., 2008; Qureshi et al., 2009). Cell death during ischemia is thought to largely arise from excitotoxicity and ionic imbalance, oxidative/nitrosative stress and activation of apoptotic pathways, however, emerging evidence also implicates other detrimental processes including inflammation, tissue acidosis and peri-infarct spreading depolarizations (Mergenthaler et al., 2004). During ischemic exposure cells may die by rupture, lysis, phagocytosis or involution and shrinkage (Lo et al., 2003). These modes of cell destruction are often classified using the terms necrosis and apoptosis to discriminate whether the cell actively participates in its destruction. Necrosis is the most prominent form of cell death during extreme ischemia and is the result of rapid disruption of the plasma membrane or organelle failure (Lo et al., 2003). Necrotic cells endanger neighboring cells due to the release of toxic or damaging molecules (Lo et al., 2003). Apoptosis in contrast represents a programmed cell death requiring adenosine triphosphate and gene expression that involves an organized degradation of the cell such that there is minimal release of cellular contents into its surrounding environment (Johnson et al., 1995). Apoptosis is triggered by an extrinsic pathway involving the activation of the aptly named death receptors such as the FAS-receptor (Mattson et al., 2001). Intrinsic factors such as elevated calcium, reactive

species, glutamate and deoxyribonucleic acid (DNA) damage also stimulate apoptosis (Lo et al., 2003). Both extrinsic and intrinsic factors can induce a mitochondrial dependent apoptotic signal involving the release of cytochrome c and subsequent caspase activation (Li et al., 1997). Activated caspases are protein-cleaving enzymes that disrupt homeostasis and disassemble cells (Dirnagl et al., 1999). Caspase-independent apoptosis occurs through the activation of the poly-(ADP ribose) polymerase 1 (PARP1) by stimulating N-methyl D-aspartate (NMDA) receptors using the signaling of apoptosis-inducing factor (AIF) (Lo et al., 2003). AIF is released from the mitochondria and binds to DNA where it promotes chromatin condensation and cell death through unknown mechanisms (Lo et al., 2003). Apoptosis's contribution to overall ischemic cell death remains subject to debate, however, it is likely milder ischemia preferentially induces apoptosis (Lo et al., 2003; Mergenthaler et al., 2004). Further, cross talk between necrotic and apoptotic pathways leads to combinatorial forms of cells death with hybrid morphological features with several cross talk-molecules having suggested roles including calpains, cathepsin B, nitric oxide (NO) and PARP1 (Lo et al., 2003).

Another important distinction in stroke pathophysiology is describing the endangered tissue by its location within the ischemic region. The ischemic zone most deprived of blood is termed the core. The core has the fastest and greatest overall loss of cells as well as exhibiting the greatest proportion of necrotic cell death (Martin et al., 1998; Banasiak et al., 2000). Tissue surrounding the ischemic core has an outward gradient of increasing blood flow and decreasing toxic elements that is collectively referred to as the ischemic penumbra. While the precise boundaries of these regions

are difficult to define each area exhibits unique patterns of cell death, gene expression, promotion/inhibition of neuronal rewiring, neurogenesis and neuronal activity that must be considered when reviewing stroke pathophysiology (Mergenthaler et al., 2004; Carmichael et al., 2005; Carmichael 2006; Popp et al., 2009).

Ionic Imbalance by Energy Failure

When blood flow sufficiently decreases during stroke, cells in the nervous system deplete their energy stores and lose their ability to maintain ionic concentration gradients. Cells experiencing low energy also fail to remove neurotransmitters from their synaptic spaces. Cells lacking sufficient energy depolarize allowing an influx of sodium, chloride and calcium ions as well as the efflux of potassium (Siesjo et al., 1991). Increased intracellular concentration of sodium and chloride ions leads to an influx of water causing cell and tissue swelling (edema) that further disturbs ion homeostasis and causes cell death by osmotic lysis. Increasing intracellular calcium concentrations promote lipolysis, proteolysis, nitric oxide production, endonuclease-mediated DNA degradation in addition to the activation of kinases and phoshatases that alter the activation states of proteins and direct certain gene expression (Emsley et al., 2008).

Excitotoxiticty

The depolarization of neurons and glia induced by insufficient energy and ionic disturbances in the extracellular space leads to the release of excitatory neurotransmitters in the absence of presynaptic and astrocytic reuptake mechanisms. The resulting excessive presence of excitatory neurotransmitter in the extracellular space results in uncontrolled postsynaptic receptor binding leading to an ion influx in postsynaptic cells. The primary excitatory neurotransmitter of the brain glutamate is thought to be a key player in this excitotoxicity. Postsynaptic binding of glutamate to

ionotropic NMDA receptors results in increased intracellular calcium concentrations while glutamate binding to □-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors and ionotropic glutamate receptors leads to increases in intracellular sodium, and chloride concentrations (Mergenthaler et al., 2004). The resulting influx of ions from glutamate binding again leads to osmotic lysis (Dirnagl et al., 1999; Lo et al., 2003). The influx of ions also results in apoptosis and inflammation (Dirnagl et al., 1999; Lo et al., 2003). Excess intracellular calcium promotes other detrimental effects to cell survival including the activation of phospholipases and proteases that degrade essential membranes and proteins (Lo et al., 2003).

Oxidative/Nitrosative Stress

Reactive oxygen and nitrogen species such as superoxide, hydrogen peroxide, hydroxyl and peroxy-nitrite radicals are dramatically increased during ischemia. These reactive species have the potential to damage membranes, DNA and almost all organelle types (Dirnagl et al., 1999; Lo et al., 2003). In particular, mitochondrial damage from reactive species results in a diminished production of ATP production as well as release of additional damaging radicals and proapoptotic molecules (Mergenthaler et al., 2004).

Acidosis

The hypoxic conditions created by low blood flow cause a switch from aerobic to anaerobic cellular metabolism leading to an accumulation of lactate via anaerobic glycolysis (Mergenthaler et al., 2004). Increased anaerobic metabolism promotes the formation of lactic acid causing intracellular pH levels to decrease (acidosis) (Sapolsky et al., 1996). This acidosis causes cellular dysfunction, including disruption to mitochondrial and glycolytic enzyme activity (Rehncrona et al., 1981). In addition,

acidosis disrupts intracellular protein synthesis and promotes the formation of damaging reactive species (Mergenthaler et al., 2004).

Peri-Infarct Spreading Depolarizations

Under conditions of low blood flow, excessive potassium ions and glutamate released in the extracellular space act to continually depolarize cells in the local area (Dirnagl et al., 1999). While cells in the core region of the infarct never repolarize, cells in the peri-infarct region do at the expense of further energy consumption (Dirnagl et al., 1999). As neurons and glia depolarize they release additional potassium ions and glutamate into the extracellular compartment causing neighboring cells to depolarize in consecutive waves. This phenomenon is known as spreading depressions/depolarizations. In experimental models of focal cerebral ischemia, peri-infarct depolarizations have been observed at a frequency of 1-4 events per hour (Busch et al., 1996; Wolf et al., 1997). These cells deplete energy reserves through continual cycles of repolarization and depolarization (Hossmann, 1996). The depletion of energy in metabolically compromised cells increases the potential of cell death and the growth of the lesion core (Iijima et al., 1992; Mies et al., 1993; Back et al., 1994; Hoehn-Berlage et al., 1995).

Inflammation

The inflammatory response to ischemia within the nervous system contributes to tissue loss, however, the detrimental role of specific inflammatory cascades remains unclear. The uncertainty in the role of inflammation during ischemia is highlighted by the fact that experimental blockades or mice knockout models of inflammatory elements may reduce or increase infarct size (Mergenthaler et al., 2004). A complete description of the localization and molecular interactions of the ischemia-induced inflammatory

response is beyond the scope of the present review (see Barone and Feuerstein, 1999; D'Ambrosio et al., 2001; Emsley and Hopkins, 2008; Emsley et al., 2008). Inflammation may alter ischemic damage through hyperthermia, activation of both the hypothalamic-pituitary-adrenal axis and sympathetic nervous system in addition to its classic acute phase response (for review see Emsley et al., 2008). Within the classic response, inflammation may promote cell damage by microvascular failure or the release of toxic molecules (Back, 1998; Jean et al., 1998; del Zoppo et al., 2001).

Ischemic damage promotes the activation and proliferation of cells derived from the mononuclear phagocytic system such as macrophages and microglia. The role of these mononuclear phagocytic cells was thought to be a repair mechanism because of their potential to restore blood flow, repair the blood brain barrier and promote general homoestasis (O'Callaghan, 1991; Norton et al., 1992). However, recent data suggest they may be detrimental to cell survival during ischemia. The activation of intracellular second messenger systems by excessive intracellular calcium levels and the increase in free radicals trigger the initiation of the inflammatory response (Ruscher et al., 1998; Emsley et al., 2008). Activated leukocytes (granulocytes, monocyte/macrophages and lymphocytes) in addition to neurons and glia (astrocytes and microglia) produce key pro-inflammatory molecules such as cytokines and chemokines (Barone and Feuerstein, 1999). Cytokines are polypeptide hormones that may have positive or detrimental effects on tissue survival through known activity in inflammation, immune activation, cell differentiation and apoptosis (Emsley et al., 2008). Chemokines are a sub-family of cytokines that direct migration and entry of inflammatory cells into tissues

thus suggesting a role where they promote the detrimental invasion of inflammatory cells such as leukocytes (Emsley et al., 2008).

Several patterns of inflammatory response are thought to promote cell death. The accumulation of leukocytes, red blood cells, fibrin deposits and platelets can lead to occlusion of microvessels resulting in the “no flow” phenomenon (del Zoppo et al., 1991; del Zoppo, 1997; Winquist and Kerr, 1997; del Zoppo, 1998; Jean et al., 1998). The inflammatory response following ischemia increases permeability of the blood-brain barrier (BBB) by endothelial components causing adhesion of leukocytes and platelets to the vascular endothelium and disruption of the basal lamina (del Zoppo and Hallenbeck, 2000). Interaction between leukocytes and adhesion molecules across the microvessel/BBB interface results in leukocyte transmigration into the brain parenchyma where further inflammatory responses may occur (Mergenthaler et al., 2004). In addition, the secretion of the enzyme inducible NO-synthase increases concentrations of NO that are cytotoxic due to their formation of peroxynitrite (Crow and Beckman, 1995) while secretion of the enzyme cyclooxygenase-2 likely causes tissue damage through the production of free oxygen radicals and prostanoids (del Zoppo et al., 2000; Emsley and Tyrrell, 2002). Collectively, the ischemic inflammatory response should be viewed as complex process with positive and negative effects on tissue survival that involves numerous targets for neuroprotective therapies.

Stroke Treatment

The three common approaches to treating stroke are prevention, neuroprotection and rehabilitation. Primary prevention involves measures that attempt to reduce the incidence of stroke in the population at large, while secondary prevention attempts to limit stroke incidence in patients who have already developed symptoms of TIA or

stroke because of the increased risk of stroke in this subpopulation (Johnston et al., 2000). Stroke prevention involves the identification of risk factors for stroke followed by attempts to limit these factors. Some risk factors are non-modifiable such as increasing age, male sex, nonwhite race, the presence of coronary heart disease or congestive heart failure and a positive family history for stroke or TIA (Sullivan and Katajamaki, 2009). Whether diabetes mellitus is a modifiable risk factor remains subject to debate (Calvin et al., 2009). Effective reductions in the expected incidence of stroke have been associated with adequate blood pressure reduction, treatment of hyperlipidemia, use of antithrombotic therapy in patients with atrial fibrillation and antiplatelet therapy in patients with myocardial infarction in both primary and secondary levels (Sullivan and Katajamaki, 2009). For secondary prevention, carotid endarterectomy in patients with severe carotid artery stenosis also reduces (Straus et al., 2002). Stroke prevention can also reduce stroke by emphasizing lifestyle changes such as avoiding tobacco, increasing physical activity and consuming a healthy diet (Sullivan and Katajamaki, 2009). While effective, stroke prevention's success is limited by the difficulty in identifying risk factors and the capacity to reduce them.

Neuroprotective strategies attempt to limit the severity of stroke by antagonizing the injurious biochemical and molecular events that results in infarction. Hemodynamic agents or techniques used to maintain circulatory patency such as thrombolytics are not typically described as neuroprotective agents although they do comprise an important line of stroke research (Cheng et al., 2004). The therapeutic window for most neuroprotective agents is within the first 4-6 hours following the onset of stroke (Gorelick, 2000). Some of the most studied neuroprotective agents include calcium

channel blockers, glutamate antagonists, GABA agonists, antioxidant/radical scavengers, phosholipid precursors, nitric oxide signaling down regulators, leukocyte inhibitors and hemodilution (Ginsberg, 2009). While some attenuation of tissue loss has been associated with the use of neuroprotective agents in human stroke patients, these strategies are limited by their narrow therapeutic window as well as difficulty in translating from animal models to human stroke patients (O'Collins et al., 2006). Nonetheless, neuroprotective strategies such as the aforementioned list and many others have strong evidence from animal models that they are capable of limiting the severity of tissue loss during stroke (Lipton et al., 2007).

Even with all of the opportunities to prevent and limit disease, healthy care systems worldwide are dominated by the treatment of people with chronic disability (Hoffman et al., 1996). In the context of stroke recovery, despite preventative and neuroprotective strategies, more than 350 000 Americans suffer a stroke and exhibit persistent motor deficits each year (Luke et al., 2004; Urton, et al., 2007). Some motor improvements occur within the first three months following the injury but these improvements are rarely complete and substantially fewer improvements occur beyond the first year of injury (Duncan et al., 2000). Rehabilitation of stroke patients aims to reduce impairments to allow these patients to perform more of the activities and participation in life situations they were capable of prior to stroke (Barak and Duncan, 2006). Unfortunately, current rehabilitative strategies only demonstrate benefit on a general level whereas individual patterns of motor improvements after stroke are highly variable and typically incomplete (Gresham et al., 1995). It is now being appreciated that parameters of rehabilitative motor training such as intensity, duration, timing, and

the nature of the training experience impact its efficacy. Further, measures of brain function after stroke are allowing an understanding of the mechanisms underlying motor improvements and allowing for therapy to be more tailored to individual stroke patients (Milot and Cramer, 2008). The increased knowledge of brain repair is also facilitating the development of adjuvant therapies that have the potential to magnify the benefits of rehabilitation (Floel and Cohen, 2009).

Cortical Plasticity and As A Neural Substrate for Motor Rehabilitation

Improvements after stroke can arise from compensation or recovery at both neural and behavior levels. It is important to distinguish between these terms because they likely represent different neurobiological or behavior processes, each with unique contributions to motor improvements (Kleim, 2009). Historically, the terms recovery and compensation have been used to describe functional improvements in conflicting ways. Levin et al. (2009) recently distinguished the two terms based on the International Classification of Function (ICF) framework proposed by the World Health organization. The ICF model recognizes post injury impairments in three levels: the pathophysiology of the body, the loss of body output (impairment) and the loss of task performance (disability) (Kleim, 2009). Neural recovery describes the return of function to nervous tissue whereas neural compensation is when residual tissue adopts function that was lost by injury (Levin et al., 2009). Behavioral recovery can involve the return of premorbid movement or premorbid task performance whereas behavioral compensation describes performing movement or activities in a different manner than the behavior prior to injury (Kleim, 2009). The closest version of neural recovery after permanent cell loss would be cell replacement therapy through the addition of exogenous precursor cells or the guided targeting of cells from endogenous adult stem cells to the injury

(Lindvall and Kokaia, 2004). Neural recovery can also occur by dysfunctional cells regaining their normal function. Stroke injury can disrupt spared tissue by many of the same ways that it causes cell death such as deinnervation, disrupted blood flow, ionic imbalances and inflammation (Nguyen and Botez, 1998). Many of these disruptions can be alleviated over time resulting in the restoration of neural function. Although the specific neurobiological mechanisms contributing to improvements are not fully understood, plasticity within residual motor brain areas supporting neural compensation is believed to be the primary factor underlying recovery. Noninvasive brain imaging studies have identified ipsilesional reorganization of primary motor and sensory areas in patients with good behavioral outcomes after stroke (Rossini et al., 1998; Cramer and Bastings, 2000; Cramer and Crafton, 2006). Motor mapping using TMS has found that motor improvements after stroke are associated with increases in the ipsilesional motor map area and motor-evoked potential (MEP) amplitudes (Wittenberg et al., 2003). In animal models, motor improvements after experimental ischemia are often associated with reorganization of movement representations within motor cortex (Nudo et al., 1996a; Kleim et al., 2003; MacDonald et al., 2007). The reorganization patterns that parallel motor improvements following ischemic injury are highly similar to the patterns observed in healthy animals (Kleim and Jones, 2008) and people (Pascual-Leone et al., 1995) during skilled motor training. This relationship suggests that the cortex has the capacity to undergo a relearning process following injury that follows principles observed during skilled motor learning.

Motor Map Plasticity and Motor Learning in the Intact CNS

The motor cortex's somatopic organization was described more than a one hundred years ago by crude stimulation of the canine precentral gyrus (Fritsch and

Hitzig, 1870; Jackson, 1874). More refined stimulation techniques such as intracortical microstimulation have more recently been used to accurately and reliably define the functional organization of the rat motor cortex (Neafsey et al., 1986; Kleim et al., 1998). This technique allows for the construction of a "map" of the topography of forelimb movement representations within the rat motor cortex. Using standard ICMS techniques (Kleim et al., 1998), the rat is anesthetized and a craniotomy is performed over the motor cortex contralateral to the movements of interest such as forelimb extension/flexion. Following this preparation, a microelectrode controlled by a hydraulic microdrive is positioned at various locations on the cortex and then lowered into cortical layer V. A small amount of current is then passed through layer V, which stimulates pools of pyramidal cells. The resulting movement pattern can then be recorded. After numerous microelectrode penetrations are made, the final result is a "motor map" of the functional representations within the motor cortex. Studies in rodents using ICMS have provided a detailed description of the rodent motor map as a fractured somatotopic mosaic, i.e., individual movements are represented multiple times and are interspersed with adjacent movement representations across separate regions of cortex (Stoney et al., 1968; reviewed in Schieber, 2001).

Motor cortex has been shown to reorganize in response to skilled motor training in healthy individuals. In non-human primates, training on a task that requires skilled digit manipulation caused an expansion of digit representations and this expansion is lost by the cessation of training or training on a skilled wrist movement task (Nudo et al., 1996a). The subsequent training on a skilled wrist task resulted in an expansion of wrist representations where digit representations previously occupied (Nudo et al., 1996a).

The motor cortex of non-human primates does not functionally reorganize when the task does not require difficult reaching movements indicating the nature of training experience is an important factor (Plautz et al., 2000). In rodents, non-skilled training such as pressing a lever (Kleim et al., 1998) or reaching for unattainable pellets (Kleim et al., 2004) does not induce map reorganization. Exercise training with running wheels does not alter motor map topography (Kleim et al., 2002b; Maldonado et al., 2008) but does induce angiogenesis and increased blood flow to motor cortex (Swain et al., 2003). Strength training does not result in map reorganization but induces synaptogenesis in the spinal cord (Remple et al., 2001). In contrast, skilled training in rodents induces functional reorganization of the motor map such that movements biased by the training subsequently increase their number of responsive sites in motor cortex (Kleim et al., 1998; Kleim et al., 2002a; Conner et al., 2003; Kleim et al., 2004). The positive shift in the proportion of “trained” sites occurs after substantial changes have occurred in reaching performance. In rodents, skilled reaching behavior is significantly improved as early as the third day of training whereas robust map reorganization is not observed until the tenth day of training (Kleim et al., 2004). The training specificity observed with motor map plasticity has also been observed by measures of dendritic hypertrophy. Training on object recognition (delayed nonmatch to sample) or visuomotor tasks does not induce dendritic plasticity within motor cortex (Kolb et al., 2008). In contrast, unilateral reach training increases dendritic length and branching in motor cortex contralateral to the training and this dendritic plasticity is observed in both motor cortices following bilateral reach training (Kolb et al., 2008). Similarly, only complex walking tasks such as acrobatic training and walking up and

down a runway increase synapse numbers within motor cortex (Black et al., 1990; Kleim et al., 1998). Finally, skilled reach training is also associated with dendritic growth (Greenough et al., 1985; Withers and Greenough, 1989; Bury and Jones, 2002; Allred and Jones, 2004), synaptogenesis (Kleim et al., 2002a; Kleim et al, 2004) and enhanced synaptic responses (Rioult-Pedotti et al., 1998; Monfils and Teskey, 2004; Hodgson et al., 2005) within motor cortex.

The invasiveness of microstimulation techniques has prevented its use for extensive study of human motor cortex, however, organized somotatopy has been identified in humans during medical procedures (Penfield and Boldrey, 1937; Woolsey et al., 1952). Reorganization of human maps has also been observed in response to skilled training or in individuals with highly skilled talents with imaging or mapping techniques using noninvasive transcranial magnetic stimulation (TMS). For example, skilled racquetball players have larger hand representations than less skilled players and naive individuals (Pearce et al., 2000). The reading hand of proficient Braille readers (Pascual-Leone et al., 1993) or five days of unilateral digit training using a piano results are associated with increased digit representations contralateral to the skilled behavior that are not observed in the ipsilateral hemisphere or in the contralateral hemisphere of control subjects (Pascual-Leone et al., 1995). Cessation of the piano training resulted in the maps shrinking back to baseline sizes (Pascual-Leone et al., 1995). Remarkably, both skilled tongue protrusion training (Svensson et al., 2003) and skilled ankle training (Perez et al., 2004) are associated with increases in their respective representations within motor cortex. Imaging studies support the relationship between cortical plasticity and skilled motor learning. For example, amateur keyboard

players exhibit a greater amount of gray volume within motor cortex compared to non-players and expert keyboard players exhibit a higher amount than either group (Gaser and Schlaug, 2003). Imaging studies have demonstrated a progression of brain activation during the stages of learning. Early motor learning appears to involve a greater activation of premotor (Grafton et al, 1992; Ghilardi et al., 2000) and cerebellar (Eliassen et al., 2001; Penhune and Doyon, 2002) cortices whereas late stages are marked with increased activation of primary motor cortex (Karni et al, 1995; Floyer-Lea and Mathews, 2005).

It is becoming increasingly clear that the brain is endowed with an exceptional capacity to reorganize itself in response to perturbation. This point is demonstrated by a study where healthy individuals were imaged using fMRI while engaging in a hand opening/closing task. Compared to a resting state, performing the task was associated with activation of contralateral motor cortex and rostral supplementary motor cortex (Walsh and Pascual-Leone 2003). Increasing activity in the contralateral motor cortex by prior application of high frequency repetitive TMS resulted in a decrease in activation of rostral SMA, i.e., more exclusive activation within motor cortex (Walsh and Pascual-Leone 2003). Interestingly, prior application of slow repetitive TMS to suppress neuronal activity in the motor cortex contralateral to the hand task resulted in increased activation of rostral SMA and motor cortex ipsilateral to the task with no decrement in behavioral performance (Walsh and Pascual-Leone 2003). Maintenance of task performance following a similar low frequency TMS procedure in healthy individuals has also been associated with an a increase in movement-related PET activity in the premotor cortex contralateral to the stimulated hemisphere (Lee et al., 2003).

Motor Cortex Plasticity and Improvement of Motor Function Following Stroke

Deficits after stroke are as much a manifestation of the loss of brain regions as they are the limit to which remaining brain structures can compensate for the lost functions (Cramer, 2004; Takahashi et al., 2004). At least some of the abnormal patterns of brain activity observed during movement tasks after stroke are the result of spared circuits attempting to establish alternate forms of intracortical, subcortical and descending projections. If stroke results in damage/disruption to motor cortex, these parallel circuits may originate from remaining active portions of this region, contralesional motor cortex, or bilaterally from secondary motor area such as SMA, PMA, somatosensory areas, cerebellum, basal ganglia, etc. It is likely cortico-cortical and cortico-subcortico-cortical circuits will continue to shift interactions across the network in an attempt to establish suitable brain activation that will produce the desired behavioral result so long as some efferent cortico-spinal output pathways exist. It is likely initial network changes after stroke aim at minimizing damage and rapid improvements in behavior arise from the amelioration of dysfunction in spared tissue or the repair of partially damaged structures (Pascual-Leone et al., 2005). The process of reorganizing residual circuits begins as the damage and these immediate changes have stabilized (Pascual-Leone et al., 2005).

Early after cortical stroke patients typically exhibit bilateral increases in fMRI activation patterns in primary and non-primary motor areas that steadily reduce with time and the timing of these reductions correlate with motor improvements (Ward et al., 2003). Acute stroke damage is associated with increased excitability in the unaffected hemisphere presumably resulting from its increased use as well as reduced intracortical inhibition and increased intracortical facilitation (Liepert et al., 2000). Transcallosal

inhibition from the unaffected to affected hemisphere during voluntary movement is also abnormal (Murase et al., 2004). Several months following stroke a normalization of the over-activity in the unaffected hemisphere is observed (Shimizu et al., 2002). Long-term poor functional outcome is associated with larger areas of cortical activation in cortical networks spanning both the intact and damaged hemisphere (Carey et al., 2002; Rossini and Dal Forno, 2004). Tombari et al. (2004) observed a progressive shift from contralesional activity in motor and somatosensory cortices to the ipsilesional hemisphere by sampling twenty days, four months and one year after stroke onset. There is a negative correlation between amount of cortical activation during movement and transcranial magnetic stimulation-derived MEPs (Ward, 2006). These MEPs are important because acute stroke patients with MEPs show better functional outcome than those without presumably because of greater CST integrity (Rapisarda et al., 1996; Escudero et al., 1998).

Subjects with the best motor outcomes typically exhibit recruitment of ipsilesional sensory and motor cortices that is highly similar to that of controls (Ward et al., 2003; Zemke et al., 2003; Cramer and Crafton, 2006). Strong activation of secondary motor areas such as PMd in the affected hemisphere was more prominent in patients with greater disability (Johansen-Berg et al., 2002; Ward et al., 2003). Chronic stroke patients with good behavioral outcomes demonstrate expanded and shifted sensorimotor fMRI activation patterns in the ipsilesional hemisphere (Cramer et al., 2000; Zemke et al., 2003). Greater motor improvements are associated with an enlargement in ipsilesional motor areas or a poster shift at the boundary between motor and sensory cortices (Rossini et al., 1998; Cramer and Bastings, 2000). Stroke damage

within the precentral gyrus is associated with a reorganization of finger and face fMRI activations after good behavioral outcome (Cramer and Crafton, 2006). Improvements in upper extremity motor performance after constraint-induced movement therapy are associated with increases in ipsilesional motor map area and MEP amplitudes (Wittenberg et al., 2003). Changes in map area and excitability are accompanied by shifts in map center of gravity indicating recruitment/reorganization of motor cortex adjacent to the original location (Liepert et al., 1998). In aphasics, greater return of language function is associated with a higher degree of left perilesional reorganization whereas contralesional reorganization was accompanied by poor language performance (Karbe et al., 1998; Cao et al., 1999; Rosen et al., 2000).

Animal models of stroke have been developed to investigate the basis for motor cortex reorganization. Focal ischemic infarcts within localized regions of squirrel monkey motor cortex result in a widespread reduction in the size of representations adjacent to the lesion (Nudo and Milliken, 1996). In primates, motor retraining of the impaired limb restores the representations adjacent to the infarct and induces an expansion into regions that formerly displayed other representations (Nudo et al., 1996a). Primates not given motor training do not show the same gains in motor function or map reorganization (Nudo et al., 1996b). Similarly, ischemic damage within the forelimb area of rodent motor cortex cause a loss of movement representations outside of the lesioned area within twenty-four hours of insult that can be restored with several weeks of rehabilitative training (Kleim et al., 2003; Monfils et al., 2005; MacDonald et al., 2007; Kleim and Jones, 2008). Map restoration is dependent upon skilled rehabilitation and does not occur in animals that experience unskilled rehabilitation

involving extensive repetition of limb movement without acquisition of skill (Kleim et al., 2003; Kleim et al., 2004).

Synaptic Plasticity Mediates Motor Map Reorganization

While the exact contribution of motor maps to movement in unclear, they appear to be surrogate markers for the capacity to produce and acquire skilled movement (Monfils et al., 2005). TMS studies in children between 2 years of age and adolescence have observed a progressive decrease in the stimulation threshold needed to produce MEPs (Eyre et al., 2001). Another study observed a decrease in TMS conduction times that was associated with the maturity of hand dexterity (Fietzek et al., 2000). Motor maps are typically absent in cats at postnatal day 45 but develop as the animals enter adulthood (Chakrabarty and Martin 2000). Armand and Kably (1993) observed that early maps contain proximal representations and then develop distal representations as well as progress from simple evoked responses to more complex responses involving multi-joint movements that co-associate with the emergence of complex forelimb movements. The stimulation used in ICMS indirectly measures the synaptic connectivity and biasing of local cortical networks because its low levels of electrical current require the recruitment of many intracortical synapses onto corticospinal neurons. Understanding the cellular basis of reorganization will aid in the development of adjuvant therapies to enhance recovery.

Stimulation experiments have revealed two important characteristics of motor cortex organization. First, the area of cortex devoted to a particular movement is related to the dexterity of the movement. Second, the proportion of the cortex devoted to any one movement is not static and can be changed in response to a variety of manipulations including motor training and/or damage. Neurons within motor cortex are

aggregated such that small groups of cells appear to encode elementary movement representations in that neighboring cells have similar output properties (Mountcastle, 1997). Further, these pools of neurons are interconnected by dense horizontal connections that can extend several millimeters (Ghosh and Porter, 1988; Keller, 1993). Intracortical microstimulation (ICMS) evokes movement via direct (Stoney et al., 1968) and indirect (Jankowska et al., 1975) activation of pyramidal tract neurons. In fact, the majority of pyramidal tract neurons that drive movement in response to stimulation are trans-synaptically activated (Cheney and Fetz, 1985; Lemon et al., 1987), presumably through activation of horizontal afferents. Any alteration in motor map topography or loss of map area after damage must therefore involve changes in the pattern of intracortical connectivity through modifications in synaptic efficacy (Hess and Donoghue, 1994; Monfils et al., 2005). In support, synaptic potentiation that occurs in response to motor skill learning (Rioult-Pedotti et al., 1998; Monfils and Teskey, 2004) is colocalized within regions of cortex that exhibit motor map reorganization (Kleim et al., 1998). In addition, learning-dependent motor map plasticity is also co-localized with synaptogenesis (Kleim et al., 2002a; Kleim et al., 2004).

