University of Alberta

Increases in corticospinal tract function by treadmill training after incomplete spinal cord injury

by

Sarah Louise Thomas 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

Master of Science

Department of Biomedical Engineering

Edmonton Alberta
Spring 2007

Your file Votre référence
ISBN: 978-0-494-30034-3
Our file Notre référence
ISBN: 978-0-494-30034-3

Dedication

For my brother, because I always want to be more like you.

Abstract

In this study, we examined if several months of intensive locomotor training increases the function of spared corticospinal tract pathways after chronic spinal cord injury (SCI). Transcranial magnetic stimulation (TMS) at incrementing levels of intensity was applied over the motor cortex supplying either the tibialis anterior or vastus lateralis muscles and the resulting peak-to-peak amplitude of the motor-evoked potentials (MEPs) were measured to obtain a recruitment curve both before and after training. In the majority of subjects (7/8), 3 to 5 months of daily intensive training increased the responses to TMS in at least one of the leg muscles tested (9/13). Across all muscles tested, MEP_{max} increased by an average of 46%. MEP_{max} was evoked at high stimulation intensities and was also paralleled by increases to motor output, demonstrated at lower stimulations intensities, thus indicating an expansion and/or increased excitability of corticospinal circuits supplying muscles to the lower leg.

Acknowledgements

The most significant portion of my gratitude must go to my supervisor, Dr. Monica Gorassini. Through out my entire graduate experience you have been a constant source of knowledge and patience, and you were never short on laughter. Thank you Monica you were a mentor; you were a friend. I would also like to extend a great appreciation for the members of my committee; Dr. Kelvin Jones and Dr. Richard Stein, both had an integral role in my formal course work, for which I am grateful. Dr. Stein also served to provide countless opportunities for me to be involved in the 'subject side' of research; this I always did enjoy. I would also like to thank Megan Rich, for copy editing my thesis manuscript.

To Jennifer Nevett-Duchcherer, thank you for your friendship, from experimental set up to volunteering in the CARRE lab, thank you for all the many ways you made achieving my Masters possible. Thank you to the physiotherapists in the CARRE lab: Scott Jamieson, Paul Doughty and Kelly Brunton, you provided excellent training and clinical assessments of the subjects, without this my thesis project would have been impossible. Also integral to my thesis project, thank you to the study participants, your dedication to recovery was a constant source of inspiration. I would also like to thank all my floor five comrades, specifically the ladies of floor five, Brenda and Carol Ann, and thank Aiko Thompson; she is the sole reason MatLab did not drive me to insanity.

To the Upper Crust Café, and all those associated with the madness that is that restaurant, thank you. Sheila specifically, you are a woman I admire to no end. Thank you for providing me with a surrogate home where I have always been able to find support, encouragement and food.

Thank you to LOMAH, the land that raised me and to the friends who pulled me through, especially Suzanne and Travis Sjovold. Also in this realm, Robin Clugston, he put up with a significant amount of harassment from both my supervisor and me.

Ryan Wright, for the past 11 years your friendship has been so much a part of who I am. Thank you for every single time you have made me feel better.

Finally, to my family: to mom and dad, thank you for the culmination of events that has shaped me into a person who makes it to the end. Steven, just thank you, as brothers go, you are a champion among men.

Table of Contents

List of Tables

Chapter 3

List of Figures

Chapter 2

Chapter 3

Chapter 4

List of Abbreviations

CNS	Central Nervous System
CPG	Central Pattern Generator
CST	Cortical Spinal Tract
EEG	Electroencephalogram
EMG	Electromyogram
FMRI	Functional Magnetic Resonance Imaging
GABA	Gamma aminobutyric acid
HAM	Hamstring
ICF	Intracortical Facilitation
LTD	Long-term Depression
LTP	Long-term Potentiation
M1	Primary Motor Cortex
MVC	Maximum Volitional Contraction
MEP	Motor Evoked Potential
MEP_h	Motor Evoked Potential Stimulation Half Maximum
MEP_{max}	Motor Evoked Potential Maximum
MEP_{Thresh}	Motor Evoked Potential Threshold
NMDA	N-methyl-D Aspartic Acid
RC	Recruitment Curve
PSTH	Post Stimulus Time Histogram
SCI	Spinal Cord Injury
SICI	Short Interval Intracortical Inhibition
SOL	Soleus
SP	Silent Period
TA	Tibialis Anterior
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation
VL	Vastus Lateralus

CHAPTER 1

Introduction and Motivation

1.1 Introduction and review of spinal cord injury

In Canada, there exist upwards of 42,000 people with spinal cord injuries (SCI) and each year there will be approximately 1,500 additional injuries. Sadly, spinal cord injuries occur largely in young adults with the average age of injury, over the past five years, being 37.6; this is a significant increase from twenty years ago when the average age was 28.7 (data from www.rickhansen.com). The increase in injury age, although still quite young, is due to multiple factors. The two main contributors to this phenomenon are an increase in awareness from drunk-driving campaigns, and an aging population, demonstrated by the more than doubling in incidence of spinal cord injury from individuals over sixty over the past 20 years (www.rickhansen.com). Regardless of when a SCI occurs, those who suffer from this type of injury are left with severe disabilities that will have both physical and emotional ramifications for the remainder of their lives. Post injury, they face living in a world where an individual's ability to walk unaided is taken for granted. For individuals to be rehabilitated, they must relearn to function with resources designed for walking; in most cases their ability to navigate their environment both at work and at home is severely compromised. (Musselman et. al, 2006 in press). The burdens associated with this expand well into the realm of personal well being, health and wellness, and financial expenditure; the bottom line cost of these will ultimately be covered by both the individual and the collective. Currently, both the

provincial and federal governments of Canada spend 750 million dollars annually on costs related to the care of spinal cord injury (data from http://www.rickhansen.com). Maximizing rehabilitation protocol after a SCI, thereby improving the self-sustainability of the individual, will give those who have been affected with this type of injury a better quality of life and also allow for a large reduction in costs to both the individual and the state (Hicks et al. 2005). Currently there is extensive rationale for pushing forward with research in SCI, particularly into restoring self-sustained locomotion by maximizing rehabilitation. Enhanced understanding into the mechanisms that are responsible for improved motor recovery following SCI is fundamental in optimizing life after injury.

1.2 **Direction of research**

Motor control in all organisms is a product of both the peripheral and central nervous system; but, it is the central nervous system (CNS) that has traditionally been recognized as 'hardwired'. During development, functional neural networks are over produced, and this is followed by an extensive period of pruning excess neural cells. The pruned neural circuitry becomes the solidified substrate of the CNS that then operates on a 'use it, or lose it' principal. Once formed, this cellular operating system has traditionally been thought to be unchangeable throughout life. If true, this would mean any damage to the CNS sustained after development would be beyond recovery; this concept takes on specific significance when confronting injury to the adult motor system. Research over the past thirty years however, has demonstrated that conceptualizing the motor system as unfixable after an injury is simply unfounded (Berlucchi 2002; Cooper 2005). The idea

that the motor cortex and spinal cord remains modifiable in adulthood in response to both spinal injury and motor training forms the major subject of inquiry in this thesis.

1.3 REFERENCES

Berlucchi G. The origin of the term plasticity in the neurosciences: Ernesto Lugaro and chemical synaptic transmission. *J Hist Neurosci* 11: 305-309, 2002.

Cooper SJ. Donald O. Hebb's synapse and learning rule: a history and commentary. *Neurosci Biobehav Rev* 28: 851-874, 2005.

Hicks AL, Adams MM, Martin Ginis K, Giangregorio L, Latimer A, Phillips SM, and McCartney N. Long-term body-weight-supported treadmill training and subsequent follow-up in persons with chronic SCI: effects on functional walking ability and measures of subjective well-being. *Spinal Cord* 43: 291-298, 2005.

CHAPTER 2

Background and Literature Review

2.1 Modifiability of the motor system

2.11 Plasticity at the synapse via long term depression and potentiation

There exist countless examples of the modifiability in the CNS well into adulthood. Currently, it is accepted that information networks in the adult brain can be 'rewired', meaning that cortical signaling can be redistributed if, for example, signaling gets disrupted. Disrupted signaling can occur as a result of damage from physical trauma or blood loss, as is what happens with a stroke. This rewiring is made possible by discrete modification of actual cellular structures such as the synapse. SCI occurs when damage is sustained to the main relay of cortical information to the periphery, whereby synaptic connections are lost. Indeed, in line with current research, if damage to spinal structures is incomplete, cortical signaling can be redirected through modification at the synaptic level and this will result in partial recovery (Rossignol 2006) This concept of reworking CNS neurons and networks has been coined cortical plasticity.

Simple reflex systems like the gill withdrawal reflex in Aplysia help in understanding the mechanisms responsible for cortical synaptic plasticity. Although first recognized by Charles Sherrington, (Sherrington 1975)(CS Sherington 1906), habituation (or depression) of simple reflexes can be seen in the cat, but are mechanistically easier to understand through studies done in Aplysia. Aplysia are preferential because they provide an uncomplicated system in which to map change in behavior, like habituation. For

example, habituation occurs when an animal is repeatedly exposed to a harmless stimulus and the response is down regulated. If an Aplysia's siphon is exposed to a harmless tactile experience their respiratory organ (gill) will contract. Repeated exposure to the same tactile stimulation will diminish this response as the Aplysia 'learns' that the stimulation is not dangerous. On the other hand, sensitization will occur if there is a negative experience from a repeated stimulus with the resultant behavior being up regulated. In this case, the Aplysia would have an even larger gill withdrawal response. The altered behavior is manifest because of a change in the effectiveness of synaptic transmission; this occurs through a modulation in the number of transmitter vesicles that are released. For example, sensitization occurs through the activation of a receptor coupled to a G protein that is released upon the binding of a specific receptor type, in this case a 5HT-receptor (Figure 1). Binding of the receptor sets off a cascade of events ending with the activation of protein kinase A, responsible for phosphorylation of potassium channels. Once a potassium channel has been phosphorylated there is a decrease in the potassium current, thereby prolonging the action potential and with it, the influx of calcium. Calcium is known to facilitate transmitter release and thus, a larger post-synaptic response (producing gill withdrawal) is produced. To add to this, L-type calcium channels are opened allowing for more calcium to flow into the cell, also occurring through the activation of a G coupled receptor, and there is an increase in excocytotic release of neurotransmitter (Kandel ER 2000).

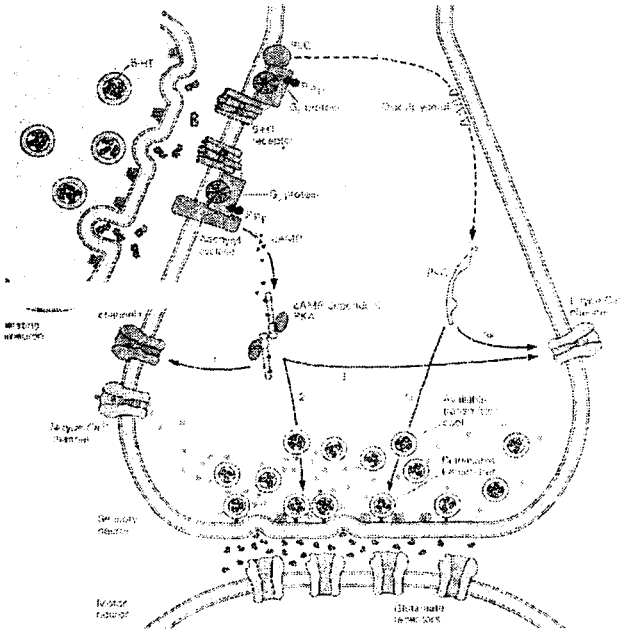

Figure 1:

Short-term sensitization of the gill withdrawal reflex in Aplysia. (adapted from Kandel et al. 2000).

Similar mechanisms have been implicated in describing the long-term potentiation (LTP) of hippocampal cells that are responsible for learning and memory in vertebrates. One of the neural components implicated in this process is the NMDA receptor. The NMDA receptor regulates cellular permeability to calcium but requires multiple synaptic events to be activated. Similar to simple reflexive models, changing the cellular concentration of calcium has a direct effect on the amount of transmitter that will be released during hippocampal synaptic transmission and will also alter cellular response. As mentioned above, activation of the NMDA receptor needs two events, as it is both ligand and voltage gated. First, the NMDA receptor must be bound by glutamate, the neurotransmitter released by the presynaptic neuron. Second, the post-synaptic neuron needs to be depolarized. This occurs after a sufficient number of non-NMDA receptors have been bound by glutamate. The drop in voltage will result in a conformational change of the already bound and active NMDA receptor, allowing for the magnesium ion, which serves to block ion flow, to be released. Once these two events have occurred,

the NMDA channel has open permeability to calcium and the ion flows into the cell. The short term ramification of NMDA activation is an increase in calcium, which in this case leads to cellular mechanisms that increases non NMDA receptors' sensitivity to glutamate, as well as acting through a retrograde messenger to enhance presynaptic transmitter release (figure 2). Experimentally, an increase in cellular calcium has been shown to be necessary for the long-term potentiation (LTP) of synaptic connections (Dunwiddie and Lynch 1979; Popov et al. 1988). An animal repeatedly exposed to a noxious stimulus will experience cellular LTP and eventually show the exaggerated behavior as the basal response (Croll 2003). In this case, behavioral learning is explained through the mechanism for LTP and synaptic plasticity and these are causally linked to morphological changes to cellular components.

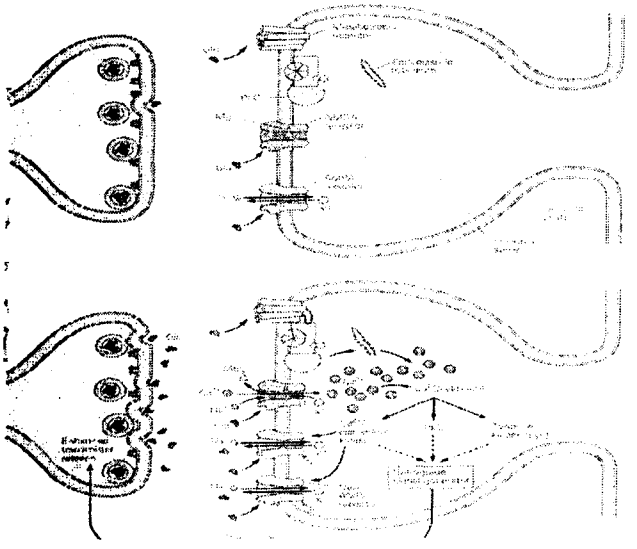

Figure 2:

Induction of the early phase of long term potentiation (adapted from Kandel et al. 2000).

Repeated activation of the NMDA receptor, responsible for continuous dumping of calcium into the cell, enables a cascade of events that will lead to gene activation and protein synthesis; therefore, long-term potentiation translates to a sustained change in behavior, which is a product of protein synthesis. Proteins are responsible for the growth of new synaptic connections between cells that have been physiologically altered from

LTP. For example, animals that show sensitized behavior will have a greater number of presynaptic terminals compared to animals that do not show sensitized behavior (Kleim et al. 1996). Conversely, animals that show habituated behavior will have a decreased number of presynaptic terminals. In summary, long term changes in behavior are due to alterations in the morphology and hence the physiology of the cell. In this particular case, the addition of presynaptyic terminals results in a different physiological environment during cellular signaling. There are changes in transmitter output, modified receptor binding sites, and ultimately, an altered behavioural response. It can be said that similar mechanisms responsible for the Aplysia's elegant behavioural adaptation in the gill withdrawal reflex are also responsible for changes seen in vertebrate hippocampal cells and this can loosely explain learning and memory in the cerebral cortex (Buonomano and Merzenich 1998).

2.12 LTP and LTD in cortex

In mammalian systems, the cortex is arranged so that information generally flows vertically through the cortical tissue. Cellular components are segmented into six layers that receive, process and output information respecting this 'vertical flow'. For each vertical column, information will generally come into the cortex from neurons projecting from the thalamus, primarily terminating in cortical layer four. Horizontal connectivity does exist but the primary function of these connections is to ensure vertical cortical columns stay connected with neighboring cortical cells. For example, in the visual cortex recording cellular activity demonstrates that horizontal connections occur only with cells that have similar functional specificity (Ts'o et al. 1986).

Similar to the occurrence of cellular conditioning in the Aplysia, it is possible to induce LTP in mammalian cortical cells. For example, an experiment that applied a spaced pairing protocol demonstrates that associative LTP can be induced in cat cortical cells (Buonomano and Merzenich 1996). In this experiment, cortical pyramidal cells were activated by horizontal inputs, the cell bodies which could be found in layer II/III. This mimicked post-synaptic depolarization by pairing the horizontal stimulation with an intracellular depolarizing pulse. Cortical layer II/III was chosen because it is the primary location for horizontal connections to the pyramidal neurons; pairing was repeated every five seconds for seven minutes. To determine if LTP was induced specifically from horizontal inputs, paired stimulation was also performed by activating pyramidal cells from vertical inputs, with bodies located in layer V1 neurons. The control (vertical) paired stimulus protocol did not produce significant potentiation, as potentiation only occurred when the pairing pathway was integrated through horizontal projections, increasing the excitatory postsynaptic potential of pyramidal neurons by 150%. It is important to note that different pairing protocols can exist and, by altering the stimulation protocol, an experimenter can produce differing physiological responses. For example, if a protocol increases the NMDA current, the more consistent the induction of long term potentiation will be. Conversely, if the NMDA current is reduced there will be a down regulation of activation, possibly leading to long term depression (LTD). This experiment and others like it signify two things; first, cortical cells are subject to similar learning mechanisms as hippocampal cells in the rat, which was the first mammalian model used

to demonstrate LTP (Classen et al. 2004). Second, in the adult mammal, associative pairing is most likely to occur through horizontal, not vertical projections (Buonomano and Merzenich 1996). This will become more important when contemplating the role of cortical interneurons in behavioural learning and, further, how mapping studies in the motor cortex of human subjects can help us understand motor learning.

2.13 Animal models: synaptic plasticity and cortical representation

Studying motor control in the mammalian system presents an opportunity to research experience driven cellular change, the existence of which will most notably be manifest to the observer as behavioural learning. In the human, this behavioural learning could equate to riding a bike, skating or learning how to do a back-handspring. Regardless of the skill acquired, the study of behaviour in the animal model presents a method of investigation into the causal link between the synapse, the cell and the cortical collective and further, how they combine to explain behaviour. For example, when rats are trained to successfully perform a complex motor task, it would follow that there should be experimental evidence to support functional changes in the synaptic organization of the corresponding motor cortex. Rats can be trained to reach through a relatively narrow slot for food pellets that have been placed on a distant rotating table, a considerably complex skill for a rat that requires a significant amount of training (Kleim et al. 1996). Rats that become efficient at this complex food retrieval task show increases in synaptic density in the motor area of the trained hand; this is compared to the hand area of rats trained to depress a lever for food retrieval, comparatively a simple skill. This study by Kleim et al. sets a significant correlation between the acquisition of a skilled behaviour and the

corresponding synaptic enrichment of cortical cells representing muscles involved in accomplishing the trained skill.

Experience will change the synaptic morphology of neural cells and it follows that numerous other cortical components in a signaling pathway will also be subject to experience induced changes. As expected, training also results in the enrichment of output cells projecting within the associated motor cortex (Rioult-Pedotti et al. 1998). This suggests that parallel increases do extend through multiple cortical components of a signaling pathway, and this must be necessary for the successful processing and propagation of information required to express a new skilled behaviour. One example demonstrates that there is a training induced increase in the dendritic arbor of pyramidal cells projecting from the corresponding motor cortex (Kleim et al. 2004). Based on these findings, the authors propose that the changed behaviour is a result of anatomical modifications, which are solidified through training, or as the case may be, experience. The anatomical changes that are seen should also be paralleled by changes to the physiological output from a given motor area.

Indeed, skilled learning has been shown to strengthen cortical output in the primary motor area of the brain and this can be qualified using microelectrode stimulation on the rat motor cortex (Kleim et al. 1998). In rats, surface electrodes record electromyogram (EMG), and can measure cortically induced responses in the musculature; this effectively gives a 'map' of cortical representation. When adult rats are allocated to perform either a skilled or unskilled reaching task there is a significant increase in the mean area of wrist

and digit representation for the rat participating in the skilled task. In this case, skill acquisition occurs as the distribution of cells dedicated to evoking a muscle response gets larger. As expected, the number of sites that produced a motor response in muscles active during the trained skill increased significantly in the cortical fields of both the wrist and digits (Kleim et al. 1998). This increase in motor cortical output constitutes cellular learning that is manifest by the changed behaviour.

The rationale from the above experiments suggest that motor training will induce synaptic plasticity on a cellular level, increasing both synaptic and dendritic density in cells specific to the trained behaviour; the net outcome is altered cortical representation and improved functional performance. Similar animal studies have directly linked modification in cortical representation to increased physiological response. A recent experiment in rats demonstrated that skilled learning is partnered by motor map reorganization through a significant increase in the number of synapses per neuron, localized to the map location showing heightened excitability (Kleim et al. 2004). Further studies have been devoted to investigating what types of experience will facilitate altered cortical representation of peripheral musculature. For example, is passive moving enough for training to be cortically altering and behaviorally beneficial? Many studies have shown that by further qualifying the experience by making it active or voluntary enhances the effect on motor cortical function.

Plasticity studies in animal models have elucidated that cortical expansion, measured by structural and physiological changes, can be facilitated by two different circumstances.

Firstly, on a use or experience dependent basis where sensory input is increased, this includes skill acquisition and motor learning as highlighted above. Secondly, decreasing sensory input from a discrete peripheral location induces heightened excitability for the remaining intact inputs. An example of sensory deprivation plasticity can be demonstrated in the barrel cortex of the rat (Xerri et al. 1994). The barrel cortex is a subset of cortical cells in the primary motor cortex of the rat and is responsible for controlling motor function in the whiskers. It is organized such that specific topographical arrangement of cortical representation corresponds to the spatial location of the actual vibrissa. When all but two neighbouring vibrissae are clipped, termed whisker pairing, two things happen: First, there is enhanced excitability in the cortex receiving the spared inputs; and second, there is a depression of excitability from the cortex receiving the lesioned inputs (Rema et al. 2003). This is similar to denervation experiments in the monkey where by transecting the median nerve, sensory information from the ventral portion of specific digits is removed and the intact cellular components from sensory cortex will become hyperactive (Recanzone et al. 1992; Wall et al. 1986).

In mammals, when sensory input no longer feeds back to cortical cells, there is an unmasking of border representations from the remaining intact sensory inputs; these unmasked inputs will 'take over' cortical tissue originally representing the body part that had the afferent input disrupted. The cortical reorganization is presumably done by similar cellular mechanisms that induced experience based LTP and LTD studied in animal models; this effectively demonstrates that when sensory input is lost, there is a "shrinking" of the cortical representation for the peripheral musculature failing to feed

back to the cortex. The de-afferented cortex is then taken over by the neighbouring cortical cells with intact afferent input.

