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Investigating the Cortical, Metabolic and Behavioral Effects of Transcranial Direct Current Stimulation in Preparation for Combined Rehabilitation

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Abstract

The goal of this thesis was to determine the cortical reorganization that occurs in patients with cervical spondylotic myelopathy (CSM) after surgical decompression and to implement this knowledge into a new rehabilitation strategy. Transcranial direct current stimulation (tDCS) is a non-invasive technique to modulate human behavior. Due to the novel electrode montage used, it was first pertinent that we determine how tDCS would modulate cortical, metabolic and motor behavior in healthy individuals.

We observed the longitudinal functional adaptations that occur in patients with CSM using functional MRI. Enhanced excitation of supplementary motor area (SMA) was observed following surgical decompression and associated with increased function following surgery. This novel finding of enhanced excitation motivated us to use a bihemispheric tDCS protocol, exciting bilateral motor areas to provide optimal motor enhancement. This novel tDCS electrode montage, targeting the SMA and primary motor cortex (M1) was implemented in healthy older adults to determine its effects on enhancing manual dexterity. Furthermore, to determine the frequency with which to apply tDCS, a single and tri session protocol was used. We observed a differential pattern of action with anti-phase and in-phase motor tasks during multisession tDCS. We used ultra-high field (7T) MRI to examine the metabolic changes that occur following tDCS. After the stimulation period we observed no significant metabolite modulation. There was a significant correlation between the change in absolute concentration of NAA and change in absolute concentration of tCrd. Finally, we examined the functional connectivity before, during and after tDCS with the use of resting-state fMRI at 7T. We observed enhanced connectivity within right sensorimotor area after stimulation compared to during stimulation. This result confirmed that cortical modulations differ during versus after tDCS, signifying that optimal modulation of behaviour may be after the stimulation period. Furthermore, we observed an enhanced correlation between motor regions and the caudate, both during and after stimulation.

In conclusion, we observed novel cortical adaptations in CSM patients after surgical decompression, which led us to believe that bihemispheric tDCS of M1-SMA network would result in optimal motor enhancement and warrants further investigation in CSM and other neurological disorders.

Keywords

Transcranial direct current stimulation, motor adaptation, cortical modulation, cervical spondylotic myelopathy, resting state functional MRI, magnetic resonance spectroscopy, manual dexterity, rehabilitation

Co-authorship

The following thesis contains material from previously published and submitted manuscripts. In addition, material from a third and fourth manuscript that are in preparation for submission. Permission was obtained from the publishers to reproduce each manuscript, and appears in Appendix B. The contributions of co-authors for each chapter are summarized below. With the exception of the co-author contributions listed below, Kayla Ryan performed all of the protocol development, participant recruitment, data acquisition and analysis, statistical analysis, interpretation of the results, and preparation and submission of manuscripts.

The material in Chapter 2 has been published in *Journal of Neurosurgery: Spine*, in a manuscript entitled 'Motor Network Recovery in Patients with Chronic Spinal Cord Compression: a Longitudinal Study Following Decompression Surgery' (28(4):379-388, 2018). Co-aurthors were Kayla Ryan, Sandy Goncalves, Robert Bartha and Neil Duggal. S Goncalves assisted in patient recruitment and data collection. N Duggal was responsible for all clinical assessments and decompression surgery in all CSM patients. N Duggal and R Bartha were responsible for the design of the study. R Bartha and N Duggal provided guidance and support in the analysis and interpretation of the data. N Duggal and R Bartha provided critical review and revision of the manuscript.

The material contained in Chapter 3 is in preparation for submission in an article entitled 'Differential Effects of Transcranial Direct Current Stimulation on Antiphase and Inphase Motor Tasks'. Co-authors were Kayla Ryan, Amy Schranz, Neil Duggal and Robert Bartha. A Schranz assisted in the study design and collection of data. R Bartha and N Duggal provided guidance and support in the analysis and interpretation of the data. N Duggal and R Bartha provided critical review and revision of the manuscript.

The material contained in Chapter 4 has been submitted to PLoS One, in an article entitled '^{1}H MR Spectroscopy of the Motor Cortex Immediately following Transcranial Direct Current Stimulation at 7 Tesla'. Co-authors were Kayla Ryan, Krzysztof Wawrzyn, Joseph Gati, Blaine A. Chronik, Dickson Wong, Neil Duggal, and Robert Bartha. K Wawrzyn prepared and analyzed temperature monitoring data. J Gati assisted in the design of the study. B Chronik provided temperature monitoring equipment. D Wong created essential analysis tools and assisted in the analysis and interpretation of the data. R Bartha assisted in the design of the study. R Bartha and N Duggal provided guidance and support in the analysis and interpretation of the data. N Duggal and R Bartha provided critical review and revision of the manuscript.

The material contained in Chapter 5 is in preparation for submission in an article entitled 'Bihemispheric Transcranial Direct Current Stimulation Acutely Modifies Resting State fMRI Measured Functional Connectivity of the Sensory Motor Network'. Co-authors were Kayla Ryan, Krzysztof Wawrzyn, Joseph Gati, Blaine A. Chronik, Ravi Menon, Neil Duggal and Robert Bartha. R Bartha, J Gati and R Menon assisted in study design. R Bartha and N Duggal provided guidance and support in the analysis and interpretation of the data. K Wawrzyn and B Chronik provided temperature monitoring equipment and analysis. N Duggal and R Bartha provided critical review and revision of the manuscript.

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tenacity for research is infectious, and is what pushed me in the direction of my thesis topic. I came to him with a crazy idea to stimulate people's brains in hopes of improving motor skill; his enthusiasm and trust was a constant motivator to persevere. Dr. Duggal, you have challenged me to become not only a better researcher, but a stronger, more confident person, and for that I will always be grateful.

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List of Abbreviations

^{13}C	Carbon
^{19}F	Fluorine
^{1}H MRS	Proton Magnetic Resonance Spectroscopy
^{1}H	Hydrogen
^{23}Na	Sodium
^{31}P	Phosphorus
7T	7 Tesla
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
BOLD	Blood Oxygen Level Dependent
Ca^{2+}	Calcium
Cho	Choline
CMMDT	Complete Minnesota Manual Dexterity Test
CNS	Central Nervous System
CO2	Carbon Dioxide
COX-2	Cyclooxygenase-2
Cr	Creatine
CSF	Cerebrospinal Fluid
CSM	Cervical Spondylotic Myelopathy
dHb	Deoxygenated Hemoglobin
DLPFC	Dorsolateral Prefrontal Cortex
DMN	Default Mode Network
ECT	Electroconvulsive Therapy
EEG	Electroencephalography
fMRI	Functional Magnetic Resonance Imaging
GABA	Gamma amino-butryic acid
Gln	Glutamine
Glu	Glutamte
Glx	Glutamate +Glutamine
GM	Grey Matter
GSH	Glutathione
Hb	Hemoglobin
HDR	Hemodynamic Response
Hz	Hertz
ISNCSCI	International Standard for Neurological Classification of Spinal Cord Injury
K^{+}	Potassium
LASER	Localization by Adiabatic Selective Refocusing
LTD	Long Term Depression
LTP	Long Term Potentiation
M1	Primary Motor Cortex
MANOVA	Multivariate Analysis of Variance
MEP	Motor Evoked Potential
MHz	Mega Hertz
mI	Myo-inositol

mJOA	modified Japanese Orthopeadic Association
MRI	Magnetic Resonance Imaging
Na^+	Sodium
NAA	*N*-acetyl aspartate
NAAG	*N*-acetylaspartylglutamate
NDI	Neck Disability Index
NMDA	N-methyl –D-aspartate
NO	Nitric Oxide
PCC	Posterior Cingulate Cortex
PCr	Phosphocreatine
PD	Proton Density
PMC	Premotor Cortex
Ppm	Parts Per Million
RF	Radio Frequency
rs-fMRI	Resting State Functional Magnetic Resonance Imaging
S1	Sensory Cortex
SCI	Spinal Cord Injury
SD	Standard Deviation
SEM	Standard Error of the Mean
SEQTAP	Sequential Tapping
SM1 (M1/S1)	Sensorimotor Cortex
SMA	Supplementary Motor Area
SMN	Sensorimotor Nework
SNR	Signal to Noise
SRTT	Serial Reaction Time Task
SVIPT	Sequential Visual Isometric Pinch Task
T_1	Longitudinal Magnetization
T_2	Transverse Magnetization
tDCS	Transcranial Direct Current Stimulation
TE	Echo Time
TMS	Transcranial Magnetic Stimulation
TR	Transverse Relaxation
TSP	Sodium 3- trimethylsilyl-propionic acid
VAPOR	Variable Pulse Power and Optimized Relaxation Delays
VOA	Volume of Area
WM	White matter

Chapter 1

1 Introduction and Goals of Thesis

1.1 Cervical spondylotic myelopathy

Cervical spondylosis is a term used to describe the degenerative changes of the spine that become increasingly more prevalent with age. In its most severe form, spondylosis can lead to compression of the spinal cord, resulting in cervical spondylotic myelopathy (CSM). CSM is a degenerative condition that results in functional decline due to narrowing of the spinal canal. It is the most common form of spinal cord dysfunction in adults over the age of 55, with males being affected at a ratio of 2.7:1.[1] The variety of symptoms caused by CSM are broad, ranging from mild dysfunction, such as numbness or difficulties with dexterity, to severe, such as quadraparesis and incontinence. [1-5] When symptoms are progressive, and patients fail to respond to conservative treatment, surgical intervention is required.[4] Although thousands of Canadians will undergo surgery every year, clinical improvement and surgical success is difficult to predict and rehabilitation strategies are scarce. There is a need for improved pre-operative screening to predict the outcome of decompressive surgery and further research into optimal rehabilitation strategies to allow for strengthened surgical success and recovery from this commonly occurring disease.

1.1.1 Pathophysiology and Pathology of CSM

Degenerative changes to the spine occur as a part of healthy aging. [4,6,7] The pathogenesis of the disease can be divided into three main components: static, dynamic and histopathological. Static factors are structural factors that cause canal narrowing. The degenerative cascade of CSM typically begins with the deterioration of the intervertebral disc. The disc collapses and the annulus bulges posteriorly causing a narrowing of the spinal canal. Decreased disc height causes the spinal column to shorten, leading to abnormal spine biomechanics.[1,4,8] The ligamentum flavum thickens and buckles into the spinal canal. Ossification of the posterior longitudinal ligament can also lead to CSM by direct compression of the cord. These changes often lead to a loss of range of motion of

the affected structures. To compensate for the decreased motion, adjacent regions of the spine become hypermobile. [8-10] Dynamic factors refer to abnormal repetitive movement of the cervical spine during flexion and extension causing nerve root and spinal cord irritation and compression. Flexion may compress the spinal cord against anterior osteophytes and intervertebral discs. [7,9,10] Hyperextension may lead to cord pinching between the posterior margin of the vertebral body anteriorly and the hypertrophied ligamentum flavum posteriorly. Lastly, mechanical compression of the spinal cord leads to vascular changes causing ischemia and inflammation.[1,4,7,9] Chronic cord compression can lead to neuronal cell loss, degeneration of the posterior columns and anterior horn cells, and endothelial damage resulting in compromised blood spinal cord barrier.[4]

1.1.2 Neuroanatomy

The central nervous system is comprised of both descending motor and ascending sensory tracts that send information either from the brain, down the spinal cord to the target muscles, or from the muscle to the brain.

1.1.2.1 Motor Tracts of the Spinal Cord

As there are two avenues of motor control in the human body, there are two separate pathways: the somatic pathways, controlling skeletal muscle, and autonomic pathways, serving smooth muscles, glands, etc. As the current thesis concentrates solely on control of skeletal muscle, only those pathways serving skeletal muscle will be discussed.[11]

The pyramidal tract is comprised of three separate tracts: corticobulbar, and the lateral and anterior corticospinal tracts. These tracts form two pyramids on either side of the medulla of the brainstem, thus giving their name. The corticobulbar tract originates in the primary motor cortex and synapses in nuclei with the lower motor neurons of cranial nerves serving conscious control over the eye, jaw and face muscles.[11] As these tracts

terminate at the level of the brain stem, they are not affected in CSM and will not be further discussed.

The corticospinal tract, serving conscious control over skeletal muscles is of large importance in CSM cases (Figure 1.1). This tract originates not only in the primary motor area, but also the primary somatosensory cortex, premotor areas and supplementary motor areas.[11] These upper motor neurons travel through the internal capsule in the forebrain and enter the cerebral peduncle at the base of the midbrain. The anterior corticospinal tract continues to descend on the ipsilateral side and decussate at the level of the spinal cord.[11] The lateral corticospinal tract, comprising the large majority of the corticospinal tract fibers, crosses the midline at the level of the medulla and traverses down the contralateral side of the spinal cord where fibres synapse on lower motor neurons in the ventral horn of the spinal cord. Neurons located in the ventral horn send axons through ventral roots to target muscles.[11]

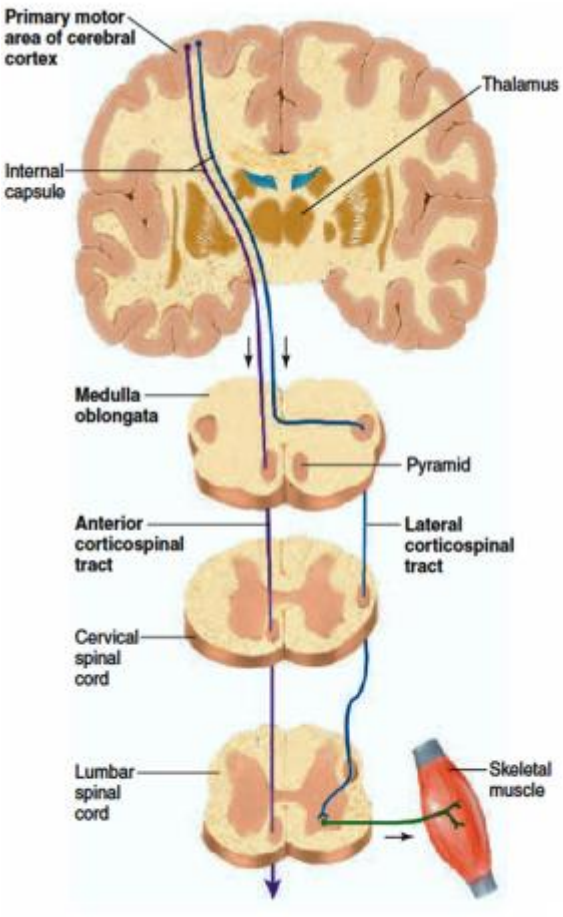

Figure 1.1 Depiction of the Corticospinal Tract. The anterior corticospinal tract (red) does not decussate, carrying information from the brain to the muscle, while the lateral corticospinal tract (blue) decussates at the medulla. Nuno A et al. (2014). From basics to clinical: A comprehensive review on spinal cord injury. *Progress in Neurobiology*. 114: 25-57. Used with permission from Elsevier.

1.1.2.2 Sensory Tracts of the Spinal Cord

The ascending pathways conduct neural input from the periphery, via the spinal cord, to the cortex. Sensory information is relayed through orders of neurons within the central nervous system. First order neurons deliver information from the muscle to the central

nervous system, the cell bodies of these neurons are found in dorsal root or cranial ganglion. Second order neurons are interneurons, with cells bodies either in the spinal cord or brain, and lastly, third order neurons transmit information from the thalamus to the cortex.[11] There are three major sensory pathways, carrying differing types of information. The posterior columns carry information on proprioception, fine touch and vibration and are divided into two separate pathways. The fasiculus gracilis carries information from the periphery to the brain from levels below T6 (eg. legs and hips). First order neurons reside in dorsal root ganglion. Axons from these neurons ascend in the posterior aspect of the spinal cord and synapse with second order neurons in the nucleus gracilis of the medulla. Axons from second order neurons cross over at the level of the medulla before entering the medial leminiscus. [11] Axons then synapse at the ventral posterolateral nucleus of the thalamus before projecting to the primary sensory cortex. The fasciculus cuneatus, carries fine touch, pressure and proprioceptive information from above T6 (eg. arms and hands).[11] This tract follows a similar pathway to the medulla, instead synapsing in the nucleus cuneatus where it crosses over before continuing the same path as the fasciculus gracilis.

The second group of ascending pathways are the lateral and anterior spinothalamic pathways.[11] The lateral pathway is located just anterior to the pyramidal tracts on the lateral side of the spinal cord and carries information pertaining to pain and temperature. Similar to the posterior column, first order neurons are located in the dorsal root ganglion and synapse on second order neurons located in the posterior gray horn of the spinal cord. [11] Axons from second order neurons decussate across the anterior commissure of the spinal cord and ascend through the brain stem and midbrain before synapsing on third order neurons located in the ventral posterolateral nucleus of the thalamus. The neurons of the thalamus project upwards through the internal capsule and carona radiata to the primary somatosensory cortex.[11] The anterior spinothalamic tract is located in the anterior aspect of the spinal cord and carries crude touch and pressure sensations. This pathway follows the same route and decussation as the lateral spinothalamic tract. [11] Lastly, the spinocerebellar tract carries information of proprioception and has both an anterior and

posterior pathway. These tracts only have first and second order neurons and synapse on the same side as stimulus on the cerebellar cortex. [11]

1.1.2.3 Integration of Motor and Sensory Commands in Primary and Secondary Motor Areas

The integration of motor commands originate from both primary and secondary motor areas, including the premotor cortex, supplementary motor area and cingulate motor areas. Although the primary motor cortex contributes a larger number of corticospinal projections to skeletal musculature, the SMA also has direct excitatory influence on the hand and arm region of the motor cortex (Figure 1.2).[12] As stated previously, motor neurons of the SMA contribute directly to the corticospinal tract innervating hand and arm muscles to control fine, dexterous and bimanual movements. The SMA has direct connections via U shaped fibers to the hand region of M1.[13] Using fMRI, the SMA has consistently been shown to have a positive influence on M1 activity. [13-15] The SMA plays an important role in bimanual coordination, and the execution of internally cued movements.[16,17] The integration of motor commands from the SMA to M1 are a crucial aspect of motor execution.

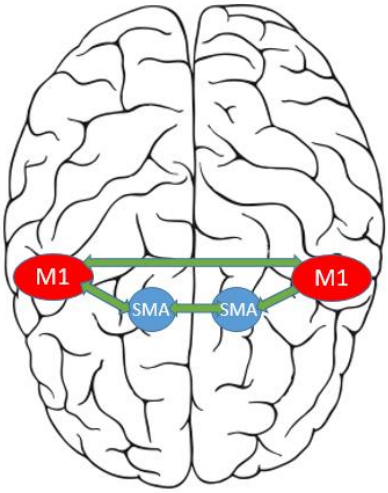

Figure 1.2 Reciprocal connectivity of M1-SMA network. Strong inter and intrahemsipheric connections create the M1-SMA network.

1.1.2.4 Consequences for CSM

Due to the pathophysiology of CSM, the most common pathways affected due to spinal cord compression are the posterior columns and the corticospinal tract. The majority of fibers from the corticospinal tract comprise the lateral corticospinal tract, which functions to carry information of fine motor control of the hands [11]. As such, manual dexterity deficits are observed in the majority of CSM cases.[10] Manual dexterity broadly defines fine motor movements of the hand that involve strength, proprioception and sensation; many activities of daily living rely on manual dexterity, such as doing up buttons, hand writing, eating with utensil, etc.[10] Quality of life can become greatly diminished when manual dexterity is compromised.

1.1.3 Clinical presentation

The clinical consequences related to compression of the cervical spinal cord are broad. The onset of symptoms is often insidious with long periods of disability and short episodes of worsening; however, symptoms can develop acutely or transiently.[10] In the early stages, CSM commonly presents as neck stiffness, changes in muscle tone, sensory loss, and deterioration in gait, balance and manual dexterity. Symptoms can progress to quadraparesis and incontinence. Patients who do progress, experience functional decline leading to a reduced quality of life.[1,8,10] For some patients, evidence of spinal cord compression may be evident from an MRI; however, clinically they present as asymptomatic. This is why a thorough history and physical examination, in addition to imaging is important when making a diagnosis.

1.1.4 Diagnostic Evaluations an Imaging

Cervical radiographs (x-rays) are often used to examine the narrowing of the disc space, presence and size of osteophyte formations and global sagittal alignment. However, x-rays provide no information regarding the condition of the spinal cord. Currently, the most useful diagnostic tool is an MRI of the cervical spine, which allows visualization of the size and shape of the spinal cord in three dimensions (sagittal, axial and coronal). Diagnostic features detected by MRI include the location and source of spinal cord compression, cord edema, and the extent of cord compression.[7,18,19] Signal changes within the cord, such as T_2–weighted image hyper intensities and T_1–weighted image hypo intensities can reflect atrophy of the spinal cord or cord edema.

Physical examination includes balance and gait assessment, cervical range of motion, and dexterity tests, to name a few; however, quantitative measurements of both gait and dexterity are severely lacking within the clinical setting. “Myelopathy hand” is a feature in patients with CSM demonstrating a decline in motor strength and sensory changes.[10,20]

Surgery for the treatment of symptomatic CSM is often considered when there is a history of a progressive decline in neurological function, concordant imaging with evidence of spinal cord compression, and limited or no response to appropriate conservative measures.[4]

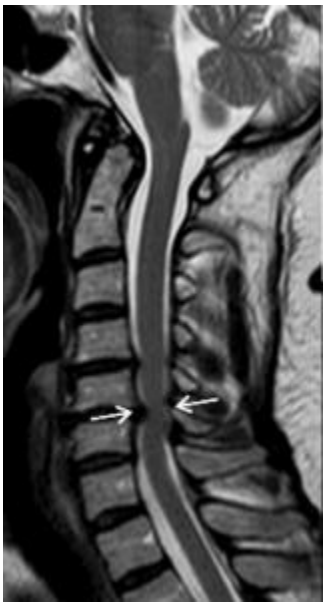

Figure 1.3 MRI of CSM; Arrows indicate presence of spinal cord compression. Kerkovsky, M *et al.* (2012). Magnetic Resonance Diffusion Tensor Imaging in Patients with Cervical Spondylotic Spinal Cord Compression: Correlations Between Clinical and Electrophysiological Findings. *Spine* 37. Used with permission from Wolters Kluwer Health Inc.

1.1.5 Clinical Measurements

Objective, quantitative measurements for assessing disability from CSM are severely lacking. Currently, the International Standard for Neurological Classification of Spinal

Cord Injury (ISNCSCI) and modified Japanese Orthopedic Association Score for CSM (mJOA) are used.[21] The ISNCSCI (Figure 1.4) is a physician derived examination of both motor and sensory function. Gross motor strength and sensation to pin prick and light touch are examined. The mJOA is a patient derived questionnaire of motor, sensory and sphincter function. The questionnaire score ranges from 3 to 18 points (18 representing the maximum score), with a score of >15 indicating mild CSM, 12-14 indicating moderate CSM and a score of <11 indicating severe CSM.[21-23] Whether or not a patient is improving, either naturally or from surgical intervention is defined by these measurements. Patient derived outcome tools commonly used for self-perceived measures of improvement include the 36-item Short Form Health Survey (SF-36) and the Neck Disability Index (NDI).[24] The SF-36 measures global health and is divided into two components: physical and mental. The NDI score has a total of 10 questions regarding neck pain that range from 0-100 (with 100 representing the maximum score).[24]

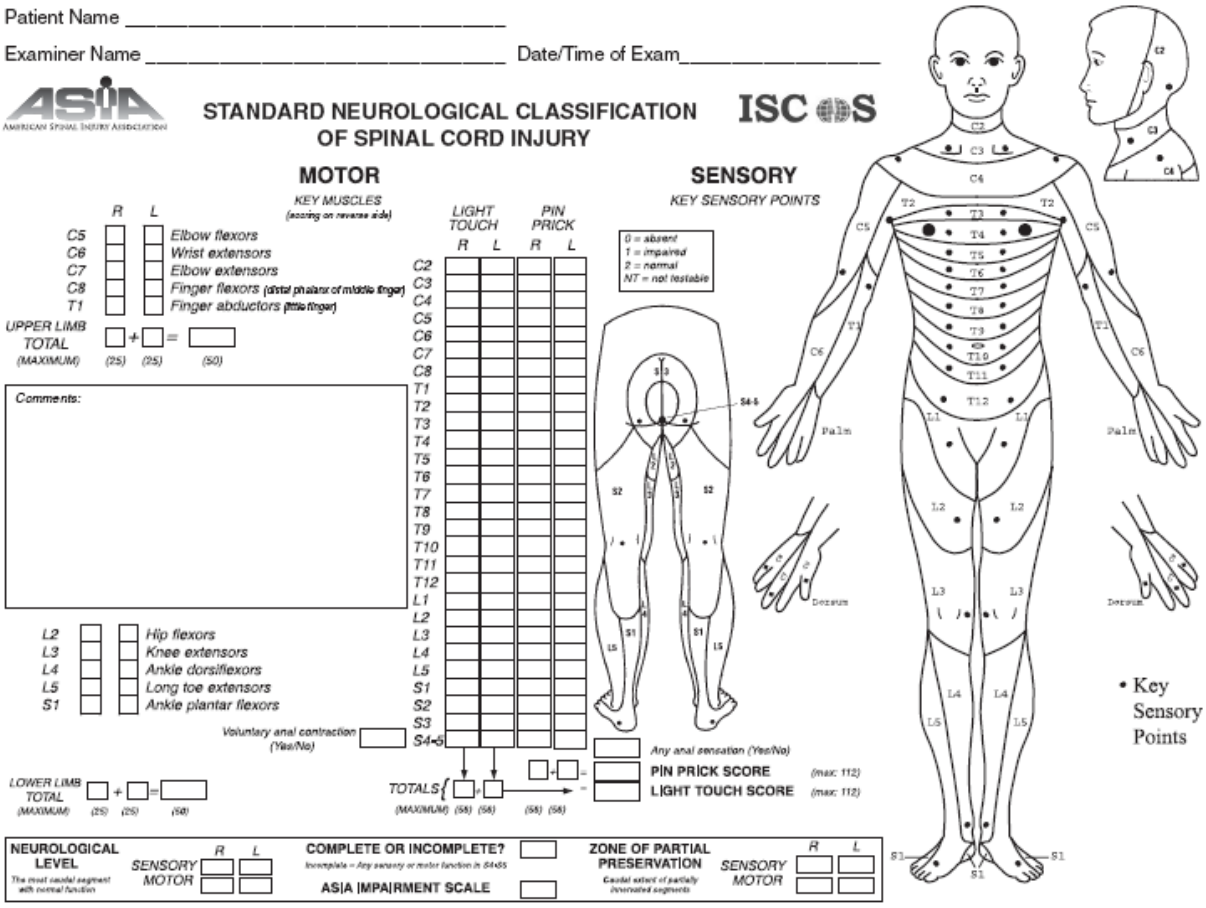

Figure 1.4 ISNCSCI Assessment Scale. Source: http://asiaspinalinjury.org/elearning/ISNCSCI.php (accessed January 2018, available for free download)

1.1.6 Conservative Treatment, Surgical Intervention and Predicting Outcome

The use of conservative versus surgical treatment of CSM has been a topic of debate. Studies have shown that some individuals with mild CSM, treated without surgery, can go as long as 10 years without progressing.[8,10,25] Conservative treatment of CSM is aimed at treating symptoms and protecting the spinal cord from additional injury. This may

include medications, and avoidance of certain activities.[25,26] Surgical treatment of CSM is often encouraged for those with more severe or progressive symptoms. The goal of surgery is to decompress the spinal cord, preserve alignment of the vertebrae and prevent any further neurological damage. However, research has shown that surgical outcomes are unpredictable. Approximately two-thirds of patients improve after surgery, while 15-30% fail to show any improvements.[3,4,8] Kadanka and colleagues performed a randomized control trial of 68 CSM patients either receiving surgery or conservative care. After a three year follow up period, they discovered no difference in mJOA scores between those treated conservatively and those who underwent surgery.[27] In contrast, another group performed a study of 43 patients, showing that surgery resulted in superior outcomes approximately 1 year later.[25] Several factors have been suggested as predictors of surgical outcome such as age, duration of symptoms, severity of myelopathy, mJOA score, cross-sectional area of spinal cord and high-intensity signal change on T_2-weighted MRI.[23,28,29]

1.1.7 Cortical Changes Due to CSM

The pathological changes in the spinal cord after CSM have been well documented; [26] however, the subsequent changes that occur in the brain are largely unknown. Although the initial insult results from compression of the spinal cord, ascending and descending cortical tracts become compromised, disrupting the communication flow between the spinal cord and brain. When neural injury is sustained, a process called Wallerian degeneration occurs wherein axons distal to the site of injury undergo progressive deterioration.[4,30] A similar process has been documented for upstream axons. Primate studies show apoptosis of corticospinal neurons in the motor cortex and decreased synaptic spine density, [31] while human studies following SCI demonstrate cortical atrophy in the somatosensory areas.[32,33] Together, this research demonstrates the need for continued examination of the cortex following SCI and how this relates to injury severity and recovery. Evidence suggests that in order to maintain motor function following injury, cortical reorganization of spared motor neuron pools occurs.[33-37] Reorganization

of the cerebral cortex due to CSM and SCI will be discussed in further detail in the following sections.

1.1.8 Enhancing CSM Outcomes

CSM is the most common form of spinal cord dysfunction in those over the age of 55. With the uncertainty of outcomes in both conservative and surgical treatment, better management of symptoms after diagnosis and surgery are required. Currently, there is no clinical standard of care that requires or even promotes a rehabilitation protocol after receiving a diagnosis of CSM or after operative treatment. With neurorehabilitation strategies gaining popularity in the literature for other neurological disorders such as stroke, it is important to determine an optimal neurorehabilitation program for individuals with spinal cord injury.

In addition, non-invasive brain stimulation such as transcranial direct current stimulation (tDCS) has also become increasingly popular in the literature for modulating cortical activity and behaviour.[38-41] Recently, it has been used in neurorehabilitation to enhance motor function in neurological disorders such as stroke. In a study by Cortes *et al.* in 2017, improved grasp function was observed after a single session of tDCS in individuals with chronic spinal cord injury.[42] Although positive results have been observed with the use of tDCS, its use remains controversial, as its exact mechanism and the optimal parameters to promote motor and cognitive enhancements are still unknown. Stagg and colleagues have performed multiple studies observing the effects of tDCS both behaviourally and physiologically.[43-47] It is thought that tDCS modulation occurs in a similar manner to long term potentiation; enhancing task specific synaptic connections.[39] The after effects of tDCS are believed to involve modulation at the level of the neurotransmitter (e.g. GABA and/or glutamate). By enhancing motor specific connections by pairing tDCS with a rehabilitation program, it is possible that recovery of CSM can be enhanced.

