MODULATION OF COMPENSATION AND RECOVERY IN A RAT MODEL OF MOTOR CORTEX STROKE: IMPLICATIONS OF TRANSCRANIAL DIRECT CURRENT STIMULATION

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Dedication

I dedicate this thesis to my mother and father, Marg and Walt Gidyk for their unwavering support; undying belief in my abilities and for managing to stay married for a zillion years.

Thesis abstract

The present thesis examines the effects of transcranial direct current stimulation and forelimb rehabilitation on motor recovery after stroke in rats. Post-stroke motor outcomes were quantified using an innovative battery of behavioural tests and high resolution, *in vivo* electrophysiology was employed to examine coherence of neural activity between hemispheres. It was shown that rats that received brain stimulation concurrently with forelimb rehabilitation displayed functional recovery, whereas rats that received rehabilitation alone partially regained motor function, but the improvements were not due to restitution of original movement patterns. Results from electrophysiological recordings showed that rats that received brain stimulation and rehabilitation regained pre-stroke levels of interhemispheric coherence, but rats that received rehabilitation alone did not. The present thesis suggests that transcranial direct current stimulation may be a viable adjunct therapy to increase the efficacy of physical rehabilitation with regard to post-stroke motor outcomes. Interhemishperic coherence between homotopic neuronal populations may represent a biomarker of genuine motor recovery after stroke.

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First, I must thank Dr. Gerlinde Metz for her support over the last two years. It was my pleasure to have the opportunity to contribute in her lab and I benefitted greatly from her expertise and patient nature. In addition to her strong mentorship, Dr. Metz is genuinely concerned about the well-being of all members of her lab and willing to go to bat for her students. Thank you again.

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Finally, but by no means lastly, I thank all my fellow graduate students at the CCBN, past and present. At times it seemed like the blind leading the blind, but between all of us, we always seemed to find the answer.

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List of Abbreviations

µA=Microamperes
µM=Micrometers
A/P=Anterior/posterior
AMPA=2-amino-3-5-methyl-3-oxo-1,2- oxazol-4-yl-propanoic acid
ANOVA=Analysis of variance
BDNF=Brain-derived neurotrophic factor
cm=Centimeters
CST=Corticospinal tract
DAPI=4',6-diamidino-2-phenylindole
DPO=Days post-operative
Hz=Hertz
i.p.=Intraperotoneal
i.v.=Intravenous
ICMS=Intracortical microstimulation
kg=Kilograms
LFP=Local field potential
LTP=Long-term potentiation
M/L=Medial/lateral
M1=Primary motor cortex
MCA=Middle cerebral artery
MCAo=Middle cerebral artery occlusion
mDCS=Modified direct current stimulation
mg=Milligrams
mm=Millimeters
mm^3=Cubic millimeters
NMDA=*N*-methyl *D*-aspartate
SEM=Standard error of the mean
SPRT=Single pellet reaching task
tDCS=Transcranial direct current stimulation
WPO=Weeks post-operative

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Chapter 1

Introduction

Stroke Pathophysiology

Ischemic stroke is the leading cause of disability in North America, and represents nearly ninety percent of all stroke cases (MMWR, 2004; American Stroke Association, 2005). This type of stroke is caused by an interruption of blood flow, transient or permanent, focal or global, to the brain. Although there are different types of stroke, for the purpose of the present thesis we will consider stroke to mean focal, permanent ischemia, which is the most common type of stroke. Shortly after stroke-induced interruption of blood flow to an area of the brain, neurological symptoms ensue. Symptoms are often location-dependent and may include headache, dizziness, bradykinesia and other motor symptoms; loss of vision, impaired vision, slurred speech and impaired cognitive processes. These initial symptoms are a result of energy starvation in the area of diminished blood flow. As soon as two minutes after the onset of ischemia, cell death occurs primarily through necrotic mechanisms (Murphy & Corbett, 2009). Since neural function is dependent on blood flow, the extent of the damage to the brain is dependent on location and severity of blood flow restriction. Secondary damage also occurs, through cascade reactions to the initial damage and can continue into the semi-acute and chronic phases of the stroke. This phenomenon has been termed evolving stroke damage (Carmichael, 2005) and involves highly complex cellular mechanisms including excitotoxicity, acidosis, inflammation, oxidative stress, peri-infarct depolarizations, diaschisis and apoptosis (for further detail, see Doyle, Simon and Stenzel-Poore, 2008; Krnjevic, 2008). After the resolution of these stroke-induced physiological phenomena, physical deficits often remain and can worsen if left untreated.

Nearly 80% of people survive ischemic stroke and two thirds of those survivors experience some degree of motor dysfunction (Carmichael, 2005). Therefore, increasing emphasis is being placed on research aimed at repairing damage and ameliorating behavioural deficits associated with the chronic phase of stroke.

Motor Dysfunction After Stroke

Those who survive stroke can display neurological deficits that range in severity and are often permanent. Some of the most common post-stroke deficits are sensorimotor in nature and can include upper limb hemiparesis, loss of tactile discrimination and abnormal movement patterns (Nudo, 2006). Some degree of resolution to these symptoms can occur spontaneously, which may be attributed to the resolution of the aforementioned evolving stroke damage in the acute phase of stroke, which includes diaschisis. Diaschisis, a term coined by Von Monakow in 1914 (Von Monakow, 1914), is the temporary spreading of depressed neural activity to remote brain areas with regard to the focus of the stroke damage. Unfortunately, in most cases the resolution of diaschisis and other post-stroke pathophysiological processes is insufficient to ameliorate the neurological motor symptoms associated with ischemic stroke. These symptoms can adversely affect the quality of life of the patient and their family and are the subject of extensive research.

The most common post-stroke motor deficit is upper limb dysfunction (Thom *et al*, 2006), which can be especially devastating. Upper limb impairment affects one's ability to be self-sufficient and can be caused by damage to the primary motor cortex which is typical in cases of middle cerebral artery (MCA) stroke (Kwakkel, Kollen and Lindeman 2004). Despite great effort by basic and clinical research, there still are no curative treatments for stroke

available. However, the gold standard in treatment for post-stroke motor deficits is physical rehabilitation therapy (Gresham *et al*, 1995), which will be discussed briefly later in Chapter 1.

Functional Organization and Plasticity in the Motor Cortex

The main function of the primary motor cortex (M1) is to execute voluntary movements (Sanes and Donoghue, 2000). M1 is organized in a loosely somatotopic arrangement of identifiable, continuous, but overlapping representations. There are six layers in the cerebral cortex and layer five of M1 gives rise to the corticospinal tract (CST, or pyramidal tract) which is the main descending motor pathway in mammals. The CST is mainly responsible for the control of skilled limb movements, and is somatotopically organized.

The first demonstration of M1 functional somatotopy was achieved by Canadian neurosurgeon Wilder Penfield in the mid-twentieth century. Penfield used low intensity electrical stimulation to identify the cortical map of movement representations, which has now come to be known as the M1 motor map (Penfield, 1950). Despite the seemingly precise nature of Penfield's work (precise for the era), it has come to light that the original somatotopy discovered in the mid-twentieth century, is in actuality far more complex with less division between functional representations than originally thought (Sanes and Donoghue, 1997). Furthermore, intracortical microstimulation (ICMS) studies have shown that functional representations in M1 are rapidly modulated by experience (Sanes and Donoghue, 2000; Kleim *et al*, 2004). In addition to being somatotopically organized and being the major motor efferent via layer five pyramidal neurons, M1 has a complex, local architecture of horizontal connections that extend to nearby cortical columns and cortical layers, up to approximately one centimetre in distance (Hess and Donoghue, 1994). No one neuron acts singularly; therefore, these complex

connections likely represent the presence of coordinated networks as opposed to the classic view of motor control which is hierarchical and thalamocentric.

M1 somatotopy is conserved to various degrees the entire length of the descending corticospinal tract and the topography of CST terminations in the spinal cord are related to the M1 map (Chakrabarty, Friel and Martin, 2009). Integrity of these corticospinal connections is required for skilled movements and loss of these connections results in the reduction of skilled movement capabilities or cessation of them entirely (Anderson, Gunawan and Steward, 2007). Thus, if connections are lost and skilled movements persists, it is logical that some physical change in connectivity has taken place to preserve the skilled movements

One of the first ideas that brain circuits were modifiable or plastic, versus the previously held view that brain structure was rigid and static, came courtesy of Hebb in 1947. Hebb observed that rats that were housed in his kitchen seemed to have better motor, learning and general cognitive abilities than rats housed in a laboratory environment and he later hypothesised that these changes in behaviour were due to changes in the brain (Hebb, 1949). At that time there was no neurophysiological or anatomical evidence to confirm Hebb's claim that the brain was plastic and capable of structural change, but it is now a fundamental tenant of neuroscience. Plasticity can be defined as the modification of neuronal physiology and structure in response to experience. Since Hebb's observations, a large number of studies have shown that the brain can be modified in virtually every cortical area through experience in a wide variety of species such as molluscs, insects, birds, rodents, and primates, including humans, with the most relevant demonstrations to the present thesis in the primary motor cortex of rodents and primates (Nudo, 2006; Adkins *et al*, 2006; Williams *et al*, 2006).

Molecular Mechanisms of Plasticity

Neuroplasticity in M1 is activity-dependent and prevalent during development, motor skill learning, and after brain damage. The most profound and comparable periods of cortical plasticity are during development and after brain damage. During development neurons grow and migrate, making widespread synaptic connections. There are highly complex and timed neurochemical events that help achieve a high precision of axonal pathfinding to assure that the proper functional circuitry is developed and maintained, which have been demonstrated by Marc Tessier-Lavigne in his work from 1990 to present (Kolokin and Tessier-Lavigne, 2011). Similarly, after injury to M1, sequential waves of the same growth-promoting molecules found in developmental processes are expressed both in the peri-infarct region and functionally related areas (Carmichael, 2006). It is important to note that a relatively high post-injury expression of growth-promoting molecules is seen after ischemic stroke, with far lower levels of expression seen in cases of traumatic brain injuries (Carmichael, 2006).

Many molecules and receptors are involved in order to make the environment in the post-stroke brain conducive to axonal growth and synapse formation. In addition to axonal plasticity, the brain is constantly remodelling synapses through dendritic changes which, like axonal changes, are even more prevalent after brain damage and stroke (Kolb, Hewson and Whishaw, 1993). However, it is unclear whether layer five M1 neurons' dendrites are highly modifiable after stroke as most research on dendrite remodelling has focused on other cortical layers (Mostany and Portera-Cailliau, 2011). A few examples of the molecules that have been shown to be upregulated in the process of axonal and dendritic plasticity are growth-associated protein 43, microtubule-associated protein 2 and cytoskeleton-associated protein 23 which are axonal growth-promoting (Wieloch and Nikolich, 2006). By contrast, ephrin A5, semaphorin 3A and Nogo A, along with others, are inhibitory to axonal growth (Wieloch and Nikolich, 2006; Murphy

and Corbett, 2009; Metz and Faraji, 2009). Many cell adhesion molecules and receptors aid in dendrite remodelling and are in the process of being identified.

Perhaps the most interesting molecule implicated in brain plasticity is brain-derived neurotrophic factor (BDNF). BDNF expression is crucial for AMPA receptor trafficking in glutamatergic neurons, which are important for increases in synaptic efficacy. Experimental demonstrations of this can be seen in rodents including long term potentiation (LTP) being BDNF-dependent (Fritsch *et al*, 2010), activity-dependent motor map reorganization being BDNF-dependent (Kleim *et* al, 2006), and BDNF being important for NMDA-mediated synaptic strengthening (Fritsch *et al*, 2010b). Taken together, research on post-stroke neuroplasticity has established that the primary motor cortex is equipped with the architecture and mechanisms required for large-scale structural modulation. The reciprocal and redundant connections, the sophisticated regulation of growth-promoting and growth-inhibiting factors, axonal growth and dendritic remodelling provide the necessary framework for significant plastic changes to take place although there are yet to be definitive causal relationships established.

Rat Models of Motor Cortex Stroke

Animal models, particularly rodent models of focal cerebral ischemia, have provided much of the information that has been collected about stroke. There are differences between a rat brain and a human brain, a human stroke and an experimentally induced rat model of stroke, but due to the high cost of non-human primate studies, and obvious impediments when studying human patients, rodent studies provide a cost-effective, logical alternative for in depth, *in vivo* study. The variability that exists between models of stroke is the subject of some controversy and should not be ignored (Gonzales and Kolb, 2003; Alaverdashvili *et al*., 2008).

However, this variability can be taken advantage of to address different characteristics of human stroke and reliably reproduce them.

No one rodent model of stroke is appropriate to mimic all aspects of human stroke. Because of this, primary stroke research must be well planned and use the appropriate model to answer the research question being asked. For example, using a large infarct rat model of stroke, such as a four vessel occlusion model, that in humans would be considered non-survivable, would be ill advised if the research question involved mild hemiparesis of the distal limb. A considerable literature base exists on most rat models of stroke, with their benefits and limitations thoughtfully discussed (Ginsberg & Busto, 1989; Carmichael, 2005). The obvious advantages of rodent-based stroke research are the wide range of behavioural, histological, immunohistochemical and *in vivo* investigations possible that are not available in human stroke patients. It is through these techniques that researchers must be able to answer questions about what is happening in the post-ischemic brain, especially when function improvements after stroke are observed.

There are fewer than ten well established rodent models of stroke (Carmichael, 2005), and of those only three are commonly used to produce focal cerebral ischemia in rats. When applied distally, the middle cerebral artery occlusion (MCAo) model provides a large, but localized and reproducible unilateral lesion by restricting blood flow via the suturing of the middle cerebral artery (MCA) either on the surface of the brain or underneath the temporalis muscle (Tamura *et al*, 1981). Both of these versions of distal MCAo require a craniotomy, a high degree of surgical skill and are invasive. Another highly used model of focal cerebral ischemia is the devascularization model, also known as the pial strip model. This model involves a craniotomy, physical removal of surface cerebral vasculature, normally achieved with a saline-soaked swab (Kolb *et al*, 1996) and is also considered relatively invasive, although the surgical

skills involved are fewer than in the MCAo model. Both of these models produce reliable, reproducible, focal cerebral lesions that are relatively large in volume and routinely cause damage to subcortical regions as well as corpus callosum fibres. A less invasive model of focal cerebral ischemia is the photothrombosis model, which does not require a craniotomy (Watson, 1985). This can be achieved by injection of a photosensitive dye, such as Bengal rose through a tail vein and shining a laser or high intensity light through the intact skull. This combination causes platelet aggregation and endothelial damage in any vasculature that is directly in the path of the light, ultimately resulting in focal ischemia similar to human stroke. Experiment 1 (Chapter 2) demonstrates that photothrombotic lesions are reproducible, localized and rarely extend past the desired stereotaxic coordinates.

One of the most important aspects of any stroke model is the behavioural deficits that are caused by the lesion, especially when examining motor recovery and compensation. Rats have been shown to be good models of motor dysfunction after stroke in particular with regards to skilled forelimb use (Whishaw, Whishaw and Gorny, 2008). Moreover, like humans, the main behavioural mechanism of functional recovery of skilled movements in rats is compensation (Kwakkel, Kollen and Lindeman 2004; Metz, Antonow-Schlorke and Witte, 2005), not recovery of original movement patterns. It has also been shown in rats that lesion size does not necessarily correlate with severity of motor dysfunction (Metz, Antonow-Schlorke and Witte, 2005; Alaverdashvili *et al*, 2008). Despite resulting in a wide range of severity with respect to motor dysfunction, human stroke produces infarcts that are most often small in size (Carmichael, 2005) which means that stroke volume does not accurately predict the severity of chronic motor deficits which is similar to findings in rats. Taken together, current research confirms a high level of translatability between human and rat studies that examine motor compensation and recovery after stroke. Therefore, when studying post-stroke compensation, recovery and M1

plasticity, it is beneficial to only damage the area of interest to control for loss of function due to peripheral damage in other brain areas.

Recovery and Compensation

The difference between recovery and compensation is distinct and often misunderstood. It is not usually the case that when a stroke patient regains the function of a previously paretic limb it is because they have recovered, in fact, the most common mechanism of functional recovery after stroke is compensation. Recovery or "genuine recovery" refers to the restitution of original function, including movement patterns; whereas, compensation is the use of altered movement patterns to achieve functional proficiency with the affected limb and is often mistakenly referred to as recovery. Of the human studies that claim observance of recovery after stroke virtually none of them have looked at quality of movement or the kinematics of the observed functional recovery (Kwakkel, Kollen and Lindeman 2004; Levin, Kleim and Wolf, 2009). Although some spontaneous improvements in post-stroke movement patterns have been documented, complete normalization of these patterns has yet to be seen in rats. Despite great effort by basic and clinical research, there still are no curative treatments for stroke available. However, the gold standard in treatment for post-stroke motor deficits is physical rehabilitation therapy (Gresham *et al*, 1995).