Cortical reorganization is made possible largely because of the cellular organization of the motor cortex. As discussed earlier, information in the cortex will flow primarily along vertical columns, running perpendicular to the surface of the cortex. This is consistent with the spatial orientation of most of the excitatory cells in the cortex, which extend vertically through the neocortex. Structurally, the columns generally measure about 50μm in diameter and contain 80-100 neurons (Mountcastle 1997). Inhibitory cells on the other hand primarily have a horizontal orientation, projecting out to form a larger 'column of influence' that will generally measure between 300-500μm in diameter. These horizontal projections will encompass between 50-80 of the vertical columns. Neurons that are linked by these horizontal projections will share common features, such as similar receptive fields in the primary sensory cortex and similar motor output in the primary motor cortex. When afferent input is removed, the lack of excitatory drive results in a down regulation of these horizontally oriented inhibitory neurons. Neurons that once received input from horizontal projections are now dis-inhibited and can be activated by afferent input that was previously "muted". Once new activation pathways are unmasked, further strengthening of these connections is possible through LTP. This is an oversimplified explanation of how de-afferentation possibly serves to re-work receptive fields in the cortex (Ziemann et al. 1998a)

As noted above, plasticity can also be facilitated dependent on use. For example, changes in cortical excitability can be seen in the receptive field of nipple bearing skin in lactating female rats (Xerri et al. 1994). Compared with non-lactating female rats, there is a near doubling in the receptive field of nipples in nursing rats due to the increased sensory input from the suckling babies. By increasing specific sensory input, the area of cortical excitability is enlarged. This phenomenon has been replicated in monkey studies by having them use specific digits to discriminate between successive vibratory stimuli differing in frequency, making it an active sensory experience (Recanzone et al. 1992). As to be expected, the population of neurons that responded to the training stimuli enlarged several-fold and demonstrated that animal models have a cortical receptive field that is highly dependent on either the maintenance or removal of afferent inputs from the periphery. Ultimately, these receptive fields are malleable through experience and it is possible for experience dependent alterations in cortical representation to occur well into adulthood. Complimentary to these studies, altered cortical excitability has also been demonstrated in the adult human by using transcranial magnetic stimulation (TMS) and imaging techniques to quantify cortical output as will be discussed below.

2.2 Cortical plasticity in humans

2.21 Techniques for measuring cortical reorganization in the human

Similar to animal studies, sensory motor experiences in the human will also induce cortical reorganization that can be associated with improvements in performance. As mentioned above, single-pulse TMS can be used to measure cortical excitability and has

been beneficial for studying the modifiability of the adult human motor system (Terao and Ugawa 2002). TMS works through electromagnetic induction to produce a depolarizing current in cortical tissue. When the stimulation is applied to a discrete location on the scalp surface, motor cortical cells deep to the area of application are activated. If the pool of cellular activation is large enough, an action potential is sent along the corticospinal tract to interneurons/motoneuorns in the spinal cord and a muscle twitch occurs in the periphery. This stimulation can be applied at incremental levels of intensity allowing for a measurable patterned output from the contralateral muscle (Devanne et al. 1997) Muscle output is measured by placing surface electrodes on the superficial skin layer and recording the voltage drop, known as the motor evoked potential (MEP). The magnitude of the MEP from increasing stimulation intensities generally fits a sigmoid function that will plateau when a maximum MEP has been reached; this is termed the recruitment curve (RC). Motor threshold refers to the lowest TMS intensity necessary to evoke a MEP in the target muscle and is demonstrated by an increase in the slope of the sigmoid fit. These specific parameters of muscle output can be compared to signify changes in motor cortical excitability. More specifically, changes in the size of MEP at comparable stimulation intensities will suggest either a strengthening or weakening of cortical sites for that particular musculature. Experiments involving non-invasive techniques provide convincing evidence in favor of the existence of cortical plasticity in the mature human CNS; this plasticity seems to be maximized when motor training is of a voluntary nature.

2.22 Skilled motor learning

There have been numerous TMS experiments that demonstrate training or experience induced reorganization in the sensory-motor cortex of healthy human subjects (Karni et al. 1995; Lotze et al. 2003a; Perez et al. 2004). The successful ability for training to alter the representational organization of the motor cortex is dependent on the type of training where the effects of active training seems to supersede that of passive training. For example, subjects who undergo short duration training (30 minutes) with either active (voluntary) or passive (non-voluntary) wrist movements showed improved behavioural performance under the active training protocol (Lotze et al. 2003a). In this case, active training includes voluntary wrist flexion and extension whereas passive training creates the same range of motion in the subject by attaching them to a torque motor. Improved behavioural performance can possibly be explained by improved cortical representation facilitated through an active training protocol. This hypothesis is supported by several complementary measures demonstrating active motor practice changes the effectiveness of cortical cell signaling. Firstly, comparative RCs from the two training conditions demonstrate improved cortical output from the active training. Secondly, intracortical facilitation (ICF), also a measure of cortical excitability, was significantly larger as a result of the active training. Finally, functional magnetic resonance imaging (fMRI) showed a larger area of activation with active as compared to passive training. FMRI is an imaging technique commonly used to measure cortical activation and it works by measuring the blood oxygen levels in the tissue, in this case the cortex; oxygen levels in cortical tissue are assumed to be an indicator of neural activity. The inference made is

that an increase in cellular oxygen content indicates an increase in cellular activation and further, a cortical strengthening in representation.

Although this study does not demonstrate passive motor training as a viable method of inducing cortical reorganization, other studies have shown that passive training will elicit an alteration in fMRI activation patterns in healthy volunteers, but only after several weeks of the training protocol (Carel et al. 2000). Despite evidence that passive motor practice can improve cortical output and therefore performance, active training is predominantly more effective. This is highlighted through numerous studies that use skilled motor training to induce changes in the organization of the primary motor cortex (M1), demonstrated by an expansion and increased excitability of cortical tissue representative of upper limb motor control (Karni et al. 1995; Lotze et al. 2003a; Lotze et al. 2003b; Pascual-Leone et al. 1993; Pascual-Leone and Torres 1993; Pascual-Leone et al. 1995). Alteration of cortical representation in the lower extremity muscles is consistent with the above-mentioned experiments. Again, active training is significantly more effective than passive training with respect to its ability to induce a reorganization in the cortex (Perez et al. 2004). In the following experiment, Perez demonstrates that active motor training, where subjects match ankle position to a moving target, increases the RCs measured from lower extremity musculature; this only occurs when the training protocol is active. To further support the cortical mechanism of training induced enhancements in muscle activation, this study also uses transcranial electrical stimulation (TES) as a test measure. RCs from TES failed to measure increases in muscle activation in either the passive or active conditions. Muscle activation from TES occurs at the axon

hillock (discussed in a future section). When activation is direct, it is not suggested to include inter-cortical contributors; therefore it would not reflect a cortical enhancement in excitability. This is different from TMS activation and further supports active training as means of driving training induced cortical reorganization, specifically because applying TES does not demonstrate increases in muscle activation. Although this study did not show an increase in ICF there was a significant reduction in short-interval intercortical inhibition (ICI) from TMS. Several studies suggest that ICI will contribute to plasticity in the primary motor cortex through horizontal cellular networks. More specifically, direct evidence from cervical epidural space recordings reveals that short term ICI has an intercortical origin, evidence for facilitation is not as conclusive (Di Lazzaro et al. 2001; Di Lazzaro et al. 2006; Nakamura et al. 1997)

Experience over time can cause long lasting alterations in the cortical organization in the human. This has been demonstrated repeatedly in various experiments. Similar results occur despite differences with test groups and training protocols; the area and extent of cortical reorganization has been shown to be skill specific. For example, Braille readers have an unusually large cortical representation of the first dorsal interosseous muscle, musculature for the reading finger (Pascual-Leone et al. 1993).The increased cortical area occurs by taking over areas that are normally associated with other finger representation comparative to a non-braille reading population. Extensive training in other muscle groups, specifically proximal muscles will cause similar phenomenon. For example, volleyball players who trained at least seven years at a national level demonstrate increased cortical representation of muscles typically involved in motor specific activity (Tyc et al. 2005). The enlarged cortical representation does not occur in athletes who

spend a comparative amount of time training an activity that is not task specific to upper limb musculature. In this case, the control activity was running. Additive to this, volleyball players show asymmetries across hemispheres. Comparisons of between-hemisphere recordings demonstrate that cortical representation is significantly larger on the side contralateral to the preferred strike. Again, these asymmetries do not present in athletes unless they are subject to specific upper arm training. The authors suggest that cortical expansion in volleyball players occurred because of an overlapping of cortical representations for distal musculature with that of proximal musculature, proximal mapping being that which expanded. Research suggests that short-term increases in excitability from actively practicing specific motor skills will lead to a long-standing restructuring of cortical representation when the practice is repeated over time. It is likely that this is maintained through anatomical changes to the neuron similar to the increases in spine density seen in rats that received skilled motor training. These studies, both in animal and human models, provide a foundation for investigating rehabilitation after injury involving the CNS.

2.23 Afferent Feedback

Much like whisker pairing in the rat motor cortex, injury to the human CNS initiates a reorganization of cortical representation. CNS damage results in a reduction of information propagation that is twofold. First, there is a reduction in muscle activation through impaired cortical signaling regardless of whether it occurs through the loss of cortical tissue, as in stroke or through disrupted descending transmission, as in SCI.

Second, due to either a compromised signaling pathways or the loss of muscle activity, there is a reduction of afferent feedback to supraspinal structures. This afferent information is crucial since it keeps the brain informed of where and how a muscle is moving. To do this, skeletal muscle contains receptors and these receptors provide feedback to the brain. The two main muscle receptors responsible for this are the muscle spindle and the golgi tendon. The first of these, the muscle spindle, has a tonic-firing rate when the muscle is at a basal length; when stretched passed that length, the muscle's firing rate will increase. If the muscle is contracted, the neuron will become silent until the muscle is either released from contraction or the basal length is reset. The muscle length that the CNS recognizes as 'normal' can be reset. To do this, muscle spindles are also interconnected with intrafusal muscle fibers. The intrafusal muscle fibers will contract or relax in order to reset the physical parameters of the muscle; thus allowing the spindle to reset for different muscle lengths. The second muscle receptor, the golgi tendon organ, responds a little differently to muscle activation. Whenever a muscle is loaded or under tension, the firing rate of the golgi tendon organ will increase and this can happen under both conditions of contraction or extension; however, this receptor is much more sensitive to a contraction. In cases of injury, if the muscle specific signaling mechanisms are disrupted, a void of sensory input will occur and the system will become unbalanced.

2.24 Injury induced plasticity - amputation and ischemic nerve block

Case studies of de-afferentation can be used to demonstrate the importance of afferent feedback and the consequence of losing that afferent feedback will have on cortical structures. Two examples of reduced sensory input can be easily explored. The first occurs in circumstances of actual limb amputations; the second is an amputation model that works by ischemic block to peripheral nerves and musculature. One speculation that explains the resultant modulation in cortical structures is the removal of sensory feedback. This is supported through experiments where ischemic nerve block is applied by inflating a blood pressure cuff above a subject's systolic blood pressure; this will cut off the blood supply to sensory nerves that feeds back to a select area of the brain. Within minutes there occurs a several-fold increase in MEP's measured from muscles adjacent to and above the ischemic block. This facilitation disappears roughly 20 minutes after the block is removed (Brasil-Neto et al. 1992; Ziemann et al. 1998b).

Limb amputation results in a similar outcome, as compared to the ischemic block of sensory input. TMS applied to motor cortical sites will evoke MEPs with a lower threshold and increased excitability in muscles immediately proximal to the site of the amputation. In addition, the cortical map expands as there is an increase in the number of stimulation sites that will evoke a muscle response, compared to the non-amputated side (Cohen et al. 1991; Fuhr et al. 1991; Ridding and Rothwell 1997). In a separate study, TMS was used in conjunction with TES and spinal electrical stimulation (SES). The results demonstrated that there was an increase in excitability with TMS, which was

absent when stimulation was done with either of the two other techniques. Again, this suggests that the reorganization will occur primarily at the motor cortical level, rather than spinal, level (Chen et al. 2002; Nudo 2003). PET studies confirm these results by showing increased regional cerebral blood flow to areas adjacent to either amputation or aschemic nerve block, again providing evidence to suggest an expanded cortical representation. This is similar to mapping studies involving SCI subjects where the number of sites that elicit a response from muscles rostral to the level of injury also increase (Levy et al. 1990; Topka et al. 1991)

In conditions involving an amputation or ischemic block (this is similar to SCI), cortical representation for muscles near an area with compromised peripheral feedback will show a facilitation of the intact area's receptive field. This expansion occurs because intact projections begin to 'claim' nearby areas suffering from reduced incoming signaling. When there is a reduced level of input from the periphery, cortical tissue compensates by increasing excitability. Those inputs from nearby intact areas will likely have extended collateral projections into the recently de-afferent areas. The reduced sensory feedback will initiate amplification for any remaining projections, since cortical tissue is no longer receiving its expected level of sensory information. It is possible that the lack of sensory feedback sets up a disinhibition in the motor cortex through the GABA(ergic) system and this is what causes the increased excitability. This has been supported by numerous studies (Hess and Donoghue 1996). Deafferentation models hold specific significance for both SCI and stroke considering both injuries result in the loss of afferent input initiating reorganization of cortical representation for neural tissue no longer receiving input from

the periphery. In either case, the net result is a taking over of quiescent tissue in favour of the intact projections. Multiple studies have suggested evidence for the reorganization of cortical output that results from a SCI. In fact, studies using TMS to measure cortical reorganization show there is an expansion in the motor map for musculature that is rostral to the level of injury, similar to deafferentation models (Davey et al. 1990; Levy et al. 1990; Topka et al. 1991). There is also an accompanied increase in the number of sites that will evoke a motor response from TMS as compared to normal. Finally, there is a shift in the influence that background muscle activity has on the motor response. When TMS is applied in the presence of a background contraction, healthy subjects will show facilitation in the amplitude of the MEP at specific stimulation intensities and this tops out at 10% of the subject's maximum voluntary contraction. In injured subjects, the size of the MEP will continue to be enhanced by background level of as much as 50% maximum facilitation (Davey et al. 1999).

Both fMRI and EEG studies demonstrate similar shifts in cortical excitability. For example, fMRIs of subjects with cervical SCI show a shift in the site of cortical activation for the control of intact tongue movement into the adjacent cortical area for the hand affected by the spinal injury (Mikulis et al. 2002). Similar, in an EEG study, surface electrodes were placed on the scalp to record changes to the motor potentials associated with finger and toe movements, control for both was below the level of injury (Green et al. 1999). Compared to EEG output from an uninjured group, motor potentials recorded during movement were more posterior, suggesting an injury-induced shift of representation. In addition, the three subjects regained movement control and, also, repeat

testing showed an associated anterior shift that correlated with their recovery. The authors were careful to note that the initial posterior shift should not lead to the assumption that the change in excitability was either beneficial or detrimental, but rather a non-quantifiable event.

2.25 Stroke rehabilitation: functional plasticity after injury

Stroke is another example where damage to the CNS results in a reorganization of cortical representation. However in stroke, unlike the examples given above, the disruption to signaling primarily occurs at cortical and or sub-cortical levels. There are two types of stroke, ischemic, when there is a blockage in neural blood flow and heamorragic, which occurs when a blood vessel breaks and blood seeps into neural tissue. Whether stroke is ischemic or heamorragic the net result is cell death due to lack of oxygen and therefore loss of neuronal tissue, or brain damage. Much like amputation, stroke is partnered with changes that will alter the excitability of cortical tissue, the extent of which can be linked to functional recovery. Further, the extent of functional recovery can be optimized with training. It is possible that this functional recovery occurs in part from cortical remodeling and this can be explained twofold. First, there could exist parallel redundant pathways that will reroute cortical commands, and second, it may be possible for new brain regions to take over the function of a damaged area via unmasking or a release of inhibition. Evidence exists to support both possibilities (Cramer et al. 1997; Seitz et al. 1998; Weiller et al. 1993). In actuality recovery is case specific and most likely a culmination of the two (Feydy et al. 2002). Studies that used fMRI on subjects who were one to six months post-stroke correlated cortical reorganization, site of

lesion, and degree of motor recovery; they showed two main patterns of cortical reorganization. The first pattern of activation, termed focussing, was characterized by widespread activation of ipsilateral and contralateral areas that would progress to a focused activation in the contralateral sensorimotor cortex as improvement occurred. The second pattern of activation, called persistent recruitment, had initial ipsilateral activation patterns that remained consistent throughout recovery. This sustained recruitment of ipsilateral activity seemingly occurred when neural damage was primarily in M1. Overall, the pattern of recruitment did not seem to have a profound effect on the quality of recovery. It was suggested that both patterns can be successful assuming the capacity for cortical reorganization is large enough. An association can be drawn between a persistent recruitment using parallel redundant pathways to mediate recovery, and similarly with focused recruitment allowing for recovery by the unmasking and strengthening of previously inhibited cells. It is important to note that the quality of recovery was not linked to either type of shift in cortical activation. However, this study demonstrates a correlation between the amount of stroke induced Wallerian degeneration to cortical tissue and the level of functional recovery. Ward et al. (2003) demonstrated that the recovery capability of motor system post injury is largely dependent on the integrity of the remaining cortocospinal system (Ward et al. 2003; Ward et al. 2006).

2.3 Rehabilitation

2.31 Driving plasticity

All of the above examples of nervous system damage are similar in that there is a reduction in sensorimotor signaling giving rise to cortical reorganization and possible

compensation for lost tissue, hence lost function. Injury induced cortical reorganization is a phenomenon that can be harnessed through recovery protocols in order to maximize rehabilitation, allowing for optimal functional recovery after an injury. Numerous stroke studies demonstrate how therapy induced improvements are largely due to cortical reorganization. For example, patients can stagnate in their recovery after suffering from a stroke; this is the chronic stage of stroke. Further recovery can occur for chronic stroke patients by treating them with constraint induced therapy (Liepert et al. 1998; Taub 2000; Taub et al. 2003). Constraint induced therapy maximizes the time the injured limb is used by dis-allowing the use of the good arm and therefore forcing practice in the bad arm, contralateral to the side of cortical damage. In one particular study there was a noticeable increase in the functional ability of the injured arm after completing just 12 days of constraint. The authors suggest a use-dependent cortical reorganization occurred and this gave rise to the therapeutic effect (Liepert et al. 2000). In this study, arm use was quantified by a motor activity log and then correlated with the number of TMS stimulation sites that evoked a muscle response. As use improved, so did the number of sites that would cause muscle activation. In addition, after training there was a closer comparison between the injured and non-injured hemispheres with respect to the number of sites that could induce a motor response.

Not only can functional cortical reorganization be encouraged through active use, but artificial stimulation protocols can also enhance functional recovery. As with stroke, patients often suffer from oro-pharyngeal dysphagia, which affects their ability to swallow and puts them at risk of aspiration. In an attempt to harness the constructive

effects of plasticity driven through sensory experience, both healthy subjects and stroke patients were exposed to pharyngeal stimulation (Fraser et al. 2002). Depending on the stimulation protocol, sensory exposure evoked either facilitation or depression in the motor response of the pharynx. Through stimulation application to the healthy subjects, the optimal stimulation protocol for facilitation was experimentally determined to have the strongest effect when it was applied at a frequency of 5hz for 10 minutes at 75% maximum tolerated intensity. Stroke patients who were exposed to the optimal stimulation protocol showed an increased excitability in response to TMS stimulation, comparative to stroke patients who were exposed to a sham stimulation protocol. There was also an overall increase in the number of cortical sites that evoked a muscle response from patients who had been treated with the stimulation procedure. Cortical outcome measures were paired with behavioural tests that suggested improved functional behaviour: a decrease in swallowing response time, pharyngeal transit time and a reduction in aspiration. Imaging data also supported the increase in excitability from sensory stimulation. Healthy subjects showed an increase in the area of motor cortical activation while swallowing after receiving the pharyngeal stimulation. The authors make an important point of noting that they did not find any evidence of pharyngeal stimulation increasing the excitability of brainstem reflexes in the pharynx. The most probable explanation for these observations is that improvements in swallowing are primarily explained by changes that occur to the cerebral cortex. Changes in the cortical representations of the adult human brain have the potential to drive functional recovery once damage to the CNS has occurred. Ultimately, this can be enhanced through increasing exposure to a sensory experience. Numerous studies have demonstrated that

nerve stimulation alone can work towards enhancing cortical control of musculature. Nerve stimulation results in an up-regulation in both muscle activity and sensory feed back (Day 2000; Ridding and Taylor 2001). For example, an experiment that included the use of functional stimulation to the common peroneal nerve resulted in promising evidence suggesting this up-regulation of both motor and sensory activity leads to cortical enhancement (Knash et al. 2003).

2.32 Body weight supported treadmill training

Body weight supported treadmill training is one way to increase the sensory exposure after injury. This technique involves suspending a patient from a motorized treadmill such that the amount of body weight a subject must support is modifiable. Reducing the weight that patients carry though each step makes it easier for them to control their limbs through the step cycle and this will allow for enhanced and patterned sensory feedback. The rationale for weight supported treadmill training arose from animal studies investigating motor learning after a complete spinal cord transection in the adult cat. These studies demonstrated that cats that have undergone a complete transection of the spinal cord are still capable of generating stepping like movements (Eidelberg et al. 1980; Grillner and Zangger 1979; Pearson and Rossignol 1991). The spontaneous recovery of function will further improve if the cats are step trained post injury (De Leon et al. 1998; Edgerton et al. 1997). This phenomenon seems to be task specific since cats that receive stand training improve in their ability to stand only, while cats that undergo step training are better steppers but fail to improve their standing ability (De Leon et al. 1998). Since

recovery of function in cats can occur with complete spinal transaction, plasticity in the remaining spinal circuitry may mediate functional locomotor recovery. Investigation into possible mechanisms responsible for recovery demonstrates that following a SCI there is an increase in the levels of inhibitory transmitters. Experimentally, it has been show that the initiation of rehabilitative training can reduce this inhibition (Edgerton et al. 2001; Robinson and Goldberger 1986; Tillakaratne et al. 2000). Similar to this, the application of pharmacological agents that artificially reduced post injury levels of inhibition can directly facilitate short term improvements in locomotor output (de Leon et al. 1999). In contrast to the short term facilitation mediated by drug application, task specific improvements from treadmill training are retained well after the training is stopped and this suggests a persistence of change beyond the reduction in inhibition, which is occurring within the spinal pathways (De Leon et al. 1999) . Unlike cats however, individuals with a SCI will only show marked improvement if their injury shows salvaged descending drive. This is indicative of the increased importance of the cortical spinal tract (CST) in humans, making it likely that functional improvements are due to a combination of both spinal and cortical enhancements.

2.4 Human Locomotion

2.41 CNS supraspinal contribution

Functional walking in a dynamic, ever-changing environment involves the convergence of information from the central nervous system including supraspinal, brainstem, spinal structures and feedback from the periphery. Supraspinal structures contributing to

locomotion include the sensory motor cortex, basal ganglia and cerebellum. It is through these structures that the brain creates a type of blue print copy of expected body movement. In part, this occurs through the latter two cortical structures forming feedback loops with the motor cortex, as this is thought to influence the creation and timing of coordinated motor programs. Benefits of this include combining complex motor programs, which will enable walking to occur in conjunction with other motor acts, like running and bouncing a ball during a basketball game. Having a blue print for motor programs is also beneficial when the body is subject to an external condition where the actual movement needs to be different from the expected movement as this capability will enable the system to recognize deviations and react efficiently.