1.2 Transcranial Direct Current Stimulation

The ability to modulate the brain through electric current is not a novel concept. As early as 1870, Fritsch and Hitzig demonstrated that injecting positive current had stimulating effects, while negative current had an inhibitory effect on the cortex.[48] In 1938, the first electroconvulsive therapy (ECT) was conducted on patients with psychiatric disorders. At that time, it was believed that by electrically inducing seizures, relief from psychiatric disorders such as major depression, mania and catatonia would occur.[49] The field of non-invasive brain stimulation has since grown and has been adapted to provide enhanced recovery of motor, language and cognitive faculties.[39,40,50] One such method, tDCS has gained popularity because of its versatility and ease of use to modulate motor and cognitive behaviour[39].

1.2.1 Neuronal Anatomy

A neuron is an electrically excitable cell that receives, processes and transmits information through electrical and chemical signals. The structure of a neuron reflects the roles carried out by each segment. A typical neuron consists of a cell body (soma), an axon and dendrites (Figure 1.5). Dendrites can be further divided into apical (those facing the cortical surface) and terminal (those extending away from the cortical surface).[11] Due to the presence of ion pumps and channels embedded in the membrane, there is a membrane voltage gradient that occurs due to difference in ion (e.g. sodium, potassium, chloride and calcium) concentration intracellularly compared to extracellularly. A change in the membrane voltage can alter the function of the voltage-dependent ion channels, allowing for a depolarization or hyperpolarization of the cell membrane. [11] If the cell becomes depolarized to a large enough degree, an action potential is generated along the axon of the neuron.

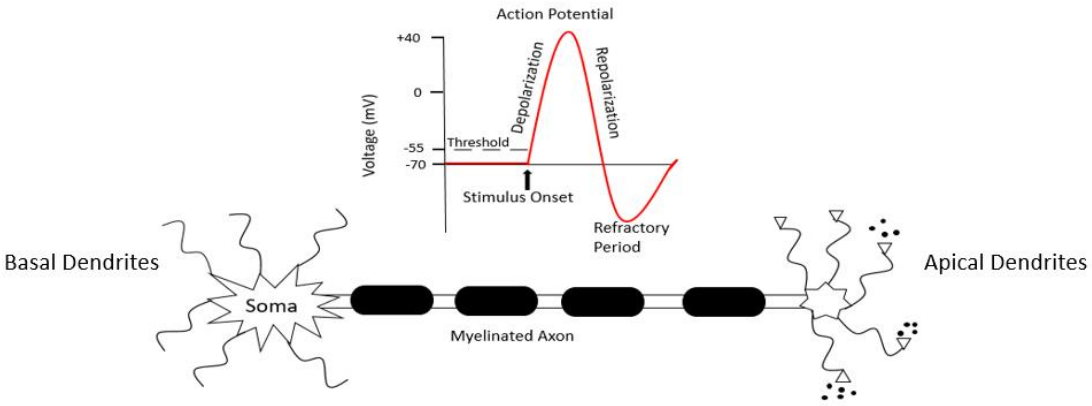

Figure 1.5 Neuronal Excitation and Action Potential Generation. Resting membrane potential of a typical neuron is -70 mV. When the distribution of ion flow changes and allows for more positive ions on the inside of the membrane, resting membrane potential can become more positive. Once a threshold of -55 mV is achieved, an action potential is generated and is carried along myelinated axons.

1.2.2 Mechanism of Action

The mechanism by which tDCS alters brain activity has been examined in both animal and human experiments. tDCS acts in a similar manner to that of a traditional battery. Current flows from the positive terminal (anode) to the negative terminal (cathode) via the path of least resistance.[45,51] As the current needs to pass through the highly resistive skull to reach the brain, a large proportion of the current is shunted through the skull. Under ideal conditions, it has been approximated that only 45% of the current will reach the brain.[52] What happens once the current reaches the brain is debated and an area of active research. Application of tDCS provides a low, continuous direct current to target areas of the brain, under the stimulating electrodes. The biophysics behind how

application of current can modulate the polarity of neuronal cells is detailed in the following sections.

1.2.2.1 Biophysics of Neuronal Polarization

Neurons maintain a resting membrane potential by shuttling three sodium ions out of the cell and two potassium ions into the cell. The resting membrane potential of a typical neuron is -70 mV, with a threshold potential of -55 mV. This means that the potential difference from the inside of the cell to the outside of the cell must increase by 15 mV in order for an action potential to fire. [11] Conventionally, nerve impulses cause an influx of Na^+ ions into the cell, causing the inside of the cell to become more positively charged. Application of tDCS electrodes to the scalp creates a similar potential difference which causes positively charged ions to be pulled toward the cathode and away from the anode; the opposite occurs with negatively charged ions.[53] This modulation in the distribution of ion flow alters the concentration gradient of K^+ and Na^+ ions, resulting in polarization of the membrane. (Figure 1.6b).[51] The magnitude and direction of membrane polarity are dependent on neuronal size and orientation in relation to the electric field. Larger, asymmetrical neurons such as layer V pyramidal neurons have an increased polarization compared to smaller, symmetrical neurons such as interneurons. Put simply, a pyramidal neuron that is oriented with apical dendrites toward the cortical surface will result in apical dendrite hyperpolarization and somatic and basal dendrite depolarization and when receiving anodal current (Figure 1.6a).[51] Electrophysiology studies using rat hippocampal slices have shown that with currents of 2 mA, the peak electric field would be between 0.4- 1 V/m, resulting in a somatic polarization of approximately 0.3 mV.[51,54,55] Based on the widely used 'somatic doctrine', the functional outcome of tDCS is based upon the polarization of the soma.[54] However, this view neglects the potential effects of dendritic polarization. While the basal dendrites are likely to be polarized in the same direction of the soma, the apical dendrites are polarized in the opposite direction.[54,55] The role of dendritic polarization will be discussed in further detail in the next section.

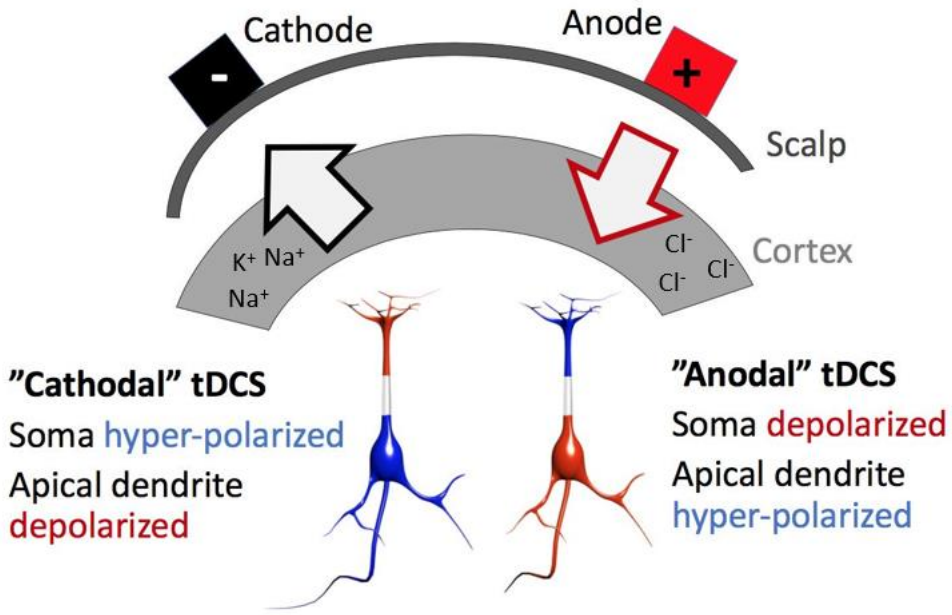

Figure 1.6 Current flow from anode/cathode and the different effect on apical and basal aspects of the neuron. Anodal tDCS allows current to flow from the anode to cathode which alters the distribution of the underlying ions. Negatively charged ions such as chloride (Cl-) are pulled towards the anode, while positively charged ions such as sodium (Na+) and potassium (K+) are pushed towards the cathode. This distribution of charged particles drives the polarity differences observed in the underlying neurons. Modified from Rahman *et al. (*2013). Used with permission from Elsevier and John Wiley and Sons.

1.2.2.2 Shortcomings of the 'Somatic Doctrine'

There are two important limitations of the somatic doctrine; the first being the cellular compartment in which it ignores, the axons and dendrites. While the orientation of the soma is important in dictating the direction of polarity (excitatory or inhibitory), there is

evidence to suggest that the orientation of the dendrites governs the magnitude of the effect of stimulation.[56] Although the soma is the central integration point for synaptic input, the dendrites play a meaningful role in synaptic processing and plasticity through glutamatergic and GABAergic receptors.[57] Furthermore, the somatic doctrine assumes not only that the electric field is perfectly radial to the cortex, but that all neurons are oriented perpendicular to the electric field.[51] Due to the high degree of cortical folding in the human brain, most of the cortex stimulated by direct current will experience both radial and tangential electric field. Tangential fields are oriented parallel to the cerebral cortex and were once believed to produce little polarization and therefore had little effect on synaptic modulation.[58] However, electrophysiology recordings on hippocampal brain slices have confirmed that tangentially applied electric fields were as effective at modulating synaptic efficacy as radially applied fields.[58,59] In addition, as tangentially applied electric fields do not modulate somatic polarization, early studies support the idea that dendritic polarization can modulate synaptic efficacy independent of soma polarization. [51,58,59] This once more solidifies the importance of dendritic polarization, as afferent axons run perpendicular to the somato-dendritic axis.[58] Brain slice studies have consistently found that terminal hyperpolarization enhances synaptic activity, whereas terminal depolarization causes an inhibition (Figure 1.6b).[56]

1.2.2.3 Network and Secondary Effects

A great deal of the work done to elucidate the physiological effects of tDCS has been on animal models, and in most cases on brain slices at the single cell level. This negates the possible effects of tDCS on multiple neuronal populations within a brain region and between brain regions. Studies have shown that tDCS effects the target neurons directly under the stimulating electrode; however, downstream, subcortical structures such as the thalamus and cerebellum have shown modulation.[53,60-62] As mentioned previously, tDCS does not induce an action potential, rather modulates the membrane potential of neuronal populations. Studies have shown that direct current modulates synaptic plasticity either through changing the rate of action potential generation or change in the timing of the

action potential. The magnitude and direction of synaptic plasticity is heavily dependent on the physiological state of the target neuronal network.[61]

tDCS is thought to act on the glutamatergic system, enhancing the release of glutamate upon anodal and reducing release upon cathodal stimulation. Rahman postulates that direct current allows the brain to receive and process a higher amount of synaptic inputs, resulting in higher synaptic efficacy.[51] Work from brain slice, as well as quantification of evoked potentials has shown that tDCS can modulate brain activity that outlasts the stimulation period.[56] Recent work by Sun *et al* showed that the after effects of tDCS are due to a molecular cascade involving glutamate, NMDA and GABA receptors.[63] Studies have shown that the plasticity that occurs during the after effects of tDCS are very similar to that of long term depression and potentiation.[45,64,65]

At the network level, Nitsche and Paulus have conducted several experiments quantifying the neuronal responses of the motor network both during and after tDCS.[64-66] With the use of TMS, the excitability of the motor cortex can be quantified using motor evoked potentials (MEP). In their 2000 study, Nitsche and Paulus measured MEPs of the right abductor digiti minimi following 1 mA of stimulation to the motor cortex representing that muscle for 5 minutes.[64] They observed an increase in MEP amplitude by 40% following anodal stimulation, and a decreased MEP amplitude of 30% following cathodal stimulation.[64] This study concluded that tDCS to the motor cortex was able to induce or inhibit cortical excitability. In a second study, they revealed that motor cortical excitability can be increased by up to 150% above resting and sustain an excitable state for up to 90 minutes after the end of 13 minutes of 1 mA of stimulation.[65]

1.2.3 Importance of Study Design

There are several parameters that can be manipulated in the study design of tDCS, each requiring careful thought and consideration. These parameters include: duration and

intensity of the simulation, electrode size and placement of the active and reference electrode, electrode montage (unihemispheric vs bihemispheric) and whether tDCS will be applied before, during or after a behavioural task. Furthermore, when possible, tDCS studies should always try to use a double-blind, within-subjects design.[53] Due to a great deal of variance in the effect of stimulation between individuals, within subject designs are superior as they control for the differences between subjects.[64,65] Blinding the subject to the condition in which they are participating is crucial; when possible, blinding the experimenter controls for bias as well. Successful blinding of the sham condition is possible by ramping the current up to the desired current for 10 seconds, and subsequently turning it off. This allows participants to feel the sensation of the electrodes in both the active and sham condition. Subjects are unable to determine which condition they are in when blinding is done in this manner.[67]

Currently research is not united on the optimal intensity of stimulation. Early tDCS work showed that the intensity of current would increase neuronal excitability in a linear manner, ie. 1 mA of current would produce twice the neuronal excitability as 0.5 mA. This was shown to be the case using very specific parameters.[64,65] Using 35 cm^2 electrodes for 13 minutes, Nitsche and Paulus have shown a linear effect of stimulation up to 1 mA.[64,65] However, recent work has discovered important physiological effects of increasing the current intensity from 1 mA to 2 mA, especially in the case of cathodal stimulation.[68] Cathodal stimulation has been popularly known to cause cortical inhibition; however, when stimulation intensity is increased to 2 mA, it instead induces excitation.[68] The reason for this polarity reversal is hypothesized to be due to an increase in calcium influx, resulting in long term potentiation. Another possible mechanism may be due to the larger intensity current causing dendritic depolarization at a sufficient level to impact neuronal excitation on surrounding neuronal structures of differing orientation.[68] Finally, an increased current may lead to neuronal excitation and recruitment of surrounding brain regions, causing an indirect change in the direction of polarity in the target brain region. When designing an experiment that is meant to inhibit a target brain region, it is crucial that this reversal in polarity of cathodal stimulation at 2 mA is accounted for.

Duration is another important stimulation parameter when designing a tDCS experiment. Nitsche and Paulus observed that 5 minutes of stimulation not only increased the size of the MEP compared to 1 minute of stimulation, but the effects lasted for a longer period of time.[64] In a follow up study, they concluded that at least 13 minutes of stimulation was required in order to observe after effects that lasted up to 90 minutes.[65]

The size of the electrode is an important parameter that does not receive the consideration it deserves. The size of the electrode determines the current density. Therefore the effect the electric field has on neuronal excitation or inhibition is not just dependent on current intensity, but also on the dispersion of the current.[54] With smaller electrode sizes, the more focal the current distribution is. This not only allows for deeper penetration of the electrical field, but also allows better specification of the target brain region.[69] In separate studies, Nitsche and Miranda demonstrated a more focal current distribution of stimulation with reduced electrode size.[69,70] Larger electrode sizes (40 cm -100 cm) are often used for the reference electrode in order to diminish the effects of the non-target regions.[71] Bastani *et al.* observed a significant difference in MEP amplitude change from baseline when altering current density from 0.029 to 0.083. Larger current densities induce larger changes in corticospinal excitability.[71]

A final factor to consider in the tDCS study design is where to place both the target and reference electrode; will a unihemipheric (one target electrode, one reference electrode) or bihemispheric (two target electrodes) montage provide optimal results for the study hypothesis.

1.2.4 Modelling current distribution

The spatial distribution of the electrodes on the surface of the scalp have important implications in tDCS efficacy. In addition to accounting for the various stimulation parameters, there are anatomic features that must be considered such as brain-scalp

distance and the concentration of CSF. The conductivity of bone is quite poor; therefore, with increased skull thickness, there is a reduced spread of current to the brain. As it is estimated that only 45% of current penetrates the scalp to reach the brain, this percentage can quickly be diminished with increasing skull thickness.[55,69] Additionally, the spacing of the electrodes plays an important role in the penetration of current to the brain. Generally, increasing the distance between two electrodes increases the current into the brain as a result of increased current density depth and decreased shunting through the scalp; however, with increased distance of electrodes, the current distribution is much less focal, and non-target brain regions may also be implicated.[54,55,69] There is a compromise between current density, electrode spatial distribution and current specificity. Rush and Driscoll found that electrodes must be at least 5 cm apart in order for effective current injection into the brain.[52]

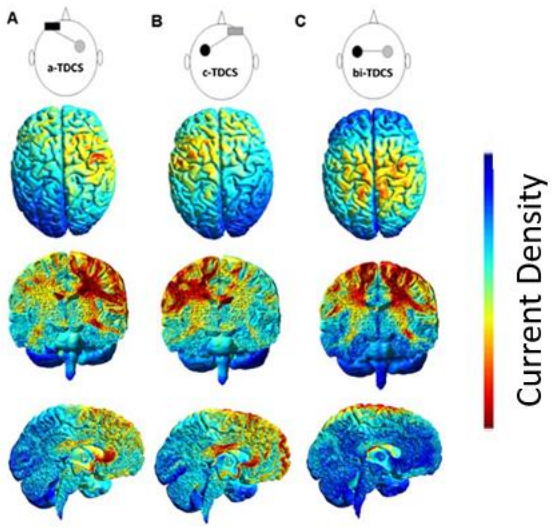

Figure 1.7 Electric Field Distribution of Different Electrode Montages. In three separate sessions, electrodes were placed on A) anode on left M1, B) cathode on right M1 and C) anode and cathode on bilateral M1. Authors concluded that bilateral tDCS is more effective than unilateral stimulation due to its polarity-specific effects on each hemisphere rather than due to its current flow direction**.** Georgios, N *et al* (2016).

Enhanced motor learning with bilateral transcranial direct current stimulation: Impact of polarity or current flow direction. *Clinical Neurophysiology*. 127:2119-2126. Used with permission from Elsevier.

The use of one active electrode and one reference electrode, commonly placed on the supraorbital bone, is the classic tDCS design (unihemispheric). However, more recently, clinical studies have begun to employ a bihemispheric electrode montage, where target areas of the brain on both hemispheres are implicated.[72-74] As one can imagine, these two different designs result in a different spatial distribution of current flow and peak current densities (Figure 1.7). The optimal design depends largely on the research question.

Both behavioural and physiological studies have shown a great deal of variability in the response to tDCS of healthy individuals. This large inter-individual variability can be largely explained by anatomical differences and has been demonstrated using computation models. Skull thickness, cortical folding patterns, GM/WM ratio and orientation of neurons will all affect the distribution of current through the brain and therefore affect the physiological and behavioural responses. A study by Song *et al.* observed as much as a 40% difference in brain volume in healthy individuals aged 18-35.[75] Datta *et al.* performed computational modelling of 1 mA of current with the anode placed over left M1 and the cathode over the right supra-orbital bone in three healthy individuals.[76] They observed a peak cortical electrical field of 0.27, 0.35, 0.4 V/m in the three individuals, which represents a 1.5 fold variation in healthy adults.[76] Furthermore, the three individuals showed variation in current flow patterns and direction. Subject specific local peaks in electrical field were observed, translating to differing neuronal pools being affected by the stimulation.[76]

Finally, the position of electrodes dictates current flow. Current flows from the anode to the cathode; therefore, the position of the cathode will determine the spatial flow of current and which brain areas will be affected. When planning a tDCS experiment, it is important to consider not only the underlying target brain region under the electrode, but

also the path in which the current will flow and how this will affect behavior and physiology.

1.2.5 Enhanced Motor Learning by tDCS

Learning of a motor skill, whether that be a simple sequential finger movement task or as complex as learning a new instrument requires repetitive practice. Motor learning is typically associated with both functional and structural change to the motor network, which includes the primary motor, premotor and supplementary motor cortex, as well as the cerebellum and basal ganglia.[77-79] Furthermore motor learning is accompanied by changes in neuronal activity and synaptic plasticity. The mechanism of motor learning is widely considered to be a long-term potentiation (LTP) or long term depression (LTD) like process.[80] LTP is based upon the adage of "neurons that fire together wire together"; continuous firing of a neuronal pool during specific activity causes a strengthening of the synapses, ultimately producing a long lasting increase in signal transmission.[80] Enhancement of LTP, and therefore motor skill learning, can be accomplished through training and practice. There have been three different, but related, processes that underlie motor skill learning, the first being online effects; improvement that occurs during the training session. Secondly, offline effects, or consolidation, occurs during a period of rest. Lastly, motor retention occurs by forming long term memory, days to months after the initial training.[81-83] tDCS is thought to work in a similar manner as LTP/LTD, thereby providing an additive, exogenous source of motor learning through all three processes; however, tDCS has shown to have its greatest effects on motor consolidation.[39,81]

Nitsche and colleagues were the first to demonstrate in humans that an increase in motor cortex excitability by anodal tDCS improved performance reaction times of an implicit motor learning task.[84] The study became the jumping off point for the now vast majority of research proposing motor enhancement by tDCS. Although tDCS has been shown to be highly polarity, timing and task specific, the application of anodal tDCS to M1 has

shown beneficial effects in a variety of motor tasks (Table 1.1) such as serial reaction time tasks (SRTT), implicit and explicit finger sequence tasks, Jebsen Taylor hand function test, ballistic thumb movements and reaching adaptation tasks, to name a few.[43,84-88] A recent review of alteration of motor behavior through tDCS has shown that single session, anodal tDCS is largely insufficient to change motor behavior outcomes both during and immediately after tDCS. However, improvements in behavior were observed 24 hours after single session, M1 anodal tDCS, suggesting tDCS acts preferentially on the consolidation of motor leaning. [83,89-91]

Table 1.1. Single session tDCS

Authors	**Brain Area Targeted**	**Current & Duration**	**Sample Size**	**Task**	**Outcome**	**Data Measurement**	**Conclusion**
Kantak et al 2012	M1 and PMd	1 mA 15 min	12	Implicit SRTT L Hand	Reaction time	Post Intervention and Retention	tDCS to M1 stabilizes motor learning and retention
Nitsche et al 2003	M1	1 mA 15 in	20	Implicit SRTT R Hand	Response Time	During stimulation	Increased performance with tDCS
Savic et al 2017	Anode and cathode DLPFC	1 mA 30 min	98 (divided into 4 groups)	Task sequence learning	Skill learning	24 hours post stimulation	No tDCS modulation
Hashemirad et al 2017	Anodal to left M1, DLPFC, PCC	20 min	51	SVIPT R Hand	Movement time, error rate, skill	15 min post intervention, 1 day post	Improvement not modulated by tDCS
Rroji et al 2015	Anode to left M1	20 min	14	Ballistic thumb movement L Hand	Movement velocity	30 min post, I day post and 1 week post stimulation	Improved long term retention with anodal tDCS

Abbreviations: M1, primary motor cortex; DLPFC, dorsolateral prefrontal cortex; PCC, posterior cingulate cortex; PMd, dorsal premotor cortex; SVIPT, sequential visual isometric pinch task; SRTT, serial reaction timed task

1.2.5.1 Muti-session tDCS

There has been a great deal of controversy over whether or not single session tDCS provides any substantial change in behavior or cognition, as there is such a high degree of variability and lack of consistency among tDCS studies.[90,92] As the modulatory effects of tDCS on healthy individuals are small compared to the large inter-individual variability in both learning and response to tDCS, observing statistically significant changes in behavior is often difficult.[93] As tDCS has been shown to preferentially effect the consolidation and retention phases of learning, recent studies have begun applying tDCS during multiple, consecutive sessions in an attempt to compound the modulatory effects (Table 1.2).[73,74,81,82] For example, Alonzo *et al.,* in a study of 12 healthy subjects, found that multiday, consecutive applications of tDCS was associated with greater increases in motor evoked potential amplitude compared to tDCS application on alternate days.[82] Multiday application of tDCS allows for consolidation of offline effects, which allow for the maintenance of an increased state of corticomotor excitability between sessions. This heightened corticomotor excitability is thought to play a major role in motor enhancement.[81,82] Reis *et al.* conducted a sequential visuo-motor isometric pinch task combined with anodal M1 tDCS for five consecutive days and compared this to sham. They observed that combined training with tDCS led to significantly greater learning at the end of the five days compared to sham. Furthermore, this effect was primarily driven through an increased consolidation of motor training in between sessions.[81] In a follow up study by the same group, they determined that the consolidation of motor training was largely dependent on the passage of time (3-6 hours), but did not necessitate the act of sleep.[94] No change in motor learning was observed when tDCS was applied after the training period, reemphasizing the need for combined tDCS and training for modulation to occur. This has further been demonstrated in brain slices; an increase in synaptic strength was induced by direct current only when paired with a second weak synaptic input. [95] Consolidation enhancement through tDCS is believed to occur through activation of neurotransmitters such as NMDA, GABA and glutamate, further perpetuating the idea of an LTP like mechanistic learning. [45,95]

Table 1.2. Multi-session tDCS

Author	**Target Brain Area**	**Current & Duration**	**Sample Size**	**Task**	**Outcome**	**Data Measurement**	**Conclusion**
Vancleef 2016	Left DLPFC or Left M1	2 mA 20 min 4 days	75	Bimanual tracking task	Skill	Post Intervention day 4 and day 11	No difference between sham and tDCS
Reis 2009	M1	1 mA 20min 5 days	12	SVIPT	Speed-Accuracy trade-off	Within, between day and 3 mon post	Enhanced online and offline learning
Reis et al 2015	Left M1	1 mA 20min 3 days	34	SVIPT	Skill	15 min, 3h, 6 h Post	Time, but not sleep required for tDCS consolidation
Saucedo Maquez et al 2013	Right M1	1mA 20 min 3 days	27	SEQTAP and SVIPT L Hand	Skill	20 min, 1 week post intervention	SEQTAP benefited from anodal-tDCS during learning, SVIPT showed improvements only at retention
Antonenko et al 2018	R tempro-parietal cortex	1 mA 20min 3 day	40	Object location memory	Skill	1 day, 1 month post training	Anodal tDCS facilitated superior recall performance after training

Abbreviations: M1, primary motor cortex; DLPFC, dorsolateral prefrontal cortex; SVIPT, serial reaction timed task; SEQTAP, sequential tapping task

1.2.5.2 Bihemispheric tDCS

As the concept of enhanced motor learning through tDCS enters into clinical research, there has been an increase in the idea that a bihemispheric approach to cortical modulation may produce greater improvements in motor learning.[74,96] A study by Vines *et al.* observed significant improvements in motor performance of a finger sequence task using the non-dominant hand after bihemispheric tDCS (anode over right M1, cathode over left M1) compared to both unihemispheric and sham tDCS.[96] In a study examining the differing effect of the two montages on corticospinal excitability, Mordillo-Mateos *et al.* observed a greater effect size of unihemipsheric tDCS on corticospinal excitability compared to bihemispheric.[97] However, the inter-subject variability observed over anodal MEP was significantly less when bihemispheric tDCS was used compared to unihemispheric. The authors hypothesized that the concomitant cathodal stimulation on the contralateral hemisphere creates a more stable environment of anodal M1 and that it is this reduction in inter-subject variability with the bihemispheric montage that is driving the differences in motor behavior between bihemispheric and unihemispheric tDCS.[97] Using a multi-session bihemispheric M1 approach, Waters-Metenier *et al.* observed increased execution and reaction times in addition to improved force synchrony in a manual dexterity task. Furthermore, this enhancement of motor performance was still present 1 month after stimulation and training sessions.[74] The bihemispheric montage may have beneficial consequences that are unavailable with a traditional unihemipsheric design. As shown in modelling studies, the current distribution in the bihemispheric montage is extended across the two hemispheres (Figure 1.7) and may target important secondary motor areas such as ipsilateral M1 and the supplementary motor area.[54,55,69,73] Consequently, Waters-Metentier showed that the bihemispheric montage allowed for facilitation of motor memory during the training period that led to enhanced performance in both hands.[74] How tDCS can enhance motor performance in clinical populations will be discussed further in section 1.5.8.

1.2.5.3 SMA as a target for motor Behavior modulation

The SMA has been largely ignored as a potential target for tDCS behavior modification. The SMA has been linked to planning of movements, grasping behavior in primates, monitoring movement errors and motor learning.[98] In addition, structurally, the SMA has strong efferent connections with both M1 and the spinal cord, making it an ideal modifier of motor behavior. There have been few studies examining the effects of anodal tDCS to SMA; however, beneficial motor effects have been identified. Carter *et al.* demonstrated an increased accuracy and speed during a bimanual dynamic phase coordination task.[99] Furthermore, Hupfield *et al.* demonstrated significantly faster reaction times in a serial reaction time task and choice reaction task, indicating more efficient movement planning of the SMA.[100] Lastly, using a visuomotor pinch force task, Vollman *et al.* demonstrated enhanced performance with anodal tDCS to SMA.[101] Together these studies provide evidence toward the need for investigation of more complex behavioural tasks with tDCS applied to SMA. There are limitations presented with tDCS to SMA that need to be addressed. Due to the size and location of the SMA (directly near the central sulcus), it is in close proximity to its contralateral counterpart. Due to the size of most electrodes, localizing and stimulating only one SMA presents a challenge. It is more likely that bilateral SMA will be targeted

1.3 MRI

MRI is an imaging technique that provides anatomical, functional and physiological information of the body. The images provide detailed information from soft tissues that can be used for diagnoses, treatment planning, and disease monitoring. MRI is a safe technique that uses magnetic fields and radio-frequency waves to create images.[102] MRI has the ability to provide soft tissue contrast, particularly between grey and white matter

in the brain, making it especially useful for the investigation of the central nervous system.[103]

Figure 1.8 7T MRI used at Robarts Research Institute

1.3.1 The MR signal

Hydrogen nuclei (protons), found in water and fat in the body, are used to produce magnetic resonance images. The interaction between the hydrogen nuclei and the magnetic field of the MRI can be conceptually explained using classical physics. Briefly, the hydrogen protons have a property known as spin, which produces a magnetic moment. Each hydrogen proton can be thought of as a small magnet that in the absence of an external magnetic field, would be randomly oriented.[102] When the hydrogen protons experience an external magnetic field, such as that produced by the MRI, the magnetic moment of the protons tend to align in the direction of that field. The magnetic moment may align in two directions, parallel (low energy) and anti-parallel (high-energy) to the external field.[102] A slight excess of protons will favour the low energy state. Although protons continually fluctuate between the two energy states, given a large enough sample, a slight majority will have their magnetic moments aligned parallel to the field.[102] With increased magnetic field, there will be a greater difference in the excess

number of protons aligned parallel to the field. The sum of all individual proton magnetic moments in a given sample is known as net magnetization, and is aligned with the magnetic field at equilibrium. The frequency at which protons precess is also dependent on the magnetic field. Higher magnetic fields cause the nucleus to precess faster, described by the Larmor Equation: $\omega=\gamma B$, where ω is the precession or the Larmor frequency in MHz, γ is the gyromagnetic ratio, a fundamental property of each nucleus (the hydrogen nucleus has a gyromagnetic ratio of 267.52×10^6 rad s^{-1} T^{-1}), and B is the external magnetic field strength in Tesla.[102]

1.3.2 Excitation

Excitation is achieved by delivering a short burst of energy in the form of a radio-frequency (RF) pulse.[102] The energy delivered must occur at the resonant frequency, which is determined by the Larmor equation. When an RF pulse is applied perpendicular to the net magnetization at the resonance frequency, the net magnetization is rotated from its equilibrium position, down towards the transverse plane.[102] If the net magnetization is rotated into the transverse plane, then a 90º RF excitation pulse has occurred. The RF pulse also aligns the phase of the protons. This RF excitation is applied through transmitter coils within the MR system. The same coils can be used to transmit and receive signals. After the RF excitation pulse is turned off, the spin system begins to return to equilibrium.[102]

1.3.3 Relaxation

To restore the system to equilibrium, the protons in the high energy state must return to the low energy state by releasing energy. When this occurs, the magnitude of the net magnetization of the transverse plane decreases and the longitudinal magnetization is restored to its original value.[102] The process whereby hydrogen nuclei lose energy and

return to equilibrium is called relaxation. The time is takes for the protons to relax is characterized by two time constants called T_1 and T_2..[102]

The energy that was absorbed by the protons during the RF pulse is dissipated back out to the surrounding tissue (lattice) in a process called longitudinal relaxation, spin-lattice-relaxation, or simple T_1 relaxation. The time that it takes for the longitudinal magnetization to recover is described by the longitudinal relaxation time constant T_1. The T_2 relaxation time constant characterizes how quickly the spins lose phase coherence[104]. One mechanism that causes T_2 relaxation is the exchange of energy between spins (spin-spin interaction), which causes the spins behavior. Once the RF pulse is turned off, the protons no longer remain in phase with one another. In tissue, T_2 signal decay typically occurs 5-10 times more rapidly than T_1 recovery[104]. T_2* is another time constant used to describe relaxation (or decay) of the signal in the transverse plane. T_2* occurs due to local inhomogeneities of the magnetic field in addition to spin-spin interactions. This type of dephasing can be reversed by applying a 180 degree refocusing pulse. The T_2* time constant is therefore shorter than T_2[104]. It is often used to produce contrast in tissue in response to changes in local magnetic fields. It is also the main source of contrast used during fMRI studies when local magnetic fields change due to altered deoxyhemoglobin concentration following brain activity and subsequent alterations in perfusion[105].