Electric Stimulation of Motor Cortex Induces Synaptic Plasticity and Motor Map Reorganization

Excitability in the central nervous system can also be altered by electrical stimulation (Blundon and Zakharenko, 2008). Indeed, electrical stimulation can induce a phenomenon known as long-term potentiation (LTP). LTP is one of the cardinal examples of plasticity and fulfils many of the criteria for a neural correlate of memory. The LTP phenomenon is a facilitation of chemical transmission between neurons that results from coincident activity of pre- and post-synaptic cells. LTP experiments typically

use high-frequency trains of electrical stimulation in pre-synaptic cells that promote action potentials post-synaptically (Cooke and Bliss, 2006). Following stimulation, enhanced postsynaptic responses are observed for hours in vitro (Bliss and Gardner-Medwin, 1973). More recently, it was demonstrated that LTP-like phenomena could be induced in vivo by repeated electrical conditioning sessions (Racine, et al., 1995) or even one session (Trepel and Racine, 1998) over several days. Decreases in postsynaptic responses, termed long term depression (LTD) can be induced by electrical conditioning using low frequency stimulation in vitro (Dudek and Bear, 1992) and in vivo (Froc et al., 2000). Manipulations that induce changes in synaptic strength also induce map reorganization. For instance, cortical kindling that drives increases in cortical excitatory postsynaptic potentials also increases motor map area (Teskey et al., 2002). Stimulation protocols that elicit LTP causes map expansion and synaptogenesis (Monfils et al., 2004) while protocols for long-term depression (LTD) induces map retraction and synaptic loss (Teskey et al., 2007). Jackson et al. (2006) used a neural implant to demonstrate that repeated conditioning with a closed loop electronic device can alter the output of motor cortex by creating stable connections between previously unconnected regions. The similarity in sensorimotor cortices' response to both electrical stimulation and behavioral experience suggest electrical stimulation can be used to facilitate the encoding of information within this tissue.

Electrical Stimulation of Motor Cortex Enhance Motor Improvement After Stroke

There is a growing body of evidence that electrically stimulating the motor cortex facilitates recovery of motor function after injury to the central nervous system (CNS). Stroke generally reduces activity in the ipsilesional hemisphere (Desrosiers et al., 2006) while increasing activity in the contralesional hemisphere (Cauraugh et al., 2000). The

strategy of many noninvasive brain stimulation therapies is to encourage activity in the ipsilesional hemisphere or discourage it in the contralesional hemisphere (Talelli and Rothwell, 2006). Transcranial magnetic brain stimulation (TMS) works by passing a strong brief electrical current through an insulated wire coil such that it generates a magnetic field that results in a secondary current within the brain (Bolognini et al., 2009). This secondary current is capable of transiently altering the membrane potential of neurons to suppress or activate cortical regions depending on the shape of the coil as well as the frequency and duration of stimulation (Pascual-Leone et al., 2002). Transcranial Direct Current Stimulation (tDCS) causes weak polarizing direct current into cortex via an active electrode placed over the target area and a reference electrode placed in the contralateral supraorbital area or a non-cephalic region (Nitsche et al., 2007). Stimulation by tDCS results in sustained changes in neuronal membrane potentials with cathodal tDCS inducing hyperpolarization (inhibition) and anodal tDCS inducing depolarization (excitation) (Nitsche et al., 2003). Low frequency rTMS applied to the contralesional hemisphere in chronic stroke patients reduces transcallosal inhibition from this hemisphere to the ipsilesional (Takeuchi et al., 2005) and increases excitability in the affected side (Fregni et al., 2006). Intermittent theta burst stimulation with rTMS increases MEP amplitudes and transiently improves behavior (Talelli et al., 2007; Di Lazzaro et al., 2008). Continuous theta burst stimulation over the contralesional hemisphere increased excitability in the affect motor cortex and resulted in motor improvements (Di Lazzaro et al., 2008). High frequency rTMS given ipsilesionally and paired with passive and active motor therapy caused greater motor improvements on a variety of scales compared to an rTMS sham group (Khedr et al.,

2005). High frequency rTMS delivered ipsilesionally and paired with a finger motor task resulted in greater movement accuracy, less movement time and greater MEP amplitudes (Kim et al., 2006). Low-frequency rTMS delivered in the contralesional hemisphere, resulted in improved acceleration and force of the affected hand (Takeuchi et al., 2008). Inhibiting the contralesional hemisphere with Low-frequency rTMS also results in other motor improvements (Fregni et al., 2006, Mansur et al., 2005). Transcranial direct cortical stimulation improves motor function in patients with chronic motor impairments when anodal current is delivered over lesioned motor cortex (Hummel and Cohen, 2005; Fregni et al., 2006). Similarly, cathodal transcranial direct current stimulation applied to contralesional motor cortex induces behavioral improvements on various scales (Fregni et al., 2005; Fregni and Pascual-Leone, 2006).

Clinical reports suggest that epidural electric stimulation of motor cortex, originally used to reduce chronic pain after sub-cortical strokes, reduces hemiparetic impairments (Tsubokawa et al., 1993), motor weakness (Katayama et al., 2002), motor spasticity (Garcia-Larrea et al., 1999), action tremor (Nguyen et al., 1998) and dystonia (Franzini et al., 2003). The efficacy of CS/RT at enhancing motor recovery after stroke has been demonstrated in rats (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Teskey et al., 2003; Adkins et al., 2006) and monkeys (Plautz et al., 2003). Furthermore, the enhanced motor recovery is associated with increased cortical dendritic hypertrophy (Adkins-Muir and Jones, 2003) and synaptogenesis (Adkins et al., 2008) in comparison to animals in standard rehabilitation. The increased post-synaptic space is accompanied by an enlargement of the polysynaptic component of motor cortical evoked potentials (Teskey et al., 2003). Finally, CS/RT also induces a greater expansion of movement

representations in rats (Kleim et al., 2003; Boychuk et al., 2009) and monkeys (Plautz et al., 2003). All of these data demonstrate that CS/RT drives significantly greater motor recovery after stroke and that the functional gains are accompanied by an upregulation of the neuroplastic changes observed with standard rehabilitation.

Translation of CS/RT into Clinical Practice

Recently a phase III clinical trial of CS/RT failed to demonstrate a significant effect of the treatment. The trial enrolled 146 patients (CS/RT=91; RT=55) and the methods were the same as the phase II clinical trial where a significant CS/RT treatment effect was demonstrated (Huang et al., 2008; Levy et al., 2008). It is likely our lack of understanding of how to translate CS/RT from preclinical basic studies to human applications contributed to the lack of CS/RT effect observed in the phase III trial (Plow et al., 2009). Interestingly, some patients in the combined CS/RT group demonstrated robust motor improvements relative to the group receiving RT alone (Plow et al., 2009). One interpretation of this finding is that CS/RT procedure used was better suited for treating a subset of the stroke patients. Identifying factors that can account for the differences between animal and human studies of CS/RT should support more effective CS/RT therapies in the future as well as contribute to our understanding of brain repair following stroke. In the present work rodent models of stroke were used in an attempt to identify several of the important factors that impact the efficacy of CS/RT therapy.

One important factor with CS/RT is the placement of stimulation on the cortex. This placement is dictated by the configuration of the CS/RT electrodes' surface contacts. Animal studies of CS/RT have used stimulation that is distributed across the motor cortex. In contrast, human studies have used very focal stimulation. Further, the focal stimulation in the clinical studies was localized to the largest fMRI activation zone

during wrist/hand/finger movement (Plow et al., 2009). In the present work a study was conducted that examined the effects of focal versus distributed cortical stimulation on both motor performance and motor map plasticity. ICMS was used to identify movement representations at the beginning and end of the study. In this study, the focal configuration applied stimulation to the largest set of wrist representations while the distributed configuration applied stimulation to non-wrist representations surrounding the large area of wrist representations located in the center of the motor map. In a second study, distributed forms of CS/RT were tested to examine the importance of the number of independent stimulation sites, i.e., to compare the importance of the density of stimulation sites within a distributed configuration.

Another important difference between animal and human studies of CS/RT is the amount of post injury motor training prior to the onset of CS/RT treatment. Human stroke patients have a minimum delay of four months between onset of stroke and enrollment into the clinical trial (Levy et al., 2008). While the amount of rehabilitative training given to these stroke patients is not reported it is expected that the majority received standard rehabilitative treatment in the months following stroke. In contrast, all of the documented animals studies of CS/RT have initiated the CS/RT-RT comparison without any preceding post injury motor training. This discrepancy raises the issue of whether CS/RT is limited by prior rehabilitative experience. It is possible that early rehabilitative training results in persistent forms of neural plasticity that cannot be altered or hinder changes by subsequent CS/RT application. It is also possible early rehabilitative training produces a ceiling effect in the amount of motor improvements that can be induced by subsequent CS/RT. Presently, a third study used a comparison

between animals receiving CS/RT and RT alone was made after the animals had received post injury motor training to identify how the behavioral effects of CS/RT are affected by weeks of prior rehabilitative training.

The subjects in animals and human studies of CS/RT also differ in the location of ischemic damage. Previous animal studies of CS/RT have exclusively modeled stroke by focal damage to motor cortex. In contrast, human stroke patients with either cortical or capsular stroke damage were enrolled in CS/RT clinical trials (Brown et al., 2006; Huang et al., 2008; Levy et al., 2008). While it is clear that both cortical and capsular stroke often result in motor impairments (Shelton and Reding, 2001; Schiemanck et al., 2008) the types of neural reorganization that are relevant to improving motor function may be different (Plow et al., 2009). It is unclear whether CS/RT can enhance motor improvements after capsular ischemic damage by the same mechanisms as those suggested following ischemic damage to motor cortex. In particular, it is not presently known whether CS/RT's enhanced reorganization of perilesional motor cortex can support greater motor improvements following ischemic damage to the internal capsule. Presently, a fourth study was performed to examine the behavioral effects of CS/RT following ischemic damage to either cortex or to the internal capsule in order to examine the efficacy of CS/RT after capsular stroke.

Thesis Outline

The goal of this dissertation is to further characterize the effectiveness of brain stimulation in enhancing motor improvements during motor rehabilitation after stroke injury. Specifically it examines how changes in the distribution of stimulation across the cortex, the effects of previous training experience and importance of locus of injury affect CS/RT. In the present experiments several rodent models are used to study the

return of motor functions after stroke. Behavioral changes in these models are assessed by performance on a skilled food-reaching task, and when possible, the physiology of the motor cortex is assessed using ICMS. Three primary questions will be addressed: First, does the configuration of the contacts on the CS/RT electrode affect its ability to drive motor improvements? Second, does prior rehabilitative experience affect CS/RT's ability to drive motor improvements? Third, will CS/RT enhance motor improvements when the ischemic injury is located in the subcortical white matter? Collectively, the configuration of CS/RT contacts, amount of prior rehabilitative experience and location of the lesion represent important factors that need to be studied in order to facilitate the translation of CS/RT from animals studies to clinical practice with human stroke patients. The data from these studies will provide information regarding the situations that CS/RT will be most effective as well as contribute to the understanding of brain repair following stroke.

CHAPTER 2
DISTRIBUTED VERSUS FOCAL CORTICAL STIMULATION TO ENHANCE MOTOR FUNCTION AND MOTOR MAP PLASTICITY AFTER EXPERIMENTAL ISCHEMIA

Introduction

Stroke remains the major source of adult disability and much of the reduction in quality of life can be attributed to motor impairments. Of the 560 000 Americans that survive stroke each year, more than 80% will have acute motor impairments and most will receive some form of motor rehabilitation (Gresham et al., 1995; Rathore et al., 2002). However, the effectiveness of motor rehabilitation is highly variable (Duncan et al., 2000) and reflects our lack of understanding of the neurobiological mechanisms underlying functional recovery. Improvements in motor function after stroke can be thought of as a relearning process whereby lost motor functions are reestablished through functional restoration and/or compensation within spared brain regions (Cramer et al., 1997; Cramer and Bastings, 2000; Nudo, 2007). Animal models of stroke show that focal ischemic damage to motor cortex results in a loss of microstimulation-evoked motor representations (Nudo and Milliken, 1996) and a decrease in synapses (Hasbani et al., 2001; Zhang et al., 2005; Murphy et al., 2008) within residual cortical areas. In the absence of rehabilitative training, the majority of lost motor representations do not reappear and the topography of the remaining map is not significantly altered (Nudo and Milliken 1996; Friel et al., 2000). With motor rehabilitation, however, improvements in motor performance are accompanied by motor map expansion and reorganization (Nudo et al., 1996b; Nudo, 2006) that is likely mediated by synaptic plasticity (Kleim et a., 2002a; Kleim et al., 2004). Thus, adjuvant therapies that promote cortical/synaptic plasticity may enhance improvements in motor performance after stroke. Indeed, several plasticity-promoting agents have been paired with motor rehabilitation to

enhance motor function after stroke. Improved motor performance has been demonstrated with pharmacological manipulations such as amphetamine and nicotine that are associated with synaptic reorganization (Stroemer et al., 1998; Adkins and Jones, 2005; Ramic et al., 2006; Papadopoulos et al., 2009). Additionally, enhancing intracellular signaling pathways known to promote synaptic plasticity augments skilled reaching ability in a rodent model of stroke that is associated with expansion of forelimb movement representations in motor cortex (Macdonald et al., 2007).

In addition to pharmacological agents, electrical stimulation has been used to treat motor impairments associated with various neurological disorders. Initial clinical studies found that epidural motor cortex stimulation, intended to reduce chronic pain after sub-cortical strokes, reduced hemiparetic impairments (Tsubokawa et al., 1993), motor weakness (Katayama et al., 2002), motor spasticity (García -Larrea et al., 1999), action tremor (Nguyen et al., 1998), and dystonia (Franzini et al., 2003). Subsequent animal models of cortical stimulation combined with rehabilitation therapy after stroke demonstrate enhanced motor outcomes relative to rehabilitative training alone (Adkins-Muir and Jones, 2003; Adkins et al., 2006). The improved motor performance is also associated with an expansion of microstimulation-evoked motor representations within residual cortical areas of rodents (Kleim et al., 2003) and primates (Plautz et al., 2003) that is accompanied by increased synapse density (Adkins et al., 2008) and enhanced synaptic potentials (Teskey et al., 2003). Together these results demonstrate the viability of CS/RT for enhancing motor improvement and concomitant cortical plasticity after stroke. However, results from a phase three clinical trial of CS/RT in stroke patients failed to show any significant effect of the treatment. This may in part be due to

our lack of understanding as to how CS/RT should be translated from animal to human applications (Plow et al., 2009). One key parameter in CS/RT is the distribution of stimulation across the cortex. Clinically, CS/RT is delivered to a focal region of motor cortex whereas animal studies have used much more distributed stimulation. This difference may account for the efficacy of the treatment in animal studies but lack thereof in human studies. Here we examine the effects of focal versus distributed cortical stimulation on both motor performance and motor map plasticity in an animal model of cerebral ischemia.

Methods

Subjects

Forty-five adult male Long-Evans hooded rats (350-420g) were housed (1 animal/cage) in standard laboratory cages. Animals were kept on a 12:12 hour light dark cycle throughout the experiment. All experimentation was conducted during the light cycle. Rats were maintained on Lab Diet 5001 (PMI Feeds, St. Louis, MO) and water ad libitum, and were handled and cared for in accordance with the National Institutes Health Guide for the Care and Use of Laboratory Animals and with the approval of the University of Florida's Institutional Animal Care and Use Committee (IACUC).

Reach Training

Over the course of several days, all animals were placed on a restricted diet until they measured 90% of their original body weight. A brief period of pretraining was then given to familiarize the rats with the reaching task. Pretraining involved placing them into test cages (10 X 18 X 10 cm) with floors constructed of 2 mm bars, 9 mm apart edge to edge. A 4 cm wide and 5 cm deep tray filled with food pellets (45 mg; Bioserv) was mounted on the front of the cage. The rats were required to reach outside the cage

and retrieve pellets from the tray. Rats were permitted to use either limb and the preferred limb was noted for each animal. All rats remained in pretraining until they had successfully retrieved 10 pellets (approximately 1 hour/day for 2 days). After pretraining, the rats were placed into a Plexiglas cage (11 cm X 40 cm X 40 cm) with a 1 cm slot located at the front of the cage. Animals were trained for 20 minutes each day to reach with their preferred limb through the slot and retrieve food pellets from a table outside the cage (Whishaw and Pellis, 1990). Each session was videotaped and later used to assess reaching performance. A successful reach was scored when the animal grasped the food pellet, brought it into the cage and to its mouth without dropping the pellet. The percentage of successful reaches [(# successful retrievals/the total # of reaches) x 100] was then calculated. All training sessions were video taped and used to measure reaching accuracy. Animals were trained for approximately two weeks on this task to establish a baseline measure of motor performance. Baseline was defined as the average accuracy across the 3 final days of training. Animals failing to achieve a mean reaching accuracy of 40% across 3 consecutive days were not used in the study. Animals were sorted by their prelesion reaching performance to create 5 groups with comparable baseline levels of reaching accuracy: nontrained (NT) (n=11), E1 configuration cortical stimulation with rehabilitative training (E1-CS/RT) (n=10), E2 configuration cortical stimulation with rehabilitative training (E2-CS/RT) (n=9), E3 configuration cortical stimulation with rehabilitative training (E3-CS/RT) (n=7), and rehabilitative training alone (RT) (n=8). All conditions were subjected to the same motor mapping and infarction procedures described below.

Electrophysiological Mapping

Within two days of baseline training, standard ICMS techniques were used to generate detailed maps of forelimb regions of the motor cortex contralateral to the trained forelimb (Kleim et al., 1998; Remple et al., 2001). Prior to surgery animals were anesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.p.). Animals received low levels of isofluorane (0.15%) and supplemental doses of ketamine (20 mg/kg i.p.) as needed. Under sterile conditions, a craniotomy was performed over the motor cortex contralateral to the trained paw of each animal. To prevent edema, a small puncture was made in the cisterna magna prior to removing the skull and dura. The exposed cortex was then covered in warm saline (37 degrees celsius). A digital image of the cortical surface was taken and a 375 mm grid was superimposed onto the image. A glass microelectrode (controlled by a hydraulic microdrive) was used to make systematic penetrations across the cortex using the cortical surface image and grid as a guide. At each penetration site, the electrode was lowered to approximately 1550 mm (corresponding to cortical layer V). Stimulation consisted of thirteen, 200 ms cathodal pulses delivered at 350 Hz from an electrically isolated stimulation circuit. Animals were maintained in a prone position with the limb consistently supported. Sites where no movement was detected at ≤ 60 mA were recorded as unresponsive. Forelimb movements were classified as either distal (wrist/digit) or proximal (elbow/shoulder) and representational maps were generated from the pattern of electrode penetrations. The caudal forelimb area (CFA) was defined by a medial boundary of vibrissa representations, a lateral and caudal boundary of non-responsive sites and a rostral boundary of head and neck representations (Kleim et al., 1998; Remple et al., 2001;

Kleim et al., 2002a). An image analysis program (CANVAS v. 3.5) was used to calculate the areal extent of the CFA.

Focal Infarction

Following ICMS, focal ischemic infarcts were created within the CFA of motor cortex via bipolar electrocauterization of the surface vasculature (Figure 2-1) (Nudo et al., 1996b; Kleim et al., 2003). The infarct targeted primarily the distal forelimb representations but in some cases included small regions of proximal representations. The coagulated vessels included fine arterial and venous capillaries as well as larger vessels but specifically avoided any bypassing arteries supplying other cortical areas. Coagulation was continued until all vessels within the targeted area were no longer visible and the tissue appeared white.

Cortical Electrode Implantation

Directly after the infarction, each animal was implanted with one of three types of electrodes that varied by the configuration of their surface contacts. Nine-pin electrode carriages (Plastics One Inc., Roanoke, VA) were implanted epidurally over top sensorimotor cortex in the hemisphere opposite each animal's preferred paw. The surface electrode was placed directly over the entire exposed cortex between 1 mm posterior to 5 mm anterior to bregma and 0.5 mm to 5.5 mm lateral to midline. The surface electrode was placed directly over the entire exposed cortex including the devascularized region, and remaining forelimb representation area of motor cortex. A return lead was fixed to the skull in a position posterior to Lambda and the craniotomy filled with gel foam. Both the electrode and gel foam were covered in non-exothermic PolyWave dental acrylic and cured with a brief pulse (50 seconds) of ultraviolet light. The electrode was then fixed to skull screws with standard dental acrylic and the

animals were given 4cc of warm ringers solution (s.c.) and metacam (0.10 mg/kg; s.c.). The electrode contacts were all 0.60 mm in diameter and placed in one of three configurations. E1 electrodes had four contacts positioned 2 mm equidistant from one another. E2 had the same four contacts but clustered in the center of the pedestal and E3 electrodes included a single contact in the center (Figure 2-2).

Determining Motor Thresholds

After surgery all animals were returned to their home cage for ten days where they were supervised for health concerns but otherwise left to recover. Animals were given full access to food until the last day where food restriction regimen (see above) was reimplemented. After the ten days, animals with implants had their individual motor thresholds (MT's) determined and then all animals (except those in the NT condition) were started in a motor rehabilitation paradigm. MT's were defined for each animal prior to training on each day as the minimum current to cause an involuntary motor response. Cortical stimulation was administered with a preclinical stimulation system (Northstar Neuroscience, Seattle WA). Briefly, each cortical electrode was connected to a remote stimulator suspended above the training cage where information was sent wirelessly to a base-station connected to a Windows-based personal computer (Dell, Roundrock TX). Current was delivered through the remote stimulator and the parameters were controlled by application software installed on the computer. Stimulation for these thresholds consisted of 3 second trains of 1 millisecond 50 Hz monopolar cathodal pulses. Current was gradually increased by 5% increments until a movement of the contralateral forelimb could be clearly detected. MT's were tested in all animals in all three CS/RT conditions. Movements could only be evoked for animals in the E1 (n =10) and E2 (n=9) conditions. Animals in the E1 showed large-scale movement of the head,

neck, forelimb and hindlimb in response to stimulation. Animals in the E2 condition showed very focal forelimb movements. This finding is consistent with the location of the electrode contacts in each of these conditions because the E1 electrode contacts were spaced throughout the motor map while the E2 contacts were localized to forelimb representations. No movements could be elicited in the E3 (n =7) animals at the maximum current (12 mA) output level. As a result, the animals from E3 remained in the study combined with the RT controls.

Cortical Stimulation and Rehabilitation Training (CS/RT)

The motor rehabilitation paradigm consisted of daily fifteen-minute training sessions on the skilled reaching task for 12 consecutive days. A probe trial was given at the end of training where all animals performed a final day of reaching but the stimulation condition received training without stimulation. During these training sessions the CS/RT animals (E1 and E2) were treated with electric stimulation via the preclinical stimulation system. Monopolar cathodal stimulation was administered continuous with a frequency of 50 Hz and a current intensity of 50% of the subject's movement threshold. Each pulse was biphasic, charged balanced and asymmetric consisting of a square phase lasting 100 $\pm$ 10 microseconds and a decaying exponential phase lasting ~19900$\pm$ 10 microseconds.

Assessing Residual Motor Map Area and Topography

Within three days of the final rehabilitation training session, ICMS was again used to generate a second map of the CFA contralateral to the trained forelimb. Prior to surgery animals were anesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.p.), receiving xylazine (0.02 mg/kg i.m.) and ketamine (20 mg/kg i.p.) as needed. Further, animals were placed on isofluorane (0.15%, 1.5% O_2) when

needed. The electrode was removed and the cortical surface covered in body temperature silicon oil. Mapping procedures were identical to those used in the initial mapping (see above). Infarct area was calculated by outlining the cauterized area on the digital image of the cortical surface obtained from the first map. The area was clearly visible as a bleached or whitened area due to the loss of blood flow. Residual CFA prior to rehabilitation (Resid-PreRehab) was defined as the total area of CFA minus the area of infarct. Residual CFA post-rehabilitation (Resid-PostRehab) was the total area of CFA after rehabilitation. The percentage change in residual CFA was then calculated as [(Res-PostRehab) – (Res-PreRehab)/100]. In addition, the percentage of residual CFA that was occupied by distal movement representations was calculated both pre and post rehabilitation. Distal movement representations were analyzed because wrist/digit movement representations expand during both normal skill learning and recovery of skill after cortical injury.

Histology and Lesion Verification

Following the rehabilitation phase the animals were given an overdose of pentobarbital and then transcardially perfused with 0.1 M sodium phosphate buffer followed by 4% paraformaldehyde solution in the same buffer. Brains were then extracted and post fixed in 4% paraformaldehyde solution in 0.1 M sodium phosphate buffer. Serial 50 μm coronal sections were then taken using a microtome. Ten sections spaced 600 μm apart and spanning approximately 2.7 mm anterior and 3.3 mm posterior to bregma were sampled for lesion verification. The same number of sections was analyzed for each animal. The sampled sections were stained with Toluidine blue (a Nissl stain) and digitally scanned (Espon Perfection V500 Photo Scanner, Long Beach, CA) for lesion verification. The area of spared tissue, characterized by

consistent Nissl staining, was traced using Image J software (Abramoff et al., 2004; Rasband, 2009) and cortical volumes were estimated with the Cavalieri's unbiased estimator method using the formula:

$$volume = d\left(\sum_{i=1}^{n} \left(y_i\right)\right) - (t)y_{\max}$$

Where "y_i" is the cross sectional area of the "ith" section through the morphometric region, "d" is the distance between sections (600 μm) and "n" is the total number of sections (12). "y_{max}" is maximum value for the area of one section and "t" is the section thickness (50 μm) and their product is subtracted from the basic question as a correction for the overprojection (Gundersen 1986; Gundersen and Jensen, 1987; Mayhew 1992). Estimated volumes for cortical and subcortical tissue were analyzed as the percent affected to unaffected side (volume lesioned hemisphere/volume non lesioned hemisphere*100) in order to account for individual differences in brain size.

Results

Reaching Accuracy

A repeated measures ANOVA with CONDITION as a between subject factor and TIME as a within subject factor revealed a significant CONDITION x TIME interaction [$F(26,403)= 15.712$; $p<0.01$] on reaching accuracy (Figure 2-3). Subsequent multiple comparisons (Fisher's PLSD; $p<0.01$) revealed that all conditions had significantly lower reaching accuracies on day 1 of rehabilitation in comparison with pre-stroke levels. Further comparisons (Fisher's PLSD; $p<0.05$) showed that while all animals receiving rehabilitation showed significant increases in reaching accuracy during the 12 days of rehabilitation, the animals receiving cortical stimulation (E1-CS/RT and E2-CS/RT) had significantly higher reaching accuracies that RT Controls on days 5 through 8. Animals

in the E1-CS/RT condition performed significantly better than the E2 and RT Controls on days 3-4 and day 6 through the remainder of the study. The RT Control condition showed a progressive increase in reaching accuracy during the first 4 days of training that was followed by a significant decrease in accuracy on days 5-7 (Fisher's PLSD; $p<0.05$). No significant improvements were seen in the stimulated conditions after 6 days of rehabilitation. NT control animals had a significantly lower final reaching accuracy than all groups with rehabilitation even though a Paired T-Test revealed a small but significant increase in motor performance in the NT controls that was indicative of spontaneous recovery [$T(10) = 5.006$; $p<0.01$]. This demonstrates that while rehabilitation induced improvements in reaching accuracy the combination of rehabilitation and cortical stimulation with a distributed contact arrangement (E1) resulted in even greater increases in relative reaching accuracy following focal cortical ischemia.

Movement Thresholds

A repeated measures ANOVA with CONDITION and TIME showed a significant CONDITION x TIME interaction [$F(2,34) = 4.115$; $p<0.05$] on mean movement threshold (Figure 2-4). The mean MT required to elicit movement was significantly higher in the E1 electrodes at all time points (FLPSD; $p<0.05$). Animals in both E1 and E2 conditions showed a progressive decrease in MT's as training continued (Figure 3). There was a trend for the E1 condition to show a larger decrease in threshold than the E2 condition [$F(1,17) = 3.736$; $p<0.07$].

Residual Motor Maps

CFA motor maps prior to infarction were 4.36 mm^2 ($\pm$0.40 mm^2) in RT controls, 4.30 mm^2 ($\pm$0.74 mm^2) in E1-CS/RT, 4.14 mm^2 ($\pm$0.38 mm^2) in E2-CS/RT, and 4.25

mm^2 ($\pm$0.45 mm^2) in NT controls. The mean infarction area was 1.08 mm^2 ($\pm$0.18 mm^2) in RT controls, 1.20 mm^2 ($\pm$0.22 mm^2) in E1-CS/RT, 1.12 mm^2 ($\pm$0.20 mm^2) in E2-CS/RT, and 1.10 mm^2 ($\pm$0.19 mm^2) in NT controls. A one-way ANOVA revealed a significant main effect of CONDITION on the area of residual CFA after rehabilitation [$F(3,41) = 120.170$; $p<0.01$]. Subsequent multiple comparisons (Fisher's PLSD; $p<0.01$) showed that the amount of residual CFA of all rehabilitation groups was greater than that of NT Controls (Figure 2-5A). The amount of residual CFA for both CS/RT groups was greater than that of either RT or NT controls (Figure 2-4A). A second one-way ANOVA revealed no significant main affect of CONDITION on the area of residual proximal representations after rehabilitation [$F(3,41) = 1.402$; $p=0.256$] (Figure 2-5B). A third one-way ANOVA revealed a significant main effect of CONDITION on the area of residual distal representations after rehabilitation [$F(3,41) = 97.243$; $p<0.01$]. Subsequent multiple comparisons (Fisher's PLSD; $p<0.01$) showed that the amounts of residual distal representations for all rehabilitation groups was greater than that of NT Controls (Figure 2-5C). The amount of residual distal representations for both CS/RT groups was greater than that of the RT and NT controls. Furthermore, a one-way ANOVA revealed a significant main effect of CONDITION on the percent of distal representations after rehabilitation [$F(3,41) = 25.732$; $p<0.01$] (Figure 2-6). Subsequent multiple comparisons (Fisher's PLSD; $p<0.01$) showed that the percent distal representations in all rehabilitation groups was significantly greater than the NT Controls. The E1-CS/RT had a significantly greater amount of residual distal representations than the RT controls (Fisher's PLSD; $p<0.01$). In addition, the RT and E2-CS/RT groups did not significantly differ in the percent of residual distal

representations (Fisher's PLSD; $p<0.01$). Finally, a significant positive correlation was found between the increase in the total CFA area and the increase in post rehabilitation reaching accuracy (Mean final 3 rehabilitation days – Mean first 3 rehabilitation days) during rehabilitation [$r=0.7838$, $p<0.0001$] (Figure 2-7). A significant positive correlation was found between the increase in the area of distal representations and the increase in post rehabilitation reaching accuracy (Mean final 3 rehabilitation days – Mean first 3 rehabilitation days) during rehabilitation [$r=0.7619$, $p<0.0001$].

Estimation of Remaining Tissue

A one-way ANOVA revealed no significant main effect of CONDITION on the estimates of remaining cortical tissue [$F(4,40) = 0.3149$; $p>0.05$]. Subsequent multiple comparisons (Fisher's PLSD; $p<0.05$) showed that the amount of residual cortical tissue was similar for all conditions (Table 2-1). A one-way ANOVA revealed no significant main effect of CONDITION on the estimates of remaining subcortical tissue [$F(4,40) = 1.3302$; $p>0.05$]. Subsequent multiple comparisons (Fisher's PLSD; $p>0.05$) showed that the amount of residual subcortical tissue was similar for all conditions.

Discussion

While the mechanisms underlying motor improvement after stroke are not fully understood, there is a growing body of evidence to support the role of functional restoration/compensation within residual neural tissue. Further, the use of adjuvant therapies that promote such reorganization by augmenting endogenous neural plasticity may serve to increase functional outcome over and above that observed with standard rehabilitation. The present study provides additional evidence that cortical stimulation in combination with rehabilitation enhances motor function and motor cortex reorganization in an animal model of cortical ischemia. In addition, the results

demonstrate the importance of the distribution of stimulation across the cortex for augmenting motor performance. In this study all groups that received rehabilitative motor training exhibited greater motor improvements than non-trained animals. Further, all animals receiving motor rehabilitation showed significant expansion/reorganization of movement representations in residual motor cortex relative to the NT group. These findings suggest that both behavioral improvements and physiological remodeling after injury are experience dependent processes and rehabilitative motor training serves as a driving factor. CS/RT increased both the motor improvement and map reorganization observed with rehabilitation. Finally, the spatial configuration of the contacts on the cortical electrode surface served as an important factor for CS/RT delivery as only the distributed configuration was associated with enhanced motor improvement over and above RT alone.