Physical Rehabilitation After Stroke

The goal of post-stroke physical rehabilitation is to provide the patient with a return to a high degree of functional motor performance. Rehabilitation normally consists of highly repetitive movements of the affected body part, either passively if there is little to no movement capabilities of the patient, or actively if the patient still has some ability to move the

affected limb. Despite rehabilitative efforts, post-stroke motor outcomes remain varied and largely incomplete with respect to functional recovery (Kwakkel, Kollen and Lindeman, 2004). Physical rehabilitation is believed to assist patients with relearning motor skills, or learning new ways to move using compensatory strategies, with therapy-induced improvements most likely being activity dependent. However, it is difficult to attribute improvements to specific treatment strategies (Aichner, Adelwoher and Haring, 2002).

One of the more publicized rehabilitative strategies for patients with upper limb motor dysfunction is constraint-induced movement therapy (CIMT) which involves the forced use of the affected limb during daily and therapeutic activities via the immobilization of the unaffected limb (Taub and Morris, 2001). Even with intensive task-specific training, it is estimated that thirty percent of patients have permanent disability (Dimyan and Cohen, 2011) and that the positive effects of rehabilitation may not be permanent.

Any rehabilitation therapy is very costly, work intensive and requires a high degree of patient compliance. These non-medical obstacles may interfere with the availability and effectiveness of physical rehabilitation in real-life clinical settings. Maximizing early results and providing the best strategies for the greatest restitution of original function are of paramount importance to circumvent these obstacles.

Electrical Stimulation to Promote Motor Cortex Function

Exogenous electrical currents, when applied to the brain, affect cortical excitability. An extremely large and convoluted literature exists on brain stimulation, beginning with the work of Fritsch and Hitzig (Fritsch and Hitzig, 1870). Here, however, we will focus on stimulation of the primary motor cortex and related areas in humans and in rats.

Human studies have shown that transcranial direct current stimulation (tDCS) applied to M1 improves motor learning and function in paretic limbs, reduces pain, and increases neuronal excitability (for a review see Nitsche *et al*, 2008). In rats, recent studies have shown that electrical stimulation increases functional recovery after focal cerebral ischemia of the forelimb area of M1 (Adkins-Muir and Jones, 2003; Kleim *et al*, 2003; Adkins, Hsu and Jones, 2008), increases cerebral blood flow (Wachter *et al*, 2011), increases BDNF-dependent cortical plasticity (Fritsche *et al*, 2010) and modulates brain activity (Ozen *et al*, 2010). Any changes in brain activity that can be elicited by other methods such as transcranial magnetic stimulation, or invasive cortical stimulation can be reproduced by tDCS with considerably less expense (Ozen *et al*, 2010). This makes tDCS a viable non-invasive and inexpensive method of brain stimulation.

The mechanisms proposed to explain the effects of tDCS on brain function include the modulation of neurotrophic factor and growth-promoting molecule expression (Fritsch *et al*, 2010) and the manipulation of sub-threshold intrinsic oscillations, termed local field potentials (LFPs) and the spiking activity (action potentials) of neuronal ensembles (Ozen *et al*, 2010). LFPs can be defined as subthreshold neuronal voltage fluctuations recorded from the extracellular space, which mainly originate from postsynaptic potentials. Due to widespread changes in brain activity after stroke and the effectiveness of tDCS to modulate cortical excitability, the current literature suggests that tDCS of the motor cortex should be investigated further as a potential therapy for stroke.

Objectives of the Present Thesis

The goals of the present thesis are threefold. The first goal, which Experiment 1 is designed to meet, is to examine the effects of tDCS on motor rehabilitation, recovery and compensation after stroke in rats. The second goal, addressed by Experiment 2, is to examine

the effects of tDCS on local field potentials generated by homotopic neuronal populations across cerebral hemispheres. The third goal is to put the results from both experiments into the context of other current findings in the field of motor compensation and recovery after stroke and attempt to draw feasible conclusions on possible mechanisms which may provide insight for future stroke research.

Chapter 2

Transcranial Direct Current Stimulation Supports Forelimb Rehabilitation After Stroke in Rats

Introduction

In humans, physical rehabilitation is currently the sole strategy for the treatment of post-stroke upper limb motor deficits with either bilateral arm training (BAT) or CIMT being the most common rehabilitation tools (Kwakkel, Kollen and Lindeman, 2004). Despite the efforts of rehabilitation experts and the adoption of designated stroke units in hospitals, stroke patients with upper limb motor deficits often experience permanent disability (Nudo, 2006). Furthermore, when stroke patients do show motor improvements, they are mostly due to the adoption of compensatory movements rather than genuine recovery (Kwakkel, Kollen and Lindeman, 2004). Often the benefits of physical rehabilitation are incomplete, or temporary, or both (Johansson, 2010). Compensation represents the majority of post-stroke motor skill improvements in rats as well (Metz, Antonow-Schlorke and Witte, 2005; Moon *et al*, 2009) which is not surprising due to the similarities in reach-to-eat movements between humans and rats (Pellis & Whishaw, 1992; Sacrey, Alaveradashvili and Whishaw, 2009) as well as the post-stroke similarities between upper limb motor dysfunction in humans and forelimb motor dysfunction in rats (Murphy and Corbett, 2009).

Recent studies have identified tDCS as a possible adjunct therapy to increase the efficacy of physical rehabilitation aimed at ameliorating post-stroke motor dysfunction (Adkins *et al*, 2008; Nowak *et al*, 2009). Despite some promising results in both humans and rats, neither the optimal stimulation parameters nor the mechanisms of the observed functional improvements have been identified. Additionally, the previous studies mentioned have only examined the effects of tDCS in conjunction with forelimb rehabilitation using a single direct

current with no additional component (Adkins-Muir and Jones, 2003; Kleim *et al*, 2003; Adkins, Hsu and Jones, 2008).

Chapter two (Experiment 1) examines the effects of tDCS and physical rehabilitation, or rehabilitation alone on skilled movement compensation and recovery after photothrombosis-induced focal cortical ischemia in rats. Experiment 1 is designed to address the hypothesis that tDCS can modulate the effects of rehabilitation. The tDCS protocol used is believed to be the first of its kind and may address some of the issues of previous stimulation protocols. Ten minutes of a cathodal, 65 μA direct current, with an additional 30 ms of 65 μA direct current applied every 5 seconds was used concurrent to forelimb rehabilitation. Similarly, quantitative and qualitative analyses of post-stroke reach-to-eat movements via the SPRT address the previous void in the literature regarding the distinction between compensation and recovery.

Methods

Animals

Eighteen Long-Evans hooded rats were received from Charles River (Ontario, Canada) at age range P70-P80 (400g-500g) and habituated to their home cage and experimenter handling for seven days. All animals had access to water and food (Purina rat chow) *ad libitum*, except for during SPRT training where they were limited to 30g once a day for motivational purposes. During food restriction, rats' weights were monitored and all rats gained weight consistently during the course of the experiment. Rats were housed singly under a 12-hour light cycle, with lights being turned on at 7:30 AM. One animal was removed from the experiment during SPRT training due to a cyst on its shoulder. All procedures were performed in accordance with the guidelines set by the Canadian Council for Animal Care and the University of Lethbridge animal welfare committee (protocol #1008).

Experimental Design

All animals were trained in the SPRT to asymptote success levels. Once trained, animals were tested for five days to establish baseline performance values and filmed on the fifth testing day to capture baseline reaching movement patterns. All rats were also filmed on the ladder rung walking task and cylinder forelimb asymmetry task. The animals were then divided into three experimental groups: Stimulation n=6; Lesion n=6; and Control n=5 which were equalized with respect to rat handedness and single pellet reaching success. All animals received transcranial electrode implantation surgeries and the Stimulation and Lesion group animals also received a photothrombosis stroke surgery concurrently. Animals were allowed to recover for seven days post-surgery. On day post operative (DPO) 7-8 all animals were tested and filmed in the ladder rung walking task, the cylinder forelimb asymmetry task and SPRT to establish post-surgery/pre-treatment values of performance. Beginning on DPO 9, post-stroke therapies were administered; tDCS and physical rehabilitation for the Stimulation group and rehabilitation only for the Lesion group. The Stimulation group received three days of tDCS and rehabilitation, then two days of rehabilitation only in the first week. In weeks two and three the Stimulation group received rehabilitation only. The Lesion group received five days of rehabilitation only in all three weeks, as did the Control group. All animals were tested weekly in the SPRT, ladder rung walking task and cylinder forelimb asymmetry task. After the third week of post-stroke therapies and testing all animals received thirty days of home cage rest before they were re-tested (Figure 2.0).

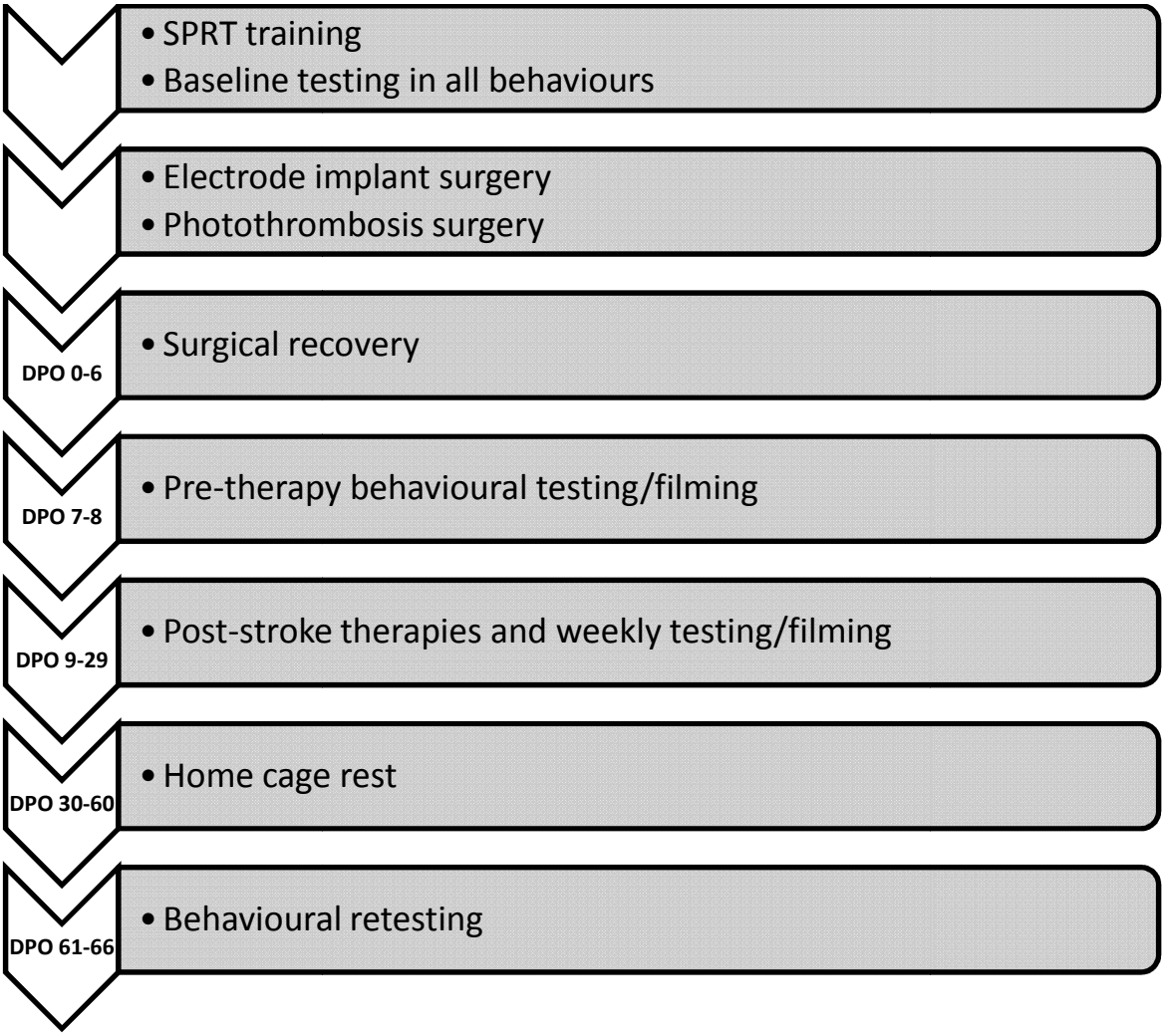

Figure 2.0. Timeline for Experiment 1.

Single Pellet Reaching Task (SPRT)

All rats were trained in the SPRT, which is a reach-to-eat task designed to quantify and qualify skilled forelimb use (Whishaw and Pellis, 1990; Whishaw *et al*, 1993). The skilled reaching apparatus was a rectangular Plexiglas box 40 cm long X 45 cm high X 13 cm wide with a 1cm slot at one end and a shelf fastened on the outside of the box which was accessible via the slot. The shelf had two symmetric indentations which were 1.5 mm deep, 1.5 cm from the slot, parallel to

each edge of the slot, to provide stability and a constant position for the food pellets to be placed. The shelf itself was 4 cm high with respect to the base of the apparatus. The food pellets used weighed 45 mg and were of uniform size (Bioserve, USA. Product # F0021).

Once trained to asymptote success levels, rats received 20 trials per day in the SPRT to establish baseline reaching success values. One trial is defined as the rat walking to the rear end of the box, turning around, walking to the front end of the box and attempting to grasp the food pellet through the slot in the front of the box with its preferred paw (Figure 2.0B). A success is defined as an animal reaching for the pellet, grasping it and placing it in its mouth/eating it on the first attempt. Success scores for each rat and each session were calculated using the following formula:

$$\text{Success rate} = \#\text{ of successful reaches} \div 20 \times 100$$

The total number of attempts over 20 trials, the time taken to complete 20 trials and the total number of pellets eaten were also recorded.

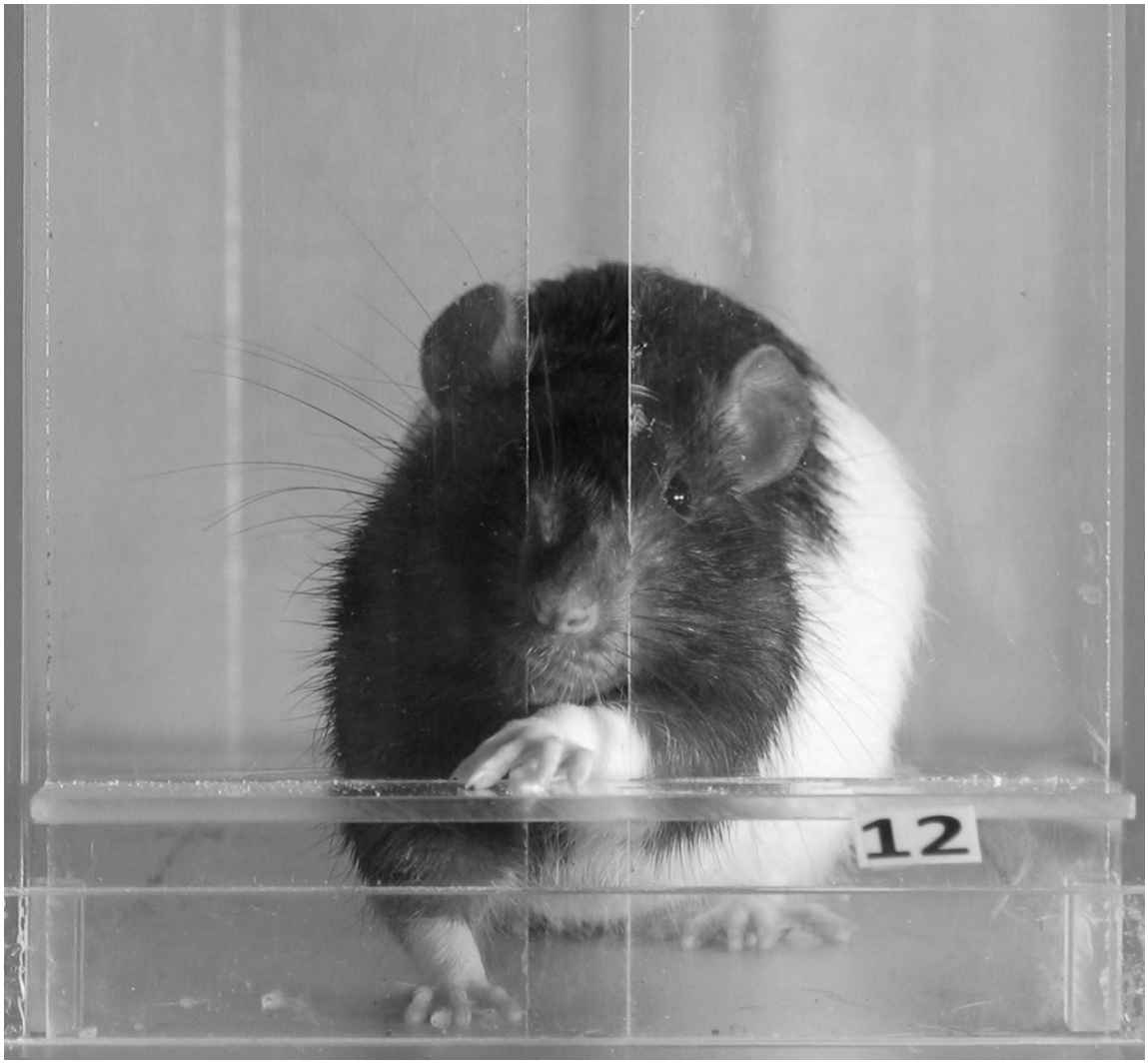

Figure 2.0B. Photograph of a rat performing the single pellet reaching task.