1.3.4 Contrasts

Different tissue types and pathologies have unique relaxation properties and can therefore be identified by adjusting image parameters. Repetition time (TR) and echo time (TE) can be manipulated to highlight specific image weightings (eg. T_1-weighted, T_2-weighted and proton density (PD) weighted)[104]. The TR is defined as the time between two successive RF pulses. Whereas, the TE is the time between the RF pulse and the collection of the MR signal. Tissue differences in the rate of recovery of the longitudinal magnetization (T_1) can be emphasized by using short TR and short TE. Fat appears bright

and fluid appears dark on T_1 weighted images. Similarly, differences in the rate of dephasing of the transverse magnetization (T_2) can be emphasized with long TR and long TE. Fat appears darker, while fluid appears bright on T_2-weighted images. PD-weighted images are acquired with a long TR and short TE, displaying the relative proton concentration in the different tissue types; tissues with higher numbers of protons produce greater (brighter) signal[104].

1.3.5 MRI in CSM and SCI

MRI is the preferred method of diagnosing CSM, as it provides excellent contrast in soft tissues and great anatomical detail of the spinal cord structure. With MRI it is relatively straight forward to determine the severity of spinal cord compression, the presence of cord edema, and intrinsic cord abnormalities by observing changes in signal intensity.[10,103] Due to ease of visualization of spinal cord structures, variations from healthy spinal cord can be observed and measured for diagnosis and treatment planning. Normal cervical spinal canal diameter (C3-C7) ranges from 17 to 18 mm. "Relative stenosis" is diagnosed when the cervical spinal canal diameter is measured to be less than 13 mm, while "absolute stenosis" is diagnosed at less than 10 mm.[106,107] Anatomical features that indicate compression of the spine can also be identified on a conventional MRI; the presence of osteophytes, loss of disc height, disc bulging, ligamentum flavum infolding and ossification of the posterior longitudinal ligament[103]. In addition to anatomical indicators, MRI will also show signal change in the spinal cord, a characteristic indicative of CSM.[26,103,108,109] Specifically, hyper-intensities on a T_2 weighted image or hypo-intensities on a T_1 weighted image have been correlated with a poorer prognosis, as it is indicative of irreversible changes in edema and ischemia.[103,109] With such a high prevalence of use as a prognostic tool in CSM cases, recent studies have found a correlation between these anatomic factors and surgical outcome. Although a recent systematic review reported that there are currently no anatomic features that are associated with outcomes of CSM, [103] several studies have shown otherwise.[26,108] Chibbaro *et al.* conducted a prospective study of 70 CSM patients, concluding that there

was a significant correlation between hyper-intense T_2 signal and hypo-intense T_1 signal with post-operative mJOA scores.[110] Conversely, in a group of 101 CSM patients, Nakashima and colleagues determined that there were no significant associations between high T_2 signal intensity and lower limb function as measured by the JOA Cervical Myelopathy Evaluation Questionnaire.[111] The discrepancy between studies may result from the heterogeneity of this patient population. Although canal size may seem pathological and signal change is present, a patient may be asymptomatic, making these anatomical features difficult biomarkers of recovery. Newer imaging techniques are emerging and have shown promise in predicting surgical outcome and recovery. These techniques will be introduced and discussed in the following sections.

1.4 Functional MRI

Functional MRI is a non-invasive technique used to measure brain activity by detecting changes in the MR signal as a consequence of alterations in local blood flow and oxygenation. The technique uses the blood oxygen level-dependent (BOLD) contrast as a means to detect local changes in blood oxygenation that have been linked to neurotransmitter release and local neuronal behavior[112]

1.4.1 Neurovascular Coupling

Neurovascular coupling refers to the relationship between neural activity, metabolism, and cerebral blood flow. An increase in blood flow occurs in tissues that are active. An increase in blood flow due to increases in neural activity requires a cascade of complex signaling mechanisms that involve neurons, astrocytes and vascular cells and an abundant energy source.[113] Under normal resting conditions, the brain's energy (ATP) demands are met almost exclusively by glucose oxidation, oxidizing glucose to water and CO_2, producing 36 molecules of ATP.[113] Neural activation increases the cerebral metabolic rate of glucose and cerebral blood flow by nearly 50%; however, the cerebral metabolic

rate of oxygen is only increased by 5%.[114] This increase in blood flow far exceeds the requirement of oxygen, which leads to a reduction in oxygen extraction and a reduction in the relative concentration of deoxy-hemoglobin, providing the basis for the BOLD signal (Figure 1.9).

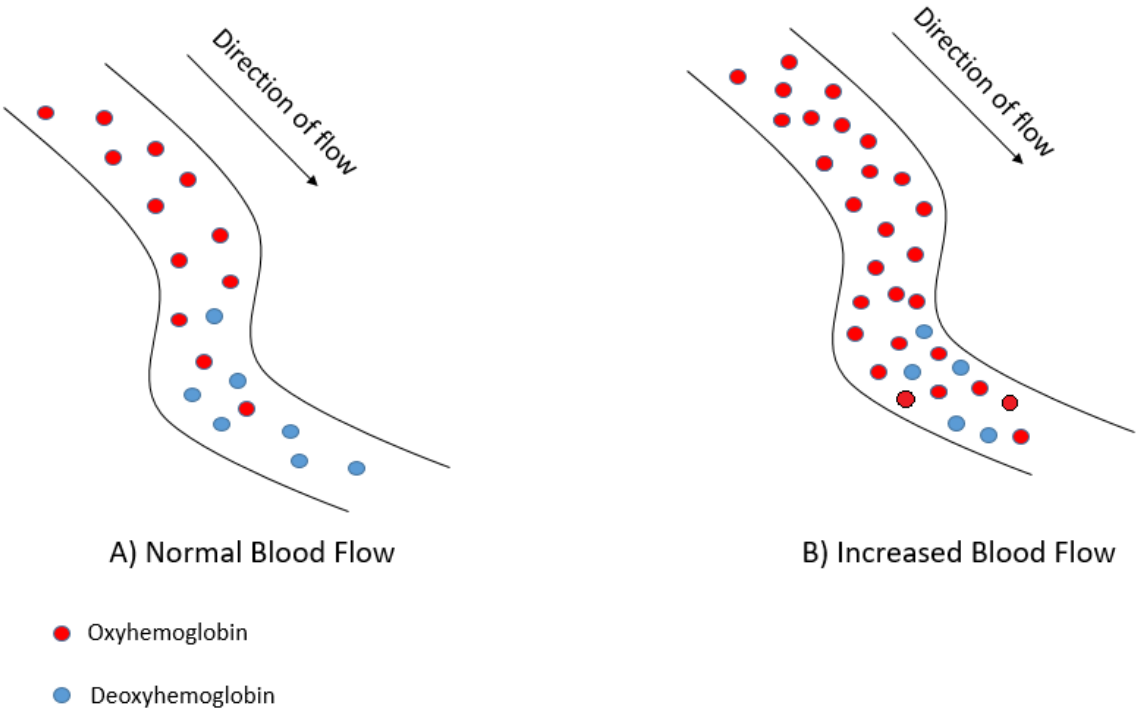

Figure 1.9 Blood Flow at rest and during neural activity. During normal blood flow, oxygen is extracted from the hemoglobin at a constant rate. B) During neural activity, the consumption of glucose and oxygen triggers an increase in blood flow, which overcompensates for the amount of oxygen being extracted. A decrease in the relative concentration of deoxyhemoglobin provides the basis or the BOLD signal.

The need for increased blood flow is communicated to the nearby blood vessels through several signaling pathways. Both neurons and astrocytes play a crucial role in releasing vasoactive messengers that alter blood flow by inducing vasoconstriction or vasodilation

of the vascular smooth muscle cells.[105,115] Astrocytes are ideally situated to participate in the coupling between neural activity and blood flow; they outnumber neurons 10:1 and are situated in close proximity to both synapses and capillaries.[105] It has been shown that dilation of arterioles triggered by neural activity is dependent on glutamate-mediated Ca^{2+} fluctuations in astrocytes. [105] This response is triggered by several vasoactive factors such as nitric oxide (NO), potassium (K^+) and adenosine. Like astrocytes, neurons also release vasodilatory factors that are regulated by glutamatergic neurotransmission. These include COX-2, nitric oxide (NO), acetylcholine and corticotropin-releasing factor. [116] It is likely that these processes work in conjunction to mediate neurovascular coupling.[105,112]

1.4.2 Hemoglobin

Hemoglobin is an iron containing protein responsible for transporting oxygen throughout the blood. In 1936 it was discovered that hemoglobin has different magnetic properties that depend on whether or not oxygen is bound.[117] Hemoglobin, in its natural state, having no oxygen molecules attached (deoxygenated hemoglobin (dHb)), is paramagnetic. In contrast, when oxygen is bound to hemoglobin (Hb), it undergoes a structural change, making it diamagnetic.[117] Therefore, in the presence of a magnetic field such as that produced by an MRI, blood vessels containing oxygenated hemoglobin create little or no distortion to the field within the surrounding tissue, while capillaries or veins containing deoxygenated blood distort the magnetic field. These small magnetic field inhomogeneities surrounding capillaries lead to T_2* based signal loss.[118] As blood flow increases in response to neural activity, the relative amount of deoxyhemoglobin falls, resulting in an increase in T_2* relaxation rate and corresponding increase in MRI signal intensity relative to the normal resting state.[118] The mechanism by which dHb modulates MRI signal intensity is termed the blood oxygen level dependent (BOLD) effect.

1.4.3 BOLD Contrast

Ogawa and colleagues were the first to recognize that deoxyhemoglobin in venous blood could act as a naturally occurring contrast agent for MRI. [119] They manipulated blood oxygen levels in rats by adjusting the amount of oxygen and carbon monoxide the rats breathed.[119] When the rodents breathed normal air, there were areas of signal loss that corresponded to blood vessels containing deoxygenated blood. With increased levels of dHb, the blood vessels became more prominent in the T_2*-weighted MR images. The cause of this contrast can be attributed to a dephasing of the tissue water signal induced by the paramagnetic deoxyhemoglobin in red blood cells. [119] The results showed that dHb decreased the MR signal on T_2* images compared to Hb and that this endogenous contrast could noninvasively monitor blood oxygenation levels in the brain.[105,119]

1.4.4 Hemodynamic Response (HDR) and Signal Generation

Cerebral hemodynamic responses rapidly alter delivery of oxygenated blood to areas of neural activity.[105,112,113] BOLD contrast is sensitive to changes in cerebral blood flow, cerebral metabolic rate of oxygen, and cerebral blood volume; collectively, this is the basis of the HDR. The predictable effect of these responses on BOLD contrast allows for the design and analysis of imaging experiments that manipulate BOLD contrast.[113]

An increase in neural activity leads to increased blood flow to that specific brain region in order to meet oxygen and glucose demands.[105,120] The onset of the HDR is typically delayed by approximately 2 seconds, reflecting the time required for blood to travel from the arteries to capillaries and draining veins. [121] An initial dip is present, which reflects the delay in the response time of cerebral blood flow relative to oxygen extraction, resulting in a higher proportion of deoxyhemoglobin, thereby reducing the MR signal.[116,121,122] The signal continues to ramp up, as there is an overcompensation of blood flow relative to oxygen extraction, and reaches a plateau after 6-12 seconds. Once

neural activity stops, cerebral oxygen metabolism remains elevated, while cerebral blood flow and blood volume return to baseline. This elevated state of oxygen extraction after cessation of stimulation results in the BOLD signal decreasing below baseline in a phenomenon known as the post stimulus undershoot.[122] (Figure 1.10)

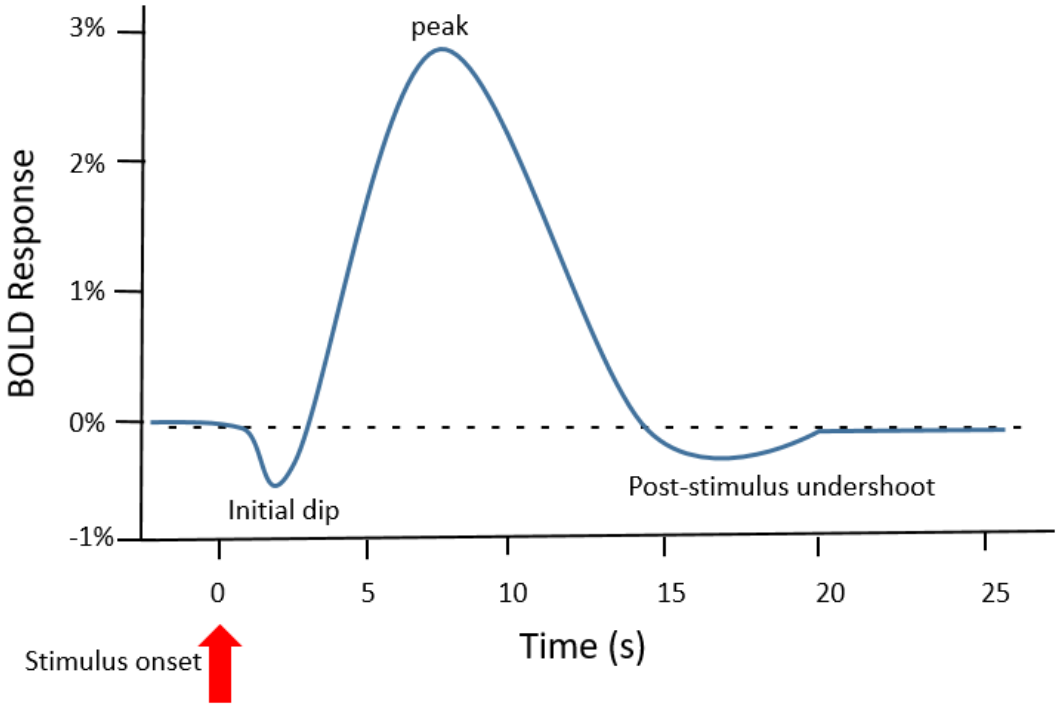

Figure 1.10 Hemodynamic Response Function. At stimulus onset there is an initial dip where oxygen consumption is increaed, resulting in more dHb. As cerebral blood flow and blood volume increase to compensate for increased demand for glucose and oxygen, there is a relative decrease in dHb, resulting in an increased BOLD signal. At stimulus offset, there is a post-stimulus undershoot as cerebral blood volume lags the decrease in oxygen consumption, resulting in increased dHb.

1.4.5 Resting state vs task based fMRI

Task-based activity of the brain can be examined using BOLD contrast in MRI. However even at rest, it has been shown that brain network activity can be monitored using BOLD contrast. Although BOLD contrast is used for signal generation in both task-based and resting state fMRI, the information obtained from these two methods differs. Resting state fMRI (rs-fMRI) measures spontaneous, low frequency fluctuations (<0.1Hz) of the BOLD signal.[123,124] The functional significance of these fluctuations was first discovered when subjects were asked to remain still and not perform any cognitive, language or motor tasks. [125] This technique identifies regions of the brain that demonstrate synchronous BOLD fluctuations at rest, known as resting state networks.[124] The first network to be observed, and the focus of a large body of research is the sensorimotor network, which encompasses the bilateral primary motor cortex, (M1) supplementary motor area (SMA) and premotor cortex (PMC). Fluctuations in resting BOLD signal between brain regions, which are temporally correlated, are thought to reflect functional connectivity of the underlying neurons. Another prominent network, the default mode network (DMN), was identified by Greicius *et al.* using fMRI and has since been the target of numerous studies. [126] Analysis of rs-fMRI data allows one to determine the relationship between two or more regions of the brain and identify spatially distinct resting state networks. Brain regions that show temporally correlated fluctuations in signal are considered to be functionally connected.[127] Studies have shown that resting state networks can be highly reliable both across imaging sessions and across different subjects.[128]

Conversely, task-based fMRI allows the detection of brain regions that are active due to the execution of a certain task (cognitive, verbal, motor, etc.). The experiment usually involves the execution of a behavioural task for a set amount of time, intermingled with periods of rest. The time series is then compared against a hypothesized model of neural function based on the task performed and the HDR. The functional map acquired identifies the difference between the functional activity at rest compared to the task.[129]

1.4.6 fMRI in CSM and SCI

fMRI is frequently used in clinical populations to identify differences in activation between two groups, or to monitor treatment/ intervention effects in a single cohort. Cortical reorganization, in response to axonal loss or neuronal injury has been shown to occur as a compensatory mechanism to preserve function.[34,35] fMRI is a valuable tool to track this reorganization in individuals with CSM and spinal cord compression.[34-36] Duggal *et al.* illustrated that reversible spinal cord compression leads to an increased activated volume within M1 in comparison to controls.[34] Surgical decompression results in cortical reorganization that is also accompanied by a significant return of clinical function.[34] Previous work from our lab has shown difference in activation patterns between mild and moderate CSM. Individuals with mild CSM demonstrated increased volume of activation of primary motor cortex compared to moderate; six months after surgery, activation patterns between the two groups were comparable.[130] In addition, Dong *et al*. demonstrated altered sensorimotor recruitment patterns during wrist and finger movements in 8 CSM patients; following spinal decompression, these patients regained motor and sensory function and demonstrated cortical maps similar to that of healthy controls.[131] The exact mechanism by which cortical reorganization occurs is not completely understood. There may be one or more processes that lead to the plasticity observed. Two possible explanations have been widely accepted. First is the modulation of preexisting connections, or the sprouting of new axonal connections.[35,132-134] For example, the dense network of horizontal connections within the primary motor cortex are not homogeneously distributed within M1. Plasticity of the motor system after injury may involve the modulation of these horizontal connections. Collateral sprouting from undamaged neurons may preserve function by taking on the role of injured neuronal pools.[135] The second proposed mechanism that leads to plasticity after spinal cord injury is a disruption in the inhibitory—excitatory balance, which produces a decrease in synaptic inhibition. The disruption of inhibitory influence has been shown to facilitate cortical reorganization through axonal sprouting.[135]

Although the majority of research has focused on the reorganization of the primary sensorimotor cortex, adjacent, non-primary motor areas should also be considered. The SMA, in particular, has enhanced activation following cortical injury to assist in maintenance and recovery of function.[16,50,136-139] This has been extensively observed in the stroke literature. Specifically, Calautti *et al*. performed a review of motor recovery after stroke and observed that recovery of motor control evolved from the unmasking of previously silent synapses, as well as an enhanced input of SMA and premotor cortex.[138] Furthermore, Holly *et al.* demonstrated an expansion of the cortical representation of the affected extremity, in CSM patients, to include neighboring motor areas such as the SMA.[36] Together, this may suggest a role for functional brain imaging as a potential biomarker for recovery in patients with spinal cord compression, and that the non-primary motor areas should be examined as an important contributor to functional recovery

1.5 Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) is a specialized technique associated with MRI that is used to study average metabolite tissue concentration changes in the brain.[140,141] This is in contrast to micro dialysis, which is used to commonly measure synaptic metabolite levels.[142] An MR spectrum can be obtained using any nucleus with a magnetic moment such as phosphorus (^{31}P), fluorine (^{19}F), carbon (^{13}C), sodium (^{23}Na) and hydrogen (^{1}H). The most commonly used nucleus is the proton (^{1}H) due to its high sensitivity and abundance in the body. Only those molecules that are freely mobile and present in relatively high concentrations (> 1 mM) can be observed by this technique.[141] MRS is a valuable tool to non-invasively study the pathologies of a variety of neurological disorders such as stroke, tumors, and spinal cord injury.[140,143,144] The MRS spectrum contains a number of peaks at different frequencies representing protons from

different metabolites. The concentration of the metabolite can be related to the signal intensity of its spectrum.[140,141,143]

1.5.1 Chemical Shift and J-Coupling

The chemical shift of the ^{1}H nucleus within a molecule determines the position of the peak in the spectrum. As described earlier, when placed in a magnetic field, the hydrogen nucleus precesses at a specific frequency determined by the Larmor equation.[141] However, the electron cloud surrounding the hydrogen nuclei causes small modulations of the main magnetic field, reducing the magnetic field experienced by the hydrogen nucleus.[141] The greater the density of the electron cloud, the more the proton is shielded from the main magnetic field, further reducing the associated Larmor frequency[146]. This results in slightly different resonant frequencies for protons in different molecules and even for protons in the same molecule but at different positions. These frequency shifts are known as the chemical shift and are expressed in parts per million (ppm).[141] The chemical shift is dependent on the strength of the magnetic field; therefore, an advantage of using high magnetic field strength is the distance between peaks in a spectrum (chemical shift dispersion) increases and allows for more accurate quantification of metabolite concentration.[147]

Another consequence of the chemical structure of a molecule is known as J coupling. J coupling results from the indirect interaction of two nuclear spins within the same molecule through their chemical bonds. J-coupling strength is measured in Hertz (Hz) and is independent of the main magnetic field strength (B_0)[146]. The result of these spin interactions is the splitting of single peaks into doubles, triplets or multiplets.[148] If a hydrogen nucleus is coupled to more than one other nucleus then triplets, quartets, and more complicated multiplets can be formed. The most prominent example of spectral splitting is that of the lactate peak, which presents as a doublet at 1.3 ppm[146].

1.5.2 Data Acquisition

The MRS spectra are usually acquired from a small, localized region of interest in the brain called a voxel. Once the voxel is placed over the area of interest, saturation bands are placed around the boundaries of the voxel to reduce unwanted signal from outside the volume of interest. Localization of the signal is achieved by exciting three orthogonal slices, at which the intersection is the volume of interest. Although increasing field strength comes with many advantages, one of the main disadvantages is the increased prevalence of non-uniform RF excitation fields[146]. Since excitation field uniformity is extremely important in MRS, the work described in this thesis used a semi localized by adiabatic selective refocusing (semi-LASER) pulse sequence. The semi-LASER sequence consists of a non-adiabatic 90° slice-selective pulse and two pairs of adiabatic hyperbolic secant pulses for refocusing.[149,150] The pairs of adiabatic pulses produce excellent slice profiles even under conditions of non-uniform RF excitation.

Approximately 80% of the brain is composed of water. As such, the water signal is much greater than that of other metabolites. Typically the water signal is suppressed in MRS acquisitions to increase the visibility of the smaller metabolite signals. There are a variety of water suppression techniques; however, the current work used the VAPOR water suppression method, which uses 8 narrow bandwidth presaturation pulses followed by signal crusher gradients to reduce the water signal.

1.5.3 The ^{1}H Spectrum

The in-vivo ^{1}H spectrum contains several distinctive frequencies that correspond to multiple metabolites. The spectrum is obtained by Fourier transformation of the acquired time domain signal (Figure 1.11). The frequency (chemical shift) of the metabolites is plotted along the x-axis and referenced to a standard, which is commonly sodium 3-trimethylsilyl-propionic acid (TSP) set to 0 ppm[146]. The most prominent peak is centred

at 2.02 ppm from *N*-aceytlaspartate (NAA). The area under each peak is proportional to the metabolite concentration.[141]

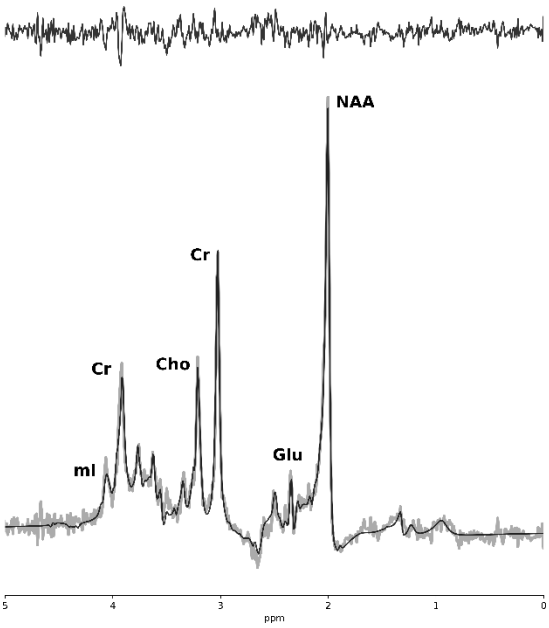

Figure 1.11 A typical semi-LASER spectrum (TE=60 ms) acquired from 7T.
The x-axis represents the frequency or chemical shift measured in ppm and the amplitude of the peak is on the y-axis. Each peak represents a metabolite. The area under the peak is calculated to determine the concentration of the metabolite within the voxel.

1.5.4 Advantages of High-Field and Ultra High-Field MRS

There are many advantages of increasing the main magnetic field to high or ultra-high field strengths (3T, 7T respectively). Primarily, with increased magnetic field there is an increased signal to noise ratio (SNR). This allows for the detection of very minute changes in metabolite concentration in both healthy and neurologically injured populations. An increased SNR also allows for shorter acquisition times, which is important in clinical applications, or a decreased voxel size, which reduces partial volume effects. When moving from 3T to 7T, the spectral resolution is also increased. This

effectively stretches the spectrum along the chemical shift axis and allows for easier discrimination of individual peaks (reduced spectral overlap). These advantages combine to increase metabolite concentration measurement precision.[151,152] It is also important to note some of the disadvantages of higher field MRS, particularly an increase in the chemical shift artefact and an increase in magnetic field inhomogeneity. Chemical shift artefacts can be reduced by increasing the bandwidth of the RF pulses.[153,154]

1.5.5 Select Metabolites Involved in Neurotransmission

1.5.5.1 *N*-Acetylaspartate

N-Acetylaspartate (NAA) is one of the most abundant and highly concentrated metabolites in the brain, with an average concentration of 9.2 mmol/g. [140,155] Its spectrum is composed of a large single peak centred at 2.02 ppm on the chemical shift spectrum, and a multiplet in the region between 2.3-2.6 ppm. NAA is synthesized in neuronal mitochondria, then transported into neuronal cytoplasm along axons and broken down in oligodendrocytes; it is found in higher concentrations in cerebral grey matter than white matter.[140,156] MRS has been shown to be sensitive enough to detect changes in NAA levels in a number of neurological disorders and conditions. A number of these studies have observed a decrease in NAA concentration after injury or damage to the brain.[157-158] These decreases in NAA concentration were hypothesized to represent irreversible neuronal damage and loss; however, recent evidence suggests that these decreases may be transient and indicate reversible neuronal or mitochondrial dysfunction[145]. One important opposition to this hypothesis is Canavan disease, in which NAA concentration increases due to the lack of the NAA catabolic enzyme. [160] NAA is also a direct precursor for the synthesis of NAAG, a neuronal peptide that acts through presynaptic glutamate receptors to modulate the release of neurotransmitters.[161] In summary, MRS measurements of NAA in-vivo provide an invaluable tool for observing and quantifying neurological function and disease.

1.5.5.2 Creatine

The creatine signal of an MRS spectrum is composed of both creatine and phosphocreatine compounds, having resonances at both 3.03 ppm and 3.91 ppm. [140,145,162] Creatine's most featured role is that of cellular metabolism and bioenergetics. Although present throughout the brain, there are higher levels in grey matter (glial cells) than white matter (neurons).[162] In the brain, there is a large tissue energy demand, especially in areas of functional activity. Creatine is therefore an important energy source for maintaining membrane potential, ion gradients, neurotransmission and intracellular behavior.[145,163] Recently, it has been suggested that creatine can act as a co-transmitter or neuromodulator in the CNS, and is therefore important to consider when discussing neurotransmission.[163] Creatine is released from the neuron in a similar manner as neurotransmitters, that is, in an action potential dependent manner. Once released, creatine modulates the activity of post synaptic receptors such as GABA.[145] A recent study by Rango *et al.* observed a transient decrease in PCr levels after functional activation, indicating the usage of creatine stores.[163] This has important implications for spectroscopy analysis. Creatine is often used as an internal reference for analysis of metabolite ratios; however, caution must be taken when doing so to ensure that creatine is stable.[140,162,165] Calculating a metabolites concentration in terms of ratios have many advantages. Tissue partial volume and tissue relaxation time constants are not taken into consideration, therefore errors in representing these are discounted.[148] In addition, ratios can provide a more sensitive measure in detecting changes when the numerator and denominator are acting in opposition (one increases and the other decreases).

1.5.5.3 Glutamate and gamma-Aminobutryic acid (GABA)

Glutamate is the most abundant excitatory neurotransmitter in the human brain and thus very important to consider when discussing neurotransmission. Although glutamate is present in the brain in relatively high concentrations (12 mmol), it is often a difficult metabolite to quantify because of its overlapping resonances with glutamine, GABA and NAA.[140,145] Glu and Gln have overlapping peaks centered at 2.05 and 2.50 ppm, respectively. Because of their similar chemical structure and metabolic role, glutamate and glutamine are often analyzed and discussed as a single entity (Glx).[140,166] Using ultra high field MRS (7T), the overlap between the glu and gln decreases and quantification of each separate metabolite is possible. Glutamine, stored in glial cells, is the precursor to both glutamate and GABA. Glutamine is released from astrocytes and converted to glutamate in neurons by a neuron-specific enzyme, phosphate-activated glutaminase.[145] Once released from the neuron, glutamate binds to post-synaptic receptors to induce activation; it is then subsequently removed by adjoining cells and converted back into glutamine in the astrocyte.[145] Glutamate has a high intracellular concentration and low extracellular concentration.