The mechanisms of CS/RT's enhanced motor recovery are not clearly understood but likely involve enhancing synaptic strength within residual cortical circuits (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Plautz et al., 2003; Teskey et al., 2003; Adkins et al., 2006; Adkins et al., 2008). The present study finds reorganization of cortical microstimulation-evoked motor representations within the affected hemisphere that occurs in parallel with behavioral recovery. Previous work using CS/RT substantiates this association between recovery and motor map plasticity in rodents (Kleim et al., 2003) and primates (Plautz et al., 2003). Learning-dependent motor map plasticity is accompanied with increases in synapse number (Kleim et al., 2002a; Kleim et al., 2004) and these same changes likely support CS/RT motor improvements as increases in synapse density (Adkins et al., 2008) and synaptic responses (Teskey et

al., 2003) have been observed in CS/RT animals relative to a RT condition. Studies with CS/RT have observed changes in the MT's used to calibrate the amount of current administered for CS/RT. MT's typically decrease across days of rehabilitation, and CS/RT conditions that display enhanced motor recovery demonstrate the greatest reductions in MT's (Kleim et al., 2003; Teskey et al., 2003). These reductions in MT suggest changes in cortical excitability that could be mediated by increased synaptic input onto corticospinal neurons (Monfils et al., 2005). In the present study, MT's decreased across days of rehabilitation and there was trend for larger threshold reductions for the E1-CS/RT condition relative to the E2-CS/RT in a manner that paralleled E1-CS/RT's enhanced motor improvements relative to E2-CS/RT. This suggests that CS/RT is associated with an increase in cortical excitability that may be mediated by increased in synapse number or strength (Adkins et al., 2008).

Although animals in both CS/RT conditions showed larger residual motor maps than RT or NT controls after training, the E2-CS/RT animals exhibited motor improvements that were markedly less than the E1-CS/RT group and similar to those observed with the RT control group. This dissociation between map restoration and behavioral motor recovery may suggest that map restoration may be necessary but not sufficient to support motor rehabilitation, at least in the context of CS/RT therapy. This finding has been reported previously in rodent studies where electric (Kleim et al., 2003) or pharmacological stimulation (Macdonald et al., 2007) induces motor map changes without robustly impacting behavior. These findings indicate that there is not a linear relationship between injured brain plasticity and final motor recovery. Map plasticity

appears necessary as all animals that demonstrated enhanced motor recovery also exhibited significantly greater motor maps within residual tissue.

The results also demonstrate that distributed cortical stimulation is more effective at enhancing motor performance than focal stimulation even when contact area was held constant. There are several possible explanations for this finding. First, the focal stimulation contacts may result in overlapping areas of cortical stimulation. That is, two electrodes may be stimulating the same cortical area resulting in a net decrease in the amount of cortex that is being stimulated as compared to the distributed contact configuration. Second, the location of cortical stimulation due to contact configuration also differed between the two electrode configurations. The focal arrangement tended to stimulate within the forelimb motor maps while the distributed stimulated both forelimb and non- forelimb (whisker, neck etc) areas. This was evident when determining MT's, focal stimulation only evoked forelimb movements whereas distributed evoked a wide range of forelimb, whisker and neck movements. Stimulation of areas outside of the forelimb representations during rehabilitation may have promoted an expansion of forelimb representations into non-forelimb areas. The distributed stimulation may have further facilitated timing-dependent recruitment of residual cortex into forelimb representations (Jackson et al., 2006). That is, having stimulation within head and neck or vibrissa areas in conjunction with forelimb movements produced during forelimb training may have recruited those areas into the forelimb motor map. The focal electrode configuration would have limited this possibility.

The present findings further support the viability of CS/RT for enhancing motor function after stroke. The results also demonstrate the importance of stimulation

distribution in CS/RT. Future clinical trials of CS/RT must consider the importance of stimulating wide areas of cortex to promote functional reorganization and motor recovery.

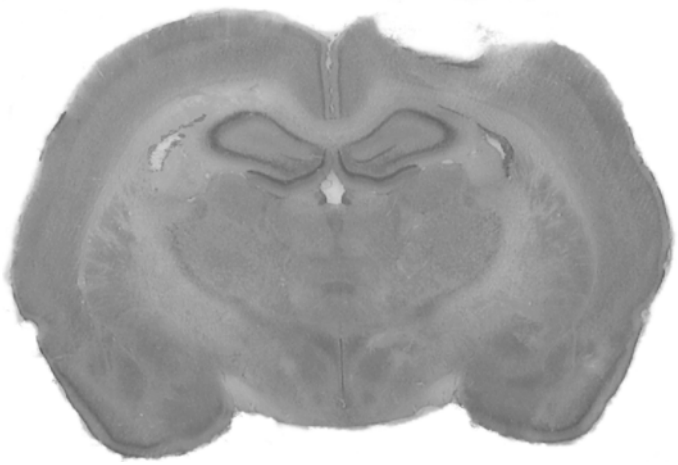

Figure 2-1. Representative Nissl stained coronal section from the MCAo lesion. The medial-lateral extent of brain damage at 24 days following ischemic insult by electrocoagulation of the surface vasculature within sensorimotor cortex is shown.

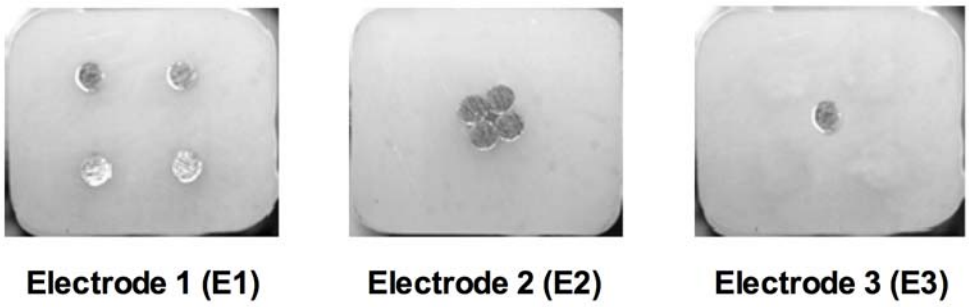

Figure 2-2. The three different electrode configurations examined. All contacts were 0.6 mm in diameter. E1 consisted of 4 contacts each in the corner of a 2mm x 2mm square. E2 clustered all four contacts into the center of the pedestal and E3 had a single contact in the center.

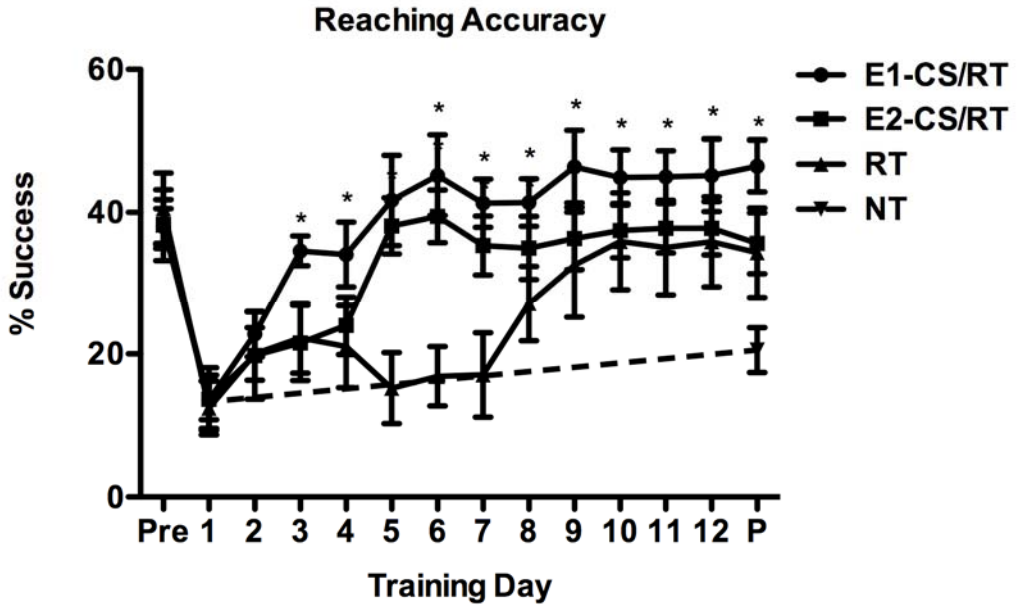

Figure 2-3. Reaching performance prestroke and during rehabilitation. Mean (±SD) percent reach accuracy both prestroke and across 12 days of rehabilitative training on the skilled reaching task. One probe session (P) was given at the end of training without stimulation. All groups receiving rehabilitation (E1, E2 and RT) demonstrated greater motor improvement than the NT animals. The RT Control condition showed a transient decrease in accuracy on days 6-8. Both CS/RT conditions (E1 and E2) showed greater reaching accuracies than RT Controls on days 5-8. Animals in the E1-CS/RT condition had significantly higher reaching accuracies than all other conditions on days 3-4 and day 6 through the remainder of the study (Fisher's PLSD; $p<0.05$; indicated by the symbol "Θ").

Figure 2-4. Mean (±SD) movement thresholds for animals in the E1 and E2 conditions. Both E1 and E2 conditions showed a progressive decrease in threshold as training continued. The mean threshold required to elicit movement was significantly higher in the E1 animals at all time points (Fisher's PLSD; $p<0.05$; indicated by the symbol "Θ"). There was a trend for the E1 condition to show a larger decrease in threshold than the E2 condition [$F(1,17) = 3.736$; $p<0.07$]

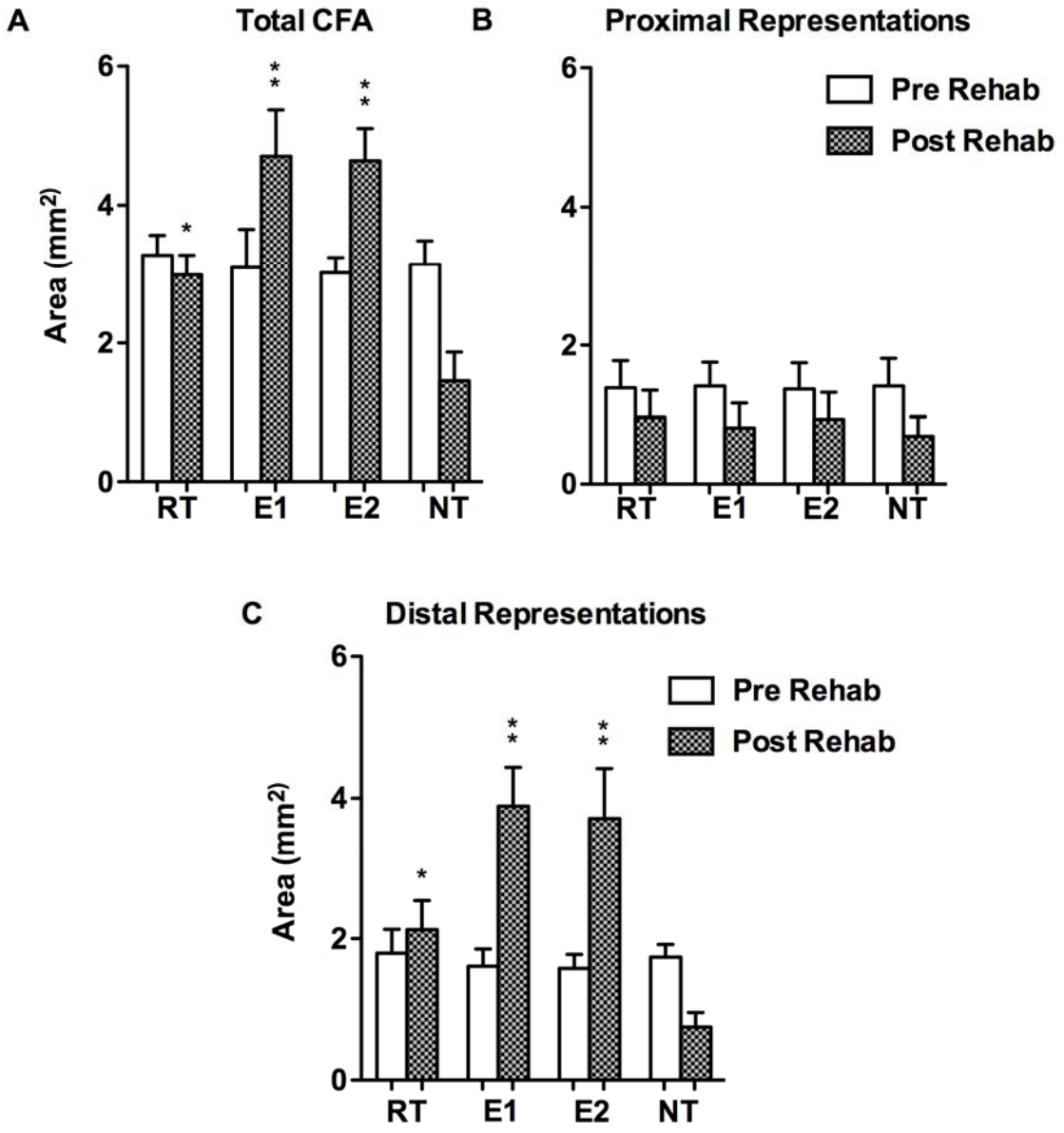

Figure 2-5. Changes in the amount of movement representations during rehabilitation. A) Mean (±SD) size of the residual CFA after rehabilitative training. CS/RT animals (E1 and E2) had a larger area of residual CFA than both the RT and NT controls (Fisher's PLSD; $p<0.05$; indicated by the symbol "Θ"). B) Mean (±SD) size of the residual proximal representations after rehabilitative training. C) Mean (±SD) size of the residual distal representations after rehabilitative training. All rehabilitation groups (RT, E1 and E2) possessed significantly greater areas of distal motor representations than NT controls (Fisher's PLSD; $p<0.05$; indicated by the symbol "Θ").

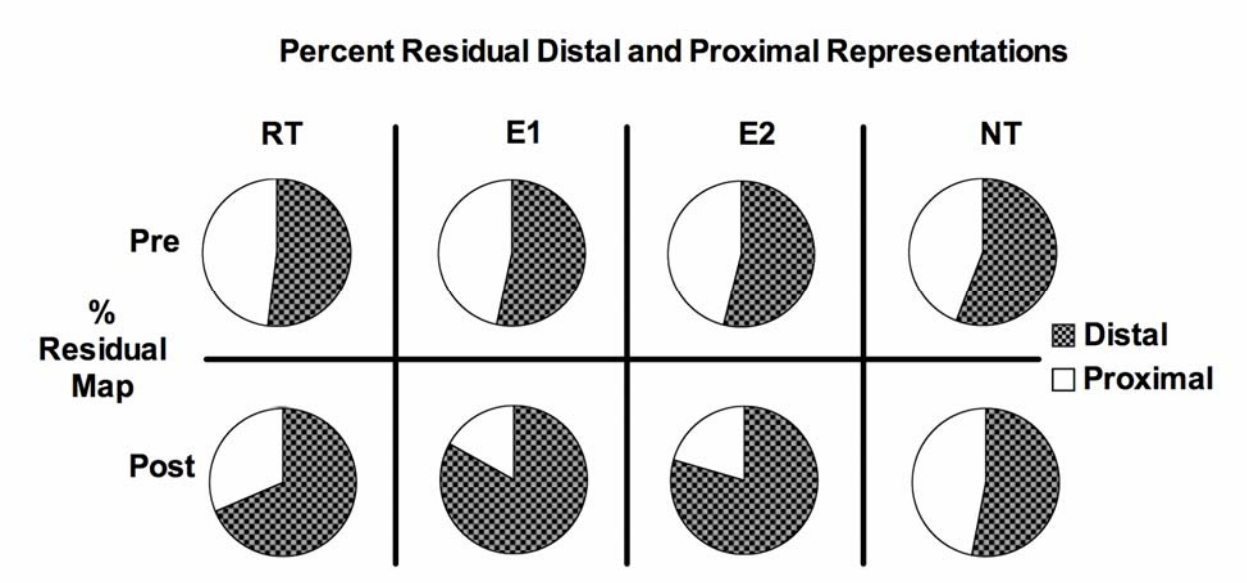

Figure 2-6. Changes in the proportion of movement representations during rehabilitation. Area of distal and proximal representations given as a percent of total residual CFA before and after rehabilitation. The percent distal representations in all rehabilitation groups (RT, E1 and E2) were significantly greater than in NT Controls. The E1-CS/RT group had a significantly larger percent of residual distal representations than the RT controls. (Fisher's PLSD; $p<0.01$). The RT and E2-CS/RT groups did not significantly differ in the percent of residual distal representation.

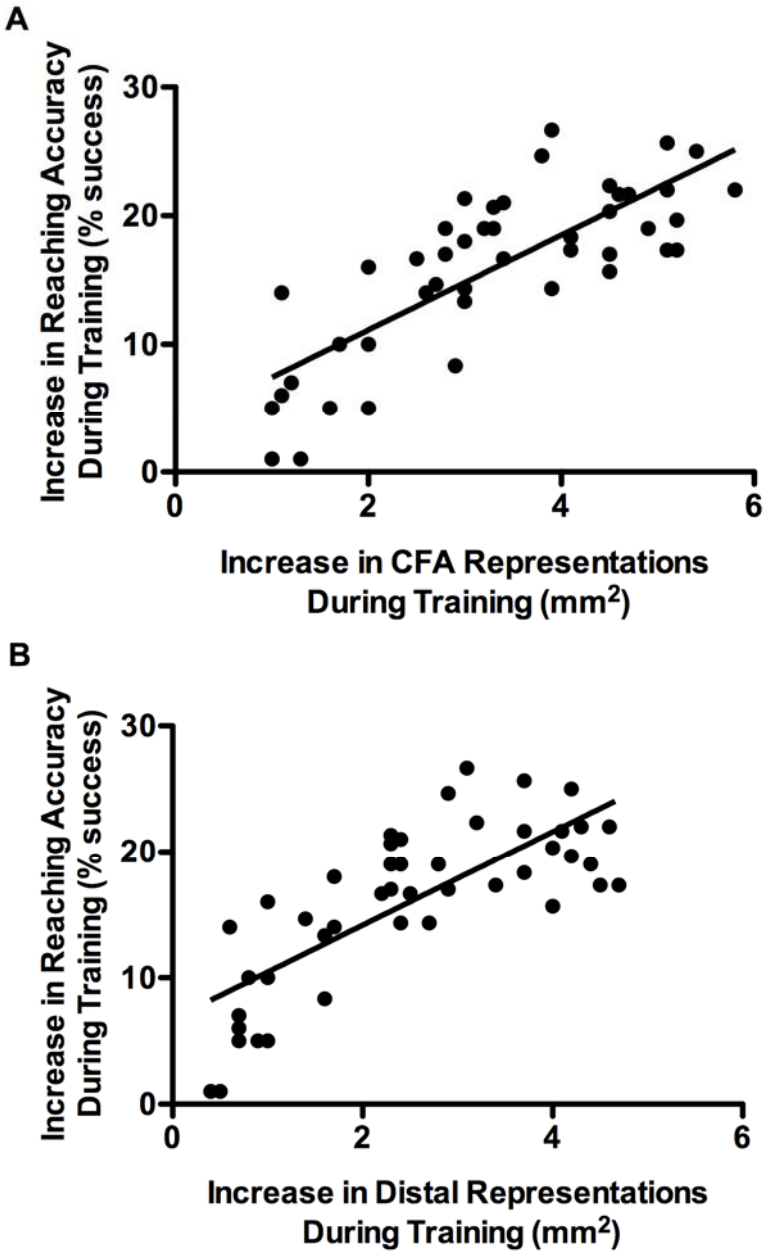

Figure 2-7. Relationship between behavior and motor map changes. A) A significant positive correlation was found between the increase in the total CFA area and the increase in post rehabilitation reaching accuracy (Mean final 3 rehabilitation days – Mean first 3 rehabilitation days) during rehabilitation [$r=0.7838$, $p<0.0001$]. B) A significant positive correlation was found between the increase in the area of distal representations and the increase in post rehabilitation reaching accuracy (Mean final 3 rehabilitation days – Mean first 3 rehabilitation days) during rehabilitation [$r=0.7619$, $p<0.0001$].

Table 2-1. Estimate of spared cortical and subcortical tissue from experiment 1. Estimated volumes for cortical and subcortical tissue were analyzed as the percent affected to unaffected side (volume lesioned hemisphere/volume non lesioned hemisphere*100) in order to account for individual differences in brain size. Data is represented as mean percent ± SEM.

Condition	Sample Size	Estimates of Spared Tissue Volume	
		Cortical (% Contralesional)	Subcortical (% Contralesional)
E1-CS/RT	10	96±2	99±1
RT	8	98±1	100±1
E2-CS/RT	9	97±1	97±1
E3-CS/RT	7	96±1	99±1
NT	11	97±1	98±1

CHAPTER 3
CORTICAL STIMULATION PLUS REHABILITATIVE TRAINING ENHANCES MOTOR FUNCTION INDEPENDENT OF THE NUMBER OF STIMULATING CONTACTS AFTER EXPERIMENTAL ISCHEMIA

Introduction

Stroke is a major contributor to adult disability and limits the quality of life of its survivors. It is estimated that more than 500 000 Americans survive stroke each year with at least 70% of these survivors exhibiting motor deficits in the upper extremities (Roth et al., 1998; Luke et al., 2004; Urton, et al., 2007). While stroke survivors often receive intensive rehabilitative practice or training, complaints of residual motor deficits are found in over half of this population (Urton, et al., 2007). In addition to being limited, motor outcomes following rehabilitation are highly variable (Duncan et al., 2000; Pedersen et al., 1998) thus reflecting our lack of understanding of the neurobiological mechanisms underlying functional recovery. Behavioral improvements following stroke are believed to be supported by the restoration and/or recruitment of spared brain regions to perform lost functions. Animal models have demonstrated that stroke is associated alterations in neural connectivity that is demonstrated by reduced dendritic arbor (Zhang et a., 2005) and synaptic atrophy (Hasbani et al., 2001; Zhang et al., 2005; Murphy et al., 2008). The disruption in neural connectivity is further demonstrated by a persistent loss of microstimulation-evoked movement representations within motor cortex (Nudo and Milliken, 1996; Friel et al., 2000). Although some motor function may be restored spontaneously after stroke (Nakayama et al., 1994; Cramer, 2008), there is considerable evidence that motor training can drive improvements in motor performance and changes in neural circuitry within motor brain regions. In non-human primates, appropriate motor rehabilitative training induces motor improvements that are paralleled

by an expansion/reemergence of microstimulation evoked movement representations that were previously lost by the ischemic damage (Nudo et al., 1996a; Kleim et al., 2003). Animals not given motor training do not show the same gains in motor function or map reorganization (Nudo et al., 1996b). Similarly, map restoration in rodents is dependent upon skilled rehabilitation and does not occur in animals that experience unskilled rehabilitation involving extensive repetition of simple limb movement (Kleim et al., 2003; Kleim et al., 2004). The functional reorganization of movement representations following stroke within motor cortex is likely mediated by a reinstitution and/reorganization of cortical circuitry involving synaptogenesis (Stroemer et al., 1995; Buonomano and Merzenich, 1998; Brown and Murphy, 2007). Therefore, adjuvant therapies that promote synaptic plasticity within motor cortex may enhance rehabilitation-dependent improvements in motor performance after stroke. Indeed, greater behavioral gains have been observed with rehabilitative training in animals receiving pharmacological interventions known to stimulate synaptic plasticity such as the administration of amphetamine (Stroemer et al., 1998; Adkins and Jones, 2005; Ramic et al., 2006; Papadopoulos et al., 2009) or nicotine (Gonzalez et al., 2005; Gonzalez et al., 2006). Pharmacological stimulation of the intracellular signaling pathways known to promote synaptic plasticity increases animals' skilled reaching ability and the size of forelimb movement representations following cortical ischemia (MacDonald et al., 2007).

Electrical stimulation can increase or decrease synaptic efficacy at a variety of sites within the nervous system (Blundon and Zakharenko, 2008) including sensorimotor cortex (Trepel and Racine, 1998; Froc et al., 2000; Teskey et al., 2002;

Monfils et al., 2004). These changes in synaptic efficacy may support the encoding of behavioral experience (Martin and Morris, 2002). For example, synapses in sensorimotor cortex are potentiated during skilled motor learning (Rioult-Pedotti et al., 1998; Monfils and Teskey, 2004). Skilled motor training also results in a reorganization of motor representations as representations for movements involved in the motor task expand their territories at the expense of others. The reorganization of motor cortex occurs later in skilled training after initial improvements in skill behavior (Kleim et al., 2004) and is temporally paralleled by increased synaptogenesis (Kleim et al., 1996; Kleim et al., 2002a; Kleim et al., 2004). Electrical stimulation protocols that can induce a form of long-term potentiation in awake behaving animals expands their motor maps in the absence of skilled motor learning (Monfils et al., 2004). Similarly, kindling-type electrical stimulation used to study of epilepsy results in large expansions of movement representations within motor cortex (van Rooyen et al., 2006). The output of the motor cortex can also be altered by facilitating the connection of previously unconnected motor regions by conditioning with an stimulating neural implant (Jackson et al., 2006). The similarities between synaptic changes due to electric stimulation and skilled motor learning have raised the possibility that electric stimulation can facilitate behavioral encoding in the nervous system. There is now evidence from animal models of stroke that CS/RT enhances restoration of motor function relative to the training alone and induces many neural plastic changes with brain tissue. These studies have assessed post injury motor performance using tasks such as single pellet retrieval (Kleim et al., 2003; Adkins et al., 2006; Adkins et al., 2008), pasta matrix (Teskey et al., 2003), Montoya staircase (Adkins-Muir and Jones, 2003), and pellet retrieval from a 5 well

apparatus in primates (Plautz, et al., 2003). Combined CS/RT therapy has resulted in a greater return of motor performance in all of these tasks following focal ischemic damage to motor cortex. CS/RT has also been associated with an expansion and/or reorganization of movement representations within motor cortices of rodents (Kleim et al., 2003) and primates (Plautz et al., 2003). In addition, CS/RT results in an increased number of synapses (Adkins et al., 2008) as well as enhanced synaptic responses (Teskey et al., 2003).

Recently, a phase three clinical trial of CS/RT in stroke patients failed to show any significant effect of the treatment. This may in part be due to clear methodological differences between the preclinical animal studies and the human clinical applications (Plow et al., 2009). Specifically, CS/RT is delivered to a much more focal region of motor cortex within human stroke patients than in animal models. A recent animal study demonstrated that CS/RT with contacts distributed across motor cortex significantly enhanced behavioral improvements relative to CS/RT that had stimulation focal to one region (Boychuk et al., 2009). While this suggests that CS/RT is more efficacious when stimulation is delivered across motor cortex the significance of the number of independent contact sites across the cortex is unknown. Here, the importance of contact arrangement was assessed by testing several types of CS/RT that differed in their number of independent stimulation sites in a rodent model of middle cerebral artery (MCA) stroke.

Methods

Subjects

Fifty-six adult male Long-Evans hooded rats (350-420g) were housed (1 animal/cage) in standard laboratory cages on a 12:12 hour light dark cycle throughout the

experiment. Rats were maintained on Lab Diet 5001 (PMI Feeds, St. Louis, MO) and water ad libitum, and were handled and cared for in accordance with the National Institutes Health Guide for the Care and Use of Laboratory Animals and with the approval of the University of Florida's Institutional Animal Care and Use Committee (IACUC).

Reach Training

Over the course of several days, all animals were placed on a restricted diet until they measured 90% of their original body weight. A brief period of pretraining was then given to familiarize the rats with the reaching task. Pretraining involved placing them into test cages (10 X 18 X 10 cm) with floors constructed of 2 mm bars, 9 mm apart edge to edge. A 4 cm wide and 5 cm deep tray filled with food pellets (45 mg; Bioserv) was mounted on the front of the cage. The rats were required to reach outside the cage and retrieve pellets from the tray. Rats were permitted to use either limb and the preferred limb was noted for each animal. All rats remained in pretraining until they had successfully retrieved 10 pellets (approximately 1 hour/day for 2 days). After pretraining, the rats were placed into a Plexiglas cage (11 cm X 40 cm X 40 cm) with a 1 cm slot located at the front of the cage. Animals were trained for 15 minutes each day to reach with their preferred limb through the slot and retrieve food pellets from a table outside the cage (Whishaw and Pellis, 1990). Each session was videotaped and later used to assess reaching performance. A successful reach was scored when the animal grasped the food pellet, brought it into the cage and to its mouth without dropping the pellet. The percentage of successful reaches [(# successful retrievals/the total # of reaches) x 100] was then calculated. All training sessions were video taped and used to measure reaching accuracy. Animals were trained for approximately two weeks on this task to

establish a baseline measure of motor performance. Baseline was defined as the average accuracy across the 3 final days of training. Animals failing to achieve a mean reaching accuracy of 40% across 3 consecutive days were not used in the study. Prior to surgery, all animals were sorted by their prelesion reaching performance to create groups with comparable baseline levels of reaching accuracy. This was done to ensure that reaching performance prior to infarction was similar across conditions.

Infarction

Following the 2 weeks of motor training, focal ischemic damage was given to the lateral aspect of sensorimotor cortex by temporary occlusion of the middle cerebral artery (MCAo). Briefly, animals were anesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.p.). Animals received low levels of isofluorane (0.15%) and supplemental doses of ketamine (20 mg/kg i.p.) as needed. Under sterile conditions, an incision was made midline and the skull exposed. Small portions of the skull were then removed over the hemisphere contralateral to each animal's trained to allow injection of the vasoconstricting peptide endothelial-1 (ET-1: 0.2µg/µL; American Peptide, Sunnyvale, CA) via the Nanolitre injection system (World Precision Instruments, Sarasota, Fl) controlled by the SYS-Micro 4 Controller (World Precision Instruments, Sarasota, Fl). Stereotaxic coordinates of the injection site with respect to bregma were as follows: anteroposterior, +0.9 mm; mediolateral, -5.2mm; and dorsoventral, -8.7 mm (Biernaskie and Corbett 2001). ET-1 (320 pMol dissolved in 0.9% sterile saline) was injected at a rate of 7nL/sec through a glass pipette and pipette was left in the injection site for 5 minutes to avoid backflow. A group of animals that never received an infarction were included as Healthy Controls (HC; n=10) and were given training on the same skilled reaching task on all days of rehabilitation.

