HEBBIAN NEUROPLASTICITY IN THE HUMAN CORTICOSPINAL TRACT AS INDUCED BY SPECIFIC ELECTRICAL AND MAGNETIC STIMULATION PROTOCOLS

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy (Ph.D.)

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Abstract

Conventional functional electrical stimulation (FES) therapy, if provided shortly after an incomplete spinal cord injury, is able to help an individual to restore voluntary hand function. This is thought to occur through the induction of neuroplasticity. However, conventional FES therapy employs a push-button-based control scheme, which does not fully require the recipient to generate volitional movements. The first study in this thesis therefore sought to determine, in an early proof-of-concept test with able-bodied participants, whether control strategies which are triggered by volitional activity (including an electroencephalography-based brain-machine interface (BMI-FES) and an electromyogram-based control scheme (EMG-FES)) might provide greater benefits to hand function. The results offer relatively weak evidence to suggest that BMI-FES, and especially EMG-FES, were able to induce greater neuroplasticity than conventional treatments in the corticospinal tract leading to the hands, but that this did not immediately translate to more functional improvements such as maximum grip force.

The second study in this thesis focussed on spinal associative stimulation (SAS), which involves paired stimulation pulses at both the head (via transcranial magnetic stimulation), and the wrist (via peripheral nerve stimulation). The purpose of this, as with the first study, was to induce neuroplasticity and upregulate the corticospinal tract leading to the hands. While limited research has suggested that it is possible to produce neuroplasticity through SAS, all such studies have provided stimulation at a fixed frequency of 0.1 or 0.2 Hz. The present study therefore sought to compare the effectiveness of a typical 0.1 Hz paradigm with a 1 Hz paradigm, and a paradigm which provided stimulation in 5 Hz "bursts". None of the paradigms were able to successfully induce neuroplasticity in a consistent manner. The increased variability in this study as compared to the previous one, despite the nearly identical assessment methodology, suggests that responses to the SAS treatment may have been highly individual. This serves to highlight a potential limitation of the treatment, which is that its effectiveness may not be universal, but rather dependent on each specific recipient. This may be a challenge faced by SAS should it continue to be tested as a novel therapy.

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List of Abbreviations

αMN: α motor neuron

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

APB: Abductor pollicis brevis

BCM: Bienenstock, Cooper, and Munro

BDNF: Brain-deriven neurotrophic factor

BMI: Brain-machine interface

BMI-FES: Brain-machine interface-controlled functional electrical stimulation

CaMKII: Ca2+/calmodulin-dependent protein kinase

CMEP: Cervicomedullary evoked potential

CST: Corticospinal tract

DAQ: Data acquisition

EEG: Electroencephalogram

EMG: Electromyogram

EMG-FES: Electromyogram-controlled functional electrical stimulation

ERD: Event-related desynchronization

ERS: Event-related synchronization

FCR: Flexor carpii radialis

FDI: First dorsal interosseus

FES: Functional electrical stimulation

ICF: Intracortical facilitation

ICI: Intracortical inhibition

ISI: Interstimulus interval

LTD: Long-term depression

LTP: Long-term potentiation

M1: Primary motor cortex

MEP: Motor evoked potential

mGluR: Metabotropic glutamate receptor

MMT: Manual muscle test

MSO: Maximum stimulator output

MVC: Maximum voluntary contraction

NMDA: N-methyl-D-aspartate

PAS: Paired associative stimulation

PNS: Peripheral nerve stimulation

RCT: Randomized control trial

ROC: Receiver operating characteristic

S1: Primary somatosensory cortex

SAS: Spinal associative stimulation

SCI: Spinal cord injury

TA: Tibialis anterior

TMS: Transcranial magnetic stimulation

UMN: Upper motor neuron

List of Equations

Equation 1. $ISI = L_{anti} - L_{cord}$

Equation 2. $L_F = L_{anti} + L_{\alpha MN}$

Equation 3. $L_{anti} = L_F - L_{\alpha MN}$

Equation 4. $L_{MEP} = L_{cord} + L_{\alpha MN}$

Equation 5. $L_{cord} = L_{MEP} - L_{\alpha MN}$

Equation 6. $ISI = (L_F - L_{\alpha MN}) - (L_{MEP} - L_{\alpha MN})$

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Part I: Introduction

I.1. Motivation

In the healthy human nervous system, voluntary movements are executed through the corticospinal tract (CST). This tract consists of upper motor neurons (UMNs), connected to α motor neurons (αMNs). UMNs, originating in the primary motor cortex (M1) of the brain, send their axons down the spinal cord until they reach the spinal segment corresponding to the muscle of interest. At this level, they connect with the αMNs, which branch off of the spinal cord, and directly innervate the target muscles. This system is outlined in Figure 1. In cases of spinal cord injury (SCI), however, this pathway (among others) is frequently compromised, and the descending signals may not be able to proceed beyond the site of injury. The effect of this is that muscles whose innervating motor neurons are below the site of injury frequently cannot receive commands from the brain, and are effectively paralyzed. The lowest injuries, in the lumbar or sacral sections of the spinal cord affect leg, foot, bowel, bladder, and sexual functions. Thoracic injuries affect all of those areas, plus trunk and abdominal control. Lastly, the highest injuries, in the cervical spinal cord, affect all of the aforementioned areas, plus arm and hand function. In the United States in 2011, an estimated 270,000 individuals live with an SCI, with approximately 12,000 new cases each year (National Spinal Cord Injury Statistical Center, 2012a). Of these, an estimated 53.6% of these injuries occur in the cervical range (National Spinal Cord Injury Statistical Center, 2012a). Low cervical injuries incur an estimated lifetime cost of US$2.0-$3.3 million (depending on the individual's age at the time of injury), and can be expected to decrease life expectancy by 12.5-19.2 years (National Spinal Cord Injury Statistical Center, 2012b).

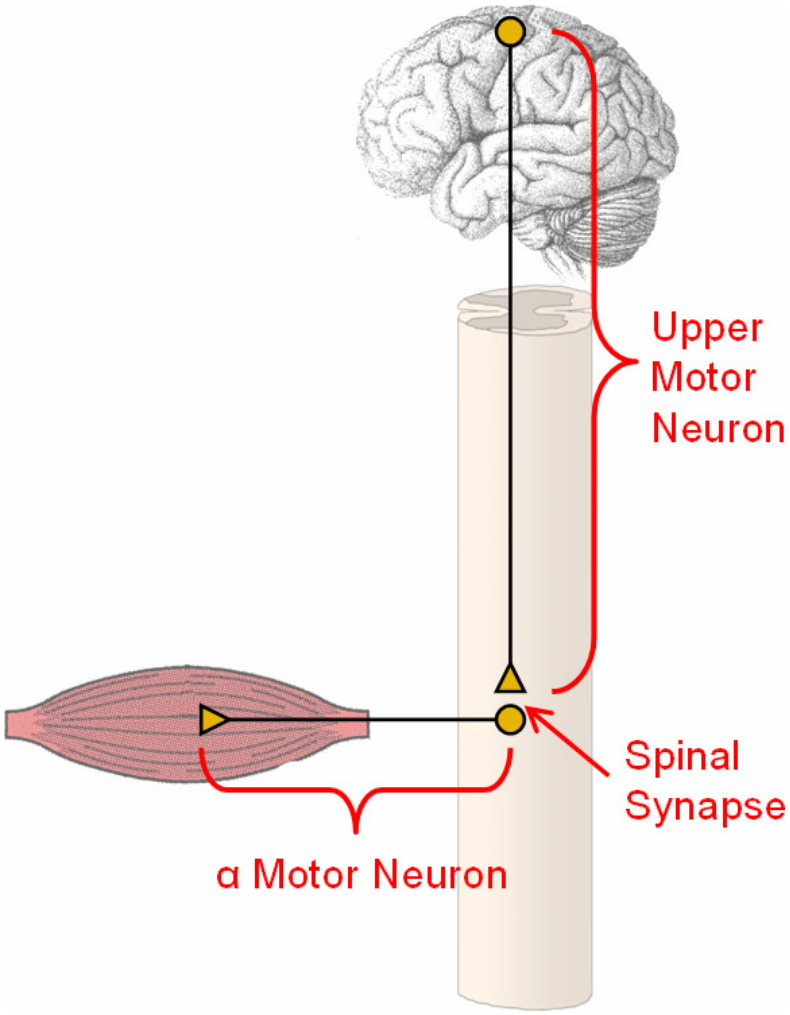

Figure 1. A diagrammatic representation of the corticospinal tract, illustrating the upper motor neuron (UMN), α motor neuron (αMN), and spinal synapse.

Among individuals with cervical SCI, hand function is considered the highest priority for recovery, as illustrated in Figure 2 (Anderson 2004). This is due to the significant contribution of arm function to independence and activities of daily living.