In contrast, GABA is the major inhibitory neurotransmitter in the brain, but present in much smaller concentrations (1 mM intracellularly and 2 μM extracellularly). The MR spectrum of GABA consists of three sets of multiplets, which correspond to the three methylene (CH_2) groups in the molecule.[144] The spectral splitting of these resonances results in a lower peak intensity and wider footprint along the chemical shift axis. Because of this, it is dominated by the NAA, Cr, Glu and Gln peaks, making quantification difficult.[145] Similar to the dispersion of glutamate and glutamine, increasing the magnetic field strength will allow for an increased spread of spectral resonances and therefore less overlap between GABA and the surrounding metabolites. The GABAergic system is involved in a variety of physiological processes in the CNS such as pain, sleep, motor control and anxiety and it is therefore a very important aspect when discussing neurotransmission.[145]

1.6 tDCS and fMRI

With the use of fMRI, we are able to observe both the online and offline functional changes that occur due to the application of tDCS. This allows a deeper level of knowledge into the mechanism behind the behavioural changes that have been observed and which brain areas are functionally affected by the stimulation. These effects can be observed either at rest, with resting state fMRI, or in combination with a task.

In combination with a serial reaction time task, Stagg *et al.* observed an increase in motor-related activity in the stimulated M1 after 1 mA of cathodal stimulation.[43] Furthermore, cathodal stimulation caused an increase in the activation of M1 and premotor cortex in the hemisphere contralateral to stimulation. On the contrary, anodal stimulation increased the activation in M1, SMA and premotor cortex on the stimulated hemisphere.[43] The use of fMRI allows for the visualization of the effects on cortical excitation and connectivity between the two tDCS montages (uni vs bihemispheric). By modelling current distribution we are able to visualize the flow of current and local hot spots; [55,122] however, with fMRI, a direct relationship between current and local and distant cortical excitation can be inferred. Lindenberg performed a study comparing the effects of bihemispheric M1 to the conventional unhemispheric approach in both task based and resting state fMRI study. [167] Stronger task-related activity was observed in bilateral M1 during bihemispheric compared with unihemipsheric tDCS. This observation was repeated by Waters *et al.* They observed a greater activation in bilateral S1 and M1 after bihemispheric tDCS. [73] Interestingly, they found a similar increase in motor learning and BOLD activation regardless of whether the anode was on the left M1 and cathode on right M1 or vice versa. This not only points towards an enhancement of motor performance and cortical activity regardless of electrode polarity, but also an active role of the ipsilateral cortex in motor control.[73] Similarly, Sehm *et al.* observed a difference in cortical activity through resting state fMRI between unihemipsheric and bihemispheric tDCS of M1.[168] Using 1 mA of current for 20 minutes, they observed specific spatial and temporal activity that differed between the two montages. During

stimulation, only bihemispheric tDCS increased activity of M1 (under the anode) and secondary motor areas such as premotor cortex and SMA. In contrast, unihemipheric tDCS increased functional activity of the cerebellum, both during and after the stimulation period.[168]

Generally, anodal tDCS to M1 has led to increase functional connectivity not only within M1, but also between M1 and motor network regions such as thalamus and SMA.[62,169] Furthermore, cathodal tDCS to M1 has also shown modulatory effects of functional connectivity. Amadi *et al.* observed increased interhemispheric connectivity between bilateral M1 hand regions and bilateral SMA following cathodal tDCS. In addition, he observed an increase in the overall strength of the default mode network following cathodal tDCS.[60]

An important area that has shown strengthened connectivity with M1 and/or increased cortical activity throughout a large majority of the literature is the SMA. Previous work has shown that the SMA is an important mediator of M1 activity.[170] Amadi *et al.* demonstrated that 1 mA of cathodal tDCS to M1 enhanced the activity of bilateral SMA.[60] In the first concurrent tDCS, rs-fMRI study, Kwon *et al.* observed increased cortical activity in left M1 and left SMA following anodal tDCS to left M1.[171] tDCS over M1 has shown whole brain effects that extend further than the target area[62]. This promotes the strengthening of not only task related synaptic connections, allowing for enhanced motor performance, but also a strengthening in the functional connectivity in resting state networks, most importantly the coupling between SMA and M1.[13]

1.7 tDCS and MRS

An alternate modality to observe the physiological mechanism of induced plasticity by tDCS is through MRS. With MRS the metabolic pathways involved in tDCS modulation can be quantified during and after the stimulation period. The most commonly measured

metabolites are GABA and Glutamate (or Glx, a combination of glutamate and glutamine), as they have been shown to play a role in human motor learning.[172,173] With the simplistic description of anodal stimulation being excitatory and cathodal being inhibitory, it has been extrapolated that anodal stimulation should decrease while cathodal stimulation will increase GABA concentration.[57,174,175] However, recent studies have shown conflicting results. Stagg *et al.* observed a significant decrease in GABA concentration in M1 following 1 mA of anodal stimulation; however, GABA concentration also decreased with cathodal stimulation. Furthermore, cathodal stimulation also decreased glutamate concentration; a strong correlation between the decrease in Glutamate and GABA has been observed.[44] Alterations of glutamate by tDCS can also be observed in other brain regions. Clark *et al.* observed a significant increase in Glx concentration of the right parietal cortex after 2 mA of anodal tDCS. In addition, he also reported a significant increase in tNAA under the stimulating electrode.[176] Finally, reduced GABA concentration has been observed after 1.5 mA of anodal tDCS to M1 at 7T; however, in this study, no other metabolite changes were observed.[176] The mechanism linking the metabolic changes observed across studies become challenging, as different current intensities and durations are used. However there are some commonalities in explaining the changes observed. Excitatory stimulation (anodal) increases neuronal firing rate, resulting in increased neuronal transmission and a subsequent increase in the rate of glutamate synthesis. Enhanced cortical activity following anodal stimulation may be largely mediated by a reduction in GABAergic inhibition.[174,178] Similarly, the inhibitory effects of cathodal tDCS may be largely mediated by a reduction in glutamate synthesis and neurotransmission.[44]

To elucidate the physiological mechanism further, previous studies have combined imaging modalities and motor performance in attempt to make correlations and draw further mechanistic conclusions. Bachtier demonstrated that at rest, before application of tDCS, GABA concentration was correlated with the strength of the motor network. However, although a reduction in GABA concentration and increased functional connectivity was observed with anodal tDCS to M1 following stimulation, these changes were not correlated.[174] This provides novel evidence that the mechanism of these two

process may not be as interconnected as once thought.[174] In relation to motor learning, tDCS induced reductions in GABA concentration are highly correlated with both motor learning and motor memory and fMRI BOLD activity.[177,178] Kim *et al.* showed that individuals who demonstrated large reductions of GABA concentration after anodal tDCS performed better on a force adaption task and demonstrated improved motor memory.[177] Together, this supports the notion that functional plasticity measured with fMRI reflects GABAergic modulation. A reduction in GABA in M1 has also been observed after motor learning in the absence of tDCS.[179] Although previous studies have shown anodal tDCS can alter cortical activity and GABA concentration, it is perhaps driven by separate underlying mechanisms in the resting brain. In the absence of task related activity, tDCS provides sub-threshold modulation that may bring a neuron closer to firing, thereby increasing the firing rate. Although GABA is found in high concentrations in presynaptic vesicles, it also accumulates extracellularly to produce GABAergic tone, or sustained inhibitory response.[180] Evidence from animal studies has shown the MRS GABA signal is primarily from extra synaptic GABA rather than presynaptic GABA, [181] as the latter may be less visible to MRS as it is bound by macromolecules. Additionally, animal studies have demonstrated that a reduction in GABAergic tone is essential for LTP-like plasticity within the motor cortex[181]. Since tDCS acts to enhance motor learning and memory through an LTP-like manner, and this process does not occur in the resting brain, it is only through the combination of tDCS and a motor task that the correlation in BOLD activity and GABA concentration share a similar mechanism.

1.8 Clinical Implications

The translation of tDCS to clinical applications has been endless. Due to its low cost, enhanced portability and user-friendly nature, tDCS has been used primarily to augment traditional treatments, whether that be behavioural or medical. Furthermore, tDCS has little to no primary side effects. The use of this tool has been used to improve impulse control in Alzheimer's disease, enhance memory in Parkinson's disease, reduce negative

thoughts in psychological disorders such as Schizophrenia, and elevate mood in depression.[182-185] Recently, tDCS has been used in conjunction with traditional rehabilitation strategies to enhance motor performance after stroke and spinal cord injury. This section will focus on clinical applications of tDCS as it relates to motor performance.

Both electrode montages have been shown to enhance motor performance after stroke.[167,186,187] Although the majority of clinical applications in spinal cord injury involving tDCS have targeted central and neuropathic pain, few have examined the beneficial effects on motor rehabilitation. Combining tDCS with both upper and lower motor training has shown beneficial effects. Raithatha *et al.* observed improvement in lower extremity motor function when tDCS was combined with multiple sessions of robot assisted gait training, [188] while Cortes *et al.* demonstrated improvement in hand motor function after a single training session with tDCS.[42] Finally, Potter-Baker *et al.* conducted a pilot study combining tDCS and motor rehabilitation for 2 hrs, 5 times a week for two weeks and assessed functional outcomes immediately and three months following the intervention.[189] Combined tDCS rehabilitation increased strength in proximal and hand muscles immediately following intervention, and these gains persisted three months following intervention.[189] These positive results indicate the need for further investigation into the appropriate dosage of tDCS combined rehabilitation and implementing it into standard clinical practice.

1.9 Limitations of tDCS

The limitations surrounding tDCS result from a lack of a standardized approach to motor or cognitive enhancement, which largely stems from the fact that the exact mechanism of action has yet to be elucidated. There is currently no method to measure the dose of tDCS. With the dosage dependent on so many variables, it is difficult to draw parallels between studies. Factors that influence tDCS dosage and effects are current intensity, electrode size, current duration, and total number of sessions.[93] Recent literature has

observed current density to be a good predictor of tDCS efficacy; however, this is dependent on current intensity and electrode size. Small changes in either of the two parameters alters current density, as well as current flow and distribution.[55,69,76,122]

A second limitation is the applicability of positive results from healthy individuals to a clinical population. When current is injected into an unhealthy or damaged brain, does this alter current distribution, flow and overall efficacy? For example, working memory has shown enhancement with 1 mA of tDCS in healthy individuals; [190] however, the same result was observed only after 2 mA of tDCS in individuals with Parksinon's disease.[182] Similar challenges have been observed in older populations. When tDCS was applied over the DLFPC, young adults demonstrated a decrease in risk behavior, [190] while older adults showed the opposite effect.[183] These studies highlight the challenges associated with translating tDCS to a clinical population.

Finally, there is a large inter-individual variability in both the effects of MEP and behavioural response to tDCS, [76] leading to difficulty identifying significant behavioural changes associated with tDCS, further increasing the variability in the literature. Determining why there is such a large variability between individuals would help to stratify results and potentially lead to greater interpretability of tDCS research.

1.10 Goals and Hypothesis

CSM is a highly prevalent and devastating disorder where the spinal cord is compressed, disrupting motor and sensory function. Although a great deal of research has been conducted to characterize this disorder and identify biomarkers for surgical and functional success, a rehabilitation strategy for both surgical and non-surgical patients has been largely ignored.

The overarching goal of this research is to develop a rehabilitation strategy involving the use of tDCS to enhance motor function, particularly hand function, in patients with CSM. Very few studies have examined the combined effects of tDCS and spinal cord injury rehabilitation. As a prerequisite to successfully applying tDCS in CSM to promote hand function, it is necessary to better understand the role of tDCS in enhancing hand function, and to examine the short term effects of tDCS on the brain. Therefore the overall goals of this thesis were to identify motor network reorganization that is correlated with recovery of function in CSM patients, and then design a tDCS paradigm that specifically enhances these cortical regions to promote enhanced recovery of hand function. Due to the novel electrode montage chosen in the current thesis, several studies on healthy individuals were done to better understand the mechanism of tDCS.

Chapter 1 one of this thesis provides an extensive literature review of cervical myelopathy, available treatment strategies and pitfalls. Furthermore, an introduction to tDCS, the controversies behind its mechanism and how its application can modulate cortical activity and human behavior is discussed.

Chapter 2 of this thesis describes the cortical reorganization that occurs in patients with CSM before and after surgical decompression of the spinal cord. Functional MRI was used to compare brain activity during a hand motor task in patients with CSM and healthy controls. Currently, the gold standard of treatment of CSM is surgical decompression of the spine; however, only approximately 35% of individuals will see neurological improvements following surgery. We hypothesize that by longitudinally examining the functional reorganization of the brain following surgery we will find direct evidence of cortical recruitment and reorganization in response to injury.

Chapter 3 describes the use of bihemispheric tDCS to enhance motor performance and consolidation in healthy older adults. A single and tri session protocol was used to determine if bihemispheric tDCS to bilateral motor areas will enhance motor dexterity.

We hypothesize that tDCS will enhance motor dexterity, preferentially using a tri session protocol compared to sham and single session due to enhanced cortical spinal activity.

Chapter 4 of this thesis sought to determine whether bihemispheric tDCS produced acute metabolite changes in the primary motor cortex in healthy individuals measured using 7 Tesla MR spectroscopy. We hypothesize that tDCS can alter metabolite concentration, specifically that of glutamate and creatine when measured immediately following 20 minutes of tDCS.

Finally, chapter 5 of this thesis extends previous literature, to determine whether bihemispheric tDCS can induce cortical modulations in resting state functional connectivity of the motor network measured using 7 Tesla resting-state fMRI. We hypothesize that tDCS will enhance the functional connectivity of the motor network both during and following tDCS.

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Chapter 2

2 Motor Network Recovery in Patients with Chronic Spinal Cord Compression: a Longitudinal Study Following Decompression Surgery

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2.1 Abstract:

We have used functional magnetic resonance imaging (fMRI) to assess cortical reorganization of the motor network after chronic spinal cord compression and characterize the plasticity that occurs post surgical intervention. A 3T MRI was used to acquire functional images of the brain in 22 patients with reversible cervical spinal cord compression and 10 controls. Controls performed a finger-tapping task on three different occasions, baseline, six weeks and six months apart, while patients performed the identical task before surgery and again six weeks and six months following spinal decompression surgery. After surgical intervention, an increased % BOLD signal and volume of activation was observed within the contralateral and ipsilateral motor network. The volume of activation of contralateral primary motor cortex was associated with functional measures both at baseline ($r = 0.55$, $p < 0.01$), and six months following surgery ($r = 0.55$, $p < 0.01$). The % BOLD signal of ipsilateral supplementary motor area six months following surgery was associated with increased function six months after surgery ($r = 0.48$, $p < 0.01$). Plasticity of the contralateral and ipsilateral motor network play complementary roles in maintaining neurological function in patients with spinal cord compression and may be critical in the recovery phase following surgery.

2.2 Key Words

cervical spondylotic myelopathy; decompression surgery; motor; rehabilitation; recovery

2.3 Introduction

Following brain or spinal cord injury, it is postulated that cortical reorganization provides a compensatory mechanism to minimize functional deficits. Cortical reorganization has previously been shown in healthy individuals.[1,2]and patients with neurological disorders[3-6] in response to finger movement tasks. Degeneration of the cervical spine, which may cause slow, progressive spinal cord compression, represents a unique model of spinal cord injury that may be reversible following decompression surgery,[7] The overwhelming majority of studies on cervical spondylotic myelopathy (CSM) have focused on local changes at the site of spinal cord compression and have neglected the intimate interconnection with the brain.[8,9]

We have previously shown altered brain function and metabolism in patients with cervical spondylosis.[4,10-13] These studies, and others, have demonstrated cortical reorganization in primary motor regions contralateral to the side of motor movements in CSM.[4,7,14] The presence and potential contributions of cortical changes ipsilateral to the side of motor movements have not been previously examined in relation to spinal cord compression, even though such contributions are well documented in stroke victims.[5,6,15-17] Specifically, the ipsilateral premotor cortex (PMC) and supplementary motor area (SMA) are known to be involved in the compensation of the loss of motor function caused by contralateral brain injury.[18,19] Extensive stroke literature has demonstrated functional reorganization of ipsilateral motor areas in response to cortical damage, supporting the idea that both hemispheres are essential for the normal integration of function and motor recovery.[6,18,20] Studying the involvement of the ipsilateral hemisphere in motor recovery in patients with CSM will allow us to further evaluate a potential imaging biomarker of motor recovery following surgery. In addition, determining the integration of both hemispheres will further our understanding of the mechanism of cortical reorganization following injury. The present study compared preoperative and postoperative brain activation during a standardized motor task, to assess both contralateral and ipsilateral motor activation. Our specific goal was to examine the

relationship between plasticity of the motor network and neurological recovery following spinal cord decompression. Neurological function, measured by validated clinical outcome scores, were also evaluated to determine whether functional magnetic resonance imaging (fMRI) could be used as an indicator of clinical outcome and recovery.

2.4 Methods

2.4.1 Participants and Study Design

Twenty-two patients (19 males, mean age 50 ± 10.9, 22 right–handed) with a clinical history of CSM of less than or equal to one year in duration and no other neurological disorders were recruited and participated in three 3.0 Tesla MR imaging sessions. Surgery for the treatment of symptomatic CSM was considered when there was a history of a progressive decline in neurological function, concordant imaging with evidence of spinal cord compression, and limited or no response to appropriate conservative measures. All CSM patients showed evidence of signal cord change in preoperative MRI. CSM patients underwent a baseline research MRI scan, surgical decompression, followed by a second research MRI scan six weeks postsurgery, and a third scan six months post–surgery. These two time points were chosen to capture important milestones in the recovery process[21] and to coincide with the time of patient clinic visits. CSM inherently affects men more commonly than women.[22] Therefore, the control group was sex matched with the patient group. Ten age-matched control subjects (48 ± 9.9 years, 7 males, 10 right–handed) with no previous history of neurological disease were also recruited. Control subjects had three MRI sessions (baseline, six weeks and six months) without surgical intervention. Informed written consent was obtained for all procedures according to the Declaration of Helinski (World Medical Association, 2008) and the study was approved by the Western University Health Sciences Research Ethics Board. All patients completed validated instruments for assessing disability resulting from myelopathy (modified Japanese Orthopedic Association (mJOA) outcome measure). This

metric was used as an indirect measure of the success of the surgery. Postoperative MRI is not acquired at our institution unless there was neurological deterioration. Controls did not participate in the assessment of neurological function, as these measures are not designed to measure variations in normal function. Given our inclusion criteria for controls, no functional impairment was expected, as premised from previous work by our group.[13] Throughout the current study, "ipsilateral" refers to brain activation on the same side as task movement, and "contralateral" indicates activation in the brain that is on the opposite side of task movement.

2.4.2 Procedures

A 3.0T Siemens Magnetom Tim Trio magnetic resonance imaging (MRI) scanner, with a 32-channel head coil including a neck and spine array was used to acquire all data. Each exam included the acquisition of sagittal T_1-weighted 3D–magnetization prepared rapid gradient echo anatomical images (192 slices, 1 mm isotropic resolution, repetition time/echo time = 2300/3.42 ms). During the functional task, blood-oxygen level dependent (BOLD) images were acquired continuously using an interleaved echo planar imaging pulse sequence (parallel imaging factor = 2, 80 × 80 acquisition matrix, 45 slices/volume, 3 mm isotropic resolution, repetition time/echo time = 2500/ 55 ms, flip angle = 90°). The total acquisition time was 5 minutes and 30 seconds for 132 volumes.

The motor pathway was activated using a block paradigm functional task, with 11 segments (6 rest and 5 active). Participants were instructed to perform finger to thumb opposition ('duck-quack') using a button box with the right hand. Participants were instructed to press the buttons simultaneously with all four fingers, followed by an extension upwards until the hand touched the roof of the box. To control the frequency at which participants performed the button tapping, visual cues instructed the participant to tap every 3 seconds during the 30 second active interval, followed by a 30 second rest period. All subjects were monitored during the fMRI scan to ensure that they could perform the "duck quack" motion in the scanner.

Anatomical and functional images were processed using Brain Voyager QX 2.1 (Brain Innovation, Maastricht, the Netherlands). The T_1-weighted images were coregistered to the functional time series and transformed to Talairach space. Processing of functional images included 3D motion correction using sinc interpolation, spatial smoothing, and temporal linear trend removal. Spatial smoothing was performed by convolving each slice with a 6 mm full-width-half-maximum Gaussian kernel.[23] A volume time course was created from anatomical and functional images and single participant analysis was completed using a general linear model. Separate predictors were created for each subject by convolving the block paradigm boxcar function with a double-Gamma hemodynamic response function. Group analysis was performed using a hypothesis-driven Random Effects Analysis of Variance. Group activation maps were created at each time point (baseline, six weeks and six months) and were adjusted for multiple comparisons using a Bonferonni correction.[24] These maps identify the average activation patterns of the group. For the purpose of this study, we report results from brain regions relevant to the stated hypothesis: the contralateral sensorimotor cortex (M1/S1), the contralateral supplementary motor area (SMA), the ipsilateral SMA and the ipsilateral premotor cortex (PMC). Significant regions of activation were quantified using beta weights and volume of activation (VOA). For all functional images, Talairach coordinates of the center of gravity of the VOA were recorded and converted into brain regions by The Talairach Daemon Client 2.0 (University of Texas Health Science Centre, San Antonio, TX). Beta weights associated with the tapping task across groups were determined by defining regions of interest from group activation maps created by an Analysis of Variance Random Effects model. Beta weights represent how much of the BOLD signal is attributed to the task and will be noted as the % BOLD signal throughout the text.[25]

2.5 Statistical Analysis

The modified Japanese Orthopedic Association scale (mJOA) was used to measure relevant functional improvement in each patient and was administered presurgery, six weeks and six months postsurgery. A One way ANOVA was used to determine if there

were functional changes between the three time points with significance set at $p < 0.05$. Paired t tests were used to determine individual activation differences in patients and controls between the three time points and unpaired t-tests were used to determine significance between the two groups (patients and controls) with significance set at $p < 0.05$. The Pearson Product Moment Correlation Coefficient was used to determine whether there were any significant correlations between clinical scores and the % BOLD signal change or VOA for patients at baseline, six weeks postsurgery, and six months postsurgery. Throughout the text, group means are provided, followed by the standard deviation (SD). All statistical tests evaluating group differences had a high level of power ($\alpha = 0.80$). The only exception was the comparison of the ipsilateral SMA between baseline and six months postsurgery in patients, which has a power of 0.72.

2.6 Results

Patient demographic and clinical information are presented in Table 2.1. All patients and controls were right handed. There were no significant differences between patients and controls with respect to age. There was a significant difference in mJOA scores between baseline and six month follow up indicating that neurological function improved in CSM patients following surgery (Table 2.1)

We examined the VOA and % BOLD signal of the contralateral and ipsilateral motor network in patients and controls at baseline and again six weeks and six months later. Specifically, the activation of the motor cortex, sensory cortex, and associated motor areas (SMA and PMC) were quantified. The ipsilateral M1/S1 did not show statistically significant activation. Relevant brain regions showing significant differences between time points are presented below; all other regions displayed no change.

Table 2.1 CSM Demographics

	Age	Gender	Site of impairment	mJOA (maxiumum 18)		
				Baseline	6WK	6MO
1	38	M	C5/C6	11	14	13
2	36	M	C5/C6	14	16	16
3	64	M	C5/C6	15	12	16
4	37	M	C5/C6	15	18	18
5	42	M	C3/C4	11	16	16
6	57	M	C5/C6	9	14	16
7	32	M	C5/C6	11	16	14
8	59	M	C5/C6	17	13	18
9	62	M	C4/C5	13	12	13
10	53	F	C5/C6	17	15	16
11	65	M	C3/C4	6	15	13
12	48	M	C5/C6	15	14	15
13	61	M	C5/C6	11	14	15
14	56	M	C3/C4	9	10	11
15	54	M	C5/C6	17	16	17
16	45	M	C5/C6	17	15	17
17	63	F	C3/C4	12	12	18
18	38	M	C3/C4	15	13	16
19	63	M	C4/C5	15	10	14
20	34	M	C6/C7	16	10	18
21	45	M	C5/C6	12	13	16
22	57	M	C4/C5	12	16	18
			Average	13 ± 3	14 ± 2	16 ± 2*

2.6.1 Longitudinal Changes in Controls

We observed longitudinal motor network changes in both the VOA and the % BOLD signal in control subjects. There was a significant decrease in the VOA at six weeks

compared with baseline in contralateral M1, PMC and SMA; additionally, there was reduced VOA at six months compared with baseline in contralateral M1/S1, PMC and SMA. ($p < 0.05$) (Table 2.2, Figure 2.1). The % BOLD signal (Table 2.3) was stable from baseline to six months in all areas except contralateral SMA (Table 2.1, Figure 2.1), which was reduced at the six month follow-up.

Changes in the ipsilateral motor network were also observed in controls. There was a significant decrease in the VOA of the ipsilateral SMA and PMC at six weeks compared with baseline, and a decrease in ipsilateral SMA at six months compared with baseline ($p < 0.05$) (Figure 2.1, 2.2, Table 2.2).

2.6.2 Longitudinal Changes in CSM Patients

Patients demonstrated unique longitudinal patterns of motor network change. The VOA of the contralateral and ipsilateral motor network remained constant over time (Figure 2.1b, 2.2b). However, there was an increase in the % BOLD signal of ipsilateral SMA six months after surgery compared with baseline (0.34 ± 0.27 and 0.41 ± 0.23 respectively, Table 2.3, consistent with the group average activation maps presented in Figure 2.1.

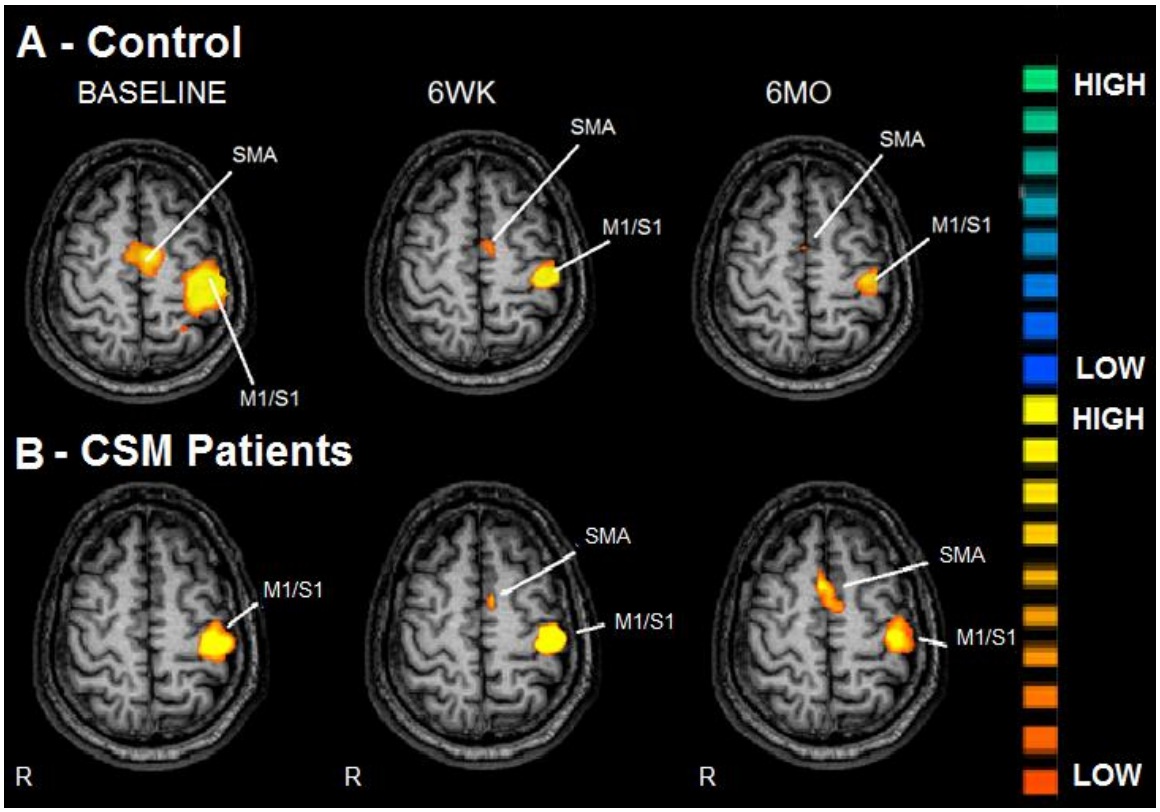

Figure 2.1 Longitudinal Cortical Activation

Functional MRI of group level activation maps in controls B) Functional MRI of group level activation maps of CSM patients at pre surgery, six weeks, and six months post decompression surgery.

Table 2.2 Volume of Activation

	Volume of Activation (voxels)		
Controls			
	PRE	**6WK**	**6MO**
Contralateral M1	1510 ± 202	970 ± 669*	1153 ± 457*
Contralateral S1	1496 ± 362	1072 ± 587	957 ± 610*
Contralateral PMC	947 ± 540	285± 400*	444± 442*
Contralateral SMA	1553± 168	703 ± 617*	846 ± 759*
Ipsilateral SMA	925 ± 551	39 ± 109*	189 ± 330*
Ipsilateral PMC	782 ± 589	140 ± 317*	290 ± 513
Patients			
Contralateral M1	1704 ±1398	1985 ± 1356†	1597 ± 1547
Contralateral S1	1121 ±2309†	1280 ± 1228	1046 ± 1416
Contralateral PMC	246 ± 554	288 ± 592	608 ± 1130
Contralateral SMA	1024 ±1225	1323 ± 920†	1117 ± 1359
Ipsilateral SMA	390 ± 636†	425 ± 743†	255 ± 504
Ipsilateral PMC	529 ± 1144	502 ± 735	576 ± 942

Abbreviations: SMA, supplementary motor area; M1, primary motor cortex; PMC, premotor cortex; S1, sensory cortex.

* Significantly different compared to baseline $p < 0.05$

† Significantly different compared to controls $p < 0.05$

Table 2.3 BOLD Activation

	% BOLD Activation		
Controls			
	PRE	**6WK**	**6MO**
Contralateral M1	0.65 ± 0.24	0.7 ± 0.29	0.58 ± 0.41
Contralateral S1	0.65 ± 0.37	0.52± 0.34	0.48 ± 0.37
Contralateral SMA	0.73 ± 0.43	0.63 ± 0.18	0.33 ± 0.31*
Contralateral PMC	0.57± 0.22	0.71 ± 0.25	0.60 ± 0.38
Ipsilateral SMA	0.37 ± 0.14	0.5 ± 0.24	0.45 ± 0.3
Ipsilateral PMC	0.22 ± 0.19	0.26 ± 0.24	0.15 ± 0.24
Patients			
Contralateral M1	0.5 ± 0.33	0.58 ±0.34	0.51 ± 0.43
Contralateral S1	0.54 ±0.37	0.48 ±0.24	0.45 ± 0.24
Contralateral SMA	0.43 ±0.33†	0.47 ±0.24†	0.45 ± 0.23
Contralateral PMC	0.41 ± 0.17	0.61 ± 0.38	0.33 ± 0.20
Ipsilateral SMA	0.34 ±0.27	0.37 ±0.30	0.41 ± 0.23*
Ipsilateral PMC	0.24 ±0.25	0.26 ±0.22	0.22 ± 0.19

Abbreviations: SMA, supplementary motor area; M1, primary motor cortex; PMC, premotor cortex; S1, sensory cortex

* Significantly different compared to baseline $p < 0.05$

† Significantly different compared to controls $p < 0.05$

2.6.3 Differences Between CSM and Controls

There were specific differences in cortical activation between patients with CSM and controls. On the contralateral side, patients exhibited a significantly smaller VOA in S1 at baseline compared with controls (Table 2.2, Figure 2.1). Six weeks following surgery, patients had a larger VOA in contralateral M1 and SMA compared with controls (Table 2.2, Figure 2.1). Patients also demonstrated a significantly lower % BOLD signal at baseline and six weeks postsurgery in contralateral SMA compared with controls (Table 2.3). When examining the ipsilateral motor network, the VOA of SMA was significantly smaller at baseline compared with controls (Table 2.2, Figure 2.1). However, six weeks following surgery, patients had a larger VOA of ipsilateral SMA compared with controls (Figure 2.1, Table 2.2).

