DOES PRIMARY MOTOR CORTEX PLASTICITY PARALLEL ADAPTIVE MODIFICATION TO HUMAN WALKING?

by

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ABSTRACT

Walking in a novel environment, such as with resistance, has been associated with changes in muscle activity (Lam, Anderschitz & Dietz, 2006), limb position (Lam et al., 2006; 2008) and cortical spinal activity (Capaday, Lavoie, Barbeau, Schneider & Bonnard, 1999; Bonnard, Camus, Coyle & Pailhous, 2002) compared to baseline walking measurements. Previous literature on locomotor adaptation, suggests that the nervous system has the ability to adapt to task demands. The location and mechanisms of these physiological and kinematic changes are still unknown. The purpose of the current study was to verify that corticospinal (CS) excitability is altered by resisted walking. Second, we explored whether CS changes are modulated by attention and lastly whether changes in excitability are muscle specific. Locomotor adaptations were induced in 40 healthy participants using a robotic gait-assisted treadmill (Lokomat). Velocity-dependent resistance was applied against hip and knee movements during walking. CS excitability was assessed by quantifying motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation immediately before and after adaptation to both resisted and nonresisted walking. Recruitment curves were collected by stimulating at increments of 5% from 105-145%AMT. To determine whether adaptation is muscle specific, MEPs were measured through random assignment of either the biceps femoris (BF) or rectus femoris (RF). To evaluate the impact of attention on adaptive walking, half the participants attended to their walking pattern via a visual feedback tracking task (post_cog). The other half watched a controlled visual stimulus (post_nocog). Results demonstrated a significant increase in MEP amplitude in the BF and not the RF following resisted walking. The post_cog condition adaptations did not reveal an increase in MEP amplitude compared the post_nocog condition. Results suggest that locomotor adaptations result in an increase in CS excitability that is muscle specific. Focused attention to motor adaptation may not be an important modulator of movement and motor learning as has been reported in past work. The current study is the first to consider both the role of the CS system in adaptations during walking and the impact of attention on CS excitability and parallels previous findings on muscle specific locomotor adaptations.

PREFACE

This thesis contains a research experiment conducted by candidate, Jeanie R. Zabukovec, under the co-supervision of Dr. Lara Boyd and Dr. Tania Lam, with guidance from Dr. Jean-Sébastien Blouin. The collection, analysis, and writing of the experiment were principally the work of the candidate. The supervisory committee and other authors of the manuscript from this study provided direction, support and critcal feedback on the design of the study. The supervisory committee provided critical feedback on the manuscript that was prepared. This thesis will be submitted for publication as a multi-authored manuscript in peer-reviewed journals. Ethical review and approval for this thesis was performed by UBC Clinical Research Ethics Board (H08-02598).

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LIST OF ABBREVIATIONS

AMT	Active motor threshold
BF	Biceps femoris
BFB	Biofeedback
CNS	Central nervous system
COMP	Compensate
CS	Corticospinal
DGO	Driven gait orthosis
EEG	Electroencephalography
EMG	Electromyography
fMRI	Functional magnetic resonance imaging
GM	Gastrocnemius
I/O curve	Input/output curve
LTD	Long term depression
LTP	Long term potentiation
M1	Primary motor cortex
MEG	Magnetoencaphalography
MEP	Motor evoked potential
MEPmax	Plateau of the relation
MH	Medial hamstring
MVC	Maximum voluntary contraction
NINT	Not intervene
PET	Positron emission tomography
RF	Rectus femoris
RMT	Resting motor threshold
ROI	Region of interest
SM1	Sensorimotor cortex
TA	Tibialis anterior
TMS	Transcranial magnetic stimulation
VBTT	Visual biofeedback tracking training

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1.0 LITERATURE REVIEW

1.1 Neuroplasticity

It is well documented that the human cortex has the ability to functionally and structurally reorganize itself in response to physiological experiences and associated changes in sensory input (Ljubisavljevic, 2006). Cortical plasticity is defined as the brain's ability to "alter the location of specific information processing function, in response to experiences of the body" by reorganizing its neuronal networks (Ljubisavljevic, 2006). Cortical reorganization can be measured at the synaptic, cellular, and/or regional level (Buonomano & Merzenich, 1998; Ljubisavljevic, 2006). Animal studies have examined the synaptic and cellular changes associated with cortical plasticity while in the human brain, cortical plasticity is investigated at a more regional level (Ljubisavljevic, 2006).

Several mechanisms that provide fast cortical plasticity have been studied. The two most commonly studied mechanisms are long-term potentiation (LTP) and long-term depression (LDP). LTP and LDP are thought to modulate synaptic efficiency during learning. LTP is a long lasting increase in synaptic efficiency between two neurons as a result of tetanic stimulation of these neurons (Teyler & Discenna, 1987). On the other hand, LTD is the lowering of synaptic efficiency following low-frequency stimulation (Ito, 1989). Other mechanisms, as reviewed by Ljubisavljevic (2006), include unmasking of pre-existing connections, awakening of existing but silent synapses, or general changes in the excitability of post-synaptic neurons. Morphological changes are also thought to occur, however more time and repetition may be required for permanent expression to take place (Ljubisavljevic, 2006; Siebner & Rothwell, 2003). Cortical changes associated with short-term training, the effects that will be elicited in this study, are most likely not due to LTP. The training is only a brief session and would not stimulate the neurons for a long enough period of time to induce long-term changes. However, neuronal changes are more likely a result of decrease inhibition or an increase in synaptic efficacy of existing neural circuits resulting in an increase cortical excitability (Pascual-Leone, 1998).

One important aspect for training-induced cortical plasticity to occur is the need for a repetitive task-specific program. A study by Pascual-Leone and Torres (1993) was one of the

first to demonstrate the effects of extensive task-specific training. This study showed that the somatosensory cortical map of the flexor digitorum indicis muscle in the reading hand of Braille readers was significantly larger than the non-reading hand (Pascual-Leone & Torres, 1993). This was concomitant with a reduction in the cortical area of the abductor digiti minimi. The non-proficient Braille readers, however, did not show this enlarged cortical area. Such representational plasticity (the change in representation of the reading finger in the somatosensory cortex) correlated with the amount of practice (Pascual-Leone & Torres, 1993). Task specific, long-term reorganization of the cortex has been also been examined in skilled violin (Elbert et al., 1995) and racquet players (Pearce et al., 2000).

1.2 Transcranial magnetic stimulation (TMS) as a tool for measuring plasticity

In humans, non-invasive neuroimaging techniques have been used to examine the spatial pattern and time course of representational plasticity. These methods include electroencephalography (EEG), positron emission tomography (PET) and magnetoencephalography (MEG). EEG measures brain activity directly, whereas PET, which measures metabolic activity, and MEG, which measures magnetic fields, are indirect markers on brain electrical activity. Functional magnetic resonance imaging (fMRI) is another non-invasive technique used to measure brain activity indirectly through the change of blood flow associated with neural activity within the brain. fMRI has many advantages including a high spatial resolution compared to EEG and MEG, and the ability to record signals from all regions of the brain. There are also disadvantages, including poor temporal resolution compared to EEG and MEG as well as the inability to move the head when inside the scanner. Transcranial magnetic stimulation (TMS) is another non-invasive technique that enables the investigation of plasticity in the human cortex. However, unlike the previously mentioned neuroimaging techniques, this technique indexes the cortical excitability of the brain by measuring the change in the amplitude of muscle activity elicited from stimulating the motor cortex. TMS is preferable for studies measuring training-induced neural changes because it allows for the comparison of corticomotor excitability immediately before and after training.

TMS is a non-invasive, painless and safe technique used to examine and alter the activity of human cortical networks. TMS works by placing a magnetic coil on the scalp that produces a rapidly changing magnetic field. The rapid change of magnetic field induced by a high-intensity

current, lasting roughly a few hundred microseconds, induces electric currents in the brain tissue exciting the cortical neurons (Rudiak & Marg, 1994). The strength of the induced current is a function of the rate of change of the magnetic field that is determined by the rate of change of the current in the coil. The stimulators currently used produce about 1.5-2 tesla at the face of the coil and are thought to activate cortical neurons 1.5-2 cm beneath the scalp (Rudiak & Marg, 1994; Wasserman, 1998). Neurons that synapse onto cells at the site of stimulation may be affected, therefore activating distant cortical and subcortical sites (Wasserman, 1998). Animal studies provide direct evidence that the effects of TMS are influenced by cortical excitability (Baker et al., 1995).

The depth at which the neurons are stimulated depends on the stimulator intensity as well as the coil being used. The double cone coil is designed to provide a stronger and less focal stimulation then the figure-of-eight coil, and is used to stimulate neurons deeper in the cortex (Rossi, Hallett, Rossini, Pascual-Leone &The safety of TMS Consensus Group, 2009). The double cone coil is most commonly used in studies involving stimulation of muscles of the leg, including the RF and BF (Bonnard, Camus, Coyle & Pailhous, 2002; Camus, Pailhous, &Bonnard, 2004; 2005), because the neurons of the leg are deeper and therefore less accessible to the figure-of-eight coil (Wheaten et al, 2009).

When TMS is applied over the motor cortex, a response can be quantified by measuring the characteristics of the motor evoked potential (MEP). Depending on the research question being asked, the TMS parameters can be set to apply a single, paired or repetitive train of stimulation. When a single pulse is applied over the motor cortex, the resting motor threshold (RMT) is defined as the minimal intensity of TMS that produces an MEP that has a peak-to-peak amplitude of at least 50 uV in 5 of 10 trials (Rothwell et al. 1999). RMT is considered a variable threshold because 1) the activated muscle is at least two synapses away from the site of the stimulation, and 2) a single pulse creates repetitive activity in the cortex creating a series of descending volleys in large diameter CS axons (Siebner & Rothwell, 2003). The RMT is therefore thought to reflect the global excitability of all the underlying cortical axons, as well as the excitability of motor neurons and their density at the location of stimulation (Ljubisavljevic, 2006). Given that MEP threshold depends on the excitability of the synaptic relays, threshold measured during an active contraction (active motor threshold, or AMT) when synaptic activity

is better defined, is thought to be a more reliable measure (Ljubisavljevic, 2006; Siebner & Rothwell, 2003). AMT is determined as the intensity of stimulator output that evokes an MEP of at least 200 uV in 5 of 10 trials while the participant isometrically contracts to 20% of his/her maximal voluntary effort. MEP amplitudes reflect the overall activity in the CS tract at the moment of stimulation, therefore reflecting the excitability of both the cortical and spinal motor neuron (Ljubisavljevic, 2006).

One method used to evaluate the cortical changes using single pulse techniques is to create recruitment or input/output (I/O) curves. Recruitment curves reflect the relationship between stimulus intensity, voluntary activation and the development of the MEP amplitude (Devanne et al. 1997). To develop an I/O curve, MEP amplitudes are measured over a range of stimulus and/or contraction intensities (Ljubisavljevic, 2006). Most I/O curves of the hand muscles are sigmoidal with a steeply rising slope that plateaus at the end whereas other muscles create curves that are more linear and never reach a plateau (Devanne et al. 1997; Siebner & Rothwell, 2003). The slope of the I/O curve depends on the distribution of excitability within the CS pathway and the spatial distribution of excitable elements (likely large diameter myelinated axons) in the cortex under the stimulation (Siebner & Rothwell, 2003). Changes in the I/O curve over time are therefore reflective of either change in the distribution of excitability in the CS system, or to changes in the spatial distribution of the excitable elements in the cortex. Common dependent measurements extracted from the I/O curve include the slope, representing the steepness or gain of the relation; the maximum value, or plateau of the relation (MEP_{max}), as well as the stimulus intensity required to obtain a response 50% of the maximum. All of these parameters can be used to interpret the excitability of the CS pathway or the number of motor neurons recruited at a given level of stimulus intensity. One downfall with the I/O curve is that it does not clearly allow one to determine where within the CS pathway the change(s) occurred. Local changes may have occurred in the intracortical segments or the segmental interneurons (Capaday et al, 1999).