Qualitative Analysis of SPRT

Reaching movements were scored qualitatively using 11 categories and a total of 35 sub-components (Metz, Antonow-Schlorke and Witte, 2005; Table 2.0), which was achieved by analysing videotaped SPRT sessions frame by frame. Each movement sub-component was given a numeric score: 0 if the movement was absent, 0.5 if the movement was present but abnormal and 1 if the movement was present and normal. The maximum total qualitative reaching score

possible was 35. The mean score of 3 successful reaches was calculated and used as the total qualitative reaching score for all rats in all testing/filming sessions.

Cylinder Forelimb Asymmetry Task

Rats were placed in a Plexiglas cylinder 50 cm in diameter and 60 cm high and filmed from a ventral aspect (Figure 2.0C; Schallert *et al*, 2000). Each forelimb lift, contact with a wall and landing after a rear was scored. A testing session consisted of 5 minutes inside the cylinder and 10 rears that involved wall contact were scored from the videotaped recordings frame-by-frame. Asymmetry was assessed by calculating the percent usage in each of the three components of a wall contact rear with respect to the preferred forelimb. Forelimb preference was determined during the SPRT training sessions.

Figure 2.0C. Photograph of a rat performing the cylinder forelimb asymmetry task.

Movement	Sub-component	Score
1. Orient	-head oriented to target	0, 0.5, 1
	-sniffing	0, 0.5, 1
2. Limb lift	-body weight shift to back	0, 0.5, 1
	-hindlimbs aligned with body	0, 0.5, 1
	-limb moves forward	0, 0.5, 1
	-digits on midline	0, 0.5, 1
3. Digits close	-palm supinated, semi-in	0, 0.5, 1
	-digits semiflexed	0, 0.5, 1
4. Aim	-elbow comes in	0, 0.5, 1
	-palm in midline	0, 0.5, 1
5. Advance	-elbow in	0, 0.5, 1
	-limb forward	0, 0.5, 1
	-limb directed to target	0, 0.5, 1
	-head and upper body raised	0, 0.5, 1
	-body weight shift front	0, 0.5, 1
	-body weight shift lateral	0, 0.5, 1
6. Digits open	-digits open	0, 0.5, 1
	-discrete limb movement	0, 0.5, 1
	-not fully pronated	0, 0.5, 1
7. Pronation	-elbow out	0, 0.5, 1
	-palm down in arpeggio	0, 0.5, 1
8. Grasp	-arm still	0, 0.5, 1
	-digits close	0, 0.5, 1
	-hand lifts	0, 0.5, 1
9. Supination I	-elbow in	0, 0.5, 1
	-palm medially before leaving slot	0, 0.5, 1
	-palm turned 90°	0, 0.5, 1
10. Sup II	-head points down	0, 0.5, 1
	-body horizontal	0, 0.5, 1
	-palm straight up	0, 0.5, 1
	-distal limb movement	0, 0.5, 1
11. Release	-open digits	0, 0.5, 1
	-puts food in mouth	0, 0.5, 1
	-head and upper body lowered	0, 0.5, 1
	-raises other paw	0, 0.5, 1

Table 2.0. Movement categories and sub-components for qualitative analysis of skilled reaching movements (Metz, Antonow-Schlorke and Witte, 2005).

Ladder Rung Walking Task

The ladder rung walking apparatus used was 100 cm in length with Plexiglas walls 19 cm high and metal rungs 0.3 cm in diameter connecting the two Plexiglas walls. Rungs were spaced in an irregular pattern with the minimum distance between rungs being 1 cm and the maximum distance between rungs being 5 cm (Figure 2.0D; Metz and Whishaw, 2002). The entire apparatus was elevated approximately 30 cm above a table surface. All animals were habituated to the apparatus for one session consisting of 5 trials. On testing days, rats were placed at one end of the apparatus (the same end for all rats on all days) and required to walk the entire length across the rungs to reach their home cage. All rats crossed the apparatus three times each testing session. Sessions were video recorded for later frame-by-frame analysis. Quantitative analysis of ladder rung walking videotape consisted of counting the number of errors based on a seven-category foot placement scoring system (Metz and Whishaw, 2002): (0) *Total miss*. The limb completely missed the rung and a fall occurred. (1) *Deep slip*. The limb was placed on the rung, but then slipped off when weight bearing and caused a fall. (2) *Slight slip*. The limb was placed on a rung, slipped off when weight bearing, but did not result in a fall that interrupted stepping cycles. In this case, the animal was able to maintain balance and continue a co-ordinated gait. (3) *Replacement.* The limb was placed on a rung, but before it was weight bearing it was quickly lifted and placed on another rung. (4) *Correction*. The limb aimed for one rung, but was then placed on another rung without touching the first one. Alternatively, a score of 4 was recorded if a limb was placed on a rung and quickly repositioned. (5) *Partial placement*. The limb was placed on the rung with either wrist or digits of the forelimb or heel or toes of a hindlimb. (6) *Correct placement*. The midportion of the palm of the limb was placed on the rung with full weight supported (taken from Metz, Antonow-Schlorke and Witte, 2005). An error (foot

fault) was defined as any limb placement that received a score of 2 or less. Total average foot placement scores were also tallied for each limb.

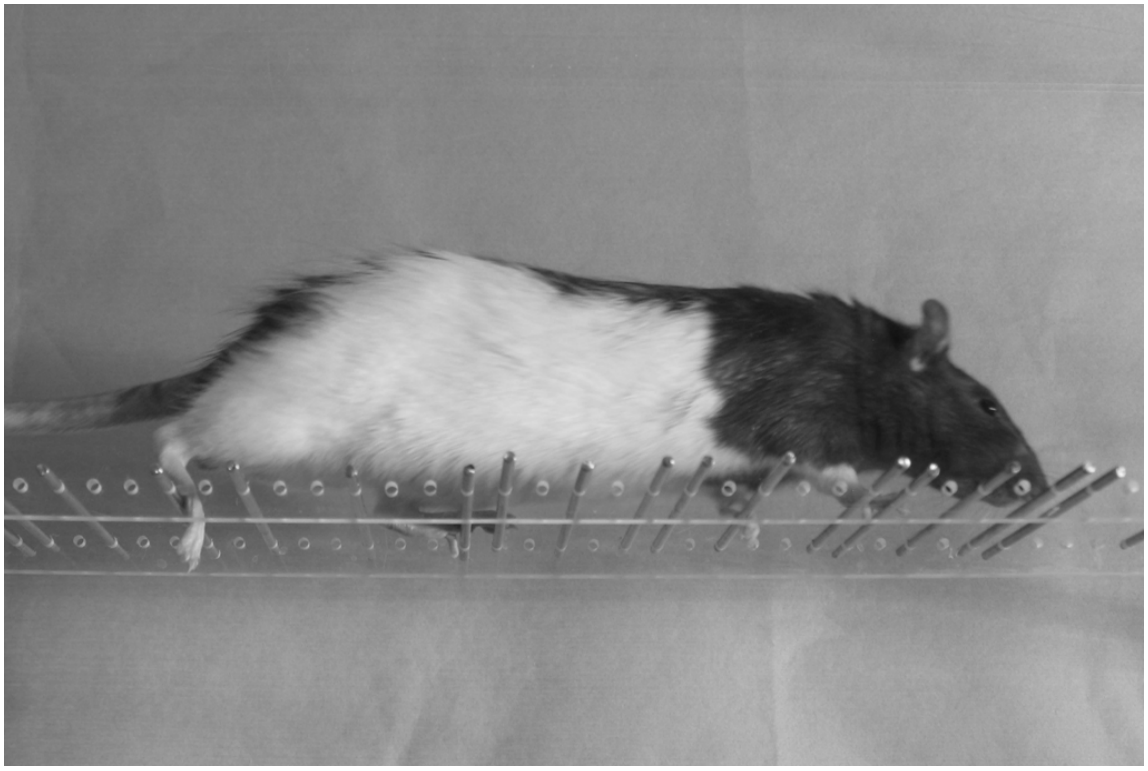

Figure 2.0D. Photograph of a rat performing the ladder rung walking task.

Photothrombosis Lesion and Electrode Implant Surgeries

Focal photothrombosis was induced in the forelimb area of M1 contralateral to the preferred forelimb (identified during SPRT task). Animals were anesthetized using 4% isoflurane in a mixture of 1.5% oxygen and their heads shaved before securing them in a stereotaxic frame (David Kopf, Germany). The scalp was transected, retracted and the underlying skull cleaned to reveal skull features for stereotaxic purposes. The skull was thinned using a fine dental burr in a rectangular shape from Bregma -1.0 to 4.0 anterior/posterior and Bregma 1.0 to 4.0 medial/lateral. A cold light source (Schott KL 1500, Germany) with an aperture of same size and

shape as the partial craniotomy was positioned over the skull. The skull was illuminated at maximum light settings for 20 minutes. During the first 2 minutes of illumination Bengal rose dye solution was injected through a tail vein (20 mg/kg, 10% solution in 0.9% saline). When the illumination period was finished, animals received transcranial stimulation, recording and ground electrode implants (Note: Control group animals did not receive photothrombosis surgeries).

Pilot holes were drilled in the skull bilaterally for stimulation and recording electrodes at coordinates: +4.5 A/P, ±1.0 M\L: -2.0 A\P, ±4.5 M/L respectively (Figure 2.1B). Pilot holes for the reference and ground screws were drilled on either side of the midline in the occipital bone. Stainless steel screws (1 mm diameter; Small Parts, USA) were then implanted in the pilot holes, with special attention not to pierce the ventral skull surface. Conductive wire (AM Systems, USA) was attached to the electrode screws and electrode plugs (AM Systems, USA). A dental acrylic skull cap was fashioned on the exposed skull, engulfing the cranial electrodes and connecting wire. The electrode plugs, which served as the interface to the stimulating and recording equipment, were inserted into a plastic electrode housing (Ginder Scientific, Canada); dental acrylic was used to fasten the entire construct to the previously hardened skull cap (Figure 2.1A). Once the acrylic was hardened, animals were placed on a heating pad and monitored until they were awake and displaying normal post-surgical behaviours.

Figure 2.1. Photograph of a skull cap construct.

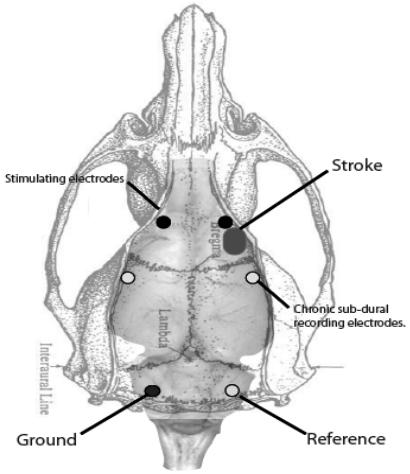

Figure 2.1B. Electrode placements.

Post-Stroke Treatments

Forelimb Rehabilitation. Stimulation, Lesion and Control groups all received forelimb rehabilitation. Forelimb rehabilitation consisted of prolonged sessions (15 minutes) of the SPRT, five days a week for three weeks. Although not quantified, it is estimated that on average rats reached for 60 pellets during the allotted time.

Transcranial Direct Current Stimulation. The tDCS stimulation protocol, consisting of 10 minutes of a cathodal, 65 μA direct current with an additional 30 ms of 65 μA direct current applied every 5 seconds, was applied to the Stimulation group rats during the first three days of post-stroke treatment concurrent with forelimb rehabilitation. LFP activity was recorded for later analysis with Neuralynx data acquisition hardware and Cheetah software (Neuralynx, USA).

Lesion Analysis

Analyses of lesion volumes, widths and depths were performed at the end of Experiment 2, but included in Experiment 1 due to greater relevance to behavioural results. DAPI-stained coronal sections (40 µm thickness), cut on a freezing microtome and mounted on microscope slides, were digitally scanned at 40x magnification (Nanozoomer, Hammatsu Photonics, Japan). The images were transferred to Image J software (NIH, USA) and the lesions were quantified. Volumes were measured by tracing lesion borders then multiplying the lesion area by section thickness and number of sections in the series (five series of each brain were taken). Lesion widths and depths were determined by superimposing a straight line connecting lesion boundaries and measuring in horizontal and vertical directions respectively. All available sections from each brain were used in the analyses and in the event of an incomplete series; volumes from missing sections were calculated as the mean of the previous and following sections. The standard method of quantifying lesions as amount of lost tissue with respect to the entire cortical volume was not used due to difficulties distinguishing cortical and subcortical boundaries and imprecise mounting technique.

Data Analysis

All behavioural data from Experiment 1 were analyzed using two-way repeated measures ANOVA or one-way ANOVA (Prism 5, GraphPad, USA). In all behavioural results figures asterisks indicate significant results (* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$). Lesion measurements and volumes were compared using T-tests (Prism 5, GraphPad, USA).

Results

Single Pellet Reaching Success

A time course plot of percent success revealed a trend of improvement in the Stimulation group's performance and an initial improvement followed by a plateau in the Lesion group's performance (Figure 2.2). Data were combined into weekly means and analyzed. Two-way repeated measures ANOVA revealed significant differences between group ($F_{2,9}=10.11$, $P \leq 0.01$) and group-by-week interaction ($F_{6,9}=8.76$, $P=<0.0001$). *Post-hoc* tests revealed the Stimulation and Control groups showed significant reduction in success rates during the first post-stroke testing session when compared to the Control group ($P<0.05$, Bonferroni). During WPO 1, the Stimulation and Lesion groups' performances returned to rates similar to Control animals. By WPO 2, the Stimulation group had improved to a rate significantly higher than Controls or Lesion animals (Figure 2.3; $P<0.05$, Bonferroni). Within-group analyses with one-way ANOVAs and *post-hoc* tests confirmed that on DPO 28 the Lesion and Control groups performed similarly to baseline measures, whereas the Stimulation group performed significantly better than the baseline success rate (Figure 2.4; $P<0.01$, Dunnett). After thirty days of home cage rest, the Lesion group had a significantly lower success rate than the Stimulation group ($P<0.001$) and the Stimulation group had declined in performance from DPO 28, but was not significantly different than Control animals and group baseline rates (Figure 2.5).