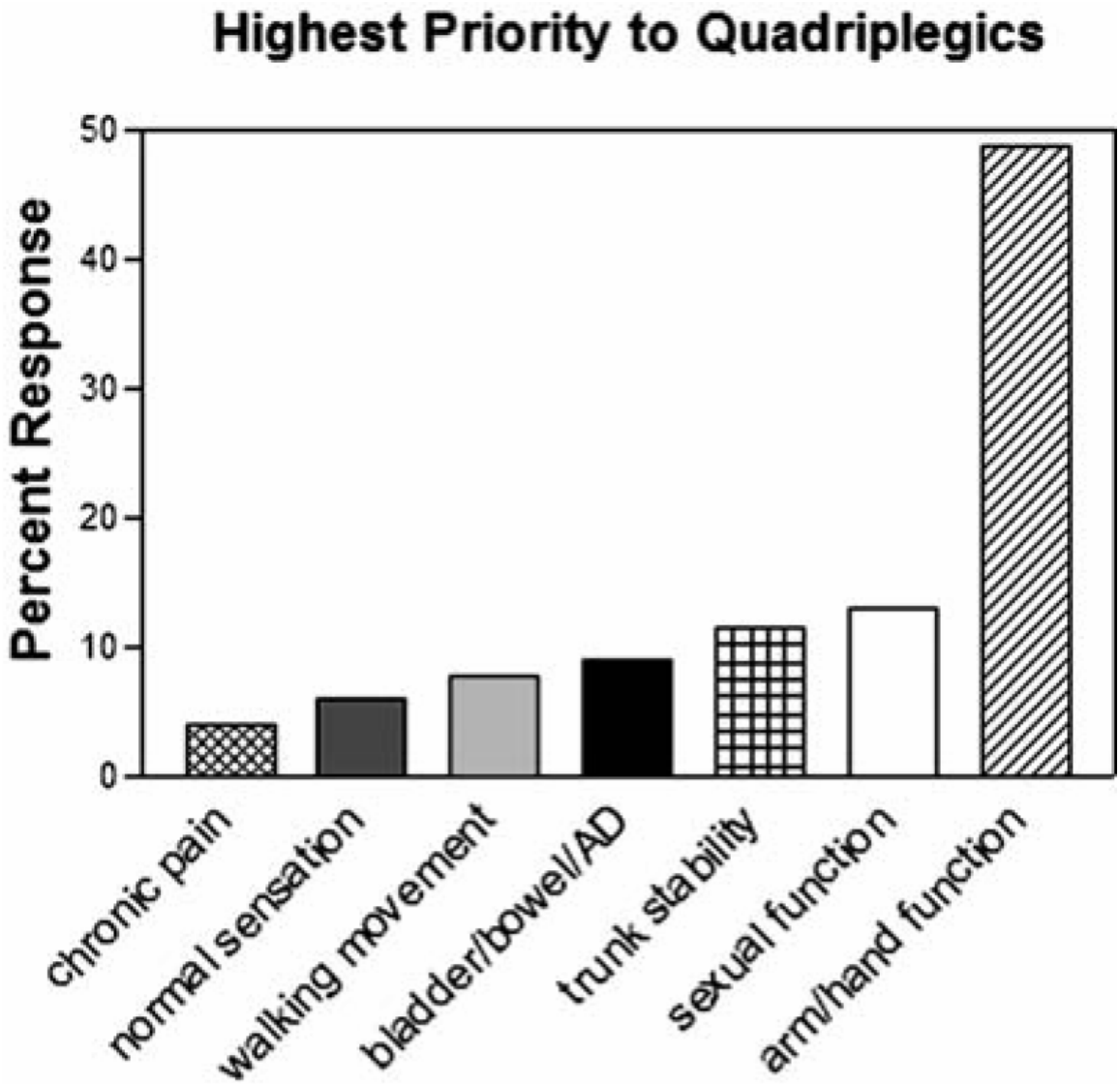

Figure 2. Reproduced from Anderson (2004). Approximately 347 individuals with quadriplegia were asked in a mail-in survey "What gain of function would dramatically improve your life?". 48.7% of responders identified arm and hand function as their top priority, far exceeding the next highest priority (sexual function, which was only identified as the top priority of 13.0% of responders).

Two broad categories of SCI exist: complete and incomplete. Complete injuries are those which damage or sever the entire cross-section of the spinal cord, preventing any

transmission of neurological information across the site of injury. By contrast, incomplete injuries are those in which some tracts are damaged, but others remain intact or merely depressed (Kern et al. 2005). In cases of incomplete SCI, there remains the potential for functional movements to be restored by upregulating the intact pathways. While the pathway itself is not generally considered "plastic", or susceptible to induced change, synapses (the connections between neurons) are. Of particular interest in cases of incomplete SCI are the so-called "spinal synapses". These are the synapses that occur where, as previously described and outlined in Figure 1, UMNs from the M1 connect to αMNs (Bear et al. 2007). Because of their potential for change, which is termed "neuroplasticity", these synapses are considered potentially promising targets for therapeutic interventions. The size of the descending voluntary command may be reduced while travelling through the site of injury, but may be increased thereafter by passing through a synapse that has had its level of output for a given level of input increased.

It is worth noting that the depiction of the CST presented above is the highly simplified. In reality, a wide range of interneuron networks, regulate the CST, including feedback from somatosensory neurons. A more through discussion of these networks is, however, considered to be outside the scope of this project.

I.2. Long-Term Potentiation and Neuroplasticity

The nature of synaptic plasticity was first proposed by Donald Hebb in his 1949 book "The Organization of Behavior". Hebb postulated that "When an axon of cell A is near

enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased" (Hebb 1949, p. 62). This effect was hence termed "Hebbian neuroplasticity". In the years since, Hebbian plasticity has been discovered in many parts of the mammalian nervous system, and more recently, its cellular mechanisms have begun to be elucidated. These will be outlined in the following section, and in Figures 3 through 9.

A synapse consists of two components: the end of the pre-synaptic cell's axon (termed the "axon terminal", and the dendrite or cell body of the post-synaptic cell. Note that in the context of the spinal synapse, the pre-synaptic cell is the UMN, and the post-synaptic cell is the αMN. These components are in close proximity, with the narrow space between the cells referred to as the "synaptic cleft". The axon terminal contains vesicles, which are small bubbles, with walls made of cell membrane. The vesicles contain extracellular fluid and large amounts of neurotransmitter chemicals. In the case of the spinal synapse, the neurotransmitter in question is glutamate, the primary excitatory neurotransmitter for the central nervous system (Curtis and Watkins 1960) (Figure 3). When an impulse coming through the axon reaches the axon terminal, it causes the vesicles to fuse with the axon's cell membrane, effectively pouring the glutamate into the synaptic cleft (Rizo and Rosenmund 2008) (Figure 4). When glutamate reaches the post-synaptic cell, it binds to receptors, which are proteins imbedded in the post-synaptic cell membrane. The exact result of this binding depends on the type of receptor involved.

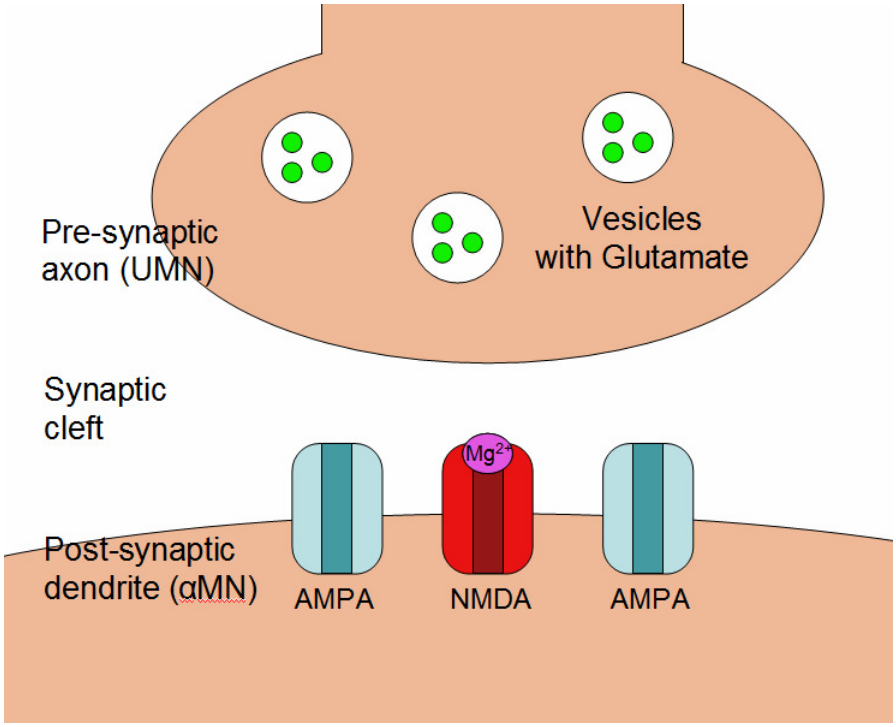

Figure 3. Outline of the spinal synapse at rest. The pre-synaptic cell (an upper motor neuron (UMN)) contains vesicles filled with the neurotransmitter glutamate. The post-synaptic cell (an α motor neuron (αMN)), has various glutamate receptors embedded in its cell membrane, including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA).