Cortical Electrode Implantation

Directly following the infarction, nine-pin electrode carriages (Plastics One Inc., Roanoke, VA) were implanted epidurally over top sensorimotor cortex in the hemisphere opposite each animal's preferred paw. The skull was removed between 1 mm posterior to 5.5 mm anterior to bregma and 0.5 mm to 5.5 mm lateral to midline to allow the electrode to be lowered onto the cortical surface. A return lead was fixed to the skull in a position posterior to Lambda and the craniotomy filled with gel foam. Both the electrode and gel foam were covered in non-exothermic PolyWave dental acrylic and cured with a brief pulse (50 seconds) of ultraviolet light. The electrode was then fixed to skull screws with standard dental acrylic and then dental cement was applied on top of the dental acrylic. The skin was sutured and given topical antibiotics and the animals were given 4cc of warm ringers solution (s.c.) and metacam (0.10 mg/kg; s.c.). The Dual Rail configuration (n=10) consisted of two parallel 0.4 mm by 3 mm stainless steel strips separated by 2 mm. This configuration was included in this study to allow comparison to previous rodent studies of CS/RT that have used similar Dual Rail-type configurations (Adkins-Muir and Jones, 2003; Teskey et al., 2003; Adkins et al., 2006; Adkins et al., 2008). The 2x2 configuration (n=10) consisted of four contacts positioned 2mm equidistant from one another each with a diameter of 0.60 mm. The 3x3 configuration (n=10) consisted of 9 contacts positioned 1.1 mm equidistant from one another each with a diameter of 0.2 mm (figure 1).

Movement Thresholds

After surgery all animals were returned to their home cage for three days where they were supervised for health concerns but otherwise left to recover. Animals were given full access to food until the last day where food restriction regimen (see above)

was reimplemented. After the three days, animals with implants had their individual motor thresholds (MT's) determined and then animals were started in a motor rehabilitation paradigm (except those in the NT condition). MT's were assessed on post lesion training days 1, 11 and 20. MT's were defined for each animal as the minimum current to cause an involuntary motor response and were tested in all animals that had received a cortical electrode. The animals were placed into a transparent cylinder and observed while 3 second trains of 1 millisecond 100 Hz monoploar cathodal pulses were given. Current was gradually increased by 5% increments until a movement of the contralateral forelimb could be clearly detected. Cortical stimulation during the post injury motor training phase was then delivered at 50% of each animal's MT during rehabilitation for the CS/RT condition.

Cortical Stimulation and Rehabilitation Training (CS/RT)

Following motor threshold testing the animals received twenty daily bouts of motor training intended to model motor rehabilitation. Each bout consisted of 20 minutes of the skilled reach training used during the training procedure described earlier or the combination of skilled reach training and cortical stimulation (CS/RT). One group of animals were included that received the stroke model and rehabilitative training alone (RT; n=10). Another group of animals were included that received the stroke model but did not receive rehabilitative training (NT; n=6). The post injury reaching behavior of the NT group was assessed by probe trials on days 1 and 20 of motor training. All sessions were video taped for analysis of reaching accuracy and number of reach attempts. Animals receiving the combination of cortical stimulation and rehabilitative training were stimulated via the Vertis Stimulation System during these sessions. The cortical electrode was connected to a remote stimulator suspended above the training cage

where information was then sent wirelessly to the rest of the system. CS/RT was delivered as monopolar cathodal stimulation and was administered continuous with a frequency of 100 Hz with a current intensity dictated by the subject's movement threshold. Each pulse was biphasic, charged balanced and asymmetric consisting of a square phase lasting 100 ± 10 microseconds and a decaying exponential phase lasting ~9900± 10 microseconds.

Histology and Lesion Verification

Following the rehabilitation phase the animals were given an overdose of pentobarbital and then transcardially perfused with 0.1 M sodium phosphate buffer followed by 4% paraformaldehyde solution in the same buffer. Brains were then extracted and post fixed in 4% paraformaldehyde solution in 0.1 M sodium phosphate buffer. Serial 50 µm coronal sections were then taken using a microtome. Ten sections spaced 600 µm apart and spanning approximately 2.7 mm anterior and 3.3 mm posterior to bregma were sampled for lesion verification. The same number of sections was analyzed for each animal. The sampled sections were stained with Toluidine blue (a Nissl stain) and digitally scanned (Espon Perfection V500 Photo Scanner, Long Beach, CA) for lesion verification. The area of spared tissue, characterized by consistent Nissl staining, was traced using Image J software (Abramoff et al., 2004; Rasband, 2009) and cortical volumes were estimated with the Cavalieri's unbiased estimator method using the formula:

$$volume = d\left(\sum_{i=1}^{n} \left(y_i\right)\right) - (t)y_{\max}$$

Where "y_i" is the cross sectional area of the "ith" section through the morphometric region, "d" is the distance between sections (600 µm) and "n" is the total number of

sections (12). "y_{max}" is maximum value for the area of one section and "t" is the section thickness (50 μm) and their product is subtracted from the basic question as a correction for the overprojection (Gundersen 1986; Gundersen and Jensen, 1987; Mayhew 1992). Estimated volumes for cortical and subcortical tissue were analyzed as the percent affected to unaffected side (volume lesioned hemisphere/volume non lesioned hemisphere*100) in order to account for individual differences in brain size.

Results

Reaching Accuracy

A repeated measures ANOVA with CONDITION as a between subject factor and TIME as a within subject factor that included all conditions revealed a significant CONDITION x TIME interaction [$F(10,100)= 12.0559$; $p<0.0001$] on reaching accuracy (Figure 3-1A). A repeated measures ANOVA with CONDITION as a between subject factor and TIME as a within subject factor that excluded the NT condition revealed a significant CONDITION x TIME interaction [$F(80,900)= 2.8347$; $p<0.0001$] on reaching accuracy. Subsequent multiple comparisons (Fisher's PLSD; $p<0.01$) revealed that all groups that received lesions had significantly lower reaching accuracies on day 1 of rehabilitation in comparison with pre-stroke levels and with the performance of the HC group. On the final day of motor rehabilitation, all groups receiving rehabilitation performed significantly higher reaching accuracies than the NT group (Fisher's PLSD; $p<0.05$). Further comparisons (Fisher's PLSD; $p<0.05$) found that the Dual-CS/RT exhibited greater reaching improvements than the RT condition on days 8, 11-13, 16 and 18-20. The 2x2-CS/RT exhibited greater reaching improvements than the RT condition on days 8, 10, 16 and 18-20. The 3x3-CS/RT exhibited greater reaching improvements than the RT condition on days 10, 12-13, 16 and 18-20. Thus, all three

CS/RT conditions had significantly higher reaching accuracies than RT Controls on several of the final days of rehabilitation (Fisher's PLSD; $p<0.05$). All CS/RT and RT groups exhibited similar number of days where significant motor improvements were observed: RT=15 days, 2x2-CS/RT=16 days, 3x3-CS/RT=10 days, Dual-CS/RT=13 days. The NT condition did not exhibit significant motor improvements between day 1 and 20 (Fisher's PLSD; $p<0.05$). These results demonstrate that while rehabilitation following focal cortical ischemia induced improvements in reaching accuracy, the combined therapy of CS/RT resulted in enhanced behavioral gains following injury.

Reaching Attempts

A repeated measures ANOVA with CONDITION as a between subject factor and TIME as a within subject factor that included all conditions revealed a significant CONDITION x TIME interaction [$F(10,100)= 5.7307$; $p<0.0001$] on the number of reach attempts (Figure 3-1A). A repeated measures ANOVA with CONDITION as a between subject factor and TIME as a within subject factor that excluded the NT condition revealed a significant CONDITION x TIME interaction [$F(80,900)= 2.0599$; $p<0.0001$] on the number of reach attempts. Subsequent multiple comparisons (Fisher's PLSD; $p<0.01$) revealed that all groups that received lesions had significantly lower numbers of reach attempts on day 1 of rehabilitation in comparison with pre-stroke levels and with the performance of the HC group. Further comparisons (Fisher's PLSD; $p<0.05$) found that none of the groups with lesions were significantly different from one another on any day except that all groups given rehabilitation performed significantly more reach attempts than the NT group on day 20. The RT condition demonstrated a significantly smaller number of reach attempts than the HC condition on days 1-14 and 16-20. The Dual-CS/RT condition demonstrated a significantly smaller number of reach attempts

than the HC condition on days 1-14 and 16-20. The 2x2-CS/RT condition demonstrated a significantly smaller number of reach attempts than the HC condition on days 1-12, 14-16, 18 and 20. The 3x3-CS/RT condition demonstrated a significantly smaller number of reach attempts than the HC condition on days 1-10, 12-14 and 17-20. Thus, all conditions with lesions performed fewer reach attempts than the healthy controls on almost all days of rehabilitation (Fisher's PLSD; $p<0.05$). No significant improvements in the number of reach attempts were seen in the RT condition after 8 days of rehabilitation, in the Dual-CS/RT condition after 9 days, in the 2x2-CS/RT condition after 10 days or in the 3x3-CS/RT condition after 9 days. The NT condition did not perform a significantly higher number of reach attempts between the assessments made on days 1 and 20. These results demonstrate that all groups receiving rehabilitation performed similar numbers of reach attempts and that all reached less than healthy controls.

Movement Thresholds

A repeated measures ANOVA with CONDITION and TIME showed a significant CONDITION x TIME interaction [$F(6,72) = 5.5221$; $p<0.001$] on mean movement threshold (Figure 3-2A). Subsequent multiple comparisons (Fisher's PLSD; $p<0.01$) revealed that all conditions had significantly lower reaching accuracies on the third assessment of MT's relative to the first. Further comparisons (Fisher's PLSD; $p<0.05$) found that the Dual-CS/RT, 2x2-CS/RT and 3x3-CS/RT groups exhibited significantly larger decreases in the percent MT between the first and final assessment relative to the RT group. Finally, a significant negative correlation was found between the percent reduction in movement threshold (MT2-MT1/MT1*100) and the increase in post rehabilitation reaching accuracy (Mean final 3 rehabilitation days – Mean first 3 rehabilitation days) [$r=-0.3861$, $p<0.01$] (Figure 3-2B). This demonstrates that the

combination of CS/RT resulted in significantly greater reductions than RT alone and reductions in MT's have a significant correlation with increased motor improvements following cortical ischemia.

Estimation of Remaining Tissue

A one-way ANOVA revealed a significant main effect of CONDITION on the estimates of remaining cortical tissue [$F(5,48) = 16.1341$; $p<0.05$]. Subsequent multiple comparisons (Fisher's PLSD; $p<0.05$) showed that the amount of residual cortical tissue was similar for all conditions except that the HC condition exhibited a significantly greater amount of remaining cortical tissue than all other conditions (Table 3-1). A one-way ANOVA revealed no significant main effect of CONDITION on the estimates of remaining subcortical tissue [$F(5,48) = 10.372$; $p<0.05$]. Subsequent multiple comparisons (Fisher's PLSD; $p<0.05$) showed that the amount of residual subcortical tissue was similar for all conditions except that the HC condition exhibited a significantly greater amount of remaining subcortical tissue than all other conditions.

Discussion

Although the neural mechanisms of stroke recovery are not fully understood, there is evidence that motor improvements can be elicited by the recruitment and/or retraining of residual tissue within motor cortex. Further, motor experience is an important mediator of the neuroplastic changes that lead to functional reorganization within motor cortex. The present experiments further show the capacity of motor rehabilitation to improve motor function post-stroke as all animals that received rehabilitative training demonstrated greater motor improvements than animals given no post injury training. This work also serves as corroborating evidence that combining rehabilitative training with adjuvant therapies can augment motor improvements. Here,

the combination of rehabilitative training and electrical stimulation of motor cortex achieved greater motor improvements than rehabilitative training alone. The benefit of CS/RT was observed regardless of the number of independent stimulating contact sites within each cortical electrode. The ability of distributed configurations consisting of either four or nine contact sites to enhance behavioral improvements was unexpected as it was thought a larger number of stimulation sites would facilitate greater neuroplastic change in motor cortex.

While a body of evidence suggests that electric stimulation of motor cortex can enhance behavioral gains during rehabilitation, the appropriate parameters to translate to human therapy are not fully understood and may contribute to the lack of efficacy observed in a recent clinical trial (Plow et al., 2009). It has previously been reported in a rodent model of stroke that CS/RT stimulation needs to be distributed across motor cortex and encompasses several ICMS-evoked movement representations in order to be efficacious (Boychuk et al., 2009). In that study, distributed CS/RT was associated with a reemergence of distal representations that had been compromised by the experimental lesion and this reemergence occurred at the expense of surrounding representations (Boychuk et al., 2009). Stimulation distributed across motor cortex targets more non-forelimb (whisker, neck etc) areas and therefore may promote a greater amount of timing-dependent recruitment of residual cortex into forelimb representations (Jackson et al., 2006; Boychuk et al., 2009). It has previously been noted that CS/RT using one stimulation contact was insufficient to drive neurons within motor cortex in order to evoke movement whereas four contact sites were sufficient (Boychuk et al., 2009).

While prior work demonstrated that a distributed configuration of CS/RT using a small number of contacts is capable of inducing enhanced motor improvements, it is not clear what number of stimulating sites in a distributed arrangement will result in the greater rehabilitative benefit. Here, two configurations of CS/RT (2x2 and 3x3) that differed in the number and proximity of stimulating contact sites were compared by their ability to enhance motor improvements in a rodent model of stroke. The 2x2-CS/RT configuration has previously been associated with enhanced behavioral gains (Boychuk et al., 2009). These two configurations were also compared to a dual rail configuration that was associated with a behavioral benefit in many previous studies of CS/RT with rodent models of stroke (Adkins-Muir and Jones, 2003; Kleim et al., 2003, Teskey et al., 2003; Adkins et al., 2006). In the current study, the electrodes for the 2x2-CS/RT condition contained very few contacts distributed across the motor cortex while the 3x3-CS/RT's electrodes contained a large number of contacts distributed across the cortex but in closer proximity to one another. It was hypothesized that 3x3-CS/RT would induce greater motor improvements than 2x2-CS/RT because of its relatively greater number of stimulation sites making contact with different areas of motor cortex. The 3x3-CS/RT configuration induced greater motor improvements than rehabilitative alone but unexpectedly conferred no additional benefit relative to 2x2-CS/RT or Dual-CS/RT. Additionally, the three types of CS/RT did not significantly differ in changes in movement threshold amongst each other but did exhibit significantly greater movement threshold reductions compared to the RT group. These reductions in movement thresholds are indicative of changes in cortical excitability and may reflect increased synaptic input onto corticospinal neurons (Monfils et al., 2005).

It is not clear why the 3x3-CS/RT conferred no additional benefit relative to 2x2-CS/RT except to say that 2x2-CS/RT provided a similar amount of necessary stimulation to drive enhanced motor improvements relative to 3x3-CS/RT. An important similarity between 2x2-CS/RT and 3x3-CS/RT is that both configurations are widely distributed across motor cortex rather than focally distributed within it. The majority of forelimb representations are located within the interior of motor map whereas non-forelimb representations are located in areas surrounding them. Boychuk et al. (2009) found that CS/RT stimulation directed at the outer regions of motor cortex (areas with predominantly non forelimb representations) induced a greater expansion of forelimb areas into these surrounding representations that was accompanied by enhanced motor improvements. In contrast, CS/RT directed at the interior of the forelimb map resulted in less expansion of forelimb areas into surrounding parts of the motor map and no behavioral benefit relative to rehabilitative training alone (Boychuk et al., 2009). It is possible the four contacts from the 2x2 configuration provided adequate stimulation of the distributed (more peripheral) areas of motor cortex such that the nine sites in the 3x3 configuration offered no additional benefit by having more independent sites of stimulation within this outer portion of the motor map. It is also possible that the additional 3x3-CS/RT contacts in the interior of the motor map are ineffective at recruiting non-forelimb areas during rehabilitation in same manner that Boychuk et al. (2009) observed with focal CS/RT. Another important consideration is that there may be an upper limit to the extent that stimulation of motor cortex can enhance motor improvements during rehabilitative training. It is possible that 3x3-CS/RT is driving

neuroplastic changes in a larger proportion of motor cortex than 2x2-CS/RT but resulting in similar behavioral benefits because of a ceiling effect.

CS/RT may be enhancing motor improvement by one or a combination of a number of processes including restoration of function within motor areas, reorganization of motor cortex, recruitment of areas outside of motor cortex, angiogenesis, glial-genesis, neuroprotection, or growth factor production. Rehabilitative training alone often induces a reorganization of motor cortex involving an expansion of movement representations when the training is specific to these representations (Nudo et al., 1996a). For example, a food-reaching task induces reemergence of distal forelimb representations (Nudo et al., 1996a). Several studies have found that CS/RT is associated with an enhanced expansion/reorganization of microstimulation evoked movement representations in ipsilesional motor cortex relative to training alone (Kleim et al., 2003, Plautz et al., 2003; Boychuk et al., 2009). CS/RT is also associated with increased synaptic density (Adkins et al., 2008) and enhanced synaptic responses (Teskey et al., 2003) within ipsilesional motor cortex that may support its functional reorganization. Further, animal studies have shown that electrical stimulation can induce the expansion/reorganization of movement representations in healthy motor cortex as well as increase synaptic densities and dendritic hypertrophy all in the absence of motor training (Teskey et al., 2002; Monfils et al., 2004; van Rooyen et al., 2006). Detailed electrophysiological measures in human stroke patients given CS/RT are limited due their invasiveness, however, Brown et al. (2006) noted that during intraoperative cortical mapping after CS/RT therapy individual finger representations were observed in some patients who were unable to perform the movements with

volitional effort. These data support that CS/RT is enhancing behavioral gains by driving functional reorganization of motor cortex above and beyond the remodeling that occurs with rehabilitative training alone. The MT's used to assign CS/RT current amplitudes also provide evidence that CS/RT induces functional reorganization. The minimum threshold to evoke movement is reduced across days of rehabilitation in animals receiving RT or CS/RT, however, CS/RT groups often exhibit the greatest reductions (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Teskey et al., 2003; Adkins et al., 2006; Boychuk et al., 2009). In the present study, significantly greater reductions in MT for the CS/RT condition relative to the RT condition were observed. These reductions in MT's suggest increased excitability in motor cortex and parallel the motor map changes observed with CS/RT (Monfils et al., 2005).

In the present study, CS/RT was associated with enhanced behavioral improvements in a rodent model of stroke. While it has previously been shown that distributed CS/RT is efficacious and focal CS/RT is not, the present work finds that increasing the number of stimulation sites within a distributed configuration imparts no additional benefit to a distributed configuration with few sites. This speaks to the robust effect of electrical stimulation of the cortex for enhancing functional improvement after stroke. These findings suggest motor cortex can respond to a small number of independent stimulation sites provided the sites are located in areas of motor cortex that can be recruited to facilitate disrupted motor patterns and behavior. This work lends further support for the use of cortical stimulation for magnifying the behavioral benefits of rehabilitation in human stroke patients.

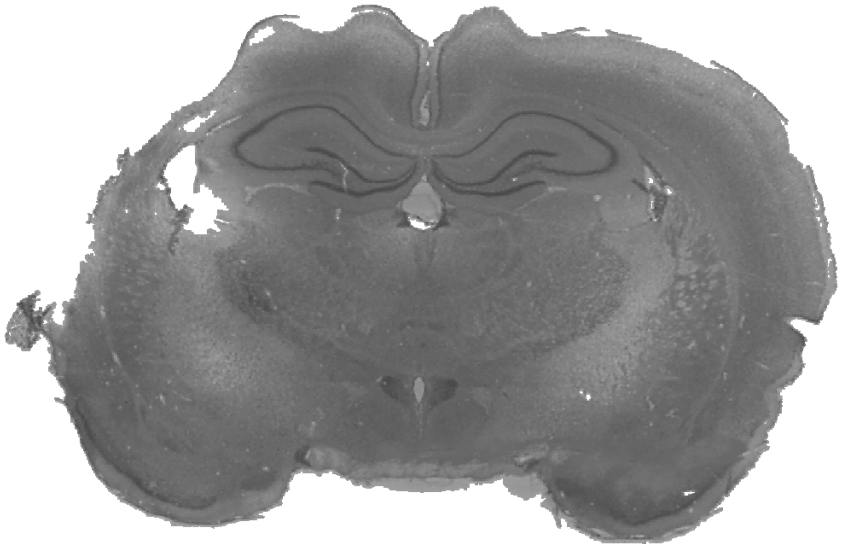

Figure 3-1. Representative Nissl stained coronal section from the MCAo lesion. The medial-lateral extent of brain damage at 24 days following ischemic insult by 4 µl injection of the vasoconstricting peptide ET-1 (0.2µg/µl) is shown.

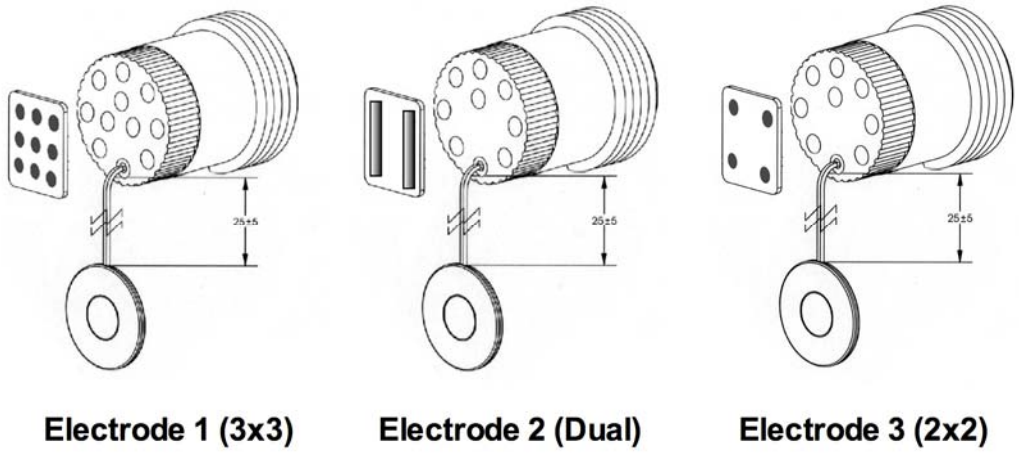

Figure 3-2. The three different electrode configurations examined. All contacts were 0.6 mm in diameter. The Dual Rail configuration consisted of two parallel 0.4 mm by 3 mm stainless steel strips separated by 2 mm. The 2x2 configuration consisted of four contacts positioned 2mm equidistant from one another each with a diameter of 0.60 mm. The 3x3 configuration consisted of 9 contacts positioned 1.1 mm equidistant from one another each with a diameter of 0.40 mm.

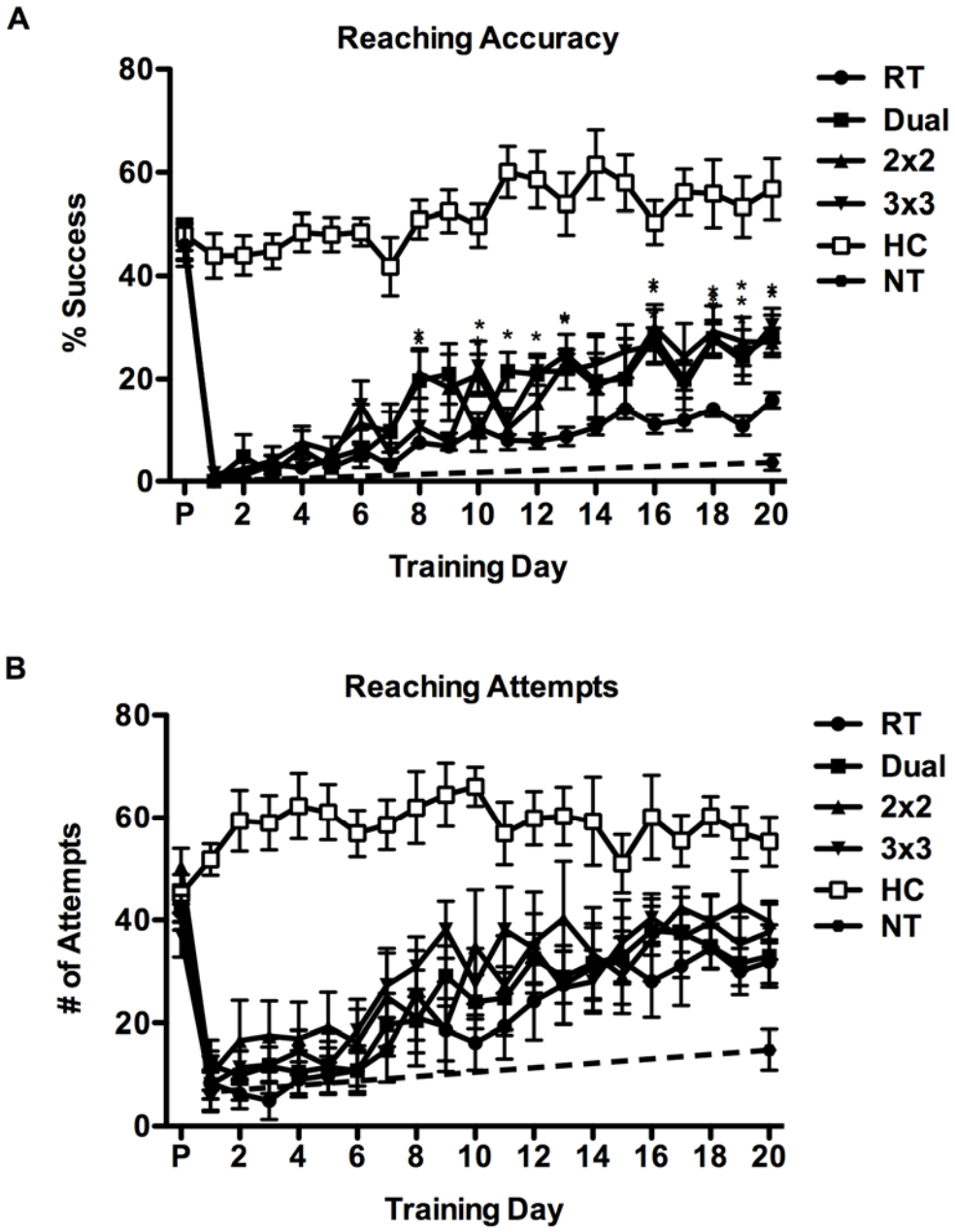

Figure 3-3. Reaching performance prestroke and during 20 days of rehabilitation. A) Mean (±SD) percent reach accuracy. All groups receiving rehabilitation performed significantly higher reaching accuracies than the NT group. All three CS/RT conditions had significantly higher reaching accuracies that RT Controls on rehabilitation days 16, 18-20 (Fisher's PLSD; $p<0.05$; indicated by the symbol "Θ"). B) Mean (±SD) number of reach attempts. Multiple comparisons (Fisher's PLSD; $p<0.05$) found that none of the groups with lesions were significantly different from one another on any day except that all groups given rehabilitation performed significantly more reach attempts than the NT group on day 20.

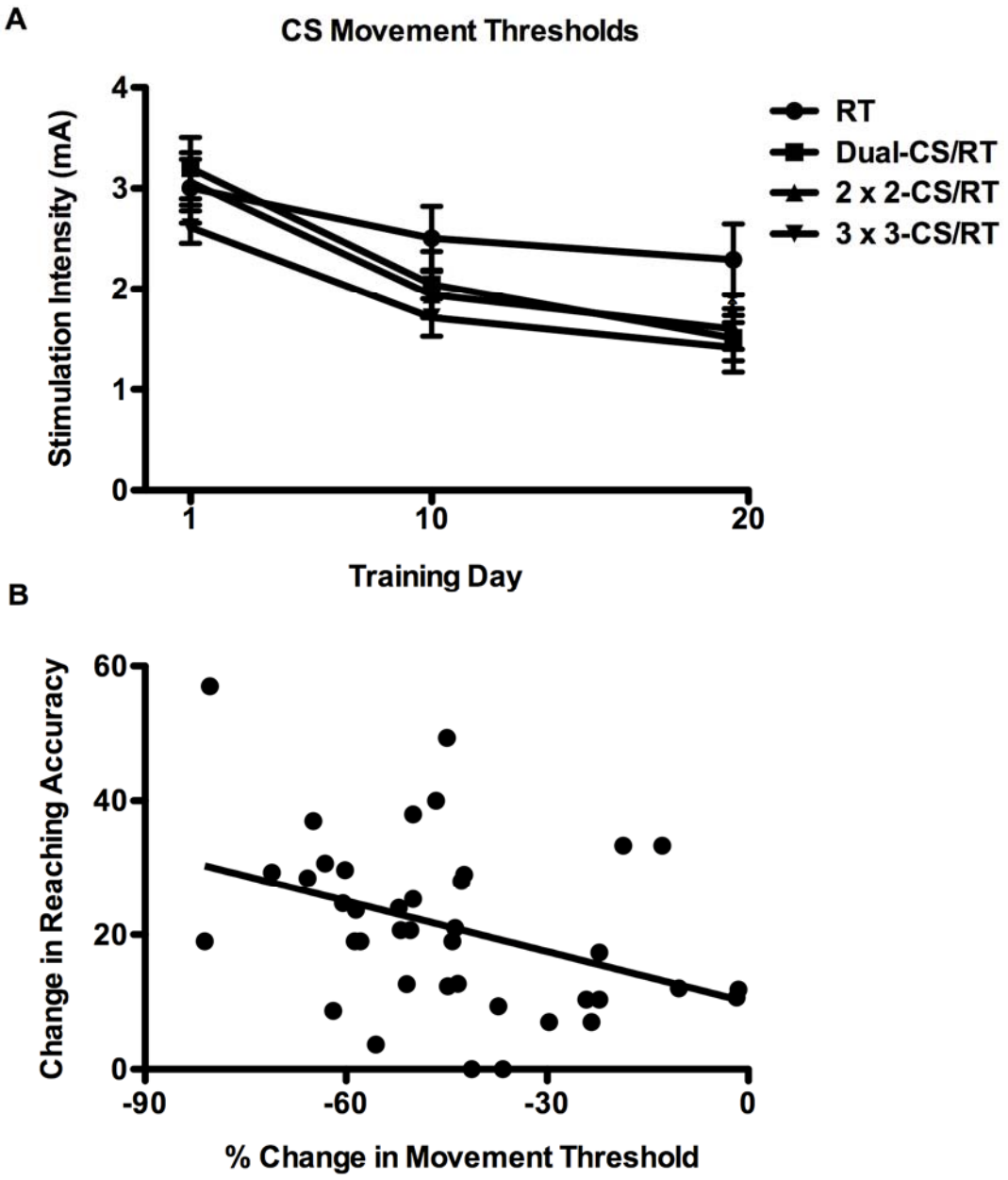

Figure 3-4. Mean (±SD) movement thresholds for animals in the RT and all CS/RT conditions during rehabilitation. The RT and all CS/RT conditions exhibited a significant decrease in threshold on the third assessment of movement threshold relative to the first (Fisher's PLSD; $p<0.05$). Further comparisons (Fisher's PLSD; $p<0.05$) found that the all CS/RT groups exhibited significantly larger decreases in the percent movement threshold between the first and third assessment relative to the RT group (Fisher's PLSD; $p<0.05$; indicated by the symbol "Θ"). B) A significant negative correlation was found between the percent reduction in movement threshold (MT3-MT1/MT1*100) and the increase in post rehabilitation reaching accuracy (Mean final 3 rehabilitation days – Mean first 3 rehabilitation days) [$r=-0.3861$, $p<0.01$].

Table 3-1. Estimate of spared cortical and subcortical tissue from experiment 2. Estimated volumes for cortical and subcortical tissue were analyzed as the percent affected to unaffected side (volume lesioned hemisphere/volume non lesioned hemisphere*100) in order to account for individual differences in brain size. Data is represented as mean percent ± SEM.