Once motor information is processed through supraspinal structures, input from these structures is then funneled to the brainstem, which then projects to spinal segments of the CNS and in turn to peripheral musculature via two pathways. First is the ventromedial pathway, which is composed of the tectospinal tract, vestibulospinal tract and the reticulospinal tract. Second is the lateral pathway, which is composed of the rubrospinal tract and the corticospinal tract (CST). Of great importance to humans, the CST is a monosynaptic pathway projecting directly from the primary motor, premotor and supplementary cortices to motorneurons of the spinal cord. It has a pertinent role in contributing to locomotion, relaying monosynaptic input from supraspinal structures to the musculature. The importance of the CST becomes apparent when comparison is made between incomplete and complete spinal injured subjects who both receive rehabilitative training. Subjects who train after suffering a complete motor SCI never regain

functionally relevant skills, in opposition to less severely injured subjects who have salvaged cortical drive and do show degrees of functional recovery (Dietz et al. 1994b; Dietz et al. 1995; Dobkin et al. 1995). This is contrary to experiments done on fully transected cats, demonstrating functional improvements with a complete cord transaction after training. For example, it is well documented that cats receiving a complete spinal transection of the thoracic cord have been shown to regain locomotor control over their hind limbs. Motor control experiments, done in the cat, can provide rationale for weight supported treadmill training in humans, as discussed above. Indeed, evidence from human subjects has demonstrated motorized treadmill training to be highly effective in inducing functional recovery in anatomically incomplete spinal injured subjects (Barbeau and Rossignol 1987; Wernig et al. 1998). Subjects, who train on a weight supporting treadmill, will show significant functional gains but only if their injury has salvaged minimal projections from the CSTs. This evidence further suggests that recovery of motor control after injury to the CST does require participation from the motor cortex.

Investigation into the exact significance of motor cortical contribution during walking has led to studies involving the intact motor system of the adult human (Capaday et al. 1999; Petersen et al. 2001). These studies demonstrate that TA activation during the swing phase of walking comes partially from the motor cortex via the corticospinal tract (CST). For example, TMS was applied to a subject's motor cortex during two conditions; the first was while the subject was walking and the second was while the subject engaged in a voluntary contraction matched in amplitude to muscle activation during walking. Both the TA and sol were tested. The motor response in the sol was 26% smaller in the

walking condition. Comparatively, the amplitude in the TA's response remained unchanged under both conditions. Evidence would suggest that in the soleus, there is a reduced contribution from the motor cortex; the TA, however, does not show this same trend. Rationale from these results suggests that in the TA there is an increased significance in contribution from the motor cortex during walking and it is comparable to when muscle activation is entirely voluntary (Nielsen 2002). Recording the size of the MEP during walking and comparatively during a matched voluntary contraction does not tell the investigators the amount of the corticospinal drive during the two tasks, but it will tell them about the corresponding cortex's relative excitability. Cortical components for TA muscle control may have a greater degree of involvement during walking. Stronger evidence into the importance of cortical activation for the TA during walking is provided by Petersen et al. (2001). Petersen demonstrates that by activating inhibitory cortical networks, a weak magnetic stimulus disrupts EMG activity in the TA during walking (Petersen et al. 2001) and suggests that EMG activity during walking is partly generated by inputs from descending cortical drive.

Increased excitability of the motor cortex while the TA is active during swing phase of walking signifies the importance of motor cortical input during walking. This also provides rationale for why individuals, having suffered a complete spinal transaction or sever stroke that resulted in maximal damage to the motor cortex, do not regain functional stepping to the same extent that an individual who has suffered an incomplete spinal injury or moderate stroke would.

2.42 CNS Spinal contribution

So why do quadruped animals demonstrate significant functional recovery even after a complete transection? Much of the patterned behaviour involved in locomotion, at least for quadrapedal mammals and in part for bipedal mammals, is dependent upon networks of interneurons within the spinal cord, loosely coined the central pattern generator (CPG). Experiments in the quadruped mammal introduces the concept of spinal control for rhythmic locomotion by demonstrating that rhythmic alternating contractions will occur in animals that have both the descending drive and sensory feedback removed (Bem et al. 1993; Grillner and Zangger 1979). This is termed fictive locomotion and was originally proposed by Graham Brown in 1911. In cats, alternating muscle contractions will occur even after eliminating afferent input through pharmacological application, again demonstrating isolated spinal drive of patterned output (Rossignol et al. 1998). Further to this, experiments involving isolated brainstem and spinal cord preparations support the notion of spinally produced locomotor-like movement patterns. For example, the rat spinal cord will show locomotor-like activation through applying neuromodulators, such as serotonin and N-methyl-D-aspartate, or by applying tonic electrical stimulation to the descending locomotor pathway.

In humans the existence of the CPG is speculative; however, there have been isolated examples of involuntary, rhythmical leg movements in human subjects (Bunge et al. 1993). The elusive evidence in humans provides only suggestive evidence of the CPG's

existence, evidence that seems to have less significance compared to proof for quadruped animals. It is important to note that in all cases, human examples of suggested fictive locomotion do occur in both complete and incomplete subjects, but only for those with some intact sensory input remaining. It should also be noted that clinically complete spinal injuries often have residual inputs and are not actually anatomically complete. Also, since fictive locomotion refers to the existence of reciprocally organized output between agonists and antagonists muscles even in the absence of descending and or afferent input, this definition does not actually apply to the human condition. Having said this, there exists human examples of locomotor control in the absence of descending drive. In complete SCI patients, stimulation to reflex afferents is followed by qualitative stepping movements and when muscle activity is recorded, EMG shows reciprocal activation of lower extremity muscles. In addition, there are historical accounts of step-like movements observed in SCI patients with a complete spinal cord lesion (Dimitrijevic et al. 1998). This witnessed spinal control over inter-limb coordination helps to support the existence of spinal networks contributing to the modulation of patterning that occurs between lower extremity limbs, seemingly essential for locomotion.

Ultimately, inter-limb coordination exists in part because of feedback between the two limbs and it is possible to demonstrate that the condition of one leg will limit the range of possible states in which the second leg can be. For example, the initiation of the swing phase is dependent on the contra-lateral limb being in stance (Lam et al. 2003a; Lam et al. 2003b; Yang et al. 1998). Further, with the appropriate afferent input, infants will display step-like co-ordination of their lower limbs. This is before acquisition of skilled

walking and prior to myelination of the CST is complete and therefore suggests innate circuitry is responsible for the complex patterned movements that are seen.

2.43 PNS afferent contribution

The final significant contributor to the overall output of locomotion comes from the periphery. Each day, as we step out into a world where we navigate an overwhelming variety of obstacles or other moving objects such as cars, bikes and other people, we need to be able to react. These are the environmental surprises that we must input and then integrate so we can jump out of the way. Gait modification is environmentally dependant, so sensory feedback during walking, even for a normal stride, needs to take both external and internal space into consideration (Fouad and Pearson 2004). Only once this external information is processed, can the overall motor output be adapted to deal with complex and variable terrain. Timing and magnitude of muscle activity during locomotion is highly dependent on input regarding the external world; walking up a flight of stairs as an example. If one were to think they were at the top before the stairs were actually finished, a person would stub their toe on the last stair. This information would result in sensory receptors in the stubbed toe sending a message back to the brain that the foot was not quite there yet. Information would then feedback to the leg muscles directing the walker to engage in further muscle activation. This would provide the extra cortical drive that would allow the stubbed toe to clear the final stair. There are of course multiple sensory systems that are important in gathering information from the environment, such as vision, hearing, balance and touch. In addition, afferents from the muscle will provide unique and essential feedback. As discussed earlier, the muscle spindles and the golgi tendon

organ are the two main contributors, gathering information on both muscle length and load; this has been well researched in both human and animal models (Grey et al. 2001; Grey et al. 2002; Hiebert and Pearson 1999).

As a function of the muscle spindle, the stretch reflex is an important feedback mechanism; it is both phase and task specific. Modifiability of the stretch reflex throughout the step cycle has been tested using the electrical analogue, the H-reflex. The H-reflex demonstrates task specificity and serves as an excellent example of how sensory input and motor output are linked. For example, running results in a smaller H-reflex modulation as compared to walking and smaller still than quiet standing. The largest attenuation occurs during running; this is important since you would not want a reflex jerk mid-stride during the 100-meter dash. The importance of afferent feedback has also been demonstrated in cases of spinal injury regardless of whether the injury is partial or complete. To review, walking is a highly modifiable process that is the product of convergent inputs from supraspinal structures, spinal networks and peripheral sensory feed back, the weight of each being highly context dependant (Dietz 1998; Dietz and Duysens 2000; Harkema et al. 1997).

The fundamental modifiability of the human motor system is largely controversial. Although common knowledge suggests that post injury modifiability is what makes functional recovery possible, there is a certain lack of consensus into exactly where and how this plasticity occurs. This incongruity makes it essential for an experimental technique that can be used to investigate the plastic nature of the adult human nervous

system. As described next, TMS provides one such tool that has revealed much about cortical plasticity and functional recovery after injury.

2.5 Transcranial Magnetic Stimulation

2.51 Historical foundation of magnetic stimulation

In 1831, Micheal Faraday discovered the principle of electromagnetic induction. Faraday placed two coils side by side, one being the primary and the other being the secondary coil. Passing a current through the primary coil causes an associated magnetic field that is defined by the coil dimensions and the strength and rise time of the current. The magnetic field's flux (change in magnetic field strength over time) will induce a second current to flow in the reverse direction of neighboring conductive material, the secondary coil. This is Faraday's Law of electromagnetic induction and is described by the following equation:

$\varepsilon = -d\Phi/dt$ *where,*

ε = Induced voltage in the secondary coil,
dt = Change in time,
$d\Phi$ = Change in Magnetic flux.

In the 1980s, the principle of electromagnetic induction was used as a non-invasive method of stimulating the brain and was coined transcranial magnetic stimulation (TMS) (Barker 1999). Typically, the stimulating coil is placed adjacent to the scalp and will run a current of approximately 8000 amps, setting up a magnetic pulse of roughly 2.5 tesla, translating to 30-k tesla/second. This magnetic field is large enough to penetrate the skin and skull, since both types of tissue offer little resistance, and will allow for a current to

be induced in the cortical tissue below. This induced current is comparable to the cellular depolarization threshold, around 20mA/cm^3, and will thus initiate action potentials. Activation of cortical tissue, specifically the motor cortex, is made manifest by muscle contraction on the contralateral side of the body and can be recorded as EMG from surface electrodes. Since its discovery, TMS has been used as a method to investigate the physiological function of the nervous system.

2.52 *Monosynaptic connections exits between the cortex and the periphery*

Studies investigating motor control in the nervous system will use TMS by applying it over the scalp to activate cells in the primary motor cortex and produce a motor response. This procedure enables TMS to be used as a method of measuring cortical change. TMS works by preferentially activating pyramidal neurons in the motor cortex. The activation is primarily 'trans-synaptic' so that any change to MEPs is likely a result of changes in cortical circuitry. This transcortical activation of TMS can be inferred from experiments involving comparative types of stimulation, such as transcranial electric stimulation (TES).(Di Lazzaro et al. 2001) It therefore follows that changes in the cortical components activated by TMS will be, at least in part, responsible for any changes that are seen in the motor output.

Before expounding on trans-synaptic activation, it is important to note that mono-synaptic connections do exist between the cortex and the motoneurons of the spinal cord (Di Lazzaro et al. 2001; Edgley et al. 1997). This is supported by anatomical evidence in

primates and shows connections running directly from cortical cells to motor neurons. For humans specifically, it is thought that our highly specialized control of distal musculature is made possible by these abundant and direct connections from the motor cortex. This is supported by the short rise time of post stimulus time histogram (PSTH), composed from motor unit recordings of TMS. Interestingly, the duration of these narrow peaks is similar to the monosynaptic peaks evoked by direct stimulation of the Ia afferent (Petersen et al. 2003). Stimulation of Ia afferents results in monosynaptic activation of the muscle, thus explaining the consistency when comparing PSTH profiles between Ia afferent stimulation and high intensity TMS that is thought to activate pyramidal cells directly. Again, this would suggest the same monosynaptic connection from motor cortical cells as is found in Ia afferents. Stimulating at different levels of the spinal cord to measure the conduction velocity from the early component of MEPs, with either electric or magnetic stimulation, confirms this monosynaptic circuitry. In either case, making a comparison of synaptic transmission times from stimulation of both Ia afferents and the descending CST, after corrections have been made for distance, show them to be of the same synaptic latency. These monosynaptic connections have been established for both upper and lower limb motorneurons (Nielsen et al. 1995).

2.53 TMS - Mechanisms of action

TMS and TES show differing activation time scales when applied directly to the motor cortex. In comparing the response times, MEPs evoked from TES are slightly shorter

(approximately 2 msec). This difference in the activation time course suggests TMS preferentially causes trans-synaptic activation, thus explaining the extra time it takes for MEPs to manifest in the muscle by using different stimulation mechanisms (Nakamura et al. 1996). Conversely, the latency for TES activation is consistent with direct activation (D-wave) of pyramidal tract neurons, occurring at the level of the axon hillock; this is followed by and preferential to, indirect activation (I-wave) by TES, which manifests MEPs after a slightly longer latency. The origin of the I-wave from TES is similar to TMS stimulation, and can also be described as trans-synaptic activation of cortical tissue, up-stream from the axon hillock. In summary, TES, applied just above threshold, causes short latency muscle activation and increasing the stimulation intensity will result in later waves of activation. This order of activation is consistent whether recording from epidural electrodes in the spinal cord or surface electrodes from the muscle. TMS causes the converse to occur, at stimulation intensities just above threshold, there is preferential activation of the longer latency I-wave volleys, followed by shorter latency activation as the stimulation intensity increases.

Current direction is thought to play a large part in the differential activation between electric and magnetic stimulation. With TMS, the induced current will run primarily in the horizontal plane and activation is most preferred for neurons that are situated as such. Cortical interneurons will generally have horizontal projections and are more prone to activation form a horizontally induced current; therefore, TMS is more likely to induce indirect activation of pyramidal tract neurons through upstream activation of interneurons. The induced current for TES on the other hand, runs primarily in the

vertical plane and as noted above, is more likely to cause activation in vertically positioned neurons, as is the case for pyramidal tract neurons. Recording directly from the spinal cord of anesthetized humans can provide a demonstration of this; descending volleys evoked from TMS are reduced by anaesthesia whereas the anaesthetic has a minimal response on neurons that respond to TES. Anaesthesia acts by preferentially depressing trans-cortical cells; therefore trans-cortical mechanisms for TMS activation is further implicated through the reduction of MEP size when a subject is under anesthetic (Edgley et al. 1997).

Recording from single motor units also lends support for trans-synaptic activation by TMS. Pooling responses from a single motor unit into a post stimulus time histogram (PSTH) gives a probability of firing at specific latencies for that motor unit. Repeated trials from TES show the first peak of firing probability occurs at a latency that corresponds to direct pyramidal tract activation, followed by subsequent peaks corresponding to I-wave activation (Day et al. 1987). Comparatively, TMS will show the first peak to occur 1.5-2.0 msec later than the first peak evoked from TES. With TES, I-waves follow the D-wave at intervals of approximately 1.5 msec, which is also comparable to the time scale for chemical synaptic transmission, each I-wave being a synapse behind its predecessor. Based on this evidence, Day et al. (1987) suggested the first peak in the PSTH from TMS be comparable to the first TES evoked I-wave, which is the second peak in the TES PSTH. This rationale leads to an understanding that TMS will preferentially induce I-waves by trans-synaptic activation of pyramidal neurons and this

is consistent with the difference in MEP latencies between the two types of stimulation (Day et al. 1987).

Comparing TMS responses from the leg and hand area are similar but not exact. The differences seen in activation are likely from differences in the anatomical location of the corresponding cortex. For example, unlike the hand area, which lies lateral to the midline and superficial, the leg area is medial and deep to the hand area. This anatomical variability changes the physical parameters of activation, such as current flow relative to the spatial location of cortical cells. Despite this, studies show that the mechanism of activation for the leg area is relatively consistent with the hand area. In general, TMS activation of leg musculature is also predominantly indirect so the same assumptions will apply (Perez et al. 2004; Terao and Ugawa 2002).

2.54 Silent period

In addition to the MEP, TMS also acts to initiate a cessation of ongoing EMG activity in muscles sustaining minimal voluntary contraction, the latter part of which is thought to be from activation of supraspinal components. Since TES does not elicit a silent period to the same extent that TMS does and spinal reflexes resume around 100ms after an MEP, it is suggested that TMS will also activate a large portion of cortical inhibitory interneurons. Activation of these inhibitory interneurons is partially responsible for the initial portion of the silent period and the main contributor to the latter part (beyond

100ms). Patients with Parkinson's, a disease involving reduced inhibition from supraspinal structures that contribute to motor control, show a shorter than normal silent period duration (Priori et al. 1995). Along with this, application of lorazepam will reduce the duration of the silent period (Ziemann et al. 1996). Lorazepam works by mimicking the effects of GABA, an inhibitory neurotransmitter, which ultimately causes a decrease in neurological activity including the inhibitory interneurons themselves. In this case, the overall reduction in cortical activity is made manifest by the shortened duration of the silent period, a double inhibition of sorts. Experimental evidence supports cortical input as a contributor to silencing voluntary EMG activation. More importantly, it seems to be the sole contributor to the late phase of the silent period (greater than 100ms). This observation can be used as a method of monitoring changes to inhibitor cortical circuits, specifically if they are altered through training as proposed in Chpt.3.

2.55 Paired pulse TMS

Mechanisms responsible for cortical facilitation and inhibition can be explored by pairing stimuli of varied intensity. For example, by applying a sub-threshold conditioning stimulus before a supra-threshold test stimulus there is an amplification or reduction of the test MEP size and this is dependent on the delay between the two stimuli (Kujirai et al. 1993). Generally, if the interstimulus interval (ISI) is less than 5 ms there is an inhibitory effect and the test MEP size is reduced, whereas if the delay is between 10-25 ms, the test MEP will be larger. It has been proposed that the mechanism of action responsible for both facilitation and inhibition is cortical in nature, since applying the first

stimulus sub-threshold does suggest the effect on the secondary stimulation must be cortical. Activity of the first stimulus does not propagate beyond the brainstem. This was confirmed for paired pulse inhibition since the conditioning stimulus had no effect on the spinal H-reflex and produced no direct motor response. Subsequently, it was demonstrated by recording from electrodes inserted into the cervical epidural space (Nakamura et al. 1997). As expected, the epidural electrodes did not record a response from the first sub-threshold stimulus; thus, proving that inhibition occurs prior to being exposed to any possible spinal component.

Paired pulse has been found to be extremely variable depending on the stimulation protocol; for example test MEP size, voluntary facilitation and stimulation intensities will all change the nature of the effect. Through experimental investigation the origin and reliability for short interval intracortical inhibition (SICI) is relatively well documented. Like the SP, SICI provides an opportunity to monitor the excitability of inhibitory circuits in the cortex. The data for intracortical facilitation is not as well founded. In a recent paper, Di Lazzaro et al (2006, in print) found that at the appropriate inter-stimulus interval for facilitation, the muscle response shows a significant increase but there is no noticeable change in the size of the descending volley as measured in the epidural space. This would suggest it is unlikely that cortical mechanisms are primarily responsible for the conditioned facilitation. The authors do provide alternate possible explanations for the measured increase in muscle response; they suggest that either there is subtle change in excitability of spinal mechanisms, or the descending volleys do not provide a good reflection of the population of neurons involved in the excitation output. Thus, the

population of neurons responsible for motor output is not inferable from the size of the volley and this limits the mechanistic understanding of paired pulse stimulation. Despite this, cortical inhibition through paired pulse stimulation remains one possible method of measuring cortical change.

2.6 Conclusion

In summary, the adult human motor system is a dynamic entity that exists in a dynamic environment, an environment that will sometimes cause damage to that system. Once damaged, the motor system is no longer capable of functioning as it was physiologically developed to do. Changed forever, the motor control system still has the capability to adapt and recover. In the following chapter, I will examine how this modifiability is subject to training, specifically after a spinal cord injury, when motor cortex output has been disrupted. As reported, subjects who suffered from a SCI were required to undergo intensive locomotor training by using body weight support on a motorized treadmill; TMS was used to measure any training induced change in spared CST connections.

2.7 REFERENCES

Barbeau H and Rossignol S. Recovery of locomotion after chronic spinalization in the adult cat. *Brain Res* 412: 84-95, 1987.

Barker AT. The history and basic principles of magnetic nerve stimulation. *Electroencephalogr Clin Neurophysiol Suppl* 51: 3-21, 1999.

Bem T, Orsal D, and Cabelguen JM. Fictive locomotion in the adult thalamic rat. *Exp Brain Res* 97: 301-304, 1993.

Brasil-Neto JP, Cohen LG, Pascual-Leone A, Jabir FK, Wall RT, and Hallett M. Rapid reversible modulation of human motor outputs after transient deafferentation of the forearm: a study with transcranial magnetic stimulation. *Neurology* 42: 1302-1306, 1992.

Bunge RP, Puckett WR, Becerra JL, Marcillo A, and Quencer RM. Observations on the pathology of human spinal cord injury. A review and classification of 22 new cases with details from a case of chronic cord compression with extensive focal demyelination. *Adv Neurol* 59: 75-89, 1993.

Buonomano DV and Merzenich MM. Associative synaptic plasticity in hippocampal CA1 neurons is not sensitive to unpaired presynaptic activity. *J Neurophysiol* 76: 631-636, 1996.

Buonomano DV and Merzenich MM. Cortical plasticity: from synapses to maps. *Annu Rev Neurosci* 21: 149-186, 1998.

Capaday C, Lavoie BA, Barbeau H, Schneider C, and Bonnard M. Studies on the corticospinal control of human walking. I. Responses to focal transcranial magnetic stimulation of the motor cortex. *J Neurophysiol* 81: 129-139, 1999.

Carel C, Loubinoux I, Boulanouar K, Manelfe C, Rascol O, Celsis P, and Chollet F. Neural substrate for the effects of passive training on sensorimotor cortical representation: a study with functional magnetic resonance imaging in healthy subjects. *J Cereb Blood Flow Metab* 20: 478-484, 2000.

Chen R, Cohen LG, and Hallett M. Nervous system reorganization following injury. *Neuroscience* 111: 761-773, 2002.

Classen J, Wolters A, Stefan K, Wycislo M, Sandbrink F, Schmidt A, and Kunesch E. Paired associative stimulation. *Suppl Clin Neurophysiol* 57: 563-569, 2004.

Cohen LG, Bandinelli S, Findley TW, and Hallett M. Motor reorganization after upper limb amputation in man. A study with focal magnetic stimulation. *Brain* 114 (Pt 1B): 615-627, 1991.

Cramer SC, Nelles G, Benson RR, Kaplan JD, Parker RA, Kwong KK, Kennedy DN, Finklestein SP, and Rosen BR. A functional MRI study of subjects recovered from hemiparetic stroke. *Stroke* 28: 2518-2527, 1997.

Croll RP. Complexities of a simple system: new lessons, old challenges and peripheral questions for the gill withdrawal reflex of Aplysia. *Brain Res Brain Res Rev* 43: 266-274, 2003.

Davey NJ, Ellaway PH, Friedland CL, and Short DJ. Motor unit discharge characteristics and short term synchrony in paraplegic humans. *J Neurol Neurosurg Psychiatry* 53: 764-769, 1990.