Devanne and colleagues (1997) investigated the I/O relation of the CS pathway of the TA. Participants were required to maintain a constant level of contraction across a range of voluntary activity levels, while researchers applied 8-16 stimuli at varying levels of stimulus intensities. Contraction levels ranged from 10-40% of the participant's maximum voluntary

contraction (MVC), and the magnetic stimulation intensity levels started at 5% below threshold and increased in intervals of 2-5% until the MEP amplitudes reached a plateau. The TA demonstrated a sigmoidal I/O relationship. Since there is a linear relationship between single motor neuron discharge and stimulus intensity (Lavoie, Cody, Capaday, 1995), Devanne et al (1997) argued that the response of the CS tract as a whole (i.e. the subliminal fringe of the cortical neurons, spinal interneurons and motor neurons), is not dependent on the I/O behaviour of a single motor neuron (Devanne et al., 1997).

Previous results also showed that as tonic background activity increased, the steepness of the I/O relationship increased up to 4-7 times the value at rest and peaked around 30-40% MVC (Devanne et al., 1997). It is believed that the increase in steepness of the I/O relation represents an increase in gain, but not a change in the order of recruitment (Devanne et al, 1997). An increase in the steepness may represent a non-linear relationship between the size of the subliminal fringe of the CS tract as a whole and the level of motor activity. This non-linear relationship may be a factor underlying the increase in slope with increasing activity (Devanne et al, 1997). In other words, cortical stimulation would activate a number of motor neurons that is proportional to the level of muscle contraction and the size of the associated subliminal fringe (Devanne et al, 1997). Devanne et al. (1997) emphasized that beyond threshold, the cortical stimuli elicits CS volley with many components and therefore, the I/O relations above threshold are a result of multiple descending volleys. Devanne and colleagues (1997) concluded that at a constant level of motor activity, the threshold, maximum slope and plateau values characterize the I/O relation of the CS pathway in a given task and changes in one of these parameters reflect task-dependent differences.

A study by Jensen and colleagues (2005) used I/O curves to assess motor learning. The authors examined the relationship between the nature of motor tasks (strength versus skill training) on motor cortex excitability. Results showed that after repeated skill training, three times a week for four weeks, MEP_{max} increased and both RMT and AMT decreased. On the other hand, strength training induced a decrease in MEP_{max} and slope of the I/O curve (Jensen et al., 2005). This decrease suggests a decrease in CS excitability. This study demonstrates that several weeks of skill training induce CS excitability while strength training induces a decrease in CS excitability (Jensen et al., 2005).

1.3 CS control of walking

The primary motor cortex lies in the precentral gyrus, anterior to the central sulcus, in the frontal lobe. This area controls movement on the opposite side of the body and is organized topographically. Of particular interest, the region of the primary motor cortex that represents the leg lies on the most medial aspect of the precentral gyrus. The thigh representation lies on top of the gyrus before the lower part of the legs and feet project down the interhemispheric (sagittal) fissure (Figure 1).

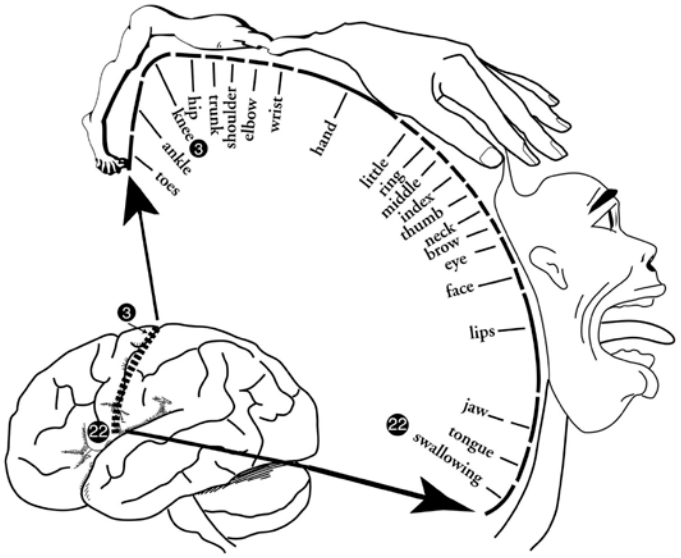

Figure 1: Motor homunculus (http://www.psywww.com/intropsych/ch02_human_nervous_system/homunculus.html)

The CS pathway in humans is essential for voluntary movement (Blumenfeld, 2002). The neuron cell bodies of this pathway originate primarily in the primary motor cortex and travel down through the posterior limb of the internal capsule and then through the brain stem. In the medulla, 85% of the fibers in the CS tract cross to the contralateral side at the pyramidal decussation. Therefore, axons originating on one side of the brain control movements on the contralateral side of the body. After the axons cross, they travel down the lateral CS tract and synapse onto lower motor neurons located in the anterior horns of the central gray matter of the spinal cord. The axons of the lower motor neurons leave the spinal cord via the anterior spinal roots or cranial nerves and synapse with the muscle cells. Therefore, when studying the CS pathway the cortical circuitry, the motor neuron pool, and its intrinsic properties, as well as

spinal interneuronal relays that influence the motor neuron pool, must be considered (Devanne, Lavoie, Capaday, 1997).

The CS tract has been demonstrated to play an important role in the control of walking (Bonnard et al., 2002; Capaday et al., 1999; Kamibayashi et al, 2009; Schubert et al., 1997; Winchester, 2005). Work done on animals has demonstrated that there is an increase in cortical involvement as terrain becomes more challenging. Motor cortex neurons increase their discharge frequency suggesting the contribution of the motor cortex to the execution of gait modifications (Drew, Kalaska & Krouchev, 2008). Drew et al. (2008) further suggest that individual CS axons in cats may branch widely in the spinal cord and possibly innervate multiple motor pools and thus could directly activate muscle synergies. Muscle synergies are "co-excited muscles acting to accelerate the segments differently to accomplish a common task goal" (Zajac, Neptune & Kautz, 2002). Synergies can include muscles that work around several joints, and can be related to distinct behavioural events during gait modification (Drew, Kalaska, & Krouchev, 2008). Muscles work together and yet vary in terms of EMG amplitude, duration and relative timing, to produce the desired movement (Drew, 1993). The nervous system has the responsibility of producing and modifying the spatiotemporal muscle activity patterns to achieve these desired movements. To achieve this, it is proposed that individual CS axons branch in the spinal cord to innervate multiple motor neuron pools and sequentially activate each synergy throughout the different phases of the step cycle (Drew, Kalaska & Krouchev, 2008).

Beloozerova, Farrel, Sirota & Prilutsky (2010) suggested that a subpopulation of motor cortical neurons is involved in control of movement accuracy in a "dose-dependent" manner. They observed a decrease in variability in motor cortical neuron discharge frequency in cats with greater accuracy paw placement on ladder cross-pieces. That is, the firing rate of the neurons was more closely related to paw placement. The authors hypothesized that the increase in precise firing of the motor cortical neurons allow for a more controlled stride length and accurate paw placement (Beloozerova, et al. 2010). Skilled precise stepping on a ladder and simple locomotion had 200 similar mechanical variables when comparing joint kinematics of the body in each task (Beloozerova, et al. 2010). Despite the similar mechanical variables, Beloozerova and colleagues (2010) found that the activity of many motor cortex neurons were different during these tasks. This study's conclusions suggest that the motor cortex may integrate incoming visual

information to provide higher level commands to lower brain and spinal centers by setting certain task-dependent parameters that alter production of steps (Beloozerova et al., 2010).

Schubert and colleagues (1997) propose that during the swing phase of walking, supraspinal centers demonstrate enhanced CS access to the motor neuron pools of the leg flexors. They investigated the effect of cortical input on the locomotor pattern by applying TMS to elicit responses in the tibialis anterior (TA) and gastrocnemius (GM) throughout the gait cycle during treadmill walking. They demonstrated that amplitude modulation throughout the gait cycle paralleled background electromyography (EMG) activity within the respective muscles during the stride cycle. TA MEPs were largest at the beginning of stance, corresponding to TA's role in slowing plantar flexion following heel contact, and also throughout the swing phase. GM MEPs, on the other hand, increased during stance and peaked at push off. The pattern of MEP responses in these two muscles therefore demonstrated an alternating reciprocal pattern (Schubert et al., 1997). This follows the idea that with increased contraction of a muscle, larger and more motor neurons are closer to firing threshold (Schubert et al., 1997). When comparing motor evoked responses in the TA prior to swing to a voluntary dynamic contraction task, there was a larger facilitation of motor responses as stimulation increased (i.e. input-output relationship). This suggests an enhancement of supraspinal input to the TA motor neurons during the swing phase. Interestingly there was no change in response latency throughout gait even with the amplitude modulation. This suggests that during gait there is an increase in activation at the motor neuron level that accompanies an enhanced synaptic transmission efficacy at the spinal level (Schubert et al., 1997). Therefore, during gait, a very low temporal summation is needed to cause the motor neuron to discharge because the efficiency of neurotransmission increased as the activation of the motor neuron also increased (Schubert et al., 1997).

Descending CS pathways from the motor cortex act on α-motor neurons and interneurons at the same time (Capaday, 2002). During motor activity, including walking, the descending pathways on spinal interneuron therefore provide a final level of adaptive control (Capaday, 2002). In the study by Lavoie and colleagues (1997), the strength of the reciprocal inhibition (inhibition of the antagonist(s) during activity of the agonist(s)) (Lavoie, Devanne & Capaday, 1997) of the soleus α-motor neuron depended on the task and was not necessarily proportional to the level of motor activity in the agonist(s). For example, during the swing phase of walking,

when the EMG activity of the TA was equal to that of a tonic voluntary contraction, the H-reflex decreased to zero in 24/30 subjects (Lavoie, Devanne & Capaday, 1997). The strength of the inhibition of antagonist(s) therefore depends on the task, as an increase in reciprocal inhibition between antagonistic muscles is much stronger during swing phase of walking than during voluntary or postural motor activities (Capaday, 2002). Although the CS tract contributes equally to the segmental motor circuit of the ankle flexors or extensors during voluntary dorsi- and plantarflexion, respectively, the authors suggest that during walking, the CS tract is more closely linked to the segmental motor circuits controlling the flexors (Capaday, Lavoie, Barbeau, Schneider & Bonnard, 1999). These findings are of importance because they not only show that the CS tract contributes to locomotion, but that the contributions of the descending commands vary depending on the task being performed.

1.4 Neural adaptive strategies during locomotion

Lokomat

The Lokomat is an excellent way to provide repetitive task specific locomotor training along with biofeedback (BFB) to help promote motor learning and recovery from brain damage or disease. The Lokomat (Hocoma AG, Volketswil Switzerland) is a robotic gait assisted device that allows for participants to receive appropriate gait-related afferent feedback needed to promote functional recovery during walking. Such feedback includes alternating flexion and extension of the hip, knee and ankle, and associated muscle-tendon length changes and loading. With the driven gait orthosis (DGO) it is possible to apply automated locomotor training by moving the legs in reproducible, rhythmical, and physiological manner. Participants are able to focus on leg movements because their body weight is supported and also have the possibility of walking faster with a reproducible, physiological gait pattern (Colombo et al, 2000). Participants are able to walk in the Lokomat without the aid of the DGO as well as against resistance. Repetitive walking in the Lokomat, against resistance, is thought to elicit locomotor adapation. This allows researchers to investigate the effects of task specific training and adaptation.

In order for movements to be performed rapidly and efficiently, the nervous system cannot rely solely on feedback mechanisms for controlling movements and adapting to changes in task dynamics. One concept is that the nervous system uses an internal model of the dynamics of the limb in order to produce the required motor output for the desired movement. When the

dynamics of the limb (and/or task) change, the internal model is updated (Shadmehr & Mussa-Ivaldi, 1994) so that the motor output suits the new demands of the task. The presence of aftereffects following training supports the formation or recalibration of the motor output for a given task when the participant is returned to the original condition. The adjusted motor outputs that were formed are no longer suitable, resulting in motor error.