Condition	Sample Size	Estimates of Spared Tissue Volume	
		Cortical (% Contralesional)	Subcortical (% Contralesional)
HC	10	98±1	101±2
RT	8	85±2	91±1
Dual-CS/RT	10	84±1	91±1
2x2-CS/RT	10	83±2	89±2
3x3-CS/RT	10	84±1	92±1
NT	6	86±3	93±1

CHAPTER 4
CORTICAL STIMULATION WITH REHABILITATIVE TRAINING CAN ENHANCE MOTOR IMPROVEMENTS AFTER EARLY APPLICATION OF REHABILITATION ALONE IN A RODENT MODEL OF ISCHEMIA

Introduction

More than half of stroke patients exhibit motor deficits after stroke (Roth et al., 1998; Luke et al., 2004; Urton, et al., 2007). While some spontaneous recovery is observed in stroke survivors (Nakayama et al., 1994; Cramer, 2008) it is estimated that a majority of this population suffers from residual motor impairments (Urton, et al., 2007). Current rehabilitative strategies demonstrate some benefit on a population level, however, individual patterns of motor improvements after stroke are highly variable and typically incomplete (Gresham et al., 1995; Duncan et al., 2000). The inability to amerliorate persistent motor impairments in chronic stroke survivors reflects our lack of understanding on how to facilitate brain repair after injury. It is expected that more effective stroke treatments will develop as the mechanisms of stroke recovery are characterized.

Human patients with a high degree of motor improvements after cortical stroke typically exhibit a reorganization of spared primary motor cortex within the ipsilesional hemisphere (Rossini et al., 1998; Cramer et al., 2000; Wittenberg et al., 2003). In animal models, ischemic damage is associated with a loss of dendritic structure (Zhang et a., 2005) as well as a loss of synapses (Hasbani et al., 2001; Zhang et al., 2005; Murphy et al., 2008). Cortical ischemia is also associated with a loss of microstimulation-evoked movement representations within motor cortex (Nudo and Milliken, 1996; Friel et al., 2000). The loss of movement representations following cortical ischemia persists in the absence of rehabilitative training (Nudo et al., 1996b).

Appropriate rehabilitative training following cortical ischemia results in motor improvements and an expansion and reemergence of movement representations that were lost by the infarct (Nudo et al., 1996a; Kleim et al., 2003). The areas of motor cortex that exhibit the expansion and reorganization of movement representations also demonstrate increased synaptogenesis (Kleim et al., 2002a; Kleim et al., 2004). Synaptogenesis following cortical ischemia is associated with behavioral improvements that are though to arise from a reinstitution of cortical circuitry (Stroemer et al., 1995; Buonomano and Merzenich, 1998; Brown and Murphy, 2007). Indeed, adjuvant therapies that promote synaptic plasticity within motor cortex such as the administration of amphetamine (Stroemer et al., 1998; Adkins and Jones, 2005; Ramic et al., 2006; Papadopoulos et al., 2009) or nicotine (Gonzalez et al., 2005; Gonzalez et al., 2006) enhance rehabilitation-dependent improvements in motor performance after stroke. Administration of a type IV-specific phosphodiesterase inhibitor (PDE4) that stimulates synaptic plasticity in a cAMP/CREB dependent manner increases animals' skilled reaching ability and the size of forelimb movement representations following cortical ischemia (MacDonald et al., 2007).

Excitability in the central nervous system can also be altered by electrical stimulation (Blundon and Zakharenko, 2008). For example, several daily bouts of stimulation in sensorimotor cortex results in potentiated synaptic responses in this tissue in awake behaving animals (Trepel and Racine, 1998; Froc et al., 2000; Teskey et al., 2002; Monfils et al., 2004). These changes in synaptic efficacy may support the encoding of behavioral experience (Martin and Morris, 2002). Interestingly, synaptic potentiation in sensorimotor cortex has also been observed following skilled motor

learning (Rioult-Pedotti et al., 1998; Monfils and Teskey, 2004). The similarity in sensorimotor cortices' response to both electrical stimulation and behavioral experience suggest electrical stimulation can be used to facilitate the encoding of information within this tissue. Jackson et al. (2006) used a neural implant to demonstrate that repeated conditioning with a closed loop electronic device can alter the output of motor cortex by creating stable connections between previously unconnected areas within this cortical region.

Electrical stimulation of motor cortex combined with rehabilitative training (CS/RT) also enhances the motor improvements normally associated with rehabilitative training in rodent (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Teskey et al., 2003; Adkins et al., 2006; Adkins et al., 2008) and primate (Plautz, et al., 2003) models of stroke. The enhanced behavioral gains with CS/RT are paralleled by increased reorganization of movement representations within motor cortices of rodents (Kleim et al., 2003) and primates (Plautz et al., 2003). In addition to motor map changes, CS/RT is associated with an increased number of synapses (Adkins et al., 2008) as well as enhanced synaptic responses (Teskey et al., 2003). CS/RT was associated with enhanced motor improvements in a case report of a single human stroke patient (Brown et al., 2003). CS/RT also induced significant improvements relative to RT alone in a human phase I clinical trial with eight participants (Brown et al., 2006) and a phase II clinical trial with twenty four participants (Huang et al., 2008; Levy et al., 2008).

A recent phase III clinical trial with one-hundred and forty-six participants failed to show enhanced behavioral gains in patients receiving CS/RT relative to RT (Plow et al., 2009). An important difference between the preclinical animal studies and clinical trials

of CS/RT is the amount of rehabilitative experience prior to the onset of CS/RT. In the clinical trials humans stroke patients were recruited a minimum of four months post stroke after conventional rehabilitation therapies had been administered (Brown et al., 2003; Brown et al., 2006; Huang et al., 2008; Levy et al., 2008). In the preclinical animal studies, CS/RT has always been initiated within two weeks of experimental ischemia in the absence of any prior rehabilitative training (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Teskey et al., 2003; Plautz, et al., 2003; Adkins et al., 2006; Adkins et al., 2008). Prior rehabilitative training may limit subsequent behavioral gains by CS/RT by reducing the brain's capacity to reorganize in response to CS/RT. Motor training before CS/RT may also interfere with CS/RT's efficacy by reinforcing connections in motor cortex that do not support behavioral improvements induced CS/RT. While both rehabilitative training and CS/RT appear to induce motor improvements through similar mechanisms, eg., reorganization of movement representations in motor cortex, it is possible that differences in their neuroplastic responses may result in interference when RT is applied prior to CS/RT. Here, rodents given experimental ischemia were given sixteen days of rehabilitative training before a comparison was made between RT and CS/RT over an additional sixteen days.

Methods

Subjects

Thirty-one adult male Long-Evans hooded rats (350-420g) were housed (1 animal/cage) in standard laboratory cages. Animals were kept on a 12:12 hour light dark cycle throughout the experiment. All experimentation was conducted during the light cycle. Rats were maintained on Lab Diet 5001 (PMI Feeds, St. Louis, MO) and water ad libitum, and were handled and cared for in accordance with the National Institutes

Health Guide for the Care and Use of Laboratory Animals and with the approval of the University of Florida's Institutional Animal Care and Use Committee (IACUC).

Reach Training

Over the course of several days, all animals were placed on a restricted diet until they measured 90% of their original body weight. A brief period of pretraining was then given to familiarize the rats with the reaching task. Pretraining involved placing them into test cages (10 X 18 X 10 cm) with floors constructed of 2 mm bars, 9 mm apart edge to edge. A 4 cm wide and 5 cm deep tray filled with food pellets (45 mg; Bioserv) was mounted on the front of the cage. The rats were required to reach outside the cage and retrieve pellets from the tray. Rats were permitted to use either limb and the preferred limb was noted for each animal. All rats remained in pretraining until they had successfully retrieved 10 pellets (approximately 1 hour/day for 2 days). After pretraining, the rats were placed into a Plexiglas cage (11 cm X 40 cm X 40 cm) with a 1 cm slot located at the front of the cage. Animals were trained for 15 minutes each day to reach with their preferred limb through the slot and retrieve food pellets from a table outside the cage (Whishaw and Pellis, 1990). Each session was videotaped and later used to assess reaching performance. A successful reach was scored when the animal grasped the food pellet, brought it into the cage and to its mouth without dropping the pellet. The percentage of successful reaches [(# successful retrievals/the total # of reaches) x 100] was then calculated. All training sessions were video taped and used to measure reaching accuracy. Animals were trained for approximately two weeks on the reaching task to establish a baseline measure of motor performance. Baseline was defined as the average accuracy across the 3 final days of training. Animals failing to achieve a mean reaching accuracy of 40% across 3 consecutive days were not used in the study.

Prior to surgery, all animals were sorted by their prelesion reaching performance to create groups with comparable baseline levels of reaching accuracy. This was done to ensure that reaching performance prior to infarction was similar across conditions. The thirty-one animals were divided into four groups. A group of animals that never received the experimental lesion was included as Healthy Controls (HC; n=8) and was given training on the same skilled reaching task on all days of rehabilitation (32 days). A second group that received the stroke model but did not receive rehabilitative training was included as non-trained controls (NT; n=7). The post injury reaching behavior of the NT group was assessed by probe trials on days 1 and 32 of motor training. The remaining animals (n=16) received the stroke model and subsequent rehabilitative training in two phases (16 days plus 16 days).

Infarction

Following the 2 weeks of motor training, focal ischemic damage was given to the lateral aspect of sensorimotor cortex by temporary occlusion of the middle cerebral artery (MCAo). Briefly, animals were anesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.p.). Animals received low levels of isofluorane (0.15%) and supplemental doses of ketamine (20 mg/kg i.p.) as needed. Under sterile conditions, an incision was made midline and the skull exposed. A small burr hole was made in the hemisphere contralateral to each animal's trained to allow a 3 µL injection of sterile saline and the vasoconstricting peptide endothelin-1 (ET-1: 0.2µg/µL; American Peptide, Sunnyvale, CA) via the Nanolitre injection system (World Precision Instruments, Sarasota, Fl) controlled by the SYS-Micro 4 Controller (World Precision Instruments, Sarasota, Fl). Stereotaxic coordinates of the injection site with respect to bregma were as follows: anteroposterior, +0.9 mm; mediolateral, -5.2mm; and

dorsoventral, -8.7 mm (Biernaskie and Corbett 2001). ET-1 (240 pMol dissolved in 0.9% sterile saline) was injected at a rate of 7nL/sec through a glass pipette and the pipette was left in the injection site for 5 minutes to avoid backflow. After the MCAo surgery all animals were returned to their home cage for three days where they were supervised for health concerns but otherwise left to recover. Animals were given full access to food until the last day where the food restriction regimen (see above) was reimplemented.

Rehabilitative Training

To examine the effect of prior rehabilitative experience on CS/RT's ability to enhance motor improvements, the animals from the remaining group, i.e., animals not placed into the HC or NT conditions, were given two phases of post injury motor training. In the first phase these animals received rehabilitative training with their affected limb on the skilled reaching task for twenty minutes/day for sixteen days. The behavior of this group was considered stable in the last five days of the sixteen-day period because no significant improvements in reaching performance were observed (days 12-16) (Fisher's PLSD; $p<0.05$). On the seventeenth day, all animals in this group were given a second surgery where surface cortical electrodes were implanted over motor cortex. The implanted animals were then divided so that half continued to receive rehabilitative training (RT; n=8), while the other half received cortical stimulation and rehabilitative training (CS/RT; n=8), each for an additional sixteen days. Animals from the third group were divided into the RT and CS/RT conditions by their reaching performance on the last three days of the first sixteen days of rehabilitation. This sorting was performed in order to create two groups with comparable levels of reaching accuracy. All sessions were video taped for analysis of reaching accuracy and number of reach attempts.

Cortical Electrode Implantation

In the second surgery a craniotomy was made over the motor cortex in the same hemisphere as the ischemic injury. The craniotomy was made between 1 mm posterior to 5.5 mm anterior to bregma and 0.5 mm to 5.5 mm lateral to midline. For each RT and CS/RT animal, a nine-pin electrode carriage (Plastics One Inc., Roanoke, VA) with a dual rail contact configuration was implanted directly on motor cortex. The Dual Rail electrode configuration consisting of two parallel 0.4 mm by 3 mm stainless steel strips separated by 2 mm was used to allow comparison to previous rodent studies of CS/RT (Adkins-Muir and Jones, 2003; Teskey et al., 2003; Adkins et al., 2006; Adkins et al., 2008). A return lead was fixed to the skull in a position posterior to Lambda and the craniotomy filled with gel foam. Both the electrode and gel foam were covered in non-exothermic PolyWave dental acrylic and cured with a brief pulse (50 seconds) of ultraviolet light. The electrode was then fixed to skull screws with standard dental acrylic and then dental cement was applied on top of the dental acrylic. The skin was sutured and given topical antibiotics and the animals were given 4cc of warm ringers solution (s.c.) and metacam (0.10 mg/kg; s.c.). After the implant surgery all animals were returned to their home cage for three days where they were supervised for health concerns but otherwise left to recover. Animals were given full access to food until the last day where food restriction regimen (see above) was reimplemented.

Movement Thresholds

After three days, the animals had their individual motor thresholds (MT's) determined. MT's were assessed for RT and CS/RT animals on rehabilitation days 17, 25 and 32 (days 1, 9 and 16 of the second phase). MT's were defined for each animal as the minimum current to cause an involuntary motor response. The animals were

placed into a transparent cylinder and observed while 3-second trains of 1 millisecond, 100 Hz, monoploar cathodal pulses were given. Current was gradually increased by 5% increments until a movement of the contralateral forelimb could be clearly detected. Cortical stimulation during the post injury motor training phase was then delivered at 50% of each animal's MT during rehabilitation for the CS/RT condition.

Cortical Stimulation and Rehabilitation Training (CS/RT)

Animals receiving the combination of cortical stimulation and rehabilitative training were stimulated via the Vertis Stimulation System during the twenty-minute training sessions. The cortical electrode was connected to a remote stimulator suspended above the training cage where information was then sent wirelessly to the rest of the system. CS/RT was delivered as monopolar cathodal stimulation and was administered continuous with a frequency of 100 Hz with a current intensity dictated by the subject's movement threshold (see above). Each pulse was biphasic, charged balanced and asymmetric consisting of a square phase lasting 100 $\pm$ 10 microseconds and a decaying exponential phase lasting ~9900$\pm$ 10 microseconds.

Histology and Lesion Verification

Following the rehabilitation phase, the animals were given an overdose of pentobarbital and then transcardially perfused with 0.1 M sodium phosphate buffer followed by 4% paraformaldehyde solution in the same buffer. Brains were then extracted and post fixed in 4% paraformaldehyde solution in 0.1 M sodium phosphate buffer. Serial 50 μm coronal sections were then taken using a microtome. Ten sections spaced 600 μm apart and spanning approximately 2.7 mm anterior and 3.3 mm posterior to bregma were sampled for lesion verification. The same number of sections was analyzed for each animal. The sampled sections were stained with Toluidine blue

(a Nissl stain) and digitally scanned (Espon Perfection V500 Photo Scanner, Long Beach, CA) for lesion verification. The area of spared tissue, characterized by consistent Nissl staining, was traced using Image J software (Abramoff et al., 2004; Rasband, 2009) and cortical volumes were estimated with the Cavalieri's unbiased estimator method using the formula:

$$volume = d\left(\sum_{i=1}^{n} \left(y_i\right)\right) - (t)y_{\max}$$

Where "y_i" is the cross sectional area of the "ith" section through the morphometric region, "d" is the distance between sections (600 μm) and "n" is the total number of sections (12). "y_{max}" is maximum value for the area of one section and "t" is the section thickness (50 μm) and their product is subtracted from the basic question as a correction for the overprojection (Gundersen 1986; Gundersen and Jensen, 1987; Mayhew 1992). Estimated volumes for cortical and subcortical tissue were analyzed as the percent affected to unaffected side (volume lesioned hemisphere/volume non lesioned hemisphere*100) in order to account for individual differences in brain size.

Results

Reaching Accuracy

A repeated measures ANOVA with CONDITION as a between subject factor and TIME as a within subject factor that included all conditions revealed a significant CONDITION x TIME interaction [$F(6,54)= 20.9405$; $p<0.0001$] on reaching accuracy (Figure 3-3A). A repeated measures ANOVA with CONDITION as a between subject factor and TIME as a within subject factor that excluded the NT condition revealed a significant CONDITION x TIME interaction [$F(64,672)= 4.3392$; $p<0.0001$] on reaching accuracy. Subsequent multiple comparisons (Fisher's PLSD; $p<0.01$) revealed that the

NT, RT and CS/RT groups had significantly lower reaching accuracies on day 1 of rehabilitation in comparison with pre-stroke levels and with the performance of the HC group. The NT condition did not significantly change its reaching accuracy between post injury training days 1 and 32. Further comparisons (Fisher's PLSD; $p<0.05$) showed that while all animals receiving rehabilitation demonstrated significant increases in reaching accuracy during the 32 days of rehabilitation, the animals receiving CS/RT had significantly higher reaching accuracies than RT Controls on days 26 and 28-32. The CS/RT condition's reaching accuracy was not significantly different from healthy controls on days 21, 24, 26 and 28-32 whereas the RT condition was not significantly different from healthy controls only on day 21 (Fisher's PLSD; $p<0.05$). No significant improvements were seen in the RT condition after 10 days of rehabilitation. In contrast, significant improvements were observed in the CS/RT condition through 25 days of rehabilitation (Fisher's PLSD; $p<0.05$). These results demonstrate that while rehabilitation following ischemic injury induced improvements in reaching accuracy, CS/RT can enhance motor improvements after early application of rehabilitation alone.

Reaching Attempts

A repeated measures ANOVA with CONDITION as a between subject factor and TIME as a within subject factor that included all conditions revealed a significant CONDITION x TIME interaction [$F(6,54)= 3.3090$; $p<0.01$] on the number of reach attempts (Figure 3-3B). A repeated measures ANOVA with CONDITION as a between subject factor and TIME as a within subject factor that excluded the NT condition revealed a significant CONDITION x TIME interaction [$F(64,672)= 1.3591$; $p<0.05$] on the number of reach attempts. Subsequent multiple comparisons (Fisher's PLSD; $p<0.01$) revealed that the NT, RT and CS/RT groups had significantly lower numbers of

reach attempts on day 1 of rehabilitation in comparison with pre-stroke levels and with the performance of the HC group. Further comparisons (Fisher's PLSD; $p<0.05$) found that the RT and CS/RT groups were not significantly different from one another on any day. On day 32 of training the NT, RT and CS/RT performed similar numbers of reach attempts (Fisher's PLSD; $p<0.05$). The RT condition demonstrated a significantly smaller number of reach attempts than the HC condition on days 1-13, 16-18, and 23-24 (Fisher's PLSD; $p<0.05$). The CS/RT condition demonstrated a significantly smaller number of reach attempts than the HC condition on days 1-13, 16-18, 20 and 26-27 (Fisher's PLSD; $p<0.05$). No significant improvements in the number of reach attempts were seen in the RT condition after 20 days of rehabilitation whereas no significant improvement were observed in the CS/RT condition after 24 days (Fisher's PLSD; $p<0.05$). The NT condition also performed a significantly higher number of reach attempts between the assessments made on days 1 and 32 (Fisher's PLSD; $p<0.05$). These results demonstrate that the RT and CS/RT groups performed similar numbers of reach attempts and that the NT, RT and CS/RT groups demonstrated significant increases in the number of reach attempts in the final days of the study.

Movement Thresholds

A repeated measures ANOVA with CONDITION and TIME showed a significant CONDITION x TIME interaction [$F(1,15) = 43.7268$; $p<0.001$] on mean movement threshold (Figure 3-4A). Animals in the RT and CS/RT conditions showed a significant decrease in MT's as training continued: RT: [$T(7) = -5.26512$; $p<0.001$] and CS/RT [$T(7) = -5.80717$; $p<0.001$]. While the MT's for the two conditions were not significantly different on the first day of assessment, the thresholds were significantly smaller in the CS/RT relative to the RT condition on the second assessment (Fisher's PLSD; $p<0.05$).

Furthermore, the CS/RT condition exhibited a significantly larger percent reduction in MT compared to the RT condition [$F(1,14) = 8.8931$; $p<0.01$]. Finally, a significant negative correlation was found between the percent reduction in MT (MT2-MT1/MT1*100) and the increase in post rehabilitation reaching accuracy (Mean final 3 rehabilitation days – Mean first 3 rehabilitation days) [$r=-0.5433$, $p<0.05$] (Figure 3-4B). This demonstrates that the combination of CS/RT was associated with the greatest reductions in MT relative to RT and reductions in MT's are significantly correlated with increased motor improvements following cortical ischemia.

Estimation of Remaining Tissue

A one-way ANOVA revealed a significant main effect of CONDITION on the estimates of remaining cortical tissue [$F(3,27) = 5.7673$; $p<0.05$]. Subsequent multiple comparisons (Fisher's PLSD; $p<0.05$) showed that the amount of residual cortical tissue was similar for all conditions except that the HC condition exhibited a significantly greater amount of remaining cortical tissue than all other conditions (Table 4-1). A one-way ANOVA revealed no significant main effect of CONDITION on the estimates of remaining subcortical tissue [$F(3,27) = 2.6208$; $p>0.05$]. Subsequent multiple comparisons (Fisher's PLSD; $p<0.05$) showed that the HC condition exhibited a significantly greater amount of remaining subcortical tissue than all other conditions except the RT condition. All of the groups that received experimental lesions (RT, CS/RT and NT) exhibited similar amounts of remaining subcortical tissue (Fisher's PLSD; $p<0.05$).

Discussion

The motor cortex is a locus for neuroplastic changes that are associated with motor improvements after cortical ischemia. Motor training after cortical ischemia results

in motor improvements along with an expansion and reemergence of movement representations that were lost by the infarction (Nudo et al., 1996a; Kleim et al., 2003). The current experiment also observed motor improvements in animals that received rehabilitative training that were not observed in animals that received the same lesion without any post injury motor training. Several studies have observed magnified behavioral gains with CS/RT relative to RT (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Teskey et al., 2003; Plautz, et al., 2003; Adkins et al., 2006; Adkins et al., 2008). In the present experiment, CS/RT magnified motor improvements after experimental ischemia in animals that had already received prior rehabilitative training. These findings indicate the nervous system can be continually remodeled following stroke in ways that support further behavioral gains. The data also suggest that CS/RT can drive behavioral gains in human stroke patients who have already received standard forms of rehabilitation following stroke.

CS/RT is likely inducing enhanced motor improvements by driving functional reorganization of ipsilesional motor cortex. Electrical stimulation in the absence of motor training can induce the expansion of movement representations in healthy motor cortex as well as increase synaptic densities and dendritic hypertrophy (Teskey et al., 2002; Monfils et al., 2004; van Rooyen et al., 2006). CS/RT is associated with increased synaptic density (Adkins et al., 2008) and enhanced synaptic responses (Teskey et al., 2003) within ipsilesional motor cortex. Relative to RT alone, CS/RT also results in a greater expansion of microstimulation evoked movement representations in ipsilesional motor cortex (Kleim et al., 2003, Plautz et al., 2003; Boychuk et al., 2009). In the present study motor maps were not analyzed, however, MT's that measure the

minimum current necessary to evoke forelimb movement by CS/RT stimulation were recorded. The CS/RT condition was associated with a significantly smaller MT on the final day of assessment relative to the RT condition. Similarly, the CS/RT condition was associated with significantly greater percent reduction in MT across days of rehabilitation. While these MT's lack the spatial resolution of ICMS, they do reflect changes in cortical excitability (Monfils et al., 2005). These data support that motor improvements can be mediated by reorganization of motor cortex and that CS/RT can magnify these processes. Other neuroplastic changes that may support CS/RT's enhanced behavioral gains include angiogenesis, gliogenesis or glial activation, neuroprotection, or secretion of growth factors.

Here, 16 days of the combined therapy of CS/RT enhanced motor improvements above and beyond 16 prior days of RT alone in a rodent model of stroke. Previous studies have found magnified behavioral gains with CS/RT relative to RT, however, CS/RT has always been initiated without any prior rehabilitative experience (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Teskey et al., 2003; Adkins et al., 2006; Adkins et al., 2008). In the present study the RT condition exhibited no increase in reaching accuracy after 10 days of rehabilitation while the CS/RT condition increased reaching accuracy for 25 days of rehabilitation. These data indicate cortical stimulation can induce functionally relevant change in the nervous system after it has already responded to rehabilitative experience and argue that rehabilitative training does not exhaust or disrupt neuroplastic changes associated with CS/RT. The data also suggest that CS/RT will not be limited in human stroke patients if they have previously received rehabilitative training.

In the experiment CS/RT was shown to enhance motor improvements in a rodent model of MCAo. Previous CS/RT using rodent models of stroke have used focal ischemic damage within motor cortex (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Teskey et al., 2003; Plautz, et al., 2003; Adkins et al., 2006; Adkins et al., 2008). These results are some of the first evidence that CS/RT can be applied to motor cortex to induce motor improvements when ischemic injury is located in remote, lateral cortex (see Chapters 3 and 5). It is not clear how CS/RT enhances motor improvements following MCAo. CS/RT may be inducing greater behavioral gains by the same mechanisms that have been observed with CS/RT after focal ischemic damage to motor cortex. It is known that MCAo disrupts forelimb movement representations within motor cortex (Gharbawie et al., 2005a; Gharbawie et al., 2008) as well as skilled reaching behavior (Gharbawie et al., 2005b; Gharbawie et al., 2008). Given that CS/RT is known to promote the reemergence of movement representations (Kleim et al., 2003; Plautz et al., 2003), in particular forelimb representations (Boychuk et al., 2009) within spared motor cortex, it is possible that CS/RT can cause enhanced motor improvements by driving reorganization of motor cortex after MCAo as well. The present study's association with CS/RT and enhanced reductions in movement threshold demonstrate a striking similarity to the same phenomenon observed with CS/RT after focal damage to motor cortex. In both cases, there appears to be changes in cortical excitability that indicate reorganization of motor cortex.

CS/RT induced motor improvements in animals that had already received rehabilitative training alone indicating that the nervous system can be continually remodeled by appropriate interventions and experience. The present findings lend

further support for the use of CS/RT in enhancing behavioral gains in human stroke patients.

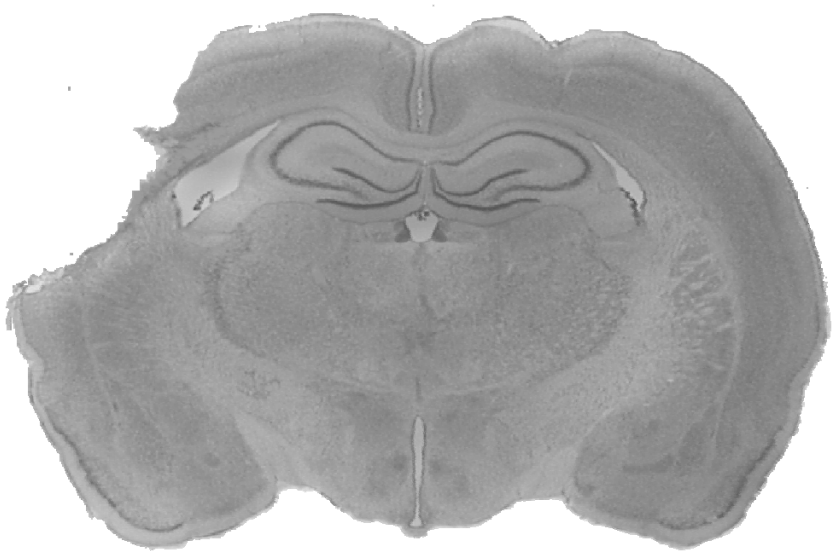

Figure 4-1. Representative Nissl stained coronal section from the MCAo lesion. The medial-lateral extent of brain damage at 40 days following ischemic insult by 3 µl injection of the vasoconstricting peptide ET-1 (0.2µg/µl) is shown.

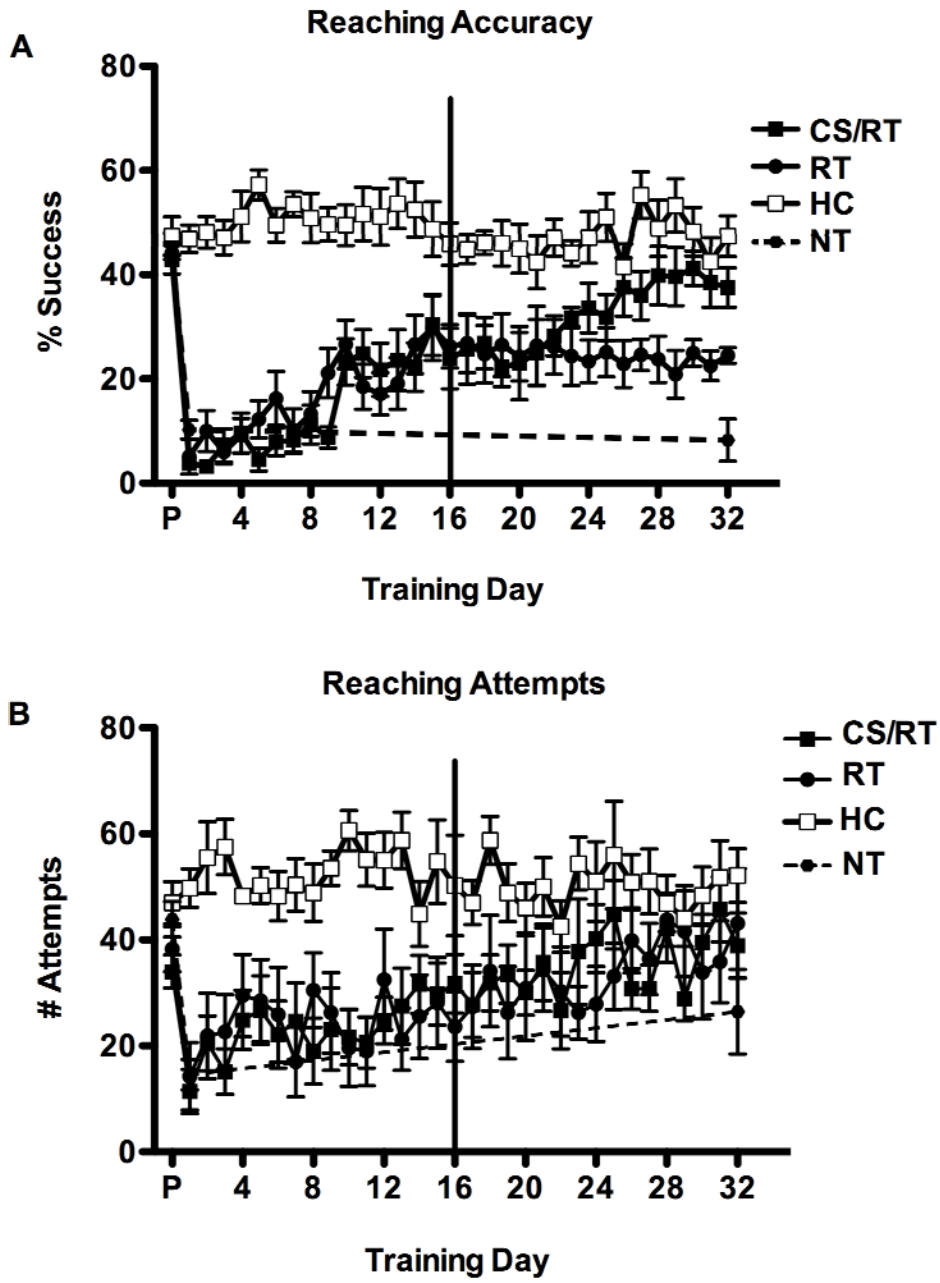

Figure 4-2. Reaching performance prestroke and during rehabilitation. A) Mean (±SD) percent reach accuracy both prestroke and across 20 days of rehabilitative training on the skilled reaching task. Multiple comparisons (Fisher's PLSD; $p<0.05$) showed that while all animals receiving rehabilitation demonstrated significant increases in reaching accuracy during the 32 days of rehabilitation, the animals receiving CS/RT had significantly higher reaching accuracies that RT Controls on days 26 and 28-32 (Fisher's PLSD; $p<0.05$; indicated by the symbol "Θ"). B) Mean (±SD) number of reach attempts both prestroke and across 20 days of rehabilitative training on the skilled reaching task. Further comparisons (Fisher's PLSD; $p<0.05$) found that none of the rehabilitation groups were significantly different from one another on any day.