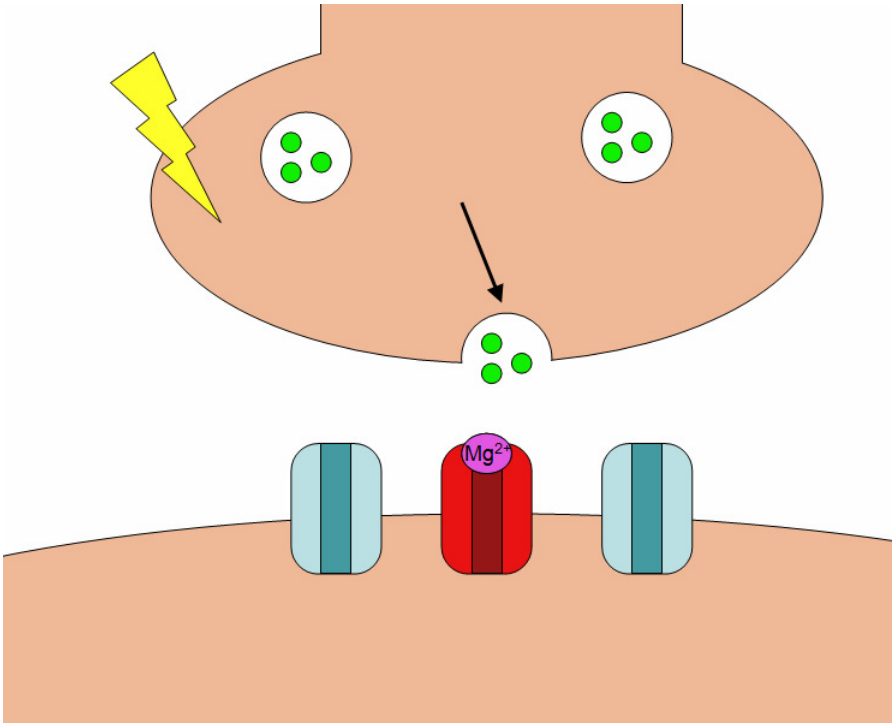

Figure 4. Activity in the pre-synaptic axon triggers vesicle fusion with the cell membrane, causing glutamate to spill into the synaptic cleft.

Glutamate receptors are broadly grouped into two categories: ionotropic and metabotropic (Palmada and Centelles 1998). Metabotropic receptors are those which act through second messenger chemicals, and will be briefly discussed later in this section. Ionotropic receptors are those which have a central pore, through which ions may travel across the cell membrane, in accordance with concentration gradients. Ionotropic receptors are further divided into three classes, named after the glutamate agonists which were first found to bind to them: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA), and kainate (Nicoll et al. 1990). As with the metabotropic receptors, kainate receptors are not presently known to play a role in neuroplasticity, and will not be discussed.

AMPA receptors are responsible for the most standard function of glutamate, which is to continue the propagation of the pre-synaptic signal. When glutamate binds to an AMPA receptor, its central pore opens, allowing Na^+ ions to flow into the cell, and K^+ ions to flow out of it, in accordance with chemical gradients (Figure 5). This depolarization is detected by voltage-gated ion channels, which propagate the potential along the cell membrane until it reaches the start of the cell's axon (Palmada and Centelles 1998).

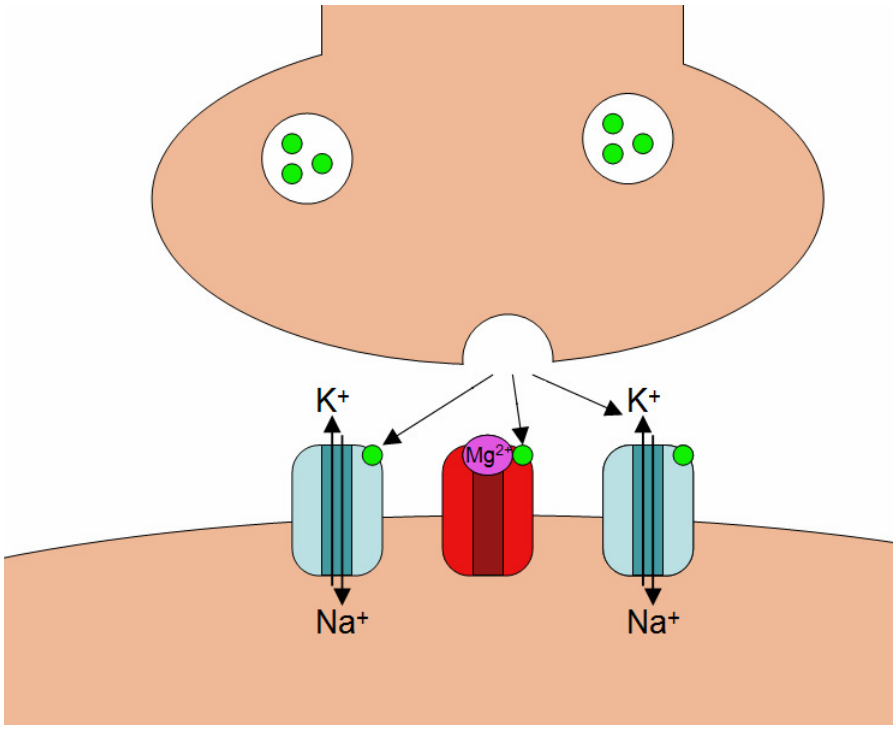

Figure 5. Glutamate binds to the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors, allowing the inflow of Na^+ and the outflow of K^+. The Mg^{2+} ion in the NMDA (N-methyl-D-aspartate) channel prevents any ion flow.

NMDA receptors differ from AMPA receptors due to the presence of a Mg^{2+} ion, which blocks the extracellular end of the synaptic pore (Nowak et al. 1984). Thus, even if glutamate binds to the NMDA receptor, the central pore does not allow the passage of ions unless the Mg^{2+} ion has first been removed. This removal is typically accomplished by the adjacent cell membrane depolarizing. When intracellular space attains a sufficient positive charge, it repels the positive Mg^{2+} ion into the synaptic cleft, leaving the central pore open for ionic flow (Mayer et al. 1984) (Figure 6 and 7).

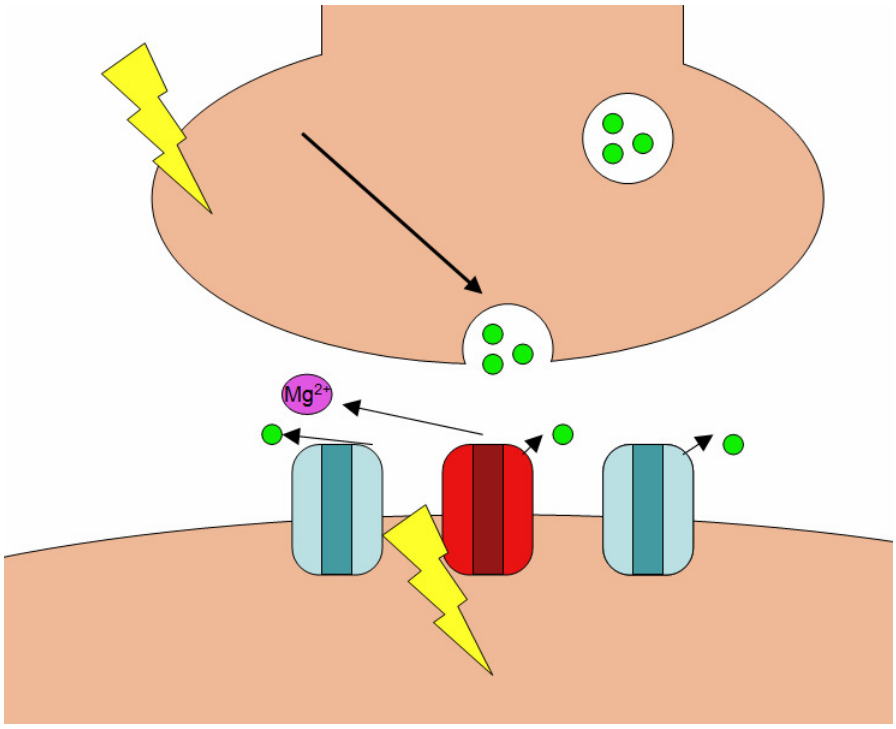

Figure 6. Depolarization in the post-synaptic dendrite repulses the Mg^{2+} ion out of the NMDA (N-methyl-D-aspartate) channel. At the same time, another impulse travelling down the pre-synaptic axon causes additional release of glutamate into the synaptic cleft.

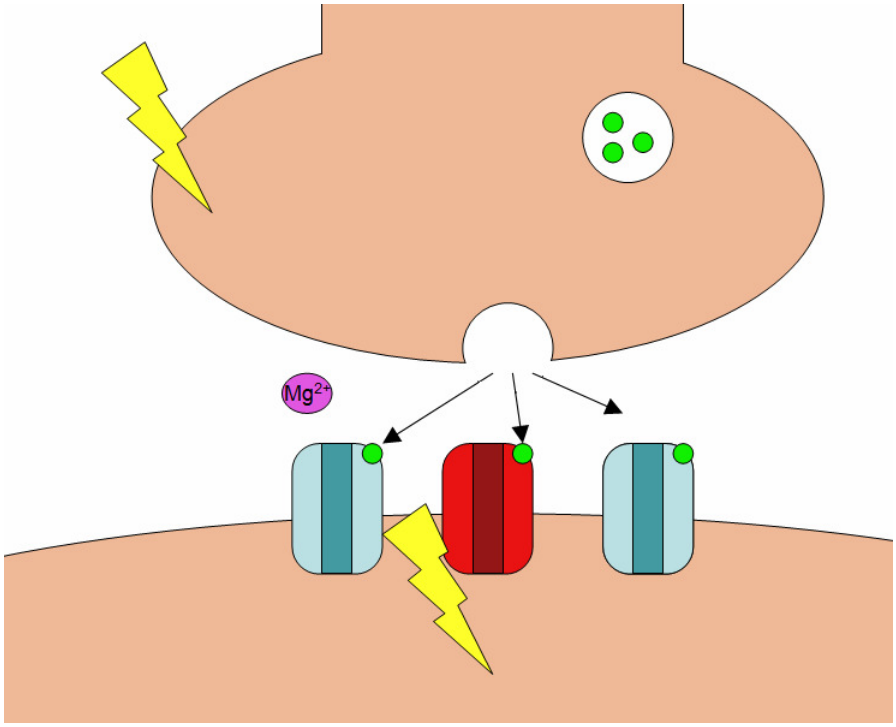

Figure 7. Glutamate binds to the post-synaptic receptors. Note that the post-synaptic cell is still depolarized, preventing the Mg^{2+} ion from returning to the NMDA (N-methyl-D-aspartate) channel.