When comparing the longitudinal changes in the VOA from baseline to six weeks, we found a difference between control subjects and patients in the contralateral PMC and SMA as well as the ipsilateral PMC (Figure 2.3a). When comparing the longitudinal changes in % BOLD signal from six weeks to six months we found a difference between control subjects and patients in contralateral SMA (Figure 2.3d).

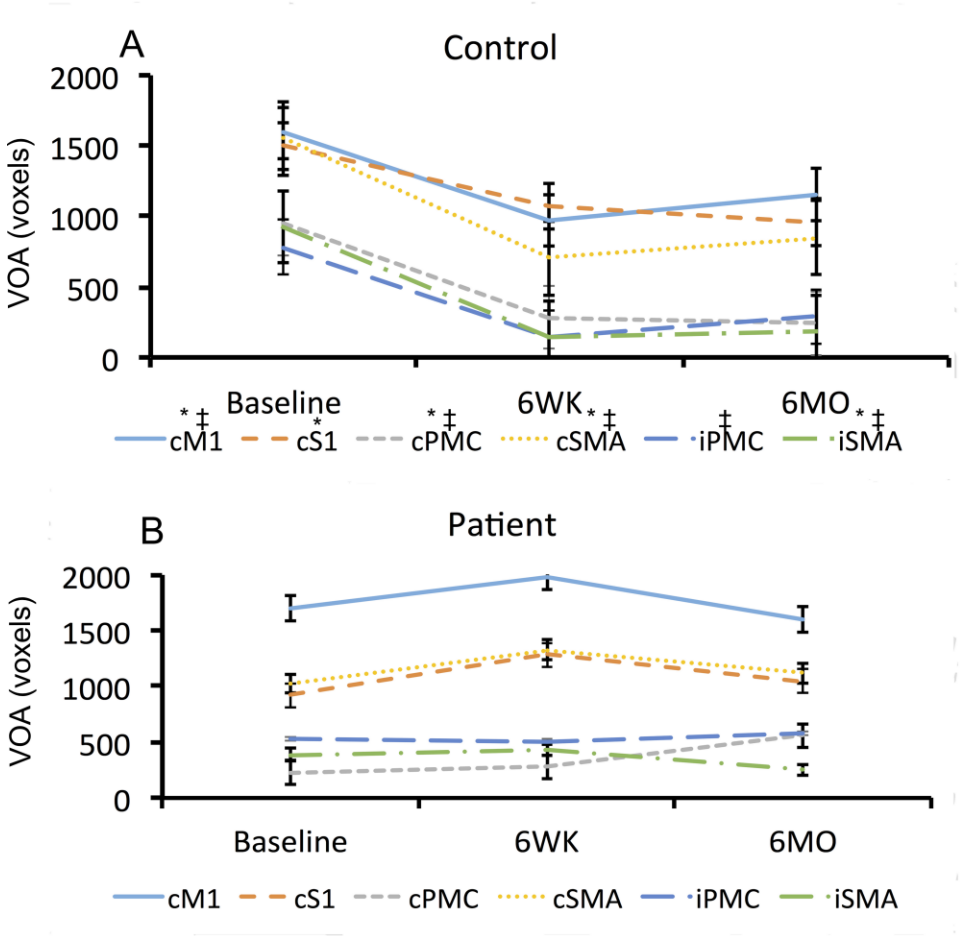

Figure 2.2 Longitudinal trends in functional cortical activation

Line graph displaying the trends in volume of activation (VOA) in controls at baseline, six weeks and six months B) Line graph displaying the trends in volume of activation in patients with spinal cord compression at baseline, six weeks and six months. *Asterisk* represents significant change from baseline to 6 month follow up. Double cross represents significant change from baseline to 6 week follow up.

2.6.4 Relationship Between Cortical Activation and Clinical Outcome

Analysis of the motor network and clinical scores (mJOA) demonstrated a significant relationship between cortical activation and function. In the contralateral M1 of patients, the VOA at baseline was significantly correlated with mJOA scores at baseline ($r = 0.55$, $p = 0.008$, Figure 2.4a) and six months following surgery ($r = 0.55$, $p = 0.008$, Figure 4b). Furthermore, a greater change (Δ) in % BOLD signal in contralateral M1 at six months following surgery was associated with higher baseline mJOA scores ($r = 0.45$, $p = 0.03$, Figure 2.4c). In the ipsilateral SMA of patients, the % BOLD signal was associated with greater mJOA scores (less impairment) at six months postsurgery ($r = 0.48$, $p = 0.03$, Figure 2.5a). Additionally, an increase in the % BOLD signal of ipsilateral SMA from baseline to six months after surgery was associated with greater mJOA scores at the six month follow-up ($r = 0.42$, $p = 0.04$, Figure 2.5b).

2.7 Discussion

This study, using a highly standardized functional task, was the first to examine and quantify longitudinal changes in the ipsilateral motor network in patients with reversible spinal cord compression, and relate these changes to functional recovery. In the current study, the motor paradigm used was not sufficiently difficult to activate the ipsilateral M1/S1. This result is consistent with previous studies that have shown contralateral M1/S1 activation during simple, visually cued motor tasks, in the absence of ipsilateral M1/S1 activation. Ipsilateral M1/S1 is activated with complex sequential finger tasks and bimanual motor tasks.[26] We observed significant changes in the ipsilateral motor network, specifically the SMA, six months after surgery, that were correlated to function, suggesting that ipsilateral reorganization and compensation may be important in functional improvements following surgery for spinal cord compression.

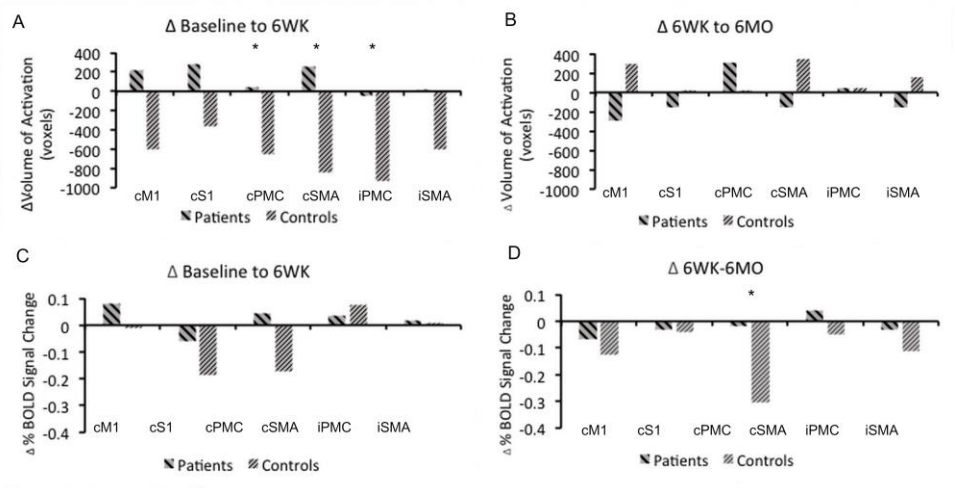

Figure 2.3 Comparison of activation between CSM and controls

Bar graph displaying the difference in the change in VOA between baseline and six weeks in controls and CSM patients over time. B) Bar graph displaying the difference in the change in VOA between six weeks and six months in controls and CSM patients. C) Bar graph displaying the difference in the change in % BOLD signal between baseline and six weeks in controls and CSM patients, D) Bar graph displaying the difference in the change in the % BOLD signal between controls and CSM patients between six weeks and six months

2.7.1 Cortical Activation as a Result of Motor Learning

In controls, there was a temporal decline in activation from baseline to six month follow up, which can be attributed to motor learning.[2,27,28] The learning of motor tasks modulates brain activity in the contralateral primary motor cortex.[28] Furthermore, as learning occurs, there is decreased activation because of the reduced requirement of neuronal resources to perform the motor task.[28] We observed a similar effect with reduced activation six months after the initial scan. The VOA of contralateral M1/S1, PMC, SMA, as well as the ipsilateral SMA were significantly decreased compared with baseline, indicating the

task had become familiar. In addition, the % BOLD signal was reduced in the ipsilateral SMA at six months compared with baseline.

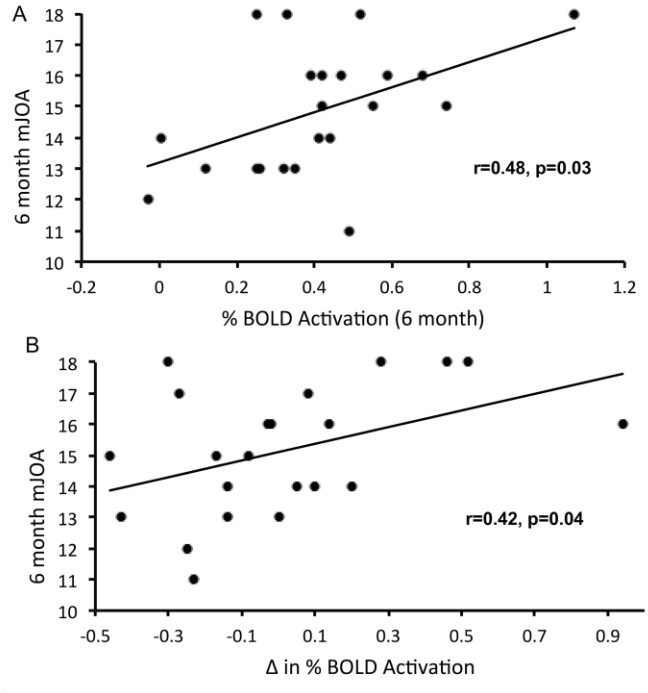

Figure 2.4 BOLD Activation and Clinical Scores

Line graph displaying the correlation between the % BOLD signal of ipsilateral SMA at six months postsurgery-and clinical outcome, measured by mJOA at six months postsurgery. B) Line graph displaying the correlation between the % BOLD signal change of ipsilateral SMA from baseline to six month postsurgery and mJOA at six months.

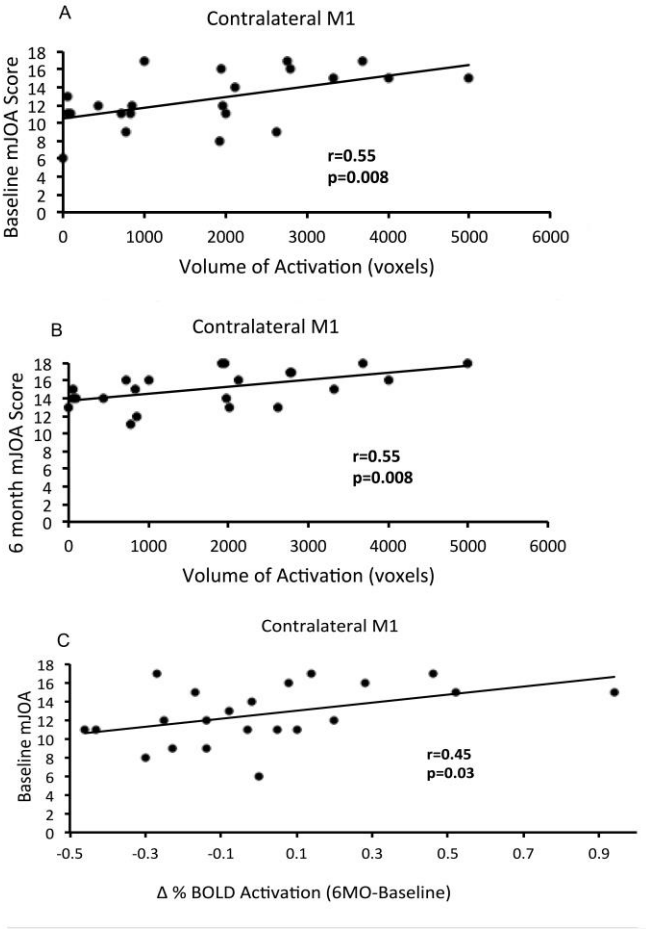

Figure 2.5 Cortical Activation Predicts Clinical Outcome

Line graph displaying the correlation between baseline (presurgery) VOA on contralateral M1 (measured by the number of activated voxels) in patients with CSM and baseline function measured by mJOA score. B) Line graph displaying the correlation between the the VOA of contralateral M1 at and mJOA at six months, C). Line graph displaying the correlation between the change in M1 BOLD activation from baseline to 6 months after surgery and baseline mJOA scores.

2.7.2 Activation Profiles in CSM Patients

Patients with CSM demonstrated a different profile of cortical activation than control subjects. When compared with controls, we found that spinal cord compression resulted in a reduced VOA of contralateral S1, PMC and ipsilateral SMA, and a reduced % BOLD signal of contralateral SMA. This reduction in activation may reflect the compression of the spinal cord with attenuating signal transduction to the cortical motor networks. Specifically, the proprioceptive sensory feedback required to modulate synaptic recruitment may be impaired, reducing the level of activation.[29]

When examining the longitudinal changes in activation, we observed a stable profile in the VOA from pre surgery to six months following surgery and a significant increase in the % BOLD signal of the ipsilateral SMA. Previous work has demonstrated that cerebral adaptations, in the form of reorganization of motor control, occur after brain and spinal cord injury to support recovery.[6,30] Freund and colleagues observed both cord and cortical atrophy following spinal cord injury. This focused atrophy of the somatosensory cortex may contribute to the cortical reorganization that occurs after decompression surgery in patients with CSM. In addition, Kriz and colleagues demonstrated that the primary motor cortex becomes partially inhibited following spinal cord injury.[29] Changes in the contralateral M1, secondary to spinal cord injury, may result in additional recruitment of both primary and secondary motor areas to assist in the cortical demand of the motor task.[15] Cortical atrophy following SCI may be a result of decreased cortical connectivity, reduced cellular activation or retrograde degeneration.[31] Our group has recently

suggested that a change in the metabolic profile of the motor cortex may trigger cortical reorganization.[10,12] Additionally, we have reported increases in the VOA within the contralateral M1/S1 following surgical intervention in CSM patients using a self-paced motor task.[4] The exact mechanisms involved in neural organization and recruitment after neurological injury is not completely understood. There may be one, or more processes that lead to the plasticity observed in the motor system. Two possible explanations have been widely accepted. First is the modulation of preexisting connections, or the sprouting of new axonal connections[32]. For example, the dense network of horizontal connections within the primary motor cortex are not homogeneously distributed within M1. Plasticity of the motor system after injury may involve the modulation of these horizontal connections.[3] Collateral sprouting from undamaged neurons may serve to preserve function by taking on the role of injured neuronal pools. The second proposed mechanism that leads to plasticity after spinal cord injury is a disruption in the inhibitory—excitatory balance, which produces a decrease in synaptic inhibition. The disruption of inhibitory influence has been shown to facilitate cortical reorganization through axonal sprouting.[32]

Following spinal cord decompression, the integrity of the spinal cord is potentially restored, and sensory feedback signals that provide updated motor plans are potentially improved. Although the source of spinal cord compression is removed, residual injury within the spinal cord and motor cortex may still exist,[10] requiring recruitment of additional areas of the brain to assist in motor performance. The current study highlights the importance of the ipsilateral motor network as it relates to neurological recovery. Six months following surgical decompression, we observed an increase in the % BOLD signal in ipsilateral SMA, which may represent the emergence of compensatory mechanisms during the recovery process. The SMA becomes preferentially activated during difficult tasks, learning, and in bimanual motor tasks, and has shown enhanced activation following cortical injury to assist in maintenance and recovery of function.[6,33] This has been extensively observed in the stroke literature. Specifically, Calautti et al. performed a review of motor recovery after stroke and observed that recovery of motor control evolved from the unmasking of previously silent synapses, as well as an enhanced input of SMA and PMC.[34] A similar process may be occurring with spinal cord

compression. For example, Holly et al. demonstrated an expansion of the cortical representation of the affected extremity to include neighboring motor areas such as the SMA.[14] These findings suggest there may be a role for functional brain imaging as a potential biomarker for recovery in patients with spinal cord compression, and that the ipsilateral motor network should be examined as an important contributor to functional recovery.

When comparing the longitudinal change in the VOA from baseline to six weeks in both controls and patients, there was a large reduction in the VOA of the motor network of controls. In contrast to controls, patients maintained or further increased their motor network activation. Although there was no difference in the change in the % BOLD signal from baseline to six weeks between controls and patients, a change emerged from six weeks to six months. When individuals have a disruption in the conduction between brain and muscle, as in cervical myelopathy, it may result in an altered perception of the magnitude of force applied to a certain task.[32] When an individual cannot sustain a certain level of force required to complete a task, additional motor units may be recruited to compensate. This has been shown in previous studies where CSM patients have an increased VOA of primary motor cortex as compared with controls, perhaps from increased neural recruitment.[4,14,35] Once the spinal cord is decompressed and signal transduction is improved, larger motor neurons are activated, allowing the volume of cortical activation to return to that of controls.[36] However, when continued cortical output is required, activated motor neurons may increase their firing rate, which may represent the increased strength in BOLD signal. We believe the increase we observe in the BOLD signal from ipsilateral SMA following surgery is the result of adaptive changes of the brain allowing for an increased synaptic efficiency and motor unit discharge rate.

2.7.3 Implications for Neural Recovery

Previous studies have demonstrated the functional significance of motor activation, demonstrating that increases in activation may be correlated with improved recovery.[7,30]

In-vivo neuroimaging biomarkers that predict surgical success and recovery are lacking. Our study demonstrated neural recruitment of motor areas that may play a role in functional recovery. Recruitment of contralateral M1 at baseline was correlated with both a maintenance of function at baseline, and greater function six months following surgery. This may indicate that contralateral M1 activation before surgery may be able to predict motor function six months following surgery. Additionally, an increase in the % BOLD signal of ipsilateral SMA six months following surgery was associated with greater function six months following surgery. The change in the % BOLD signal from baseline to six months following surgery was correlated with improved function. This suggests that the % BOLD signal of the ipsilateral SMA may be an important indicator for recovery after chronic spinal cord compression.

2.7.4 Limitations

Limitations of the current study are important to note. There may have been subtle differences in finger tapping between subjects. Although we attempted to control for the rate of tapping by providing subjects with a visual cue, which is a standard technique reported previously by our group,[4] alternations in the force of tapping may lead to variability in activation patterns. The severity of spinal compression in CSM patients will also lead to variability in function, sensory perception and consequently activation patterns between individuals. The variability in patient presentations and neurological recovery limits the interpretation of average group activation maps within the motor network between baseline and postsurgical time points.

2.8 Conclusions

Patients with spinal cord compression demonstrate the ability to recruit secondary motor regions six weeks and six months after surgery to assist in the cortical demands of neurological recovery. Cortical reorganization of the motor network may have a strong

influence on motor control and may be important in the maintenance of function after injury, and in the recovery process.

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Chapter 3

3 Differential Effects of Transcranial Direct Current Stimulation on Anti-phase and In-phase Motor Tasks

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3.1 Abstract

Ageing is associated with a general decline in motor function that critically interferes with activities of daily living involving bimanual motor movements. Cortical adaptation may preserve the function of these hand movements in aging adults. Transcranial direct current stimulation (tDCS) is a form of non-invasive brain stimulation that has been shown to enhance manual dexterity in healthy aging adult. The supplementary motor area (SMA) is involved in motor preparation and bimanual control; bihemispheric tDCS incorporating the SMA may therefore preferentially enhance bimanual motor movements in healthy older adults. The aim of the current study was to determine if tDCS incorporating SMA in a bihemispheric approach could improve manual dexterity in older adults. Twenty-four healthy older adults, aged 67-84 participated in this double-blind, randomized, cross over design study. One group of participants (n=17) were randomized to receive stimulation or sham on their first visit and received the contrary on their second visit, seven days later. A second group of participants (n=10) received three consecutive days of tDCS while performing a motor task. In all cases, participants performed the Complete Minnesota Dexterity Task, which utilizes five motor tasks to assess unimanual and bimanual dexterity while receiving 2 mA of tDCS. The total time for participants to complete three trials of each of the five tasks was recorded. No significant differences in performance were observed between single session tDCS and sham conditions. Furthermore, tDCS did not enhance the execution of the motor task after three consecutive days. However, tDCS had opposing effects on the motor consolidation of antiphase and in-phase bimanual tasks. Application of bihemispheric tDCS did not improve unimanual task performance speed during a single session or tri session paradigm. However, during the tri session paradigm, healthy older adults improved performance learning of antiphase bimanual movements more quickly than inphase bimanual movements, suggesting a different mechanism of action of these two movements.

3.2 Keywords

Transcranial direct current stimulation, motor task, motor enhancement, antiphase task, inphase task, supplementary motor area

3.3 Introduction

Reduced accuracy and speed of motor movements, and a loss of fine motor control are common in healthy aging [1,2]. To compensate for these age related declines, studies have shown increased cortical activation of the ipsilateral motor cortex when completing both unimanual and bimanual motor tasks [3,4]. The execution of bimanual finger coordination tasks involves increased activation of the supplementary motor area (SMA) and pre motor cortex (PMC) compared to unimanual tasks [5,6]. These regions also play an important role in the dynamic integration of both limbs. This adaptive plasticity of the motor cortex is well documented in neurological injury such as stroke and spinal cord injury [7-9]. Motor related cortical excitability can be naturally enhanced through practice or rehabilitation [10]. Therefore, mechanisms that exogenously enhance cortical excitability may improve motor performance or slow the deterioration of motor skills in both healthy aging adults and those with neurological injury.

Transcranial direct current stimulation (tDCS) is a form of non-invasive brain stimulation that has been shown to alter motor behavior by applying continuous, weak current to the motor cortex during training of a motor task [11-14]. By targeting the motor areas of the brain, cortical plasticity can be manipulated to enhance motor functions such as manual dexterity and motor skill learning [13,15]. As bimanual hand movements have been shown to involve a complex organization of cortical activity in both hemispheres, providing stimulation to both cortices may provide optimal enhancement [6,16,17]. Previous studies using bihemispheric tDCS, have shown enhanced motor behavior in complex and simple motor tasks compared to a traditional unihemispheric paradigm [10,16-19]. In addition, consecutive, multisession tDCS, in combination with motor practice, is associated with increased corticospinal excitability compared to single session tDCS or application on alternate days [20].

The SMA has become a recent target of tDCS to enhance motor dexterity due to its direct role in motor planning and execution and strong connections to the primary motor cortex

(M1)[6,21-26]. In the current study, we investigated the effect of applying bihemispheric tDCS for three consecutive days compared to single session tDCS, while healthy older adults completed a motor dexterity task. We hypothesized that applying tDCS during the execution of a motor task would increase cortical excitability and result in improved motor dexterity in healthy older adults. Furthermore, based on previous findings, we hypothesized that bihemispheric tDCS targeting the SMA would preferentially improve the execution of bimanual tasks compared to unimanual tasks [6,25]. In addition, three consecutive days of tDCS and motor practice were expected to enhance motor performance compared to a single tDCS session.

3.4 Methods

3.4.1 Participants

Twenty-four healthy older adults participated in this study (10 female, average age 73 ±5, all were right handed). None of the subjects reported any history of neurologic or psychiatric disorders. The study was approved by the Western University Health Sciences Research Ethics Board, conducted in accordance with the Declaration of Helsinki and written informed consent was obtained from all participants.

3.4.2 Transcranial Direct Current Stimulation

tDCS was applied by a DC-Stimulator (Neuroconn, Germany) for 20 minutes. Direct current (2 mA) was applied to bilateral motor areas while the participant performed the prescribed motor task. Rubber electrodes were 3x3 cm^2, providing a total current density of 0.22 mA/cm^2 and a total charge with respect to time of 0.27 C/cm^2. Electrodes were placed in saline soaked sponge pockets and positioned on each participant using the EEG 10-10 system, which has been shown to be a reliable localization tool [27]. The cathode was placed on the left primary motor cortex (C3), and the anode was placed on the right

supplementary motor area (FC_2). For stimulation, current was ramped up over 10 seconds to reach 2 mA and held constant for 20 minutes, followed by a 10s ramp down period. During sham stimulation, current was ramped up over 10s and then immediately turned off, as it has been shown that subjects are unable to distinguish between sham and true tDCS using this paradigm [13,28].

3.4.3 Motor Task

The Complete Minnesota Manual Dexterity Test (CMMDT) is a standardized measure of both gross and fine dexterity [29]. The CMMDT is composed of two thick cedar boards measuring 23.5 x 85 x 3 cm^3 with 58 wells for cylindrical blocks. The test is composed of five subtests. The Placing Test, the Displacing Test, the One-Hand Turning and Placing Test are all executed using the dominant hand only (unimanual). The Turning and the Two-Hand Turning and Placing Test are executed using both hands (bimanual). Together, these tasks measure both fine and gross unimanual and bimanual dexterity. The test has a high two-trial reliability of 0.91 and correlates well with alternate tasks of gross and fine dexterity [30].

Standardized instructions were given to the participant before each of the subtests at every session and participants were able to practice each subtest before timing began. All subtests were performed with the subject standing at a waist high table. Each of the five tasks were completed three times, the order of the tasks was the same for all participants. The amount of time to perform each trial was recorded and averaged over the three trials. In the ready position, with the participants hand touching the first block, time started when the experimenter gave a count down from ‘3-2-1 Go’ and stopped when the hand left the final block placement. If the participant dropped a block at any point, they were told to retrieve it as time continued; drops were noted during each test.

3.4.4 Experimental Protocol

The current study explored two different methodological designs of tDCS motor enhancement: single session and multiple session tDCS. Seventeen (6 females) individuals participated in the single session (Experiment 1) and ten (4 females) participated in the multi-day sessions (Experiment 2). There were three individuals who participated in both. In this crossover, double-blind, within-subjects design, subjects were pseudo-randomized to have a balanced number across the groups. Subjects completed the motor task while concurrently receiving tDCS. Sham and stimulation sessions were separated by one week to prevent carry-over effects.

3.4.4.1 Single-session tDCS

Each of the seventeen subjects participated in two sessions, where they received either sham or bihemispheric tDCS in a randomized order. Subjects completed the CMMDT, which is comprised of the five sub tasks listed above. Participants were given a practice trial of each of the five motor tasks before performance was timed. tDCS began before the first practice trial of the first task and ended after 20 minutes.

3.4.4.2 Multi-day tDCS (3 consecutive days)

Each of the 10 participants were randomized to receive either three consecutive days of motor training plus tDCS, or motor training and sham. Every subject participated in both sham and tDCS, resulting in six sessions altogether. Session one, two, and three took place on consecutive days; session four, five and six took place on consecutive days one week later. All other aspects of Experiment 2 were identical to Experiment 1.

3.4.5 Data Analysis

Data were analyzed using SPSS (version 22). The average time of the three trials for each of the five sub tasks were calculated. For the single-session tDCS, a 2 (condition) x 5 (task) MANOVA was performed. Since tDCS may have differing effects for unimanual and bimanual dexterity, we performed a 2 (condition) x 3 (unimanual tasks) MANOVA and a 2 (condition) x 2 (bimanual tasks) MANOVA. For multi-day tDCS, a 2 (condition) x 5 (task) x 3 (day) MANOVA was performed. To determine the differential effects on unimanual and bimanual dexterity over a three day session we also performed a 2 (condition) x 3 (unimanual task) x 3 (day) MANOVA and a 2 (condition) x 2 (bimanual task) x 3 (day) MANOVA. Post-hoc t-tests were performed for each subtask for sham and stimulation. Significance was set at $p < 0.05$.

3.5 Results

3.5.1 Single Session tDCS

All participants tolerated tDCS without any adverse effects. The overall MANOVA (including all 5 subtasks) showed no significant effect of condition ($F_{(15,1)} = 0.938$, p=0.35). However, there was a main effect of task ($F_{(12,4)} =316.9$, $p< 0.001$), which was expected, as the tasks were not equal in difficulty or time to completion. There was no significant interaction between condition and task, ($F_{(12,4} = 1.36$, p=0.30, Figure 3.1).

3.5.1.1 Unimanual Motor Tasks

Three of the five subtests involved unimanual dexterity. There was no main effect of condition ($F_{(15,1)} =1.57$, p=0.22). However, a main effect of task was observed ($F_{(14,2)}$= 729.8, p<0.0001). There was no significant interaction effect ($F_{(14,2)}$=2.38, p=0.12).

3.5.1.2 Bimanual Motor Tasks

Two of the five subtests involved bimanual dexterity. There was no main effect of condition ($F_{(15,1)}$ =0.26, p=0.61). However, a main effect of task was observed ($F_{(15,1)}$ = 9.67, p<0.01). There was no significant interaction ($F_{(15,1)}$ = 0.51, p=0.48).

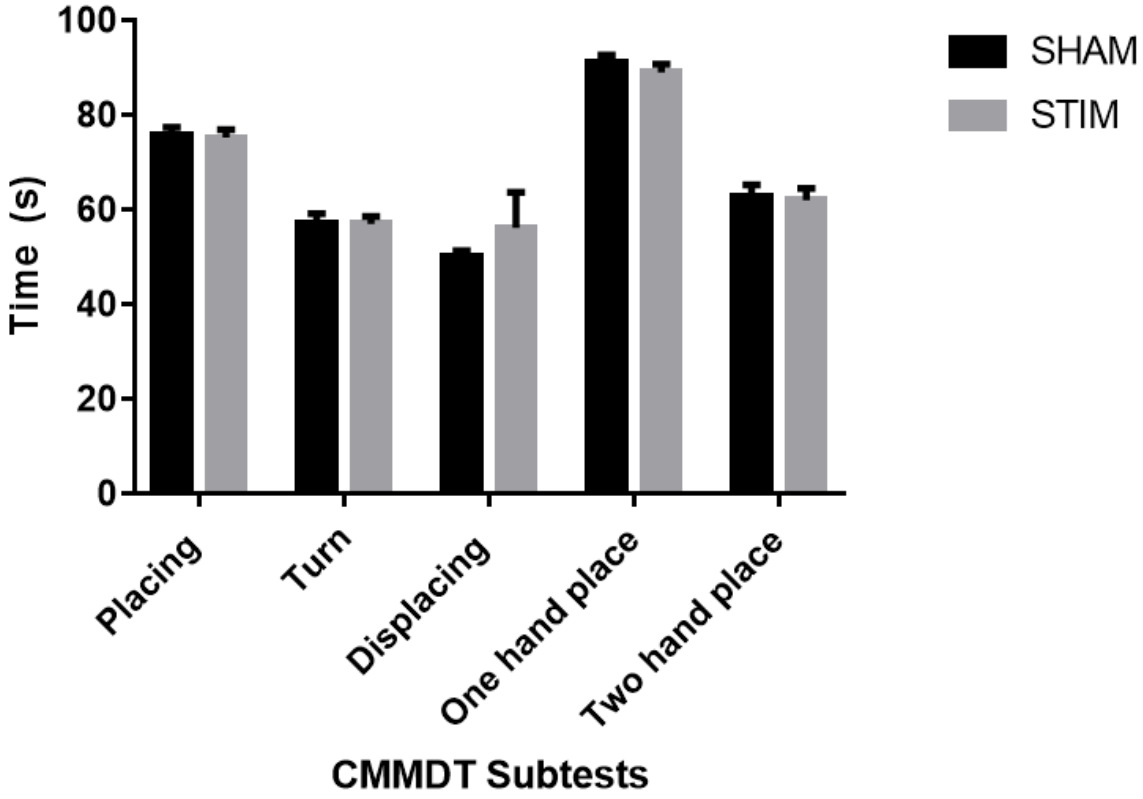

Figure 3.1. Single session tDCS versus sham. No differences were observed between tDCS and sham in any of the tasks.