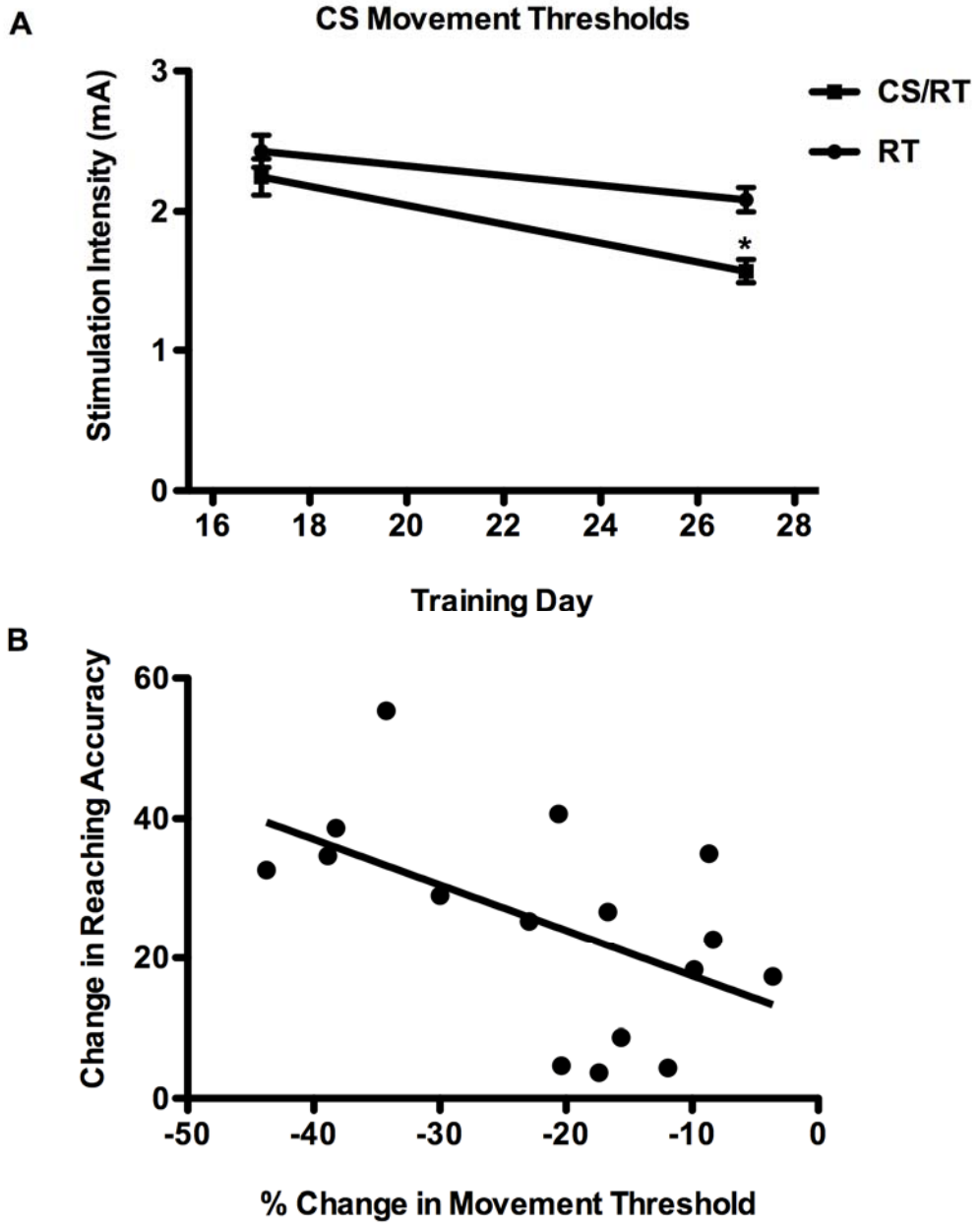

Figure 4-3. Movement thresholds during rehabilitation. A) Both the RT and CS/RT conditions showed a progressive decrease in mean (±SD) movement threshold as training continued. The CS/RT condition exhibited a significantly larger percent reduction in movement threshold compared to the RT condition ([$F(1,14) = 8.8931$; $p<0.01$] indicated by the symbol "Θ"). B) A significant negative correlation was found between the percent reduction in movement threshold (MT2-MT1/MT1*100) and the increase in post rehabilitation reaching accuracy (Mean final 3 rehabilitation days – Mean first 3 rehabilitation days) [$r=-0.5433$, $p<0.05$].

Table 4-1. Estimate of spared cortical and subcortical tissue from experiment 3. Estimated volumes for cortical and subcortical tissue were analyzed as the percent affected to unaffected side (volume lesioned hemisphere/volume non lesioned hemisphere*100) in order to account for individual differences in brain size. Data is represented as mean percent ± SEM.

Condition	Sample Size	Estimates of Spared Tissue Volume	
		Cortical (% Contralesional)	Subcortical (% Contralesional)
HC	8	99±2	100±1
CS/RT	8	90±1	93±1
RT	8	89±2	94±3
NT	7	92±2	93±1

CHAPTER 5
CORTICAL STIMULATION DOES NOT ENHANCE MOTOR FUNCTION AFTER SUBCORTICAL STROKE

Introduction

In the United States approximately 795, 000 new or recurrent strokes occur each year (American Heart Association, 2009). The nature and severity of the functional deficits observed after stroke are widely varied and reflect the fact that infarctions occur virtually anywhere in the brain (Tekin et al., 2002; Saczynski et al., 2009). Because of the cerebral cortex represents the largest brain area, most strokes involve loss of cortical tissue. However, more than 20% of all ischemic stroke cases involve damage to subcortical structures (Bryan et al., 1999; Saczynski et al., 2009). Many of the motor impairments present after subcortical stroke occur as a result of damage to descending white matter connections (Norrving, 2003; Arakawa et al., 2006). Indeed, stroke damage in the posterior region of the internal capsule has been associated with relatively severe impairments and little restoration of motor function (Morecraft et al., 2002; Lie et al., 2004; Wenzelburger et al., 2005). In some cases, upper extremity impairments show less functional improvement after damage within the posterior limb of the internal capsule than after damaged to motor cortex (Shelton and Reding, 2001; Schiemanck et al., 2008). Recent advances in MR imaging have allowed measurement of the degree of damage in white matter in human stroke patients based on impairments of water diffusion within these tracts (Werring et al., 2000). Reduced fractional anisotropy, one measure of axonal integrity, within the first weeks after stroke correlates with further loss of fractional anisotropy and motor function in patients with poor motor outcome (Møller et al., 2007). More symmetrical bilateral fractional anisotropy after stroke damage with the coronal radiata or internal capsule is correlated with improved

motor function at a three-month assessment (Jang et al., 2005). MR spectroscopic measurements of the neuronal marker compound N-acetyl aspartate in the posterior limb of the internal capsule found that the extent the stroke damage intersected with motor pathways exhibited a stronger relationship with the resulting motor deficit than the total size of the lesion (Pineiro et al., 2000). Functionally, the absence of TMS responses within the first forty-eight hours is associated with complete hand palsy (Pennisi et al., 1999).

In patients with some motor recovery, there is also evidence for cortical reorganization after subcortical damage within the corticospinal tract. For instance, there is a positive correlation between the extent of subcortical CST damage and the recruitment of the contralesional motor and sensory cortices (Schaechter et al., 2008). The recruitment of secondary motor areas in the contralesional hemisphere is also associated with motor improvements in cases of subcortical stroke involving white matter damage (Ward et al., 2003; Ward et al., 2006) including capsular stroke (Gerloff et al., 2006; Lotze et al., 2006). Motor improvements after ischemic damage to subcortical white matter have also been associated with the recruitment of several ipsilesional primary and secondary motor areas (Loubinoux et al., 2003). Further, Weiller et al. (1993) found a ventral shift in PET activation of motor cortex during a hand movement of stroke patients with damage to the posterior limb of the interior capsule. While studies of human patients with subcortical white matter damage suggest ipsilesional reorganization of motor areas may have a functionally significance, the similarity to functional reorganization of motor cortex following cortical ischemia is not known. Similarly, it is not clear whether stroke injuries to subcortical white matter will

respond to the same treatments as stroke injuries to cortex. The unique injury profiles of cortical and subcortical stroke suggest that the underlying mechanisms of recovery/compensation may not be the same. A greater of the understanding of the importance of lesion location will help guide the appropriate selection of treatments.

Stroke patients with good recovery can demonstrate fMRI activations patterns with reorganized activity around the central sulcus (Cramer, 2000). Cortical stroke followed by a high degree of motor recovery exhibits an enlargement and posterior shift of the sensorimotor areas (Rossini et al., 1998). Patients given constraint-induced movement therapy after cortical stroke exhibit decreased PET activation during motor tasks and increased TMS evoked motor maps in the ipsilesional hemisphere (Wittenberg et al., 2003). In animal studies, ischemic damage to motor cortex results in a loss of synapses (Hasbani et al., 2001; Zhang et al., 2005; Murphy et al., 2008) and a loss of ICMS evoked movement representations (Nudo and Milliken, 1996; Friel et al., 2000). In the absence of rehabilitative training the majority of lost motor representations do not reappear and the topography of the remaining map is not significantly altered (Nudo and Milliken, 1996; Friel et al., 2000). Appropriate motor training after cortical ischemia results in motor improvements and an expansion and reemergence of movement representations (Nudo et al., 1996a; Kleim et al., 2003). The reemergence of perilesional motor maps is also associated with synaptogenesis (Kleim, 2009). Thus, cortical ischemia results in a loss of movement representations that are likely restored by rehabilitative training through synaptic plasticity including the formation of new synapses (Stroemer et al., 1995; Buonomano and Merzenich, 1998; Kleim et al., 2002a; Kleim et al., 2004; Brown and Murphy, 2007). It is unclear whether these same forms of

cortical plasticity also occur in response to motor rehabilitation after subcortical stroke Animal studies comparing neuroplastic changes within motor cortex after cortical versus subcortical stroke are lacking because most methods for experimentally inducing stroke involve damage to the cortex.

In addition to the potentially different neural substrates for both motor impairment and functional improvement after cortical versus subcortical stroke, these two different lesions may respond differentially to plasticity promoting, adjuvant therapies. Several adjuvant therapies that promote synaptic plasticity within motor cortex such as amphetamine (Stroemer et al., 1998; Adkins and Jones, 2005; Ramic et al., 2006; Papadopoulos et al., 2009) and nicotine (Gonzalez et al., 2005) have magnified behavior improvements in models of cortical ischemia. Further, enhanced motor improvements and expansions in forelimb movement representations have been demonstrated after cortical ischemia by pharmacological stimulation of intracellular pathways that participate in synaptic plasticity (MacDonald et al., 2007). Electrical stimulation of sensorimotor cortex increases synaptic efficacy (Trepel and Racine, 1998) and expands movement representations (Teskey et al., 2002; Monfils et al., 2004). In animal models of cortical ischemia electric stimulation of motor cortex during rehabilitative training (CS/RT) enhances behavioral improvements (Adkins-Muir and Jones, 2003; Teskey et al., 2003; Adkins et al., 2006; Adkins et al., 2008). CS/RT also results in further expansion and reorganization of movement representations (Kleim et al., 2003; Plautz, et al., 2003; Boychuk et al., 2009). In addition, CS/RT results in a greater increase in the number of synapses (Adkins et al., 2008) as well as enhanced synaptic responses (Teskey et al., 2003). However, none of these treatments have

been used to enhance motor performance after subcortical stroke. Specifically, all of the animal studies have examined the efficacy of CS/RT after cortical stroke where stimulation is being applied to both damaged and healthy tissue. This is an important clinical question given the recent finding that CS/RT failed to confer enhanced motor improvements in a large population of stroke patients (Plow et al., 2009). An important difference between the basic and clinical studies is the location of ischemic damage. Clinical studies of CS/RT have enrolled patients with cortical and capsular ischemic damage (Brown et al., 2006; Levy et al., 2008). In contrast, preclinical animal studies have used focal cortical models of ischemia (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Teskey et al., 2003; Adkins et al., 2006; Boychuk et al., 2009). Without animal studies of subcortical white matter stroke to compare the effects of CS/RT or even more generally the functional reorganization patterns to cortical stroke damage the efficacy of CS/RT after capsular infarct is not known. Here, a model of predominantly cortical stroke and a model of subcortical capsular stroke were used to assess how lesion location impacts the benefits of combined CS/RT therapy.

Methods

Subjects

Forty adult male Long-Evans hooded rats (350-420g) were pair housed (2 animals/cage) in standard laboratory cages on a 12:12 hour light-dark cycle within the University of Florida's Communicore Research Building vivarium. All experimentation was conducted during the light cycle. Rats were maintained on Lab Diet 5001 (PMI Feeds, St. Louis, MO) and water ad libitum, and were handled and cared for in accordance with the National Institutes Health Guide for the Care and Use of Laboratory

Animals and with the approval of the University of Florida's Institutional Animal Care and Use Committee (IACUC).

Reach Training

Over the course of several days, animals were placed on a restricted diet until they measured 90% of their original body weight. A brief period of pretraining was then given to familiarize the rats with the reaching task. Pretraining involved placing them into test cages (10 X 18 X 10 cm) with floors constructed of 2 mm bars, 9 mm apart edge to edge. A 4 cm wide and 5 cm deep tray filled with food pellets (45 mg; Bioserv) was mounted on the front of the cage. The rats were required to reach outside the cage and retrieve pellets from the tray. Rats were permitted to use either limb and the preferred limb was noted for each animal. All rats remained in pretraining until they had successfully retrieved 10 pellets (approximately 1 hour/day for 2 days). After pretraining, the rats were placed into a Plexiglas cage (11 cm X 40 cm X 40 cm) with a 1 cm slot located at the front of the cage. Animals were trained for 15 minutes each day to reach with their preferred limb through the slot and retrieve food pellets from a table outside the cage (Whishaw and Pellis, 1990). Each session was videotaped and later used to assess reaching performance. A successful reach was scored when the animal grasped the food pellet, brought it into the cage and to its mouth without dropping the pellet. The percentage of successful reaches [(# successful retrievals/the total # of reaches) x 100] was then calculated. All training sessions were video taped and used to measure reaching accuracy. Animals were trained for approximately two weeks on this task to establish a baseline measure of motor performance. Baseline was defined as the average accuracy across the 3 final days of training. Animals failing to achieve a mean reaching accuracy of 40% across 3 consecutive days were not used in the study.

Animals were sorted by their prelesion reaching performance to create groups with comparable baseline levels of reaching accuracy. This was done to ensure that reaching performance prior to infarction was similar across conditions.

Infarction

Following the 2 weeks of motor training, focal ischemic damage was induced in either in the area of the proximal branches of the middle cerebral artery (MCA) or in the subcortical territory associated with the internal capsule (IC). Briefly, animals were anesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.p.). Animals received low levels of isofluorane (0.15%) and supplemental doses of ketamine (20 mg/kg i.p.) as needed. Under sterile conditions, an incision was made midline and the skull was removed overtop the appropriate injection site. The vasoconstricting peptide endothelin-1 (ET-1: 0.2µg/µL; American Peptide, Sunnyvale) was then injected either adjacent to the middle cerebral artery to induce cortical ischemia or adjacent to the internal capsule to induce subcortical white matter ischemia. ET-1 was delivered through a glass pipette via the Nanolitre injection system (World Precision Instruments, Sarasota, Fl) controlled by the SYS-Micro 4 Controller (World Precision Instruments, Sarasota, Fl). Stereotaxic coordinates of the injection site for the middle cerebral artery occlusion (MCAo) were: anteroposterior, +0.9 mm; mediolateral, -5.2mm; and dorsoventral, -8.6 mm with respect to bregma (Biernaskie and Corbet 2001). Stereotaxic coordinates of the injection site near the internal capsule were: anteroposterior, +0.9 mm; mediolateral, -5.2mm; and dorsoventral, -6.5 mm with respect to bregma (Frost et al., 2006). For the MCAo 3 µl of ET-1 (240 pMol dissolved in 0.9% sterile saline) were used while for the IC stroke model 1 µl of ET-1 (80 pMol dissolved in 0.9% sterile saline). For all injections, the ET-1 was delivered at a rate of 7nL/sec

through a glass pipette and the pipette was left in the injection site for 5 minutes to avoid backflow. After the surgery all animals were returned to their home cage for three days where they were supervised for health concerns but otherwise left to recover. Animals were given full access to food until the last day where food restriction regimen (see above) was reimplemented.

Cortical Electrode Implantation

Following the infarction procedure nine-pin electrode carriages (Plastics One Inc., Roanoke, VA) were implanted epidurally over sensorimotor cortex in the hemisphere contralateral to each animal's preferred paw. The surface electrode was placed directly over the entire exposed cortex between 1 mm posterior to 5 mm anterior to bregma and 0.5 mm to 5.5 mm lateral to midline. A return lead was fixed to the skull in a position posterior to Lambda and the craniotomy filled with gel foam. Both the electrode and gel foam were covered in non-exothermic PolyWave dental acrylic and cured with a brief pulse (50 seconds) of ultraviolet light. The electrode was then fixed to skull screws with standard dental acrylic and the animals were given 4cc of warm ringers solution (s.c.) and metacam (0.10 mg/kg; s.c.).

Movement Thresholds

After surgery all animals were returned to their home cage for three days where they were supervised for health concerns but otherwise left to recover. Animals were given full access to food until the last day where food restriction regimen (see above) was reimplemented. After the three days, animals with implants had their individual motor thresholds (MT's) determined and then animals were started in a motor rehabilitation paradigm. MT's were assessed on post lesion training days 1, 10 and 19. MT's were defined for each animal as the minimum current to cause an involuntary

motor response and were tested in all animals that had received a cortical electrode. The animals were placed into a transparent cylinder and observed while 3 second trains of 1 millisecond 100 Hz monopolar cathodal pulses were given. Current was gradually increased by 5% increments until a movement of the contralateral forelimb could be clearly detected. Cortical stimulation during the post injury motor training phase was then delivered at 50% of each animal's MT during rehabilitation for the CS/RT condition.

CS/RT and RT

Three days after surgery began receiving daily twenty-minute sessions of motor rehabilitation. Each session consisted of skilled reach training using the same training parameters described earlier. All sessions were video taped for analysis of reaching accuracy and number of reach attempts. Animals receiving the combination of cortical stimulation and rehabilitative training were stimulated via the Vertis Stimulation System during these sessions. The cortical electrode was connected to a remote stimulator suspended above the training cage where information was then sent wirelessly to the rest of the system. CS/RT was delivered as monopolar cathodal stimulation and was administered continuous with a frequency of 100 Hz with a current intensity dictated by the subject's movement threshold. Each pulse was biphasic, charged balanced and asymmetric consisting of a square phase lasting 100 + 10 microseconds and a decaying exponential phase lasting ~9900+ 10 microseconds. Half the animals that received MCAo were given rehabilitative training alone (MCA-RT; n=8) while the other half received the combination of cortical stimulation and rehabilitative training (MCA-CS/RT; n=8). Similarly, in the two groups that received subcortical ischemic damage near the internal capsule, half of the animals received rehabilitative training alone (Sub-RT; n=8) while the other half received the combination of cortical stimulation and rehabilitative

training (Sub-CS/RT; n=8). A group of animals that never received an infarction were included as Healthy Controls (HC; n=8) and were given training on the same skilled reaching task on all days of rehabilitation.

Histology and Lesion Verification

Following the rehabilitation phase the animals were given an overdose of pentobarbital and then transcardially perfused with 0.1 M sodium phosphate buffer followed by 4% paraformaldehyde solution in the same buffer. Brains were then extracted and post fixed in 4% paraformaldehyde solution in 0.1 M sodium phosphate buffer. Serial 50 µm coronal sections were then taken using a microtome. Ten sections spaced 600 µm apart and spanning approximately 2.7 mm anterior and 3.3 mm posterior to bregma were sampled for lesion verification. The same number of sections was analyzed for each animal. The sampled sections were stained with Toluidine blue (a Nissl stain) and digitally scanned (Espon Perfection V500 Photo Scanner, Long Beach, CA) for lesion verification. The area of spared tissue, characterized by consistent Nissl staining, was traced using Image J software (Abramoff et al., 2004; Rasband, 2009) and cortical volumes were estimated with the Cavalieri's unbiased estimator method using the formula:

$$volume = d\left(\sum_{i=1}^{n} \left(y_i\right)\right) - (t)y_{\max}$$

Where "y_i" is the cross sectional area of the "ith" section through the morphometric region, "d" is the distance between sections (600 µm) and "n" is the total number of sections (12). "y_{max}" is maximum value for the area of one section and "t" is the section thickness (50 µm) and their product is subtracted from the basic question as a correction for the overprojection (Gundersen 1986; Gundersen and Jensen, 1987;

Mayhew 1992). Estimated volumes for cortical and subcortical tissue were analyzed as the percent affected to unaffected side (volume lesioned hemisphere/volume non lesioned hemisphere*100) in order to account for individual differences in brain size.

Results

Reaching Accuracy

A repeated measures ANOVA with CONDITION as a between subject factor and TIME as a within subject factor revealed a significant CONDITION x TIME interaction [$F(80,700)= 2.1027$; $p<0.0001$] on reaching accuracy (Figure 4-1A). Subsequent multiple comparisons (Fisher's PLSD; $p<0.01$) revealed that groups given either experimental stroke had significantly lower reaching accuracies on day 1 of rehabilitation in comparison with pre-stroke levels and with performance levels of the HC group. No significant improvements were seen in the Sub-RT condition after 13 days of rehabilitation and in the Sub-CS/RT condition after 12 days of rehabilitation. No significant improvements were seen in the MCA-RT condition after 5 days of rehabilitation and in the MCA-CS/RT condition after 8 days of rehabilitation. The Sub-RT and Sub-CS/RT groups were not significantly different from one another on any day (Fisher's PLSD; $p<0.05$). In contrast, multiple comparisons of the MCA-RT and MCA-CS/RT groups found that the MCA-CS/RT performed significantly greater reaching accuracy than MCA-RT on days 8-9 and 18-20. The Sub-RT condition demonstrated significantly smaller percent reach accuracies than the HC condition on days 1-20. The Sub-CS/RT animals demonstrated significantly smaller percent reach accuracies than the HC condition on days 1-14 and 16-20. The MCA-RT group demonstrated significantly smaller percent reach accuracies than the HC condition on days 1-20. The MCA-CS/RT condition demonstrated significantly smaller percent reach accuracy than

the HC condition on days 1-15 and 17-20. These results demonstrate that while the combined therapy of CS/RT following MCAo magnified motor improvements, the combined therapy of CS/RT conferred no additional benefit following subcortical ischemia.

Reaching Attempts

A repeated measures ANOVA with CONDITION as a between subject factor and TIME as a within subject factor revealed a significant CONDITION x TIME interaction [$F(80,700)= 1.6819$; $p<0.001$] on the number of reach attempts (Figure 4-1B). Subsequent multiple comparisons (Fisher's PLSD; $p<0.01$) revealed that all groups that received lesions had significantly lower reaching accuracies on day 1 of rehabilitation in comparison with pre-stroke levels and with performance levels of the HC group. Further comparisons (Fisher's PLSD; $p<0.05$) found that the Sub-CS/RT and Sub-RT conditions performed significantly similar numbers of reach attempts on all days. Similarly, multiple comparisons found that the MCA-CS/RT and MCA-RT performed significantly similar numbers of reach attempts on all days. The Sub-RT condition demonstrated a significantly smaller number of reach attempts than the HC condition on days 1-20 while the Sub-CS/RT condition demonstrated a significantly smaller number of reach attempts than the HC condition on days 1-16 and 18-20. The MCA-RT condition demonstrated a significantly smaller number of reach attempts than the HC condition on days 1-13 and 16-20 while the MCA-CS/RT condition demonstrated a significantly smaller number of reach attempts than the HC condition on days 1-11, 13 and 18-20. No significant increases in reach attempts were seen in the Sub-RT condition after 11 days or in the Sub-CS/RT condition after 10 days of rehabilitation. No significant increases in reach attempts were seen in the MCA-RT condition after 8 days or in the MCA-CS/RT

condition after 9 days of rehabilitation. These results demonstrate that while all groups increased the number of number of reach attempts during rehabilitation, no differences in the number of reach attempts were observed between the RT and CS/RT conditions following subcortical ischemic damage near IC or MCAo.

Movement Thresholds

A repeated measures ANOVA with TIME revealed a significant effect of TIME [$F(6,56) = 17.5017$; $p<0.0001$] on mean movement threshold (Figure 4-2). Subsequent multiple comparisons (Fisher's PLSD; $p<0.01$) revealed that all conditions had significantly lower MT's on the third assessment relative to the first. No significant difference in MT was found between the Sub-CS/RT and Sub-RT conditions during any of the three assessments. In contrast, the MCA-CS/RT group exhibited a significantly lower MT relative to the MCA-RT group on the third threshold assessment. Further comparisons (Fisher's PLSD; $p<0.05$) found that all four groups exhibited significant decreases in the percent MT between the first and final assessment. Again, no significant difference was found between the Sub-CS/RT and Sub-RT conditions while the MCA-CS/RT condition exhibited a significantly greater percent decrease in MT relative to the MCA-RT condition (Fisher's PLSD; $p<0.05$). Multiple comparisons (Fisher's PLSD; $p<0.05$) also found the Sub-CS/RT and Sub-RT groups to have significantly higher thresholds relative to both the MCA-CS/RT and MCA-RT groups at all time points.

Estimation of Remaining Tissue

A one-way ANOVA revealed a significant main effect of CONDITION on the estimates of remaining cortical tissue [$F(4,35) = 10.3268$; $p<0.05$]. Subsequent multiple comparisons (Fisher's PLSD; $p<0.05$) showed that the amount of residual cortical tissue

was similar for the HC, Sub-RT and Sub-CS/RT conditions (Table 5-1). The amount of residual cortical tissue was also similar between the MCA-RT and MCA-CS/RT conditions (Fisher's PLSD; $p<0.05$). In addition, the HC, Sub-RT and Sub-CS/RT conditions exhibited a significantly greater amount of residual cortical tissue relative to the MCA-RT and MCA-CS/RT conditions (Fisher's PLSD; $p<0.05$). A one-way ANOVA revealed a significant main effect of CONDITION on the estimates of remaining subcortical tissue [$F(4,35) = 3.0039$; $p<0.05$]. Subsequent multiple comparisons (Fisher's PLSD; $p<0.05$) showed that the amount of residual subcortical tissue was similar for the HC, Sub-RT and Sub-CS/RT conditions. The amount of residual cortical tissue was also similar between the MCA-RT and MCA-CS/RT conditions (Fisher's PLSD; $p<0.05$). Finally, the HC, Sub-RT and Sub-CS/RT conditions exhibited a significantly greater amount of residual cortical tissue relative to the MCA-RT and MCA-CS/RT conditions (Fisher's PLSD; $p<0.05$).

Discussion

Animal studies have provided clear evidence that cortical stimulation in combination with motor rehabilitation can significantly enhance both motor performance and cortical plasticity after cortical ischemia (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Teskey et al., 2003; Plautz, et al., 2003; Adkins et al., 2006; Adkins et al., 2008). The present study examined the efficacy of this treatment for augmenting motor performance after subcortical ischemia. The animals received either a predominantly cortical infarct by MCAo or subcortical infarct involving damage to the internal capsule. Although both injury models resulted in forelimb motor impairments, motor improvements during twenty days of rehabilitative training were greater in the MCA-CS/RT condition than the Sub-CS/RT group. Indeed, the Sub-CS/RT animals showed

no significant benefit of CS/RT over rehabilitation training alone. This suggests that the efficacy of adjuvant therapies may be dependent upon lesion location.

Prior animal studies of CS/RT have modeled stroke by inducing focal ischemic damage within motor cortex (Adkins-Muir and Jones, 2003, Kleim et al., 2003; Teskey et al., 2003). The present finding that CS/RT can also enhance behavioral gains following MCAo has not been previously published but is supported by other experiments in this dissertation (see Chapters 3 and 4). The MCAo model in rodents results in a disruption of movement representations (Gharbawie et al., 2008) that is likely due to a loss of synapses and dendrites within motor cortex (Zhang et al., 2005). After ischemic damage within motor cortex the reemergence of perilesional motor maps following rehabilitative training is associated with synaptogenesis (Kleim, 2009). CS/RT after focal ischemic damage within motor cortex results in magnified motor map reorganization (Kleim et al., 2003; Plautz et al., 2003; Boychuk et al., 2009) and synaptogenesis (Adkins et al., 2008). Rehabilitative training after MCAo may result in similar motor map reorganization and synaptogenesis that can be facilitated by CS/RT in a similar fashion to what has been observed in the focal cortical models. Indeed, Stroemer et al. (1995) found an association between behavior improvements and synaptogenesis within sensorimotor cortex following MCAo that indicates a functional importance for restoring synaptic input in motor cortex.

Sparing of the CST is an important predictor of motor outcome following subcortical stroke (Pennisi et al., 1999; Pineiro et al., 2000; Werring et al., 2000). In the present study animals receiving subcortical lesions were given ischemic damage near the internal capsule. The integrity of the CST following ischemic insult near the internal

capsule was not directly measured but can be inferred by the animals' responses during MT assessments. Movement could be elicited in animals given the subcortical lesion, however, the amounts of current needed was markedly higher than in animals given the cortical lesion. These responses suggest the CST was intact but damaged in the subcortical lesion animals. Behaviorally, animals that received the subcortical lesion exhibited smaller initial impairments than animals that received the cortical lesions by MCAo. These behavioral results also suggest that the subcortical lesions did not eliminate all descending motor output through the internal capsule with the consideration that other motor tracts or uncrossed CST fibers from the unaffected hemisphere may have also contributed to the post injury reaching behavior.