The second way in which NMDA receptors differ from AMPA receptors is that, in addition to Na^{+} and K^{+}, their central pores allow the passage of Ca^{2+} into the cell (Ascher and Nowak 1988, Jahr and Stevens 1987). This Ca^{2+} then binds to Ca^{2+}/calmodulin-dependent protein kinases II (CaMKII) (Figure 8). Activated CaMKII is thought to be responsible for the subsequent upregulation of the synapse, through mechanisms that are not yet fully understood (Yamauchi 2005). Early phases of the upregulation are thought to involve insertion of previously-intracellular AMPA receptors into the cell membrane and/or phosphorylation of the existing AMPA receptors. Later mechanisms may involve the synthesis of new proteins, including but not limited to additional receptors (Nayak et

al. 1998), and/or morphological changes which increase the cell's surface area at the synapse (Desmond and Levy 1988).

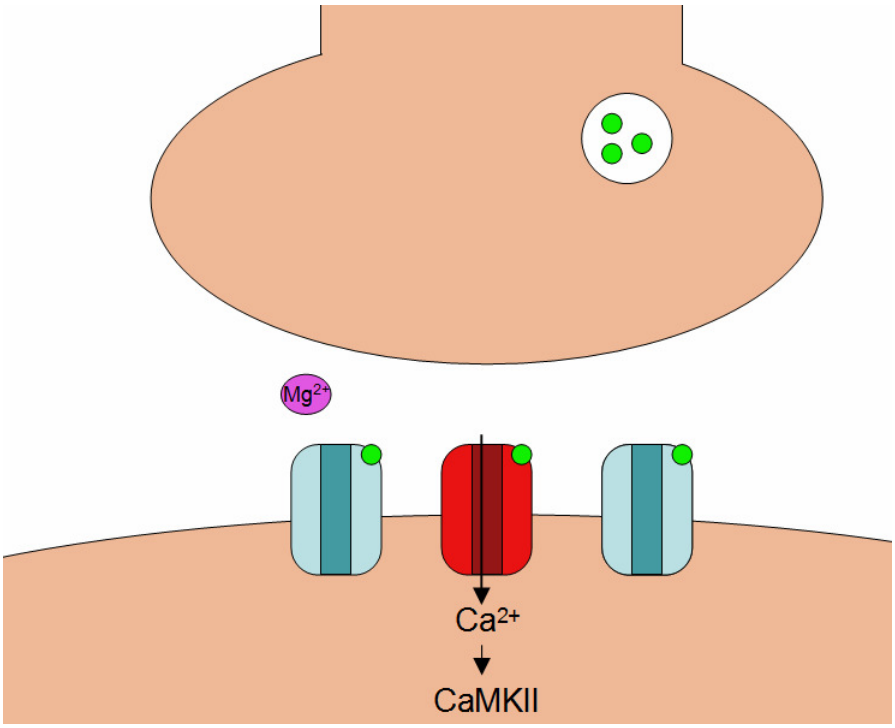

Figure 8. Ca^{2+} flows through the now-open NMDA (N-methyl-D-aspartate) channel and binds to Ca^{2+}/calmodulin-dependent protein kinases II (CaMKII).

Regardless of the mechanism, the end effect of CaMKII activation is that the post-synaptic cell becomes more sensitive to excitation from the pre-synaptic cell. This effect is termed long-term potentiation (LTP) (Figure 9). In essence, LTP can be thought of as an increase in the efficiency or sensitivity of the synapse. If the pre-synaptic axon provides a given level of stimulation both before and after the occurrence of LTP, the post-synaptic cell will produce a larger response after LTP. By the same token, after

LTP, a lesser amount of pre-synaptic activity should be required to produce a given level of post-synaptic activity (Bear 1996).

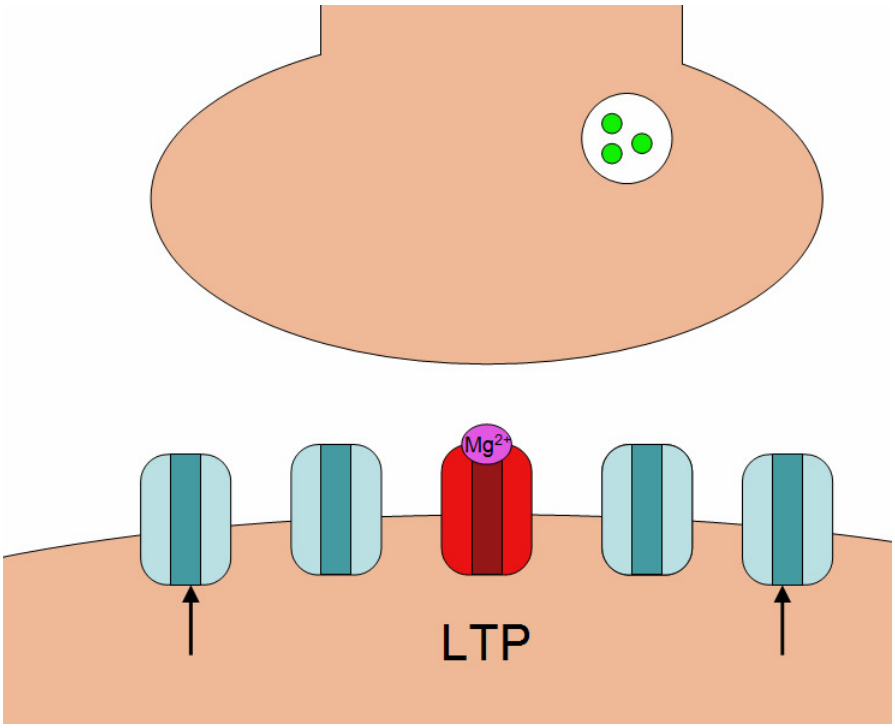

Figure 9. The downstream effects of the activated Ca^{2+}/calmodulin-dependent protein kinases II (CaMKII) trigger various mechanisms of long-term potentiation (LTP). The mechanism shown here involves the insertion of additional AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors into the cell membrane. Note, however, that other mechanisms of LTP are possible.

Recent research has been able to show that the mechanisms of LTP are, in fact, more complex than what has been outlined above. For example, metabotropic glutamate receptors (mGluRs), particularly mGlu1 and mGlu5, have been shown to modulate Ca^{2+} currents through the NMDA receptor channels, and thereby modulate both the induction

and the maintenance of LTP (Mukherjee and Manahan-Vaughan 2013). By contrast, other mGluRs work to prevent LTP and promote long-term depression (LTD) (Gladding et al. 2009). This work, as with most LTP research, has primarily been conducted on hippocampal slices, more rarely on cortical slices, and only in a few cases in the spinal cord, specifically the dorsal column sensory pathways (Gerber et al. 2000). Since receptor expression can vary from one region of the CNS to another (Feragutti and Shigemoto 2006), it cannot be guaranteed that metabotropic receptors will have similar effects in the motor pathways of the spinal cord. A full discussion of the role of mGluRs in LTP is therefore not warranted in the context of this study, but is raised as an example of the complex nature of LTP in the CNS.

From the previously described mechanisms, it can be seen that in order to induce LTP, the post-synaptic cell membrane must already be activated when a new signal is received from the pre-synaptic cell. Only in these circumstances will the NMDA channel be able to respond to glutamate binding with a Ca^{2+} influx. We can consider three circumstances through which these conditions may be met.

The first circumstance involves the pre-synaptic axon firing at a high frequency. This is the scenario outlined in Figures 3 through 9 above. Bursts of high-frequency stimulation are termed tetanus, or tetanic stimulation. A first action potential causes glutamate release, which activates AMPA receptors. This in turn causes a depolarization of the cell, which ejects the Mg^{2+} ion from the NMDA channel. If a second action potential causes glutamate release before the Mg^{2+} ion has had time to resettle into the channel, it will

cause Ca^{2+} influx. In rodent visual cortex tissue samples, the relationship between stimulation frequency and LTP was determined to be non-linear. While frequencies above 20 Hz were found to induce LTP, lower frequencies produced no effect (at 0 Hz), or LTD, with the maximum LTD occurring at 1 Hz (Bear 1996). This relationship, which is outlined in Figure 10, is termed the BCM curve, after its discoverers (Elie Bienenstock, Leon Cooper, and Paul Munro) (Bienenstock et al. 1982). Later research found that this curve is itself subject to change, with repeated LTP induction increasing the frequency necessary to produce further potentiation. This effect is termed "homeostatic metaplasticity" (Ziemann and Siebner 2008). This circumstance is evoked during repetitive transcranial magnetic stimulation of the brain, which has been proposed as a neuroplasticity-based treatment for a variety of conditions (Fitzgerald et al. 2006, Belci et al. 2004).