Davey NJ, Smith HC, Savic G, Maskill DW, Ellaway PH, and Frankel HL. Comparison of input-output patterns in the corticospinal system of normal subjects and incomplete spinal cord injured patients. *Exp Brain Res* 127: 382-390, 1999.
Day BL, Rothwell JC, Thompson PD, Dick JP, Cowan JM, Berardelli A, and Marsden CD. Motor cortex stimulation in intact man. 2. Multiple descending volleys. *Brain* 110 (Pt 5): 1191-1209, 1987.
Day M. Neuromodulation: spinal cord and peripheral nerve stimulation. *Curr Rev Pain* 4: 374-382, 2000.
De Leon RD, Hodgson JA, Roy RR, and Edgerton VR. Full weight-bearing hindlimb standing following stand training in the adult spinal cat. *J Neurophysiol* 80: 83-91, 1998.
de Leon RD, Hodgson JA, Roy RR, and Edgerton VR. Locomotor capacity attributable to step training versus spontaneous recovery after spinalization in adult cats. *J Neurophysiol* 79: 1329-1340, 1998.
De Leon RD, Hodgson JA, Roy RR, and Edgerton VR. Retention of hindlimb stepping ability in adult spinal cats after the cessation of step training. *J Neurophysiol* 81: 85-94, 1999.
de Leon RD, Tamaki H, Hodgson JA, Roy RR, and Edgerton VR. Hindlimb locomotor and postural training modulates glycinergic inhibition in the spinal cord of the adult spinal cat. *J Neurophysiol* 82: 359-369, 1999.
Devanne H, Lavoie BA, and Capaday C. Input-output properties and gain changes in the human corticospinal pathway. *Exp Brain Res* 114: 329-338, 1997.
Di Lazzaro V, Pilato F, Oliviero A, Dileone M, Saturno E, Mazzone P, Insola A, Profice P, Ranieri F, Capone F, Tonali PA, and Rothwell JC. Origin of facilitation of motor-evoked potentials after paired magnetic stimulation: direct recording of epidural activity in conscious humans. *J Neurophysiol* 96: 1765-1771, 2006.
Di Lazzaro V, Oliviero A, Profice P, Meglio M, Cioni B, Tonali P, and Rothwell JC. Descending spinal cord volleys evoked by transcranial magnetic and electrical stimulation of the motor cortex leg area in conscious humans. *J Physiol* 537: 1047-1058, 2001.
Dietz V. Evidence for a load receptor contribution to the control of posture and locomotion. *Neurosci Biobehav Rev* 22: 495-499, 1998.
Dietz V and Duysens J. Significance of load receptor input during locomotion: a review. *Gait Posture* 11: 102-110, 2000.
Dietz V, Zijlstra W, and Duysens J. Human neuronal interlimb coordination during split-belt locomotion. *Exp Brain Res* 101: 513-520, 1994.
Dietz V, Zijlstra W, Prokop T, and Berger W. Leg muscle activation during gait in Parkinson's disease: adaptation and interlimb coordination. *Electroencephalogr Clin Neurophysiol* 97: 408-415, 1995.
Dimitrijevic MR, Gerasimenko Y, and Pinter MM. Evidence for a spinal central pattern generator in humans. *Ann N Y Acad Sci* 860: 360-376, 1998.
Dobkin BH, Harkema S, Requejo P, and Edgerton VR. Modulation of locomotor-like EMG activity in subjects with complete and incomplete spinal cord injury. *J Neurol Rehabil* 9: 183-190, 1995.
Dunwiddie TV and Lynch G. The relationship between extracellular calcium concentrations and the induction of hippocampal long-term potentiation. *Brain Res* 169: 103-110, 1979.

Edgerton VR, de Leon RD, Tillakaratne N, Recktenwald MR, Hodgson JA, and Roy RR. Use-dependent plasticity in spinal stepping and standing. *Adv Neurol* 72: 233-247, 1997.
Edgerton VR, Leon RD, Harkema SJ, Hodgson JA, London N, Reinkensmeyer DJ, Roy RR, Talmadge RJ, Tillakaratne NJ, Timoszyk W, and Tobin A. Retraining the injured spinal cord. *J Physiol* 533: 15-22, 2001.
Edgley SA, Eyre JA, Lemon RN, and Miller S. Comparison of activation of corticospinal neurons and spinal motor neurons by magnetic and electrical transcranial stimulation in the lumbosacral cord of the anaesthetized monkey. *Brain* 120 (Pt 5): 839-853, 1997.
Eidelberg E, Story JL, Meyer BL, and Nystel J. Stepping by chronic spinal cats. *Exp Brain Res* 40: 241-246, 1980.
Feydy A, Carlier R, Roby-Brami A, Bussel B, Cazalis F, Pierot L, Burnod Y, and Maier MA. Longitudinal study of motor recovery after stroke: recruitment and focusing of brain activation. *Stroke* 33: 1610-1617, 2002.
Fouad K and Pearson K. Restoring walking after spinal cord injury. *Prog Neurobiol* 73: 107-126, 2004.
Fraser C, Power M, Hamdy S, Rothwell J, Hobday D, Hollander I, Tyrell P, Hobson A, Williams S, and Thompson D. Driving plasticity in human adult motor cortex is associated with improved motor function after brain injury. *Neuron* 34: 831-840, 2002.
Fuhr P, Agostino R, and Hallett M. Spinal motor neuron excitability during the silent period after cortical stimulation. *Electroencephalogr Clin Neurophysiol* 81: 257-262, 1991.
Green JB, Sora E, Bialy Y, Ricamato A, and Thatcher RW. Cortical motor reorganization after paraplegia: an EEG study. *Neurology* 53: 736-743, 1999.
Grey MJ, Ladouceur M, Andersen JB, Nielsen JB, and Sinkjaer T. Group II muscle afferents probably contribute to the medium latency soleus stretch reflex during walking in humans. *J Physiol* 534: 925-933, 2001.
Grey MJ, Larsen B, and Sinkjaer T. A task dependent change in the medium latency component of the soleus stretch reflex. *Exp Brain Res* 145: 316-322, 2002.
Grillner S and Zangger P. On the central generation of locomotion in the low spinal cat. *Exp Brain Res* 34: 241-261, 1979.
Harkema SJ, Hurley SL, Patel UK, Requejo PS, Dobkin BH, and Edgerton VR. Human lumbosacral spinal cord interprets loading during stepping. *J Neurophysiol* 77: 797-811, 1997.
Hess G and Donoghue JP. Long-term potentiation and long-term depression of horizontal connections in rat motor cortex. *Acta Neurobiol Exp (Wars)* 56: 397-405, 1996.
Hiebert GW and Pearson KG. Contribution of sensory feedback to the generation of extensor activity during walking in the decerebrate Cat. *J Neurophysiol* 81: 758-770, 1999.
Kandel ER. Principles of Neuroscience, Chapter 63. au. Kandel ER, Schwartz JH, Jessel TM. McGraw/Hill Companies, NY, 2000.
Karni A, Meyer G, Jezzard P, Adams MM, Turner R, and Ungerleider LG. Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature* 377: 155-158, 1995.
Kleim JA, Barbay S, and Nudo RJ. Functional reorganization of the rat motor cortex following motor skill learning. *J Neurophysiol* 80: 3321-3325, 1998.

Kleim JA, Hogg TM, VandenBerg PM, Cooper NR, Bruneau R, and Remple M. Cortical synaptogenesis and motor map reorganization occur during late, but not early, phase of motor skill learning. *J Neurosci* 24: 628-633, 2004.
Kleim JA, Lussnig E, Schwarz ER, Comery TA, and Greenough WT. Synaptogenesis and Fos expression in the motor cortex of the adult rat after motor skill learning. *J Neurosci* 16: 4529-4535, 1996.
Knash ME, Kido A, Gorassini M, Chan KM, and Stein RB. Electrical stimulation of the human common peroneal nerve elicits lasting facilitation of cortical motor-evoked potentials. *Exp Brain Res* 153: 366-377, 2003.
Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P, and Marsden CD. Corticocortical inhibition in human motor cortex. *J Physiol* 471: 501-519, 1993.
Lam T, Wolstenholme C, van der Linden M, Pang MY, and Yang JF. Stumbling corrective responses during treadmill-elicited stepping in human infants. *J Physiol* 553: 319-331, 2003a.
Lam T, Wolstenholme C, and Yang JF. How do infants adapt to loading of the limb during the swing phase of stepping? *J Neurophysiol* 89: 1920-1928, 2003b.
Levy WJ, Jr., Amassian VE, Traad M, and Cadwell J. Focal magnetic coil stimulation reveals motor cortical system reorganized in humans after traumatic quadriplegia. *Brain Res* 510: 130-134, 1990.
Liepert J, Bauder H, Wolfgang HR, Miltner WH, Taub E, and Weiller C. Treatment-induced cortical reorganization after stroke in humans. *Stroke* 31: 1210-1216, 2000.
Liepert J, Miltner WH, Bauder H, Sommer M, Dettmers C, Taub E, and Weiller C. Motor cortex plasticity during constraint-induced movement therapy in stroke patients. *Neurosci Lett* 250: 5-8, 1998.
Lotze M, Braun C, Birbaumer N, Anders S, and Cohen LG. Motor learning elicited by voluntary drive. *Brain* 126: 866-872, 2003a.
Lotze M, Scheler G, Tan HR, Braun C, and Birbaumer N. The musician's brain: functional imaging of amateurs and professionals during performance and imagery. *Neuroimage* 20: 1817-1829, 2003b.
Mikulis DJ, Jurkiewicz MT, McIlroy WE, Staines WR, Rickards L, Kalsi-Ryan S, Crawley AP, Fehlings MG, and Verrier MC. Adaptation in the motor cortex following cervical spinal cord injury. *Neurology* 58: 794-801, 2002.
Mountcastle VB. The columnar organization of the neocortex. *Brain* 120 (Pt 4): 701-722, 1997.
Nakamura H, Kitagawa H, Kawaguchi Y, and Tsuji H. Direct and indirect activation of human corticospinal neurons by transcranial magnetic and electrical stimulation. *Neurosci Lett* 210: 45-48, 1996.
Nakamura H, Kitagawa H, Kawaguchi Y, and Tsuji H. Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans. *J Physiol* 498 (Pt 3): 817-823, 1997.
Nielsen J, Petersen N, and Ballegaard M. Latency of effects evoked by electrical and magnetic brain stimulation in lower limb motoneurones in man. *J Physiol* 484 (Pt 3): 791-802, 1995.
Nielsen JB. Motoneuronal drive during human walking. *Brain Res Rev* 40: 192-201, 2002.

Nudo RJ. Adaptive plasticity in motor cortex: implications for rehabilitation after brain injury. *J Rehabil Med*: 7-10, 2003.
Pascual-Leone A, Cammarota A, Wassermann EM, Brasil-Neto JP, Cohen LG, and Hallett M. Modulation of motor cortical outputs to the reading hand of braille readers. *Ann Neurol* 34: 33-37, 1993.
Pascual-Leone A and Torres F. Plasticity of the sensorimotor cortex representation of the reading finger in Braille readers. *Brain* 116 (Pt 1): 39-52, 1993.
Pascual-Leone A, Wassermann EM, Sadato N, and Hallett M. The role of reading activity on the modulation of motor cortical outputs to the reading hand in Braille readers. *Ann Neurol* 38: 910-915, 1995.
Pearson KG and Rossignol S. Fictive motor patterns in chronic spinal cats. *J Neurophysiol* 66: 1874-1887, 1991.
Perez MA, Lungholt BK, Nyborg K, and Nielsen JB. Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. *Exp Brain Res*, 2004.
Petersen NT, Butler JE, Marchand-Pauvert V, Fisher R, Ledebt A, Pyndt HS, Hansen NL, and Nielsen JB. Suppression of EMG activity by transcranial magnetic stimulation in human subjects during walking. *J Physiol* 537: 651-656, 2001.
Petersen NT, Taylor JL, Butler JE, and Gandevia SC. Depression of activity in the corticospinal pathway during human motor behavior after strong voluntary contractions. *J Neurosci* 23: 7974-7980, 2003.
Popov NS, Reymann KG, Schulzeck K, Schulzeck S, and Matthies H. Alterations in calmodulin content in fractions of rat hippocampal slices during tetanic- and calcium-induced long-term potentiation. *Brain Res Bull* 21: 201-206, 1988.
Priori A, Berardelli A, Mercuri B, Inghilleri M, and Manfredi M. The effect of hyperventilation on motor cortical inhibition in humans: a study of the electromyographic silent period evoked by transcranial brain stimulation. *Electroencephalogr Clin Neurophysiol* 97: 69-72, 1995.
Recanzone GH, Merzenich MM, and Jenkins WM. Frequency discrimination training engaging a restricted skin surface results in an emergence of a cutaneous response zone in cortical area 3a. *J Neurophysiol* 67: 1057-1070, 1992.
Rema V, Armstrong-James M, and Ebner FF. Experience-dependent plasticity is impaired in adult rat barrel cortex after whiskers are unused in early postnatal life. *J Neurosci* 23: 358-366, 2003.
Ridding MC and Rothwell JC. Stimulus/response curves as a method of measuring motor cortical excitability in man. *Electroencephalogr Clin Neurophysiol* 105: 340-344, 1997.
Ridding MC and Taylor JL. Mechanisms of motor-evoked potential facilitation following prolonged dual peripheral and central stimulation in humans. *J Physiol* 537: 623-631, 2001.
Rioult-Pedotti MS, Friedman D, Hess G, and Donoghue JP. Strengthening of horizontal cortical connections following skill learning. *Nat Neurosci* 1: 230-234, 1998.
Robinson GA and Goldberger ME. The development and recovery of motor function in spinal cats. II. Pharmacological enhancement of recovery. *Exp Brain Res* 62: 387-400, 1986.
Rossignol S. Phil Trans R Soc B 361: 1647-1671,2006

Rossignol S, Chau C, Brustein E, Giroux N, Bouyer L, Barbeau H, and Reader TA. Pharmacological activation and modulation of the central pattern generator for locomotion in the cat. *Ann N Y Acad Sci* 860: 346-359, 1998.
Seitz RJ, Hoflich P, Binkofski F, Tellmann L, Herzog H, and Freund HJ. Role of the premotor cortex in recovery from middle cerebral artery infarction. *Arch Neurol* 55: 1081-1088, 1998.
Sherrington CE. Charles Scott Sherrington (1857-1952). *Notes Rec R Soc Lond* 30: 45-63, 1975.
Taub E. Constraint-induced movement therapy and massed practice. *Stroke* 31: 986-988, 2000.
Taub E, Uswatte G, and Morris DM. Improved motor recovery after stroke and massive cortical reorganization following Constraint-Induced Movement therapy. *Phys Med Rehabil Clin N Am* 14: S77-91, ix, 2003.
Terao Y and Ugawa Y. Basic mechanisms of TMS. *J Clin Neurophysiol* 19: 322-343, 2002.
Thomas SL and Gorassini MA. Increases in corticospinal tract function by treadmill training after incomplete spinal cord injury. *J Neurophysiol*, 2005.
Tillakaratne NJ, Mouria M, Ziv NB, Roy RR, Edgerton VR, and Tobin AJ. Increased expression of glutamate decarboxylase (GAD(67)) in feline lumbar spinal cord after complete thoracic spinal cord transection. *J Neurosci Res* 60: 219-230, 2000.
Topka H, Cohen LG, Cole RA, and Hallett M. Reorganization of corticospinal pathways following spinal cord injury. *Neurology* 41: 1276-1283, 1991.
Ts'o DY, Gilbert CD, and Wiesel TN. Relationships between horizontal interactions and functional architecture in cat striate cortex as revealed by cross-correlation analysis. *J Neurosci* 6: 1160-1170, 1986.
Tyc F, Boyadjian A, and Devanne H. Motor cortex plasticity induced by extensive training revealed by transcranial magnetic stimulation in human. *Eur J Neurosci* 21: 259-266, 2005.
Wall JT, Kaas JH, Sur M, Nelson RJ, Felleman DJ, and Merzenich MM. Functional reorganization in somatosensory cortical areas 3b and 1 of adult monkeys after median nerve repair: possible relationships to sensory recovery in humans. *J Neurosci* 6: 218-233, 1986.
Ward NS, Brown MM, Thompson AJ, and Frackowiak RS. Neural correlates of motor recovery after stroke: a longitudinal fMRI study. *Brain* 126: 2476-2496, 2003.
Ward NS, Newton JM, Swayne OB, Lee L, Thompson AJ, Greenwood RJ, Rothwell JC, and Frackowiak RS. Motor system activation after subcortical stroke depends on corticospinal system integrity. *Brain* 129: 809-819, 2006.
Weiller C, Ramsay SC, Wise RJ, Friston KJ, and Frackowiak RS. Individual patterns of functional reorganization in the human cerebral cortex after capsular infarction. *Ann Neurol* 33: 181-189, 1993.
Wernig A, Nanassy A, and Muller S. Maintenance of locomotor abilities following Laufband (treadmill) therapy in para- and tetraplegic persons: follow-up studies. *Spinal Cord* 36: 744-749, 1998.
Xerri C, Stern JM, and Merzenich MM. Alterations of the cortical representation of the rat ventrum induced by nursing behavior. *J Neurosci* 14: 1710-1721, 1994.

Yang JF, Stephens MJ, and Vishram R. Transient disturbances to one limb produce coordinated, bilateral responses during infant stepping. *J Neurophysiol* 79: 2329-2337, 1998.
Ziemann U, Corwell B, and Cohen LG. Modulation of plasticity in human motor cortex after forearm ischemic nerve block. *J Neurosci* 18: 1115-1123, 1998a.
Ziemann U, Hallett M, and Cohen LG. Mechanisms of deafferentation-induced plasticity in human motor cortex. *J Neurosci* 18: 7000-7007, 1998.
Ziemann U, Lonnecker S, Steinhoff BJ, and Paulus W. The effect of lorazepam on the motor cortical excitability in man. *Exp Brain Res* 109: 127-135, 1996.

CHAPTER 3

Increases in corticospinal tract function by treadmill training after incomplete spinal cord injury

3.1 Introduction

Animal and human studies have shown that following damage to the cerebral cortex the recovery of motor function induced by intensive training is associated with structural and functional changes in the intrinsic circuitry of spared motor-related cortices (reviewed in Buonomano and Merzenich 1998; Nudo 2003). Such cortical reorganization is hypothesized to ultimately produce an expansion of and/or increase in the excitability of cortical networks supplying the affected muscles, as revealed by imaging and transcranial magnetic stimulation (TMS) techniques (Karni et al. 1995; Levy et al. 2001; Liepert et al. 2000). After spinal cord injury (SCI) where only the downstream *output* of motor-related cortices are disrupted, evidence both for and against increases in the expansion and excitability of corticospinal pathways rostral to the lesion have been reported (Brouwer and Hopkins-Rosseel 1997; Levy et al. 1990; Topka et al. 1991). It has been postulated that the increased expansion and/or excitability of cortical networks innervating the unaffected muscles results from the exaggerated use of these muscles during motor compensation (Levy et al. 1990; Topka et al. 1991). From these initial observations in SCI and from the training studies described above for stroke, we wished to study whether intensive training of muscles affected by SCI can increase the function of associated spared corticospinal pathways.

To examine the effects of training on spared corticospinal function directly, rather than from spontaneous motor recovery, we tested human subjects with injuries that were typically 1 year or more and whose motor function had reached a plateau. To produce long-lasting improvements in leg motor function, subjects with incomplete SCI were trained 5 days per week, for 3 to 5 months using treadmill therapy (Barbeau et al. 1987; Wernig et al. 1998) which consisted of body weight support combined with manual assistance of leg movements over a motorized treadmill (Barbeau et al. 1987). Although our ultimate goal was to produce functional locomotor recovery and not to examine the efficacy of treadmill therapy per se, it remains unknown if treadmill training in chronic SCI is better than other approaches in restoring locomotor function (Dobkin and Havton 2004). We hypothesized that any form of locomotor training in humans would increase corticospinal tract function because the corticospinal tract, along with spinal circuits, provides a part of the drive to lower leg muscles during walking when examined in non-injured control subjects (Capaday 2002; Petersen et al. 2001).

To assess changes in corticospinal tract function from training, we measured motor-evoked potentials (MEPs) in response to incrementing levels of TMS over the leg area of the primary motor cortex. The resulting TMS-evoked recruitment curves recorded in muscles of the leg were typically well represented by a sigmoid function (see Fig. 1 and also Devanne et al. 1997). We measured changes in the threshold, slope and maximum amplitude of the recruitment and sigmoidal curves (see Methods) to determine if training affected the excitability, expansion and/or functional connectivity of spared corticospinal networks activated by the TMS (Devanne et al. 1997; Ridding and Rothwell 1997). As

this was a longitudinal study, we also compared the reproducibility of these parameters in non-trained SCI and non-injured controls to verify that any changes observed in the trained SCI group were indeed due to training.

The second purpose of our study was to examine if improvements in walking function from intensive treadmill training were related to increases in corticospinal tract function. In studies where quadrupedal animals are re-trained to walk after a complete SCI, the recovery of walking function has been postulated to be mediated by modifying the activation of locomotor and inhibitory networks intrinsic to the spinal cord (Edgerton et al. 2001; Ribotta et al. 2000; Tillakaratne et al. 2002). In contrast, in humans, attainment of independent walking from training only occurs in subjects having some preservation of voluntary leg movements (Dietz et al. 1994a; Wernig and Muller 1992). Furthermore, functional gains made while walking on a treadmill in subjects that have gained a moderate walking capacity are transferable to over-ground walking. Thus, in humans, training-induced improvements in walking function may not only require increased activation of spinal locomotor networks that are entrained by locomotor-generated sensory inputs. Increases in the activation of spared descending pathways, such as the corticospinal tract, may also be necessary components to achieve attainment of functional independent walking. To test this, we examined if increases in locomotor recovery were correlated to increases in corticospinal tract function as assessed with TMS. Parts of this study have been presented in abstract form (Thomas and Gorassini 2004).

3.2 Methods

3.1 Subjects

Ten subjects (8 male) with incomplete SCI aged 29-78 years (54.4 ± 14.8) volunteered with informed consent for this study (Table 1). Eight of them participated in TMS experiments both before and after receiving intensive treadmill training. Inclusion criteria for treadmill and TMS experiments were that subjects must have some ability to move one or more of their limb joints in both legs (ASIA C and D). Exclusion criteria were: 1) damage to the nervous system other than the spinal cord; 2) previous brain surgery; 3) bone density that was 30% or less of age-matched, non-injured subjects; 4) impaired mental capacity; 5) history of epilepsy; 6) spinal implants above C2; 7) severe depression; 8) other medical contraindications to treadmill training (cardiovascular problems, back or joint injury, arthritis, pulmonary disorders, history of deep vein thrombosis, etc.). Motor function scores (see Clinical measures below) assessed between the time of initial screening and immediately before the start of training (typically a 2-month period) did not change indicating that all subjects were not in a process of spontaneous motor recovery before the start of treadmill training. Five non-injured control subjects [3 of them male, 21-36 years (26 ± 6)] and 6 non-trained SCI subjects, 4 of whom were subsequently trained (subjects 3M, 6M, 7M, 8F, see Table 1) were also tested for day-to-day variability of the motor-evoked potentials (MEPs). Ethics approval for this study was obtained from the Human Ethics Research Committee at the University of Alberta.

Code/ Sex	Age	Cause	Years post injury	Injury Level	Asia Score	Weeks Trained	# of sessions	Muscle Tested	Hot Spot (cm)	
									Before	After
1M	44	Trauma	3	T11/12	C	21	74	*rVL* lVL*	1, -1 .5, -2.5	1, -1 1, -2
2M	52	Trauma	28	C5/6	C	16	78	*rTA**	1, -3	1, -2
3M	66	Trauma	5	C5/6	C	13	48	lTA	1, -2	1, -1
4M	60	Trauma	3	L1	C	19	60	*rVL**	5, -2.5	3, -2
5M	71	VE	0.8	T5/9	D	13	64	rTA* *lTA**	1, -4 2, -4.5	1, -4 1, -4
6M	78	Tumor	2	T4/5	D	23	74	*rTA** lTA	2, -1 2, -1	2, -1 2, -1
7M	41	Trauma	0.6	C3/5	C	18	89	*rTA** lTA*	1, -1 1, -1	2, -1 2, -1
8F	48	Trauma	24	T12/L2	D	10	40	*rTA* lTA*	1, -1 1, -1	1, -1 1, -1
9M	29	Trauma	5	C5/6	C	NA	NA	rTA lTA	1, -1 1, -1	1, -1 1, -1
10F	55	TM	7	T1/5	C	NA	NA	lTA	1, -1	1, -1

Table 1

Demographic, injury, training and experimental details for all spinal cord injured subjects. For cause of injury, VE = viral encephalitis, TM = transverse myelitis. In subjects where trauma was not the initial diagnosis, damage to the spinal cord was verified by MRI. Subject 5M became paraplegic after surgical removal of a thoracic epidural abscess, subject 6M became paraplegic after removal of posterior elements of C4-T2 and subject 10F only had lesions in the spinal cord, with the brain and brainstem MRI being unremarkable. For muscle tested, r = right, l = left, * denotes muscles where there was an increase in MEP_{max} after training with more affected muscles in bilateral recordings marked in italics. For hot spot location, the first co-ordinate denotes the lateral distance from vertex and the second co-ordinate denotes the posterior distance from vertex, i.e., 1, -1 for the right VL muscle of subject 1M signifies the hotspot was 1 cm left of and 1 cm posterior to vertex. NA = not appropriate.