There is no immediate explanation for the lack of enhanced behavioral gains with CS/RT after ischemic damage to the internal capsule. The motor impairments observed in the present study are supported by previous reports where focal ischemic damage to the internal capsule resulted in impairments in forelimb placing (Frost et al., 2006) and forelimb exploration within a cylinder (Lecrux et al., 2008). Injuries to the CST result in a loss of cells in motor cortex (Pernet and Hepp-Reymond, 1975; Hains et al., 2003) and a retraction of damaged pyramidal cell axons (Galea and Darian-Smith, 1997). Injury to the CST is also associated alterations in motor cortex pyramidal cells including cell shrinkage and a decrease in Nissl staining (Wannier et al., 2005). While ICMS has not been performed on a model of capsular infarct, motor mapping has been performed on a more downstream portion of the CST within the pyramid tracts. Complete unilateral lesions of the pyramid tract (pyramidotomy) results in impaired forelimb function and a loss of forelimb movement representations within motor cortex (Piecharka et al, 2005).

In contrast to cortical ischemic insult, rehabilitation after pyramidotomy does not result in reorganization of movement representations (Piecharka et al, 2005). Similarly, large ischemic lesions in primate motor cortex result in an expansion of movement representations within the secondary motor area PMv rather than within primary motor cortex (M1) (Nudo, 2007). In a primate model of stroke a linear relationship between increasing lesion size within M1 and increasing reorganization of PMv was observed (Dancause et al., 2005). Collectively, these studies suggest that post infarct reorganization of movement representations within motor cortex requires sufficient sparing of this motor area and its axonal projections. In the absence of sufficient remaining motor cortex, postinfarct reorganization of the motor system may depend on the recruitment of secondary motor areas outside of motor cortex. For example, human stroke patients with damage to the CST often demonstrate a recruitment of secondary motor areas (Ward et al., 2003; Ward et al., 2006). It is possible then that CS/RT was ineffective in enhancing behavioral improvements following experimental damage within internal capsule because its promotion of reorganization of movement maps within motor cortex was not as functionally relevant as recruitment of secondary motor areas. Even though the model of capsular infarct used here likely spared many pyramidal cells it is possible they were in insufficient quantities to support behavioral gains through reorganization of motor cortex.

Enhanced motor improvements with CS/RT have been observed in several animal studies but the mechanisms underlying the enhancement are not clear. Cortical stimulation may have neuroprotective, angiogenic, anti-inflammatory or growth factor-releasing properties (Baba et al., 2009). CS/RT is also associated with increased peri-

infarct reorganization of movement representations. Appropriate rehabilitative training following cortical ischemia results in motor improvements that are accompanied by an expansion and reemergence of movement representations that are specific to the training (Nudo et al., 1996b; Nudo, 2006) that is likely mediated by synaptic plasticity (Kleim et al., 2002; Kleim et al., 2004). Cortical stimulation is associated with greater reorganization of the motor map than RT alone (Kleim et al., 2003; Plautz et al., 2003; Boychuk et al., 2009). In particular, CS/RT results in increased distal forelimb representations that have a strong positive correlation with skilled reaching ability (Boychuk et al., 2009). CS/RT after cortical ischemia is also associated with a greater number of synapses (Adkins et al., 2008) and increased synaptic responses (Teskey et al., 2003) that likely support the increased reorganization of perinfarct motor maps.

The present findings further support the viability of CS/RT for enhancing motor function after cortical stroke. The results also demonstrate the importance of lesion location in CS/RT as cortical stimulation was ineffective in magnifying behavioral improvements in a model of subcortical ischemia involving white matter damage. Future clinical trials of CS/RT must consider the location of stroke to promote functional reorganization and motor recovery.

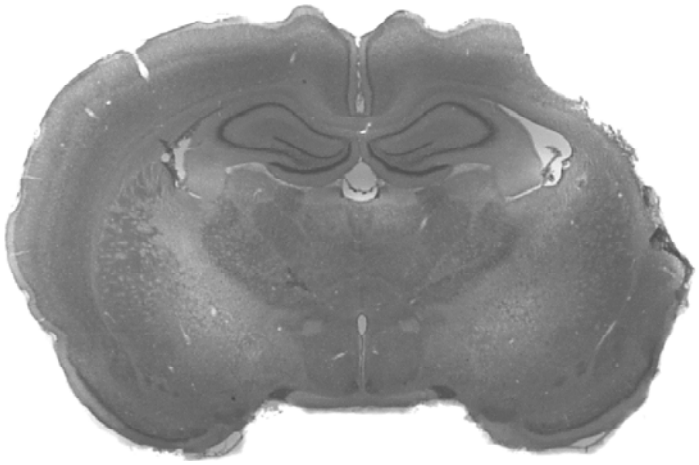

Figure 5-1. Representative Nissl stained coronal section from the MCAo lesion. The medial-lateral extent of brain damage at 24 days following ischemic insult by 3 µl injection of the vasoconstricting peptide ET-1 (0.2µg/µl) is shown.

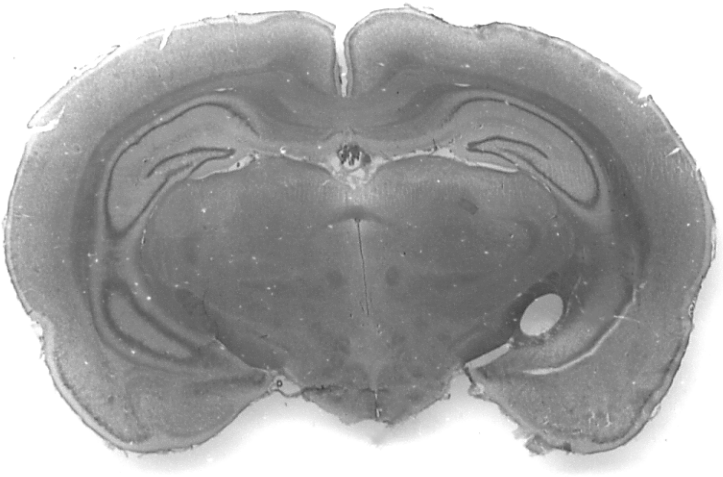

Figure 5-2. Representative Nissl stained coronal section from the capsular lesion. The subcortical white matter damage at 24 days following ischemic insult by 1 µl injection of the vasoconstricting peptide ET-1 (0.2µg/µl) is shown.

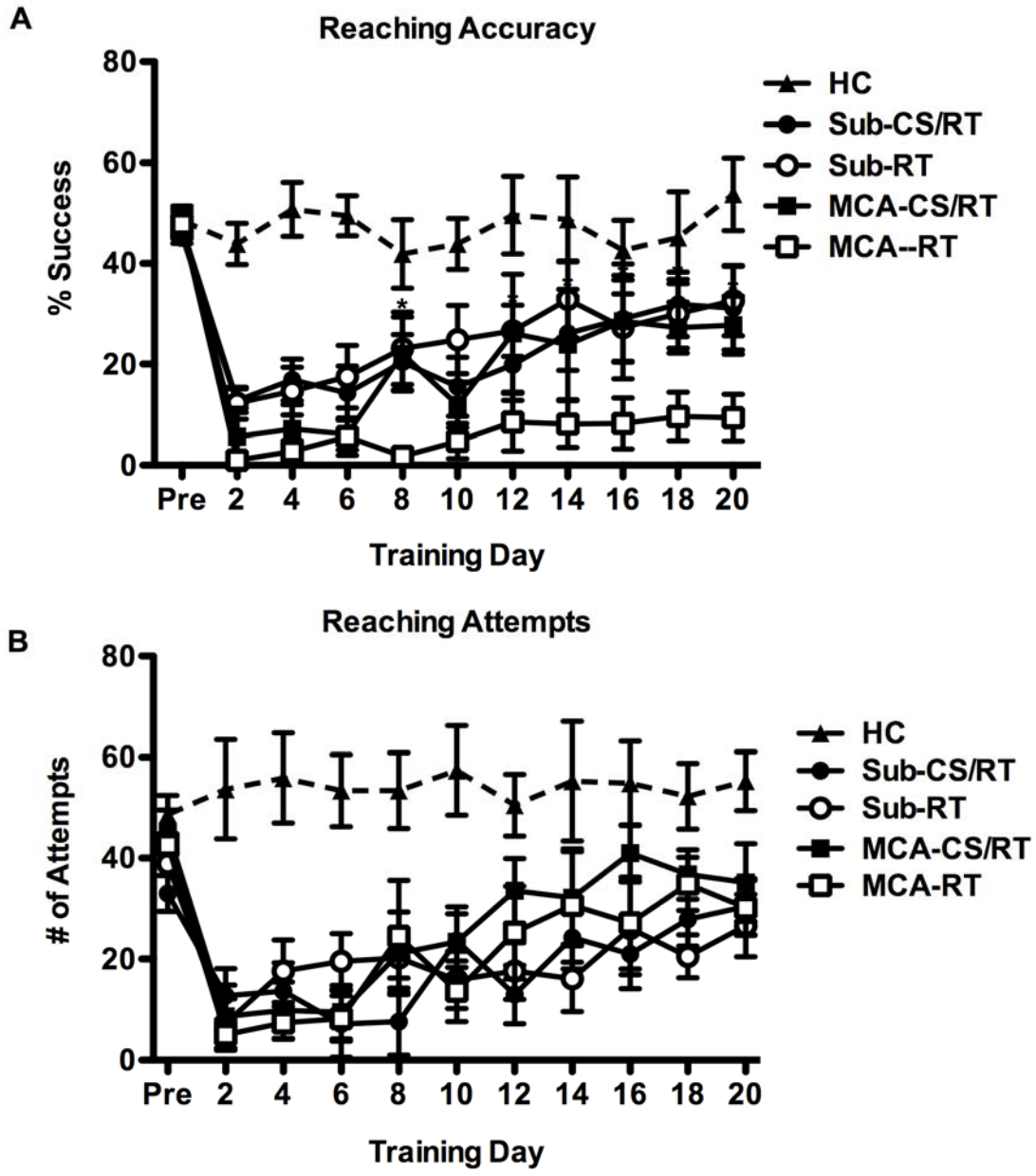

Figure 5-3. Reaching performance prestroke and during 20 days of rehabilitation. A) Mean (±SD) percent reach accuracy. The Sub-RT and Sub-CS/RT groups were not significantly different from one another on any day (Fisher's PLSD; $p<0.05$) while the MCA-CS/RT exhibited significantly greater reaching accuracies than MCA-RT on days 8-9 and 18-20 (Fisher's PLSD; $p<0.05$; indicated by the symbol "Θ"). B) Mean (±SD) number of reach attempts. The Sub-CS/RT and Sub-RT conditions did not exhibit significantly different number of reach attempts on any day (Fisher's PLSD; $p<0.05$). Similarly, the MCA-CS/RT and MCA-RT groups not exhibit significantly different number of reach attempts on any day (Fisher's PLSD; $p<0.05$).

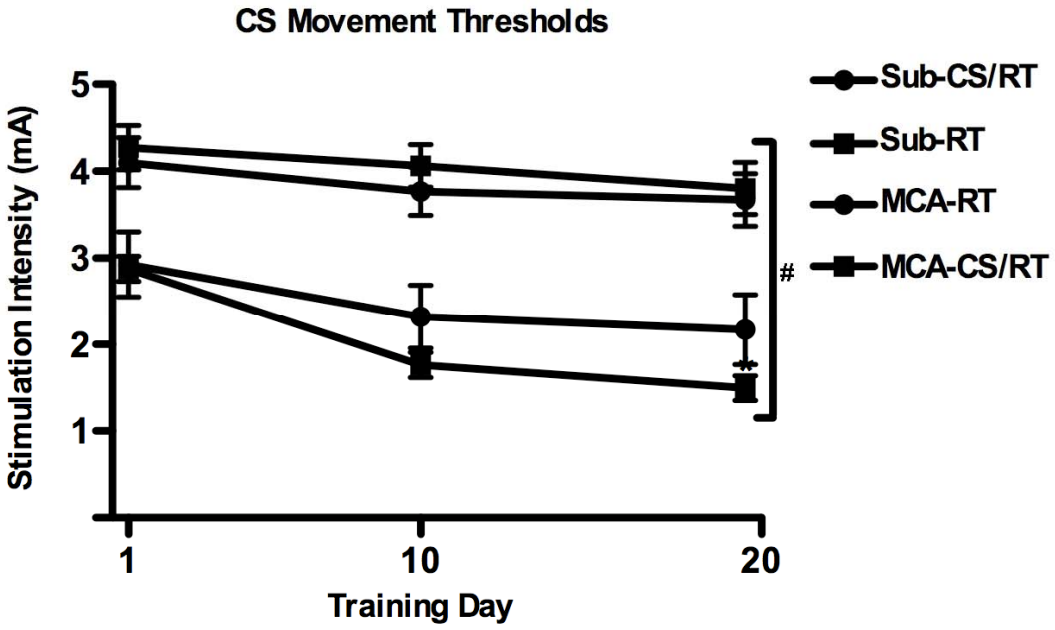

Figure 5-4. Mean (±SD) movement thresholds after cortical or subcortical ischemia. All conditions had significantly lower movement thresholds on the third assessment relative to the first (Fisher's PLSD; $p<0.01$). The Sub-CS/RT and Sub-RT groups exhibited significantly higher thresholds relative to both the MCA-CS/RT and MCA-RT groups at all time points (Fisher's PLSD; $p<0.01$; indicated by the symbol "#"). No significant difference in movement threshold was found between the Sub-CS/RT and Sub-RT conditions. In contrast, the MCA-CS/RT group exhibited significantly lower movement thresholds relative to the MCA-RT group on the third threshold assessment (Fisher's PLSD; $p<0.05$; indicated by the symbol "Θ").

Table 5-1. Estimate of spared cortical and subcortical tissue from experiment 4. Estimated volumes for cortical and subcortical tissue were analyzed as the percent affected to unaffected side (volume lesioned hemisphere/volume non lesioned hemisphere*100) in order to account for individual differences in brain size. Data is represented as mean percent ± SEM.

Condition	Sample Size	Estimates of Spared Tissue Volume	
		Cortical (% Contralesional)	Subcortical (% Contralesional)
HC	8	99±2	98±1
MCA-RT	8	91±1	94±2
MCA-CS/RT	8	89±1	94±1
Sub-RT	8	100±2	98±2
Sub-CS/RT	8	99±1	99±1

CHAPTER 6
GENERAL DISCUSSION

Summary

The present set of experiments explored the use of electrical stimulation of motor cortex as a treatment adjuvant to magnify the behavioral benefit of rehabilitative training after ischemic brain damage. The experiments have produced several important findings. First, the distribution of stimulating contacts was identified as an important factor for cortical stimulation and rehabilitative training (CS/RT). CS/RT with stimulating contacts distributed across motor cortex resulted in enhanced motor improvements while CS/RT with focal stimulation conferred no benefit. Second, within distributed configurations, the number of independent contact points did not alter the efficacy of the treatment. Third, prior rehabilitative training did not prevent enhanced motor improvements with CS/RT. Finally, the location of injury influenced the effectiveness of CS/RT. Animals with subcortical stroke did not respond differently to the combined therapy relative to animals that recieved RT alone. The goal of these studies is to have biologically relevant basic science inform potential clinical applications. To that end, these results are highly relevant. While these and other studies show that cortical stimulation paired with rehabilitative training has demonstrated a behavioral benefit in preclinical animal studies, a recent phase III of the therapy in stroke patients failed (Plow et al., 2009). This demonstrates the complexity of translating such animal data into clinical application. The results of the present set of studies demonstrate that there are critical parameters of both delivery of CS/RT (electrode distribution) and the nature of the injury (cortical versus subcortical) that influence the efficacy of the therapy.

Study #1: Distributed Versus Focal Cortical Stimulation To Enhance Motor Function And Motor Map Plasticity After Experimental Ischemia.

The first two studies were performed to identify the most effective configuration of stimulating contacts for CS/RT. The first experiment determined how the distribution of cortical stimulation affects motor improvement, and motor map plasticity, by comparing focal versus distributed electrode configurations. The total contact surface area between the distributed and focal configurations was kept constant and focal ischemic damage within motor cortex was used as the experimental lesion. Distributed CS/RT stimulated a greater proportion of non-distal forelimb representations relative to focal CS/RT based on the location of contacts within the residual motor map as well as by the responses to motor threshold testing. CS/RT with a distributed configuration of stimulating contacts induced greater motor improvements than RT alone or CS/RT with a focal distribution of contacts. Distributed CS/RT was also associated with a greater expansion of movement representations compared to RT alone and the size of the post rehabilitation motor map positively correlated with motor improvements. CS/RT has previously been associated with magnified expansion of the motor map (Kleim et al., 2003; Plautz et al., 2003). CS/RT in this first experiment was specifically associated with an expansion of distal representations. Changes in distal representations in the intact motor cortex reflect changes in the ability to perform skilled wrist and forelimb movements during skilled reach training (Monfils et al., 2005). The size of post rehabilitation distal movement representations positively correlated with motor improvements. Similarly, the increase in distal representations during rehabilitation also positively correlated with motor improvements. There was also a trend for distributed CS/RT to exhibit a greater decrease in the current needed to evoke a movement with CS/RT stimulation (MT)

relative to focal CS/RT. While lacking the spatial resolution of intracortical microstimulation (ICMS), these changes in movement threshold reflect changes in cortical excitability and reorganization of motor cortex (Monfils et al., 2005). It is possible that distributed CS/RT's association with greater motor improvements was due to its stimulation in movement representations adjacent to the representations that were lost by the ischemic damage. Appropriate rehabilitative training alone results in some reemergence of movement representations that were lost by the injury (Nudo et al., 1996a; Kleim et al., 2003). Stimulation may be facilitating the reemergence of movement representations by reinforcing coincident activity during appropriate rehabilitative training (Jackson et al., 2006). Distributed CS/RT's stimulation of neighboring representations may facilitate the invasion of representations that are emphasized by motor training into these areas.

Although CS/RT with focal and distributed stimulation resulted in greater expansions of movement representations only distributed CS/RT resulted in enhanced motor improvements. The dissociation between motor map reorganization and behavioral improvements suggests that these measures share a non-linear relationship. This finding has previously been reported in rodent studies where electric (Kleim et al., 2003) or pharmacological stimulation (Macdonald et al., 2007) induces motor map changes without robustly impacting behavior. It is possible that motor map restoration is necessary but not sufficient to support motor improvements and that other neuroplastic changes are needed to induce behavioral gains. Map plasticity appears necessary as all animals that demonstrated enhanced motor recovery also exhibited significantly greater motor maps within residual tissue. It is also possible that certain patterns of motor map

reorganization are more relevant than others. In a primate model of focal ischemia within motor cortex, Plautz et al. (2003) observed a 410% expansion of hand representations after CS/RT using a pellet retrieval task that required fine hand movement. These hand expansions occurred in areas of motor cortex that had previously been occupied by proximal forelimb representations and the greatest expansions of hand representations occurred near the stimulating contacts of the CS/RT electrodes (Plautz et al., 2003). In the present study comparing focal versus distributed types of CS/RT, both types of CS/RT resulted in map expansions, however, only distributed CS/RT was associated with a greater proportion of distal representations compared to RT controls while the focal was not. Further, the total area and expansion of distal representations observed by ICMS at the end of the study positively correlated with post injury reaching improvements. Electrical stimulation of motor cortex in the absence of behavioral training expands motor maps, however, it is does not alter the proportion of different types of movement representations (Monfils et al., 2004). The close relationship between the expansion of distal forelimb representations and motor improvements with distributed CS/RT suggests that changes in specific movement representations are an important measure of functional reorganization after injury.

Study #2: Cortical Stimulation Plus Rehabilitative Training Enhances Motor Function Independent of The Number of Stimulating Contacts After Experimental Ischemia.

The results of the first study showed that distributed stimulation was more effective than focused stimulation at enhancing motor improvement and inducing motor map expansion. To further examine the importance of how the topography of stimulation influences motor improvement, the second study used a distributed contact

configuration but varied the number of independent contacts. The total electrode contact area was held constant at (1.2 mm^2) but distributed through either a 2x2 array (four contacts) or a 3x3 array (nine contacts). The 2x2 array had previously demonstrated enhanced behavioral gains (see chapter 2). The stimulation from CS/RT is likely driving motor improvements by inducing reorganization of motor cortex through the facilitation of coincident activity during rehabilitative training (Jackson et al., 2006). It was expected that the 3x3-CS/RT configuration would drive greater behavioral gains than 2x2-CS/RT because its additional contact sites placed within independent areas of motor cortex should promote greater reorganization of motor cortex. The result show that 3x3-CS/RT induced greater behavior gains compared to RT alone but not relative to 2x2-CS/RT. Instead, the 2x2 and 3x3 arrays were associated with highly similar patterns of motor improvements in rodents given middle cerebral artery occlusion (MCAo). The 2x2-CS/RT and 3x3-CS/RT also exhibited similar patterns of motor improvement as a Dual Rail-CS/RT condition that was included in the study to allow comparison to previous CS/RT work in rodent models of stroke (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Teskey et al., 2003; Adkins et al., 2006; Adkins et al., 2008). The close similarity in patterns of behavioral improvements between 2x2-CS/RT, 3x3-CS/RT and Dual Rail-CS/RT suggests the number of contact sites does not alter the effectiveness of CS/RT at least within the range of contact sites examined here. There is likely a limit to the smallest number of contacts that can support CS/RT. For instance, CS/RT through one contact is unable to evoke involuntary movement during motor threshold testing (see chapter 2). The similarity between 2x2-CS/RT and 3x3-CS/RT indicate there is also an upper limit to the number of CS/RT stimulating sites needed to enhance motor

improvements. There is also the caveat with the model used in these studies, in that because of the small size of the motor cortex relative to the electrodes, there may not be sufficient space in which to test for subtle differences in the number of contacts. For example, in an animal with a larger cortex more dramatic differences in contact number could be compared. Regardless, the efficacy of both the 2x2 and 3x3 electrodes speaks to the robustness of the therapy.

Study #1 demonstrated that distributed CS/RT magnifies behavioral gains while focal CS/RT does not, i.e., focal CS/RT directed at the center of the motor map does not enhance motor improvements (see chapter 2). This suggests that stimulation of the borders of the motor cortex may be critical. This is consistent with the finding that no additional behavioral benefit is observed with the Dual Rail and 3x3 configurations that both stimulate a larger proportion of the center of motor cortex than the 2x2 configuration. In other words, the 3x3 and Dual Rail configurations may confer no additional benefit relative to the 2x2 configuration because they are not stimulating additional sites on the edges of the forelimb map where forelimb areas could expand into non-forelimb areas such as whisker, jaw or neck. Indeed these border areas have been shown to be malleable in response manipulations that increase neural excitability including the application of GABA antagonists (Sanes and Donoghue, 2000) and intracortical stimulation (Nudo et al., 1990). The similarity of behavioral outcomes with these three configurations is also likely the result of all of them sufficiently stimulating motor cortex in a distributed manner that targets the more peripheral movement representations. While the exact similarities in the neuroplastic changes induced by these three configurations are not known, all three of these CS/RT configurations

resulted in similar reductions in motor thresholds relative to RT alone. The motor thresholds represent a general measure of excitability as well as the ability of CS/RT stimulation to activate the corticofugal projections of motor cortex. Reductions in motor thresholds also positively correlated with motor improvements during the rehabilitation phase of this experiment.

Study #3: Cortical Stimulation With Rehabilitative Training Can Enhance Motor Improvements After Early Application of Rehabilitation Alone in a Rodent Model of Ischemia

Currently most stroke interventions focus on the acute phase and involve trying to reduce the amount of tissue loss by promoting reperfusion with mechanical or chemical clot busters. While these treatments can successfully reduce infarction size and reduce functional impairment, most stroke victims fail to receive the treatment in time and suffer motor deficits of varying degrees. In these patients the only current treatment is motor rehabilitation and most patients receive several weeks of rehabilitation after the insult. Thus there is a large population of individuals with chronic stroke that have undergone motor rehabilitation that, regardless of the efficacy, has likely induced changes within residual neural tissue. These changes may influence the efficacy of CS/RT. In all previous CS/RT studies, the animals receive treatment after stroke but without prior rehabilitation experience. The third study was performed to investigate whether prior rehabilitation interacted with CS/RT. Animals were given an experimental model of MCAo stroke followed by a phase of RT and then a phase of RT or CS/RT. The results showed that even after extensive motor rehabilitation and significant gains in motor performance, CS/RT could induce further motor improvements.

It is important to note that the same neurophysiological and neuroanatomical changes that occur in residual motor cortex after RT alone also occur with CS/RT.

Specifically, rehabilitative training results in an expansion of movement representations within motor cortex (Nudo et al., 1996a; Kleim et al., 2003) and CS/RT results in an even greater expansion (Kleim et al., 2003; Plautz et al., 2003). While the areas of motor cortex that exhibit expansions of movement representations also exhibit synaptogenesis in response to motor training (Kleim et al., 2002a; Kleim et al., 2004), CS/RT is associated with even greater increases in synaptogenesis (Adkins et al., 2008). Finally, CS/RT is also associated with enhanced synaptic responses following therapy (Teskey, et al., 2003). By these measures CS/RT appears to be enhancing behavioral improvements by magnifying the innate neuroplastic changes that occur in response to standard RT. These data suggest that CS/RT and RT are generally complimentary to one another and that the nervous system can be continually remodeled after injury by experience and adjuvant therapies. Motor mapping was not performed in this third experiment but MT's were assessed. RT was associated with a reduction in motor thresholds while CS/RT was associated with enhanced reductions in MT's. Reduced MT's suggest increased synaptic strength consistent with prior observations of enhanced synaptic responses (Teskey et al., 2003) and synaptogenesis (Adkins et al., 2008).

Study #4: Cortical Stimulation Does Not Enhance Motor Function After Subcortical Stroke

The fourth experiment examined the significance of lesion location for CS/RT's ability to enhance motor improvements after brain ischemia. In the clinical trials of CS/RT, patients are enrolled who have suffered either cortical or capsular ischemic damage (Brown et al., 2006; Levy et al., 2008). In contrast, preclinical animal studies have only used cortical models of ischemia to study CS/RT (Adkins-Muir and Jones,

2003; Kleim et al., 2003; Teskey et al., 2003; Adkins et al., 2006; Boychuk et al., 2009). For the fourth experiment a rodent model of capsular ischemia was developed in order to test CS/RT's ability to drive motor improvements in a rehabilitation phase. The effects of CS/RT following damage to the internal capsule (IC) were compared to the effects of CS/RT following a model of middle cerebral artery (MCA) stroke. RT following ischemic damage in the internal capsule or MCAo resulted in motor improvements. Compared to RT alone, CS/RT enhanced motor improvements in animals that had received MCAo but conferred no benefit in animals that had received capsular ischemia. MT's were significantly higher following capsular ischemia compared to thresholds after MCAo. After MCAo, animals that received RT exhibited a reduction in their MT's. Animals given CS/RT after MCAo exhibited an even greater decrease in MT's. Animals given CS/RT also exhibited a reduction in MT's following capsular ischemia, however, the reduction was statistically similar to the reduction observed in animals given the same type of lesion and RT alone. Potential reasons for CS/RT's lack of effect in this model of capsular stroke are described below.

Possible Neural Bases for CS/RT's Enhanced Motor Outcomes

The effect of CS/RT appears to be mediated through an enduring change in the synaptic connectivity of the motor cortex as evidenced by motor map reorganization (Kleim et al., 2003), enhanced synaptic responses (Teskey et al., 2003) and synaptogenesis (Adkins et al., 2008). Persistent enhancements in motor performance have been observed four to fourteen weeks post CS/RT therapy in primates (Plautz et al, 2003) and as long as nine to ten months post CS/RT therapy in rodents (A. O'Bryant, A. A. Sitko, and T. A. Jones, unpublished observations). Studies with human stroke patients have observed enhanced motor performance in individuals given three weeks

of CS/RT as far as twelve weeks later (Brown et al., 2006). There are numerous sites of plasticity in the nervous system that support motor improvements after injury. Following ischemic injury, CS/RT may be inducing brain plasticity such as peri-infarct reorganization (Nudo and Milliken 1996), recruitment of ipsilesional (Loubinoux et al., 2003; Ward et al., 2003) or contralesional areas (Johansen-Berg et al., 2002), shifts in interhemispheric interactions (Murase et al., 2004; Duque et al., 2005;) or shifts in bihemispheric connectivity (Seitz et al., 2004). These brain changes during CS/RT may be the result of processes including expansion of motor maps, angiogenesis, alterations in synaptic strength, neuroprotection, synaptogenesis, dendritic hypertrophy, release of growth factors or suppression of inflammatory responses.

Direct Effects of CS/RT Stimulation

Little is known of the direct actions of CS/RT stimulation in human motor cortex. The electrical stimulation used in CS/RT is administered at a frequency in the range of fifty to one-hundred pulses per second. Each pulse is biphasic, charged balanced and asymmetric consisting of a square phase lasting 100-250 microseconds followed by a decaying exponential phase. Models of CS in human M1 have given some details of the location of current density. CS electrodes placed directly above a model of the precentral gyrus result in half of the current flow entering the gyrus while the remainder passes down the banks of the sulci (Wongsarnpigoon and Grill, 2008). Neuronal activation with this electrode placement is thought to be isolated to the crown of the gyrus whereas cells further down the banks of sulcus would have increased membrane potentials that are not sufficient for activation (Wongsarnpigoon and Grill, 2008). The magnitude of the activation is inversely related to the thickness of CSF (Wongsarnpigoon and Grill, 2008). Changes in the electrode placement on the modeled

precentral gyrus or changes in the thickness of the gyrus alters the activation pattern largely by changes in the orientation of neurons beneath the electrode (Wongsarnpigoon and Grill, 2008). Neuronal axons have the highest probability of becoming activated by electrical stimulation because they have the highest density of ion channels (Huerta and Volpe, 2009). Thus, the predominant effect of electrical stimulation in the cortex is the presynaptic release of neurotransmitter onto postsynaptic cells by axonal stimulation. Most cortical neurons release glutamate while a smaller portion release GABA. However, electric stimulation of axons in motor cortex may lead to the presynaptic release of glutamate, GABA, acetycholine, dopamine, norepinephrine or serotonin (Huerta and Volpe, 2009). The response to even a single pulse of electrical stimulation is likely mixture of activation in excitatory, inhibitory and neuromodulatory pathways from intracortical afferents. Direct responses by activation of axons of corticofugal fibers are also possible by stronger electrical stimuli, however, the primary responses observed by electric stimulation of the cortex demonstrate latencies more indicative of indirect responses by the activation of intracortical afferents (Jankowska et al., 1975). Serial trains of electrical stimuli in motor cortex have the capacity to activate entire networks over wide areas of cortex based on the projections of postsynaptic cells receiving neurotransmitter release within motor cortex (Fetz and Cheney, 1980). However, weak electrical stimuli in motor cortex are thought to activate “organized groups of inputs to pyramidal tract neurons” (Porter and Lemon, 1995). Finally, the temporal and spatial facilitation of electrical stimulation within the cortex may support coincident activity of incoming afferent signals that does not originate from the electrical stimulation resulting in the strengthening of neuronal connections (Jackson et al., 2006).