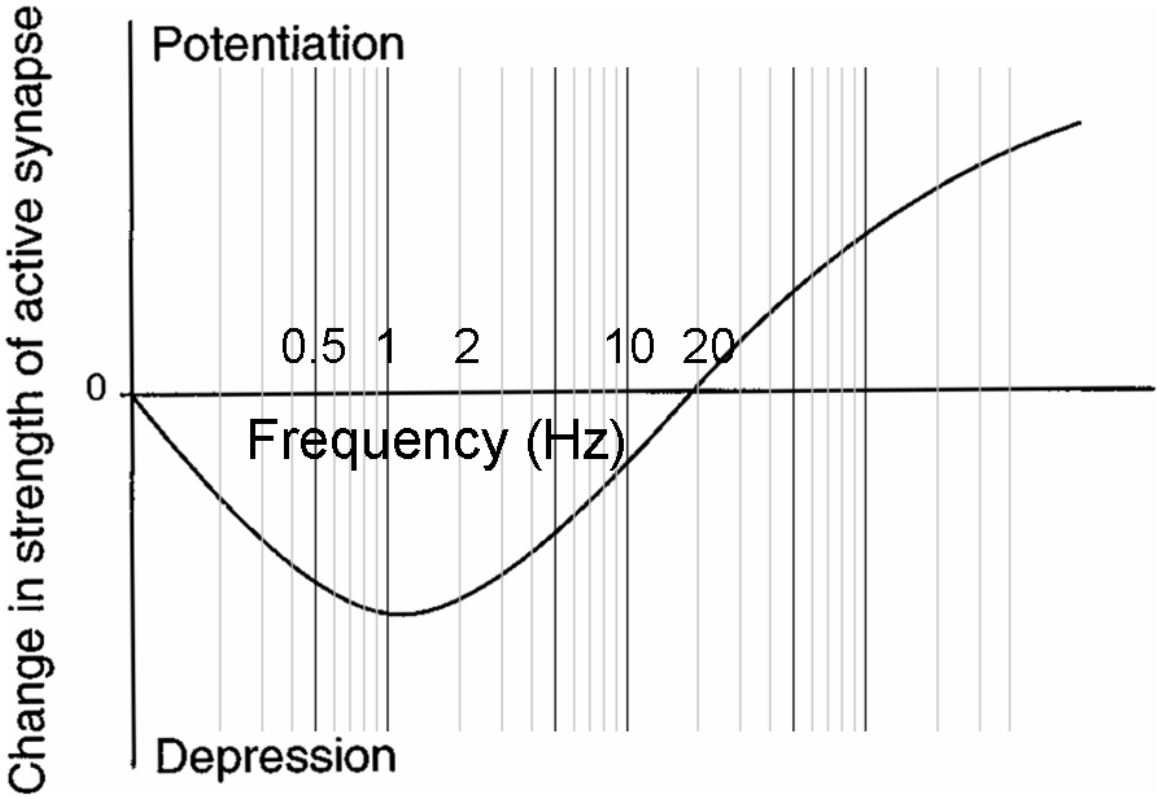

Figure 10. Outline of the Bienenstock, Cooper, Munro (BCM) curve. Repetitive stimulation of rat visual cortex produced long-term potentiation (LTP) only above 20 Hz. Peak long-term depression (LTD) occurred in response to 1 Hz stimulation. Modified from Bear (1996).

The second circumstance involves two or more adjacent axons synapsing onto the same part of the post-synaptic cell. The first cell to fire activates AMPA channels, and causes a depolarization which ejects the Mg^{2+} ion from the NMDA channel. If the second cell fires soon enough after the first, its glutamate release will occur before the Mg^{2+} has returned to the channel, and will cause Ca^{2+} influx. This mechanism allows the synapse to function as a sort of "coincidence detector". Tracts in which cells fire synchronously together will undergo upregulation. Strong evidence currently suggests that this

circumstance may be the neural basis for associative learning and memory, and may be essential in the formation of functional networks within the nervous system (Cain 1997, Rison and Stanton 1995). It was, in fact, in an effort to explain associative learning that Donald Hebb first proposed his model of neuroplasticity, and more modern research has served to validate his hypotheses. This principle was recently exploited in the development of an implantable device which induces neuroplasticity by recording activity in one part of the brain, and reproducing it in another part, thereby associatively linking the two (Jackson et al. 2006).

The final circumstance is one in which the conditions necessary for LTP are artificially induced. The post-synaptic cell may be activated through exogenous stimulation, after which subsequent activity in the pre-synaptic cell (whether natural or artificial) will be likely to result in a Ca^{2+} influx. It is this circumstance which will be examined herein as a means of upregulating the spinal synapse for potential therapeutic benefit. Both of the studies in this thesis (outlined separately in Parts II and III) employ various electrical stimulation modalities in order to try to artificially establish the conditions necessary for LTP.

Part II: Brain-Machine Interface Functional Electrical Stimulation Study

Chapter II.1: Introduction

II.1.1. Functional Electrical Stimulation and Its Control

Functional electrical stimulation (FES) describes the external application of electrical stimuli to a muscle or motor nerves, with the purpose of inducing functional muscle contractions (Popovic and Thrasher 2004). FES differs from other forms of electrical stimulation in that the user is typically not passively receiving the stimulation. Rather, they are using it to facilitate movements which they themselves are attempting to perform. This technique has long been proposed as a way of restoring motor function in individuals with neurologically-based motor disabilities, such as those caused by stroke or spinal cord injury (SCI). Debate still persists, however, about whether FES should be employed as a permanent prosthetic, or as a means of assisting physiotherapy and retraining voluntary function. Recent studies provide evidence to support the use of FES for therapeutic rehabilitation. The majority of these studies focus on stroke patients, and have generally had positive results (Everaert et al. 2010, Thrasher et al. 2008, Popovic et al. 2002, etc.). Information in SCI cases is much sparser and less clear. For example, Popovic et al. (2006) found that when FES was applied to high-level SCI patients to retrain grasping function, the functional measurements showed strongly positive perceptions from the FES users, and a clear trend towards functional improvement in the FES group, but without statistical significance. While more recent studies have been able to show further functional improvement (Popovic et al. 2011), there is still a need for its improvement.

Since FES must, by definition, be timed and coordinated so as to facilitate existing or attempted movements (as opposed to passive electrical stimulation which is simply left turned on), different control schemes exist, either by which a user may activate their own FES, or by which another individual may activate it for them. The simplest such strategy involves the use of external controls, such as push-buttons to switch between modes of stimulation, or potentiometers to control the level of stimulation. More advanced strategies can use sensors to control the stimulation. This is most common in walking aids, where pressure sensors or accelerometers may be used to determine different phases of gait, and stimulate at appropriate times. Finally, novel and experimental strategies can be explored, including the detection of muscle activity through electromyography (EMG), or the direct detection of a user's intention to move through brain recordings. It is these last two control strategies which will be discussed in more detailed below.

II.1.2. Brain-Machine Interface

Brain-machine interface (BMI) is another technique that is being developed to assist individuals with neurological disabilities. In general, a BMI records brain activity through one of several different modalities, then extracts or derives from these recordings a control signal, which can be applied to any number of assistive devices (Pfurtscheller et al. 1993). FES has been considered as a possible target for these control signals. This combination is ideal in cases of quadriplegia, such as a high-level SCI, since a quadriplegic patient would be unable to use conventional control strategies, such as push-buttons or EMG signals, to access and control their FES stimulation (most likely for upper-limb function). The feasibility of combining BMI and FES technologies in real-

time has been demonstrated in neurologically intact humans (Bentley et al 2008, Gollee et al. 2010, Tavella et al. 2010, Do et al. 2011), humans with SCI (Pfurtscheller et al. 2003, Muller-Putz et al. 2005, Muller-Putz et al. 2009), humans with stroke (Caria et al. 2010, Tan et al. 2010), and macaque monkeys (Moritz et al. 2008, Pohlmeyer et al. 2009, Ethier et al. 2012). These studies, however, focussed on the use of BMI-controlled FES as a permanent prosthetic. To date, only a handful of studies have investigated how a BMI-controlled FES system might be used as a tool for rehabilitation – these are discussed below in the "Critical Review" section.