3.5.2 Multi-session tDCS

When all five subtasks were combined, we observed no significant main effect of condition ($F_{(9,1)}$ =0.004, p=0.95). However a main effect of task ($F_{(6.4)}$ =81.2, p<0.0001) and day were observed ($F_{(8,2)}$ = 464.5, p<0.0001). There were no interactions between condition, day, or task. (Figure 3.2).

3.5.2.1 Unimanual Motor Tasks

When tasks were stratified based on unimanual dexterity, we observed a significant main effect of task ($F_{(8,2)}$ = 180.9, p<0.0001) and day ($F_{(8,2)}$ =95.5, p<0.0001). There was also a significant interaction between task and day, ($F_{(6,4)}$ =5.33, p=0.035). There was no main effect of condition ($F_{(9,1)}$ =0.001, p=0.97).

3.5.2.2 Bimanual Motor Tasks

A main effect of task ($F_{(9,1)}$ =11.57, p<0.01) and day ($F_{(8.2)}$ =68.18, p<0.0001) were observed for bimanual tasks. In addition, we observed a significant interaction of condition and task, ($F_{(9,1)}$ =4.98, p<0.05) and a significant interaction of condition, day, and task ($F_{(8,2)}$ =5.32, p<0.05). Post hoc t-tests revealed significant motor consolidation from day one to day two in the sham condition for the two hand turn and place task (in-phase) compared to the stimulation condition. Participants improved their completion time by an average of 12.5 seconds in the sham condition and only 5.1 seconds in the stimulation condition (p<0.05). There was no main effect of condition ($F_{(9,1)}$ =0.26, p=0.87).

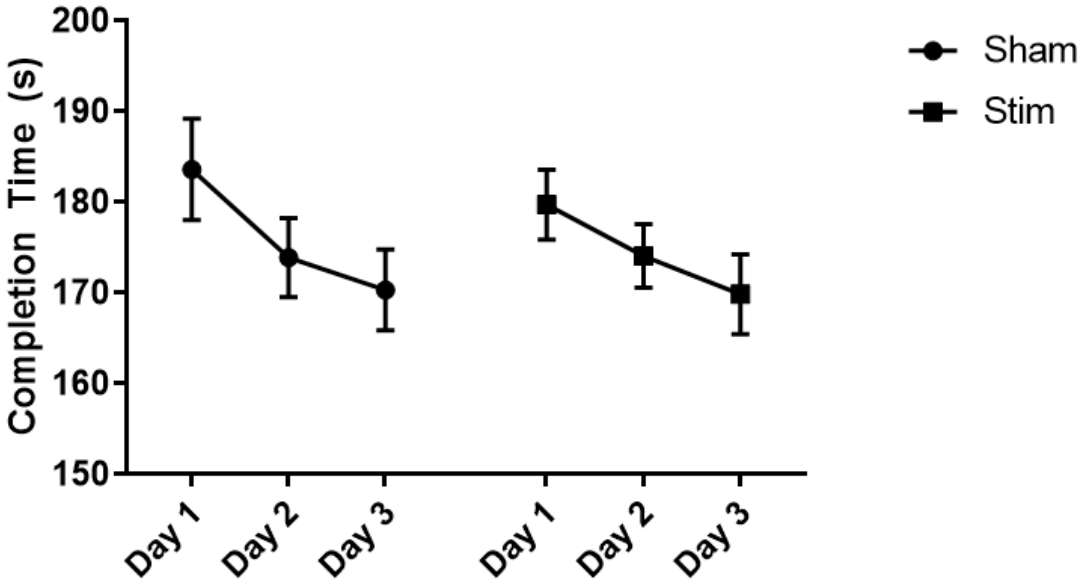

Figure 3.2. Multisession tDCS versus sham. Significant learning effect over the three days was observed for both sham and stimulation condition.

3.6 Discussion

The aim of the current study was to examine the effects of bihemispheric M1-SMA tDCS on manual dexterity in healthy older adults. Although the effect of tDCS on unimanual dexterity has been previously reported, the possible beneficial effect of tDCS on bimanual dexterity is understudied. We observed no effect of single session bihemispheric stimulation on unimanual or bimanual dexterity. However, tri session tDCS, had opposing effects on motor consolidation of antiphase and in-phase bimanual movements. Coordinated bimanual movements form the basis of many everyday motor skills and can become impaired in older adults [2]. Previous research has shown cortical adaptation during motor skill learning occurs in healthy older adults. Specifically, the

SMA, which is involved in bilateral movement coordination, movement initiation and sequential motor learning, is preferentially active during the learning of a motor task [6,22,23,31]. By applying tDCS over SMA, studies have observed improved reaction times, early response initiation and improved bimanual coordination [22-25]. Recently, it has been shown that a bihemispheric electrode montage provides optimal motor improvements, especially for those involving bimanual control [16,32]. Specifically, bihemispheric M1-M1 provides optimal motor activation and improved motor performance compared to the traditional unihemispheric approach [16,32]. It has been suggested that by stimulating SMA, enhancement of motor preparation can occur via alterations in the cortico-cortical connections with M1 [22]. By stimulating both SMA and M1, as in the current study, we aimed to enhance both intra and inter hemispheric connections between SMA and M1 to provide enhancement of bimanual motor tasks.

Cortical representations for unimanual and bimanual motor tasks are different [5,6]. Therefore, it is reasonable to expect that these tasks may be differentially affected by the application of tDCS, particularly when applying bihemispheric tDCS, as in the current study. To ensure one aspect of manual dexterity was not driving our results, we chose to observe unimanual and bimanual dexterity separately. In single session tDCS, we did not observe any significant enhancements or diminutions of motor performance compared to sham in our cohort of healthy older adults. The literature has been highly variable regarding the effect of single session tDCS on motor performance [33-36]. Using 15 different single session tDCS paradigms, Horvath *et al*. found no significant difference between any stimulation paradigms compared to sham using a simple visuomotor reaction time task [35]. This study concluded that short duration, or single session tDCS over M1 may not have a reliable effect on simple visuomotor reaction times [35]. Considering the studies showing motor performance differences after single session tDCS, most report no differences *during* the task. Rather, the effect was observed after the stimulation period had ended, indicating tDCS may have more prominent effects on motor consolidation and retention rather than motor execution [36,37]. This interpretation is consistent with our results, which showed no differences in motor performance during a single session of bihemispheric tDCS.

It is thought that tDCS assists in the retention and consolidation of motor tasks, therefore optimal motor enhancement may be achieved by multiple tDCS and training sessions [10,13,38]. In a study of twelve healthy subjects, Alonzo *et al*. found that multiday, consecutive applications of tDCS were associated with greater increases in motor evoked potential amplitude compared to tDCS application on alternate days [20]. Multiday application of tDCS allows for the consolidation of offline effects (motor improvements that occur after the motor task is completed), and maintains an increased state of corticomotor excitability between sessions. This heightened corticomotor excitability is thought to play a major role in motor enhancement [20,38]. In the current study, a significant learning effect was observed in both sham and stimulation conditions. However, bihemispheric tDCS did not have an additive effect on unimanual tasks, even after three consecutive days. Hupfield *et al*. obtained a similar result using anodal tDCS applied to the SMA over three consecutive days. They did not find enhanced performance on the grooved pegboard task, a test of unimanul dexterity [25]. This result may indicate that anodal tDCS to the SMA preferentially enhances bimanual motor tasks. A previous study by Gomes-Osman observed enhanced performance on a bimanual typing task after a five day course of bihemispheric M1-M1 tDCS. The change in motor performance was significantly enhanced from pre-task day 1 to post task day 5 compared to sham, indicating bihemispheric tDCS may increase motor consolidation of bimanual tasks [10]. Furthermore, Reis and colleagues applied anodal tDCS to M1, assessing the impact on both within day (online) and between day (offline) effects [38]. They observed a greater total (online plus offline) skill acquisition with tDCS compared to sham suggesting this result was primarily mediated through the enhancement of offline effects [38]. Using a three consecutive day paradigm, the present study did not observe a main effect of bihemispheric tDCS on motor performance. This may be due to our measurement of motor skill *during* the task. It is possible that motor performance measured after the cessation of tDCS, could better elucidate an offline effect.

When controlling for unimanual compared to bimanual hand movements, we observed a differential effect of tDCS for in-phase and anti-phase bimanual hand movements. In the CMMDT, there are two tasks requiring bimanual motor movements, the two hand turn

and place task, and the turning task. The two hand turn and place task required both hands to act symmetrically, mirroring the action of the other (in-phase), whereas, the turning task required each hand to perform synchronized movements in the same direction (antiphase), therefore out of phase by 180 degrees (supination / pronation of the hand). Previous literature has shown that anti-phase movements become more variable and less accurate when participants are required to perform at a faster pace [23]. In addition, in a study using functional magnetic resonance imaging, the magnitude of SMA activation was greater during antiphase tasks relative to in-phase, indicating that SMA is important in bimanual control, and antiphase coordination may be preferentially enhanced with anodal tDCS of SMA [6]. In the current study, there was a significant differential learning effect of tDCS on antiphase and inphase tasks. Offline learning from day one to day two was significantly worse for in-phase motor tasks in the stimulation condition compared to sham (Figure 3). Carter *et al*. observed a similar result; in their study using anodal tDCS to SMA, participants were able to perform antiphase movements more accurately and consistently compared to sham. Furthermore, anodal tDCS had no effect on in-phase motor performance. The authors concluded that in-phase patterns are more stable and less likely to be affected by enhancing SMA [23]. In the current study, there was an inhibition of offline motor consolidation for the in-phase task (Figure 3 B). As in-phase motor movements are not dependent on SMA activation, its exogenous excitation in the current study may have induced unnecessary noise into the system, altering reciprocal inhibition patterns, thereby slowing motor consolidation. In healthy individuals, each motor cortex provides reciprocal inhibition to the other to promote coordinated movements [39]. By providing excitatory stimulus to both hemispheres, the complex communication between the motor networks and SMA may have disrupted motor consolidation for in-phase tasks. Further research is necessary to elucidate the mechanism of motor consolidation in bimanual motor movements. By the third day of motor learning, there was no difference in motor performance or consolidation between sham and tDCS. The SMA is preferentially active during motor skill learning and decreases in activity when the skill has been successfully learned [25,39]. Perhaps by day three of motor learning, the task was no longer novel, and no longer required recruitment of SMA. Therefore enhancing SMA activity resulted in no further

increases in motor consolidation. It is also possible that participants reached a ceiling effect and no further enhancements we observed.

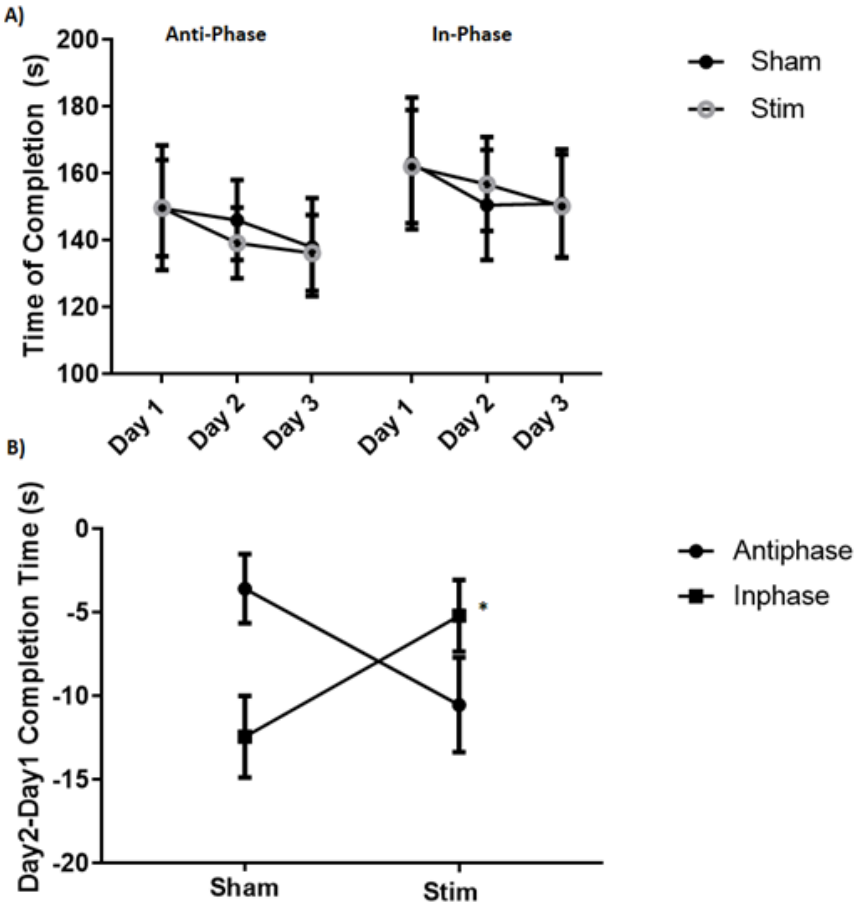

Figure 3.3. Differing effects of stimulation on in-phase and antiphase tasks. Time of completion of anti-phase and in-phase motor tasks over the three consecutive day period of both sham and stimulation protocols. Anti-phase and in-phase bimanual movements show opposing pattern of learning during sham and stimulation. B) The effects of stimulation on learning from Day 1 to Day 2 had different effects on anti-phase and in-phase tasks. We observed a significant reduction in learning from day one to day two in the stimulation condition for in-phase tasks, such that participants performed an average of 12.5 seconds faster in the sham condition and only 5.1 in the stimulation condition (*$p<0.05$).

3.6.1 Limitations

There are a several limitations that should be considered in the current study. First, a baseline pre-test of motor performance was not completed before stimulation or sham began. Therefore, contributions of motor learning through practice cannot be isolated. As there was a significant effect of day, indicating practice did have an effect on motor performance, the additive effect of stimulation would need to be greater to observe a significant effect of stimulation. Furthermore, recent tDCS literature has shown the presence of "non-responders", individuals who do not respond at all, or in the opposite direction of intended tDCS effect. A study by Davidson *et al.* observed high variability in MEP amplitude due to 40-50% of individuals classified as "non-responders"[40]. An increase in the sample size, and the inclusion of a baseline measurement may have decreased the variability observed in the current study. Additionally, all measurements of motor enhancements were completed during the application of bihemispheric tDCS, with no inclusion of a post-test measurement. We therefore cannot determine whether the stimulation paradigm had an effect on long term motor retention. A further limitation of the present study was that we did not include direct neurophysiological measures of cortical excitability, and therefore can only infer that there were changes based on published evidence. [12]

3.7 Conclusion

Bihemispheric tDCS incorporating the SMA has the potential to modulate bimanual motor performance in healthy, aging adults. Bimanual hand movements are a part of many aspects of daily living and are important to maintain. The paradigm used in the current study has the potential to assist in maintaining bimanual motor dexterity in healthy aging adults, and may be extended to neurorehabilitation. Further research that incorporates SMA in tDCS paradigms is required to fully elucidate the mechanism behind motor training consolidation and retention in older adults.

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Chapter 4

4 ^{1}H MR Spectroscopy of the Motor Cortex Immediately Following Transcranial Direct Current Stimulation at 7 Tesla

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4.1 Abstract

Transcranial direct current stimulation (tDCS) is a form of non-invasive brain stimulation that may modulate, cortical excitability, metabolite concentration, and human behaviour. The supplementary motor area (SMA) has been largely ignored as a potential target for tDCS neurorehabilitation but is an important region in motor compensation after brain injury with strong efferent connections to the primary motor cortex (M1). The objective of this work was to measure tissue metabolite changes in the human motor cortex immediately following tDCS. We hypothesized that bihemispheric tDCS would change levels of metabolites involved in neuromodulation including *N*-acetylaspartate, glutamate, and creatine. In this single-blind, randomized, cross-over study, fifteen healthy adults aged 21-60 participated in two 7T MRI sessions, to identify changes in metabolite concentrations by magnetic resonance spectroscopy. Immediately after 20 minutes of tDCS, there were no significant changes in metabolite levels or metabolite ratios comparing tDCS to sham. There was a strong, positive correlation between the change in the absolute concentration of NAA and the change in the absolute concentration of tCr ($p<0.001$), suggesting an effect of tDCS. Both NAA and creatine are important markers of neurometabolism. Our findings provide novel insight into the modulation of neural metabolites in the motor cortex *immediately following* application of bihemispheric tDCS.

4.2 Key Words

Transcranial direct current stimulation, magnetic resonance spectroscopy, creatine, *N*-acetyl-aspartate

4.3 Introduction

Transcranial direct current stimulation (tDCS) is a form of non-invasive brain stimulation that has shown promise in modulating cortical excitability and behavior in humans [1-4]. However, many facets of its use remain controversial. For example, the optimization of stimulation parameters (current level, duration, electrode montage, etc.), characterization of individual response variability, and the physiological mechanism of action are still under active investigation.

Several potential mechanisms of action have been proposed based on pharmacological, behavioural, and imaging studies [1-3,5,6]. At current levels of 1mA, tDCS is thought to induce alterations of the membrane potential, with anodal tDCS making it more likely, and cathodal tDCS making it less likely, for an action potential to fire [7,8]. However, the level of current may also impact the neuronal response to stimulation. At 2 mA, cathodal stimulation has been shown to have an excitatory influence on membrane potential. This has been thought to occur due to an increased release of Ca^{2+} at the higher current. [9] Furthermore, current penetrates deeper into cortical tissues, potentially causing dendritic depolarization at a sufficient level to excite adjacent neuronal structures.[9] Magnetic resonance spectroscopy (MRS) provides a means to investigate the effects of tDCS on cellular metabolism and synaptic transmission as it can be used to non-invasively quantify cerebral metabolites *in vivo,* including glutamate (Glu) and gamma-aminobutyric acid (GABA). Previous MRS studies have shown changes in excitatory and inhibitory neurotransmitter levels, minutes after tDCS, with current levels ranging from 1-2 mA [6,7,10-14]. Other studies have suggested that creatine may have an important role in bioenergetics and neuromodulation[15-17]. For example, Rae *et al.* found an increase in adenosine-triphosphate (ATP) synthesis, with a decrease in the concentration of phosphocreatine in the left temporo-frontal region following anodal tDCS to the left dorsolateral prefrontal cortex[17]. In another study, 2mA of anodal tDCS to the right parietal cortex caused an increase in both Glx and total *N*-aceytl-aspartate (NAA + NAAG) relative to sham, measured from the parietal cortex [10], while a study by Stagg

and colleagues [7] found that 1 mA of cathodal stimulation to left M1 decreased Glx under the electrode. Other studies have found no effect. For example, Kim *et. al.* found no changes after 1.5 mA of cathodal tDCS to left M1 in any metabolite measured under the stimulating electrode.[18] Similarly, using 1mA of current in an M1-M1 bihemispheric montage, Tremblay *et. al.* found no significant changes in any metabolite in left M1[19]. These conflicting results are difficult to interpret, and leads to uncertainty with regards to the implementation of an optimum stimulation paradigm.

The application of tDCS to improve motor performance and recovery in neurological disorders requires optimization of stimulation parameters. Bihemispheric tDCS can enhance both behavior and physiological responses in healthy and neurologically injured individuals [20-22]. The supplementary motor area (SMA) has proven to be an important area of the brain during the execution of bimanual hand movements[23], and plays a compensatory role during the recovery of both stroke and spinal cord injury [24-26]. With its strongest efferent projections to M1 and the corticospinal tract, SMA is a unique target for tDCS [27]. Support for this notion come from a recent study that showed enhanced motor performance by targeting the left SMA with 0.4 mA of anodal tDCS for 90 min over three days.[28] By targeting *both SMA and M1* with 2mA of tDCS, it may be possible to induce additive effects on M1 excitability via interhemispheric connections, which are thought to be more focal than those associated with M1-supraortibal stimulation [19,20].

The purpose of the current study was to demonstrate the feasibility of concurrent tDCS and 7T MRI, and to determine whether targeting both SMA and M1 using a bihemispheric tDCS montage would produce immediate changes in metabolite concentrations in M1 measured using ultra high-field (7T) MRS. To our knowledge, this is the first study to examine the metabolic changes after bihemispheric tDCS, delivered in the MR environment, at an ultra-high magnetic field strength. Based on previous studies, we hypothesized that 2 mA bihemispheric tDCS would enhance synaptic and metabolic activity[10,11,15,17]. As such, metabolites involved in neurometabolism such as NAA, glutamate, and creatine would be altered by stimulation.

4.4 Methods

4.4.1 Participants and Study Design

15 healthy adults aged 21-60 years (mean ± standard deviation: 28 ± 10, 9 female), with no reported history of mental or neurological illness, participated in two sessions on a 7 Tesla (Siemens, Erlangen) head–only MRI scanner. All participants had ^{1}H MRS in this single blind, sham controlled, cross-over design. Participants were randomized to receive tDCS stimulation or sham stimulation on their initial visit, and the contrary on their second visit, at least 7 days apart. Informed written consent was obtained for all procedures according to the Declaration of Helinski (World Medical Association, 2008) and the study was approved by the Western University Health Sciences Research Ethics Board.

4.4.2 tDCS Stimulation

Using an MR-compatible DC-STIMULATOR (NeuroConn, Germany), 2 mA of current was applied to bihemispheric motor areas in the MRI scanner, for a total of 20 minutes. Electrodes were 3x3 cm^2, providing a total current density of 0.22 mA/cm^2 and a total charge with respect to time of 0.27 C/cm^2. For use inside the scanner, electrodes were fit with 5 kOhm resistors placed next to the electrode to minimize the possibility of eddy currents induced in the leads during MRS acquisition. Electrodes were positioned on each participant outside the magnet using the EEG 10-10 system, which has been shown to be a reliable localization tool [29]. The cathode was placed on the left primary motor cortex (C3), anode on the right supplementary motor area (FC_2). For stimulation, current was ramped up over 10 seconds to reach 2 mA and held constant for 20 minutes, followed by a 10 s ramp down period. During sham stimulation, current was ramped up over 10 s and then immediately turned off. As it has been shown that subjects are unable

to distinguish between sham and true tDCS using this paradigm, we used this measurement as a baseline comparison [3,30].

4.4.3 Temperature Monitoring

To ensure the safety of the participants during tDCS in the MRI, temperature was monitored on all subjects throughout the duration of the scan (approximately an hour and 15 minutes). Specifically, four T1C 1.7 mm diameter fibre optic temperature sensors (Neoptix, Quebec, Canada) were located under both electrode pads and the nearest cable chokes. Temperature was monitored in real time with a calibrated Reflex signal conditioner (Neoptix, Quebec, Canada) and a custom data collection program written in LabVIEW 2010 (National Instruments).

4.4.4 Magnetic Resonance Image Acquisition and Analysis

A 7 Tesla Siemens (Erlangen, Germany), head-only MRI (Magnetom) was used to acquire spectroscopy and imaging data. Data were acquired using an 8 channel transmit and 32 channel receive coil array. T_1-weighted MP2RAGE anatomical images (TE/TR = 2.83/6000 ms and 750 µm isotropic resolution) were acquired and used for voxel positioning. These images were also used to estimate white-matter (WM), gray-matter (GM) and cerebrospinal fluid (CSF) fractions for partial volume correction when determining metabolite concentration. The MRS acquisition began immediately following the completion of the stimulation to capture alterations in metabolite concentration due to tDCS. Water suppressed (64 averages) and unsuppressed (8 averages) ^{1}H MR spectra were acquired from a single voxel ($1.6\times2.0\times1.8$ cm^3) located in the left primary motor cortex (under the cathode) (Figure 4.1) using the semi Localization by Adiabatic Selective Refocusing (semi-LASER) pulse sequence [31]: TE/TR = 60/7500 ms, voxel size=$1.6\times2.0\times1.8$ cm^3,total MRS acquisition time was approximately 10 minutes. A localized B_0 and B_1 shim were applied prior to data acquisition. The B_0 shim

was optimized using a two-echo gradient recalled echo (GRE) shimming technique [32] and the B_1 field was optimized such that the phases of the transmit channels added constructively within the MRS voxel. Spectra were lineshape corrected using combined QUALITY deconvolution and eddy current correction (QUECC) with 400 QUALITY points [33]. Simulated prior knowledge metabolite lineshapes were fitted to post-processed spectra using the fitMAN software developed in-house (Figure 4.2) [34]. Metabolite concentrations were examined as ratios normalized to creatine and also as absolute concentrations using unsuppressed water as an internal reference standard as previously described [35]. Measurement of tissue partial volume with the voxel was made using the MP2RAGE images in FMRIB Software Library (FSL) [36] to obtain the fraction of WM, GM and CSF within the voxel. In addition, relaxation rates of the metabolites were incorporated into the quantification to correct for T_1 and T_2 relaxation induced signal loss [37-40].

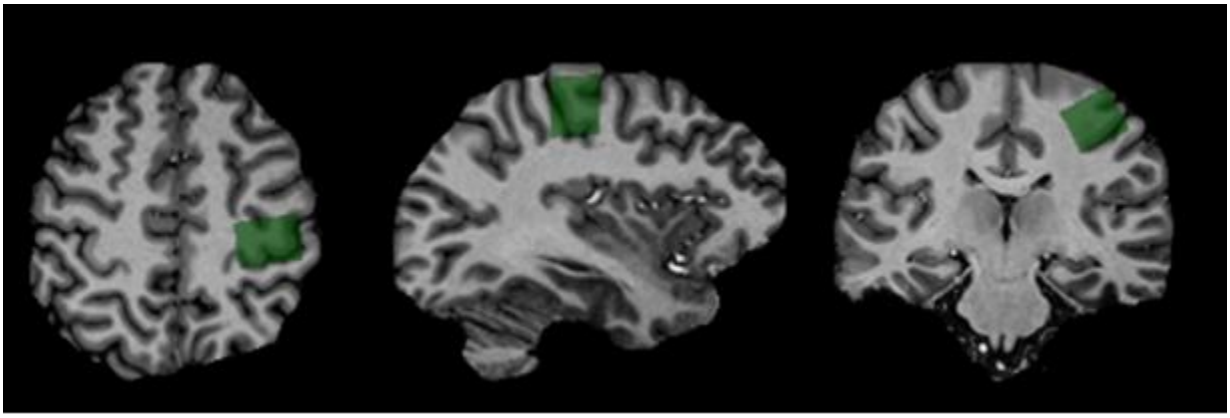

Figure 4.1. Voxel Position: Typical MP2RAGE anatomical images used for voxel placement were brain extracted using FSL. The voxel shown in green ($2x2x2cm^3$) was placed over the left primary motor cortex (under the cathode).

Metabolites measured with a group coefficient of variation of less than <30% in the sham condition were included in statistical analyses. To identify differences in metabolite levels and metabolite ratios between sham and tDCS conditions, repeated measures MANOVA was performed in SPSS (IBM SPSS Statistics Version 25). The main factors were the type of stimulation (two levels: sham and tDCS) and the metabolite (six levels: *N*-acetyl aspartate (NAA), myo-inositol (mI), creatine (Cr), choline (Cho), glutamate (Glu), glutathione (GSH) or metabolite ratio (five levels: NAA/Cr, mI/Cr, Cho/Cr, Glu/Cr, GSH/Cr) measured. In addition, differences between sham and tDCS conditions were compared separately for each metabolite and metabolite ratio using paired t-tests.

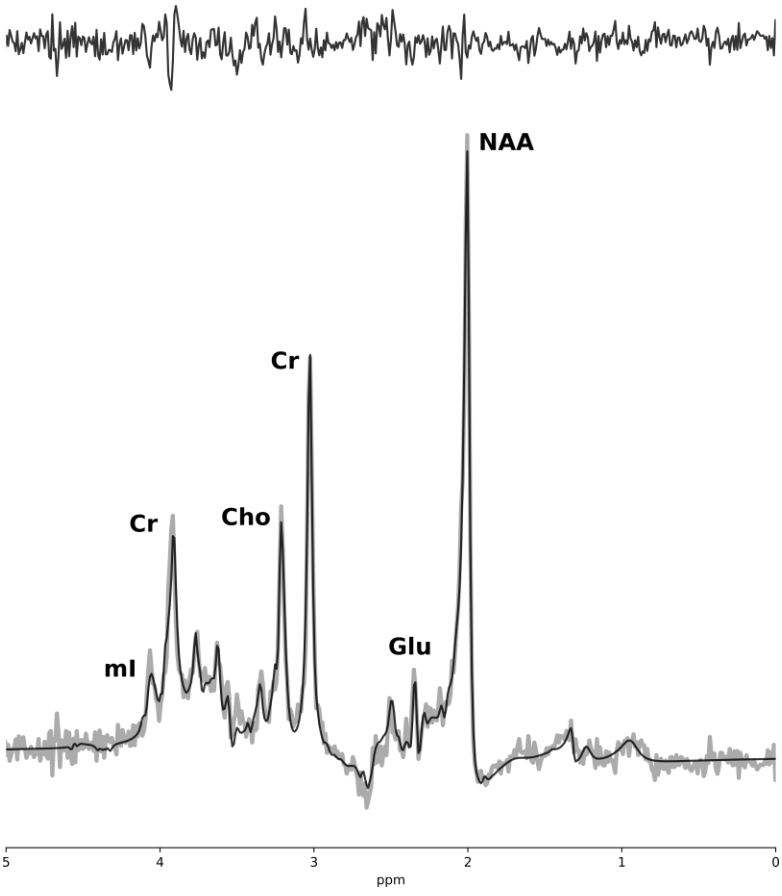

Figure 4.2 7T MRS Spectrum. Semi-LASER ^{1}H MRS (TE=60 ms) of the left primary motor cortex. The spectrum (grey) is overlaid on the fitted result (black) with the residual shown above (black). Select metabolite peaks are identified.

4.5 Results

4.5.1 Temperature Monitoring

The tDCS was safely and successfully applied in the 7T MRI environment in all subjects. The average temperature change in all four probes was 4.3 ± 0.2 ºC throughout the duration of the experiment. This temperature increase was largely due to warming of the bore and from the participant's natural body heating. Once equilibrium was established, small fluctuations on the order of 1 ºC were observed during periods when RF was turned on.