There is evidence that such control strategies are also used for adaptive strategies during human walking (Lam, Anderschitz, Dietz, 2006). Lam and colleagues (2006) had participants walk in the Lokomat and adapt to a velocity-dependent resistance against hip and knee movement during walking. Patterns of muscle activity in the lower leg during resisted walking and catch trials (when the resistance was removed for a single step) suggested that both feedforward and feedback strategies were utilized to adapt to the resistance. There was an increase in EMG activity in the rectus formis (RF) and TA muscles in the initial steps of resisted walking and was not present in the catch trails. The immediate adaptive modifications in locomotor pattern suggest that feedback strategies were used to accommodate RF and TA EMG activity to the resistance (Lam et al., 2006). In contrast, the BF and medial hamstring (MH) demonstrated enhanced EMG activity only after a few steps against resistance were taken. Increased EMG activity of the BF and MH persisted in the first four catch trails and did not return to baseline levels until 40 and 30 steps, respectively, during a period of no resistance (Lam et al., 2006). These after effects following the removal of the resistance suggests that feedforward strategies were also implemented to adapt the locomotor pattern. This provides insight that both feedback and feedforward mechanisms are used quickly to modify adaptive locomotor strategies and ensure stability and safety during walking.

Bonnard et al. (2002) investigated the role of the CS pathway involved in voluntary walking compared to that of constrained walking. Researchers had participants walk on a treadmill under both a constrained situation, where extensor muscles were impeded throughout the swing phase of walking, and an unconstrained situation. While walking in these conditions, participants were cortically stimulated via TMS every six (±1) strides, with only one stimulus per stride. Depending on the subject, 15 to 25 stimulus intensities were used to plot an I/O curve under each walking condition for the RF and BF. Results showed co-facilitation of MEPs in the two antagonist thigh muscles throughout the swing phase of voluntary controlled walking

compared to unconstrained walking. When comparing the two groups, the constrained groups showed an increase in MEP amplitudes that increased with the stimulus intensity (Bonnard et al., 2002). These results suggest that although the muscle activity was normal prior to stimulation, the adaptation in the constrained condition affected the excitability of the CS pathways to these muscles.

1.5 Influence of attention on motor skill learning

In the review of recent developments in biofeedback for neuromotor rehabilitation, Huang and colleagues (2006) identified that a key ingredient for promoting motor recovery is the active engagement of patients throughout therapy. Providing BFB is one way of engaging patients in the rehabilitation process and is thought to promote motor function and control by **reinforcing attention** to the sensory cues associated with the movement to be trained. Biofeedback (BFB) can be defined as the process of changing physiological processes into a meaningful form (Bradley, Hart, Mandana, Flowers, Riches, Sanderson, 1998; Huang et al., 2006; van Dijk, Jannink, & Hermens, 2005). The characteristics of BFB can vary by the type of physiological signals provided to the patient as well as how meaningful these signals are to the patient. EMG, joint angle, position, and pressure or ground reaction force are some of the signals that can be used to augment feedback during training through visual display, auditory pitch or volume, or mechanical tactile stimulation (Huang et al., 2006).

Reports on the effectiveness of BFB are varied. Different findings reached in BFB studies may stem from the type of information to which participants are instructed to attend. Early BFB studies used “static” BFB where participants were required to “control a specific parameter through a quantified cue (12)”, or the movement being performed was unrelated to activities of daily living (Huang et al., 2006). Most results from studies using static BFB do not demonstrate significant improvements in motor function recovery. However, studies that used functional task-specific “dynamic” BFB have yielded more promising results (Huang et al. 2006). Task-oriented BFB allows for better interaction between the neuromuscular system and the environment. Task-oriented training allows patients to explore the environment and solve movement problems online. In order for task-oriented BFB to be effective, feedback should reflect the physiological processes that will be informative without overwhelming the patient’s perceptive and cognitive ability. Factors such as the size or site of brain lesion, patient’s motivation during therapy, as

well as cognitive ability are all variables that may impinge on the effectiveness of BFB throughout rehabilitation. These considerations point to the importance of motivation and attention for motor function recovery (Huang et al., 2006).

The importance of reinforcing attention throughout therapy has been made obvious by the work of Camus and colleagues (Bonnard, Camus, deGraaf & Pailhous, 2003; Camus, Pailhous & Bonnard, 2004; 2006). They have shown how the cognitive tuning of CS excitability adapts to evoked-flexion/extension movement in both the upper (Bonnard, Camus, deGraaf & Pailhous, 2003) and lower extremities (Bonnard, Camus, & Pailhous, 2003; Camus, Pailhous & Bonnard, 2004). Participants were required to perform a flexion/extension movement of their wrist (Bonnard, Camus, deGraaf & Pailhous, 2003) or continuously walk on a treadmill (Bonnard, Camus, & Pailhous, 2003; Camus, Pailhous & Bonnard, 2004). Participants were each assigned to a group that was instructed to cognitively prepare to either not intervene (NINT) or to compensate cognitively without coactivating their muscles (COMP), for the evoked movements caused by TMS. The stimulation was applied during wrist flexion (Bonnard, Camus, deGraaf & Pailhous, 2003), or during the stance and swing phase of the gait cycle (Camus, Pailhous & Bonnard, 2004; 2006). The results demonstrated that participants were able to cognitively prepare themselves to resist a TMS-induced central perturbation (Bonnard, Camus, deGraaf & Pailhous, 2003; Bonnard, Camus, & Pailhous, 2003; Camus, Pailhous & Bonnard, 2004). This conclusion was supported by an increase in the MEP of the RF and BF in the COMP group in the stance and swing phases, respectively, compared to the group that was instructed to yield to the perturbation (Bonnard, Camus, deGraaf & Pailhous, 2003). Results also demonstrated that participants were able to compensate for random TMS-evoked movements, by using the same preparation strategy- i.e. selectively increasing the CS excitability of the muscles counteracting the central perturbation, as the fixed-phase condition (Camus, Pailhous & Bonnard, 2004). These results provide evidence that participants were able to continuously tune CS excitability throughout the gait cycle (Camus, Pailhous & Bonnard, 2004). All three studies emphasized the importance of providing explicit instructions on what the participant had to do and encourage devoted attention to the task required.

A systematic review by van Dijk et al. (2005) focused on “augmented” feedback, defined as “adding to or enhancing task-intrinsic feedback with an external source,” and the effects on

motor function of the affected upper extremity in rehabilitation patients. Training with visual BFB has been used as a tool for motor learning by enabling self-correction during a repeated pattern of a motor pattern, continuously stimulating motor planning and motor control (Cho et al., 2007). With devoted attention to the visual BFB and repetition of the motor pattern, motor learning and/or motor control ability is improved and may induce neural plasticity (Cho et al., 2007). However, of the twenty-six studies that met their inclusion criteria, only nine of the studies showed a positive effect of augmented feedback throughout therapy, whereas thirteen showed no difference. A definitive answer regarding the effectiveness of enhancing attention throughout therapy via augmented feedback could not be reached. This was attributed to the variation in the duration of exercise treatment as well as differences in feedback characteristics. Similar conclusions were reached in the review by Huang, Wolf & He (2006). No relationship between patient characteristics or types of feedback (EMG, kinetic, kinematic, or knowledge of results) on reported effects has been stated (van Dijk et al., 2005). Although the theory behind BFB supports motor recovery, a solid conclusion on the effectiveness could not be formed from these systematic reviews of the literature. Notably, out of the twenty-six articles reviewed by van Dijk et al., (2005), none of them looked at cortical measurements as an outcome measure.

The study by Jonsdottir and colleagues (2010) provided task-oriented BFB to improve gait velocity in a population with hemiparetic stroke. Task-oriented BFB was applied by providing information during gait activities that became more variable and challenging as training progressed. This approach was consistent with theories of motor learning where feedback was progressively reduced towards the end of the training. When a target level of gastrocnemius lateralis muscle activation was reached, an acoustic sound was provided for the participants. The outcome goal was to increase the power production of the ankle during the push off phase to increase gait velocity. In comparison to a control group receiving usual rehabilitation care without BFB, the group receiving task-oriented BFB showed a significant improvement in velocity and stride length that persisted at the follow up assessment six weeks later (Jonsdottir et al., 2010). This study demonstrates that stroke participants were able to adapt their motor output in response to feedback during task-oriented training and learned to incorporate it in their normal motor repertoire (Jonsdottir et al., 2010). Although the authors focused on functional changes of speed, force and distance, it would have been interesting to track the cortical changes associated with these improvements in the group receiving BFB

compared to the group that did not, and more interestingly to see whether these changes persisted at follow-up.

Cho et al. (2007) investigated whether cortical reorganization was associated with improved gait function in stroke patients who participated in a knee-joint visual biofeedback tracking training (VBTT) program. Ten stroke patients were randomly assigned to either the training group, trained to follow computer generated sine waves with a knee joint electrogoniometer on their affect limb, or a control group, who did not receive the VBTT program. Pre and post-training measurements showed an improvement in gait speed, assessed by the 10-meter walk test, and motor function, assessed by the walking items on the modified motor assessment scale, in the training group compared to the control group (Cho et al., 2007). These improvements with the VBTT coincided with a shift in activation of the primary sensorimotor cortex (SM1) from the unaffected side to the affected side, when participants performed the same tracking task in the magnetic resonance scanner (Cho et al., 2007). The SM1 was the only region of interest (ROI) that was showed a significant shift following training. Other ROI included the premotor cortex, supplementary motor area, posterior cortex, cerebellar hemisphere and the vermis. Unfortunately, the primary motor cortex was not included in the analysis. The results in this study also demonstrated that improvements were enhanced when tracking motion was increased (ie. greater knee flexion and extension) (Cho et al., 2007). They hypothesized that VBTT, with a larger tracking motion, promotes increased attention to the task and greater active motion therefore providing more information for motor skill acquisition (Cho et al., 2007).

As already illustrated, motor skill training induces neuroplastic changes and cortical excitability, for example in the skilled violin players (Elbert et al, 1995). However, the mechanisms underlying these changes are still unknown. Perez, Lungholt, Nyborg, and Nielsen (2004) investigated the contribution of motor skill training of ankle muscles to cortical excitability by comparing motor cortical outputs of three groups. Participants were randomly assigned to one of 3 groups: 1) a motor skill group, who received 32 minutes of visuo-motor tracking training involving making a cursor follow a series of target lines on a computer screen by performing voluntary ankle dorsi- and plantar-flexion movements; 2) a non-skill group, who performed continuous voluntary ankle dorsi- and plantar flexion at a comfortable speed; or 3) a passive group, where one of the investigators moved their ankle in the same motion as group 2.

Cortical measures, including single and paired pulse TMS, were performed before and after training to measure cortical changes associated with the TA muscle. Results showed improved performance that was associated with an increase in TA MEPs evoked by TMS following motor skill training. The non-skill and passive training groups did not demonstrate this increase (Perez et al., 2004). There was an increase in the slope and the maximum MEP of the recruitment curves but MEP threshold was unchanged. This suggests that there is likely an enlargement of the representation area of the TA in the motor cortex, however only neurons with a higher threshold to TMS showed large excitability changes (Perez et al., 2004). Paired pulse analysis showed that there was a decrease in intracortical inhibition following training with the visuo-motor tracking task. This suggests that after training, cortical inhibition may be removed, activating more functional connections and promoting increased representation of the TA muscle (Perez et al, 2004). The findings in this study are promising for providing evidence of increasing cortical excitability by devoting participant's attention to a visuo-motor, task-specific training.

Although visual feedback is important for movement skill learning and motor function rehabilitation, the varying parameters of the visual feedback, including gain, frequency and delay (Foulkes & Miall, 2000), affects motor output (Hou et al., 2006; Winstein, 1991). A behavioural study by Hou and colleagues (2006) investigated the effects of two visual feedback parameters, gain and frequency, on grasp force output. Participant's ability to control grasp force, as measured by force deviation and error rate, increased under conditions with higher gain (Hou et al., 2006). Enhanced gain decreased error rate on both the slow and fast conditions compared to the same frequency conditions with low gain (Hou, et al., 2006). These results demonstrate that visual feedback affects the control of force output and that the ability to control force can be enhanced with increased gain. Engaging participant's attention to the physiological states throughout movement may optimize participant's control of force output and achieve the desired movement.