II.1.3. Brain-Controlled Functional Electrical Stimulation

There is reason to believe that BMI-controlled FES (BMI-FES) may be superior to other forms of FES in terms of retraining motor function. The basis of this is the common theory that FES facilitates functional recovery by inducing neuroplasticity and a general reorganization of the nervous system (Rushton 2003). This is suggested to operate through classical Hebbian LTP, as was previously outlined (Baranyi and Feher 1981). Rushton (2003) suggests that FES may send electrical impulses back up the αMNs to the spinal cord (termed "antidromic firing", since they travel in a direction opposite to normal motor commands), which, if paired with a descending command from the brain (such as attempted voluntary muscle activation), should induce LTP-like plasticity at the spinal synapse. The necessity of descending commands for FES-induced neuroplasticity has been experimentally confirmed by Barsi et al. (2008). In current FES studies for SCI patients, however, the descending command from the brain is only indirectly involved. For example, the FES may be controlled by a push-button held by an accompanying

physiotherapist (Thrasher et al. 2008), which the physiotherapist presses if they notice the participant attempting a movement. LTP-like plasticity may therefore not be as strongly induced as it would be if descending commands from the brain were more directly involved. The potential advantage of controlling FES with a BMI is that descending commands from the brain can be more readily paired with the peripheral stimulation. The same pattern of neural activity should always induce peripheral stimulation, allowing for a greater induction of LTP-like plasticity between those two activities. In addition to the aforementioned studies which actually apply this theory, several publications have mentioned the theoretical plausibility of it in passing (Daly and Wolpaw 2008, Do et al. 2011, Tan et al. 2010). Note that, given this mechanism, recovery is most likely to be seen in cases of incomplete SCI, where some of the spinal tracts are spared, since it should only be through these tracts that descending commands from the brain may reach the level of the spinal synapse. However, some precedent exists to suggest that individuals with complete SCI may also undergo functional recovery as a result of FES therapy (Popovic et al. 2006), though the mechanisms behind this remain unclear.

It is worth noting that even BMI use alone, without electrical stimulation, has been shown to have neuroplastic effects (Shindo et al. 2011, Jarosiewicz et al. 2008, Ros et al. 2010), although contrary results exist (Ang et al. 2010). The principle behind this is suggested to be similar to biofeedback, in that visualization of one's own neural rhythms gradually allows a user to control them through subtle operant conditioning (Grosse-

Wentrup et al. 2011). For this reason, any proper investigation of the neuroplastic effects of BMI-controlled FES will also need to investigate the effects of BMI use itself.

II.1.3.1. Critical Review

To date, only 3 studies have examined how brain-controlled electrical stimulation may be able to induce improved neuroplasticity for the purposes of rehabilitation. These are summarized in Table 1.

Table 1. Summary of the literature concerning the use of brain-controlled functional electrical stimulation for inducing rehabilitative neuroplasticity.

Authors	Year	Muscle	Number of participants	Population	Treatment duration	Outcome measures
Daly et al.	2009	Finger extensor	1	Stroke	9 x 45 min.	Range of motion
Takahashi et al.	2011	Tibialis anterior	1	Stroke	30 min.	MVC, range of motion
Niazi et al.	2012	Tibialis anterior	16	Able-bodied	10-26 min.	MEP

The first of these (Daly et al. 2009) was a case study with a chronic stroke patient (10 months post-injury), who received 9 sessions, each lasting approximately 45 minutes, of BMI-FES for index finger extension over 3 weeks. No comparison or control group was presented, and the functional outcome measure of the study was limited to the observation that the participant had recovered some range of motion in the targeted finger (as reproduced in Figure 11). Furthermore, subsequent attempts to reproduce the results were unsuccessful in multiple subjects (Daly, personal communication). The interpretation of this study is therefore highly limited.

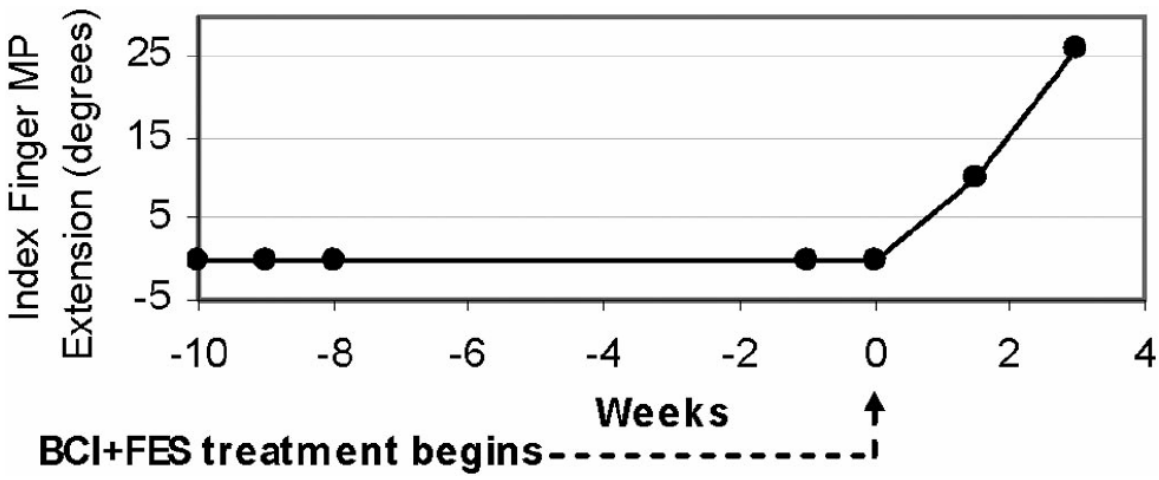

Figure 11. Reproduced from Daly et al. (2009). The figure indicates increased index finger range of motion shortly at the metacarpal phalangeal (MP) joint following the beginning of a BMI-FES training regime. The participant received 9 sessions of BMI-FES training, each approximately 45 minutes long, over 3 weeks.

A more recent case study was presented by Takahashi et al. (2012). The participant presented with chronic hemiparesis as the result of a brainstem stroke 2.4-2.8 years prior to the test sessions. Separate sessions, separated by 4 months, were used to determine the effect of the BMI-FES intervention and the effect of a standard FES intervention (in which stimulation was applied based on a predetermined pattern), with both interventions being approximately 30 minutes in duration. Both of these test sessions targeted the tibialis anterior (TA) muscle, and included pre- and post-intervention assessment. Unlike the aforementioned study by Daly et al. (2009), this study therefore represented short-term effects of the stimulation protocol (that is, immediately following the single treatment session) rather than persistent effects (that is, over the course of weeks following repeated treatment sessions). The BMI-FES was shown to result in a

significantly greater pre-to-post improvement in peak TA EMG than the predetermined FES treatment (approximately 7-fold as compared to 2-fold), and a significantly greater ankle range of motion (approximately 20 degrees as compared to 11 degrees). The EMG and range of motion figures from this paper are reproduced in Figure 12. While this study provides a comparison between BMI-FES and conventional FES, it is still limited by single-participant design and the low number of experimental sessions. Furthermore, the control group used does not properly represent the manner in which FES therapy is typically conducted. In this study, the stimulation is not in any way related to the participant's perceived voluntary effort. Barsi et al. (2008) have shown this type of passive stimulation to be less effective at inducing neuroplastic changes than stimulation which is controlled by a therapist to coincide with the recipient's attempted movements (such as that employed in recent clinical trials (Kapadia et al. 2011)).