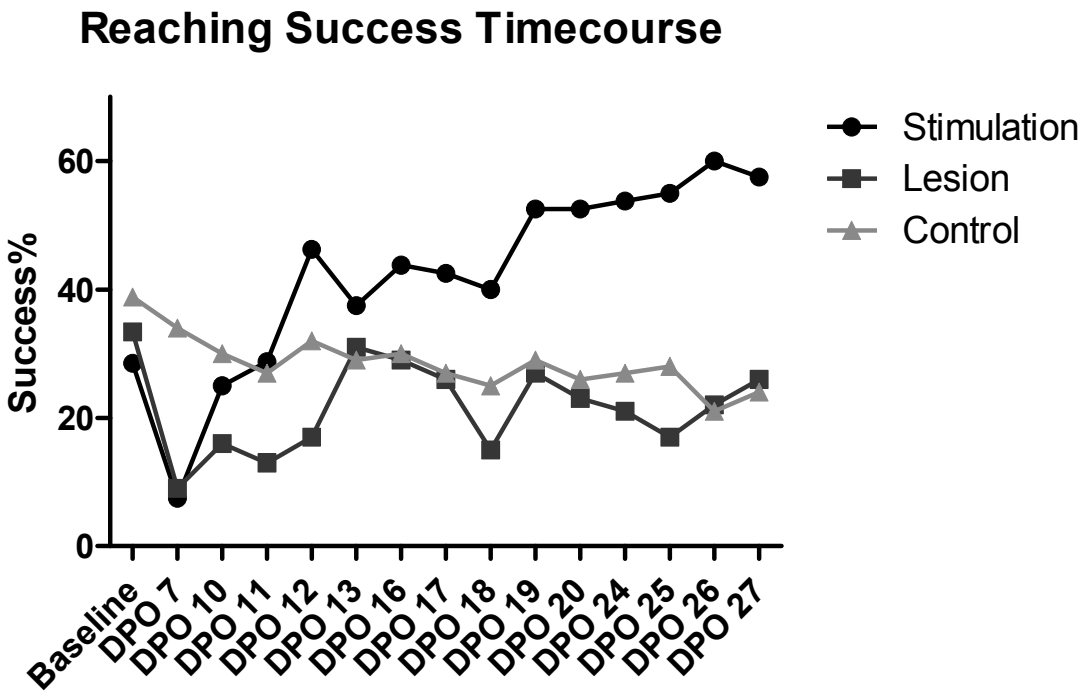

Figure 2.2. Performance in the single pellet reaching task; time course. The Stimulation and Lesion groups both showed a reduction in success on DPO 7 and a return to success rates not statistically different than the Control group on DPO 11 and DPO 13, respectively. The Stimulation group continued to improve over time, whereas the Lesion group remained not statistically different to the Control group. All data represented as group mean.

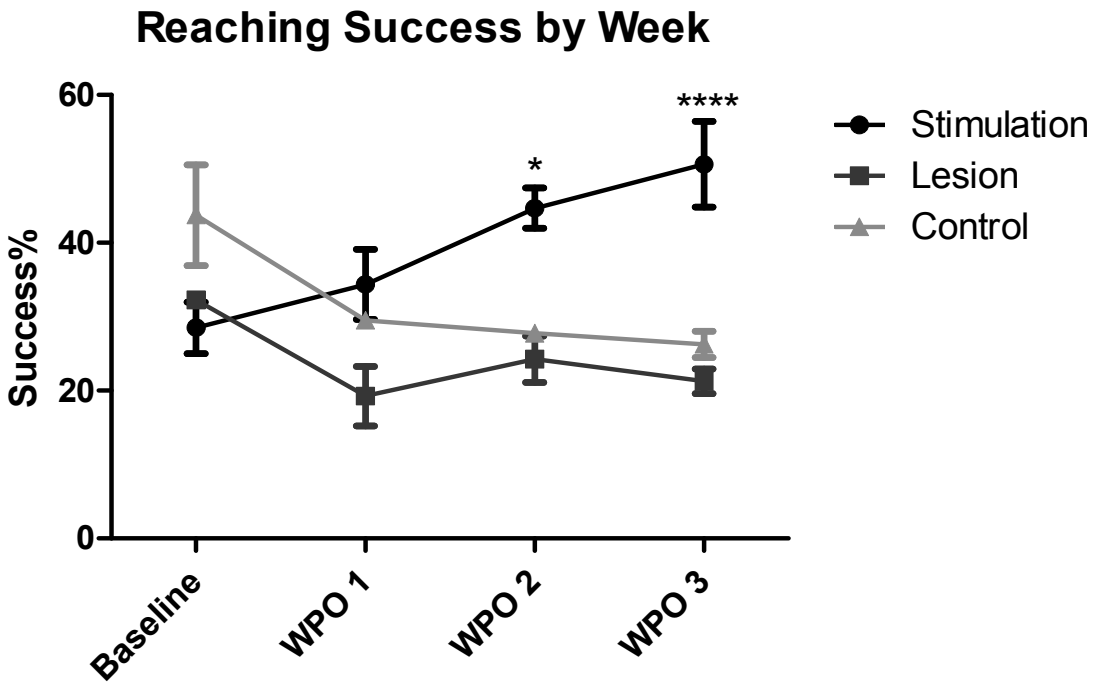

Figure 2.3. Quantitative results in the single pellet reaching task, weekly averages. The Lesion group returned to pre-stroke success levels by the end of the first week of post-stroke rehabilitation. The Simulation group showed a statistically significant improvement in the second and third weeks (denoted by asterisks; WPO 2, $P<0.05$; WPO 3. $P<0.0001$) post-stroke compared to the Control and Lesion groups. Comparison between groups using two-way repeated measures ANOVA with all data presented as group means ± SEM.

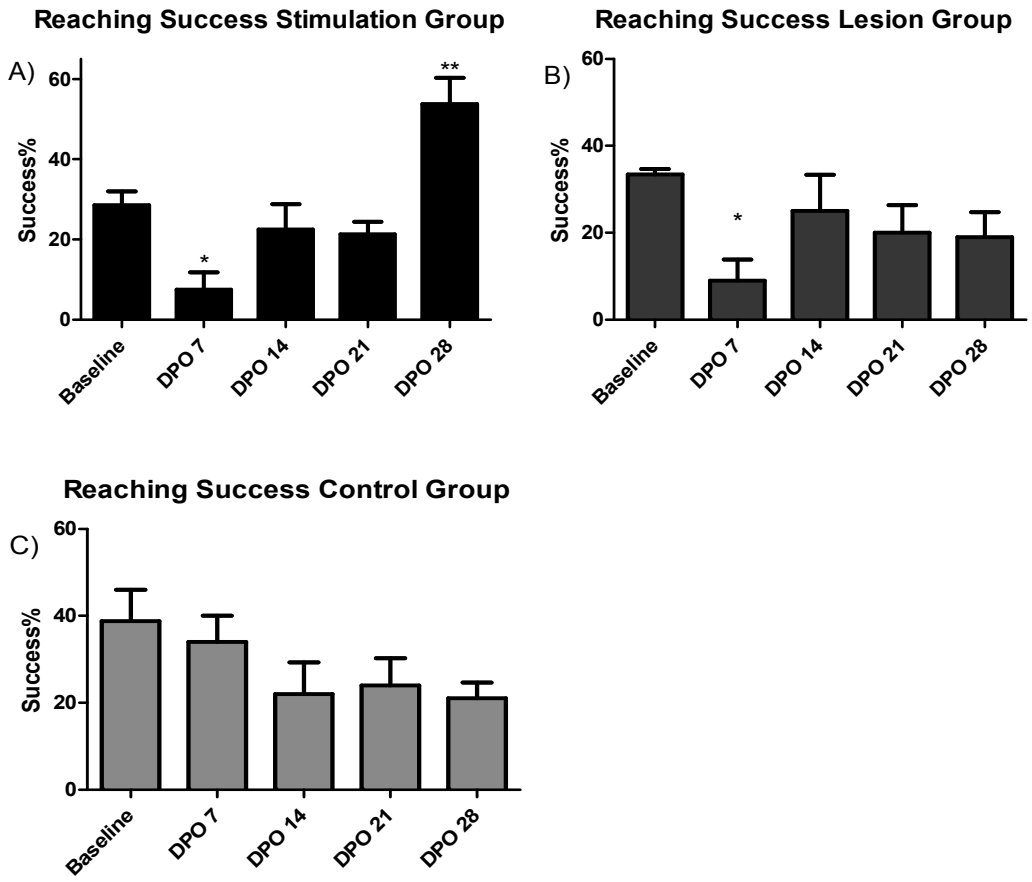

Figure 2.4A-C. Quantitative results in the single pellet reaching task; within-group comparisons. A) The Stimulation group showed significant reduction in reaching success on DPO 7 ($P<0.05$) and returned to levels not significantly different to baseline levels of success by DPO 14. On DPO 28 the Stimulation group showed a significant gain in success when compared to baseline measures ($P<0.01$). B) The Lesion group showed significant reduction in success on DPO 7 ($P<0.05$), with a return to levels not significantly different to baseline values on DPO 14-DPO 28. C) The Control group showed no significant difference in success on any day baseline to DPO 28. Comparisons within-group using one-way ANOVA. All data shown as group mean ± SEM.

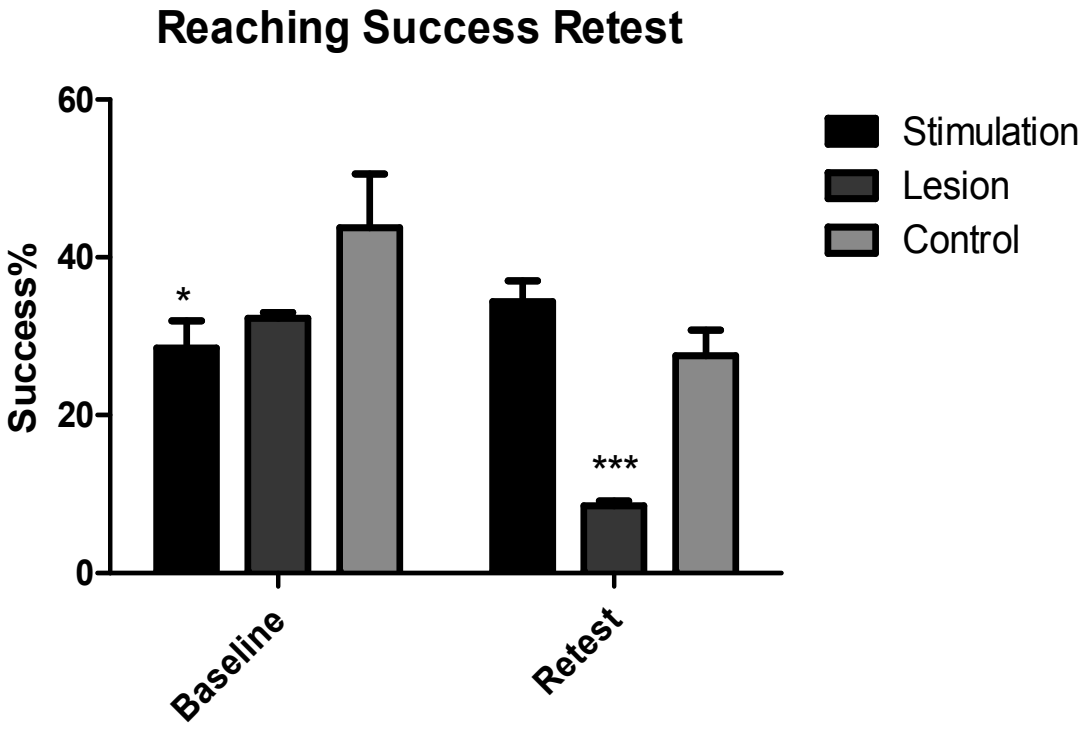

Figure 2.5. Comparison between baseline success rates and retest success rates. The Lesion group showed a significantly lower success rate than Stimulation and Control groups during the retest period ($P<0.001$). Comparison between groups using two-way repeated measures ANOVA. All data shown as group mean ± SEM.

Reaching Movement Analysis

Two-way repeated measures ANOVA revealed differences in day, group and day-by-group interaction ($F_{4,11}=23.78, P<0.0001$; $F_{2,11}=15.10, P=0.0007$; $F_{8,11}=8.68, P<0.0001$). *Post-hoc* tests showed significant reductions in mean reach rating scores on DPO 7 in the Stimulation and Lesion groups ($P<0.01$, Bonferroni). The Stimulation group returned to a mean rating score similar to Control animals on DPO 14, whereas the Lesion group's rating scores remained significantly lower than both the Control and Stimulation groups on all post-stroke filming days (Figure 2.6; $P<0.01$, Bonferroni). Two-way repeated measures ANOVA analyses were also performed on all individual components of the reaching movement scale which revealed significant differences in the measures of Orient, Limb lift, Advance, Digits open and Supination 1. *Post-hoc* tests showed that in these components, there were significant reductions in the endpoint mean rating scores of the Lesion group when compared to the Control and Stimulation groups (Figure 2.7; Orient $P<0.001$; Limb lift $P<0.001$; Advance $P<0.01$; Digits open $P<0.01$; Supination 1 $P<0.01$, Bonferroni). Analysis of reach rating scores after thirty days of home cage rest revealed that the previous improvements in the Stimulation group's movement patterns were conserved and the Lesion group showed persistent deficits (Figure 2.8). General qualitative observations made during scoring of reaching movements will be discussed in Chapter 4.

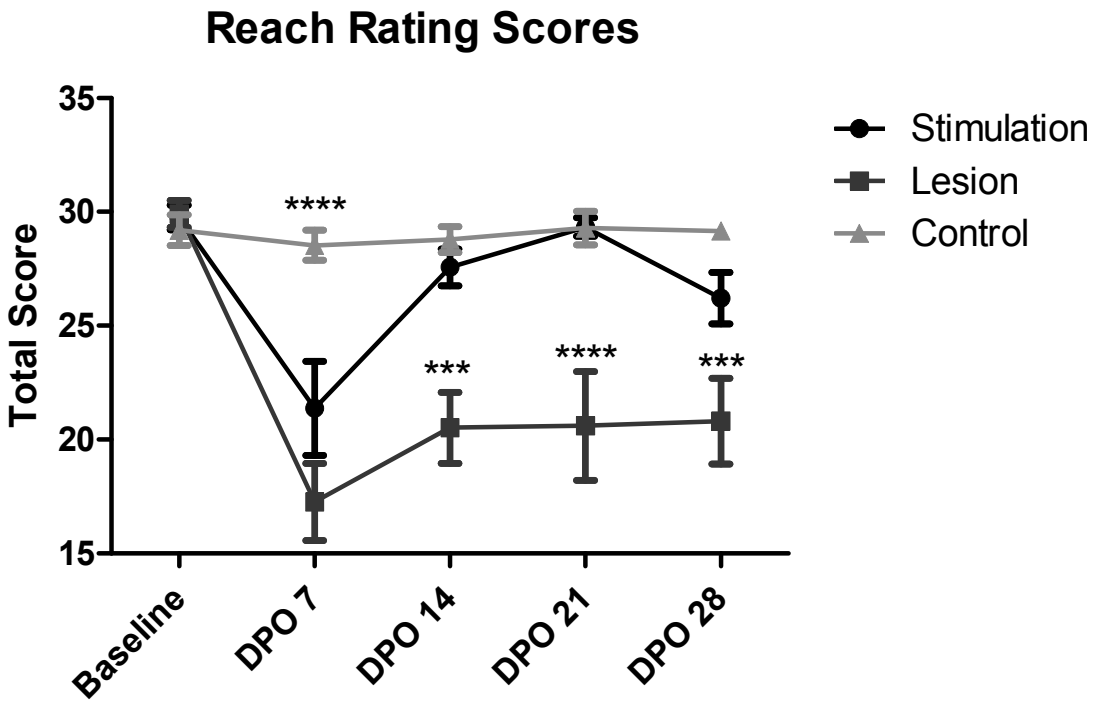

Figure 2.6. Single pellet reaching task movement quality scores. The control group performed significantly better than the Stimulation and Lesion groups on DPO 7 ($P<0.0001$). The Stimulation group regained movement quality not statistically different than the Control group after the first week of tDCS+Rehab, whereas the movement deficits in the Lesion group persisted for the entire treatment period (DPO 14, $P<0.001$; DPO 21, $P<0.0001$; DPO 28, $P<0.001$). Comparison between groups using two-way repeated measures ANOVA with all data presented as group means ± SEM.