When visually guided movements are being performed, the brain converts visuo-spatial information into the appropriate motor commands to produce the desired movement. Co-contraction and viscoelastic properties are used initially when learning new dynamics. After practice, muscle activity is reduced and an internal model of the limb dynamics and kinematics is modified to reach the visible goal under the new conditions (Paz, Boraud, Natan, Bergman &

Vaadia, 2003). Paz et al. (2003) demonstrated that a decrease in muscle activity in rats was followed by neuronal changes, when learning a visuo-motor skill. Results suggested that the primary motor cortex (M1) stores newly acquired skills in working memory before consolidation where the skill is transferred into long-term memory (Paz et al., 2003). After the initial stages of learning, the premotor areas, which are thought to be involved in coding kinematic components of movement, become more active (Paz et al., 2003). This is supporting evidence that neuronal activity in the M1 may be increased during the initial stages of using visual information to guide movements and create an internal model, as demonstrated by aftereffects (Paz et al., 2003), to achieve a desired goal.

2.0 RATIONALE

Although theories behind BFB support motor recovery, the conclusions from studies vary due to different types of information provided (van Dijk et al., 2005), parameters of the feedback provided (Hou et al., 2006; Winstein, 1991), the population of participants being tested, as well as the motivation and attention of individual participants (Huang et al., 2006). However, based on what we know, providing BFB reinforces participant's attention to important sensory cues throughout motor skill training, and is thought to update the internal model in the CNS. The initial adaptation to training is thought to involve feedback mechanisms (Paz et al, 2003; Lam et al., 2008). With repetition of the task and devoted attention, the internal model is updated and used to form feedforward strategies (Lam et al., 2008) to produce the desired movement (Paz et al., 2003). Several studies have demonstrated that task specific, or "dynamic," BFB promotes an increase in cortical excitability that coincides with an improvement in motor performance (Cho et al., 2007; Paz et al., 2003; Perez et al., 2004; Roche & O'Mara, 2003).

Based on what we know about providing participants with adapted locomotor training in the Lokomat, our goal was to first, investigate changes in cortical excitability immediately following adaptive walking with resistance. We predicted, based on the findings from the study by Bonnard et al. (1998), that both the RF and BF will demonstrate an increase in MEPs following resisted Lokomat walking. Secondly, the novelty of this study was to determine if directing participant's attention to visual force output BFB will influence the extent of CS changes. It was hypothesized that the upregulation in CS excitability would be further increased in the post_cog condition, where participants focused on the resistance being applied through the swing phase, compared to the post_nocog condition, when participants watched a nonsense visual stimulus and were not provided information about the resistance. We predicted that transmission along the CS tract would be further facilitated when attention was devoted to the task specific, attention driven training. Additionally, given what we know about the mechanisms of locomotor adaptations to the BF and RF specifically (Lam et al., 1998), we further investigated the possibility that adaptations in the RF and BF activity to resistance is mediated via different neural pathways. The RF has not shown to alter activity without the presence of resistance, unlike the BF that demonstrates increased activity following the removal of resistance for several steps (Lam et al., 2006). It was therefore predicted that the BF would demonstrate a greater increase in MEP amplitude compared to the RF following the removal of resistance.

The purposes of this study were to:

1) determine if CS excitability was altered during adapted walking with resistance
2) investigate whether CS changes were dependent on directing the participant's attention to motor control throughout adaptation
3) determine whether changes were specific to the BF compared to the RF

It was hypothesized that:

1) MEPs would increase immediately after the Lokomat walking with resistance, demonstrating an upregulation in CS excitability;
2) the change in CS excitability would be greater after the condition where participants were explicitly focusing on adapting their walking pattern to the applied resistance compared to when they are not explicitly aware of the applied resistance;
3) the BF would have a larger effect of resistance on MEP amplitude compared to the RF and therefore show a greater increase in CS excitability following the removal of resistance.

This study is the first to consider both the role of the CS system in adaptations during walking and the impact of attention on CS excitability. As such, it is important for determining how to enhance the central nervous system's ability to integrate adaptive strategies during walking. Also, it is important for research and clinicians to understand the role of attention during locomotion. Exercises provided to patients may only be beneficial if the patient is cognitively aware and focusing on the therapy provided. In addition, our results may be used by clinicians to form novel methods of rehabilitation for individuals with gait pathology.

3.0METHODS

3.1 Participants

Forty healthy individuals (M=13, F=27; age= 25.85 years, SD=4.47, range [19-36] participated in this study. Recruitment was predominately from students at the University of British Columbia. Participants were randomized to one of two groups. In one group, muscle recordings were taken from the BF (BF group, *n*=20) and in the other group, recordings were made from the RF (RF group, *n*=20). The two groups were further sub-divided, randomly, to either receive an additional walking condition with resistance and provided with an attentional/cognitive task, or one that received resistance with a controlled visual stimulus (http://www.youtube.com/watch?v=YzFDCsCscyo). A schematic of the groups is shown in Figure 2. All participants were free of any neurological or motor disabilities and without family history of epilepsy or personal history of seizure. Participants signed an informed consent (see Appendix 2) in accordance with the University of British Columbia (UBC) Human Ethics Committee. All procedures were approved by the University of British Columbia Behavioural Research Ethics Board (Approval # H08-02598).

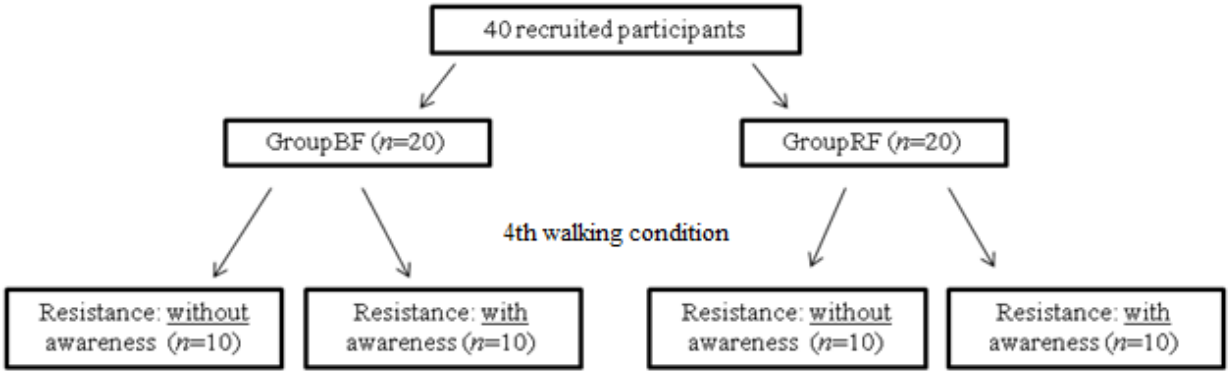

Figure 2: Schematic of participant allocation into groups. All group assignments were made randomly.

3.2 TMS

To investigate the CS excitability in the left lower limb muscles, application of TMS was performed with a 70mm Double Cone coil (Magstim Super Rapid², Magstim Company, Ltd.). TMS has been shown to be a reliable and valid measurement tool of cortical excitability (Cacchio et al., 2009).

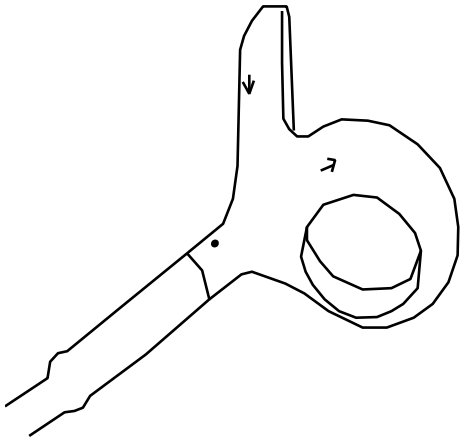

Figure 3: Double cone TMS coil

Depending on the assigned group, surface bipolar Ag-AgCl electrodes (10mm diameter) were placed 25mm apart on the belly of the left biceps femoris (BF) or rectus femoris (RF). A ground electrode was placed on the knee. The skin was shaved, cleaned and abraded prior to application of the electrodes to attempt to achieve impedances at or below 5 kΩ. During stimulation the TMS coil was positioned in the best location for eliciting MEPs in the assigned muscle on a subject-wise basis. The coil was positioned so that the current flowed in an anterior-posterior direction. The participant wore a swim cap to allow the coil position that elicited motor evoked potentials (MEPs) to be traced onto the head; the procedure enabled rapid re-assessment of MEPs throughout the experiment. The optimal stimulation location for the specified muscle and initial threshold was found while the participant was seated. The Lokomat harness was then placed on the participant and they were set up in the Lokomat. From this point on, all TMS measures were done while the participant was standing in the Lokomat in an upright position. Participants were asked to relax unless told otherwise. The experimenter stood on a platform one meter high, located to the right of the Lokomat, to be able to position the coil over top of the participant's head when delivering stimulations.

Once the optimal cortical location was found, participant's maximal voluntary contraction (MVC) for their assigned muscle was determined. Active motor threshold (AMT) was determined while standing in the Lokomat. Participants were asked to contract their left BF (BF group) or RF (RF group) to 20% of their maximal voluntary contraction (MVC). Online EMG recordings were provided via visual feedback on a computer monitor. AMT was defined

as the lowest stimulator intensity that generated 5 MEPs across 10 trials each with a peak-to-peak amplitude of at least 200μV. To generate a recruitment curve, the motor cortex was stimulated at 105, 115, 120, 125, 130, 135 and 145% AMT (Ljubisavljevic, 2006. Ten stimuli were collected at each intensity with approximately 1 sec to 4 sec between each stimuli. An Input-Output (I/O) curve (i.e., stimulus-response) of the BF or RF was then produced by averaging the 10 MEPs elicited at each level of stimulus intensity. This TMS protocol was performed before and after each walking condition, for a total of five collections, to allow us to compare measures of short term CS excitability (Figure 3). EMG was filtered with a high pass filter (0.3Hz) and notch filter (60Hz) using Power Lab (AD Instruments, Colorado Springs). MEPs were recorded and saved using LabChart software (AD Instruments, Colorado Springs) for later analysis.

In accordance with established and institutional safety guidelines each participant was screened for history of seizure, medication use, metal implants in the brain or head, and history of neurological diagnosis prior to inclusion in this study (Wasserman, 1998; Rossi & Hallett, 2009). The risks associated with single pulse TMS are very low. Even with high frequency repetitive TMS, the risks of inducing a seizure in healthy participants is less than 1% and only increases to 1.4% with epileptic participants (Rossi et al., 2009). There has been an incidence of secondarily generalized or partial motor seizures induced by single-pulse TMS in patients with history of other disorders involving the central nervous system, including epilepsy (Wasserman, 1998). Participants who have a history of seizure should therefore not undergo TMS and none were enrolled in this study.

3.3 Lokomat

The lower limbs of participants were measured to ensure a proper fit within the Lokomat robotic gait device (Hocoma AG, Volketswil Switzerland). This system incorporates a body weight support system suspended over a treadmill with a pair of robotic actuators which attach to the subjects legs. Participants were strapped into the exoskeleton with thick Velcro cuffs around the mid-thigh, upper shank and lower shank while a chest strap provided trunk support. Inside the Lokomat, hip and knee flexion/extension was permitted while the ankle joint was allowed free movement without constraint.

Both groups, BF & RF, completed four Lokomat walking conditions (Figure 3). The treadmill speed was set at 2.0km/hr for all four conditions. Prior to initial walking on the treadmill, AMT was determined and a baseline recruitment curve was collected (pre_loko). Participants walked for 2 trials of 3 minutes each, to familiarize themselves with the device (Baseline conditions). Each baseline walking condition was followed by the collection of recruitment curve data (post_loko1 & post_loko2). The Lokomat was programmed to then apply a velocity-dependent force against hip and knee movements, defined by:

$$\begin{bmatrix} \bar{M}_{\mathrm{H}} \\ \bar{M}_{K} \end{bmatrix} = -\begin{bmatrix} B_{\mathrm{H}} & 0 \\ 0 & B_{K} \end{bmatrix}\begin{bmatrix} \dot{\theta}_{\mathrm{H}} \\ \dot{\theta}_{K} \end{bmatrix}$$

Where M is the instantaneous amount of torque applied, B is the viscous (or damping) coefficient, and ϴ is the instantaneous angular velocity of the hip (H) and knee (K) joints (Lam, et al., 2006). When B is set to zero, no force is applied (null field). B values to apply a velocity-dependent force and was based on 10% of the participant's maximal voluntary hip and knee flexor contraction as determined within the Lokomat device. Two conditions of resisted walking were performed. In the first condition with resistance, participants were not explicitly told that resistance was being applied during walking (post_resist) and were asked to "walk in the Lokomat for another three minutes, continuing to match right heel contact with the tone." In the second, and final, condition with resistance participants were randomly divided into two groups. Half of the participants were assigned to a cognitive group in which they knew resistance was being applied via a biofeedback program (Post_cog); whereas the other half repeated another resisted walking condition without being explicitly told resistance was being applied (Post_nocog).