3.22 Treadmill training

Eight of the 10 SCI subjects were asked to take part in five training sessions/week. On average, subjects participated in 4.1 ± 0.76 sessions per week for an average of 16.6 ± 4.4 weeks (see Table 1 for individual training times and sessions completed). Each training session was one hour in duration and consisted of body weight support combined with manual assistance of leg movements while the subject walked on a motorized treadmill. Depending on the need, one or two people were positioned at the lower limb in order to provide stepping assistance by lifting the foot through swing, flexing the knee at the start of swing and/or stabilizing the leg during stance. A bungee cord tied to the harness and overhead support frame was sometimes used to help stabilize the subject's posture. The base of support, weight shift between the two legs, step length, postural alignment, hip extension at the start of swing, and foot contact during stance (heel to toe) were all monitored. Subjects were encouraged to arm swing when possible (one or two) and horizontal bars positioned at chest level were used to aid in balance control only. Subjects walked at a slow pace (≈ 1.5 km/hr or 1mph), enabling them to concentrate on voluntarily activating their muscles during walking. Rests were taken when needed but subjects were encouraged to walk/rest at ten-minute intervals. When the physical therapist noticed improvements in cardiovascular tolerance, proper limb kinematics throughout the step cycle, weight transition between limbs and upright trunk alignment, the amount of body weight support and/or stepping assistance were gradually decreased. On average, body weight support decreased from 44.3 ± 25.2% at the start of training to 13.4 ± 18.1% at the end of training. Subjects were not allowed to wear lower-extremity orthoses while

training. In 3 subjects (6M, 7M, 8F), training of over-ground walking after a treadmill session was also performed once or twice a week during the last one or two months of training. Training was stopped when improvements in walking ability, as assessed by the WISCI II score, the distance a subject could walk in 6 minutes, and the time to walk 10 meters, remained constant over a 4-week period.

3.23 Transcranial magnetic stimulation

TMS was produced by a Magstim Model 200 stimulator (The Magstim Company Ltd) and a large double-cone coil (P/N 9902-00) having an inside diameter of 96 mm and an outside diameter of 125 mm for each individual coil. MEPs were evoked in the tibialis anterior (TA) muscle; however, in two subjects (1M and 4M) MEPs were evoked in the vastus lateralis (VL) muscle because it was not possible to maintain a constant background contraction of TA (see below). To determine the optimal location on the scalp to elicit a MEP in either the TA or VL muscle, vertex (Cz with 0 medio-lateral and 0 antero-posterior co-ordinates) was first marked on the scalp and a transparent grid with 1 cm x 1 cm markings was centered over the vertex. Different locations on the grid, usually centered around 1 cm lateral and 1 cm posterior to Cz (1, -1), were stimulated at an intensity that was approximately 1.2 times the motor threshold. The location where the largest and most consistent MEP was elicited, i.e., hot spot, was marked on the scalp and the mid-point of the coil was positioned over this spot to obtain a TMS recruitment curve (described below). When possible, a hot spot was identified over both the right and left motor cortices (see Table 1). The position of the coil was maintained flush with the subject's head by the experimenter and the position and orientation were periodically

checked throughout the experiment to ensure that the handle was perpendicular to the scalp and that the grid did not move from its original location.

2.24 Electromyogram recording

Surface electromyogram (EMG) activity was recorded from leg muscles on the right and left side of the body, including the (TA) and soleus (SOL) muscles or the (VL) and hamstring (HAM) muscles. The skin surface over each muscle was shaved, cleaned with alcohol and allowed to dry. Two disposable silver/silver chloride-recording electrodes (Kendal Soft –E H59P) were placed 1.5 cm apart over the prepared area. EMG signals were led to an isolated pre-amplifier/amplifier (Octopus, Bortec Technologies, Calgary, AB) with a bandpass of 10 to 1000 Hz and the signal was amplified between 1000 and 5000 times. The EMG signal was digitized with a 5-kHz sampling rate using AxoScope hardware and software (DigiData 1200 Series, Axon Instruments, Union City, CA), and displayed on a personal computer.

3.25 Experimental protocol (TMS)

For each pre-training experiment, subjects were instructed to maintain a background voluntary contraction that was approximately 10% of their maximum (MVC) in either the TA or the VL muscle. MEP responses were measured during muscle activation to control for both cortical and motoneuron pool excitability and to ensure that changes in response to TMS were not simply a result of differences in sub-threshold activation of

neurons (Ridding and Rothwell 1995). To help subjects maintain a 10% MVC, the surface EMG signal was rectified, low-pass filtered (3 Hz) and displayed on an oscilloscope with a fast time scale. The subject was asked to contract their leg muscle until the rectified EMG signal reached a horizontal line on the oscilloscope that corresponded to approximately 10% of MVC. To produce a TMS recruitment curve (Fig. 1) the applied stimulation intensity was increased, from below motor threshold to maximum MEP (MEP_{max}), in increments of 5 or 10% of the maximum stimulator output (MSO). Typically, stimulation intensities ranged from 30 - 80% of MSO, with four stimuli given at each intensity every 5-6 seconds. Brief rest periods were given between the different stimulation intensities. MEPs were collected from both sides of the body unless the patient could not maintain a steady background contraction in the target muscle (e.g., subjects 2M, 3M and 4M, Table 1). The identical experimental protocol was used post-training. Subjects did not train on the day of a TMS experiment and all subjects were tested within one week before or after training was concluded. The location of the hot spot was re-identified and typically similar co-ordinates were used (see Table 1). The maximum amplitude of the rectified and smoothed EMG signal during a MVC was also re-measured after training. Care was taken to place the surface EMG electrodes in the same location and similar background EMG activity was maintained to ensure that larger background EMG was not used on the post-training recording day. For example, when a subject's MVC increased post-training, background contractions less than 10% MVC were needed in order to match levels reached during pre-training testing.

Similar procedures as described for the trained SCI subjects were used with non-injured and non-trained SCI control subjects where recruitment curves were recorded on different experimental days. A non-trained SCI group was included to determine if the amount of day-to-day variability of recruitment curves was larger in subjects with SCI given that maintaining a constant background EMG would be more difficult for these subjects. Specifically, the absolute background EMG of the target muscle and the location of the hot-spot were the same on different recording days. Recording days were separated by 1 week in both non-injured and non-trained SCI subjects except for 2 non-injured subjects that were tested 2 and 9 months apart. The non-injured controls were younger than the SCI trained group (see *Subjects* above). Because age does not affect the threshold, slope or MEP_{max} values of TMS recruitment curves (although peak slope and MEP_{max} of the recruitment curve occurs at lower stimulation intensities in younger subjects), we felt that age would not appreciably affect the day-to-day variability in TMS recruitment curves (Pitcher et al. 2003). Both non-injured and non-SCI subjects were instructed to not engage in motor activities that were outside of their normal, weekly routine. All subjects were re-tested at the same time of day, either mid-morning or early afternoon.

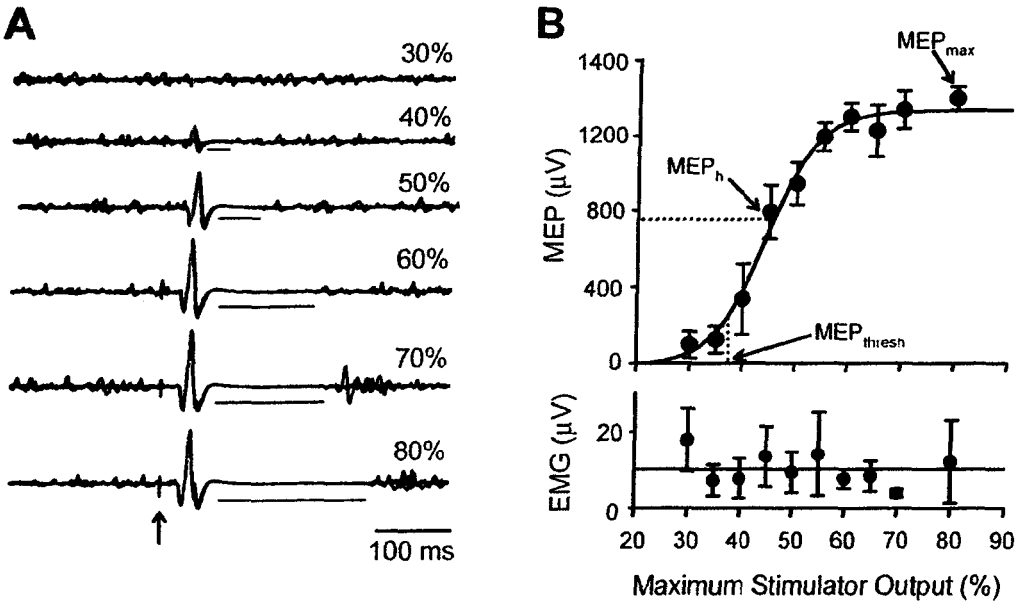

Figure 1
A) Four superimposed raw EMG responses at incrementing levels of TMS from the VL muscle in subject 1M. Time of stimulation marked by arrow. At each stimulation intensity, the average peak-to-peak amplitude of the motor evoked potential (MEP) was calculated. The duration of the silent period was measured as the period of time from the end of the MEP to the earliest re-emergence of background EMG in two of the four TMS trials (marked by solid horizontal lines). B) Top graph: mean peak-to-peak MEP at each stimulation intensity (recruitment curve). A 4-parameter sigmoid function (Boltzman) was fit to the data. MEP_{max} was the largest MEP response in the recruitment curve. MEP_h was measured as the mid-point of the minimum and maximum value of the sigmoid curve. MEP_{thresh} was the stimulation intensity at 5% of maximum of the sigmoid curve. Bottom graph: The average background EMG measured 27 ms prior to the stimulation was plotted for each stimulation intensity with the mean background EMG for the entire recruitment curve marked by the solid horizontal line. Data points represent trial mean ± standard deviation.

3.26 Data analysis: Recruitment curves

Axoscope files were imported into Matlab 6.5 (The Mathworks, Inc., Natick MA) for offline analysis. Custom Matlab software was used to obtain a peak-to-peak value for the MEP response by setting a time window around the MEP and calculating the maximum

and minimum amplitude for the un-rectified EMG signal in this window. At a given stimulation intensity, the four peak-to-peak MEPs were measured and then averaged together. The averaged MEP response was plotted against the corresponding stimulation intensity as per Capaday et al. (1999) to produce a recruitment curve (Fig. 1B). A 4-parameter sigmoid function (Boltzman) was fit to this recruitment curve (see also Knash et al. 2003) that included a parameter for background EMG activity given that there was a measurable peak-to-peak EMG response when no MEP was present. Four specific parameters of the recruitment and sigmoid curves were measured: 1) MEP_{max} which was the largest MEP evoked in the facilitated muscle and typically occurred at the largest stimulation intensities; 2) MEP_h which was measured from the sigmoid curve at the stimulation intensity that produced a half-maximum response in the before-training condition (Carroll et al. 2001; Knash et al. 2003); 3) MEP_{thresh} which was the stimulation intensity that produces 5% of maximum of the sigmoid curve (similar to Carroll et al. 2001); and 4) the slope of the steepest region of the sigmoid curve (measured near MEP_h) (Devanne et al. 1997). In addition to measuring the peak-to-peak values of the MEP, the mean rectified value of EMG over the period of measurement of the MEP was also calculated. To calculate the average background EMG activity at each stimulation intensity, the mean amplitude of the rectified EMG was calculated over a 27-ms window prior to the stimulation with the mean of all points for a given recruitment curve marked by a horizontal line (see lower graph in Fig. 1B).

3.27 Silent period

The silent period, a cessation of background EMG activity after the MEP, was calculated at each stimulation intensity by measuring the time from the end of the MEP to the earliest re-emergence of background EMG in at least two of the four TMS trials that best showed a clear silent period (see horizontal lines in Fig. 1A, Garvey et al. 2001). The duration of the silent period was plotted against the corresponding stimulation intensity to produce a silent-period recruitment curve (e.g., Fig. 6A). The threshold of the silent period was measured as the lowest stimulation intensity where a cessation of background EMG activity, lasting for at least 20 ms, could be observed in at least two of the four TMS trials. The duration of the silent period measured at MEP_{max} and the threshold of the silent period were compared before and after training using paired Student's *t*-tests (see below).

3.28 Locomotor EMG and clinical motor measurements

Surface EMG activity from the TA, SOL, VL and HAM muscles and ankle and knee joint angle trajectories (via goniometry, Biometrics Ltd) were measured both before and after training as subjects walked on a motorized treadmill (5 kHz sample rate). Using custom Matlab software, EMG signals were rectified and low-pass filtered (100 Hz), and 25 steps or more were averaged together using joint angle trajectories to align the averages. The peak amplitude of the locomotor EMG burst, minus background noise, was compared before and after training. In addition, the distance a subject could walk in a 6-

minute period and the time it took to walk 10 m (both measured at preferred walking speeds) were measured along with a qualitative locomotor score, the WISCI II (Dittuno and Dittuno Jr 2001), which is a 21-point scale that measures the degree of assistance and gait aids required to walk 10 m. Each was measured both before and after training by the physical therapist performing the treadmill training. An 11-point manual muscle strength score was measured for the ankle dorsiflexors or knee extensors before and after training, where: 0 = no palpable or visual muscle contraction, 1 = muscle contraction palpable or visible, 2 = complete range of movement with gravity eliminated, 3 = complete range of movement against gravity, 4 = complete range of movement with moderate resistance, 5 = complete range of movement with maximum resistance. Sub-scores were given between the 1 and 4 scales if subjects were able to partially perform a given movement, e.g., 2+ = < 50% initiation of motion against gravity and was given a value of 2.3. Along with MVC measurements, the maximum evoked response to direct nerve stimulation (M_{max}) was tested in the TA or SOL muscle by applying incrementing levels of percutaneous stimulation (1 ms pulse width) over the common peroneal or tibial nerve until a maximal M-wave response was produced. The average peak-to-peak amplitude of 3 M_{max} responses was compared both before and after training.

3.29 Statistical analyses

Paired Student's t-tests were used to compare differences between the various parameters of the recruitment/sigmoid curves (MEP_{max}, MEP_h, MEP_{thresh}, slope and silent period) before and after training in SCI subjects using SigmaPlot 8 software. Similarly, paired

Student's t-test were used to compare differences between muscle strength scores, percent changes in MVC, M_{max}, distance walked in 6 minutes, time to walk 10 m and peak locomotor EMG. A Wilcoxon signed rank test was used to compare ordinal WISCI II scores before and after training using SPSS11 software. To compare the changes in recruitment/sigmoid curve parameters between the different recording days in trained SCI, non-trained SCI and non-injured controls, a one-way ANOVA for repeated measures was used with *post hoc t*-test analysis (Bonferroni-corrected for multiple corrections) using SPSS11 software. The percentage increase in MEP_{max} was correlated with changes in WISCI II score, distance walked in 6 minutes and peak locomotor EMG activity using a Pearson's (r) correlation coefficient. For all tests, a p value of < 0.05 was used to indicate statistical significance. All values are expressed in terms of mean ± standard error (SE) unless otherwise stated.

3.3 Results

3.31 Training-induced changes in TMS recruitment curves: MEP_{max} and MEP_h

Before training, recruitment curves obtained from plotting the mean peak-to-peak MEP against the corresponding TMS intensity were well fit by a sigmoid function in both TA and VL muscles as a large percentage of the variance in the MEP (R^2) was accounted for by the sigmoid curve (solid circles, Fig. 2, median $R^2 = 0.82$). After several months of intensive treadmill training, the size of the MEP increased as reflected by a vertical shift of the recruitment curves (open circles, Fig. 2, median R^2 of sigmoid curves = 0.88). Various patterns of recruitment shift occurred and ranged from cases where MEPs were mainly enhanced at high levels of stimulation intensity (as shown for the TA muscle in Figure 2) to cases where MEPs were enhanced starting at low stimulation intensities (as shown for both VL muscles in Figure 2). Note that the changes in recruitment curves after training occurred even though subjects maintained similar background EMG activity compared to before training trials (see lower graphs in Figure 2).

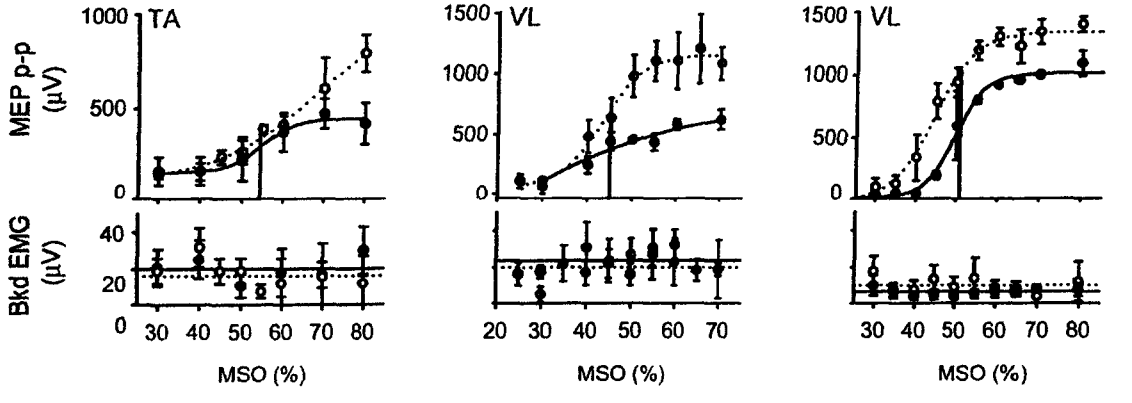

Figure 2
Representative recruitment curves from 3 different subjects before (solid circles, solid lines) and after (open circles, dotted lines) treadmill training. Top graphs: recruitment curves from the TA (subject 5M, left graph) and VL muscles (subject 4M, middle graph; subject 1M, right graph). In all three examples, the recruitment curves after training rested above the recruitment curves before training, especially at intermediate and high stimulation intensities. The slope of the steep portion of the sigmoid line fit to the recruitment curve also increased after training although MEP_{thresh} did not change in most cases. Bottom graphs: The mean background EMG after training (dotted horizontal line) was similar to the background EMG before training (solid horizontal lines). Data points represent trial mean ± standard deviation.

In the 8 trained subjects, MEP responses were measured in 13 muscles, with 5 of these subjects being tested on both legs. Of the 8 subjects tested, 7 demonstrated an appreciable training-induced increase in the maximum MEP (MEP_{max}) in at least one of their leg muscles, with 2 of the 5 subjects tested bilaterally having increases in both leg muscles (9/13 muscles, Table 1). When comparing across all 13 muscles from the 8 subjects, MEP_{max} increased from a mean of 729.0 ± 78.3μV before training to a mean of 1000.9 ± 84.3 μV after training (left bars in Fig. 3A, significantly different) when measured at similar levels of background EMG (right bars in Fig. 3A). When comparing the percentage increase of MEP_{max} in each muscle before and after training to normalize for differences in absolute MEP_{max} size across subjects [(after MEP_{max} – before MEP_{max})/ before MEP_{max} x 100%], there was an average percent increase of 46.3 ± 12.3% across all muscles (left bar in Fig. 3B, significantly different from zero).

We also compared MEPs that were evoked during mid-range stimulation intensities, i.e., MEP_h, which was measured at the stimulation intensity that produced half the maximum response before training (see solid vertical lines in Fig. 2). Similar to MEP_{max}, the mean MEP_h in all 13 muscles increased from 428.5 ± 41.5 before training to 571.4 ± 65.7 μV after training (left bars in Fig. 3C, p = 0.07, not significantly different) when compared at similar levels of background EMG (right bars in Fig. 3C). Correspondingly, the average percentage increase in MEP_h was 45.4 ± 20.3% (left bar, Fig. D, significantly different from zero).

Comparable training-induced increases in MEP responses were also observed when measuring the mean rectified values of the MEP as opposed to measuring the peak-to-peak MEP. For example, the mean rectified MEP_{max} increased from 61.1 ± 8.3μV before training to 95.0 ± 11.7 μV after training (significantly different), a 55.7% increase.

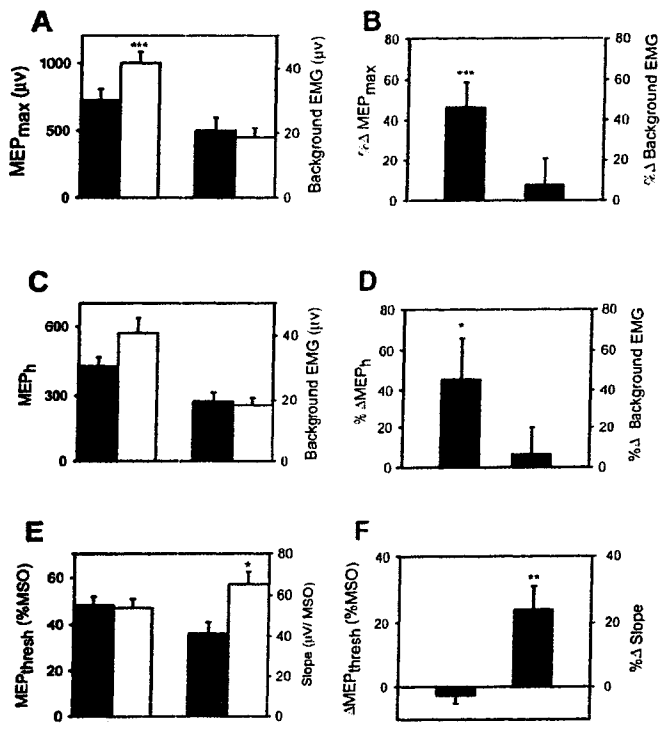

Figure 3
Mean MEP and threshold responses before (solid bars) and after (open bars) treadmill training for all 13 muscles tested. Mean peak-to-peak MEP_{max} (**A**, left bars) and corresponding mean percentage increase of MEP_{max} (**B**, left bar) was larger after training when measured at similar mean background EMG (right bars in A show mean background EMG at MEP_{max}, mean percentage change in B). Likewise, MEP_h increased after training (**C**, left bars) and the mean percentage increase in MEP_h was significant (**D**, left bar)

at comparable background EMG (right bars in C show mean background EMG for entire recruitment curve, mean percentage change in D). MEP_{thresh} did not change after training (left bars in **E**, average change in **F**) whereas the slope of the sigmoid curve did increase after training (right bars in **E**, average percentage increase in **F**). Bars represent group mean ± SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

3.32 Training-induced changes in TMS recruitment curves: Slope and MEP_{thresh}

On average, the slope region of the sigmoid fit to the recruitment curve was steeper after training compared to before, as shown for all muscles in Figure 2. When compared across all muscles the average slope of the sigmoid increased from 41.2 ± 5.7 to 65.3 ± 6.0 μV/MSO (significantly different, right bars in Fig. 3E), suggesting an increase in the excitability and/or expansion of corticospinal networks after training (Ridding and Rothwell 1997). Correspondingly, the average percent increase in peak slope was 23.9 ± 7.1% (right bar in Fig. 3F, significantly different from zero). Changes in the threshold to evoke a MEP response (MEP_{thresh}), a measure of the excitability of the lowest-threshold corticospinal tract networks, were quite variable amongst subjects. When compared across all muscles, MEP_{thresh} (see Methods for definition) did not change after training as shown for the TA and VL muscles in Figure 2 (left and middle graphs). The mean MEP_{thresh} across all muscles was 48.7 ± 3.9% MSO before training and 47.2 ± 3.9% MSO after training (left bars in Fig. 3E, not significantly different) and corresponded to an average absolute decrease in MEP_{thresh} of only -2.9 ± 2.2% MSO (left bar in Fig. 3F, not significantly different from zero).