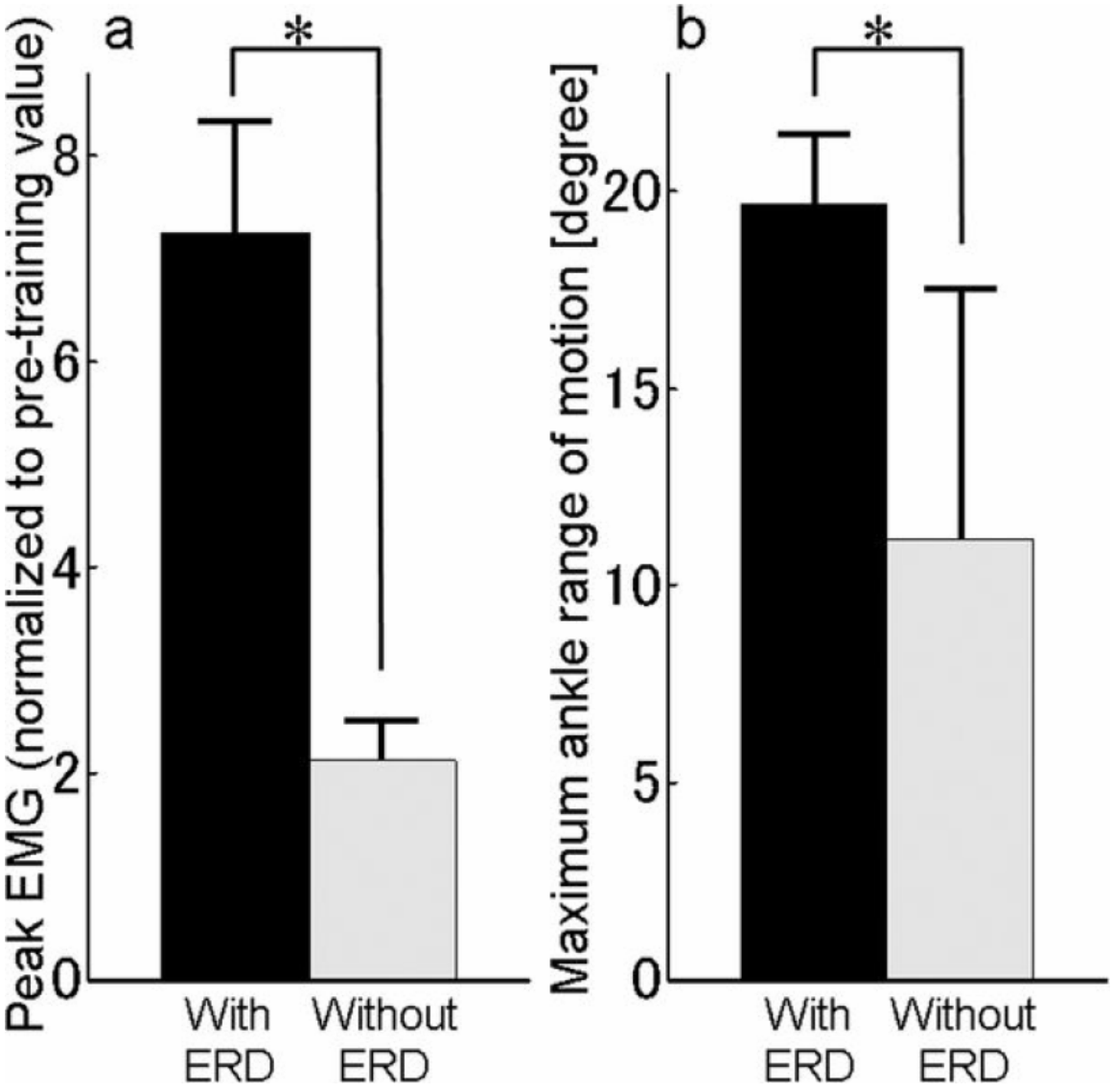

Figure 12. Reproduced from Takahashi et al. (2012). (a) shows increases in peak EMG, and (b) shows increases ankle range of motion, in brain-controlled FES, as compared to a pre-determined pattern of passive FES. “ERD” (event-related desynchronization) refers to BMI control strategy employed, in which FES is activated in response to a decrease in EEG bandpower from 24-26 Hz. The ERD and non-ERD interventions were tested in two separate sessions, separated by 4 months, with each intervention lasting approximately 30 minutes. Assessments were conducted immediately prior to an immediately following the intervention.

The final study (Niazi et al. 2012) involved a much greater sample size (n=16), but enrolled able-bodied participants rather than ones with neurological impairments. Like the study by Takahashi et al., it focussed on short-term effects by applying only a single brief session (averaging 26 minutes) of BMI-controlled TA stimulation, with pre- and post-intervention assessments. Two control treatments were also applied: self-paced motor imagery without feedback, and randomly-timed stimulation. The stimulation applied consisted of only single pulses, rather than the high-frequency trains used in FES. The primary outcome measure was motor evoked potential (MEP), which measures the EMG response to single pulses of transcranial magnetic stimulation (TMS) applied over the primary motor cortex, thereby representing the excitability of the CST. BMI-controlled stimulation was found to result in a significantly greater increase in the maximum TA MEP amplitude, as compared to either of the control groups (the figure representing these results is reproduced in Figure 13). While the larger sample size lends this study statistical power, it sacrifices applicability by using able-bodied participants. The choice of control groups also leaves certain confounding factors, namely the lack of feedback in the motor imagery condition (since someone receiving BMI-controlled stimulation is effectively receiving somatosensory biofeedback about their neural patterns), and the lack of volitional user intention in the random stimulation condition, (since conventional FES therapy involves pairing stimulation with volitional user intention (Kapadia et al. 2011), which has been shown by Barsi et al. (2008) to be essential for inducing neural plasticity). Furthermore, the stimulation applied, which uses only single pulses, is not representative of the high-frequency FES employed in recent

clinical trials (Kapadia et al. 2011). Lastly, a potential bias in these results comes from the duration of the BMI-controlled stimulation (an average of 26 minutes) exceeding that of the control interventions (approximately 10 minutes).

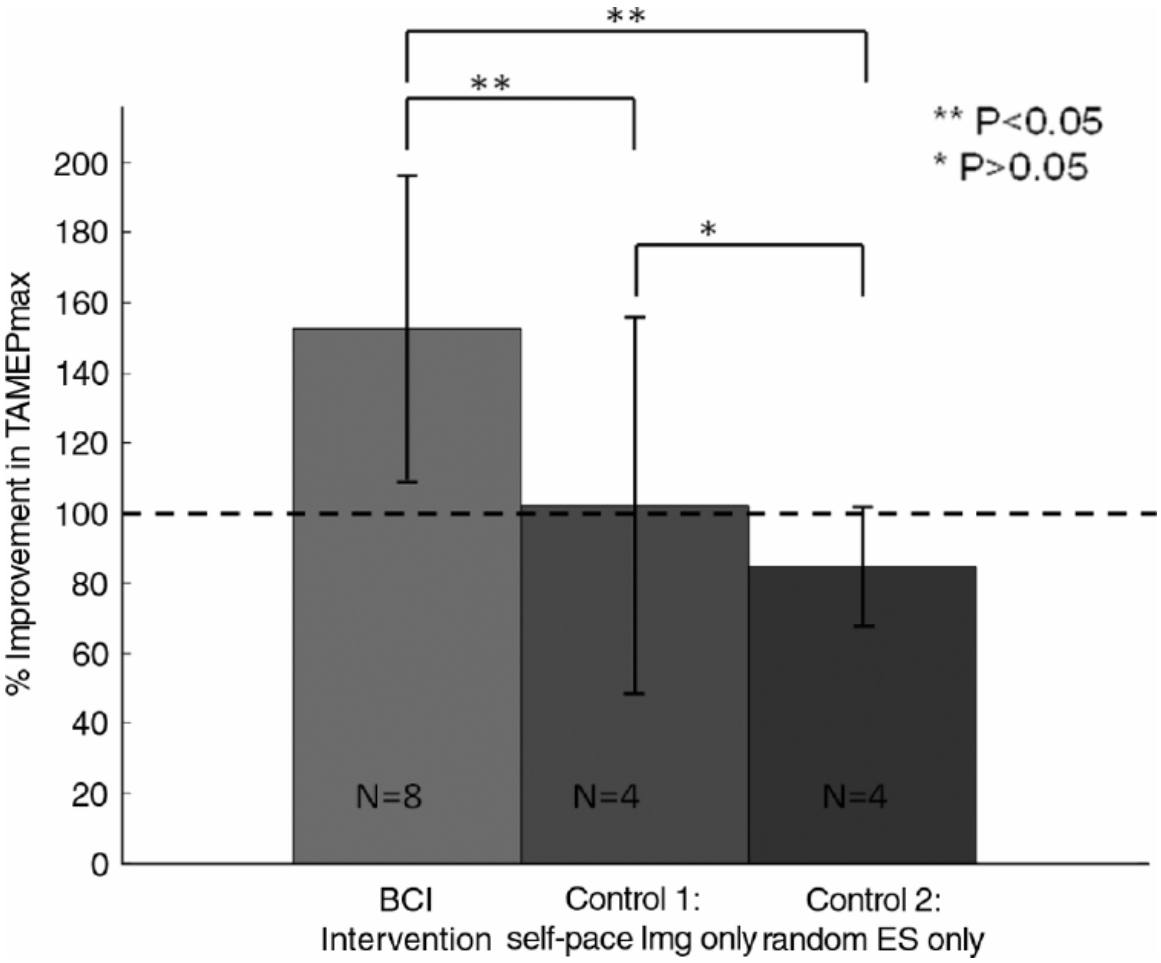

Figure 13. Reproduced from Niazi et al. (2012). Increased tibialis anterior motor evoked potential is indicated with the BCI (brain-computer interface, a term used interchangeably with BMI)-based stimulation paradigm, as compared to either of two control groups. The intervention lasted an average of 26 minutes, while the duration of the control tests is not specified, but is estimated at approximately 10 minutes.

II.1.3.2. Summary

Taken as a whole, the BMI-controlled FES literature can be considered promising but insufficient. The key problems stem from small sample sizes (single case studies in 2 of the 3 aforementioned studies), lack of reproducibility, inappropriate control groups, and a focus on short-term effects, rather than persistent changes. While it is worthwhile to investigate the effects of these treatments in stroke populations, the SCI population has gone unattended to in this field. While a long-term study on individuals with SCI would be considered ideal, and was initially proposed for this thesis, that approach is now considered impractical, given difficulties in recruiting individuals with SCI. Given the aforementioned limitations of the field at present, and within the framework of a short-term study, key points of focus for the BMI-FES studies included herein will be, using appropriate control groups and investigating a significant sample size of individuals with SCI .

II.1.4. Muscle-Controlled Functional Electrical Stimulation

A similar suggestion can be made about the use of EMG to control FES (EMG-FES). In the same way that changes in the EEG activity in primary motor cortex indicate that descending commands are being sent, EMG activity indicates that these descending commands have reached the muscles. Each muscle contraction can therefore be taken as a clear indicator that a descending command has taken place. Using EMG activity to trigger FES may therefore ensure that the antidromic firing reaches that spinal synapse at a time when descending commands are coming from the brain, ensuring a proper contingency for Hebbian plasticity.