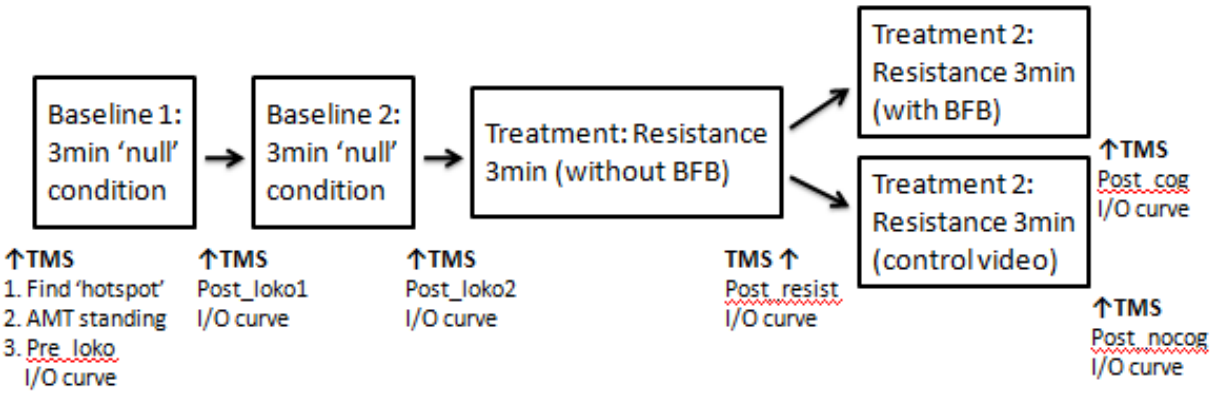

Figure 4: Experimental outline. MEPs were recorded from either the BF (GroupBF) or RF (GroupRF).

3.4 Biofeedback

Every participant walked in the Lokomat with resistance twice. However, to assess the impact of attention on excitability in the CS tract during resistance the groups (BF and RF) were further sub-divided. Half of the BF group (*n*=10) received a second walking treatment of resisted walking without awareness while watching a nonsense youtube video (CulturePub, 2008) (post_nocog). Participants in the nocog group were instructed to "walk for another 3 minutes, however this time while focusing on the video shown on the computer monitor infront of you." The other half (*n*=10) were provided with an online task-specific visual biofeedback (BFB) displayed on a computer screen while walking, with awareness, against resistance (post_cog). Likewise, the RF group was also randomly divided to receive either one of the second resistive walking condition (post_nocog or post_nocog). Participants receiving BFB were provided with a visual representation of their lower limb trajectory in real time and were asked to keep the line that represents their knee trajectory within ±1SD of their initial normal walking pattern before resistance was applied. Specifically participants were told "to walk for another three minutes, however this time resistance will be applied to your leg while you are walking. It will feel as though you are walking through water. During these three minutes you will be shown a display with a red line that represents your previous leg position before resistance was added, and would like to match the blue line, that represents your current leg position with resistance, to the red line. This will be challenging so just try the best you can to try to keep the blue line within the two black lines." When resistance was applied, participants were asked to maintain the same walking pattern by matching a tone with heel strike as well as using the visual BFB. This allowed the participants to be cognitively aware that resistance was applied without being

overwhelmed with information. Participants were verbally reminded throughout the cognition condition that the blue line represents their knee trajectory and were encouraged to try their best to match the blue line to the red line to keep their motivation and attention levels high. An example of a knee angle trace is shown in Figure 4. Both of the final walking conditions (post_nocog & post_cog) received the same amount of resistance they received in the first walking condition with resistance (post_resist) and walked against this resistance for the same amount of time (3 minutes). The task-specific biofeedback program allows us to examine whether a change in cortical excitability following Lokomat walking is a product of knowing and thinking about the intervention (post_cog) or not (post_resist). Comparing the results within the groups (BF and RF) that walked with resistance twice without knowing (post_nocog), to the results from the groups that walk in the cognitive condition (post_cog), allowed us to determine if the effects of the cognitive condition are simply an additive effect of resistive walking (Hypothesis 2).

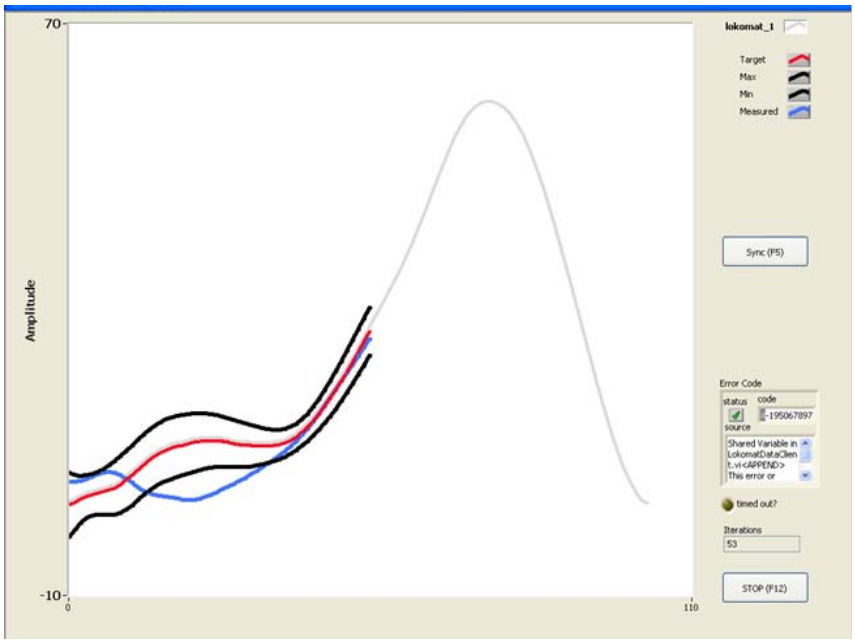

Figure 5: An example of a knee angle trace that will be provided to participants while walking with resistance in the Lokomat during the cognitive condition. The red line represents the participant's knee angle and the black lines represent ± 1SD. The blue line represents the participant's knee angle in real time. Participants were asked to match the blue line to the red line, and trying their best to stay within the two black lines.

3.5 Data analysis

Descriptive statistics regarding age, height and weight of each subject in BF group and RF group were calculated.

MEP amplitudes were calculated to determine peak-to-peak amplitude using a custom program (LabView, National Instruments, Austin, TX). Ten MEP amplitudes for each level of stimulus intensity were averaged for each subject. Input/output curves were formed by plotting the averaged MEP amplitudes across stimulus intensities. For each participant, the MEP amplitude at each stimulation intensity (6 levels) was normalized to the average MEP amplitude at 105% AMT from the pre_loko condition. Subsequently, for each analysis the dependent measure was the normalized mean MEP amplitude at each intensity. All statistical tests were set with an alpha level of .05 and a significant level of $p<0.10$ would be interpreted as a trend. Follow tests employed Bonferroni correction where necessary.

Hypothesis 1: Normalized MEP amplitude will increase immediately after resisted walking in the Lokomat, demonstrating an upregulation in CS excitability, with significant increase noted in both the BF and RF

To test Hypothesis 1, a condition [pre_loko, post_resist] by stimulus intensity [115, 120, 125, 130, 135, 145% AMT] repeated measures ANOVA, for each muscle group (RF, BF), was performed. It was expected that results would reveal a larger increase in CS excitability in the post_resist condition than the pre_loko condition. It was also hypothesized that post hoc tests would reveal increases at the higher level intensities (135 and 145 %AMT) compared to the lower intensities.

Hypothesis 2: Facilitation in CS excitability will be further increased in the cognitive condition (post_cog), where participants are focusing on the resistance being applied throughout the swing phase, compared to the condition when resistance is applied without the participant's awareness (post_nocog).

To determine the impact of explicitly directing attention during Lokomat training between condition [post_cog, post_nocog] by stimulus intensity [115, 120, 125, 130, 135, 145% AMT] repeated measures ANOVA was conducted separately for each muscle group (BF, RF). The dependent measure were the change scores, [post_cog-post_resist] or [post_nocog-post_resist] at each stimulation intensity. It was expected that this analysis would reveal the

change in MEP amplitudes between the post_resist and post_cog condition to be greater than the post_nocog condition. In addition, it was expected that MEPs would be further increased at the higher range of stimulus intensities (135 and 145% AMT).

Hypothesis 3: The RF will not demonstrate significant increases in MEPs following walking with resistance in the Lokomat and the BF will show a larger effect of resistance on MEP amplitude as compared to the RF

Hypothesis 3 was tested using a condition [pre_loko, post_resist] by stimulus intensity [115, 120, 125, 130, 135, 145% AMT] repeated measures ANOVA with muscle [BF, RF] as a between groups factor. Post hoc tests to determine how the groups differ would consist of an intensity [115, 120, 125, 130, 135, 145% AMT] by group [BF, RF] repeated measures ANOVA for each condition (pre_loko, post_resist). It was expected that the BF, compared to the RF, would demonstrate greater increases (135 and 145%) in MEP amplitudes in the post_resist condition.

4.0RESULTS

Aim 1: To determine whether CS excitability is altered during adapted walking with resistance

Figure 6 illustrates the change in average MEP amplitude, normalized to pre_loko 105%AMT for each participant, across the 6 intensities between baseline and resisted walking. A 2 [condition: pre_loko, post_resist] by 6 [intensity: 115, 120, 125, 130, 135, 145% AMT] repeated measures ANOVA was conducted. Two separate ANOVAs were run, one for each muscle group (BF and RF). For the BF (fig 6A), there was a statistically significant condition by intensity interaction $F(3.04, 57.75) = 4.314$, $p = .008$. Paired sample T-tests were conducted to determine at what intensities the normalized MEP amplitudes differed between conditions. There was a statistically significant difference between pre_loko and post_resist at 115, 130, 135, and 145% AMT. The means, standard deviations and significant values are presented in Table 1. There was also a main effect of condition $F(1, 19) = 18.28$, $p < .0001$ and intensity $F(2.21, 41.89) = 41.95$, $p < .0001$. Raw MEPs of the BF at three intensities pre and post resistance are shown in Appendix 1.

Although the RF (fig 6B) did show a slight increase in MEP amplitude following resisted walking compared to baseline walking, there was no main effect of condition $F(1, 19) = 3.57$, $p = .74$. There was a main effect of intensity $F(2.25, 42.76) = 31.62$, $p<.0001$; MEP amplitudes varied across intensity levels.

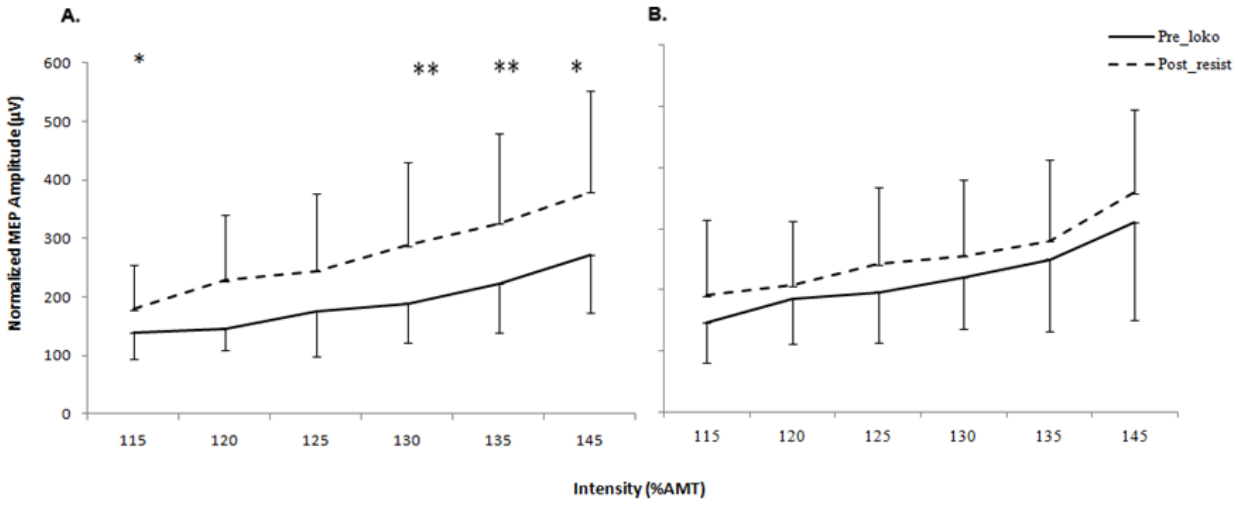

Figure 6: average normalized MEP amplitudes for the BF (A) and RF (B) before (solid line) and after resisted walking (dashed line) across stimulus intensity. *p<.05; **<.001

Table 1: Differences in normalized MEP amplitudes (uV) in the biceps femoris between walking with (post_resist) and without (pre_loko) resistance across TMS intensities.