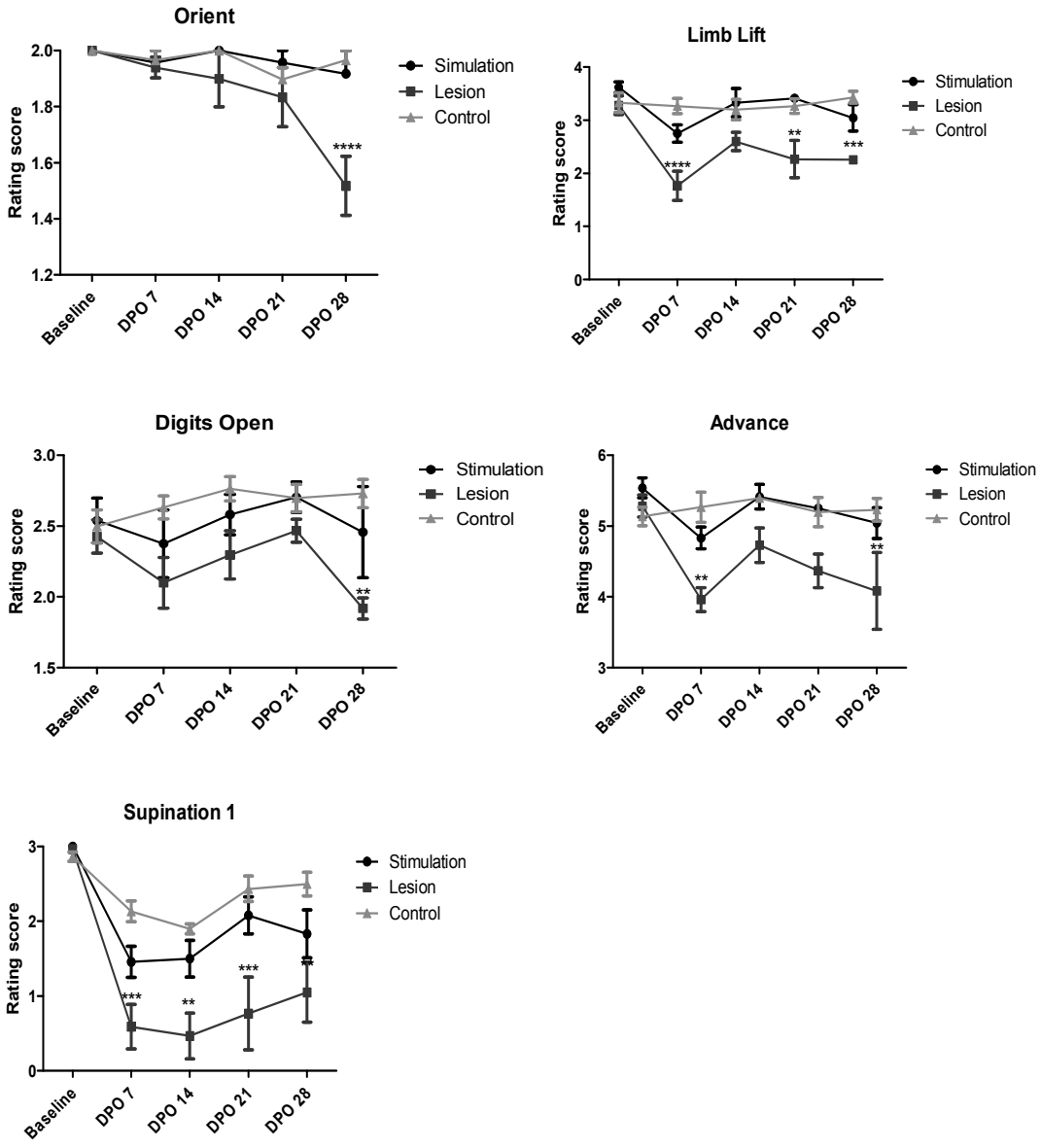

Figure 2.7. Rating scores in Orient, Limb lift, Digits open, Advance and Supination 1 components of the reaching movement rating scale. The Lesion group displayed significant impairments in all components shown at endpoint (DPO 28; Orient, $P<0.001$; Limb lift, $P<0.001$; Advance, $P<0.01$; Digits open, $P<0.01$; Supination 1, $P<0.01$)when compared to Control animals, whereas the Stimulation group showed movement rating scores not statistically different to Control animals DPO 14-DPO 28. Comparison between groups using two-way repeated measures ANOVA with all data presented as group means ± SEM.

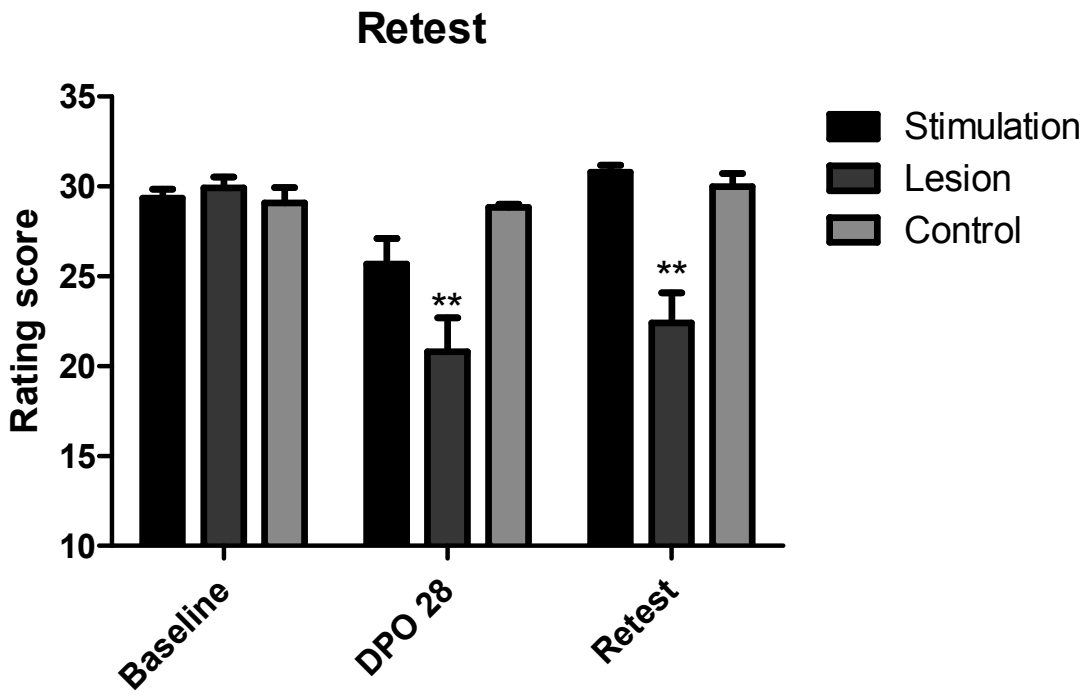

Figure 2.8. Movement quality scores; Baseline, Endpoint and Retest. The Stimulation group regained movement quality to levels not statistically different to the Control group and Stimulation group baseline values and maintained the improvement after thirty days of home cage rest, whereas the Lesion group showed permanent reduction in movement quality (DPO 28, $P<0.01$; Retest, $P<0.01$). Comparison between groups using two-way repeated measures ANOVA with all data presented as group means ± SEM.

Ladder Rung Walking Task

Two-way repeated measures ANOVA revealed significance in measures of day, group and day-by-group interaction in foot faults committed with the preferred forelimb ($F_{4,11}=17.40$; $F_{2,11}=9.90$; $F_{8,11}=4.01$). *Post-hoc* tests revealed that on DPO 8 the Stimulation and Lesion groups committed significantly more foot faults than controls ($P<0.01$, Bonferroni) with the Stimulation group returning to rates not statistically different to Control animals by DPO 15, whereas the Lesion group did not return to a rate comparable to the Control group until DPO 29 (Figure 2.9). Analyses of mean forelimb scores, digit scores, mean hindlimb scores and hindlimb foot faults yielded no significant results.

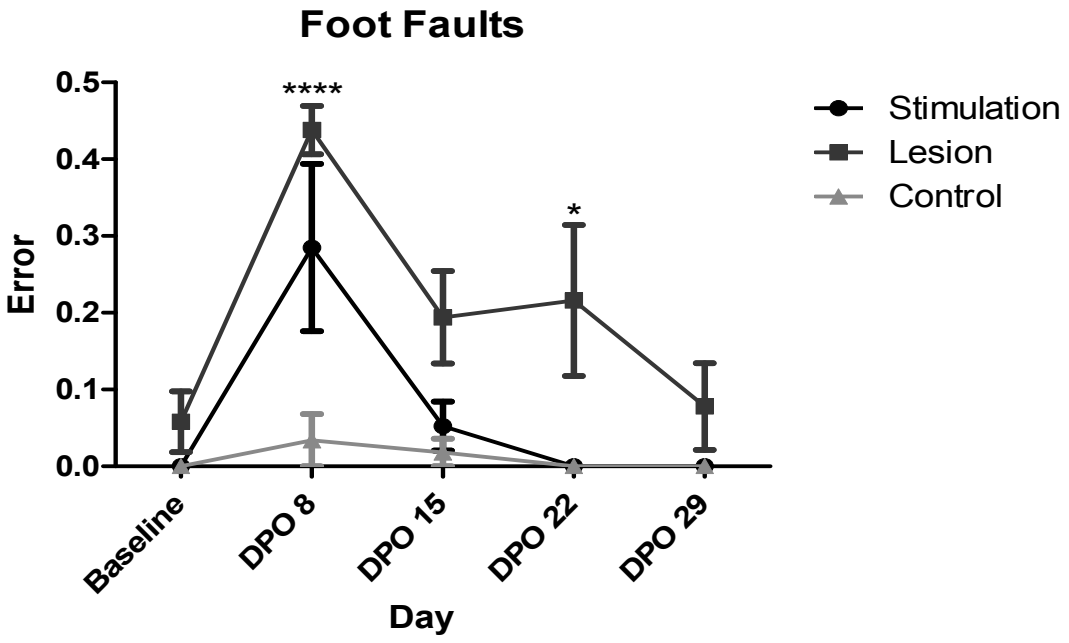

Figure 2.9. Foot faults of the preferred forelimb during the ladder rung walking task. The Lesion and Stimulation group animals committed significantly more foot faults than Control group animals on DPO 8 ($P<0.0001$). The Stimulation group returned to near baseline performance by DPO 15, whereas the Lesion group did not return to near baseline performance until DPO 29.

Cylinder Forelimb Asymmetry Task

There were no significant differences between groups in any measurement in the cylinder task (data not shown).

Lesion Volumes

Analyses of lesion volumes, widths and depths with t-tests revealed no significant differences between Stimulation and Lesion groups (Figure 2.10, A-C.). The Stimulation and Lesion groups had similar means: 4.92 mm^3; 5.42 mm^3 (Figure 2.11), standard deviations: 3.61 mm^3; 4.02 mm^3 and standard errors: ±1.80 mm^3; ±2.01 mm^3. There was electrode damage in two Stimulation group animals and one Lesion group animal on the contralesional side of the cortex. These volumes could not be compared statistically. However, by visual inspection there appeared to be slightly more electrode damage in the two Stimulation group animals.

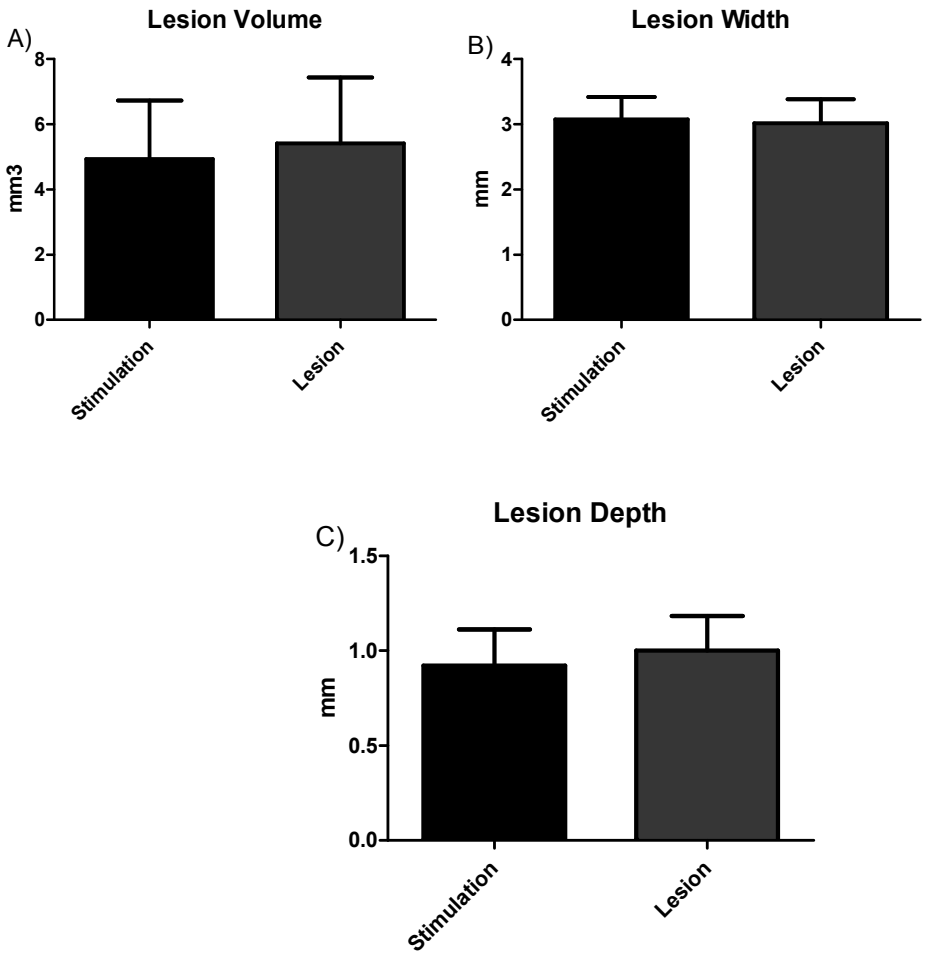

Figure 2.10. A) Lesion volume; B) Lesion width; C) Lesion depth. There were no statistical differences between groups in any measure of lesion damage. All data shown as group mean ± SEM.

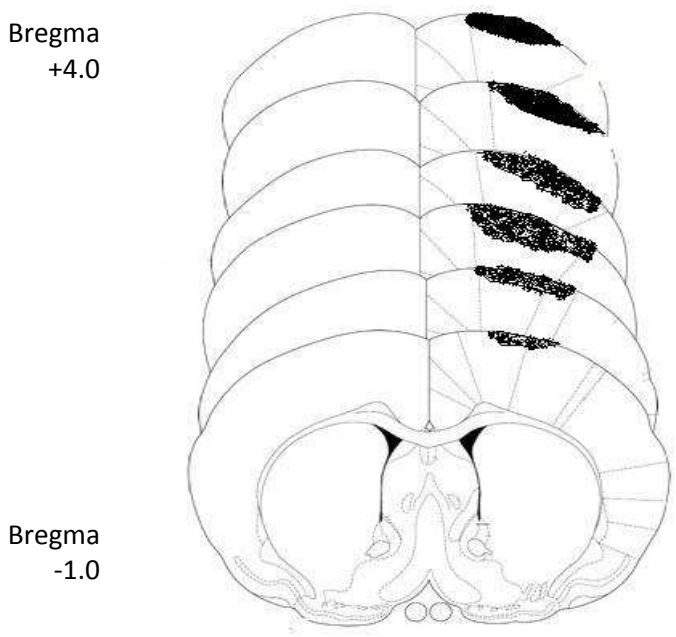

Figure 2.11. Graphic representation of mean lesion volume across the Stimulation and Lesion groups. Figure 10 was produced with actual lesion measurements using Image J and MS paint software.

Discussion

Summary

Chapter 2 examined the effects of tDCS + forelimb rehabilitation or forelimb rehabilitation alone on motor compensation and recovery after photothrombosis lesion of the forelimb area of the primary motor cortex in rats. The data show that forelimb rehabilitation alone is sufficient to increase post-stroke performance in skilled motor tasks. Moreover, supplementary tDCS in addition to forelimb rehabilitation increases the efficacy of this therapy. Furthermore, tDCS + rehabilitation improved skilled movement patterns of the impaired forelimb, whereas rehabilitation alone failed to achieve the same results. It was also shown that tDCS combined with forelimb rehabilitation produce long-lasting results, as demonstrated during retesting after thirty days of home cage rest. Whereas, the beneficial effects of rehabilitation alone were shown to be less robust. These findings suggest that therapy-induced motor recovery after stroke in rodents can be promoted. It is important to note that lesion volume did not correlate with performance on any behavioural task.

Forelimb Rehabilitation After Stroke Improves Performance in a Skilled Motor Task

The results show that forelimb rehabilitation can be used to improve performance in a skilled reaching task. Animals that received forelimb rehabilitation alone returned to baseline success rates in the SPRT which demonstrates task-specific motor improvements. These findings are similar to other studies that have examined the effects of stroke on skilled forelimb use (Metz, Antonow-Schlorke and Witte, 2005; Moon, *et al*, 2009; Knieling, *et al*, 2009). However, it appears that the beneficial effects of rehabilitation are not long lasting as seen in the decreased success rates of the Lesion group after thirty days of home cage rest. The Lesion group's decrease in performance after thirty days of rest was not likely due to forgetting (Whishaw,

Alaverdashvili and Kolb, 2008), or the need to relearn the task, as the Control animals' success rates during the SPRT retest period were not statistically different to their performances at baseline and DPO 28. Taken together, rehabilitation alone appears to increase reaching success, but this effect may be impermanent.