In 5 subjects, TMS measurements from muscles on different sides of the body in same subject were included in the overall averages because we considered them to be relatively independent measures given that they were produced from two separate corticospinal pathways that need not have been under simultaneous control during locomotor training. In fact, in the majority of subjects from which bilateral recordings were made (3/5 subjects), changes in MEP responses from the left corticospinal tract were different than the changes measured from the right corticospinal tract. None-the-less, when comparing the various recruitment curve parameters (MEP_{max} , MEP_h, MEP_{thresh} and slope) in only 8 muscles from each of the 8 subjects (grouped by either right or left muscles from subjects with bilateral recordings), differences before and after training were still statistically significant for MEP_{max} and slope, similar to that shown for the 13 muscle averages.

3.33 Latencies at MEP_{thresh} and MEP_{max}

The mean latency at which a notable ($\approx$ 50 μV) MEP response was visible in the raw EMG signal (i.e., near MEP_{thresh}) was similar to the latency of the MEP evoked at MEP_{max}, both before (39.4 $\pm$ 1.1 ms vs. 38.6 $\pm$ 2.2 ms) and after training (37.8 $\pm$ 1.7 ms vs. 38.2 $\pm$ 2.1 ms, $p > 0.66$). Moreover, there were no statistical differences between corresponding threshold and MEP_{max} latencies before and after training ($p > 0.20$).

3.34 Changes in antagonist MEP responses

When eliciting MEP responses in the facilitated target muscles (TA or VL), MEP responses were often evoked in the corresponding antagonist muscle, i.e., SOL or HAM

respectively, even though these muscles were electrically silent just prior to the TMS. Similar to the target muscles, there was also a training-induced increase in MEP_{max} of the antagonist muscles as shown for the SOL muscle in Figure 4. Interestingly, in the 9 target muscles that demonstrated a training-induced increase in MEP_{max}, there was also an associated post-training increase of 107.3% in MEP_{max} of the corresponding antagonist muscle when averaged across all 9 muscles. However, the absolute MEP values in antagonist muscles were much smaller than in target muscles, increasing from 102.6 ± 29.7 before training to 212.7 ± 37.5μV after training (significantly different). In the 4 target muscles that did not show an increase in MEP_{max}, the average MEP_{max} of the corresponding antagonist muscles also did not increase after training (from 145 ± 62.3 μV to 123.0 ± 50.6 μV, not significantly different). The parallel changes in MEP_{max} of target and antagonist muscles after training suggest that there was a general increase in the connectivity of corticospinal inputs to muscles of the leg.

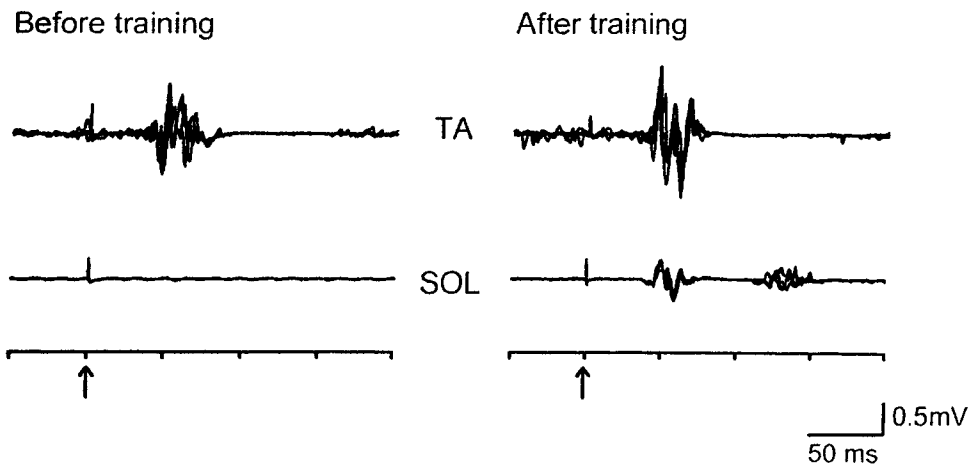

Figure 4
Antagonist MEP's (four superimposed raw EMG traces) of the SOL muscle (bottom traces) to TMS while the subject (7M) produced a background contraction of the target (agonist) TA muscle (top traces). Before training (left graphs) MEPs were not evoked in the electrically silent SOL muscle. After training (right graphs), the MEP in the facilitated TA muscle increased and a MEP emerged in the SOL muscle with a similar latency to that of the TA muscle (40 ms TA vs. 41 ms SOL). Arrows mark the time of the 80% MSO stimulation.

3.35 Maximum voluntary contractions and M-waves

Following training, the peak of the rectified and smoothed EMG activity of the target muscles reached during a maximum voluntary contraction, or MVC, increased, on average, by 16.0% from 164.3 ± 19.0 μV before training to 191 ± 24.7 μV after training (statistically different from zero). Likewise, the manual muscle strength score (see

Methods) increased from 3.1 ± 0.3 before training to 4.0 ± 0.3 after training (significantly different, $p < 0.0002$). In contrast, the maximum evoked response from direct motor axon stimulation, or M_{max}, did not change when tested in the SOL (n = 5) and TA (n = 2) muscles (1.2 ± 0.4 mV before vs. 1.3 ± 0.5 mV after, peak-to-peak M_{max} values, tested in 7 subjects, not significantly different), suggesting that training-induced changes in MEP responses were of central, and not muscle, origin. Moreover, stable M_{max} responses before and after training verifies that comparison of raw EMG values (e.g., peak-to-peak MEP or mean rectified MEP) was valid.

3.36 Reproducibility of MEP measurements: MEP_{max} and MEP_h

To ensure that the changes in recruitment curves observed after training were not simply due to day-to-day variability in the TMS evoked MEP responses, experiments in non-injured control and non-trained SCI subjects, i.e., subjects who should be neurologically stable, were performed on different experimental days. Because non-injured control subjects had much higher MEPs than SCI subjects, we compared the percentage change [(experiment 2 – experiment 1)/ experiment 1 x 100%] in MEP_{max} and MEP_h between the different groups. The mean percentage change in MEP_{max} from day-to-day in non-injured control (-1.2 ± 1.6%) and non-trained SCI subjects (4.2 ± 9.2%) was significantly smaller than the percentage increase measured in trained SCI subjects (46.3 ± 12.3%, Fig. 5A, see also Fig. 3B, one-way ANOVA: $F = 5.29$, $DF = 2, 24$, $p < 0.05$). Likewise, the mean percent increase in MEP_h was greater for SCI trained (45.4 ± 20.2%, Fig. 5B) compared

to non-injured (7.0 ± 12.0%, p = 0.12) and non-trained SCI subjects (-4.5 ± 15.8%, p = 0.38, F = 2.14, DF = 2, 24), although the increase in MEP_h did not reach significance.

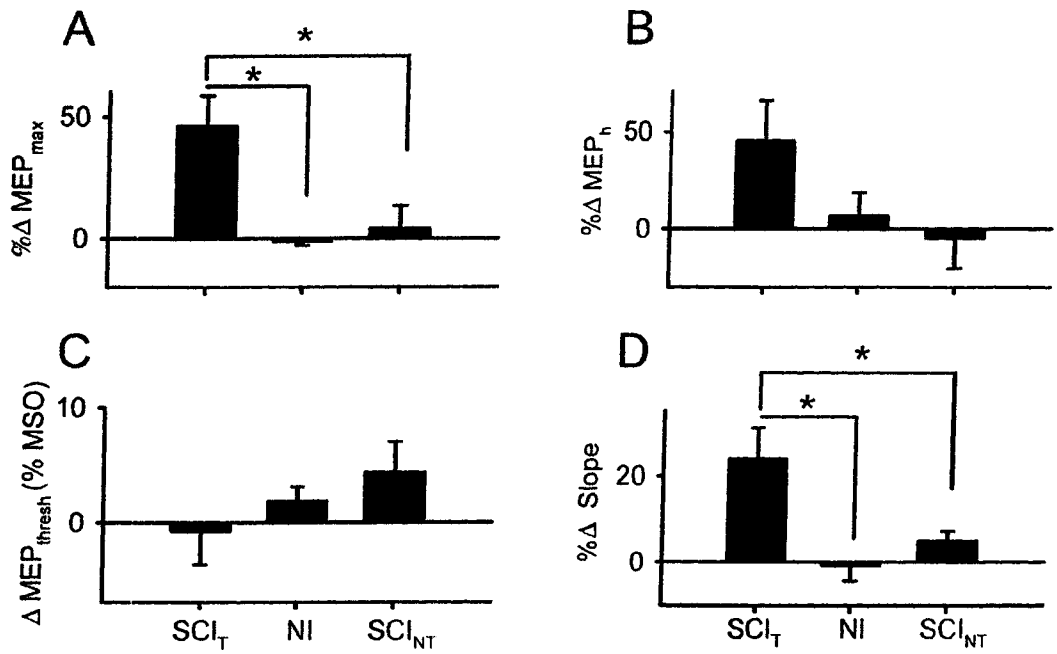

Figure 5
Comparison of mean percentage increases [(day 2- day 1)/ day 1 x 100%] in MEP and slope values and absolute mean differences in threshold responses before and after training in SCI subjects that were trained (SCI_T), and between different experiments days in non-injured control subjects (NI) and in non-trained SCI subjects (SCI_{NT}). (**A**) The mean percentage increase in MEP_{max} in the SCI_T group was significantly greater than the NI and SCI_{NT} groups when compared with one-way ANOVA. The average percentage change in NI subjects tested at 2 and 9 month intervals (-1.75%) was similar to the percentage change in NI subjects tested at a 1 week interval (0.83%). (**B**) The mean percentage increase in MEP_h was greater in trained SCI compared to NI and SCI_{NT} but the difference did not quite reach significance. (**C**) The mean threshold to elicit a MEP after training was lower after training but not statistically different compared to the NI and SCI_{NT}. It was not possible to measure MEP threshold in one SCI_T subject because stimulation intensities used after training were too high. (**D**) Increase in slope was greater in SCI_T compared to both NI and SCI_{NT}. Bars represent group mean ± SE. * $p < .05$. ** $p < 0.01$.

3.37 Reproducibility of MEP measurements: Slope of sigmoid and MEP_{thresh}

The mean percent increase in the slope of the sigmoid fit to the recruitment curve in trained SCI subjects (23.9 ± 7.1%, Fig. 5D) was statistically greater compared to non-

injured (-0.8 ± 9.1%) and non-trained SCI subjects (6.6 ± 2.4%, $F = 4.74$, $DF = 2, 24$, $p < 0.05$). Mean changes in the absolute MEP_{thresh} between different experiment days in non-injured (1.8 ± 1.3% MSO) and non-trained SCI subjects (4.4 ± 2.6% MSO) were not statistically different to the mean decrease in MEP_{thresh} in trained SCI subjects (-2.9 ± 2.2% MSO, $F = 3.04$, $DF = 2, 23$, $p > 0.06$, Fig. 5C).

3.38 Training-induced changes in silent period EMG

Similar to the MEP responses, the duration of the silent period increased after training, as shown for subject 6M in Figure 6A. The larger MEP responses after training should be associated with longer silent periods due to concomitant increases in activation of inhibitory spinal interneurons and/or motoneuron refractory periods (Trompetto et al. 2001). However, silent periods lasting beyond 100 ms, where influences from spinal mechanisms are relatively small (Fuhr et al. 1991; Ikeda et al. 2000), still showed training-induced effects. For example, the average silent period measured at MEP_{max} increased from 130.1 ± 19.9 ms before training to 178.4 ± 27.9 ms after training (left bars in Fig. 6B, significantly different). In addition, the threshold to evoke a silent period (see Methods) decreased from 49.1 ± 3.2% MSO to 41.8 ± 2.7% MSO (right bars in Figure 6B, significantly different), with the incidence of a sub-threshold silent period increasing from 42% of all muscles tested before training to 75% after training.

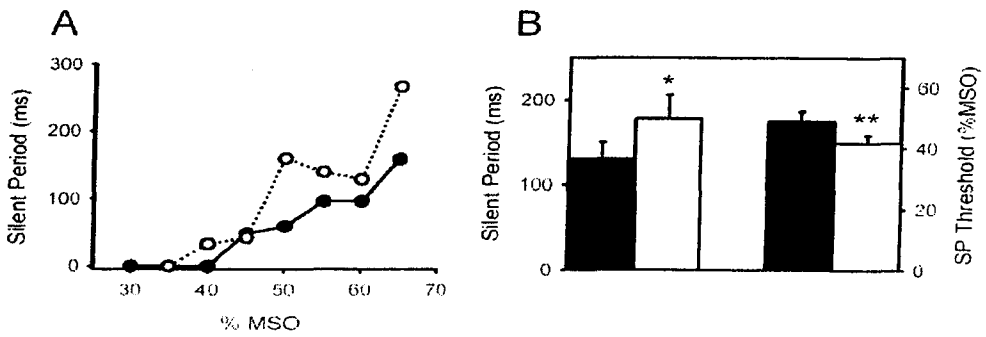

Figure 6
A) Duration of the silent period measured at each stimulation intensity before (solid circles) and after (open circles) training in the TA muscle from subject 6M. **B)** Left bars: the mean duration of the silent period measured at MEP_{max} before (solid bars) and after (open bars) training across all 13 muscles. Right bars: the mean % MSO threshold to evoke a silent period. Bars represent group mean ± SE. * $p < 0.05$, ** $p < 0.01$.

3.39 Correlation between changes in corticospinal tract connectivity and locomotor function

All but one subject (3M) demonstrated improvements in walking function after training as reflected in the increase in WISCI II scores from a mean of 6.4 ± 2.2 before training to a mean of 9.8 ± 2.4 after training (Fig. 7A, $p = 0.027$, Wilcoxon signed ranks test). Interestingly, subject 3M also did not exhibit an appreciable (> 20%) increase in MEP_{max} in response to training. Thus, we wanted to examine if there was a correlation between increases in MEP_{max} and increases in locomotor function gained from training. We found that there was a significant correlation within patients between the percent increase in

MEP_{max} and the absolute increase in WISCI II score (Fig. 7B, r = 0.71, r = correlation coefficient). Subject 8F who had a high WISCI II score before training (17, the upper end of the scale) was excluded from the analysis because we have found previously that such subjects can improve in walking function from training (endurance, speed, etc.) but tend not to advance in their WISCI II scores (personal observations) Similar to that shown for the WISCI II scores, the distance that a subject could walk in 6 minutes increased significantly from 34.2 ± 17.7 m before training to 167.6 ± 51.9 m after training (Fig. 7C) and the peak locomotor EMG activity reached during walking on a treadmill increased from 82.4 ± 25.6 μV to 137.1 ± 10.9μV (Fig. 7E, not significant at p = 0.06). When examining overground walking speed, in the 4 subjects that were able to walk before training (2M, 6M, 7M, 8F), the average time it took to walk 10 m was lower after training (18.8 ± 6.4 s) compared to before (62.6 ± 40.5 s), resulting in an average percentage decrease of 42% (significantly different from zero). Both the increase in distance walked and the increase in peak locomotor EMG correlated significantly to the percent increase in MEP_{max} (r = 0.90 and 0.79, respectively, Figs. 7D and F). There was no significant correlation between the percentage increase in MEP_{max} and the age of the injury (r = 0.47).

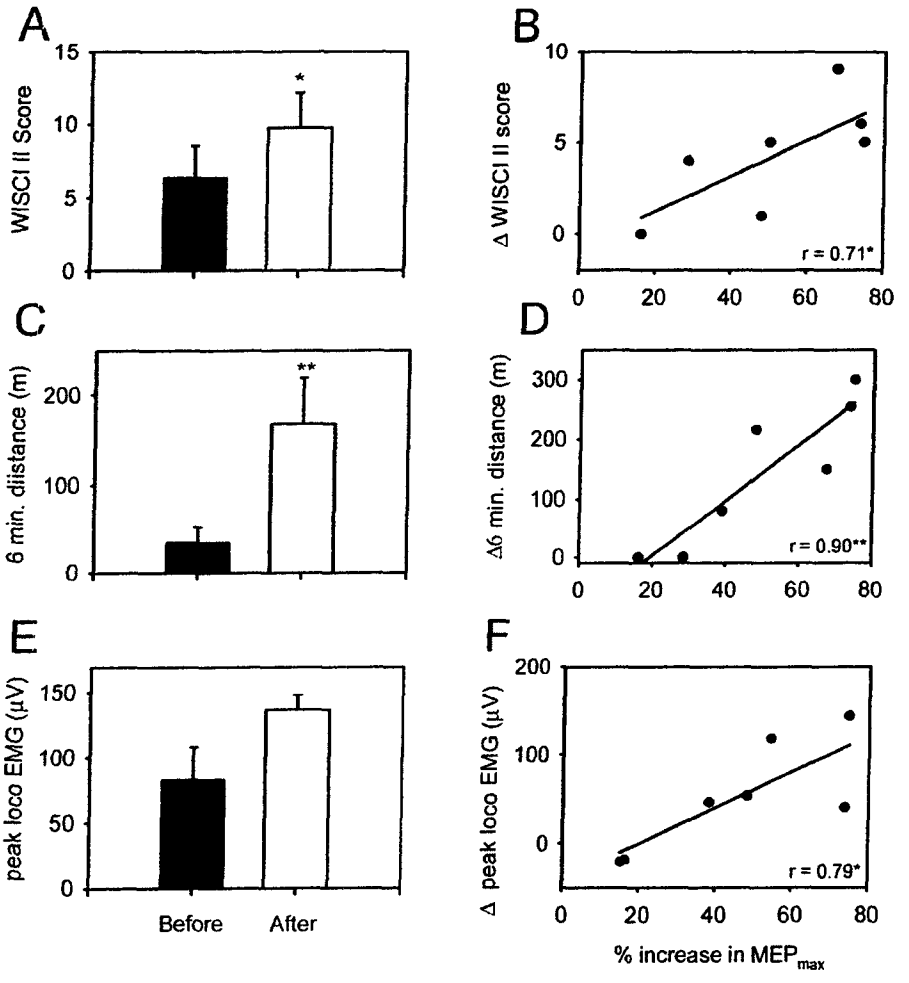

Figure 7
WISCI II score (**A**), distance walked in 6 minutes (**C**) and peak locomotor EMG (**E**) averaged across all subjects before (solid bars) and after (open bars) training. Each of these measures was positively and significantly correlated to the percentage increase in MEP_{max} as shown in **B**, **D** and **F** (r = correlation coefficient). In subjects where MEPs were recorded bilaterally, MEP_{max} from the leg that had the largest increase was used when comparing to WISCI II and 6 min distance scores in B and D. In F, locomotor EMG from both legs were included when recorded. Data was excluded if walking speeds were not matched before and after training. Bars represent group mean ± SE. * $p < 0.05$, ** $p < 0.01$.

In two subjects, 4M and 5M, it was possible to re-measure recruitment curves 2.5 years after training. Both subjects maintained the gains in walking function that were obtained from treadmill training. As shown in Figure 8, the recruitment curves recorded a few years after training (open squares) remained well above the recruitment curves measured

before training (solid circles) and were similar to the recruitment curves recorded immediately after training (open circles).

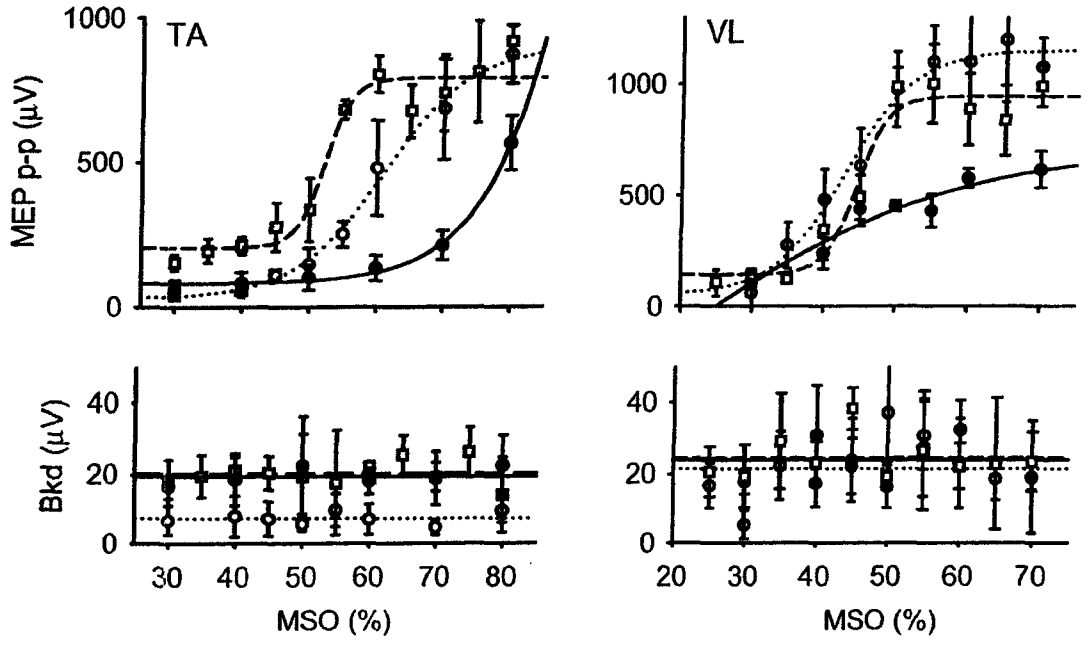

Figure 8
Recruitment curves recorded in subjects 4M (**A**) and 5M (**B**) 2.5 years after receiving treadmill training (solid squares). The follow-up recruitment curves remained above the recruitment curvesrecorded before training (solid circles) and were similar to the recruitment curves recorded immediately after training (open circles). Data points represent trial mean ± standard deviation.

3.4 Discussion

This is the first study to examine the effects of long-term (3-5 months) motor training on spared corticospinal function in subjects with incomplete SCI. We found that intensive treadmill training, and in some cases over-ground training, produced a generalized increase in the connectivity of spared corticospinal pathways, as noted by increases in MEP_{max} of both target (TA and VL) and antagonist (SOL and HAM) muscles. Training-

induced increases in the excitability and/or expansion of corticospinal networks were also suggested by increases in the slope of the sigmoid function fit to the recruitment curve. However, training did not produce increases in the excitability of the lowest-threshold corticospinal tract pathways, as MEP_{thresh} was not changed post-training. We also observed that the duration of the silent period increased after training, even for silent periods lasting longer than 100 ms, where beyond this point, silent periods are mainly produced by cortical mechanisms. One of the striking results from this study was the strong relationship between the percent increase in MEP_{max} (corticospinal connectivity) and the improvement in walking function achieved as a result of daily intensive training, suggesting that recovery of walking was, in part, mediated by the corticospinal tract. Finally, when measured 2.5 years after training, increases in corticospinal tract function were maintained in subjects that continued to use the new locomotor abilities gained from intensive training.