	Pre_loko		Post_resist		
Intensity (AMT%)	M	SD	M	SD	t(df)
115	144.24	45.48	190.30	78.11	-2.92*
120	151.12	32.95	251.21	125.42	-3.82
125	183.35	72.52	273.22	157.13	-2.69
130	200.96	68.83	320.03	164.51	-4.53**
135	239.77	88.13	362.33	178.31	-4.68**
145	283.82	94.32	414.31	184.46	-4.01*

*p<.05; **p<.001

Aim 2: To determine whether CS changes are dependent on directing the participant's attention to motor control throughout adaptation

For each muscle (BF, RF), a between groups 2 [post_cog vs. post_nocog] X 6 [115, 120, 125, 130, 135, 140%AMT] repeated measures ANOVA was conducted to explore the impact of cognition on MEP amplitudes, at varying intensities (Fig 7). After comparing change scores (post_cog/nocog – post_resist) for focused cognition and non-focused conditions there were no significant effect of condition (post_cog vs. post_nocog) in either the BF or RF group, [$F(1, 18) = .044$, $p = .836$ and $F(1, 18) = .035$, $p = .854$] respectively. There was a significant main effect of intensity for the RF group [$F(3.75, 67.51) = 2.64$, $p = .044$]. A priori comparisons between intensities 135 and 145%AMT and all the lower intensities [adjusted t= .00056] revealed a statistical significance between intensities 145 and 130%AMT [t(19)= -3.210, p=.005]. There was a trend between the mean amplitude difference of 145%AMT and lower intensities, 115, 120, 125 and 135 [p=.027, p=.017, p=.30, p=.025] respectively. Table 3 provides the mean, standard deviation, std error mean, t scores and significant values across intensities for the a prior planned comparisons.

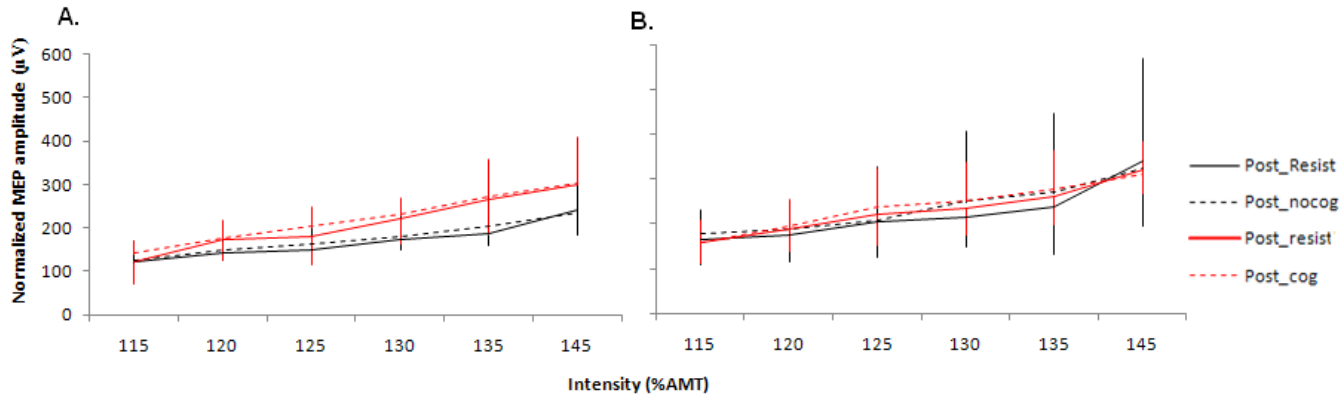

Figure 7: Averaged normalized MEP amplitude (µV) for the BF (A) and RF (B) after resisted walking (black and red solid lines) and following the second resisted walking condition either without (black dashed lines) or with (red dashed lines) awareness. The dependent measure was the difference between the post_nocog or post_cog (dashed lines) subtracted from the post_resist condition (solid line) of the same colour.

Table 2: A prior pairwise comparisons between intensities

Intensity	Intensity	Mean	Std. Deviation	Std. Error Mean	t	Sig. (2-tailed)
145	115	-44.50	82.83	18.52	-2.40	.027
	120	-53.48	91.83	20.53	-2.60	.017
	125	-39.43	75.36	16.85	-2.34	.030
	130	-50.22	69.97	15.65	-3.21	.005*
	135	-42.63	78.05	17.45	-2.44	.025
135	115	-1.86	78.15	17.48	-.107	.916
	120	-10.84	92.16	20.61	-.526	.605
	125	3.20	64.16	14.50	.221	.827
	130	-7.59	56.32	12.59	-.602	.554

*p<.005

Aim 3: To determine whether change in CS excitability is specific to the RF or BF

A repeated measures between groups ANOVA was conducted to compare the effect of condition [pre_loko, post_resist] on MEP amplitudes in the RF and BF muscle groups across intensities [115, 120, 125, 130, 135, 145%AMT]. the statistically significant condition by intensity by group interaction on MEP amplitude [$F(5, 2.69, 102.1) = 2.78$, $p = .05$; power=.62].

This suggests MEP amplitudes vary across condition and intensity levels by muscle group (BF vs. RF). Figure 8 illustrates a statistically significant Group X Condition Interaction [F(1.0, 102.1)=2.25, p=.049].To determine the direct difference between conditions, two separate intensity [115, 120, 125, 130, 135, 145%AMT] by group [BF, RF] repeated measures ANOVAs were conducted, one for pre_loko and one for post_resist. There was no significant interaction intensity by group interaction for the pre condition [F(2.52, 85.6)=.448, p=.664]; however there was a trend for a significant interaction in the post condition [F(2.63, 99.86)=2.43, p=.078; power=.55]. There was a significant main effect of intensity for both the pre and post condition, [F(2.25, 85.6)=39.99, p<.001] and [F(2.63, 99.86)=57.72, p<.001] respectively.

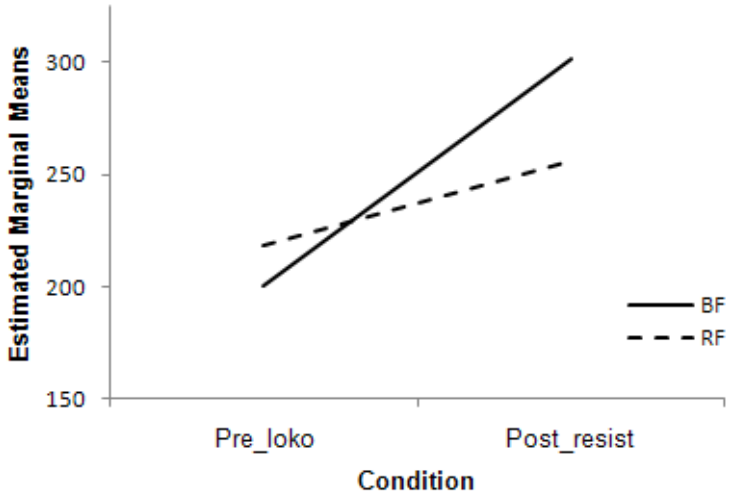

Figure 8: Group*Condition interaction

5.0DISCUSSION

The aims of this study were to determine whether there is an increase in CS excitability following locomotor adaptation and whether attention to the locomotor task influences CS excitability. Adaptation to locomotion was induced by the Lokomat, a robotic gait device, by applying velocity-dependent resistance against hip and knee joint movements during walking. The results show that resistance at the hip during walking increases excitability in the CS tract as illustrated by significant increases in MEP amplitude in the BF. However, this effect appears to be muscle specific as there were no changes in RF MEP amplitude. The results also demonstrated that attention to the locomotor task did not have an effect on MEP amplitude.

Changes in CS excitability are muscle-specific

We demonstrated that change in CS excitability following locomotor adaptation is muscle specific. There was an increase in CS excitability for the BF but not the RF following resisted walking. The difference in muscle-specific CS pathway adaptation may be a result of the functional role of these muscles during gait. Throughout the gait cycle the role of the quadriceps and hamstrings differ; their unique roles are reflected in the adaptation strategies used during locomotion adaptations. When resistance is applied to the limb, the role of the RF is to flex the hip in the beginning of the swing phase and it is powerful enough to cause a rotational force to lift the knee and foot throughout this phase (McFayden, Lavoie & Drew, 1999; Mcfayden & Winter, 1991). The increase in RF activity is an appropriate response to resistance during the swing phase because if the leg is not flexed high enough, a trip may occur. During normal, undisturbed gait, the BF's role at the end of swing phase is to decelerate the limb and prepare for landing. In the presence of resistance however, a new motor pattern emerges; the BF assists in raising the leg to prevent tripping by flexing the knee. Unlike the RF, the increase in BF muscle activity does not occur immediately (Lam et al., 2006).

Afferent information is an essential element in motor control. When resistance is applied against the hip, BF activity aids limb movement by increasing knee flexion and therefore decreasing inertia to compensate for enhanced RF activity. However, as demonstrated by Lam et al. (2006), BF adaptation to resistance takes time to adapt. The increase in EMG activity observed in the BF with resistance, takes place prior to swing. Interestingly, it takes several steps with afferent feedback regarding the applied force before the BF demonstrates increases in activity (Lam et al., 2006). This increase was associated with larger pre-swing knee flexion

activity that prepared the leg to swing through resistance (McFayden, Lavoie & Drew, 1999; Mcfayden & Winter, 1991). The findings by Lam et al (2006) suggests that the BF has adapted or 'learned' the motor patterns necessary to support walking under new conditions in the most efficient way. This is the idea of muscle memory, or motor learning, that with repetition of a new task, long term muscle memory for that task is created (Krakauer & Shadmehr, 2006). With practice the movement is performed more efficiently without conscious effort (Krakauer & Shadmehr, 2006). In the present study, although the increase in BF muscle activity was delayed, it facilitates a functionally appropriate adaptation to resistance throughout the swing phase.

The degree of involvement of the CS tract during adapted control of the RF and BF during walking varies by phase of the gait cycle. Camus and colleagues (2004) stimulated the primary motor cortex of the RF and BF during either the stance of the swing phase of walking. Participants were asked to either not intervene with the voluntary movement evoked by the TMS, or to cognitively prepare themselves for the stimulation so that movements would not occur. The RF and BF demonstrated increased MEP amplitude when the participant asked to compensate for a TMS-evoked hip extension (during stance) and hip flexion (during swing), respectively (Camus et al., 2004). Participants demonstrated that they were able to cognitively prepare themselves (by a decrease in MEP amplitude) for a centrally evoked-movement via "selective anticipatory modulation of the CS excitability to the muscle counteracting the potential central perturbation" (Camus et al., 2004). This lends further support that the roles, and possibly the readiness, of the RF and BF differ throughout the gait cycle, reflecting phase dependent motor adaptations. In the current study, although resistance was applied throughout the gait cycle, the effect of the resistance was greatest during the swing phase. The resistance applied was velocity dependent and the angular velocity of the limb is relatively the greatest throughout the swing phase. Thus, the increase in CS excitability in the BF and not the RF following resisted walking may be a result of the phase of the gait cycle that the resistance most affected.