Forelimb Rehabilitation Fails to Improve Quality of Reaching Movements After Stroke

Using the reaching rating scale described earlier in this chapter, it was demonstrated that although forelimb rehabilitation improves success rates in the SPRT, it is insufficient to overcome the post-stroke decrease in reaching movement quality. These results are similar to results in recent studies that used detailed reaching movement analyses, which also showed that post-stroke improvements in SPRT success are primarily due to compensation rather than genuine recovery (Metz, Antonow-Schlorke and Witte, 2005; Moon, *et al*, 2009; Knieling *et al,* 2009). Taken together, these results suggest that the Lesion group animals used movements during the post-stroke testing period that differed from their baseline, or normal movement patterns. The Lesion group animals showed significantly lower reaching rating scores at endpoint in five out of ten movement categories: Orient, Limb Lift, Digits Open, Advance and Supination 1. Other studies have demonstrated similar results with regard to post-stroke movement deficits, although some differences exist in the exact deficits (Metz, Antonow-Schlorke and Witte, 2005; Alaverdashvili *et al*, 2009; Knieling *et al*, 2009; Moon *et al*, 2009). Of paramount importance are the similarities between Experiment 1 and these other studies with regard to postural and rotatory components of reaching movements as these deficits appear to be mostly conserved in humans (Cristea and Levin, 2000). Recently, Kwakkel *et al.* hypothesized that adjustments in movement patterns, including reaching patterns after stroke (i.e. compensation) may be a result of "the reducing of the number of independent elements to be

controlled" (Kwakkel *et al*, 2004). Simply stated, compensation may indeed involve the formulation of new movement patterns, but more so the limiting of previous components of movement that are not longer available due to stroke-induced damage. It then follows that although it is clear that compensation occurs after stroke, the measurement of deficits within the context of pre-stroke behaviour may not provide a complete an accurate analysis of compensatory movements. Conversely, a return to pre-stoke movement patterns may still be an accurate indication of genuine recovery.

Transcranial Direct Current Stimulation Increases the Efficacy of Forelimb Rehabilitation

Animals that received tDCS and forelimb rehabilitation after stroke improved in the SPRT and ladder rung task faster than animals that received rehabilitation alone. Moreover, the increase in performance in the animals that received tDCS and rehabilitation continued throughout the post-stroke testing period, such that at endpoint (DPO 28), the Stimulation group animals had surpassed their own pre-stroke levels of reaching success. Due to the low variability in stroke volume and location, these large improvements cannot be attributed to differential effects of stroke location or size between groups. The observed relationship between tDCS and motor improvements after stroke has been demonstrated in other studies (Adkins-Muir and Jones, 2003; Kleim *et al*, 2003; Adkins, Hsu and Jones, 2008). However, the effects in Experiment 1 appear to be more pronounced. This is possibly due to the novel stimulation protocol applied in Experiment 1. The other studies mentioned all used a constant direct current with no additional component. These studies also used daily or weekly movement thresholding protocols to determine the amplitude of tDCS (or similar cortical stimulation) that was to be administered rather than keeping the stimulation consistent between sessions. Together, the differences between simulation protocols in Experiment 2 and previous studies

may contribute to the lower efficacy of electrical stimulation with regard to motor improvements after stroke seen in these studies. Possible mechanisms for the quantitative improvements in SPRT success in Experiment 1 will be discussed in Chapter 4.

Transcranial Direct Current Stimulation and Forelimb Rehabilitation Improves Reaching Movement Patterns After Stroke

Initially, the significantly faster return to baseline reaching success rates and the eventual eclipsing of these rates at endpoint in the animals that received both tDCS and forelimb rehabilitation was attributed to compensation. However, after frame-by-frame video analyses of reaching movements, it was found that the improvements were less likely due to compensatory movements, but rather due to recovery of original reaching movement patterns as elucidated by the return to baseline reaching rating scores. In contrast, animals that received forelimb rehabilitation alone did not regain original reaching movements despite their increase in reaching success. It is likely that the combination of post-stroke physical rehabilitation and the application of electrical stimulation created a beneficial summation of effects which ultimately facilitated recovery of original movement patterns in the Stimulation group, whereas in the Lesion group forelimb rehabilitation alone was sufficient to produce a return to near baseline skilled reaching rates, but insufficient to restore original reaching movement patterns. Other studies have shown limited recovery of reaching movement patterns after stroke (Knieling *et* al, 2009; Moon *et al*, 2009) and cortical stimulation and rehabilitation after stroke (Adkins, Hsu and Jones, 2008). However, the observed recovery in these studies was either partial, or measured in a less precise manner. Taken together, tDCS and rehabilitation protocol applied in Experiment 2 appears to facilitate genuine motor recovery. Although other studies have demonstrated improvements in movement quality after stroke are possible, Experiment 1 appears to have

demonstrated that tDCS and rehabilitation may be able to facilitate relatively complete restitution of skilled movements after stroke.

Effects of Transcranial direct Current Stimulation and Forelimb Rehabilitation are Long Lasting

After thirty days of home cage rest, all animals were retested in the SPRT. The Lesion group animals' previous near return to baseline reaching success levels was not conserved at the time of retest, whereas the stimulation group animals were still performing at pre-stroke levels of success. Although the Stimulation groups' performance had declined between DPO 28 and the retest, the previously observed normalization of reaching movement patterns was conserved. This indicates that the effects of tDCS and forelimb rehabilitation are robust and perhaps permanent. Conversely, forelimb rehabilitation alone, which did originally improve motor performance through compensatory movement strategies, did not produce long lasting benefits with respect to SPRT performance. This suggests a highly activity-dependent component to physical rehabilitation, with the cessation of treatment resulting in a return to early post-stroke motor impairments. Other studies that have demonstrated similar endpoint improvements in skilled motor function after cortical stimulation and rehabilitation have not addressed the permanency of the effect (Adkins-Muir and Jones, 2003; Kleim *et al*, 2003; Adkins, Hsu and Jones, 2008). Altogether, results from the SPRT retest sessions in Experiment 2 may provide needed insight into the impermanent nature of rehabilitation-based improvements after stroke and the possibility that tDCS alleviates this shortcoming.

Transcranial Direct Current Stimulation Accelerates Post-lesion Improvement in the Ladder Rung Walking Task

Both the Stimulation and Lesion groups returned to pre-stroke levels with respect to preferred forelimb foot faults in the ladder rung task. However, the Stimulation group returned to baseline levels two weeks faster than the Lesion group. Again, this is most likely due to the previously discussed effects of tDCS and is consistent with our findings in the recovery of skilled reaching success. The latency to return to baseline performance may be interpreted as a direct effect of treatment efficacy as endpoint measures are restricted by a ceiling effect, meaning that any animal cannot commit less than zero foot faults. Analyses of hindlimb foot faults and average hindlimb placement scores revealed no differences between groups although other studies have documented significant deficits followed by returns to pre-stroke performance (Metz and Whishaw, 2002; Metz, Antonow-Schlorke, and Witte, 2005; Knieling *et al*, 2009). This could be due to the combination of relatively small lesion volumes and the focal positioning of photothrombotic damage in M1 forelimb area, or differences in rung patterns which reflect the difficulty of the task (Metz, Antonow-Schlorke, and Witte, 2005). Overall, results in the ladder rung walking task in Experiment 1 may not mirror other studies, but are consistent within the context of other observed effects within Experiment 1.

Failure of tDCS and Rehabilitation to Modulate Performance on the Cylinder Forelimb Asymmetry Task

Results from the cylinder task in Experiment 1 showed no differences and ambiguous fluctuations in performance not seen in previous studies that used this task as a measure of post-stroke motor improvements. This is possibly due to differences in cylinder diameter, or

experimenter error. It is also possible that performance was affected by the fact that multiple behavioural tasks were performed on testing days.

Conclusion

The present study may provide a preliminary demonstration of the recovery of original skilled movement patterns after stroke in rats. Additionally, decreased latency to return to baseline success levels in the SPRT and an increase in success rates with respect to baseline measures at endpoint as well as a normalization of reaching movement patterns indicate a high degree of effectiveness of tDCS and forelimb rehabilitation after stroke in rats. Also of note is the long-lasting nature of these improvements as seen during the SPRT retest after thirty days of home cage rest. Taken together, these results may indicate large-scale cortical reorganization presumably due to the combinatory effects of post-stroke, behavioural and tDCS-induced plasticity. There are similarities and differences between the results of Experiment 1 and other related studies. As discussed earlier, these differences in results may be attributed to slightly different methodologies, especially with regard to stimulation protocols and qualitative measures of reaching movements. However, due to the differences in lesion volumes in The Stimulation and Lesion groups being non-significant, it is unlikely that any statistical differences revealed in the analyses of post-stroke behaviours were due to a lesion effect. A general discussion about technical considerations such as implications of inter-rater reliability as related to these findings is provided in Chapter 4. The next chapter examines the effects of tDCS + forelimb rehabilitation or rehabilitation alone on large-scale neuronal populations.

Chapter 3

Transcranial Direct Current Stimulation Improves Coherence Between Interhemispheric Local Field Potentials After Stroke in Rats (Experiment 2)

Introduction

Cortical stimulation has been shown to improve motor function after stroke in rats (Adkins, Hsu and Jones, 2008) and humans (Hummel and Cohen, 2006), but the mechanisms responsible for the improvements and which stimulation protocol provides the most benefit are still unclear. Synchronization of LFPs across brain regions is believed to be important in cortical processing and neural plasticity (Fell and Axemacher, 2011). It can be argued that the coordination of neural activity is of paramount importance in order to produce behaviours. In a recent experiment (Luczak, 2010; unpublished), rats that had stroke-induced M1 lesions showed lower interhemispheric coherence of LFPs than intact animals weeks after the damage as well as displaying only minor motor dysfunction. This observation suggests that following stroke, the brain does not function normally, regardless of severity of physical deficits. Recent work by Ozen *et al* (Ozen *et* al, 2010) showed that transcranial electric stimulation can entrain populations of cortical neurons, providing evidence that it is possible to manipulate brain activity with non-invasive electrical stimulation.

In vivo cortical recordings are a useful tool to explore the activity-based relationships of neuronal populations; including, LFPs and spiking activity. One of the most effective methods to record from a large number of neurons and monitor multiple local neuronal circuits simultaneously is using silicon electrode arrays (Buszaki, 2004). The advantages of using silicon probe arrays are that they are very small in size, which limits structural damage during probe insertion and they have a large number of recording sites. The 32 multiple sites used in the current experiment were arranged such that recordings in multiple cortical layers and in

multiple cortical columns were possible. This geometrically precise arrangement of shanks and recording sites allows the determination of spatial relationships between single neurons (Bartho *et al*, 2004). As no one neuron acts in isolation, increasing importance is being placed of the activity of entire networks of neurons and how they interact and ultimately produce behaviours through their coordinated activity (Buzsaki, 2004).

Chapter 3 (Experiment 2) examines the effects of the experimental conditions in Experiment 1 on neuronal activity through *in vivo* electrophysiological recordings as well as the direct effects of tDCS on spontaneous brain activity on the timescale of minutes. The results of Experiment 2 may help further the understanding of how electrical stimulation affects the intact and damaged brain and how these effects may contribute to recovery after stroke in rats.

Methods

Animals

After the completion of Experiment 1, the same animals were used in Experiment 2. All surgical techniques were approved by the University of Lethbridge Animal Welfare Committee (protocol #1008) and were consistent with requirements set by the Canadian Council for Animal Care.

Electrophysiological Surgery and Recordings

Detailed descriptions of surgery and recording procedures have been published in Schjetnan and Luczak 2011 and Buzsaki, 2004. Briefly, after the last day of retesting in the SPRT (Experiment 1), rats were anaesthetized with urethane (1.5 g/kg, i.p.) and were placed in a stereotaxic frame. Two craniotomies were opened in the skull over the forelimb somatosensory cortex (AP: +0.5 to -2.0; ML: ± 2.5 to ± 3.5) and the dura mater removed. Extracellular signals

were recorded with silicon probes (NeuroNexus Technologies, USA) consisting of eight shanks with 32 channels per probe and four recording sites on each shank. The location of the recording sites in the cortex was determined to be layer V with fluorescent dye-based histological reconstruction of the electrode tracks, electrode depth, and firing patterns (Bartho et al., 2004). Silicon probes were connected to a headstage, the output of which was conducted via a lightweight, multi-wire, tether cable and through an 82-channel, slip-ring commutator to a data acquisition system containing 64 digitally programmable analog amplifier/filter modules (Neuralynx, USA). Unit activity was amplified by a factor of 3,000–5,000 and band-pass filtered from 600 to 6,000 Hz. Spike waveforms above a threshold set by the experimenter (55–80 μV) were time-stamped and digitized at 32 kHz for 1 ms.

The electrophysiological recordings consisted of the acquisition of 5 minutes spontaneous activity in the forelimb somatosensory area, followed by a 10 min period of tactile stimulation applied to the preferred forelimb (300 trains 95 Hz/1s, 1 second between trains). A 5 minute period of spontaneous activity was recorded following the tactile stimulation. This protocol was repeated once in conjunction with the electrical stimulation protocol which was the same as in behaving animals in Experiment 1 (65 μA of continuous direct current with 65 μA pulses of 30 ms duration every 5 seconds added). At the end of the acute electrophysiology animals were deeply anaesthetized with pentobarbital and perfused transcardially in order to perform histological analysis on the extracted brains. (Note: all electrophysiological recording surgeries were either performed by Dr. Schjetnan or Darryl C. Gidyk under the direct supervision of Dr. Schjetnan)

Silicon Probe Placement

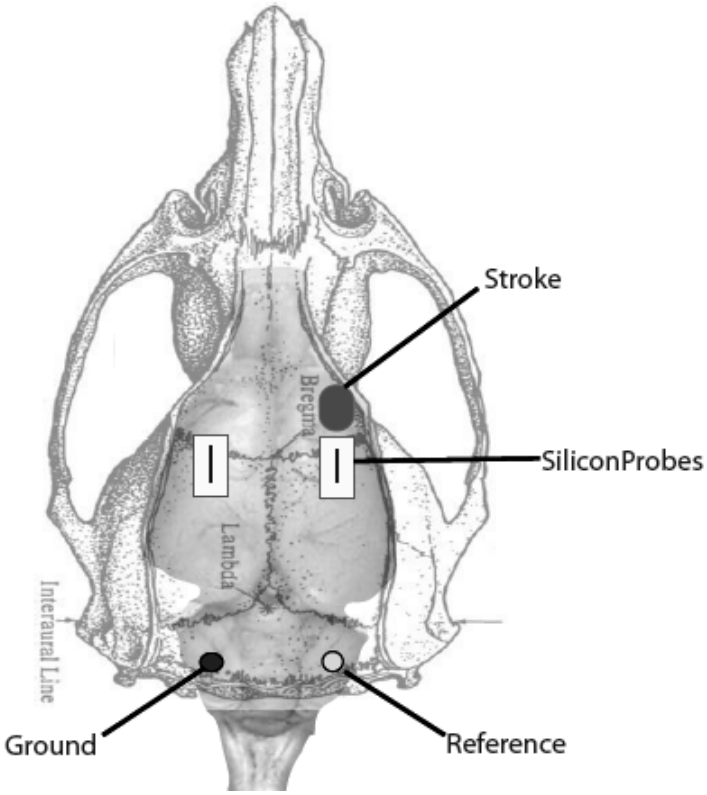

Figure 3.0. Graphic representation of silicon probe placement during acute electrophysiological recordings.

Data Analysis

For the purposes of the present thesis, the main focus was placed on the analysis of differences in the LFPs recorded in the somatosensory cortex between hemispheres. Local field potentials were defined as neuronal voltage fluctuations recorded from the extracellular space, which mainly originate from postsynaptic potentials. First, power spectral density estimates via

Welch's method were performed. Later, a magnitude squared coherence estimate between hemispheres was performed. Coherence was determined by a function of frequency that indicates how well the activity in one hemisphere corresponds to the other hemisphere at every frequency. Finally, a correlation coefficient analysis was performed to investigate the correlation between LFP coherence and reaching success (all analyses performed by Dr. Luczak using Matlab software, Mathworks, USA).

Results

Acute Electrophysiology

Preliminary analysis of spontaneous LFPs in both hemispheres revealed that the Stimulation group animals displayed higher interhemispheric coherence than Lesion group rats, especially over the frequency range of 20-40 Hz (Figure 3.1). Additionally, Stimulation group animals displayed a higher level of coherence than Control group animals over lower frequencies (1-10 Hz).

Preliminary analysis of recordings during and after the stimulation protocol revealed that in general, higher levels of interhemispheric LFP coherence were observed after stimulation when compared to before stimulation (Figure 3.2). Finally, it was observed that animals displaying higher coherence also generally performed better in the SPRT in Experiment 1 (Figure 3.3). Note that all analyses are preliminary and more in depth analyses are currently being performed by Dr. Luczak and Dr. Schjetnan.