Potential Neuroplastic Responses to CS/RT

Electric stimulation of sensorimotor cortex immediately after MCAo results in neuroprotection, angiogenesis, the release of growth factors, and suppression of inflammatory responses (Baba et al., 2009). CS/RT is typically initiated beyond the acute phase of ischemic injury, however, each of these processes merit attention because of their potential to alter the brain's response to stroke damage. Of this list, only the role of neuroprotection in CS/RT has been investigated. Early application of electrical stimulation after ischemic insult decreases cell death and infarct volume (Glickstein et al., 2003; Maesawa et al., 2004). Some animals CS/RT studies have not reported spared tissue volumes (Kleim et al., 2003; Teskey et al., 2003). Of those that do report spared tissue volume, most observe enhanced motor improvements with no differences relative to RT groups (Adkins-Muir and Jones, 2003; Adkins et al., 2008; Boychuk et al., 2009). In the present experiment no differences in spared tissue volumes were observed. Adkins et al. (2006) found that relative to an RT group, one group of CS/RT initiated 10-14 days after ischemia was associated with enhanced motor improvements and a greater perilesional neuronal density that was not due to an increase in newly born BrdU-labeled cells. In this same study, a second group with CS/RT initiated 10-14 days after ischemia was associated with enhanced motor improvements and no elevated perilesional neuronal density relative to the RT group (Adkins et al., 2006). These authors also observed a CS/RT group that demonstrated enhanced motor improvements and relatively less spared tissue (a greater difference in contralesional versus ipsilesional cortical volume) but the decreased spared tisse was attributed to the CS/RT group tending to have the greatest contralesional and lowest ipsilesional volumes (Adkins et al., 2006). Further, studies have reported enhanced

motor improvements when CS/RT is initiated 3 months after experimental ischemia in primates (Plautz et al., 2003) or in human patients at least 4 months post stroke with average delays of 28 months (Brown et al., 2006) and 32.8 months (Levy et al., 2008). In these studies CS/RT ability to enhance motor improvements in a more chronic phase of stroke suggests that the underlying mechanism of CS/RT is not neurprotection.

The relationship between CS/RT and the release of growth factors deserves attention. Many growth factors have demonstrated neuroprotective effects when administered acutely in animal models of stroke. For instance, administration of BDNF early after ischemic injury reduces the loss of cells and infarct volume (Wu and Pardridge, 1999; Zhang and Pardridge, 2006). Administration of vector expressing glial-derived neurotrophic factor (GDNF) during MCAo is associated with smaller infarcts and less apoptotic markers such as TUNEL staining (Tsai et al., 2000; Yagi et al., 2000). Intravenous administration of vascular-endothelial growth factor (VEGF) within two days after ischemic damage induces angiogenesis and restores neuronal function (Zhang et al., 2000). The chronic effects of increasing neurotrophic factor levels are less understood making it difficult to characterize their relationship with CS/RT. For example, administration of HSV-amplicon-based vector encoding GDNF promoted behavioral improvements after MCAo when delivered four days prior but not three days after the lesion (Harvey et al., 2003). Chronic VEGF administration after stroke is cautioned because of its secondary effects of increasing vessel permeability, edema and the activation of inflammatory responses (Shibuya, 2009). Understanding the relationship between BDNF and CS/RT is becoming increasingly important because delayed administration of BDNF following ischemic injury enhances motor recovery most likely

by increasing synaptic connections within the brain (Schabitz et al., 2004; Schabitz et al., 2007; Muller et al., 2008). It is known that motor learning increases BDNF levels in the motor cortex (Klintsova et al., 2004). Further, blocking BDNF signaling by infusions of antisense BDNF oligonucleotide attenuates motor improvements during motor training after MCAo (Ploughman et al., 2009). BDNF may facilitate reorganization of motor cortex by influencing neuroplastic changes such as synaptogenesis or dendritic hypertrophy (Biernaskie and Corbett, 2001; Kleim et al., 2003; Monfils et al., 2005; Müller et al., 2008). Given that no differences infarct volume were observed in the present studies, if growth factors are involved they are likely promoting plasticity rather than neuroprotection.

The relationship between CS/RT and angiogenesis, glial responses and the suppression of inflammatory responses has not been examined. Glial responses are of interest as interventions such as skilled reach training or exposure to enriched environments result in behavioral improvements that are associated with suppressed proliferation of microglia/macrophages and increased proliferation of astrocytes in perilesional cortex (Keiner et al., 2008). Angiogenesis and the suppression of inflammatory responses are particularly important in the acute phase. Lowering inflammatory responses may increase the number of cells that survive brain ischemia by blocking the production of cytokines and NO and preventing neutrophil and macrophage infiltration into brain parenchyma (Vexler and Yenari, 2009). Angiogenesis may be supporting neurons that are endangered by low blood supply in the ischemic penumbra (Noshita et al., 2001). It is likely CS/RT is initiated outside of the window to fully influence acute mechanisms such as neuroprotection, angiogenesis, and

suppression of inflammatory responses. However, because CS/RT is administered relatively earlier in preclinical animal studies than in clinical trials using human stoke patients it is important to study these responses as important translational issues.

Reorganization of Motor Cortex With CS/RT

Many animal studies of CS/RT have reported reorganization at the site of stimulation: the ipsilesional motor cortex. The patterns of reorganization in motor cortex after CS/RT share a high degree of similarity with patterns in the same area during healthy motor training. Skilled motor training results in a reorganization of motor representations as representations for movements involved in the motor task expand their territories at the expense of others. The reorganization of motor cortex occurs later in skilled training after initial improvements in skill behavior (Kleim et al., 2004) and is temporally paralleled by increased synaptogenesis (Kleim et al., 1996; Kleim et al., 2002a; Kleim et al., 2004). Following the discovery that LTP can be induced in the neocortex of awake behaving rodents (Racine et al. 1995, Trepel and Racine, 1998). Rioult-Pedotti et al. (1998) demonstrated that the acquisition of skilled motor behavior results in LTP-like potentiation in the horizontal afferents within motor cortex. This potentiation of motor cortex occurs late in training after initial improvements in skill behavior (Monfils and Teskey, 2004). Interestingly, motor cortex plasticity is task specific with neither strength training (Remple et al., 2001) nor endurance training (Kleim et al., 2002b) resulting in reorganization of motor cortex. Further, reaching on an unskilled task does not result in motor map reorganization (Kleim et al., 2004).

In animal studies, ischemic damage to motor cortex results in a loss of synapses (Hasbani et al., 2001; Zhang et al., 2005; Murphy et al., 2008) and a loss of movement representations (Nudo and Milliken, 1996; Friel et al., 2000). In the absence of

rehabilitative training the majority of lost motor representations do not reappear and the topography of the remaining map is not significantly altered (Nudo and Milliken, 1996; Friel et al., 2000). Appropriate motor training after cortical ischemia results in motor improvements and an expansion and reemergence of microstimulation-evoked movement representations (Nudo et al., 1996a; Kleim et al., 2003). The reemergence of perilesional motor maps is also associated with synaptogenesis (Kleim et al., 2002a; Kleim et al, 2004). CS/RT studies of experimental ischemia have observed a heightened increase in reorganization of movement representations within motor cortex during rehabilitation (Kleim et al., 2003; Plautz et al., 2003; Boychuk et al., 2009). Increases in the size of movement representations following CS/RT on a skilled reaching task positively correlated with increases in motor improvements (Boychuk et al., 2009). CS/RT appears to expand motor maps by emphasizing the reemergence of representations that were lost by the ischemic injury such as hand representations in a primate model of stroke (Plautz et al., 2003) or distal forelimb representations in a rodent model of stroke (Boychuk et al., 2009). Electrical stimulation of motor cortex in the absence of behavioral training expands motor maps, however, it is does not alter the proportion of different types of movement representations (Monfils et al., 2004). In chapter 2, increases in the proportion of distal forelimb representations following CS/RT on a skilled reaching task positively correlated with increases in motor improvements (Boychuk et al., 2009). While the invasiveness of ICMS prevents its use in human stroke patients, the possibility of cortical reorganization was raised in a study that noted individual finger movements in response to intraoperative cortical mapping that patients

were not capable of voluntarily making before cortical stimulation and rehabilitative training (Brown et al., 2006).

CS/RT is also associated with synaptic changes that may support the functional reorganization of movement representations. CS/RT results in an increased density of axodendritic synapses including increases in (presumed efficacious) perforated synapses and multiple synaptic boutons within layer 5 of motor cortex (Adkins et al., 2008). In addition, CS/RT is associated with enhanced polysynaptic responses in motor cortex (Teskey et al., 2003). In CS/RT studies, MT's are typically assessed on the first day of rehabilitation following experimental stroke as well as every seven to ten days subsequent. It is common to find that all conditions receiving RT or CS/RT exhibit decreases in MT's through days of rehabilitation (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Teskey et al., 2003; Adkins et al., 2006; Boychuk et al., 2009). In chapters 2-5 of this dissertation the CS/RT groups that demonstrate enhanced motor improvements also exhibited the greatest reductions in MT. In several of the present experiments, decreases in MT positively correlated with motor improvements after brain ischemia. While these MT's lack spatial resolution compared to ICMS they do reflect general levels of excitability in motor cortex (Monfils et al., 2005). Excitability can be altered by the number of cells or the intrinsic excitability of individual cells and the strength of their synaptic connections (Redecker et al., 2000). Given that CS/RT is not typically associated with neuroprotection or the proliferation of new cells it is likely that affects the intrinsic excitability of cells within motor cortex (Adkins et al., 2006).

Shifts in Excitability With CS/RT

CS/RT may be mediated through a resolution of cortical hyperexcitability or hyperinhibition. Ischemic insult results in a disruption of excitability at many levels such

as through a loss of cells, their connections and the expression of their receptors (Redecker et al., 2002). Early after ischemia, many regions of cortex become hyperexcitable as NMDA receptors are upregulated and GABA-subtype A receptors are downregulated (Redecker et al., 2000). For example, the PMv motor area demonstrates hyperexcitability and reduced motor maps suggesting that increases in excitability may disrupt the function of cortical areas (Nudo, 2007). After the acute phase of stroke there is typically a reduced output or activation of the affected hemisphere (Desrosiers et al., 2006) whereas the opposite case is found in the contralesional hemisphere (Cauraugh et al., 2000). The contralesional hemisphere may also be exerting excess inhibition on the affected hemisphere (Platz et al., 2005). Many stroke treatments using noninvasive stimulation employ a general strategy of trying to encourage activity in the ipsilesional hemisphere or discourage it in the contralesional hemisphere (Talelli and Rothwell, 2006). In the ipsilesional hemisphere, stimulation protocols that facilitate neuronal activity such as high-frequency repetitive transcranial magnetic stimulation (rTMS) (Khedr et al., 2005, Kim et al., 2006) anodal transcranial direct current stimulation (Hummel and Cohen, 2005) or intermittent theta-burst stimulation delivered ipsilesionally (Talelli et al., 2007, Di Lazzaro et al., 2008) result in motor improvements. Similarly, low-frequency rTMS (Fregni et al., 2006, Mansur et al., 2005) cathodal transcranial direct current stimulation (Fregni et al., 2005) or continuous theta-burst stimulation (Talelli et al., 2007, Di Lazzaro et al., 2008) delivered contralesionally also result in behavioral improvements. The enhanced synaptic responses, synaptogenesis and motor map expansions with CS/RT suggest that it is facilitating or activating the ipsilesional hemisphere. It is possible that CS/RT is enhancing motor improvements

after brain ischemia by encouraging the activity and output of the motor cortex in the injured hemisphere.

The Effect of CS/RT in New Lesion Models

CS/RT After MCAo

In chapters 3-5, CS/RT enhanced motor improvements following a middle cerebral artery occlusion type stroke model. Enhanced behavioral improvements following MCAo have not been reported in the literature. The original animals studies that found enhanced behavioral gains with CS/RT induced ischemic damage focally to motor cortex by electrocoagulation of surface vasculature (Kleim et al., 2003; Plautz et al., 2003), pial stripping (Teskey et al., 2003) or topical application of the vasoconstricting peptide ET-1 (Adkins-Muir and Jones, 2003; Adkins et al., 2006; Adkins et al., 2008). The present results suggest that CS/RT can induce functional benefits when ischemic damage is outside of motor cortex. It is unclear how CS/RT affects brain repair following MCAo. The MCAo model in rodents results in forelimb motor impairments (Gharbawie et al., 2005a; Gharbawie et al., 2005b; Gharbawie and Whishaw, 2006) and a disruption of movement representations within motor cortex (Gharbawie et al., 2008). Depending the extent of MCAo lesion, motor map dysfunction may be the result of disruption of the cortico-basal ganglia-thalamo-cortical loops (Karl et al., 2008), however, neurotoxic striatal damage affecting cells but sparing axons in the striatum did not alter motor map topography (Karl et al., 2008). Further, smaller MCAo lesions that spare basal ganglia, motor cortex and the CST still resulted in a disruption of the motor maps (Gharbawie et al., 2005b). The loss of motor maps following MCAo may be due to a loss of cells or synaptic connections within motor cortex (Garcia et al., 1997). MCAo is associated with decreased excitability within motor cortex indicated by a reduction in field potential

amplitudes (Neumann-Haefelin and Witte, 2000). MCAo also results in a loss of large neurons in layer five of motor cortex (Moisse et al., 2008) as well as a loss of synapses and dendrites within sensorimotor cortex (Zhang et al., 2005). Stroemer et al. (1995) found an association between behavior improvements and synaptogenesis within sensorimotor cortex following MCAo that indicates a functional importance for restoring synaptic input in motor cortex. Further, the ICMS techniques that produce motor maps rely on both direct stimulation of pyramidal cell axons as well as indirect stimulation of pyramidal cells by transynaptic recruitment (Jankowska et al., 1975; Fetz and Cheney, 1980). Using ICMS, Bolay and Dalkara (1998) found a recovery of the direct wave whereas a persistent deterioration of the indirect (transynaptic) wave in the pyramidal tract following MCAo. The persistent dysfunction of the indirect wave suggests the associated map dysfunction is due to a loss of intrahemispheric connections within motor cortex rather than a loss of pyramidal cells. Given the damage within motor cortex following MCAo, CS/RT may be enhancing motor improvements by restoring connections within motor cortex. This may be mediated by strengthening residual connections within motor cortex by facilitating coincident activity (Jackson et al., 2006). In support of this notion, in chapters 3-5 where MCAo was used to cause ischemic damage, CS/RT was associated with greater reductions in motor threshold than RT alone suggesting that cortical stimulation did increase excitability within the motor cortex.

CS/RT After Capsular Infarct

The motor impairments observed in the present study are supported by previous reports where focal ischemic damage to the internal capsule resulted in impairments in forelimb placing (Frost et al., 2006) and forelimb exploration within a cylinder (Lecrux et

al., 2008). Capsular infarct is typically associated with increased motor thresholds to electric stimulation that are likely due to a loss of or dysfunction within pyramidal tract cells (Liepert et al., 2005). Injuries that damage the corticospinal tract at the level of either the pyramids (Wohlfarth, 1932; Pernet and Hepp-Reymond, 1975) cervical spinal cord (Holmes and May, 1909; Levin and Bradford, 1938) or thoracic spinal cord (Hains et al., 2003) result in a loss of large pyramidal neurons in the motor cortex. The loss of pyramidal cells following CST injury is a graded response. For example, unilateral pyramidotomy affecting 60%, 90% or 100% results in a decrease in the proportion of pyramidal cells in motor cortex of 27%, 35% or 53% respectively (Pernet and Hepp-Reymond, 1975). Injury to the CST is also associated with alterations in surviving pyramidal cells including cell shrinkage and a decrease in Nissl staining (Wannier et al., 2005). Retrograde tracing experiments have demonstrated a reduction in CST axons to the hemicord caudal to a C3 hemisection (Galea and Darian-Smith, 1997). While detailed histological assessment of motor cortex following internal capsule has not been performed to confirm similar findings as the spinal cord injury models, it is expected that internal capsule damage results in a loss and disruption of pyramidal tract neurons from layer five of motor cortex.

Ischemic damage in motor cortex is associated with a loss of neural connections that is demonstrated by a loss of dendritic arbor (Zhang et a., 2005) as well as by a loss of synapses (Hasbani et al., 2001; Zhang et al., 2005; Murphy et al., 2008). The disruption in neural connectivity is further demonstrated by a persistent loss of microstimulation-evoked movement representations within motor cortex (Nudo and Milliken, 1996; Friel et al., 2000). Stroke damage to the lateral aspect of the brain by

occlusion of the middle cerebral artery also causes a disruption of movement representations within motor cortex (Gharbawie et al., 2008). The disruption of movement representations within motor cortex was due to a loss of intracortical synapses indicated by the persistent impairment of the indirect (transynaptic) response to ICMS in the pyramidal tract (Bolay and Dalkara, 1998). In support, histological examination of sensorimotor cortex following MCAo shows a loss of synapses and dendrites (Zhang et al., 2005) and the restoration of these synapses is associated with behavioral improvements (Stroemer et al., 1995). Rehabilitation after focal ischemic injury to motor cortex or MCAo results in motor improvements that are likely mediated by a restoration of local cortical circuitry in motor cortex involving synaptogenesis (Stroemer et al., 1995; Buonomano and Merzenich, 1998; Kleim et al., 2002a; Kleim et al., 2004; Brown and Murphy, 2008). Given CS/RT's enhancement of motor map expansions (Kleim et al., 2003; Plautz et al., 2003; Boychuk et al., 2009), synaptogenesis (Adkins et al., 2008) and reductions in MT (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Teskey et al., 2003; Adkins et al., 2006; Boychuk et al., 2009) it is likely CS/RT is enhancing motor improvements by magnifying the brain's response to rehabilitation.

CS/RT's inability to enhance motor improvements after capsular infarct in the present rodent model suggests it is not able to facilitate the restoration of local circuitry in motor cortex. One possibility is that the damage with this model does not spare enough pyramidal cell fibers to permit a restoration of local circuitry because there is insufficient corticofugal output to allow appropriate feedforward and feedback mechanisms. However, the animals' motor improvements in response to rehabilitative

training and CS/RT's ability to evoke movement during MT testing indicate that there was substantial sparing of CST fibers. Another possibility is that focal ischemic motor cortex damage and MCAo result in a predominant loss of intracortical afferents that can be restored with RT and CS/RT whereas capsular infarct predominantly results in a loss of pyramidal tract cells and their arbor. In this case, it is not that pyramidal tract cells have been completely eliminated, but rather, that CS/RT cannot reinforce connections to the remaining pyramidal cells as it may be doing with the other two injury types. CS/RT may be unable to reinforce spared connections when the loss of synapses is due to a reduced number of postsynaptic pyramidal cells rather than due to a loss of presynaptic intracortical afferents.

There are many other possibilities for the lack of CS/RT effect after capsular infarct. Ischemic damage within the internal capsule may be damaging an additional motor tract besides the CST that is important for CS/RT-enhanced motor improvements. Capsular infarct may also be damaging subcortico-cortical connections such as thalamic inputs into motor cortex that are necessary for CS/RT-enhanced motor improvements (Axer and Keyserlingk, 2000). If CS/RT is magnifying behavioral improvements by increasing the excitability of the ipsilesional motor cortex, perhaps its inability to do so after capsular infarct is because of a unique pattern of aberrant excitability following this injury. Cortical stroke typically results in acute patterns of hyperexcitability (Redecker et al., 2000) followed by a loss of excitability involving decreases in output and neural activation (Desrosiers et al., 2006). The presumed loss of pyramidal cells following capsular infarct would result in a net loss of excitability. Relative to the cortical lesion models used here, the net loss of pyramidal cells coupled

with the preservation of inhibitory interneurons may result in a state of hyperinhibition. If CS/RT is facilitating motor improvements after cortical stroke by relieving inhibition it is possible a state of hyperinhibition following capsular stroke prevents CS/RT from increasing cortical excitability to sufficient levels. Contrary to this possibility, however, stroke patients with motor cortex lesions demonstrate higher rates of intracortical inhibition than patients with internal capsule lesions (Liepert et al., 2005). Motor improvements after partial stroke damage within the coronal radiata have been associated with a reorganization of the residual fibers (Jang et al., 2005). If motor improvements are mediated through the reconfiguration of CST fibers, CS/RT may lack efficacy in treating these injuries because it cannot facilitate this type of plasticity. It is also possible that brain repair after capsular infarct is best accomplished by the recruitment of secondary motor areas that project to spinal cord motor neurons. In contrast to cortical ischemic insult, rehabilitation after pyramidotomy does not result in reorganization of movement representations (Piecharka et al, 2005). Similarly, large ischemic lesions in primate motor cortex result in an expansion of movement representations within the secondary motor area PMv rather than within M1 (Nudo, 2007). In this primate model of stroke, a linear relationship between increasing lesion size within M1 and increasing reorganization of PMv was observed (Dancause et al., 2005). Finally, some reports from non-invasive imaging of human stroke patients have observed an association between stroke damage to the CST and the recruitment of secondary motor areas (Ward et al., 2003; Ward, 2006).

Translation of CS/RT From Animal to Clinical Studies

The lack of a CS/RT treatment effect in a recent phase III clinical trial was unexpected given the many previous preclinical and Phase II demonstrations of CS/RT-

enhanced behavioral gains after brain ischemia. Preclinical animal studies of CS/RT have also demonstrated a CS/RT-induced enhancement of motor improvements on tasks such as single pellet retrieval (Kleim et al., 2003; Adkins et al., 2006; Adkins et al., 2008), pasta matrix (Teskey et al., 2003), Montoya staircase (Adkins-Muir and Jones, 2003), and pellet retrieval from a 5 well apparatus in primates (Plautz, et al., 2003). Clinical reports have indicated that epidural motor cortex stimulation, used to reduce chronic pain after sub-cortical strokes, reduces hemiparetic impairments (Tsubokawa et al., 1993), motor weakness (Katayama et al., 2002), motor spasticity (Garcia-Larrea et al., 1999), action tremor (Nguyen et al., 1998) and dystonia (Franzini et al., 2003). CS/RT resulted in substantial behavioral gains in a single case report of human stroke patient (Brown et al., 2003). CS/RT has been associated with greater behavioral gains in both phase I (Brown et al., 2006) and phase II clinical trials (Huang et al., 2008; Levy et al., 2008) using the same procedures as the phase III trial. Interestingly, some patients in the combined CS/RT group of the phase III clinical trial demonstrated robust motor improvements relative to the group receiving RT alone (Plow et al., 2009). These studies serve as evidence that electrically stimulating the brain during rehabilitative training can magnify the training's benefit. Difficulty in translating CS/RT from the preclinical basic studies to its application in human patients likely contributed to the lack of CS/RT effect observed in the phase III trial (Plow et al., 2009).

One of the present experiments identified the placement of CS/RT stimulation as an important predictor of CS/RT efficacy. CS/RT distributed across motor cortex enhanced motor improvements while CS/RT administered focally within motor cortex conferred no benefit in a rodent model of stroke (see chapter 2). Distributed CS/RT was

also associated with a significantly greater reemergence of distal forelimb representations relative to RT while focal CS/RT was not. Furthermore, the reemergence of distal forelimb representations positively correlated with motor improvements during rehabilitation. Distributed CS/RT stimulated a greater proportion of non-distal forelimb representations relative to focal CS/RT based on the location of contacts within the residual motor map as well as by the responses to motor threshold testing. These data suggest that stimulation for CS/RT should be targeted to movement representations surrounding and adjacent to the representations that are have compromised by brain ischemia. CS/RT electrodes in animal studies are sufficiently large enough to cover the entire motor cortex whereas the CS/RT electrodes in clinical studies only cover a portion of human motor cortex. The volume of human motor cortex is approximately 10.87 cm^3 based on histological estimates (Rademacher et al., 2001). The total contact area of the CS/RT electrodes in human clinical trials is constrained by the capacity of the power supplies and has been between 28.26 mm^2 and 42.39 mm^2 (Brown et al., 2006; Huang et al., 2008; Levy et al., 2008). Given that CS/RT in human stroke patients will be administered to only a portion of motor cortex, an understanding of the most effective location within motor cortex to administer the stimulation will likely improve CS/RT's effectiveness in human stroke patients. The clinical trials of CS/RT have localized the placement of the stimulating contacts based on fMRI activation during hand/wrist/finger movement (Brown et al., 2006; Huang et al., 2008; Levy et al., 2008). More detailed localization with fMRI using craniometer landmarks and phase reversal to localize the central sulcus will likely provide better localization of the appropriate target for CS/RT stimulation movements (Plow et al., 2009). Further, TMS

mapping of ipsilesional motor cortex may provide more optimal placement of stimulating contacts by providing details of the size and location of each motor map (Plow et al., 2009). If the present data are correct and stimulation for CS/RT should be directed to movement representations surrounding the representations compromised by brain ischemia, then it will be necessary to perform TMS motor mapping in order to identify each individual's movement representations.

Additionally, proper localization of CS/RT stimulation will require a greater understanding of the current distributions within human motor cortex after stroke. Current distribution modeling in rodents is informative but limited due to species differences that affect current such as the size of cortex and dura as well as lissencephalic nature of rodent cortex (Wongsarnpigoon and Grill, 2008). Using MRI-derived finite head modeling to examine the effects of TMS stimulation, Wagner et al. (2006) demonstrated that cortical stroke in the human brain displaces the location of maximal current density, alters the magnitude of maximal current density and causes the current density to be disjointed and multifocal around the infarct. More current modeling using noninvasive techniques in human as well as studies with non-human primate motor cortices should permit better localization of stimulation for CS/RT. The use of fMRI localization of hand/wrist/finger movement to identify the site of CS/RT stimulation in the clinical studies have ignored the type of rehabilitative training used for CS/RT as many of the rehabilitative tasks trained proximal limb movements (Plow et al., 2009). The animal studies have observed little transference of CS/RT-induced motor improvements from the trained task to other motor tasks suggesting that the localization of stimulation and rehabilitative training should be directed at the same type of

movements (Adkins et al., 2006). The activation site of hand/wrist/finger movement was used as the site for CS/RT stimulation regardless of the location of each individual's stroke or its clinical manifestations. Greater clinical benefits of CS/RT will likely be observed if the location of stimulation and rehabilitative training are directed at each patient's motor impairments.

In one of the present experiments the location of stroke injury was shown to be an important predictor of CS/RT efficacy. Here, CS/RT conferred no additional behavioral benefit relative to RT alone in a rodent model of capsular infarct. These data indicate that CS/RT may not be a viable therapy for human stroke patients with capsular damage yet the clinical studies of CS/RT have enrolled patients with cortical and capsular ischemic damage (Brown et al., 2006; Levy et al., 2008). While it is not known if CS/RT can enhance motor improvements in human stroke patients with capsular infarct, CS/RT's lack of effect in the present animal model suggests that the inclusion of these patients in the phase III clinical study may have contributed to the failure to observe enhanced motor improvements with CS/RT. The data also offer the possibility that there may be other locations of stroke that do not respond to CS/RT therapy. Additional animal studies with other stroke models should contribute a better understanding to how lesion location affects CS/RT therapies. Clinical studies of CS/RT should also include lesion location as a covariate in analysis. In order to better characterize each patient's stroke, the integrity of the descending motor pathways could be assessed using TMS prior to enrollment for CS/RT therapy because viability of these pathways is an important factor for motor prognosis (Stinear et al., 2007). The integrity of the descending motor pathways after stroke is also an important predictor of brain

activation patterns that may indicate different mechanisms of brain repair (Ward, 2006). Interestingly, only 16% of the participants in the CS/RT group in the third clinical trial exhibited evoked movements during intraoperative stimulation compared to 100% in the phase I and 42% in the phase II clinical trial (Plow et al., 2009). The portion of participants in the phase III trial that did show evoked movements during surgery also responded to CS/RT by exhibiting greater motor improvements compared to participants receiving RT (Plow et al., 2009).

There are several other issues concerning the translation of CS/RT from animal studies to clinical studies that were not addressed in the present experiments. For example the most effective versions of the timing of stimulation during CS/RT, the amount of CS/RT given daily and the intensity of CS/RT given across days are not known (Plow et al., 2009). It is likely that information from noninvasive brain stimulation studies such as TMS and tDCS can guide CS/RT studies (Plow et al., 2009). Additionally, many of the stimulation parameters used in CS/RT such as polarity (Kleim et al., 2003; Adkins et al., 2006) and frequency (Adkins-Muir and Jones, 2003; Teskey et al., 2003) of stimulation were identified in rodent models of stroke. The stimulation parameters for CS/RT in the clinical trials have deviated from those recommended by the animal work without providing rationales for the parameters selected (Brown et al., 2006; Huang et al., 2008; Levy et al., 2008). Lastly, individual characteristics of each person with stroke may impact responsiveness to CS/RT therapy. Malcolm et al. (2007) demonstrated the importance of individual responses to brain stimulation in a report where only a subgroup of stroke patients demonstrated the desired effects of rTMS in the affected hemisphere. No animal studies of CS/RT have investigated effects in

females and human clinical trials of CS/RT have not reported sex as a covariate. There is some evidence that sex is predictive of poor functional outcome after stroke even after correction for prognostic factors (Gargano et al., 2007; Gray et al., 2007). The sexes may also respond differently to stroke treatments (Saini and Shuaib, 2008). Finally, there appears to be a genetic component to cortical plasticity and responsiveness to brain stimulation. For example, the BDNF val66met polymorphism is associated with less alleviation of drug resistant depression by rTMS (Bocchio-Chiavetto et al., 2008). The val66met polymorphism is also associated with lower training-induced cortical reorganization (Kleim et al., 2006). These individual factors may also be limiting overall effectiveness of CS/RT in clinical studies. Improvements in translating CS/RT from animal studies to human clinical trials as well as appreciating individual differences in responsiveness to brain stimulation will likely improve CS/RT therapeutic efficacy in individuals with stroke.

Conclusion

The variables associated with neural recovery/compensation after stroke are well understood. An understanding of the mechanisms that produce motor improvements after stroke will lead to more effective treatments strategies. After brain ischemia, the motor cortex has the capacity to reorganize in response to rehabilitative experience. Adjuvant therapies such as cortical stimulation have the potential to magnify cortical reorganization and behavioral improvements after stroke. The present studies investigated the importance of several parameters of CS/RT. Distributed configurations of CS/RT electrodes resulted in enhanced motor improvements and motor map plasticity that were not found with focal configurations of CS/RT. Among different types of distributed CS/RT, the number of independent contact sites did not affect CS/RT's

ability to augment motor improvements following cortical ischemia. Early rehabilitative training after cortical ischemia did not affect CS/RT's ability to augment improved motor performance. In contrast, lesion location predicted CS/RT outcome. CS/RT produced enhancements in motor performance in two separate models of cortical stroke that were absent in a model of subcortical stroke involving white matter damage. The present data support that a more detailed understanding of how to translate CS/RT findings from preclinical animals studies to its clinical application in human stroke patients will improve its use in this stroke population. These and future studies will provide a greater understanding of how the brain repairs itself after injury that will guide the development of more effective post-stroke treatment interventions and ultimately improve the quality of life for stroke victims.

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BIOGRAPHICAL SKETCH

Jeffery A. Boychuk was born and raised in Lethbridge, Alberta, Canada. He graduated from Lethbridge Collegiate Institute in 2001. He then eared his Bachelor of Science in neuroscience from the University of Lethbridge (U of L) in May of 2005. During his time at the U of L, he worked as a research assistant in Professor John Vokey's laboratory studying cognitive processes such as the psychophysics of fingerprint identification. In the spring of 2005, he joined Professor Jeffrey Kleim's laboratory to investigate mechanisms of stroke recovery. Upon receiving his bachelor's degree, he moved with Dr. Kleim to the University of Florida (UF) in Gainesville. In the fall of 2005, he began pursuit of a doctoral degree in the UF College of Medicine's Interdisciplinary Program in Biomedical Sciences.