3.41 Changes in MEP response due to motor training

The changes in MEP responses recorded months after the onset of training could have resulted from day-to-day variability in MEP recordings known to be associated with TMS (summarized in Carroll et al. 2001). However, the fact that percent increases in MEP_{max} and the slope of the sigmoid curve were statistically different from the percent changes measured on different recording days in non-injured control and non-trained SCI subjects strongly suggests that the increases in corticospinal tract function were indeed a result of intensive daily training. Although the control subjects were re-tested at shorter time

intervals compared to the trained SCI subjects (1 week vs. 4 months), the day-to-day variability in TMS parameters in 2 non-injured control subjects that were tested 2 and 9 months apart were not different from non-injured controls tested at the 1 week interval (see legend in Fig. 5 for values). Likewise, we would expect that non-trained SCI subjects that had long-standing injuries and/or stable clinical motor scores before training to not demonstrate marked changes in TMS parameters over time if they were not trained. In fact, in SCI subjects that were tested on two separate recording days before training, differences in MEP_{max} and slope were statistically greater after training than before, but not between the different pre-training recording days. Values of MEP_{thresh} proved to be more variable across the different recording days but as a whole, threshold did not change after training. An unchanging MEP_{thresh} suggests that the effective stimulus to the motor cortex from TMS did not change appreciably before and after training so the observed increases in MEP and silent period values were truly a result of training-induced changes in corticospinal tract function rather than a result of greater stimulation currents reaching the cortex.

The highly reproducible responses in non-injured controls and non-trained SCI subjects may have been due to the fact that we used a large stimulating coil, which produces MEP responses that are not sensitive to small changes in coil position and orientation (see also Carroll et al. 2001). As long as subjects maintained a constant level of background EMG facilitation, MEP responses measured on different recording days were very stable when subjects were not being trained. In contrast, motor maps produced by stimulating a 6-by-6 cm grid over the leg region of the primary motor cortex were very variable from day to

day in both non-injured and SCI subjects as it was more difficult to maintain a constant level of background EMG activity throughout the many stimulation trials required to produce a motor map (unpublished results, see also Wolf et al. 2004).

Increases in MEP responses were also observed after training in electrically quiet antagonist muscles. It is possible, therefore, that increases in MEP responses after training may have been due to systematic increases in the sub-threshold depolarization of the respective motoneuron pools or cortical neurons. For example, imagined motor movements that have the potential to increase the sub-threshold level of activation of cortical and spinal neurons have been shown to facilitate MEP responses in lower limb muscles at rest (Tremblay et al. 2001). However, systematic increases in the sub-threshold activation of antagonist cortical or spinal neurons after training are unlikely for several reasons. First, the background level of EMG activation in the target (agonist) muscles was the same or even lower after training; thus, one would expect that the sub-threshold level of activation of cortical and neuron pools of the antagonist muscles would also be about the same or lower. Second, training tended to decrease the amount of co-contraction between flexor and extensor muscles during walking (unpublished results) so that co-activation of antagonist muscles during a steady contraction of a target muscle should also be reduced after training. Finally, when MEP_{max} increased in a target muscle after training, it also increased in the corresponding antagonist muscle; likewise, when MEP_{max} remained constant or slightly decreased in a target muscle after training, its response in the antagonist muscle followed suit. Thus, the corticospinal tract supplying antagonist muscles followed the general excitability changes of the corticospinal tract

supplying agonist muscles whose level of excitation was controlled for both before and after training.

The increases in TMS responses observed post-training could potentially arise from a transient increase in corticospinal tract excitability after a single bout of walking in the SCI subjects and thus, confound our interpretation of the data, especially in subjects that walked into the lab post-training but wheeled in pre-training. However, this was only the case in 2 of the 8 SCI subjects (6M and 7M) and these subjects did not demonstrate increases in TMS responses that were greater than the other subjects using the same modality of transport to the experiment. Moreover, subjects were not trained on the day of the TMS experiments to ensure that we were not examining transient effects from a single bout of training.

3.42 Origin of changes in corticospinal tract function

Given that the evoked responses from direct nerve stimulation (e.g., M_{max}) did not change after training, the increases in TMS-evoked MEPs, and thus, increases in corticospinal tract function in this study were presumably central in origin. However, by only using TMS, it is not possible to determine if the observed increases in spared corticospinal tract function were cortical or spinal in origin (or both). Studies employing short-term motor skill training in non-injured controls have demonstrated that training-induced increases in TMS-evoked MEPs are cortical in origin because MEPs elicited by transcranial electrical stimulation, which mainly activates the axon hillock of corticospinal fibers, are not

affected by training (Perez et al. 2004). Likewise, early reports of imaging studies in incomplete SCI subjects reveal treadmill training can induce reorganization in cortical leg representations (Dobkin 2000). In line with these two studies, we found that silent periods lasting longer than 100ms (as occurred at MEP_{max}) increased even further in duration after training. Since the silent period beyond 100 ms is mainly cortically mediated (Fuhr et al. 1991; Ikeda et al. 2000), long-term motor training likely affected the excitability of cortical tissue that was being activated by the TMS. In addition, we did not observe increases in the amplitude of H-reflexes evoked in either the SOL or TA muscles after training (Monica Gorassini and Jaynie Yang, unpublished observations, see also Schneider and Capaday 2003; Trimble et al. 2001) suggesting that the excitability of spinal circuits did not change appreciably (although different spinal networks may be activated by TMS vs. H-reflexes). Finally, the lack of change in MEP_{thresh} and the concomitant increase in slope of the sigmoid curve is consistent with an expansion of cortical sites activating the muscles of the lower leg as a result of training (Perez et al. 2004; Ridding and Rothwell 1997).

TMS can activate pyramidal axons directly (D waves), especially at high stimulation intensities, and indirectly via interneurons (I waves), especially at low stimulation intensities (Di Lazzaro et al. 2001; Edgley et al. 1997). If MEP responses at high stimulation intensities were activated predominantly by D-waves, then increases in MEP_{max} from training would most likely result from increases in spinal excitability. However, the latency of the MEP at threshold (low stimulation intensities) was similar to the latency at MEP_{max} (high stimulation intensities), indicating that we were not

consistently evoking D waves at high levels of stimulation intensity. Thus, increases in MEP_{max} after training were perhaps a result of increased I-wave activation as a result of increased excitability of intrinsic cortical networks or their inputs (Ridding and Rothwell 1995). In line with this, MEPs that were activated during mid-range TMS intensities (at MEP_h), where there is most likely to be I-wave activation, were also increased after training and provide supportive evidence for the excitability/expansion of cortical networks. Regardless of the location, the important finding is that the function of the spared corticospinal tract can be enhanced after SCI following several months of intensive training, even for injuries that have occurred many years before training (see Table 1). Such increases in corticospinal tract function appear to be long-lasting when the trained locomotor function is maintained; however, it remains to be tested if corticospinal tract function decreases when locomotor function cannot be maintained after training.

If increases in the strength and recruitment of cortical networks/inputs excited by TMS occurred, training probably affected existing spared connections rather than recruiting, on a large scale, new areas of motor-related cortices. This is supported by the observation that the location where the largest MEP response could be evoked, or hot spot, did not change appreciably after training (see Table 1). In most cases, hot spot co-ordinates were centered over the leg area of the primary motor cortex (1 cm lateral and 1 cm posterior to vertex, Petersen et al. 2001), suggesting that training increased the function of spared projections from the primary motor cortex and did not recruit, on a large scale, pre-motor and supplementary motor areas as seen for the affected hemisphere during stroke (Liepert et al. 1998; Trompetto et al. 2000). However, assessing the loci of plasticity with TMS,

especially for the leg area, is very difficult and these other nodes of the sensorimotor network, in addition to cerebellar, reticulospinal and propriospinal networks, most likely contributed to enhancing corticospinal and motor function (Dobkin 2000)

3.43 Correlation between improvements in walking function and corticospinal tract function

The percent increase in connectivity of the corticospinal tract, or MEP_{max}, was positively and significantly correlated to the amount of improvement in locomotor function as assessed by the increase in WISCII II score, the distance a subject could walk in a 6-minute period and the increased peak locomotor EMG activity. Although the above correlation does not prove unequivocally that increases in the strength of the corticospinal tract *cause* improvements in walking function, the data do provide strong evidence that increases in corticospinal tract function have relevance to the trained motor behavior (see also Fraser et al. 2002). Information as to the relationship between the time course of MEP increases and improvements in locomotor function would help to determine the role of the corticospinal tract in mediating locomotor recovery from training. For example, in one subject (7M) who was tested at 2 months into a 4.5-month training trial, both MEP_{max} and WISCI II scores reached maximal values at 2 months even though walking distance and weight bearing continued to improve over the next 2.5 months. These initial observations suggest that the corticospinal tract plays a larger role in improving walking skill rather than walking endurance and strength. Future studies will examine the time-course of the increases in MEP responses in relation to functional improvements.

If increases in the function of the spared corticospinal tract do mediate, in part, training induced improvements in walking then subjects should concentrate on voluntarily activating their leg muscles during treadmill training. By using slow walking speeds and allowing subjects to activate their muscles independently as much as possible, subjects in this study not only improved in their over-ground walking function but also in their ability to voluntarily activate their muscles in contrast to that shown in other studies (Wernig et al. 1999). Maximum voluntary intervention may occur when training is conducted at low speeds of walking (≈ 1.6 km/h) as was done in this study. However, training stroke patients at fast speeds of walking on the treadmill results in faster self-selected velocities of overground walking (Pohl et al. 2002; Sullivan et al. 2002). In addition, the more normal gait produced at higher velocities of walking may allow subjects to concentrate more on the overall gait pattern rather than on individual muscles (Pepin et al. 2003). Thus, further studies examining the effect of training speed on locomotor recovery in both acute and chronic injury are required.

In summary, our results demonstrate that improvements in corticospinal tract function and motor recovery can occur with intensive daily training, even many years after a SCI. These increases in corticospinal tract and motor function are long-lasting when tested in subjects that continue to use the new locomotor abilities gained from intensive training.

3.4 REFERENCES

Barbeau H, Wainberg M, and Finch L. Description and application of a system for locomotor rehabilitation. *Med Biol Eng Comput* 25: 341-344, 1987.

Brouwer B and Hopkins-Rosseel DH. Motor cortical mapping of proximal upper extremity muscles following spinal cord injury. *Spinal Cord* 35: 205-212, 1997.

Buonomano DV and Merzenich MM. Cortical plasticity: from synapses to maps. *Annu Rev Neurosci* 21: 149-186, 1998.

Capaday C. The special nature of human walking and its neural control. *Trends Neurosci* 25: 370-376, 2002.

Carroll TJ, Riek S, and Carson RG. Reliability of the input-output properties of the cortico-spinal pathway obtained from transcranial magnetic and electrical stimulation. *J Neurosci Methods* 112: 193-202, 2001.

Devanne H, Lavoie BA, and Capaday C. Input-output properties and gain changes in the human corticospinal pathway. *Exp Brain Res* 114: 329-338, 1997.

Di Lazzaro V, Oliviero A, Profice P, Meglio M, Cioni B, Tonali P, and Rothwell JC. Descending spinal cord volleys evoked by transcranial magnetic and electrical stimulation of the motor cortex leg area in conscious humans. *J Physiol* 537: 1047-1058, 2001.

Dietz V, Colombo G, and Jensen L. Locomotor activity in spinal man. *Lancet* 344: 1260-1263, 1994.

Ditunno PL and Ditunno Jr JF, Jr. Walking index for spinal cord injury (WISCI II): scale revision. *Spinal Cord* 39: 654-656, 2001.

Dobkin BH. Spinal and supraspinal plasticity after incomplete spinal cord injury: correlations between functional magnetic resonance imaging and engaged locomotor networks. *Prog Brain Res* 128: 99-111, 2000.

Dobkin BH and Havton LA. Basic advances and new avenues in therapy of spinal cord injury. *Annu Rev Med* 55: 255-282, 2004.

Edgerton VR, Leon RD, Harkema SJ, Hodgson JA, London N, Reinkensmeyer DJ, Roy RR, Talmadge RJ, Tillakaratne NJ, Timoszyk W, and Tobin A. Retraining the injured spinal cord. *J Physiol* 533: 15-22, 2001.

Edgley SA, Eyre JA, Lemon RN, and Miller S. Comparison of activation of corticospinal neurons and spinal motor neurons by magnetic and electrical transcranial stimulation in the lumbosacral cord of the anaesthetized monkey. *Brain* 120 (Pt 5): 839-853, 1997.

Fraser C, Power M, Hamdy S, Rothwell J, Hobday D, Hollander I, Tyrell P, Hobson A, Williams S, and Thompson D. Driving plasticity in human adult motor cortex is associated with improved motor function after brain injury. *Neuron* 34: 831-840, 2002.

Fuhr P, Agostino R, and Hallett M. Spinal motor neuron excitability during the silent period after cortical stimulation. *Electroencephalogr Clin Neurophysiol* 81: 257-262, 1991.

Garvey MA, Ziemann U, Becker DA, Barker CA, and Bartko JJ. New graphical method to measure silent periods evoked by transcranial magnetic stimulation. *Clin Neurophysiol* 112: 1451-1460, 2001.

Ikeda A, Ohara S, Matsumoto R, Kunieda T, Nagamine T, Miyamoto S, Kohara N, Taki W, Hashimoto N, and Shibasaki H. Role of primary sensorimotor cortices in generating inhibitory motor response in humans. *Brain* 123 (Pt 8): 1710-1721, 2000.

Karni A, Meyer G, Jezzard P, Adams MM, Turner R, and Ungerleider LG. Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature* 377: 155-158, 1995.

Knash ME, Kido A, Gorassini M, Chan KM, and Stein RB. Electrical stimulation of the human common peroneal nerve elicits lasting facilitation of cortical motor-evoked potentials. *Exp Brain Res* 153: 366-377, 2003.

Levy CE, Nichols DS, Schmalbrock PM, Keller P, and Chakeres DW. Functional MRI evidence of cortical reorganization in upper-limb stroke hemiplegia treated with constraint-induced movement therapy. *Am J Phys Med Rehabil* 80: 4-12, 2001.

Levy WJ, Jr., Amassian VE, Traad M, and Cadwell J. Focal magnetic coil stimulation reveals motor cortical system reorganized in humans after traumatic quadriplegia. *Brain Res* 510: 130-134, 1990.

Liepert J, Bauder H, Wolfgang HR, Miltner WH, Taub E, and Weiller C. Treatment-induced cortical reorganization after stroke in humans. *Stroke* 31: 1210-1216, 2000.

Liepert J, Miltner WH, Bauder H, Sommer M, Dettmers C, Taub E, and Weiller C. Motor cortex plasticity during constraint-induced movement therapy in stroke patients. *Neurosci Lett* 250: 5-8, 1998.

Nudo RJ. Adaptive plasticity in motor cortex: implications for rehabilitation after brain injury. *J Rehabil Med*: 7-10, 2003.

Pepin A, Norman KE, and Barbeau H. Treadmill walking in incomplete spinal-cord-injured subjects: 1. Adaptation to changes in speed. *Spinal Cord* 41: 257-270, 2003.

Perez MA, Lungholt BK, Nyborg K, and Nielsen JB. Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. *Exp Brain Res*, 2004.

Petersen NT, Butler JE, Marchand-Pauvert V, Fisher R, Ledebt A, Pyndt HS, Hansen NL, and Nielsen JB. Suppression of EMG activity by transcranial magnetic stimulation in human subjects during walking. *J Physiol* 537: 651-656, 2001.

Pohl M, Mehrholz J, Ritschel C, and Ruckriem S. Speed-dependent treadmill training in ambulatory hemiparetic stroke patients: a randomized controlled trial. *Stroke* 33: 553-558, 2002.

Ribotta MG, Provencher J, Feraboli-Lohnherr D, Rossignol S, Privat A, and Orsal D. Activation of locomotion in adult chronic spinal rats is achieved by transplantation of embryonic raphe cells reinnervating a precise lumbar level. *J Neurosci* 20: 5144-5152, 2000.

Ridding MC and Rothwell JC. Reorganisation in human motor cortex. *Can J Physiol Pharmacol* 73: 218-222, 1995.

Ridding MC and Rothwell JC. Stimulus/response curves as a method of measuring motor cortical excitability in man. *Electroencephalogr Clin Neurophysiol* 105: 340-344, 1997.
Schneider C and Capaday C. Progressive adaptation of the soleus H-reflex with daily training at walking backward. *J Neurophysiol* 89: 648-656, 2003.
Sullivan KJ, Knowlton BJ, and Dobkin BH. Step training with body weight support: effect of treadmill speed and practice paradigms on poststroke locomotor recovery. *Arch Phys Med Rehabil* 83: 683-691, 2002.
Thomas SL and Gorassini MA. Training-induced plasticity in corticospinal pathways after spinal cord injury. *Can Phys Soc Meeting and RB Stein Symposium* 36, 2004.
Tillakaratne NJ, de Leon RD, Hoang TX, Roy RR, Edgerton VR, and Tobin AJ. Use-dependent modulation of inhibitory capacity in the feline lumbar spinal cord. *J Neurosci* 22: 3130-3143, 2002.
Topka H, Cohen LG, Cole RA, and Hallett M. Reorganization of corticospinal pathways following spinal cord injury. *Neurology* 41: 1276-1283, 1991.
Tremblay F, Tremblay LE, and Colcer DE. Modulation of corticospinal excitability during imagined knee movements. *J Rehabil Med* 33: 230-234, 2001.
Trimble MH, Behrman AL, Flynn SM, Thigpen MT, and Thompson FJ. Acute effects of locomotor training on overground walking speed and H-reflex modulation in individuals with incomplete spinal cord injury. *J Spinal Cord Med* 24: 74-80, 2001.
Trompetto C, Assini A, Buccolieri A, Marchese R, and Abbruzzese G. Motor recovery following stroke: a transcranial magnetic stimulation study. *Clin Neurophysiol* 111: 1860-1867, 2000.
Trompetto C, Buccolieri A, Marinelli L, and Abbruzzese G. Differential modulation of motor evoked potential and silent period by activation of intracortical inhibitory circuits. *Clin Neurophysiol* 112: 1822-1827, 2001.
Wernig A and Muller S. Laufband locomotion with body weight support improved walking in persons with severe spinal cord injuries. *Paraplegia* 30: 229-238, 1992.
Wernig A, Nanassy A, and Muller S. Laufband (treadmill) therapy in incomplete paraplegia and tetraplegia. *J Neurotrauma* 16: 719-726, 1999.
Wernig A, Nanassy A, and Muller S. Maintenance of locomotor abilities following Laufband (treadmill) therapy in para- and tetraplegic persons: follow-up studies. *Spinal Cord* 36: 744-749, 1998.
Wolf SL, Butler AJ, Campana GI, Parris TA, Struys DM, Weinstein SR, and Weiss P. Intra-subject reliability of parameters contributing to maps generated by transcranial magnetic stimulation in able-bodied adults. *Clin Neurophysiol* 115: 1740-1747, 2004.

CHAPTER 4:

General Discussion and Conclusion

4.1 Changes in motor response due to training

Task specific motor training after SCI will result in an increase in the connectivity of spared cortical pathways and will generally manifest as long-term functional improvements in the mobility of individuals who receive the training (Thomas and Gorassini 2005). This is suggested by post training increases in MEPmax, occurring in both the facilitated muscle and the partnered antagonists. The improvements seen in muscle activation are most likely from active motor training, which can encourage cortical facilitation from a variety of different locations along the CNS. Primarily, training induces an increase in excitability of cortical cells which can cause a further expansion in the cortical network of cells devoted to muscle signaling. Increases in spinal networks have also been implicated, but are probably not the sole contributor for numerous reasons. Firstly, cortical excitability was measured with TMS and the increase in MEPmax was paired with an increase in the overall duration of the silent period (SP), of which the maximum SP duration was greater than 100ms. Studies have demonstrated that the length of the SP in excess of 100 ms is mediated by the activation of inhibitory cortical circuits (Fuhr et al. 1991; Ikeda et al. 2000). Since increases in MEP were paralleled by an increase in the cortically mediated component of the SP, it is suggestible that training induced changes, inclusive of increases to the excitatory cortical networks responsible for producing the MEP, were cortical in origin. Secondly, the onset latency of

MEP threshold and MEP max did not change. As discussed earlier, TMS can induce MEPs either directly or indirectly. At lower stimulation intensities, TMS will produce predominantly I-wave activation, meaning activity will be primarily cortical in origin. In this study there was no apparent change in latency at either threshold or MEP_{max}, the occurrence of which would suggest indirect activation switched to direct activation as stimulation intensity increased. Further, there was no notable change in latency when comparing between before training and after training motor responses. This lends support to TMS producing MEP activation that is primarily intercortical in action, and this would seem to apply throughout the experiment. Although these results were calculated as average latencies, the assumption can be made that there was minimal D-wave activation throughout. This is supported by minimal variation in MEP latencies when comparing single sweep responses from the same individual receiving stimulation, both before and after training. Finally, unpublished observations failed to note an accompanied increase in the amplitude of the H-reflex (Yang and Gorassini personal communication) and further suggest that increases in MEP responses were not likely from facilitation of spinal excitability. Arguably, inter-cortical facilitation provides the best explanation for improvements seen in CST signaling and this can be further linked to the functional improvements that result from motor training.

4.2 Motor mapping

In an attempt to further quantify corticospinal changes as a result of motor rehabilitation we also set out to map cortical activation both before and after training (unpublished

results). Similar to the RC, measurements were taken with a small background contraction allowing for facilitation of the subjects' muscle response (Davey et al. 1999). Attempts were made to ensure the background excitability of cortical and spinal neurons was constant both before and after training. Mapping followed a cross hair formation that was centered on the hot spot; this was done to reduce the number of applied stimulations, therefore reducing the overall time a subject was required to maintain a sustained contraction. Since we were most interested in results from the RCs, motor mapping was always done at the latter end of the experiment. In this set of experiments, a complete RC would generally necessitate 10 different stimulation intensities, with four stimulations being applied at each intensity level. In each experiment the RC was repeated two or three times on each leg, done for the purpose of testing inter and intra experimental reproducibility. Stimulations were also applied to subjects in order to locate the area on the scalp defined as hot spot, further requiring the patient to contract to the required background level. This would translate to over 120 TMS applications where a background contraction had to be maintained by the subject before mapping even began; mapping then required a further 44 stimulation applications. It is likely that in many cases, the duration of time subjects were required to keep steady background facilitation was too great and fatigue may have introduced instability in the required level of background muscle contraction. In combination to possible fatigue, subjects with SCI show a greater degree of variability in the motor response from a larger magnitude range of background facilitation (Davey et al. 1999). For example, non-injured subjects generally cease to show facilitation past 10% MVC, where the facilitated maximum MEP reaches a plateau. Unlike the control subjects, those with a SCI will show a

comparatively reduced MEP at 10% MVC, which is considerably more unstable, and the MEP of a SCI subject will continue to show facilitation with as much as 50% MVC, also increasing the probability for inconsistent output measures. This is clearly demonstrated in figure 4-1, where the subject was tested both before and after training with the same stimulation intensity while attempting to maintain similar levels of background facilitation. The map after training does show an increase in excitability; however, the argument that this is due to a cortical increase in excitability is weakened by the associated increase in background facilitation. (Figure 4-1). Finally, this study mapped at a stimulation intensity that was 1.1X pre-training threshold. Motor map responses, evoked from TMS applied just above threshold, may be more prone to variability, as compared to measurements taken at MEP_{max}. In conjunction with this, results from the recruitment curve did not demonstrate significant MEP enhancement at threshold levels. For this reason, increases in excitability, measured from mapping at stimulation intensities just above threshold may not show a measurable change from cortical enhancement. A handful of subjects demonstrated an increase in motor map excitability after training, even with notably smaller background facilitation post training (Figure 4-2). Despite this, changes that were seen in the cross hair maps recorded from subjects who received motor training are possibly a reflection of variability in mapping, which does explains why both increases and decreases in motor cortical mapping were noted. Non-injured subjects also demonstrated map instability; however, when background levels were closely monitored mapping was generally reproducible. Motor maps from normal subjects were generally recorded from stimulating scalp locations on a 5 cm X 5 cm grid. The addition of extra stimulation points possibly contributed to a more complete

and apparently stable map. This is in compliance with other mapping studies done in non-injured populations.