Verification of Recording Sites

Probe placement was verified through examination of DAPI-stained coronal sections with probe tracts labelled with dye I. Both recording shank depth and stereotaxic position were confirmed to be correct with respect to coordinates described in the methods section (Figure 3.4)

Interhemispheric Coherence

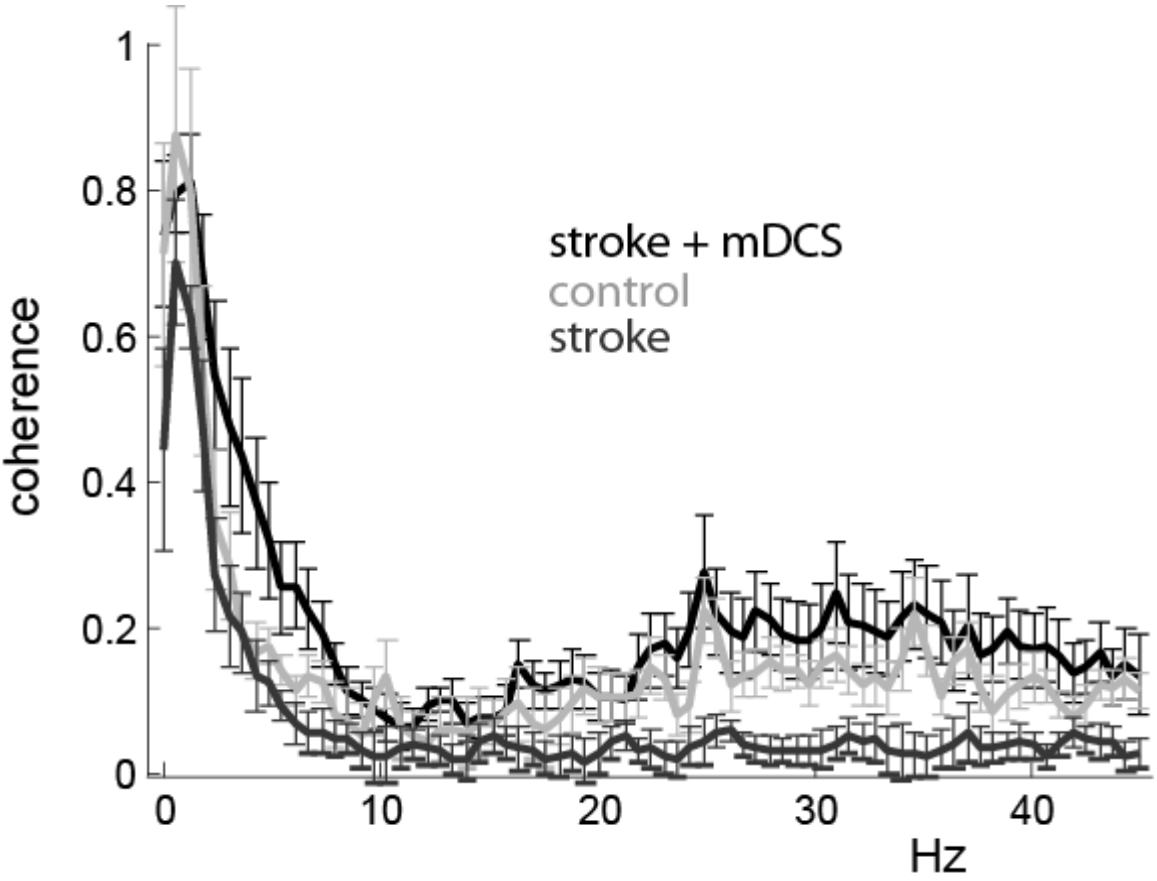

Figure 3.1. Values of interhemispheric coherence of LFPs during spontaneous activity in acute electrophysiological recording sessions. The Stimulation group (stroke+mDCS) displayed higher coherence of LFPs than the Lesion Group (Stroke) over the frequency range of 20-40Hz. Additionally, the Stimulation group showed higher coherence of LFPs than the Control animals over the frequency range of 1-10 Hz. (Note: mDCS is defined as modified direct current stimulation and this term is interchangeable with tDCS)

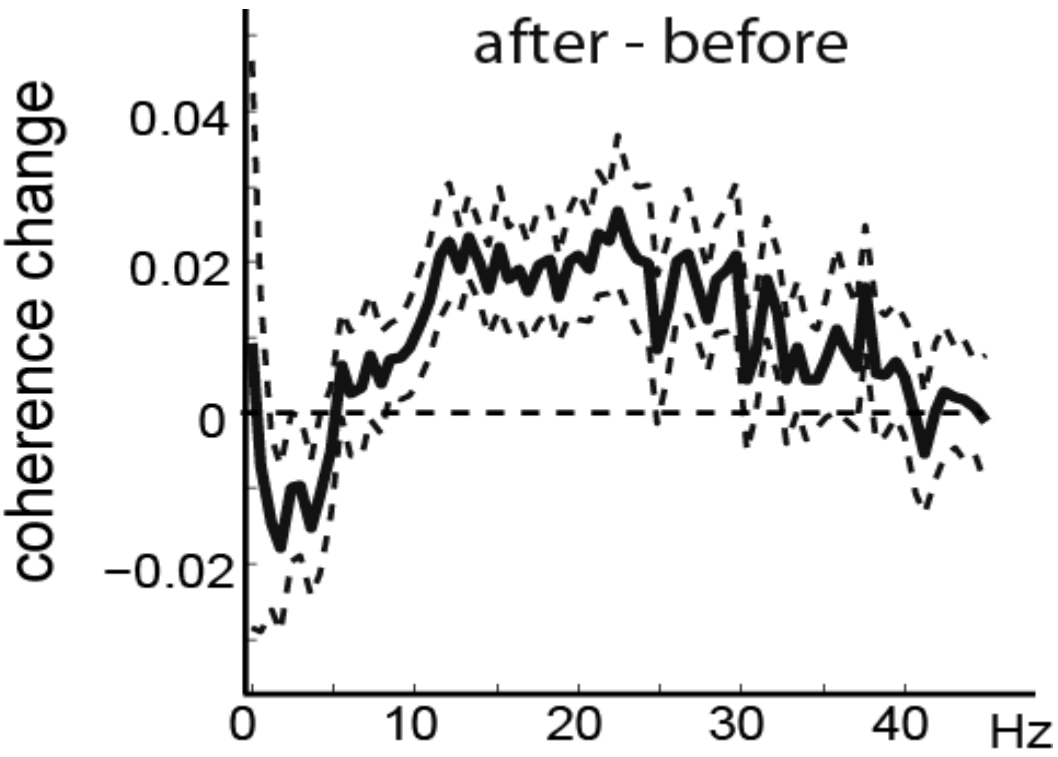

Figure 3.2. Change in interhemispheric LFP coherence as a direct result of application of mDCS (tDCS) stimulation protocol during acute electrophysiological recordings. A general increase in coherence after the tDCS stimulation protocol was applied during the acute electrophysiological recording sessions was observed. The coherence values after stimulation were subtracted from the coherence values before stimulation (After-Before) and the mean plotted at each frequency from 1-40 Hz. The solid blue line represents the mean and the broken lines represent the SEM.

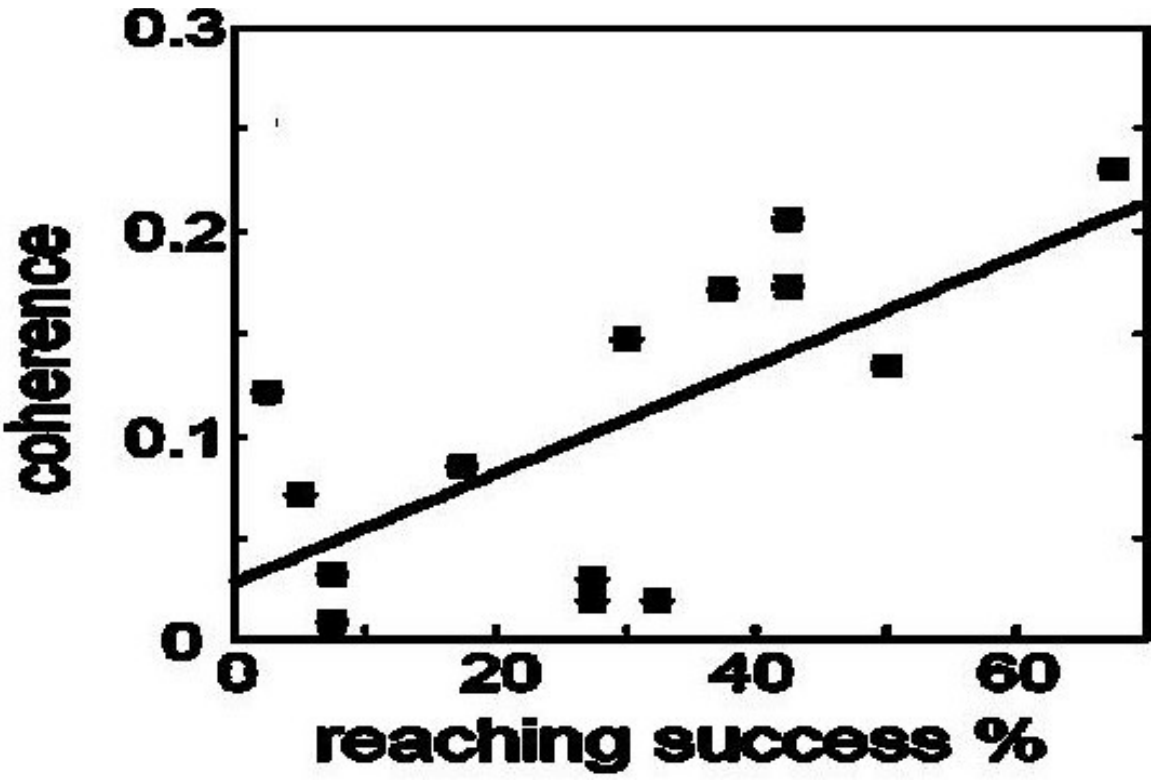

Figure 3.3. Correlation between SPRT reaching success in Experiment 1 and interhemispheric coherence of LFPs. Black, Red and Green squares represent individual Stimulation, Lesion and Control group rats respectively. In general, Stimulation group animals displayed better success rates in the SPRT and this correlated with level of interhemispheric LFP coherence. The Control group animals showed a similar trend. The Lesion group's success rates in the SPRT also correlated with level of LFP coherence. However, both coherence and success rate were lower than the Stimulation and Control groups.

Silicon Probe Verification

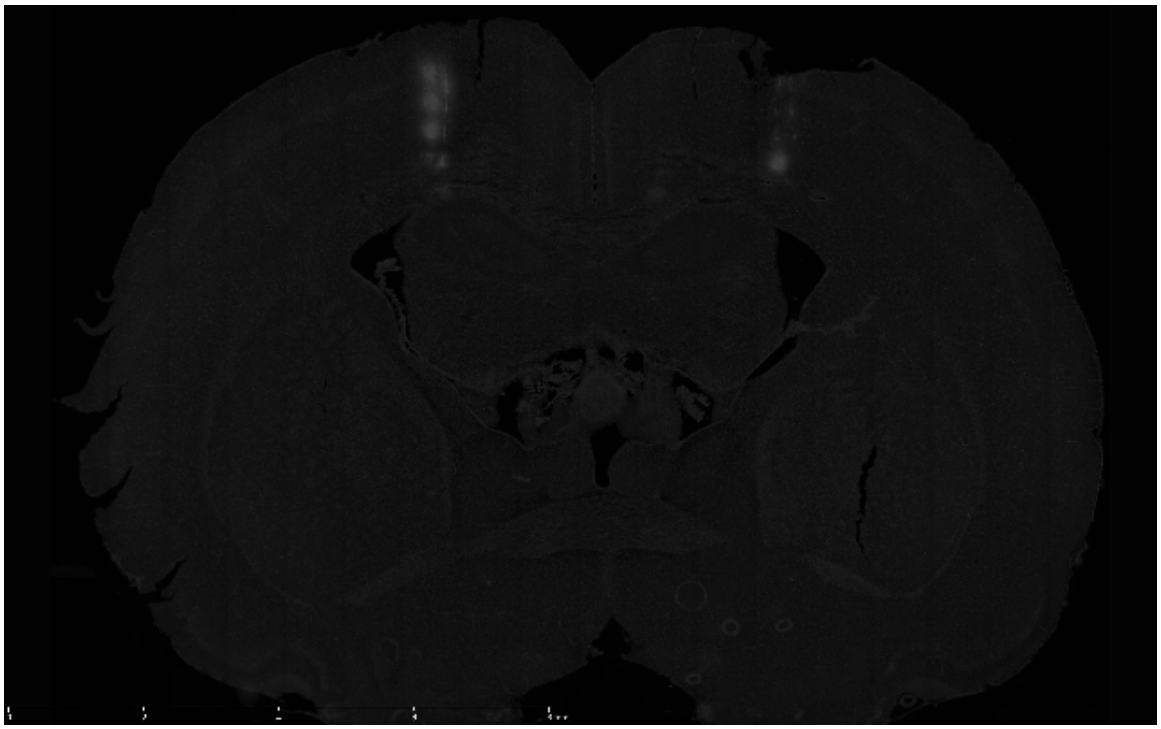

Figure 3.4. Representative example of histological verification of probe depth and coordinates.

Discussion

Summary

Chapter 3 (Experiment 2) examined the effects of the experimental conditions of Experiment 1 (tDCS + rehabilitation and rehabilitation alone after stroke) on interhemispheric coherence of LFPs through acute, *in vivo* electrophysiological recordings in anesthetized rats. Preliminary analysis of local field potentials suggest higher interhemishperic coherence in Stimulation group animals than Lesion group animals in the frequency range of 20-40 Hz. Additionally, Stimulation group animals displayed higher coherence than Control animals over

the frequency range of 1-10 Hz. Analysis of LFPs after the stimulation protocol was applied showed that in general, rats displayed higher coherence tDCS than before. Taken together, the results from Experiment 2 suggest that not only does tDCS and rehabilitation increase interhemishperic coherence after stroke in rats, but this increase may be a direct effect of tDCS.

tDCS and Rehabilitation Facilitates Interhemispheric Coherence After Motor Cortex Stroke in Rats.

After experimentally induced focal stroke of the forelimb area of M1, rats appeared to display lower coherence of brain activity between hemispheres. Motor dysfunction in the limb contralateral to the infarct is well documented and was demonstrated in Experiment 1 (Whishaw, Whishaw and Gorny, 2008). Increases in skilled motor performance take place over a timescale of weeks after the insult and are most often attributed to compensation (see Chapter 1 and 2). This can been seen in a variety of motor tasks, but one of the best and most reproducible demonstrations of this gradual post-stroke improvement in motor function can be seen in the SPRT. Experiment 2 suggests that coherence of LFPs differ between intact rats, rats that received rehabilitation alone after stroke and rats that received tDCS and rehabilitation after stroke. The increased coherence of interhemispheric coherence of LFPs seen in the Stimulation group animals suggests that with respect to LFPs in normal rats, the rats that received tDCS and rehabilitation were virtually indistinguishable although they had significant tissue loss via photothrombotic M1 lesions. These results support the emerging idea that the ensemble behaviour of neuronal populations may provide one key to brain function (Buszaki, 2004). Additionally, it can be hypothesized that the synchrony of brain oscillations produced by populations of neurons may be predictive of normal or abnormal brain function.

Increased Interhemispheric LFP Coherence is a Direct Effect of tDCS

Naturally the first question that follows the observed increase in the Stimulation group animals' LFP coherence in response to tDCS and rehabilitation after stroke is whether the positive change in synchrony of LFPs is due to a combination of rehabilitation and tDCS or due to the stimulation alone. The preliminary analysis of LFP coherence after tDCS applied during the acute recording sessions suggests that tDCS may be directly responsible for the synchrony of LFPs across hemispheres, at least on a short temporal scale. In general, LFP recordings during the acute surgery sessions displayed higher correlation between hemispheres after tDCS than before. This suggests the possibility that not only does tDCS in conjunction with rehabilitation after stroke improve synchrony of LFPs, but tDCS alone may be able to facilitate increased synchrony of interhemispheric LFPs in animals with normal or abnormal interhemispheric coherence on an immediate temporal scale.