4.5.2 Metabolite Ratio Changes

Spectral quality measures including signal to noise ration and linewidth are summarized in Table 1 for all participants. There were no age or gender related effects. When examining the metabolite ratios the repeated measures MANOVA indicated no effect of stimulation ($F_{(1,14)}$ =3.52, p=0.08). There was a significant main effect of metabolite ($F_{(12,3)}$ =343.35, p<0.001). There was no main interaction effect ($F_{(12,3)}$=1.25, p=0.33) (Table 4.2). *Post-hoc* t-tests (uncorrected for multiple comparisons) were performed to confirm alterations in metabolite ratios. Our results showed that NAA/tCr was 4% higher in the tDCS condition compared to sham, but this was not significant (p=0.08, Cohen's d =0.52), no significant changes in any other metabolite ratios were observed (Figure 4.3, Table 4.2).

Table 4.1. Spectral Quality:

	Sham	**tDCS**
Signal to noise ratio	78.9 ± 5.2	82.3 ± 4.6
Linewidth (Hz)	13.5 ± 1.2	13.6 ± 1.3
Water Area	4.8 ± 0.16	4.9 ± 0.02
GM Fraction	0.37 ± 0.01	0.37 ± 0.02
WM Fraction	0.53 ± 0.02	0.53 ± 0.03
CSF Fraction	0.09 ± 0.008	0.10 ± 0.009

Characterization of spectral quality and voxel tissue composition. Signal to noise ratio represents the intensity of the NAA_{CH3} peak divided by the standard deviation of the baseline noise after Fourier transformation of the initial 0.3 seconds. The linewidth represents the full width at half maximum (FWHM) of the unsuppressed water signal. The water area represents the area of the unsuppressed water spectrum. The voxel tissue partial volume is provided for gray matter (GM), white matter (WM) and cerebral spinal fluid (CSF). Data are presented as mean ± standard error of the mean. Repeated measured t-tests were conducted on all spectral parameters; no significant changes between sham and stimulation were observed.

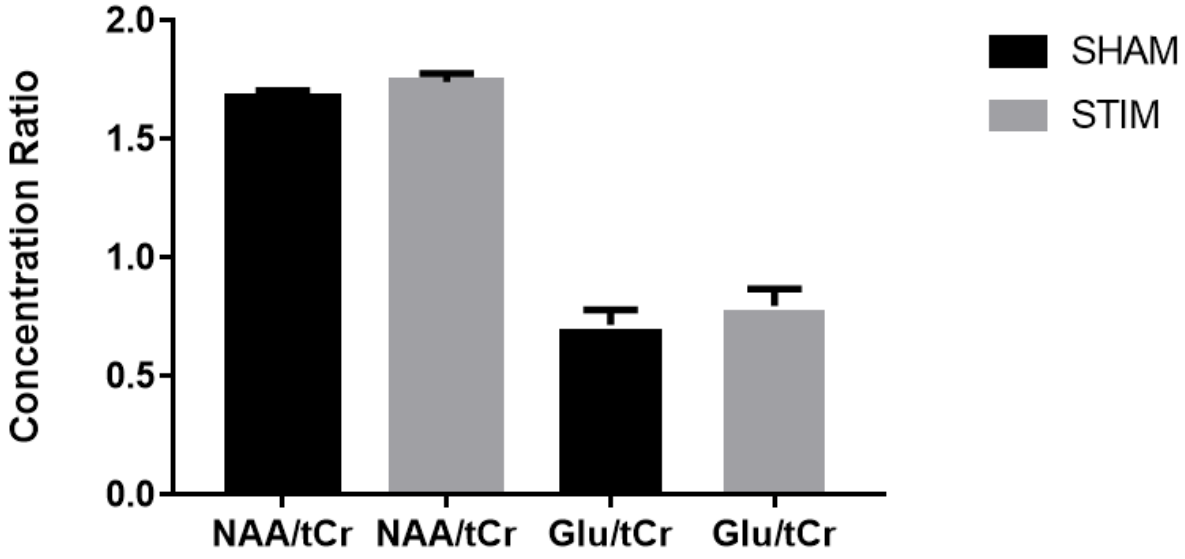

Figure 4.3. Metabolite Ratios. tDCS does not alter metabolite ratio concentration when measured immediately following 20 minutes of tDCS. Error bars indicate SEM.

Table 4.2. Metabolite Ratios.

	Sham	**tDCS**	***p*-value**
NAA/Cr	1.67 ± 0.03	1.73 ± 0.03	0.08
Cho/Cr	0.68 ± 0.02	0.67± 0.02	0.78
Myo/Cr	0.72 ± 0.02	0.72 ± 0.02	0.79
Glu/Cr	0.71 ± 0.06	0.79 ± 0.07	0.21
GSH/Cr	1.28 ± 0.05	1.40 ± 0.06	0.21

Metabolite ratios relative to total creatine. Values indicate means ± SEM. The *p*-values were calculated in post-hoc analysis of individual metabolite ratios using paired t-tests.

4.5.3 Metabolite Concentration Changes

Using repeated measures MANOVA, there was no main effect of bihemispheric M1-SMA tDCS on the absolute concentration of any metabolite ($F_{(1,14)}$ =1.55, p=0.23). There was also no significant interaction effect of metabolite and condition ($F_{(4,11)}$ =1.42, p=0.29). Table 3 displays the absolute metabolite concentrations. *Post-hoc* comparisons using paired t-tests (uncorrected for multiple comparisons) showed no significant changes in any metabolites. However, tCr (p=0.07, Cohen's d =0.42) and mI (p=0.08, Cohen's d=0.48) were close to threshold for significance.

Table 4.3. Absolute Metabolite Concentration.

Metabolite	**Sham tDCS**	**tDCS**	***p*-value**
NAA (mM)	16.2 ± 0.65	15.6 ± 0.46	0.40
Cho (mM)	2.4 ± 0.1	2.3 ± 0.11	0.14
Myo (mM)	6.4 ± 0.28	5.9 ± 0.24	0.08
tCr (mM)	10.9 ± 0.4	10.1 ± 0.3	0.07
Glu (mM)	7.9 ± 0.71	8.2 ± 0.79	0.69
GSH (mM)	2.4 ± 0.18	2.4 ± 0.13	0.88

Absolute concentration of metabolites measured by MRS. Values indicate means ± SEM. The *p*-values were measured by post-hoc analysis of individual metabolites using paired t-tests.

4.5.4 Correlation between NAA and tCr

We observed a strong, positive correlation between the change in the absolute concentration of NAA and the change in the absolute concentration of tCr (stimulation – sham, $R^2 = 0.64$, $p < 0.001$, Figure 4.4).

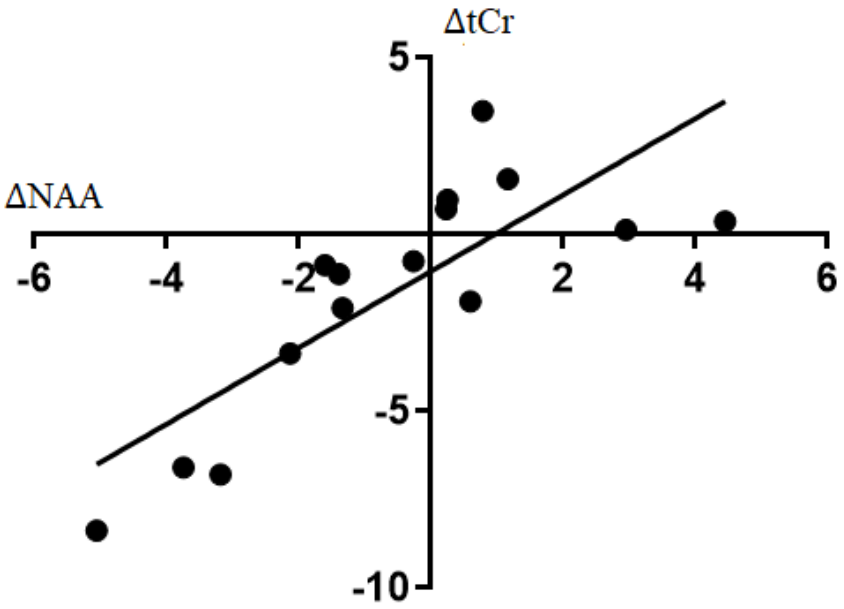

Figure 4.4. NAA and tCr Coupling. The association between the Δ in the absolute concentration of NAA and tCr (stimulation – sham). We observed a strong, positive correlation, indicating NAA and tCr both change in the same direction after stimulation ($R^2 = 0.64$, $p < 0.001$).

4.6 Discussion

Bihemispheric tDCS was safely and successfully performed in the 7T MRI environment with minimal heating effects. However, the bihemispheric tDCS of M1-SMA produced no significant metabolite level changes in the left primary motor cortex (M1) immediately after 20 minutes of stimulation measured by 7T MR spectroscopy. Although, we observed a strong association between the change in absolute concentration

of NAA and the change in absolute concentration of tCr that may indicate a coupling between these metabolites following tDCS.

Brain activity has been shown to decrease both NAA and tCr levels. Specifically, NAA is associated with metabolic and mitochondrial activity [16,41]. Following visual stimulation, Baslow and colleagues found that the concentration of NAA decreased by approximately 13% [42] in the visual cortex. Similarly, Castellano and colleagues observed a 20% decrease in NAA after visual stimulation [43]. This decrease in NAA was attributed to a lower rate of NAA synthesis compared to hydrolysis during periods of cortical activation, suggesting that the brain used NAA faster than it could be synthesized [42,43]. NAA is the precursor for the synthesis of *N*-acetylaspartylglutamate (NAAG), a modulator of glutamate and GABA neurotransmitter release. When neural activity is increased, there is an increased release of NAAG from the synapse [43,44]. It has been suggested that the reduction in NAA upon neural activation is due to increased demand for NAAG. In support of this hypothesis, both Landim *et al*. and Castellano *et al*. observed a decrease in NAA concentration with a subsequent increase in NAAG upon stimulation [43,44].

Creatine (Cr) may also be altered as a consequence of neuronal stimulation due to its role in energy metabolism through its conversion to phosphocreatine (PCr) [15,45]. In the central nervous system, Cr and PCr are involved in maintaining the high energy levels necessary for the maintenance of membrane potentials, ion gradients, calcium homeostasis, and intracellular behavior [46]. Cr has also been observed as a potential modulator of neurotransmission [15,45]. The Cr peak measured by MR spectroscopy represents intracellular contributions from both Cr and PCr (tCr). Although the tCr peak is often used as an internal reference for metabolite concentration, we cannot discount the possibility that tDCS may modulate tCr concentration. In areas of high energy demand, PCr is used to convert ADP to ATP. As such, intracellular stores of PCr will transiently decrease, consistent with the trend toward decreased tCr observed in the current study. Rango *et. Al.* have also discovered a transient decrease in PCr after short bursts of visual

stimulation, concluding that functional activation reduces PCr [47]. Furthermore, Cr is released from the neuron in an action potential dependent manner. An increase in the resting membrane potential, induced by tDCS, may result in release of Cr from the neuron to act as a co-transmitter. Early studies on rodents indicate Cr may modulate postsynaptic neurotransmitters such as GABA, inhibiting its action [48,49]. Release of Cr from intracellular stores would decrease the measureable concentration of Cr.

The observed association between ΔNAA and ΔtCr indicates that individuals that had a decrease in NAA following tDCS relative to sham also had a decrease in tCr levels (Figure 4.4). The increase in NAA/tCr after tDCS (Figure 4.3) suggests that the concentration of tCr decreased relative to NAA. As reported earlier, reduction in NAA upon neural activation is due to increased demand for NAAG, a modulator of GABA and glutamate. As Cr and NAAG are both released from the neuron in an action-potential dependent manner to act as neuromodulators, their correlated decrease in concentration is feasible. This data supports the notion that tDCS increases cortical activation, resulting in an increased neuronal energy demand, which subsequently decreases tCr and NAA.

The after effects of tDCS are thought to be dependent on alterations of the membrane potential and changes in glutamate and GABA signaling, relating to synaptic plasticity [3,6], As such, we expected to observe changes in glutamate and GABA following tDCS. However, the literature presents conflicting results [7,10-12,17,19,50,51]. In a study observing metabolite concentration both during and after tDCS, Bachtiar *et. al.* observed a significant decrease of GABA concentration in left M1 after anodal tDCS to the same area compared to sham, but no differences between sham and anodal tDCS *during* the stimulation period [50]. This indicates that the alteration of neurotransmitters that occurs due to tDCS is predominantly evident after the stimulation period. It is possible that our measurement of metabolite concentration occurred outside the optimal window of neurotransmitter modulation, and instead we observed upstream events. Further studies are required to identify the mechanism by which GABA and Glu are altered to enhance

or depress synaptic activity and the optimal time to observe the peak change in these neurotransmitters.

The current study is the first to measure metabolite concentrations of the motor cortex using a bihemispheric montage in an ultra-high-field MRI (7T). Currently, there are only two studies that have examined the metabolism of the motor cortex following tDCS at 7T, both using the conventional M1-supraorbital (unihemispheric) montage, and both stimulating outside of the scanner [7,18]. Both studies examined the effects of 1 mA of cathodal stimulation over left M1 for 15 [18] and 10 [7] minutes with differing results. Stagg *et. al.* found a decrease in Glu/Cr after cathodal stimulation, while Kim and colleagues reported no significant change in Glu concentration following cathodal stimulation. Kim *et. al.* did report a significant reduction in GABA following anodal stimulation, and no changes in other key metabolites [18]. The current study applied 2 mA of current for 20 minutes. It has recently been shown that cathodal stimulation, which is believed to be inhibitory, reverses its polarity at 2mA and becomes excitatory [9]. The higher current used in our study compared to previous studies may explain the differing results. Increasing the current to 2 mA delays the time of peak metabolic change from immediately after stimulation, to up to 90-120 minutes after stimulation [9]. This delay may explain why we did not observe changes in Glu and GABA in the current study.

Only one other study has examined bihemispheric (M1-M1) tDCS on motor cortex metabolism [19]. Using this montage, and 1mA of current for 20 minutes, Tremblay and colleagues reported no significant modulation in any metabolite concentration at 3T [19], consistent with the current study. They concluded that the complex relationship between excitatory and inhibitory mechanisms within and between the primary motor cortices resulted in high inter individual variability and response to tDCS stimulation. The ultra-high field MRS used in the current study provided greater signal to noise ratio and spectral dispersion compared to Tremblay *et al.* [19], increasing metabolite measurement precision.

Although there have been few studies observing the metabolic and functional changes following M1-M1 tDCS, the current study is the first to incorporate the SMA as a potential target for bihemispheric tDCS. The SMA has been studied as a potential tDCS target in behavioural studies of posture and visuomotor learning [28,52]. Its anatomical positioning and strong connections to M1 make it a well-suited target for motor network modulation. In addition, the SMA has strong efferent connections to the corticospinal tract, making it an ideal candidate as a target for neurorehabilitation [27]. Various studies have shown the importance of the SMA and associated non primary motor areas after brain or spinal cord injury [24-26]. Neural recruitment is an important aspect of recovery. SMA should be considered to enhance synaptic connections of bilateral M1, subcortical structures, and further downstream to the corticospinal tract.

4.6.1 Limitations

One important limitation of the current study was the omission of a within session baseline measurement. The MRS data acquired for this study was part of a longer imaging protocol that incorporated anatomical and resting-state fMRI measurements (to be published elsewhere). Therefore, time constraints prevented the inclusion of a baseline spectroscopy measurement. However, the cross-over design of this study including a separate spectroscopy measurement during sham stimulation on a separate day has previously been shown to be an acceptable approach [30]. However, the inclusion of a baseline measurement in the future would likely reduce inter-subject variability. A recent study at 7T has provided estimates of the reliability of metabolite measurements taken on separate days[31]. In addition, a comparable protocol, using a sham control without baseline measurements observed no changes in metabolite concentration after 1mA of bilateral dorsolateral prefrontal cortex (DLPFC), measured from the left DLPFC[11]. Another limitation of the current study is that metabolite measurements were not made in the SMA, again due to time constraints. Future studies would also benefit from examining metabolite changes in this brain region following stimulation. Finally, the current study was not designed to examine GABA levels. Although, previous studies

have shown changes in GABA concentration, we were not able to measure GABA with sufficient reproducibility using the spectroscopy method applied in the current study. Future studies using GABA editing methods could help elucidate the modulation of GABA by tDCS.

4.7 Conclusion

In conclusion, bihemispheric transcranial direct current stimulation with anode over SMA and cathode over M1 was safely applied during 7T MRI for 20 minutes at 2 mA. Immediately following stimulation there were no changes in metabolite levels measured by ^{1}H MR spectroscopy of the left primary motor cortex in this sham controlled cross-over study. However, when comparing stimulation to sham conditions, there was a significant positive association between the change in *N*-acetyl aspartate and the change in creatine in the same region.

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Chapter 5

5 Bihemispheric Transcranial Direct Current Stimulation Acutely Modifies Resting State fMRI Measured Functional Connectivity of the Sensory Motor Network

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5.1 Abstract

Enhancement of motor function after neurological injury or disease is an area of active research. Recent studies aimed at improving rehabilitation and motor outcomes have combined traditional rehabilitation strategies with transcranial direct current stimulation (tDCS). This approach has shown variable results because the mechanism by which stimulation enhances cortical activity and the optimal stimulation paradigm to enhance motor function and network connectivity is still unknown. Resting-state functional magnetic resonance imaging provides a non-invasive measure of brain network connectivity. We hypothesized that tDCS targeting the left primary motor (M1) and contralateral supplementary motor areas (SMA), would increase functional connectivity within the sensorimotor network during and after stimulation. In this single-blind, randomized, cross-over study, fourteen healthy adults aged 21-60 participated in two 7T MRI sessions to measure differences in sensorimotor network connectivity before, during, and after tDCS. We observed a temporal effect of stimulation on the sensorimotor network. No differences were observed during stimulation, however after stimulation we observed an increase in connectivity of the right sensorimotor area. Furthermore, during stimulation we observed an increase in the functional connectivity between bilateral sensorimotor cortex and right caudate, as well as right supplementary motor area and right caudate, which persisted after stimulation. The observed results indicate that bihemispheric tDCS is capable of modulating sensorimotor network activity. The temporal pattern of activity observed indicates there may be an optimal time for peak

cortical enhancement. However, further work is needed to define the optimal timing of rehabilitation strategies in relation to the applied tDCS.

5.2 Keywords

Functional MRI, resting-state fMRI, transcranial direct current stimulation, enhanced connectivity, primary motor cortex, supplementary motor area, caudate, cortico-striatal connectivity, bihemispheric tDCS

5.3 Introduction

Enhancement of neurological function following disease or trauma, particularly to improve motor performance in conjunction with rehabilitation, is an area of active research. The use of transcranial direct current stimulation (tDCS) to enhance the effects of training have had mixed results[1-5] because the current path and depth depends on the current level, the electrode position,[6,7] and duration of stimulation. For example, 1 mA of cathodal stimulation has an inhibitory effect on cortical excitability[8-10]. However, increasing the current to 2 mA has been shown to have an excitatory effect.[11]
Understanding how tDCS influences brain network functional connectivity will be critical to designing paradigms that maximize the effectiveness of this technology in promoting functional recovery in conjunction with rehabilitation.
Brain network functional connectivity can be measured using resting state functional magnetic resonance imaging (rs-fMRI). Specifically, in the absence of task performance, rs-fMRI can detect spontaneous, low frequency fluctuations in blood oxygen level dependent (BOLD) contrast[12,13]. At rest, spontaneous activity that is highly correlated between specific brain regions is indicative of the degree of functional connectivity[12,14,15]. These highly correlated regions have been grouped into distinct resting state networks (RSN)[12,16]. The first network to be observed, and the network most relevant to the enhancement of motor performance is the sensorimotor network (SMN), which encompasses the bilateral primary motor cortex (M1), supplementary motor area (SMA), and premotor cortex (PMC)[14]. In particular, the right and left sensorimotor cortices demonstrate coherent BOLD fluctuations during rest[12,14].
Using rs-fMRI, tDCS has been shown to induce changes in functional connectivity both during and after stimulation[17-19]. In particular, cathodal tDCS to left M1 increased the inter-hemispheric connectivity between right and left SMA, and right and left M1 following stimulation[20]. Additionally, anodal tDCS applied to the pre-motor cortex strengthened the connection between pre-motor cortex and M1[21]. It is thought that by placing electrodes across hemispheres, the activity of the underlying cortex will be

increased in one hemisphere, while simultaneously decreased in the opposite hemisphere[22,23]. Sehm and colleagues demonstrated the effects of 1 mA bilateral tDCS to M1 on functional connectivity both during and post stimulation [19]. During stimulation, there was a decrease in inter-hemispheric functional connectivity (IHFC) between the right and left M1; however, after stimulation, an increase in the intra-cortical functional connectivity of right M1 was observed[19].

Although previous studies have focused on stimulating PMC and M1[20,24], there are no imaging studies examining the modulatory effects of stimulating SMA. The SMA is preferentially active during the learning of a complex task[25,26], during bimanual coordination[25,27], and in brain and spinal cord injury as a compensatory mechanism[28,29]. Furthermore, due to the strong structural and functional connections between SMA and M1, modulation of SMA influences the activity of M1[30]. Therefore, stimulating both SMA and M1 may enhance motor network strength, which could benefit neurorehabilitation techniques, particularly those aimed at improving manual dexterity. Furthermore, tDCS of the M1-SMA network using 2 mA of current, could modulate subcortical regions such as caudate, as observed in previous studies[17,31].

To our knowledge, ultra high magnetic field strength (7 Tesla) MRI has not been previously used to study the modulation of resting state connectivity both during and after tDCS. Using ultra high-field strength provides increased sensitivity to the low frequency BOLD contrast oscillations observed in the brain at rest. The purpose of the current study was to determine the online and after effects of bihemispheric M1-SMA tDCS on the inter and intra-hemispheric functional connectivity of the motor network at rest. We hypothesized that functional connectivity within the SMN would be enhanced during and post tDCS; specifically, the right SMA and left M1 would show increased functional connectivity. Furthermore, region of interest analysis would show increased intra and inter-hemispheric functional connectivity between right SMA and bilateral M1. In addition, increased connectivity between motor cortical and subcortical structures such as caudate would also occur due to stimulation. `

5.4 Methods

5.4.1 Experimental Design

Fourteen healthy adults aged 21-60 (mean ± standard deviation: 28 ± 10, 8 female) participated in two sessions on a 7 Tesla Siemens head-only MRI scanner. Participants were randomized to receive tDCS stimulation or sham stimulation on their initial visit, and the contrary on their second visit, at least 7 days apart, in this single blind, sham controlled, cross-over design. Informed written consent was obtained for all procedures according to the Declaration of Helinski (World Medical Association, 2008) and the study was approved by the Western University Health Sciences Research Ethics Board.

5.4.2 tDCS Application

Using an MR-compatible DC-STIMULATOR (NeuroConn, Germany), 2 mA of current was applied to bihemispheric motor areas, for a total of 20 minutes. Electrodes were 3x3 cm^2, providing a total current density of 0.22 mA/cm^2 and a total charge with respect to time of 0.27 C/cm^2. For use inside the scanner, electrodes were fit with 5 kOhm resistors placed next to the electrode to minimize the possibility of eddy currents induced in the leads during image acquisition. Electrodes were positioned on each participant outside the magnet using the EEG 10-10 system, which has been shown to be a reliable localization tool [32]. The cathode was placed on the left primary motor cortex (C3), and the anode on the right supplementary motor area (FC_2). Electrolyte gel was used on each electrode pad to provide optimal conductance to the scalp. For stimulation, current was ramped up over 10 seconds to reach 2 mA and held constant for 20 minutes, followed by a 10s ramp down period. During sham stimulation, current was ramped up over 10s and then immediately turned off as it has been shown that subjects are unable to distinguish between sham and true tDCS using this paradigm[33].

5.4.3 Temperature Monitoring

To ensure the safety of the participants during the concurrent tDCS administration and image acquisition in the MRI, temperature was monitored on all subjects throughout the duration of the scan (approximately an hour and 15 minutes). Specifically, four T1C 1.7 mm diameter fibre optic temperature sensors (Neoptix, Quebec, Canada) were located under both electrode pads and the nearest cable chokes. Temperature was monitored in real time with a calibrated Reflex signal conditioner (Neoptix, Quebec, Canada) and a custom data collection program written in LabVIEW 2010 (National Instruments).

5.4.4 Image Acquisition

A 7 Tesla Siemens, head-only MRI (Magnetom), using an 8 channel transmit and 32 channel receive coil array was used to acquire all rs-fMRI data. Each session included the acquisition of sagittal T_1-weighted 3D-magnetization prepared rapid gradient echo anatomical images (TE/TR = 2.83/6000 ms and 750 μm isotropic resolution). During the resting state functional exam, blood-oxygen level dependent (BOLD) images were acquired continuously for 10 minutes using an interleaved echo planar imaging pulse sequence (58 slices/volume, 2 mm isotropic resolution, repetition time/echo time = 1500/ 20 ms, flip angle = 35º). Three resting state functional time series were collected through each examination: baseline (immediately prior to stimulation), during stimulation (beginning immediately after the start of stimulation), and after stimulation (beginning 10 minutes after the end of the stimulation period). Subjects were instructed to lie still, keep their eyes open, stay awake, and think of nothing in particular.

5.4.5 rs-fMRI Pre-processing

All preprocessing steps were completed using the Functional Connectivity (CONN) toolbox of SPM 8 (http://web.mit.edu/ swg/software.htm). Preprocessing of individual 4D datasets included motion correction, slice-timing correction, functional segmentation and normalization to MNI space. In addition, segmentation of gray matter, white matter and cerebrospinal fluid (CSF) were completed; BOLD signals in white matter and CSF were added as covariates and removed as confounding factors. Images were spatially

smoothed using a 6 mm full-width half-maximum Gaussian kernel and band-pass filtered between 0.008 Hz – 0.09 Hz to reduce the influence of noise.

5.4.6 Statistical Analysis

Data were analyzed using SPSS (version 22). To determine changes in connectivity of SMN between the three time courses, a 3 (time) x 3 (brain region) MANOVA was performed. Similarly, to identify changes in connectivity between ROIs, a 3 (time) x 5 (brain region) MANOVA was performed. *Post-hoc* paired t-tests were performed for each specified brain region between the three time points. Significance was set at $p < 0.05$. To ensure reliability across sham and baseline scans, intraclass correlations (ICC) were computed for ROIs and at the network level. ICC were categorized based on five common intervals 0< ICC <0.2 (slight); 0.2 < ICC, 0.4 (fair); 0.4< ICC<0.6 (moderate); 0.6 < ICC <0.8 (substantial); and 0.8 <ICC <1.0 (almost perfect)[34].

5.4.6.1 Network level analysis

BOLD signal time series for each participant were compressed through principal component analysis (PCA). Independent component analysis (ICA) was used to decompose rs-fMRI signals into functionally related groups of voxels[35]. Using criteria built into the CONN toolbox, twenty maximally independent components were identified and separated based on spatial and temporal patterns[15,16]. Spatially independent patterns were further assessed at the group level. Network connectivity was compared between baseline, stimulation, and post stimulation time points. Differences in RSN were corrected using the False Discovery Rate (FDR).

5.4.6.2 Region of Interest Analysis

Regions of interest were identified a priori based on the stated hypotheses (right and left sensorimotor (SM1), right and left SMA, and right caudate). The CONN toolbox allows

for definition of seed areas based on Broadmann areas. Functional connectivity was measured between each pair of defined ROIs, and the average BOLD time series was produced using all voxels within each ROI[36]. ROI based analyses were performed for all subjects with a general linear model to determine significant resting state connections at the individual level. Individual level results were converted into standard scores and group differences were examined between baseline, during stimulation, and after stimulation and between sham and baseline.

5.5 Results

5.5.1 Temperature Monitoring

Bihemispheric tDCS was safely and successfully applied at 7T in all subjects. The average temperature change in all four probes was 4.3 ± 0.2 ºC from the beginning of image acquisition to completion of the experiment (1hr 15 min). The increase in temperature was largely due to warming of the bore and from the participant's natural body heating. Once equilibrium was established, small fluctuations on the order of 1 ºC were observed during periods when RF was turned on.

5.5.2 Stability of Sham and Baseline Scans

Substantial reliability was found between sham and baseline scans in region of interest analyses including the five a priori brain regions, with a mean ICC of 0.75. Furthermore, at the network level, mean ICC of 0.58 was observed for the SMN.

5.5.3 Modulation of Resting –State Networks

Network connectivity in the SMN and the DMN at baseline and during subsequent sham stimulation is displayed in Figure 1a; no differences were observed for SMN or DMN between sham and baseline. Similarly, network connectivity in the SMN and the DMN

at baseline, then during stimulation, and after stimulation is shown in Figure 1b. A 3 (condition: baseline, during stimulation and post stimulation) x 4 (brain region: right SM1, left SM1, right SMA and left SMA) MANOVA showed a significant effect of condition ($F_{(12,2)}$ =6.95, p<0.01) and brain region ($F_{(12,2)}$ =22.35, p<0.001). *Post hoc* analysis indicated a significant increase in functional connectivity of right SM1 after stimulation compared to during stimulation (4.81±0.09, 4.56±0.10 respectively, p<0.03). No other changes were observed during or after stimulation.

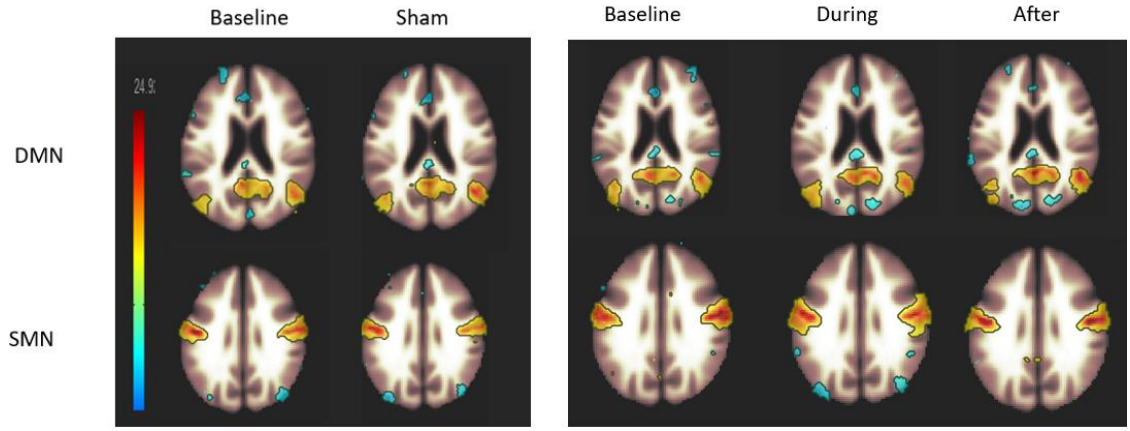

Figure 5.1 Resting State Network Modulation by tDCS. Sensorimotor (SMN) and Default Mode (DMN) comparing A) baseline and sham and B) baseline, during stimulation and after stimulation (p <0.0001). Colour bar indicates the degree of correlation within the network, with red colours indicating a strong correlation, and blue a relative anti-correlation.