The idea that there is a functional difference in the BF and RF, and that the difference in neural control of the flexors and extensors is dependent on the task being performed, is also supported by Mrachacz-Kerting and colleagues (2006). TMS was applied to the motor cortex during a flexion perturbation timed with the response of one of the three stretch reflex bursts, short, medium or long latency responses. MEPs were recorded from the RF, vastus lateralis and

medialis, as well as the BF. Of the three quadriceps muscles, the RF was the only one to demonstrate an increase in MEP amplitude when TMS was timed with the long latency burst. It is suggested that as a biarticular muscle the RF may be under separate control, with a greater degree of cortical input, compared to the pure knee extensors (Mrachacz-Kerting, Grey & Sinjaer, 2006). The BF did not show an increase in MEP amplitude when TMS was imposed on the knee flexion perturbation (Mrachacz-Kerting, Grey & Sinjaer, 2006). Although the BF is also a biarticular muscle, flexing the knee and extending the hip, only the knee extensors were activated in this sitting task. The lack of increase in the long latency response therefore emphasizes that the selective control of the CS tract is task dependent.

Capaday et al (1999) suggested that during walking the CS tract is more closely linked with the segmental motor circuits controlling flexors rather than the extensor. However during voluntary tasks requiring attention to the level of motor activity, it is equally linked with the segmental motor circuits of ankle flexors or extensors. Considering previous work by Lam et al (2006) suggest that the BF adaptation to resistance is not reflexive, it is possible that during walking the extensors (RF) are more driven by online changes in afferent feedback whereas the flexors (BF) may be more cortically driven. It is possible that our findings in this specific task also provide evidence of a heightened connection from the CS to the knee flexor following our specific task. Both the knee extensor (RF) and flexor (BF) had equivalent EMG activity during the time of stimulation however, only the knee flexor demonstrated a significant increase in MEP amplitude. An older study by Bates (1953) involved stimulating 45 separate motor cortical points in nine participates. Results revealed that the primary movements evoked were predominantly hip and knee flexion over extension. Due to their deep, medial location the distinct representation of the BF and RF muscles is still unknown. However research suggests, the motor circuits controlling the flexors and extensors may be more closely linked to the CS tract depending on the role of these two muscle groups during the task provided.

Cortical contributions to attentional movement

Attention to motor training has been shown to enhance CS excitability associated with improved performance. Perez et al (2004), demonstrated that participants who performed voluntary dorsi- and plantar-flexion movements to move a cursor with a visuo-motor tracking task, showed significantly increased TA MEPs following training compared to the groups that

continuously moved their ankle at a preferred speed or whose ankles were passively moved. These results suggested that motor training requiring attention leads to improved motor performance accompanied by an increase in MEP amplitude compared to performing the training with no attention (Perez et al., 2004). Other work has demonstrated that MEP amplitude can be influenced by performing an attention-demanding task (Conte et al., 2007). Based on this previous work on the impact of attentional processes in motor memory, motor learning and movement performance (Conte et al., 2007), it was surprising that our results did not correspond to these findings. We found no significant differences in MEP amplitude between the cognitive and non-cognitive group. This was the case for both the BF and RF. Together, these findings suggest that attention to the walking patterns during adaptation does not alter CS excitability.

Previous work has indicated that attention to movements is associated with greater activation of several brain areas, compared to motor execution without attention. These areas include the prefrontal, cingulate, supplementary motor cortex, premotor and cerebellum (Johansen-Berg & Matthews, 2002; Rowe et al., 2002). The prefrontal areas is thought to be of importance during early motor skill acquisition followed by the involvement of the premotor, posterior parietal and cerebellum areas (Shadmehr & Holcomb, 1997). We assume that our BFB intervention would have enabled recruitment of these cortical areas involved in attention and learning, as well as areas involved in integrating signals associated with the provided feedback; given this, it is surprising that there was not an increase in MEP amplitude. By providing a cognitive task-specific BFB, the primary motor area would be thought to contribute to motor preparation (Chen et al., 1998). The parietal and premotor regions of the cortex are involved in motor planning and visual feedback and relay signals to the primary motor cortex (Scott, 2004). The premotor cortex may also be involved in ‘cross-modal interaction’ between tactile stimulation of a particular body region and visual perception of the stimulus to that area (Graziano & Gross, 1994).

While the data from the current study do not support a role for BFB in motor learning and/or motor control ability, several methodological issues may have hampered our capability to investigate this factor. It is possible that there was a ceiling effect associated with testing healthy participants. Although all 20 participants found the task challenging, the desired amount of prefrontal contribution needed to achieve target position may have been minimal due to the

integrity of the CNS and its ability to accomplish the motor task. One possible reason why the cognitive group did not show an increase in MEP amplitude compared to the non cognitive group could be due to the attentional task provided to them. Having participants focus on a matching their current knee pattern with resistance to their previous knee pattern without resistance is not equivalent to requiring them to learn a new movement/task. The movement required in the current study would be similar to walking in water, something most of us has experienced. If the task oriented BFB instead required the participants to walk in a novel movement pattern, for example exaggerating abduction throughout swing, we may have detected changes in CS excitability. It is also possible that attention was an important factor but that it was not detected by stimulating the primary motor cortex. Motor cortex areas associated with the RF or BF were stimulated to measure the excitability of the entire CS tract. There may have been changes elsewhere in the cortex (i.e. premotor areas) that demonstrated an increase in activation however that was undetectable by our methods.

Possible site of origin of adaptations

There is a notion that adaptation in BF to resisted walking involves feedforward strategies. During resisted walking, there is an increase in muscle activity in both the RF and BF (Lam et al., 2006). However, when the resistance was removed unexpectedly, only the BF demonstrated an increase in activity (Lam et al., 2006) suggesting that the adaptation in this muscle involved a feedforward, or 'predictive' strategy for locomotor adaptation. The increase in MEP amplitude in the BF demonstrated in our study may also support the contribution of cortical networks involved in this feedforward strategy.

Predictive strategies usually refer to the anticipatory portion of movement that is planned prior to the consequence of afferent information causing motor pattern changes. Several areas of the brain, including the cerebellum and M1, are involved in adaptive motor learning. The cerebellum is thought to be involved in motor control, coordination and learned movement sequences (Maill, Wier, Wolpert & Stein, 1993). It has a significant role in online feedback control and is thought to function as a sensorimotor predictor (Bastian, 2006). Experimental evidence and observation on patients with cerebellar damage suggest the importance of the cerebellum in predictive movement strategies. Those with cerebellar damage are able to use 'reactive' control strategies to make on-line corrections during movements by using peripheral

feedback however are unable to use 'predictive' control (Bastian, 2006). It is considered that the combination of ascending proprioceptive information, as well as input from M1, allows the development of a prediction for the best possible motor output related to motor periphery. It can be suggested that the cerebellum was involved in the increase in muscle activity (Lam et al, 2006) and MEP amplitude (in our study), following the removal of resistance. In the current study it may be that the cerebellum had planned movement prior to the consequence of afferent information regarding resistance. Early work on tremor in monkeys also suggests that the cerebellum provides the motor cortex with predictive signals, prior to movement, to the antagonist muscle (the BF in our study) to prevent overshooting the movement (Vilis & Hore, 1980). This predictive, feedforward contribution from the cerebellum to M1 would have been important in response to the external force on the perturbed limb in the present study.

There is also evidence that the M1 plays a role in forming internal models, neural processes that reflect the association between motor commands and limb movements. Internal models are thought to convert the overall movement goal into motor commands. In addition, they are updated with experience (Scott & Norman, 2003). The M1 likely converts trajectory goals into detailed motor patterns to control limb muscular through the correlation of patterns of movement and activation of the M1 (Scott, 2004). These patterns demonstrating M1 activity prior to the onset of muscle activity (Scott, 2004), suggest that the role of the M1is in forming at least part of an internal model.

5.1 Methodological considerations

One limitation with using TMS and I/O curves as a dependent measure, is that it does not allow us to determine, except at the level of the α-motor neuron, where within the CS pathway change(s) have occurred (Capaday et al., 1999). Changes may have occurred cortically in the intracortical circuits or in the spinal cord within the segmental interneurons. In order to determine whether the changes occur at the level of the spine, H-reflexes need to be performed. However, H-reflexes can be influenced by other inputs, independent of what is occurring in the CS tract, and therefore may not providing an obvious indicator of changes in the spinal pathways. H-reflexes would also be challenging to collect given the muscles of interest in the current study.

Recruitment curves in the present work were collected while participants held a constant contraction following walking. This task only requires participants to monitor and maintain 20% of their MVC, which does not have significant repercussions if precise and specific control is not met, when compared to walking. In Bonnard's study (2002) on the other hand, participants received TMS while walking, a task where falling or tripping are consequences of inaccurate muscle activation. The fact that MEPs were elicited in a static, not a walking state, could have changed the results of the current study. Nevertheless, the fact that we showed specific changes in BF MEP amplitude, even while subjects were in a static position, demonstrates that the methodology used in the present work was appropriate for assessing short-term motor cortical plasticity associated with locomotor adaptations.

Variation in MEP amplitude across stimulation bouts within each testing session could have occurred due to 1) inconsistent contractions, 2) varying stimulation sites and/or 3) a change in electrode position/impedance. To address these possibilities, participants were required to maintain a muscle contraction at 20%MVC in their assigned muscle group. Possibility of varying contraction levels across stimulation sessions was minimized by providing the participants with online visual feedback during contraction. Participants were also required to wear a tight swim cap throughout the experiment. This allowed the coil position that elicited MEPs to be traced onto the head to help maintain consistency of coil position across conditions. Participants were asked to let us know if they felt the cap shift at anytime throughout the session. The swim cap however was quite tight and only moved on one subject, whose data was removed from the analyses. After the skin was cleaned and abraded, electrodes were taped to the skin to ensure that they stayed in the same place. A tensor bandage was also wrapped around the leg to ensure that the Lokomat did not pull on the wires or move the electrodes. The skin was cleaned and abraded prior to application of the electrodes to reduce impedance. The participants completed 4 walking conditions, therefore if the participants sweated, this could have caused the impedance to change. However, each condition lasted only four minutes and it is unlikely that excessive sweating is a consideration. When removing the electrodes all of the electrodes were in the same location and the skin did not seem moist from perspiring.

Leg dominance was not recorded in this study. This could potentially be seen as a hindrance to the study if, for example, only the non dominant leg demonstrated adaptation

changes. If this were the case and the non-dominant leg was only measured on a few participants, important information may have been missed. However, walking is considered a coordinated, repetitive task that may not involve preference of one leg over the other. In a review paper by Sadeghi and colleagues (2000) on symmetry and limb dominance in able-bodied gait concluded that although an action with a goal is carried out by the preferred leg, more work is needed to assess gait symmetry and its possible relationship to laterality. Leg dominance therefore may not be present during locomotion and therefore affected this study.

Although we asked the participants how hard they focused on the knee angle trace or youtube video, we did not measure how well the participants in the cognitive group followed the knee trace. This limits us to the participant's feedback regarding their focus of attention. We are unable to reliably say whether or not they improved on matching their knee angle position with resistance, to their previous knee angle trace without resistance.

5.2 Significance

This is one of the first studies to investigate changes in transmission along the CS pathway associated with adaptations during walking and the impact of attention on CS excitability. Although research provides evidence of plastic changes associated with motor skill training, the mechanisms that are involved are still uncertain. Providing motor skill training is important for the rehabilitation of patients with spinal cord or cortical damage. It is therefore important to determine the impact of attention throughout rehabilitation. It is still under debate whether providing patients with BFB, and therefore additional information, promotes functional improvements or prevents the CNS from naturally adapting to the locomotor training.

5.3 Future studies

Future work should consider how neuropathology shifts the findings of the current study. Work testing patients with incomplete spinal cord injuries (SCI) as well as those with cerebellar stroke lesions may be of particular interest. Recent work demonstrated that individuals with motor-incomplete SCI (grade D on the American Spinal Injury Association Impairment Scale) did show after-effects in kinematic patterns following the removal of resistance (Houldin, Luttin

& Lam, 2011). It would therefore be interesting to note if the after-effects corresponded to increased modifications in MEP amplitude following adaptation in this population when compared to that of healthy age matched controls. If the increase in MEP amplitude was a result of input from the feedforward, cerebellar circuits, it can be assumed that cerebellar stroke patients would not exhibit this adaptive response. Both of these populations might also benefit from the provided BFB by reinforcing sensory cues that are not as evident to them as healthy controls. An increase in MEP amplitude following the cognitive condition may therefore be observed in these two populations.