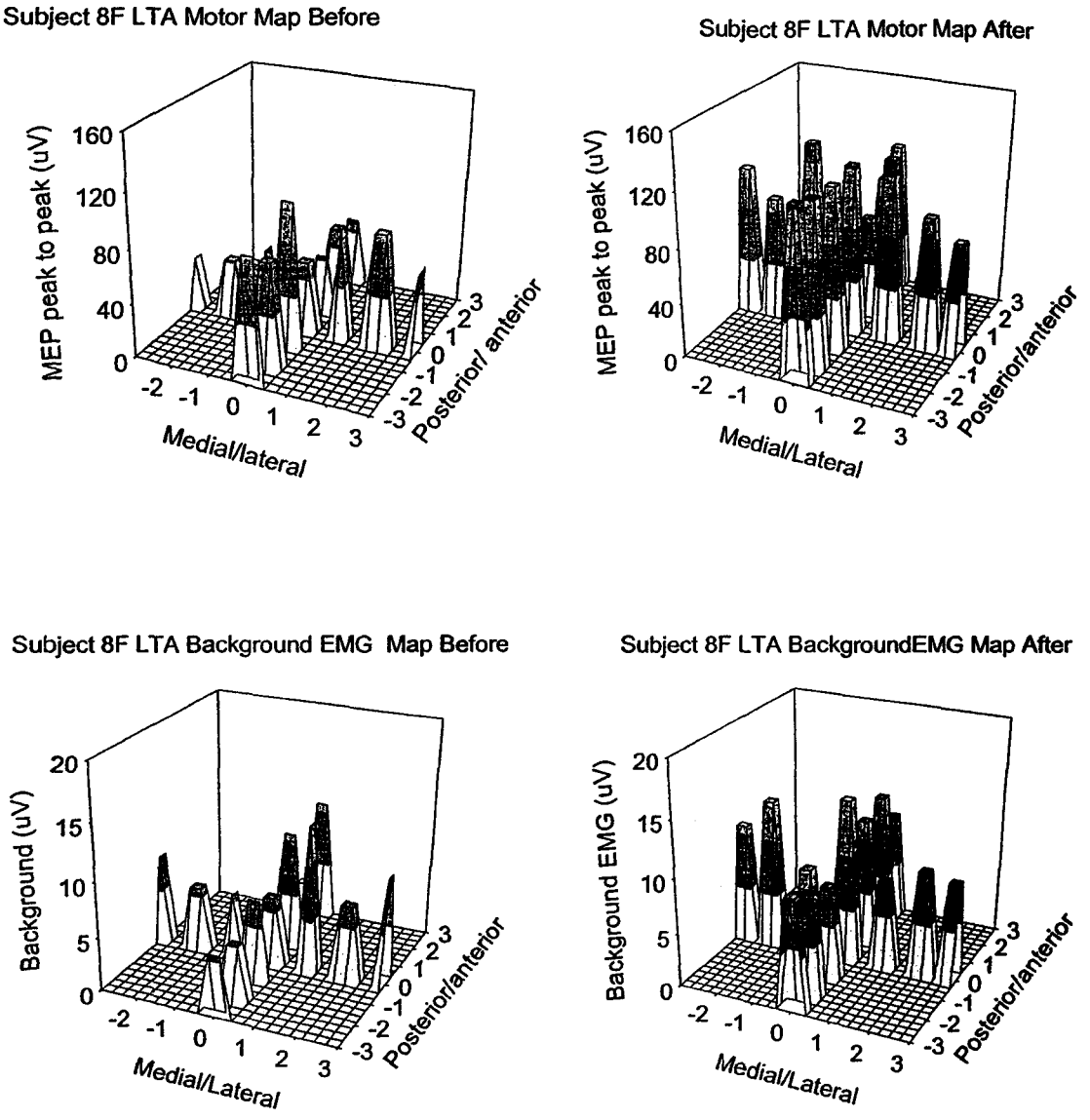

Figure 4-1
Motor maps (top graphs) with associated background contraction (bottom graphs), recorded in one subject (8F) both before (left) and after (right) training. Mapping was done at 54% MSO centering on one-cm lateral and one-cm posterior of vertex. Each value represents the mean of four peak to peak MEP responses to stimulation applied to that representative spatial location.

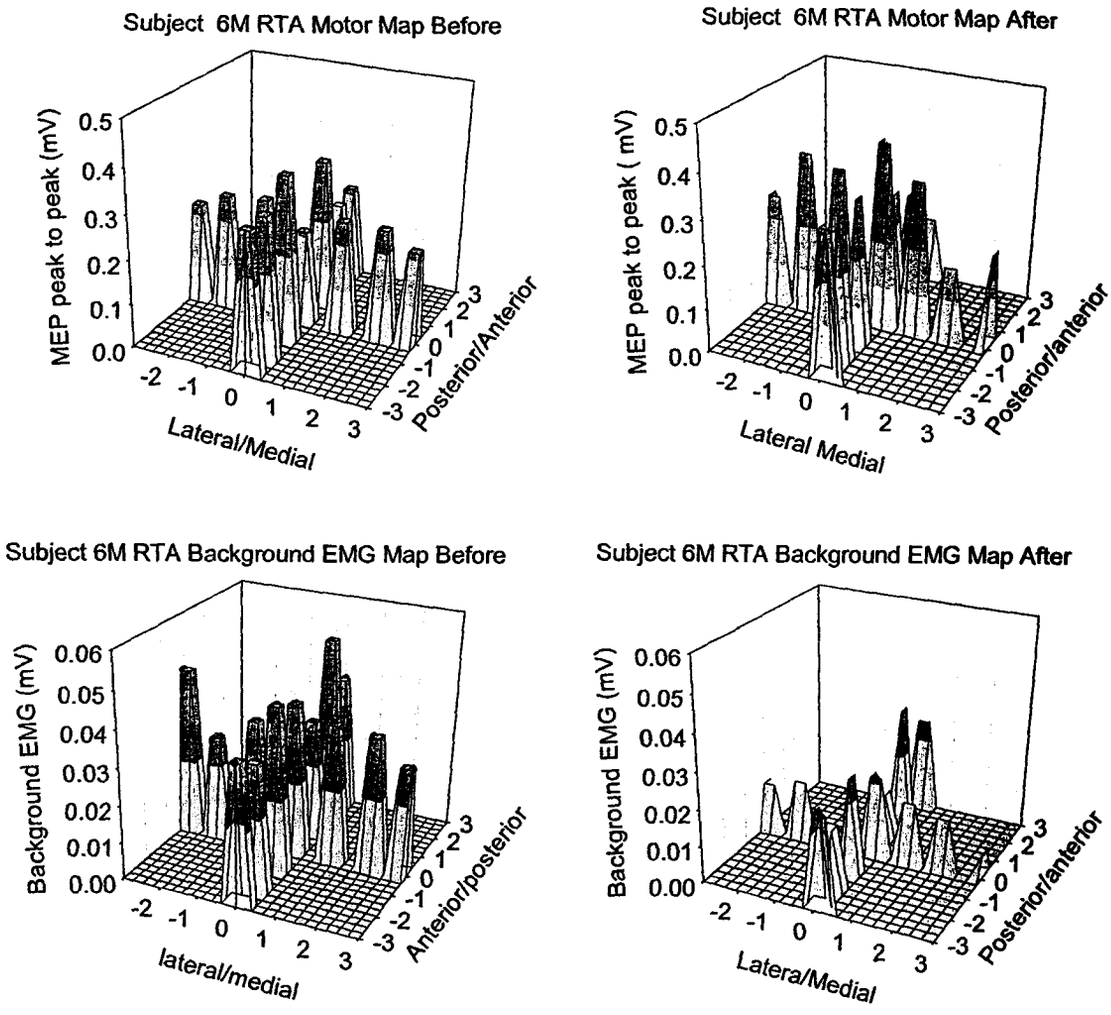

Figure 4-2
Motor maps (top graphs) with associated background contraction (bottom graphs), recorded in one subject (6M) both before (left) and after (right) training. Mapping was done at 54% MSO centering on two-cm lateral and one-cm posterior of vertex. Each value represents the mean of four peak to peak MEP responses to stimulation applied to that respresentative spatial location.

Comparing our study to studies that have investigated map stability can provide a further rationale as to why our study failed to provide reliable mapping data. Uy et al (2002) researched the stability of motor maps and concluded that minimal variability is inherent to motor maps. Uy suggests that any observed shift in mapping could be the result of continuous reorganization of the motor cortex, occurring in response to the everyday functional demands of the muscle in question (Uy et al. 2002). They also added that noise in the measurement process would contribute to variability. The final conclusion from Uy's study was that motor maps of the intrinsic hand muscles are comparatively stable for intervals of no longer than two weeks. A few points should be made respecting the validity of our mapping data as compared to this study. Firstly, comparative analysis from our study necessitates measurement intervals of at least three months and this is far outside Uy's experimentally derived limit. Secondly, Uy's study measured map stability in a non-injured population with muscles recorded at rest. This study was done in an injured population and required background contractions to ensure a measurable response at lower stimulation intensities. MEP variability is demonstrated to be significantly increased when measured in an injured population, especially when evoked with a background contraction. Finally, our study measured responses from lower extremity muscles and although TMS recorded from lower extremity muscles has been shown to behave similar to upper extremity muscles, responses are a result of activation from different motor neuron pools that are subject to different levels of variability with different experimental protocols. Unfortunately, both the experiment length and the heightened variability found with variations in background facilitation and hence motor output, made mapping an unreliable method to compare cortical change from training as

applied in this particular study. Despite this, RC data collected from SCI subjects remain a reliable and conclusive source of evidence supporting improvements in corticospinal function.

4.3 Muscle coherence and corticospinal tract function

This study demonstrated a positive correlation between increased corticospinal excitability with functional improvements, as seen in locomotion patterns of subjects who undergo intensive treadmill training. Although the experimental protocol specifically had TMS being applied to subjects while sitting and maintaining a background contraction, the results still suggest that general increases in excitability occur and contribute to functional improvements in walking. To confirm that increases in excitability during task specific activity occurred, a subsequent study estimated the changes in corticospinal drive during the recovered walking, showing that training alters the inter-muscular coherence profiles, but only in subjects with incomplete spinal cord injury who demonstrated functional improvements (Norton and Gorassini 2006). Muscle coherence patterns are derived from EMG activity and can serve to measure the similarity in the frequency domain between antagonist muscle pairs (Challis and Kitney 1991; Gross et al. 2000; Grosse et al. 2002; Salenius and Hari 2003). There are two types of frequency domains that can be measured from musculature, higher frequency "beta" bands (20-40 Hz), which are thought to originate from a cortical source and lower frequency bands (<20 Hz) that are most likely mediated by a sub cortical source such as spinal networks. Studies have shown individuals who suffer from anatomically complete spinal cord injury will

only show muscle activation with a coherence profile in the lower frequency band. Further, this particular study had subjects segregated by pre-training parameters, those with moderate volitional motor strength in their leg muscles compared with those having little to no strength in the same leg musculature. Interestingly, only subjects who showed moderate volitional motor strength had notable improvements in their high frequency coherence profile. This observation is further compounded by post training results that demonstrate a correlation between increases in the magnitude of the high frequency coherence profile and measurable locomotor recovery. Subjects who did not respond to the training had little or no change in either of the muscles frequency profiles.

4.4 Imaging data and corticospinal tract function

Results from the Norton study reflect significantly on the TMS study discussed in this paper, as consistent overlap occurs in the results of both studies. For example, each of the subjects who demonstrated a significant increase in MEPmax, and consequently improved in locomotor function, were among the subjects who showed post training increases in high frequency coherence. The two studies taken together demonstrate a training induced improvement in CST function where the most probable origin for behavioural change is cortical. In complement, imaging studies also support the concept of training induced cortical plastic change (Fraser et al. 2002; Karni et al. 1995; Lotze et al. 2003a). One particular case study used imaging techniques as an indicator of cortical change after having SCI subject engage in similar treadmill rehabilitation. Results were complimentary and demonstrated that functional improvement were accompanied by

associated changes to brain activation. Imaging techniques localized the areas of altered cortical activity to both cortical and cerebellar regions (Winchester et al. 2005).

4.5 Mechanisms of Corticospinal plasticity

Two main contributors can explain functional recovery occurring largely at a cortical level. In the initial stages after a central motor injury occurs, there is an acute stage of heightened excitability in neural cells with salvaged signaling. These highly excitable cells are hypersensitive to any possible input that could replace the lost signaling. This could be considered recovery using parallel or redundant pathways. Or, there may be long-term recruitment of new cellular regions in order to 'replace' those cells that previously received input through the now disrupted system. These cells new 'take over' the function of the disabled area. The most probable explanation is of course both. When cortical signaling is disrupted, this leads to a phenomenon described as unmasking, meaning cortical cells originally responsible for muscle control from the lost input will now be hyper responsive to any remaining intact collateral input. The activity of these cells will then be able to influence other surrounding cells through horizontal projections and this can lead to a 'shift' in cortical representation, making up for lost function.

The evidence suggests training induced changes in cortical excitability and hence signaling does not necessitate a change in threshold (Smith et al. 2000). In the acute stage, post injury there may be a slight modulation in the cellular threshold of activation; however, this does not seem to be as pronounced as when it occurs in ischemic models or

peripheral amputation. In fact, following corticospinal function over time post SCI will reveal that there is minimal deviation in threshold levels, specifically when a muscle is engaging in a slight contraction. This evidence further supports the possible recruitment of new cells contributing to the recovery of muscle activation and is contrary to their merely being an increase in the excitability of the remaining cells. Although this study does not provide conclusive evidence to support increased map area, the increase in the slope of RCs from individuals who did show significant functional improvement suggest a broadening of cortical cells concerned with the activation of specific muscle groups (Ridding and Rothwell 1997). Likewise in amputation models, the expanded motor map could really be a ramification of increased cellular activity, which is common under acute injury conditions.

4.6 Other methods of inducing cortical change and optimizing rehabilitation after injury

In conjunction to maximizing a training protocol, future studies should endeavor to explore combining rehabilitation strategies, each of which have been independently shown to improve post injury outcome. For example, alternate methods of inducing cortical activity do exist and can include artificial stimulation protocols and the application of pharmacological agents. Looking first at stimulation as a means of enhancing rehabilitation post injury, both peripheral and cortical stimulation have been used to drive cortical change. In each of these cases, subjects demonstrate improvements

in their functional ability to perform motor tasks dependant on muscle groups associated with the stimulation. For example, patients suffering from the effects of stroke who were exposed to pharyngeal stimulation improve their functional ability to swallow; the functional improvements that are seen can be linked with improvements in cortical representation and are supported by measures indicating an increased cortical excitability and an expanded motor map. Other studies also support the use of peripheral nerve stimulation, as it enhances the cortical excitability of the targeted area. Indeed, this has been used repeatedly to supplement recovery from injury. Peripheral stimulation likely works by feeding back through the same pathways that are active when voluntary practice is used as a means of enhancing recovery.

Taking this one step further, recent studies have applied stimulation directly to the cortex in order to facilitate excitability (Huang et al. 2005). One such study, using what the researchers define as a theta burst stimulation protocol, applied rTMS directly to the motor cortex of healthy subjects in order to induce stimulus dependant alterations in cortical excitability. Measured motor responses could be either facilitated or suppressed with varied cortical stimulation parameters, and the functional outcome of hand reaction time could be decreased when corticospinal excitability was increases. Again, it is important to note that different neuronal groups will possibly respond differently to similar stimulation parameters, making it important to investigate into outcome specific stimulation protocols. Walking-specific cortical cells can be targeted with a stimulation protocol that will enhance excitability and further facilitate active activity dependant

rehabilitation; this could serve to further improve recovery of functional motor control after injury (Stinear and Hornby 2005).

Similar to stimulation, the application of pharmacological agents will also work towards the same outcome of improved functional motor recovery. Neurotransmitter systems such as noradrenergic, serotonergic, glutamate and glycine are involved in modulating basic locomotor rhythm; hence, artificial application of agonists should serve to enhance functional motor control in subjects who suffer from a motor system injury (Grillner 2003). In animal models, the correct application of different neurotransmitters such as N-methyl-D-aspartate (NMDA), 5-hydroxytryptamine (5-HT) or dopamine, which all act on these neurotransmitter systems, will result in a stable rhythmic motor output (Edgerton et al. 2004). Clinical studies where patients are exposed to pharmacological application after a motor injury seems to be inconclusive with respect to the level of facilitation that pharmacological application will bring to recovery. Studies do exist that would suggest that pharmacological application could hold much promise for motor control rehabilitation (Unwin and Walker-Batson 2000). Further investigation into pharmacological benefits enhancing motor recovery will be needed before they can be used as a recognizable method to ensure locomotor improvements. Streamlining an approach to combine these methods of enhancing motor control with currently proven physio-motor approaches will be beneficial in any scenario where an individual is relearning, or even enhancing a motor task. This is certainly no different for individuals who are recovering from spinal cord injury. Currently, the exact mechanisms that will

provide for the best rehabilitation are resoundingly debatable and consensus among researchers will mark an important stage in motor control rehabilitation.

Preliminary studies pairing motor practice and peripheral stimulation to hand muscles in subjects who have cervical spinal cord injury demonstrate improvements in both the strength and functional quality of hand control in addition to what was seen with motor practice only. This study did not measure any significant difference between cortical measures; however, increased functional improvements were paralleled by an increase in strength scores (Beekhuizen and Field-Fote 2005).

4.7 Future directions for rehabilitation, optimizing neural plasticity

Our results suggest that opportunities exists to enhance rehabilitation methods and therefore, functional outcome. Task dependent motor training is beneficial and thus active volitional attention is important for successful training. Active motor practice generally encourages a larger degree cortical involvement, hence more cortical plasticity. If cortical improvements generate functional recovery, training should be based on cognitive attention above and beyond simple motor repetition. Slower speeds, with a greater focus to attention, should enhance the benefits seen from current rehabilitative strategies, such as treadmill training. This is not to say that spinal mechanisms do not enhance recovery. Certainly, sensory feedback derived from motor repetition is beneficial; however, optimal rehabilitation protocols should target the most influential source for enhancing motor control, which has been demonstrated by this, and many

other studies, to be at the level of cortical signaling. The type of therapy needed for optimum recovery may be subject specific. To this end, the existence or absence of high frequency inter-muscular coherence has the potential to serve as a detection mechanism for whether a subject will respond to attention based motor training. The quality of residual CST signaling in subjects who are post SCI will probably alter the type of training that will best serve to maximize an individual's recovery from SCI. To this end, more research will be needed to ensure patients receive a properly tailored regime for their individual road to recovery.

4.8 References

Beekhuizen KS and Field-Fote EC. Massed practice versus massed practice with stimulation: effects on upper extremity function and cortical plasticity in individuals with incomplete cervical spinal cord injury. *Neurorehabil Neural Repair* 19: 33-45, 2005.

Challis RE and Kitney RI. Biomedical signal processing (in four parts). Part 3. The power spectrum and coherence function. *Med Biol Eng Comput* 29: 225-241, 1991.

Davey NJ, Smith HC, Savic G, Maskill DW, Ellaway PH, and Frankel HL. Comparison of input-output patterns in the corticospinal system of normal subjects and incomplete spinal cord injured patients. *Exp Brain Res* 127: 382-390, 1999.

Edgerton VR, Tillakaratne NJ, Bigbee AJ, de Leon RD, and Roy RR. Plasticity of the spinal neural circuitry after injury. *Annu Rev Neurosci* 27: 145-167, 2004.

Fraser C, Power M, Hamdy S, Rothwell J, Hobday D, Hollander I, Tyrell P, Hobson A, Williams S, and Thompson D. Driving plasticity in human adult motor cortex is associated with improved motor function after brain injury. *Neuron* 34: 831-840, 2002.

Fuhr P, Agostino R, and Hallett M. Spinal motor neuron excitability during the silent period after cortical stimulation. *Electroencephalogr Clin Neurophysiol* 81: 257-262, 1991.

Grillner S. The motor infrastructure: from ion channels to neuronal networks. *Nat Rev Neurosci* 4: 573-586, 2003.

Gross J, Tass PA, Salenius S, Hari R, Freund HJ, and Schnitzler A. Cortico-muscular synchronization during isometric muscle contraction in humans as revealed by magnetoencephalography. *J Physiol* 527 Pt 3: 623-631, 2000.

Grosse P, Cassidy MJ, and Brown P. EEG-EMG, MEG-EMG and EMG-EMG frequency analysis: physiological principles and clinical applications. *Clin Neurophysiol* 113: 1523-1531, 2002.

Huang YZ, Edwards MJ, Rounis E, Bhatia KP, and Rothwell JC. Theta burst stimulation of the human motor cortex. *Neuron* 45: 201-206, 2005.

Ikeda A, Ohara S, Matsumoto R, Kunieda T, Nagamine T, Miyamoto S, Kohara N, Taki W, Hashimoto N, and Shibasaki H. Role of primary sensorimotor cortices in generating inhibitory motor response in humans. *Brain* 123 (Pt 8): 1710-1721, 2000.

Karni A, Meyer G, Jezzard P, Adams MM, Turner R, and Ungerleider LG. Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature* 377: 155-158, 1995.

Lotze M, Braun C, Birbaumer N, Anders S, and Cohen LG. Motor learning elicited by voluntary drive. *Brain* 126: 866-872, 2003.

Norton JA and Gorassini MA. Changes in cortically related inter-muscular coherence accompanying improvements in locomotor skills in incomplete spinal cord injury. *J Neurophysiol*, 2006.

Ridding MC and Rothwell JC. Stimulus/response curves as a method of measuring motor cortical excitability in man. *Electroencephalogr Clin Neurophysiol* 105: 340-344, 1997.

Salenius S and Hari R. Synchronous cortical oscillatory activity during motor action. *Curr Opin Neurobiol* 13: 678-684, 2003.

Smith HC, Savic G, Frankel HL, Ellaway PH, Maskill DW, Jamous MA, and Davey NJ. Corticospinal function studied over time following incomplete spinal cord injury. *Spinal Cord* 38: 292-300, 2000.

Stinear JW and Hornby TG. Stimulation-induced changes in lower limb corticomotor excitability during treadmill walking. *J Physiol*, 2005.
Thomas SL and Gorassini MA. Increases in corticospinal tract function by treadmill training after incomplete spinal cord injury. *J Neurophysiol*, 2005.
Unwin H and Walker-Batson D. No side effects after low-dose amphetamine administration in stroke rehabilitation. *Stroke* 31: 1788-1789, 2000.
Uy J, Ridding MC, and Miles TS. Stability of maps of human motor cortex made with transcranial magnetic stimulation. *Brain Topogr* 14: 293-297, 2002.
Winchester P, McColl R, Querry R, Foreman N, Mosby J, Tansey K, and Williamson J. Changes in supraspinal activation patterns following robotic locomotor therapy in motor incomplete spinal cord injury. *Neurorehabil Neural Repair* 19: 313-324, 2