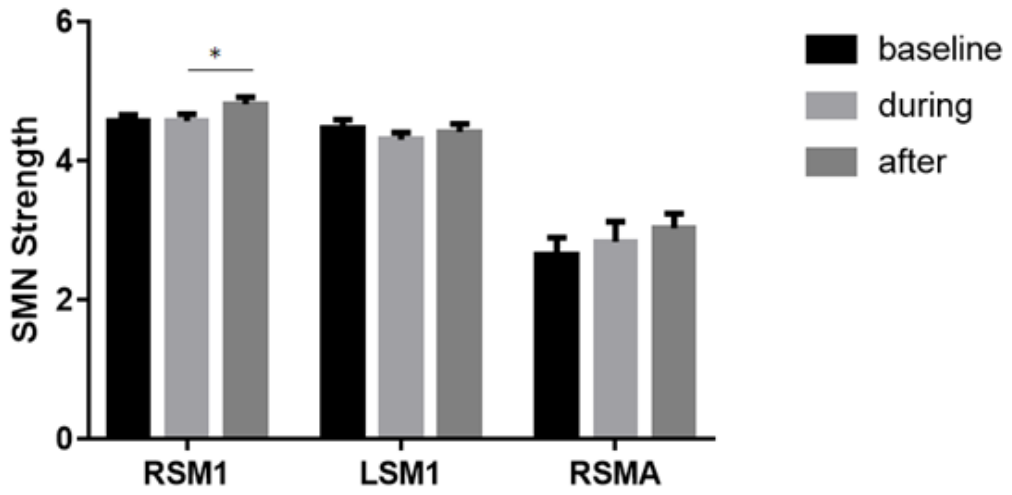

Figure 5.2 Temporal Dynamics of tDCS Modulation on SMN. Connectivity within right SM1 increased between stimulation and after stimulation period (p<0.05). No observed differences in any other regions were observed.

5.5.4 Region of Interest Analysis

To compare functional connectivity between motor cortical regions and caudate, a 3 (condition) x 3 (brain region) MANOVA was performed on the strength of connectivity between right SM1 and right caudate, left SM1 and right caudate, and right SMA and right caudate at baseline, during stimulation, and post stimulation. There was a significant effect of condition ($F_{(12,2)}$=4.86, p=0.02) and brain region ($F_{(12,2)}$ =6.53, p=0.01). *Post-hoc* analysis showed increased functional connectivity between right caudate and the right SM1 (correlation=0.03 ± 0.03,-0.09 ±0.04, p=0.03), the left SM1 (0.12±0.03, 0.003±0.03, p=0.04 and the right SMA (0.03±0.02,-0.11±0.04, p=0.007) during stimulation compared to baseline. Furthermore, increased connectivity was observed in right SM1 (correlation =0.05 ±0.04, p=0.01), left SM1 (0.12 ±0.03, p=0.04) and right SMA (0.04±0.05, p=0.03) post stimulation compared to baseline. A significant temporal increase in functional connectivity was observed between the three time points (Figure 3).

To observe the influence of M1-SMA tDCS on the connectivity between motor regions, a 3 (condition) x 3 (brain region) MANOVA was performed, including the right SM1, left SM1 and left SMA, using right SMA as the primary region of interest. The MANOVA revealed a main effect of brain region ($F_{(11,2)}$=21.31, p<0.001); however, no significant effect of condition ($F_{(11,2)}$ =0.26, p=0.77).

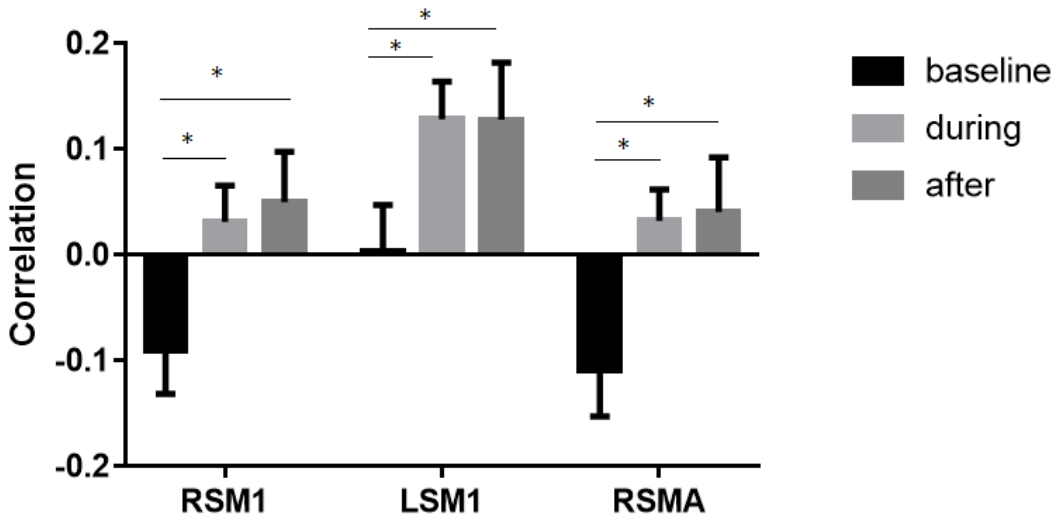

Figure 5.3 Region of Interest Analysis with right caudate. At baseline, all regions shown above displayed an anti-correlation with right caudate. During stimulation and after stimulation, increased functional connectivity (positive correlation) between right M1, left M1 and right SMA with right caudate was observed, indicating an enhanced synchronicity of neural activity between these regions.

5.6 Discussion

Combining M1-SMA tDCS and rs-fMRI, the current study shows that tDCS modulates motor network connectivity, with differing effects during stimulation compared to after stimulation. Furthermore, functional connectivity analysis revealed significant modulation in down-stream, subcortical regions. These results are consistent with previous studies that have observed widespread changes in functional connectivity at

both local and distant cortical regions relative to the area of stimulation[17-20,37]. Using rs-fMRI to monitor the effects of tDCS is a relatively novel approach that examines modulatory effects at a whole brain level and may provide insight into the mechanism by which tDCS modulates brain activity. Comparing sham and baseline conditions showed moderate to substantial reliability between the two scans. This result supports our hypothesis that sham stimulation did not lead to cortical modulation. The current study is the first to examine the online and immediate after effects of tDCS using ultra-high field (7T) MRI, which increases sensitivity to the BOLD contrast used to measure the functional connectivity changes induced by tDCS.

5.6.1 Modulation of SMN by tDCS

During the stimulation period we observed no difference in SMN compared to baseline. However, following tDCS, there was enhanced connectivity of SMN, specifically in the right SM1. Similar results have been obtained previously. Specifically, Amadi *et al*. observed increased coherence between left M1 and right M1 following 1 mA of cathodal tDCS[20]. Similarly, using an M1-M1 bihemispheric montage with the anode placed over right M1, Sehm and colleagues also observed increased functional connectivity of the right M1 after tDCS with 1 mA of stimulation[19]. There is strong evidence that the SMN may be involved in movement initiation and planning, readying the brain to perform and coordinate motor tasks. The results from the current study suggest that this network can be modulated, perhaps to enhance task performance, with M1-SMA tDCS.
Furthermore, we observed a trend towards increased connectivity within right SMA after stimulation compared to baseline, with no changes observed during stimulation. Amadi *et al.* observed increased coherence between right and left SMA following 1 mA of cathodal stimulation to M1, with no observed changes following anodal stimulation[20]. It has been suggested that enhanced connectivity by cathodal tDCS is the result of an increased signal to noise ratio[38]. At 1mA, cathodal stimulation causes hyperpolarization of the neuron, decreasing neuronal firing rate and effectively reducing neuronal noise. Polania *et al.* proposed that this would result in an increased neuronal signal to neuronal noise ratio within the stimulated region, promoting increased synchronization with other

areas[39]. The current study used 2 mA of bihemispheric current, potentially causing neuronal depolarization and increased neuronal firing. Although connectivity within right SMA may have been increased following the stimulation period, the additional noise introduced into the system by tDCS could have reduced our ability to measure such changes.

5.6.2 Modulation of subcortical regions

Stimulation of the motor cortex has also been shown to modulate down-stream, cortical regions [17,31]. Furthermore, SMA has fibres that project directly to the caudate nucleus[40,41]. This cortico-striatal connection is important in different aspects of motor control including initiation, and modulation of voluntary movement[42,43]. Our results extend previous research by showing an effect of bihemispheric tDCS on the connection between motor related areas and the caudate during and post stimulation. During the stimulation period, we observed enhanced functional connectivity between the right caudate and bilateral SM1 as well as the right SMA.

A recent study by Hone-Blanchet observed an increase in the concentration of glutamate + glutamine (Glx) in caudate during bihemispheric tDCS to dorsolateral prefrontal cortex (DLPFC)[44]. The authors concluded that excitatory stimulation may have an excitatory effect over striatal regions leading to an elevation in Glx[44]. Due to the lack of research observing the functional activity of the brain by rs-fMRI during application of tDCS there are few studies to validate our results. However, post-mortem studies have shown that fibers from SMA project directly to the caudate[40]. Therefore the excitatory stimulation of SMA could penetrate these descending fibres, leading to enhanced connectivity between these two regions.

As the M1-SMA network is strongly connected, stimulation of left M1 and right SMA further strengthens this network, resulting in enhanced connectivity between these regions and the caudate. This enhanced cortico-striatal network was also observed post stimulation, an observation that has previously been reported. Anodal stimulation of M1 has been shown to modulate subcortical activity, specifically in the ipsilateral caudate nucleus[17]. Striatal activity has been shown to precede cortical activity in the executive

corticostriatal loop, as supported by fMRI studies in humans[45]. The executive loop has been implicated in motor feedback processing and is active during task performance, especially when coordinating two tasks at once[45]. During baseline rs-fMRI, before stimulation, we observed an anti-correlation, or "decoupling" of the right caudate to the right SMA and bilateral SM1. However during stimulation, there was an enhanced correlation between these regions that continued after the stimulation period had ended (Figure 3). We speculate that by stimulating SMA, cortical projections to the caudate activated the executive loop, providing feedback to the cortex, thereby enhancing the functional connectivity between bilateral M1 and caudate. Polania *et. al* observed a polarity effect of stimulation on cortico-thalamic connectivity. Cathodal tDCS over M1 decreased the functional connectivity between left M1 and contralateral putamen, where anodal tDCS to M1 enhanced the connectivity between left caudate and parietal association areas, as well as left M1 and ipsilateral thalamus[17].

Together, these results provide evidence for strong cortico-caudate connections that can be modulated by tDCS. This result is of particular clinical relevance, as anodal stimulation over M1 has been shown to improve gait and bradykinesia in Parkinson's patients[46]. We speculate that the clinical improvements observed in Parkinson's patients may be driven by the enhanced connectivity between cortical and subcortical structures. In fact, it has previously been shown that repetitive TMS to prefrontal cortex induces dopamine release in the caudate nucleus. The current study provides evidence that stimulating the M1-SMA[47] network may provide optimal enhancement of cortico-striatal connectivity both during and post stimulation, which may provide beneficial clinical improvements for Parksinon's patients. Further research is warranted to determine if this electrode montage is optimal for motor enhancement.

5.6.3 Limitations

The rs-fMRI measurement concluded approximately 30 minutes after the end of stimulation. Therefore, further studies are needed to determine the full time course of cortio-cortico and cortico-striatal functional connectivity modulations. However, a

previous study has shown that 20 min of tDCS produces cortical modulations that outlast the stimulation period for up to 90 minutes[48].

5.7 Conclusions

Our results support our hypothesis that targeting the M1-SMA network with bihemispheric tDCS modulates functional connectivity in both cortical and subcortical regions. The effects of tDCS during the stimulation period differed from those observed post stimulation, which may be an important factor when designing protocols to enhance behaviour modulation using tDCS. Additionally, M1-SMA tDCS enhanced the connectivity between cortical and subcortical regions, which may further enhance motor control in both healthy and neurologically injured populations.

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6 Conclusions and Future Directions

The goal of the current thesis was to examine the functional, metabolic and behavioural changes associated with a novel tDCS montage that may specifically enhance manual dexterity in patients with CSM.

tDCS is a form of non-invasive brain stimulation that has recently become popular due to its ability to modulate cortical excitability and alter brain biochemistry. In light of these physiological changes that occur with tDCS, its use in enhancing cognitive and motor performance gas gained increased attention over the years. Although there is strong evidence that tDCS can enhance cognitive and motor performance and alter cortico-spinal activity, the variability in methods used in the literature call to question the mechanism behind tDCS modulation and the stimulation parameters that optimize motor performance. This is particularly important when determining the effects of a combined tDCS and rehabilitation protocol. The use of tDCS in rehabilitation has shown promise in stroke and depression populations. However, recent studies have emerged attempting to enhance traditional rehabilitation with the use of tDCS. Of interest to our group, is spinal cord injury, specifically, CSM. CSM is a devastating disorder affecting dexterity, gait, and in severe forms can lead to quadraparesis. Rehabilitation strategies in this population are severely lacking. This thesis provides novel insights into the functional reorganization that occurs following surgical decompression of the spinal cord in patients with CSM. We observed the recruitment of SMA may be an important aspect in recovery of motor function following spinal cord decompression. This knowledge was the motivation to examine a novel tDCS montage, incorporating SMA and M1, with the goal of integrating its use into rehabilitation protocols. To determine its validity and gain further insight into this montage, we first examined the behavioural effects on healthy older adults. Additionally, we examined the functional and metabolic effects of this montage to further elucidate the mechanism by which it may improve recovery following CSM. In addition, we observed the effects on manual dexterity in older adults using a one day and three day tDCS protocol.

Chapter 2 describes the results of the first study to longitudinally observe brain functional changes, both contralateral and ipsilateral that occur in patients with CSM before, six weeks and six months following decompression surgery. With the use of task based fMRI, we observed an increase in % BOLD and volume of activation within the contralateral and ipsilateral motor network (six weeks after surgery). In addition, we observed the importance of the ipsilateral SMA six months following surgery, as it was associated with an increase in function as measured by the mJOA six months following surgery. This study concluded that plasticity of the contralateral and ipsilateral motor network play complementary roles in maintaining neurological function in patients with spinal cord compression; furthermore, the SMA may play a critical role in the recovery of motor function following surgery. The results of this study served as our motivation to determine if enhancing SMA activity would result in enhanced recovery. To do so, we fostered a novel electrode montage to provide stimulation to right SMA and left M1, with the aim of enhancing motor recovery.

Chapter 3 details the first study to use SMA-M1 tDCS in combination with a motor task to examine whether this montage can enhance unimanual and bimanual dexterity in healthy older adults. We used both a single session and multiple day (3 consecutive day) session to observe the effects of motor execution and motor learning that may be enhanced by tDCS. Although we observed no significant improvements in motor execution or motor learning in either a single or tri session tDCS protocol, an opposing effect was observed for in-phase and antiphase bimanual tasks. SMA-M1 significantly worsened motor learning from day one to day two for in-phase tasks; however, a relative improvement was observed from day one to day two for antiphase tasks. Previous literature has shown that the SMA is preferentially activated for antiphase tasks as they are more complex and difficult to perform, especially when performed at a quick pace. Previous research has shown preferential enhancement of motor learning of antiphase tasks over in-phase when anodal stimulation to SMA is applied. The current study provided further insight into how SMA-M1 tDCS may modulate behavior, and further perpetuated the notion that the action of tDCS is highly task specific.

Chapter 4 details one of the very few studies to examine the metabolic changes that occur in the brain due to tDCS at ultra-high field (7T). Due to the critical role of the SMA observed in CSM recovery of function, the current study examined a novel bihemispheric tDCS electrode montage that targeted the SMA and M1. By targeting the interhemispheric connections of this network, we speculated that excitation of right SMA would provide enhanced excitability of left M1. We acquired spectroscopy data from a voxel over left M1 to determine the metabolic changes that occur after 20 minutes of tDCS. We observed no significant metabolite modulation after the stimulation period. However a significant correlation between the change in NAA and the change in tCr from stimulation to sham was observed. Cr is an important neuromodulator and is released from the neuron in an action potential dependent manner to act on GABA receptors. Additionally, previous research has shown a significant decrease in phosphocreatine following stimulation, in response to an increased energy demand. As the time course of metabolite modulation by tDCS has not been fully elucidated, it is possible that our measurement was outside the peak window of metabolite change.

Chapter 5 details the first study to examine the temporal functional changes that occur due to bihemispheric tDCS at ultra-high field (7T). Using three rs-fMRI measurements, we observed the resting state brain connectivity in the sensorimotor network (SMN) before, during, and post 20 minutes of bihemispheric tDCS. In a previous study from our group we observed enhanced activation of SMA in CSM patients following decompression surgery that was associated with enhanced learning. The current study aimed to observe the cortical modulations associated with application of bihemispheric tDCS to the M1-SMA network. We observed a temporal effect of stimulation. During stimulation, we observed no changes in connectivity of SMN. However, following stimulation, connectivity within the SMN, specifically right SM1 was significantly enhanced compared to baseline. Furthermore, bihemispheric tDCS resulted in enhanced connectivity within the cortico-striatal network. Specifically, connectivity between bilateral SM1 and right SMA to right caudate was significantly strengthened during and post stimulation compared to baseline. This study provides novel insight that tDCS can

modulate excitability at both the cortical and subcortical level and can modulate striatal connectivity.

6.1 Limitations

The limitations of each of the studies presented in this thesis have been discussed in detail in their respective chapters. However, a general limitation of observing cortical and behavioural modulation through tDCS is the variability with which individuals respond to tDCS. Recent literature suggests that as many as 50% of individuals have a very minor or no response to tDCS, as measured by motor evoked potential amplitude. Potential reasons for the lack of response to stimulation has not yet been uncovered. This creates unpredictable modulations by tDCS, resulting in difficulty interpreting data both within and between studies. Furthermore, the variability in tDCS protocols leads to a fragmented picture of the mechanism behind tDCS modulation. Parameters such as current level, stimulation duration, and electrode montage all have varying effects on cortical and behavioural modulation. Continued research is required to fully elucidate the mechanism behind cortical stimulation in order to translate its use into rehabilitation.

6.2 Future Directions

This thesis contributed to the current tDCS literature by examining network connectivity and metabolic modulation using ultra-high field (7T) MRI. The goal of the current thesis was to examine the functional reorganization that occurs after spinal cord decompression and areas of the brain that can be attributed to enhanced recovery. Furthermore, we aimed to determine the modulatory role of stimulating the M1-SMA network in preparation for its use in motor rehabilitation for spinal cord injured populations. The novel approach of stimulating the bihemispheric M1-SMA network was motivated by the results described in Chapter 2, where we observed enhanced SMA activity in CSM patients following surgical intervention that was associated with enhanced recovery. We hypothesized that

by further enhancing the M1-SMA network with tDCS, in combination with traditional rehabilitation, we could promote enhanced recovery in this population. Due to the novel electrode montage, it was first pertinent that we determine how this protocol would modulate cortical, metabolic and motor behavior in healthy individuals. Although the current thesis provided novel insight into the mechanism by which tDCS may modulate the M1-SMA network, continued research is required to fully understand this novel electrode montage. We examined the metabolic changes that occur following tDCS of left M1 (under the cathode). Future studies to determine the metabolic profile of the right SMA (under the anode) and subcortical regions such as caudate would help understand if and how different brain regions have altered metabolic profile and how these regions are working together.

Secondly, observing the difference between how resting brain networks respond to tDCS versus modulation during and after a motor task may uncover additional information about how to enhance motor performance and function. As the goal is to combine tDCS and rehabilitation protocols, determining the cortical adaptations that occur due to tDCS and a combined motor task will help refine the parameters of tDCS. Finally, correlating metabolic and functional changes, in addition to correlating imaging results with motor performance will allow for further clarification of the mechanism through which tDCS can enhance motor performance and function.

Although the current thesis extends the current knowledge of bihemispheric tDCS, continued research is required before implementing this technique into a rehabilitation protocol. In Chapter 3 we observed the effects of bihemispheric tDCS on different manual dexterity tasks, during a single and tri session protocol. We observed a conflicting action of tDCS on bimanual dexterity tasks; although a slight improvement in motor consolidation was observed for anti-phase tasks, tDCS may have resulted in diminution of motor consolidation of in-phase tasks. Previous research has shown that the action of tDCS is highly task dependent; therefore, further research into how bihemispheric tDCS would affect the motor tasks to be used in a rehabilitation protocol

would need to be carried out. Furthermore, tDCS has been shown to alter functional activity and behavior differently in healthy and neurologically injured individuals. To determine if the action of bihemispheric tDCS has similar results to that observed in the current thesis, observation of the functional changes in CSM both during rest and during task based fMRI with concurrent tDCS would be advised.

6.3 Conclusions

Each of the presented studies offered novel information of the cortical and behavioural modification of bihemispheric tDCS and how this montage may be specifically useful to patients with CSM. The most important findings presented in this thesis are as follows: 1) patients with CSM undergo plastic functional changes to support the maintenance and recovery of function; the SMA is involved in recovery of function six months following decompression surgery; 2) tDCS is highly task specific and has differing effects on antiphase and in-phase motor tasks. 3) with the use of ultra-high field MRS we did not observe significant local metabolic changes after stimulation; however, there was a trend towards an increase in NAA/Cr ratio in the left M1; 4) bihemispheric M1-SMA tDCS is capable of modulating motor network connectivity not only locally, but at the subcortical level, where connectivity between caudate and motor network was strengthened during and after stimulation. Each of these studies provided novel insight into the mechanism by which tDCS may enhance motor function and translate into a neuromodulatory role. The goal is to gain further information of the optimal tDCS protocol to enhance motor function in spinal cord injury in hopes of extending its use into a clinical role. Due to the variability between individuals and task, tDCS rehabilitation may have to be tailored to the individual to optimize motor recovery.

Appendix A: Research Ethics Board Approval

Research Western

Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Neil Duggal
Review Number: 18118
Review Level: Full Board
Approved Local Adult Participants: 46
Approved Local Minor Participants: 0
Protocol Title: Metabolic and Functional Correlates in Spinal Cord Compression Measured by Magnetic Resonance Imaging
Department & Institution: Clinical Neurological Sciences,London Health Sciences Centre
Sponsor: Canadian Institutes of Health Research

Ethics Approval Date: August 24, 2011 **Expiry Date:** March 31, 2015

Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
UWO Protocol		
Letter of Information & Consent		2011/07/25
Advertisement		

This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The UWO HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

[redacted]

Signature

Ethics Officer to Contact for Further Information

Janice Sutherland (jsutherl@uwo.ca)	Grace Kelly (grace.kelly@uwo.ca)	Shantel Walcott (swalcot@uwo.ca)

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Western Research

Research Ethics

Western University Health Science Research Ethics Board
HSREB Full Board Initial Approval Notice

Principal Investigator: Dr. Robert Bartha
Department & Institution: Schulich School of Medicine and Dentistry\Medical Biophysics,Robarts Research Institute

HSREB File Number: 106178
Study Title: Enhancing manual dexterity using transcranial direct current stimulation in healthy individuals
Sponsor:

HSREB Initial Approval Date: April 24, 2015
HSREB Expiry Date: April 24, 2016

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Other	screening form	2015/04/02
Recruitment Items	recruitment poster	2015/04/02
Recruitment Items	recruitment email	2015/04/02
Letter of Information & Consent		2015/04/02
Western University Protocol		

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

[redacted]

Ethics Officer, on behalf of Dr. Joseph Gilbert, HSREB Chair

Ethics Officer to Contact for Further Information

✓ Erika Basile ebasile@uwo.ca	Grace Kelly grace.kelly@uwo.ca	Mina Mekhail mmekhail@uwo.ca	Vikki Tran vikki.tran@uwo.ca

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Appendix C: Curriculum Vitae

EDUCATION/TRAINING			
INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Western University, London, ON	PhD	2013 - Present	Medical Imaging
Western University, London, ON	MSc	2011 - 2013	Neuromuscular Integration
University of Waterloo, Waterloo, ON	BSc (Hons)	2006 - 2011	Science and Psychology

A. RESEACH INTERESTS

Kayla Ryan is a PhD student in Medical Biophysics, with a concentration in medical imaging. Her research is primarily focused on enhancing recovery from chronic spinal cord compression through the use of innovative methods and technologies such as transcranial direct current stimulation, functional magnetic resonance imaging and magnetic resonance spectroscopy. Recently she has adapted the use of transcranial direct current stimulation as a training tool to improve and/or recover manual dexterity in older adults, in the hopes to translate this technique to spinal cord injury rehabilitation.

B. GRANTS,AWARDS AND HONOURS

Grants Awarded:

2017 Lawson Internal Research Fund, Translation of a manual dexterity task to assess motor function and dexterity in spinal cord compression, $30 000 over two years

2014 Collaborative Research Seed Grant, **Improving Outcomes in Patients with Reversible Spinal Cord Compression: Development and Validation of a Novel Rodent Model for Interventional Testing, $50 000**

Honours and Awards:

May 2015	Robarts Research Institute Oral Presentation Award
September 2008-2011	Dean's Honour Award, University of Waterloo, Waterloo, ON

C. PEER-REVIEWED PUBLICATIONS & CONFERENCE ABSTRACTS

Ryan, K., Goncalves, S., Bartha, R., Duggal, N. *Motor network recovery in patients with chronic spinal cord compression using fMRI: a longitudinal study.(2018) Journal of Neurosurgery:Spine 28(4):379-388*

International Society of Magnetic Resonance in Medicine, Honolulu, Hawaii, *7 Tesla ^{1}H MR Spectroscopy of the Motor Cortex following Transcranial Direct Current Stimulation.* Kayla Ryan, Dickson Wong, Kryzstof Wawrzyn, Blaine Chronik, Neil Duggal, Robert Bartha, April 28-May 4, 2017

Imaging Network of Ontario, London, ON *Does transcranial direct current stimulation alter glutamate concentration in healthy adults?* Kayla Ryan, Dickson Wong, Kryzstof Wawrzyn, Blaine Chronik, Neil Duggal, Robert Bartha, March 15, 2017

Robarts Retreat, Western University, London ON, *Enhancing Manual Dexterity in Older Adults with the use of Transcranial Direct Current Stimulation,* Kayla Ryan, Amy Schranz, Robert Bartha, Neil Duggal, June 13, 2016

London Healthy Research Day, London, ON *Enhancing Manual Dexterity in Older Adults with the use of Transcranial Direct Current Stimulation,* Kayla Ryan, Amy Schranz, Robert Bartha, Neil Duggal, March 29th, 2016

CNS Research Day, Western University, London, ON, *Enhancing Manual Dexterity in Older Adults with the use of Transcranial Direct Current Stimulation,* Kayla Ryan, Amy Schranz, Robert Bartha, Neil Duggal, April 1, 2016

Robarts Retreat, Western University, London, ON, *Visualizing Ipsilateral Activation in Patients with Cervical Myelopathy using Functional Magnetic Resonance Imaging,* Kayla Ryan, Sandy Goncalves, Robert Bartha, Neil Duggal, June 8th, 2015.

International Society for Magnetic Resonance in Medicine, Toronto, ON, *Cortical Plasticity of the Ipsilateral Motor Areas in Cervical Myelopathy following Decompression Surgery,* Kayla Ryan, Sandy Goncalves, Robert Bartha, Neil Duggal, May 30th – June 5th, 2015

Imaging Network of Ontario, London Convention Centre, *Visualizing Ipsilateral Activation in Patients with Cervical Myelopathy using Functional Magnetic Resonance*

Imaging, Kayla Ryan, Sandy Goncalves, Robert Bartha, Neil Duggal, March 30th-31st 2015

CNS Research Day, Western University, *Activation of Ipsilateral Non Primary Motor Areas in Cervical Spondylotic Myelopathy following Spinal Decompression Surgery,* Kayla Ryan, Sandy Goncalves, Izabela Aleksanderek, Robert Bartha, Neil Duggal, March 10th, 2015

London Imaging Day, Victoria Hospital, London, ON, *Cortical Plasticity in Cervical Myelopathy as Measured by Functional Magnetic Resonance Imaging*, Kayla Ryan, Sandy Goncalves, Izabela Aleksanderek, Robert Bartha, Neil Duggal, June 26th 2014

Robarts Retreat, Bellamere Winery, London, ON, *Cortical Plasticity in Cervical Myelopathy as Measured by Functional Magnetic Resonance Imaging*, Kayla Ryan, Sandy Goncalves, Izabela Aleksanderek, Robert Bartha, Neil Duggal, June 9th 2014

Southwestern Ontario Neuroscience Association, Western University, *Cortical Reorganization in patients with Cervical Myelopathy before and after decompression surgery*, Kayla Ryan, Sandy Goncalves, Izabela Aleksanderek, Robert Bartha, Neil Duggal, May 5th 2014

CNS Research Day, Ivey Business Centre, London, ON, *Cortical Plasticity in Cervical Myelopathy Measured by Functional Magnetic Resonance Imaging*, Kayla Ryan, Sandy Goncalves, Robert Bartha, Neil Duggal, March 11th 2014

London Health Research Day, London Convention Centre, London, ON *Cortical Plasticity in Cervical Myelopathy Measured by Functional Magnetic Resonance Imaging*, Kayla Ryan, Sandy Goncalves, Robert Bartha Neil Duggal, March 18th, 2014

D. WORK EXPERIENCE

Research Consultant — Centre for Family Medicine, Waterloo, ON

2017-Present

- Assist in grant writing and ethics applications

- Assist in the creation of new tools to enhance primary care in spinal cord injury
- Develop, plan and execute new research avenues in primary care for spinal cord injury

Exercise Instructor Retired Researchers Association, London, ON
2015-Present

- Create and instruct appropriate workout routines for older adults

Lab Coordinator Western University, London, ON
2012-2013

- Instructed graduate level teaching assistants effective and useful ways to teach labs to university students
- Coordinated bi-weekly meetings to ensure teaching assistants were prepared for labs
- Trained teaching assistants to use pertinent equipment and calculations, and answered questions when necessary

Teaching Assistant Western University, London, ON
2011-2012

- Instructed university students in exercise physiology using lab based exercises
- Answered student questions pertaining to course and lab work
- Proctored examinations
- Graded student examinations and provided helpful feedback

Research Assistant University of Waterloo, Psychology Department, Waterloo, ON
2008

- Conducted computerized experiments to UW students to measure cognitive processing of boredom and spatial working memory

- Ensured students received all information needed to execute experiment safely and correctly
- Calculated scores on surveys by forward and reverse scoring
- Performed statistical analysis of the collected data using SPSS

E. VOLUNTEER EXPERIENCE

Emergency Room Information Grand River Hospital, Emergency Department, Kitchener ON 2008-2009

Thames Valley School Board Science and Engineering Fair Judge, London ON 2012-2015

Co-Chair: Raising Hope Division of Strong Bones, Strong Muscles, Strong Minds 2015-2017