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APPENDICES

A: Raw MEPs of the BF in one participant

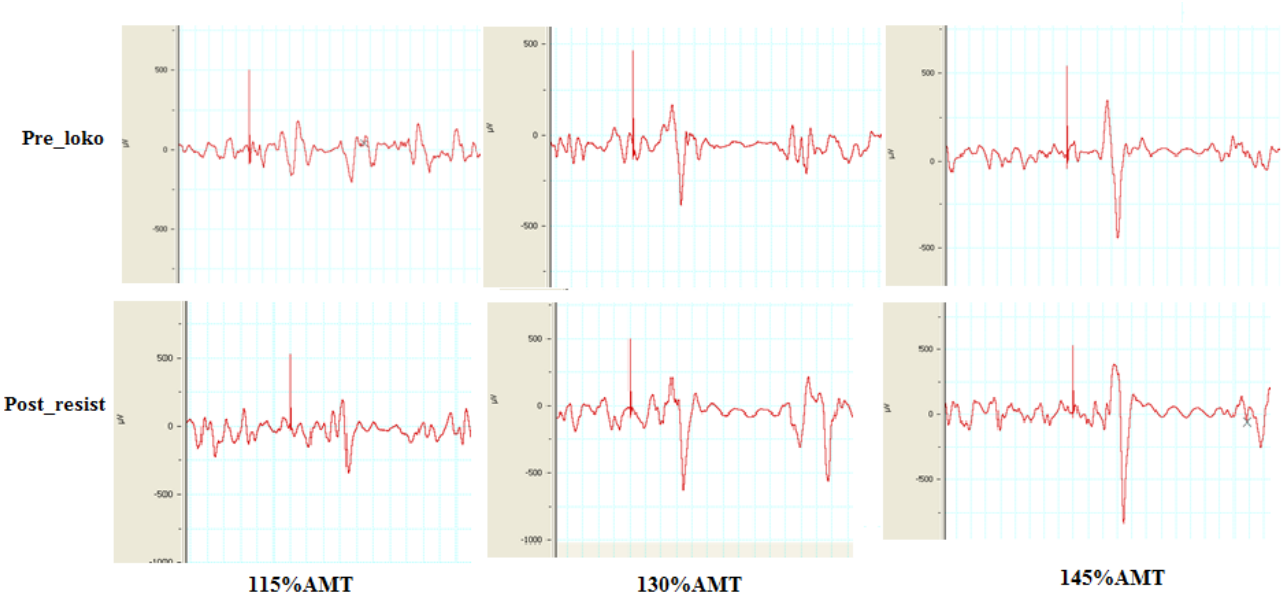


Figure 9: Raw MEPs of the BF from one participant before (pre_loko) and after (post_resist) resisted Lokomat walking at three intensities (115, 130 and 145%AMT).

B: Consent form

SUBJECT INFORMATION

Title of Study:	Contribution of corticospinal input to adaptive strategies to Lokomat-applied perturbations during walking
Principal Investigator:	Dr. Tania Lam (Assistant Professor, School of Human Kinetics, ICORD)
Co-Investigator:	Dr. Lara Boyd (Associate Professor, Department of Physical Therapy)
Graduate Student:	Jeanie Zabukovec, MSc student

Introduction

We know from personal experience how easy it is for us to adapt and walk through different environments. We will encounter innumerable types of walking environments and need to cope with changes to the physical properties of our bodies throughout life. We are interested in how our nervous system deals with these ever changing demands to ensure that we can move accurately and safely. You are being invited to take part in this research study because we are looking for able-bodied volunteers to participate in a walking study.

Purpose

The main purpose of this study is to evaluate how motor commands from your brain to your leg muscles changes when after you walk against a resistance. In addition, we are interested in how attention plays a role in locomotor adaptation.The resistance will be applied by the Lokomat system, a specially designed rehabilitation device for treadmill walking. This research is currently being supported by the Natural Sciences and Engineering Research Council of Canada (NSERC). The infrastructure for this research was supported by the Canada Foundation for Innovation.

Who can participate in this study?

You can participate in this study if you are between 20 and 50 years old, in good general health and are able to walk without difficulty.

Who should not participate in the study?

You should not participate in this study if any of the following applies to you:

- You have a history of seizure, epilepsy, neurodegenerative disorder, head trauma, or a psychiatric diagnosis.
- You have metal implants in your body.
- You have a neurological condition that affects your ability to move.

- You have a medical condition that prevents you from exercising (e.g. heart problem, muscle pain, broken or weak bones).
- You are pregnant.
- You have a Body Mass Index (BMI) > 30 (BMI = your weight (in kg) divided by your height^2 (in meters))

Study Procedures

This study will take place on the 3rd floor of the ICORD Blusson Spinal Cord Injury Centre (818 West 10th Ave, Vancouver). This study will require you to make 1 visit to our laboratory lasting 3-4 hours. If you agree to take part in this study, you can expect the following:

Before You Begin the Study

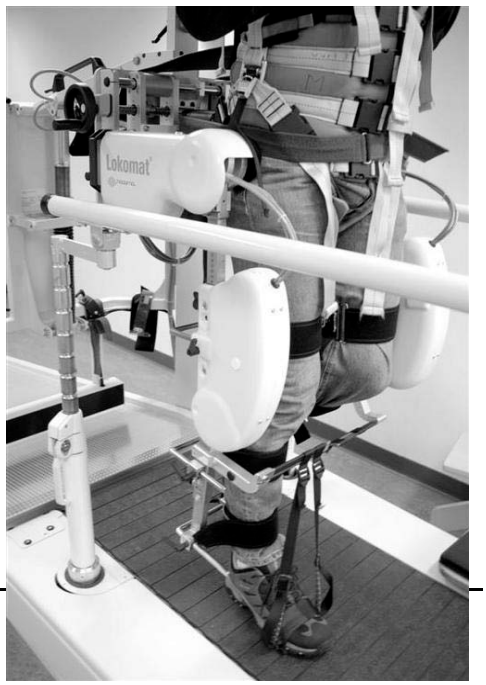
Before beginning the study, we will contact you by telephone to confirm your eligibility to participate in this study.

Laboratory Visit

We will arrange a time that is convenient for you to come to the laboratory. Please bring a pair of comfortable walking shoes. Because we will need to tape various instruments to your shoes, it is preferable if you bring running shoes or an older pair of shoes.

Muscle Activity and Leg Movement Recordings: This analysis will allow us to evaluate your leg movements during walking. Using skin-sensitive tape, we will attach pairs of small disks to the lower leg and thigh area to record the activity in your leg muscles. Before taping the disks on your skin, we will first shave the hair and clean the skin area with a light abrasive cream or fine-grade sandpaper and alcohol swabs, which will allow us to obtain better recordings of your muscle activity. Small disks will be taped on your shoes and legs. Special sole inserts to measure the times when your feet contact the ground during walking will be placed in your shoes. All of these instruments will be connected through thin, lightweight wires to a computer system. All equipment attached to you is battery powered or electrically isolated. You will not feel any electrical sensations from the equipment attached to your body. These procedures should take about 30-45 minutes.

After the instruments have been taped to your legs, we will have you stand on the treadmill within the Lokomat. The Lokomat is a computer-controlled gait rehabilitation system that consists of a pair of exoskeletal legs to which your thighs and lower legs are strapped. The Lokomat will be adjusted according to the length and size of your legs. You will be secured to the Lokomat by leg cuffs around the mid-thigh, upper shank, and lower shank as well as a waist belt to provide trunk support. Each exoskeletal leg is attached to a central horizontal frame that



The Lokomat

secures you around the hip. There are motors embedded within the exoskeletal legs that can be programmed to control the Lokomat.

The treadmill will be set to a speed that is comfortable for you and we will give you time to become familiar with walking in the Lokomat. The Lokomat will be set to a “passive” mode and you should walk as you normally do.

After this familiarization period, you will undergo two more walking trials. During these walking trials, you may feel the Lokomat applying some resistance against the movements of your hip and knee once in a while. This will feel similar to walking underwater.

In between these trials, we will use a technique called transcranial magnetic stimulation (TMS) to map the excitability of your brain before and after your participate in walking trials. A double cone coil will be held over your head. A total of 40 pulses will be applied to the head with increasing intensity. This magnetic stimulation will last about 3 minutes and repeated 4 times throughout the session. This technique is painless but may become uncomfortable at the higher intensities. You may feel a tugging or tingling feeling on your scalp during this time.

The total time (not including rest breaks) that you will be in the Lokomat will be a maximum of 45 minutes. You should always try to walk as normally as you can throughout the trial. We may videotape your leg movements while you walk with the Lokomat. The video camera will be positioned so that only your lower body will be filmed in order to protect your identity.

Possible harms and side effects

The risks are not greater than the risks in everyday life. The TMS procedures will be conducted according to published safety standards by Dr. Boyd who has completed procedural and safety training for these procedures at Harvard Medical School and certified in their use. Dr. Boyd or her associates have discussed this research with you and have described them as follows:

> There is a potential risk of seizure induction in people with a history of seizures (e.g. epilepsy). You will not be eligible to participate in this study if you have such history. There is also a small but real risk of seizure in people who do not have epilepsy during TMS brain mapping and treatment. Safety standards for the application of TMS have been developed and will be followed during this study to minimize the risk. In this study, we are using single-pulse TMS, which poses very little risk of seizure in healthy people. In addition, Dr Boyd has been trained in the safe application of TMS and will administer all brain stimulation sessions.

Some people may feel uneasy walking on a treadmill or with the Lokomat. However, we will provide ample time to allow you to become accustomed to the treadmill and Lokomat. The Lokomat also has several safety features that will automatically shut it down should it experience excessive forces (e.g. if you were to stumble on the treadmill). There are also emergency stop switches on the treadmills that you can press if you feel uncomfortable at any time of the study. It is also possible that you may feel tired after the testing sessions. You will have rest from walking in between each trial

while we do the TMS mapping. However, because of the way the Lokomat is set-up, you won't be able to sit-down during the rest breaks.

Confidentiality

Your confidentiality will be respected. The data collected from this study will be disseminated in the form of publications and conference or teaching presentations. However, no information that discloses your identity will be released or published without your specific consent to the disclosure. All records which identify you by name or initials and all video data will be kept in a secured place (locked cabinets and password-protected electronic data) in the principal investigator's office or laboratory. The results of this study, once completed, can be provided to you at your request.

Remuneration/Compensation

You will be provided with $10 for your participating in this study. You will be given this money to you in cash at the end of your visit.

What are the benefits of participating in this study?

We do not expect any direct benefits to you from taking part in this study. We hope the information learned from this study will further our understanding of the control of walking, supporting further research into rehabilitation therapy to improve walking in people with neurological damage such as stroke and spinal cord injury.

Contact for information about the study

If you have any questions or desire further information about this study before or during participation, you can contact Tania Lam at (604) 827-3165.

Contact for concerns about the rights of research subjects

If you have any concerns about your rights as a research subject and/or your experiences while participating in this study, contact the Research Subject Information Line in the UBC Office of Research Services at (604) 822-8598.

Consent

Your participation in this research is entirely voluntary. You may refuse to participate or withdraw from this study at any time without penalty or consequences

CONSENT FORM

Title of Study:	Contribution of corticospinal input to adaptive strategies to Lokomat-applied perturbations during walking
Principal Investigator:	Dr. Tania Lam (Assistant Professor, School of Human Kinetics, ICORD)
Co-Investigator:	Dr. Lara Boyd (Assistant Professor, Department of Physical Therapy)
Graduate Student:	Jeanie Zabukovec, MSc student

Your signature below indicates that you have received a copy of this consent form for your own records.

Your signature indicates that you consent to participate in this study.

_____ _____

Subject Signature Date

_____

Printed Name of Subject

Please indicate whether or not we may contact you in the future to let you know about other research studies you may be interested in.

☐ Yes, I may be interested in other research studies. Please keep my contact information on file to let me know of other studies I may be eligible for.

_____ _____

Subject Signature Date