Interhemispheric Coherence of LFPs Correlates with SPRT Success Rates

To investigate whether interhemispheric coherence is related to success on a skilled motor task, a preliminary correlation analysis was performed on LFP coherence coefficients and SPRT success rates. The preliminary analysis suggests that there may be a positive trend between brain synchrony and skilled motor performance. In general, rats that had higher coherence of LFPs had higher success rates in the SPRT. This trend was preserved within each group with respect to individual rats' performances vs. coherence. Additionally, the highest levels of coherence and SPRT success appear to be in the Stimulation and Control groups, with the lowest being in the Lesion group. These preliminary results suggest that interhemispheric coherence of LFPs may be predictive of SPRT success, or vice versa. Moreover, coherence of LFPs may provide a biomarker of functional motor recovery after stroke in rats.

Conclusion

The results of preliminary analyses from Experiment 2 suggest that normalization of interhemispheric coherence of LFPs may be elicited by the application of tDCS and rehabilitation after stroke in rats; whereas rehabilitation alone is insufficient to produce a similar effect. Additionally, tDCS appears to increase coherence on an immediate temporal scale. Finally, it was observed that correlation of LFPs between hemispheres may be positively correlated with skilled motor performance and within the context of Experiment 1 and 2, recovery of original movement patterns (i.e. genuine recovery). Chapter 4 will address possible mechanisms of the observed results in Experiment 1 and 2 as well as place them into context with respect to, stroke-induced M1 damage, compensation, recovery, neural networks and synchronized activity across related brain areas.

Chapter 4

General Discussion

Summary

The present thesis examined the effects of tDCS and physical rehabilitation on motor recovery and compensation and brain activity after stroke in rats. Experiment 1 demonstrated that tDCS and rehabilitation was sufficient to reinstate pre-stroke performance in the SPRT and ladder rung walking task. Conversely, rehabilitation alone was insufficient to facilitate a return to pre-stroke success rates in the SPRT, although eventually decreasing foot faults in the ladder rung walking task to pre-stroke rates. The combination of tDCS and rehabilitation also improved reach-to-eat movement patterns, which is a novel finding. Conversely, rehabilitation alone failed to reinstate original reaching movement patterns. Furthermore, it was demonstrated that the positive effects of rehabilitation are not permanent, but the combination of rehabilitation and tDCS produced long-lasting benefits.

Experiment 2 suggests that LFPs recorded bilaterally from somatosensory cortex in rats with M1 stroke damage are less coherent than those in undamaged rats. Additionally, preliminary analysis suggests that rehabilitation alone failed to improve interhemispheric coherence of LFPs whereas; tDCS and rehabilitation applied concurrently increased LFP coherence. During acute recording surgeries it was observed that increases in interhemispheric coherence of LFPs may directly result from the novel tDCS stimulation protocol used in Experiments 1 and 2. tDCS also improved interhemispheric coherence on an immediate temporal scale during the acute recording surgeries.

Taken together, the results of Experiments 1 and 2 reiterate the possibility that tDCS may be a potential post-stroke therapy as suggested by other studies (Adkins-Muir and Jones, 2003; Kleim *et al*, 2003; Adkins, Hsu and Jones, 2008). However, with the addition of

improvements in stimulation protocol, skilled moved analysis and preliminary analyses of interhemispheric LFP coherence, the current experiments may provide new insight and address voids in the current stroke literature.

Motor Dysfunction After Photothrombotic Lesion of M1 forelimb Area

In Experiment 1, photothrombosis-induced stroke in the forelimb region of M1 caused similar motor deficits in both the Stimulation and Lesion group animals. Initial measurements of post-stroke motor performance were not statistically different between groups in any measure of any test, including reaching movement patterns. Similarly, lesion volumes, depths and widths showed some variation within group, but were not statistically different between groups. Additionally, photothrombosis reliably damaged the area of interest (M1) and spared underlying structures including the corpus callosum and subcortical tissue. Although it has recently been shown that lesion volume is not necessarily an accurate predictor of chronic motor deficits after stroke in rats (Metz, Antonow-Schlorke and Witte, 2005; Knieling *et al*, 2009), it is still beneficial to limit variability in stroke volumes so only the area of interest is damaged as in Experiment 1. Taken together, it can be concluded that photothrombosis is an effective lesion method when studying post-stroke motor deficits arising from focal damage to the primary motor cortex in rats.

The Effects of tDCS and Rehabilitation Versus Rehabilitation Alone on Compensation and Recovery After Stroke

Experiment 1 examined the effects of tDCS and rehabilitation and rehabilitation alone on recovery and compensation after stroke in rats. Results from endpoint analyses of SPRT success rates revealed that the benefits of tDCS and rehabilitation eclipsed those of

rehabilitation alone. tDCS and rehabilitation induced an improvement in post-stroke SPRT success rates. Mean SPRT success rates of the Stimulation group in the last week of Experiment 1 were higher than pre-stroke success rates. Histological verification of similar stroke damage across groups and an initial, statistically significant drop in SPRT success rates further rule out differential effects of lesion size and the possibility that the lesion surgeries failed to cause damage to M1.

Rehabilitation alone was sufficient to induce positive changes in post-stroke SPRT success rates in the Lesion group. However, the improvements were modest and endpoint success rates remained below pre-stroke success rates. This demonstrates the inability of physical rehabilitation alone to ameliorate post-stroke motor deficits despite the therapy being applied long into the chronic phase of stroke. Taken together, these results indicate there may be differential effects of tDCS and rehabilitation and rehabilitation alone on the post-stroke recovery processes.

Reaching movement analysis was employed to differentiate between compensation-based improvements in SPRT success rates and improvements based on genuine recovery. Results from the endpoint analysis of reaching movements in Lesion group animals revealed the persistence of abnormal reaching movements. This demonstrates that although Lesion group animals showed quantitative improvements in SPRT success rates after stroke, these improvements were not due to the restitution of original reaching patterns. Altogether, these results are consistent with the current view that post-stroke motor improvements are mediated by compensatory behavioural mechanisms, not by genuine recovery (Metz, Antonow-Schlorke and Witte, 2005).

After analysis of reaching movement patterns of the Stimulation group animals the opposite effect was found. Rats that received tDCS and rehabilitation displayed a similar

reduction of reaching movement quality after stroke. However, at endpoint (and as early as WPO 2) they displayed recovery of normal reaching movement patterns statistically similar to pre-stroke movements. Together, the improved endpoint SPRT success rates and normalization of reaching movement scores suggest that tDCS and rehabilitation applied concurrently may facilitate genuine recovery of skilled motor function.

Possible Mechanisms of Motor Improvements: Compensation, Recovery and Plasticity Processes

As discussed in Chapter 1, M1 in rats has been shown to consist of a highly complex, distributed architecture equipped with mechanisms for substantial and rapid plastic changes (Sanes and Donoghue, 1997). Due to the existence of a large body of literature on M1 plasticity in rats, it can be concluded that M1 does in fact undergo large changes in functional topography as a result of experience and especially after stroke (Nudo, 2001; Nudo 2006). With the intrinsic mechanisms for plastic change in M1 upregulated after stroke, it follows logically that it may be possible to modulate these mechanisms to be adaptive for recovery from post-stroke motor dysfunction.

Some of the molecular mechanisms of post-stroke plasticity have been identified. These mechanisms are very similar to those during development and are responsible for axonal sprouting, axonal guidance, and dendritic modifications (Wieloch and Nikolich, 2006; Murphy and Corbett, 2009; Metz and Faraji, 2009; Kolokin and Tessier-Lavigne, 2011). This conservation of mechanisms suggests that there may be just one “plan” regarding the modulation of expression of the molecules implicated in brain plasticity in intact and stroke-damaged brains. Plastic changes observed in M1 after stroke in rats are not confined to tissue proximal to the infarct, suggesting that entire networks of neurons may play a role in post-stroke plasticity

processes (Nudo, 2006). Recently, tDCS has been shown to increase synaptogenesis, expression of BDNF and NDMA-dependent synaptic strengthening (Fritsch *et al*, 2010; Fritsch *et al*, 2010b). Additionally, tDCS has been implicated in motor improvements after stroke in rats (Adkins-Muir and Jones, 2003; Kleim *et al*, 2003; Adkins, Hsu and Jones, 2008). With the knowledge that M1 plasticity is dependent, at least in part, on BDNF expression (Kleim *et* al, 2006), that tDCS facilitates synaptic strengthening as well as BDNF expression (Fritsch *et al*, 2010; Fritsch *et al*, 2010b) and that there is an upregulation of intrinsic plasticity-inducing events post stroke, it is reasonable to assume that these intrinsic and extrinsic factors may interact.

An interaction of intrinsic post-stroke plasticity processes and tDCS-induced effects may provide insight into the results of Experiment 1 in the present thesis. Experiment 1 suggests that intrinsic post-stroke mechanisms are adaptive to motor improvements and may be modulated in part by rehabilitation. However, it appears that these improvements are small in magnitude and incomplete. Together with findings in similar studies, it can be suggested that rehabilitation may be sufficient to facilitate post-stroke plastic processes required for compensatory behavioural mechanisms to develop. Conversely, it can be suggested that rehabilitation alone may be insufficient to facilitate post-stroke plastic mechanisms that are robust enough to facilitate genuine recovery. Moreover, the benefits of rehabilitation after stroke (i.e. compensation-based motor improvements) seen in Experiment 1 do not appear to be long-lasting. This is also consistent with the prospect of rehabilitation alone being insufficient to facilitate genuine recovery and with recovery and compensation perhaps representing different magnitudes of the same plasticity "plan".

In contrast to the effects rehabilitation appears to have on post-stroke motor improvements, it appears that tDCS + rehabilitation facilitated more robust motor improvements. The results of Experiment 1 suggest that tDCS improved motor improvements

which may be attributed to genuine recovery rather than compensation. Support for this idea comes from the observation that both quantitative and qualitative motor performance improved after stroke as a result of the addition of tDCS to physical rehabilitation. Improvements in reaching success alone, as seen after rehabilitation in Experiment 1, represent compensation due to the fact the improvements were not accompanied by the normalization of movement quality. Conversely, tDCS appears to have fostered a return to similar movement quality as seen during the pre-stroke period and in intact animals. Additional evidence that tDCS may facilitate genuine recovery and that in turn may represent a greater magnitude of modulation of post-stroke plasticity processes was provided by the reaching retest. The beneficial effects of tDCS + rehabilitation were still seen one month after cessation of its application, which is inconsistent with at least one similar study that used similar methodology but a very different stimulation protocol (Adkins, Hsu and Jones, 2008). In addition, it can be suggested that the effects of tDCS are robust due to its limited application (three days) during the first week of post-stroke therapy only. Up to this point, some general inferences can be made: 1) compensation represents the primary behavioural mechanism of motor improvements after stroke; 2) tDCS and rehabilitation applied concurrently may facilitate greater benefits than rehabilitation alone and perhaps genuine recovery; 3) compensation and recovery after stroke may represent two different magnitudes of the same processes, i.e. structural and physiological plasticity, regardless whether they are induced by stroke or the interaction with extrinsic factors or interventions.

Interhemispheric Coherence as a Possible Biomarker of Compensation and Recovery After Stroke

As described in Experiment 3, coherence of interhemispheric LFPs differed between rats with stroke-induced M1 lesions and intact rats. Similarly, Experiment 2 suggests that coherence also differs between animals that received post-stroke tDCS + rehabilitation versus animals that received rehabilitation alone. It also suggests that increases in coherence may be a direct effect of tDCS. Although these results came from preliminary analyses, some inferences can be made in light of the previous discussion of compensation, recovery and plasticity after stroke.

The idea that improvements due to compensation and recovery are a result of different magnitudes of the same plasticity processes may be reflected in the correlation of brain activity across hemispheres. Animals that received rehabilitation alone displayed "abnormal" (different from intact animals) coherence of LFPs, which is consistent with previous observations of stroke damaged animals showing lower coherence (Luczak, unpublished, 2010). From this it can be suggested that rehabilitation alone may have little effect on post-stroke coherence of LFPs. When combined with behavioural observations it can be inferred that animals which displayed compensation after stroke may have LFPs that are more consistent with damaged animals than intact animals. Conversely, animals that received tDCS + rehabilitation displayed "normal" (similar to intact animals) coherence of LFPs. Combined with behavioural observations it can also be suggested that animals which displayed genuine recovery displayed coherence of LFPs that were closer to intact rats than stroke-damaged animals. Taken together, it can be inferred that coherence of LFPs may possibly provide a biomarker of recovery and compensation after stroke, with recovery represented by a return to "normal" brain synchrony and compensation represented by a persistence of "abnormal" brain synchrony. Although there may be other possible interpretations of the combination of results from Experiment 1 and preliminary results

from Experiment 2, this interpretation appears to be parsimonious, and congruent with the current understanding of plasticity processes and with the current literature cited in the present thesis.

Experimental Considerations

Reaching Rating Scale

Although it can be argued that the version of the rat reaching rating scale used in Experiment 1 is the best available tool for measuring qualitative differences in reaching movement patterns, there is a possibility that the scale is insufficient to track changes in gait and weight bearing movements. Pure observation during SPRT sessions of stroke damaged rats seems to indicate that quantification of trunk rotation and body posture in general may need to be added to the reaching scoring system to further the understanding of compensatory movements. Additionally, it may be beneficial to quantify general types of movements as degrees of freedom and compare the number of degrees to elucidate whether animals with forelimb dysfunction are adding or subtracting movements from the normal reaching patterns seen in intact animals.

Ladder Rung Walking Task

In the hindlimb ipsilateral to the impaired forelimb, foot fault scores failed to show a similar effect seen in forelimb foot faults. This may be due to: 1) the photothrombotic lesion not extending into the hindlimb region of M1; 2) different effects of stroke damage with regard to forelimb versus hindlimb stepping precision; 3) hindlimb stepping movements being less vulnerable to disruption in general.

Cylinder Task

Results from the cylinder forelimb asymmetry task failed to show any significant results. This may be due to inaccurate or inconsistent scoring performed by a blind observer. It may also be a combination of effects such as the cylinder task primarily measuring non-skilled motor movements, and/or the lesion model used in Experiment 1 not producing profound enough motor dysfunction to be quantified by the task.

BDNF Immunofluorescence

To examine the possible role of BDNF in the results of Experiment 1 and 2, BDNF expression is in the process of being quantified. This process was not complete in time to be included in the present thesis.

Experimenter Bias

After being partially re-scored by blind observers, there appears to be a systematic bias in the results of the SPRT. When plotted next to experimenter results the scoring completed by blind observers through repeated inspection of video material appears lower in the Stimulation group. This apparent bias was also identified in qualitative SPRT results. However, upon visual inspection, the Stimulation group appears to have performed better than the Lesion group and managed to reach success rates similar to the Control group. These differences are yet to be statistically analyzed, therefore no graph is included. This inconsistency may be explained by the fact that in Experiment 1, most quantitative SPRT scoring was done live and therefore subject to greater error. A solution to this potential problem would be to video record all SPRT sessions in their entirety and have three blind observers score the recordings both quantitatively and

qualitatively to ensure negation of experimenter bias and provide measures of inter-rater reliability.

SPRT Limitations

In Experiment 1 it is important to note that any claims involving the effects of rehabilitation alone are made in the context of comparisons between the Stimulation and Lesion groups or within the Lesion group itself. One limitation of the SPRT in Experiment 1 is the inability to distinguish between SPRT rehabilitation-induced effects and the effects, if any of spontaneous recovery due to the need to apply the task on a daily basis.

Future Directions

In the future, it would be beneficial to continue to further elucidate the facilitation of post-stroke plasticity mechanisms by tDCS + rehabilitation. Of note is the idea that the re-synchronization of related neuronal populations contributes to already heightened mechanisms of plasticity after stroke and this may possibly be reflected in behavioural recovery. The current literature suggests that M1 has the structure and substrates necessary for significant plastic changes, especially after stroke. The mechanisms implicated in observed changes may interact, thereby producing different effects within the context of compensation and recovery. Together, it can be suggested that focus should remain on combining methods that have been shown to elicit plastic changes in the brain after stroke, particularly brain stimulation and rehabilitation as the combination of effects may eventually provide a key to understanding and facilitating genuine recovery after stroke.

Conclusion

In conclusion, the present thesis reiterates the current view that compensation is the main behavioural mechanism of motor improvements after stroke. Additionally, it suggests that brain stimulation and rehabilitation when combined may produce greater benefits than rehabilitation alone in the chronic phase of stroke. The present thesis also suggests that the synchrony of brain activity either plays a role in recovery from stroke or may be a biomarker of compensation and recovery. Finally, although the exact mechanisms that facilitate genuine recovery from stroke are yet to be understood, it is clear that post-stroke motor improvements are possible and are modulated by extrinsic factors such as, rehabilitation and electrical stimulation of the brain.

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