The chief advantage of using EMG for this purpose rather than a BMI is that EMG signals are much clearer and less ambiguous – that is, they have a higher signal-to-noise ratio. Whereas most BMI systems require complex signal processing in order to find discernable events, and typically require extended training periods to allow the user's brain to learn the skill of neuromodulation, periods of activity and inactivity in EMG signals from able-bodied users can often be discerned even by simple visual inspection (this is, in at least some cases, considered the "gold standard" of EMG event detection (Vaisman et al. 2010)).

One disadvantage of EMG-based systems is that small amounts of volitional command from the brain need to actually reach the target muscles. This means that this approach is only truly appropriate for individuals with incomplete SCI who have partial control of the targeted muscles, rather than individuals with a complete SCI or incomplete SCI who have no control of the targeted muscles, since those incomplete injuries spare some tracts and can result in diminished, rather than abolished muscle contractions. As mentioned above, however, at least some descending signals must cross the lesion site in both conventional FES therapy and BMI-FES therapy in order to induce plasticity, although these need not necessarily be strong enough to induce voluntary muscle activity.

Another disadvantage is that EMG signals will only be detected after the descending command has already passed across the spinal synapse. If we conceive of both descending commands and antidromic firing as being single pulses of activity, this means

that the two will not meet at the synapse, or anywhere else. However, neither descending commands nor FES are truly single pulses, but rather, sustained, high-frequency trains. An EMG-based system could therefore detect the earliest part of this train and provide stimulation for most of its duration. While not perfect, this protocol would ensure that the contingency would still be properly trained a majority of the time.

II.1.4.1. Critical Review

On the whole, the field of EMG-FES is much more well-developed than that of BMI-FES. A considerable number of EMG-FES systems have been designed, implemented, and even commercialized (such as the Zynex NeuroMove [http://www.zynexneuro.com/neuromove]), with the intention of using FES to assist movements when users are able to exert partial muscle contraction (Saxena et al. 1995, Thorsen et al. 2001, Chiou et al. 2008, etc.). However, as with the BMI-FES literature, the emphasis is largely on the use of everyday use of such systems, in the manner of an orthosis or assistive device, rather than their potential use as therapeutic aids. When the therapeutic potential of EMG-FES is investigated, it is compared to a non-FES condition such as standard physiotherapy (Cauraugh et al. 2002, Cauraugh and Kim 2000, Francisco et al. 1998, Hara et al. 2008, Hummelsheim et al. 1996). Benefits seen in these studies may therefore simply be that seen with all FES, and no clear statement can yet be made about what potential effect the control scheme itself may or may not be having.

II.1.5. Objectives

The primary objective of the investigations into brain-controlled and muscle-controlled electrical stimulation is to compare these two treatments to conventional FES control strategies, in terms of their ability to induce neuroplastic changes. A secondary objective is to furthermore compare these treatments to additional controls in an effort to determine if any particular aspect of the treatments are responsible for any observed effects, or whether the components of the treatment are synergistic. This study will be termed the "brain-machine interface functional electrical stimulation" (BMI-FES) study.

Chapter II.2: Hypotheses

- Participants are hypothesized to have improved grip force and maximum voluntary contraction (MVC) in the abductor pollicis brevis and/or flexor pollicis brevis (for brevity's sake, this will be abbreviated as APB) muscles, following one hour of electrical stimulation to those muscles, or neurofeedback from the primary motor cortex area relevant to the control of APB muscles.

- These improvements are hypothesized to be greater when the stimulation is controlled by electrical measures that indicate voluntary attempts at motor performance (either change in the electroencephalogram (EEG) activity over primary motor cortex (M1) or EMG activity over the APB muscle).

- These improvements are hypothesized to coincide with alterations in the APB MEP, either in terms of a lowered motor threshold, lowered stimulation intensity required to elicit a half-maximal contraction, or an increased maximal contraction.

Chapter II.3: Methods

II.3.1. General

All procedures presented herein were approved by the Research Ethics Boards of the University of Toronto and the University Health Network, Toronto Rehabilitation Institute. All procedures were conducted in an electromagnetically shielded room (Raymond EMC, Canada).

II.3.2. Participant Population

The BMI-FES study sought to enrol able-bodied participants. Volunteers were subject to the following inclusion/exclusion criteria:

Inclusion criteria:

- 18 to 85 years of age
- Participants must have a clear understanding of the goal of the study, which is to examine the use of brain-controlled functional electrical stimulation therapy for the upper limb and its possible effects on functional recovery (which may or may not enhance recovery).
- Able to understand English

Exclusion criteria:

- Participants must not have any contraindications for FES. These include:
  - cardiac pacemakers
  - skin lesions, allergy, wound or rash at potential electrode sites

o pressure ulcers

o cardiovascular conditions such as hypertension that is uncontrolled

- Participants must not have a history of stroke, multiple sclerosis, amyotrophic lateral sclerosis Parkinson's disease, Alzheimer's disease, Huntington's disease, brain injury, or spinal cord injury
- Participants must not have a previous seizure, epilepsy, or a history of seizure or epilepsy in any first-degree relatives.
- Participants must not have had a previous arm surgery
- Participants must not be taking any medications which lower the seizure threshold (these include: antiasthmatics, antibiotics, antidepressants, insulin, estrogen, stimulants, neuroleptics, antihistamines, baclofen, lithium, and oxytocin).
- Participants must not have an allergic reaction to alcohol.
- Participants must not have aneurysm clips.
- Participants must not have heart or vascular clips.
- Participants must not have prosthetic valves.
- Participants must not have any intracranial metal prostheses or metal in the head.
- Participants must not be pregnant or be unsure of whether or not they may be pregnant.
- Participants must not have tinnitus.
- Participants must not have bipolar disorder.
- Participants must not have increased intracranial pressure or a history thereof.

II.3.3. Overall Study Design

The overall study design is outlined in Figure 14 below.

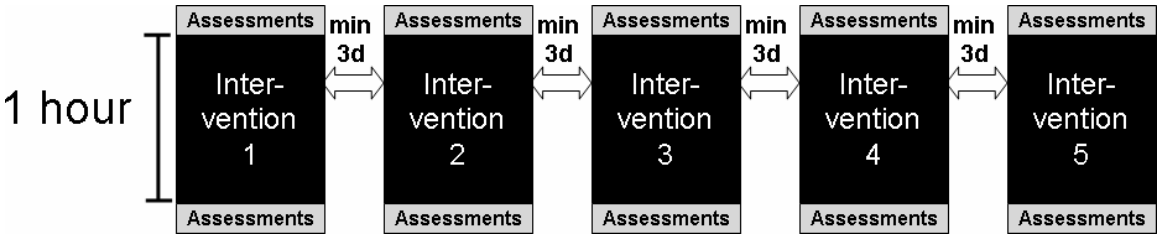

Figure 14. Overall design of the BMI-FES study for each participant. 5 interventions were applied, each for one hour, with assessments both pre- and post-intervention. Each session was separated from the others by a minimum of 3 days.

Each participant took part in 5 sessions, each of which was used to test one of the 5 interventions, which are described in the "Intervention/Treatment" section below. Since each participant was undergoing each of the 5 interventions, this was a within-subjects design. Each session was separated from the others by a minimum of 3 days in order to minimize carryover effects from one session to the next. Evidence to suggest that this period was sufficient to allow for the washout of any induced neuroplasticity comes from studies which showed MEP upregulation following a protocol of paired peripheral nerve stimulation and TMS (similar to the protocol applied in the study described in §III). These studies showed that MEP values returned to baseline between 1.5 and 24 hours after the cessation of treatment (Stefan et al. 2000, Wolters et al. 2003, Stefan et al. 2006). In all cases, both interventions and assessments pertained to the non-dominant hand (in able-bodied individuals) or the more-impaired hand (in individuals with SCI).

II.3.4. Intervention/Treatment

Each session's intervention involved 1 hour of grasping and manipulating small objects. The duration of this training was primarily chosen so as to mimic a single session of the FES training conducted with individuals with SCI in current clinical trials of FES therapy (Kapadia et al. 2011). The suggestion that this duration should be sufficient to induce detectable neuroplasticity comes from literature showing that as little as 10 minutes of FES therapy can produce significant changes in MEP in able-bodied participants (Stein et al. 2013). The 5 different interventions dictated the manner in which grasping was performed and the devices used during the session. The order in which the 5 interventions were applied (in separate sessions) was randomized for each participant, with the exception that the first session did not involve the brain-machine interface (since it will need to be calibrated at the end of the first session). Details of the individual components of the treatments are provided in several sections below ("FES Therapy", "BMI System", and "EMG System"). A graphical representation of the groups is provided in Figure 15, and the experimental setups used in each group are shown in Figure 16.

Grasp	Control Method: Voluntary	Control Method: EEG	Control Method: EMG
Voluntary	VOL	BMI	
FES	FES	BMI-FES	EMG-FES

Figure 15. The 5 intervention groups in the BMI-FES study. The groups are "voluntary" (VOL), "functional electrical stimulation only" (FES), "brain-machine interface only" (BMI), "brain-machine interface-controlled functional electrical stimulation" (BMI-FES), and "electromyography-controlled functional electrical stimulation" (EMG-FES). Vertical categories indicate the manner of grasp production, and horizontal categories indicate the strategy by which this grasping was controlled. EMG control with voluntary grasp was not investigated, as it was not considered meaningful. Full descriptions are found in §II.3.4. Each participant was tested with each of the 5 interventions, with experimental session per intervention.

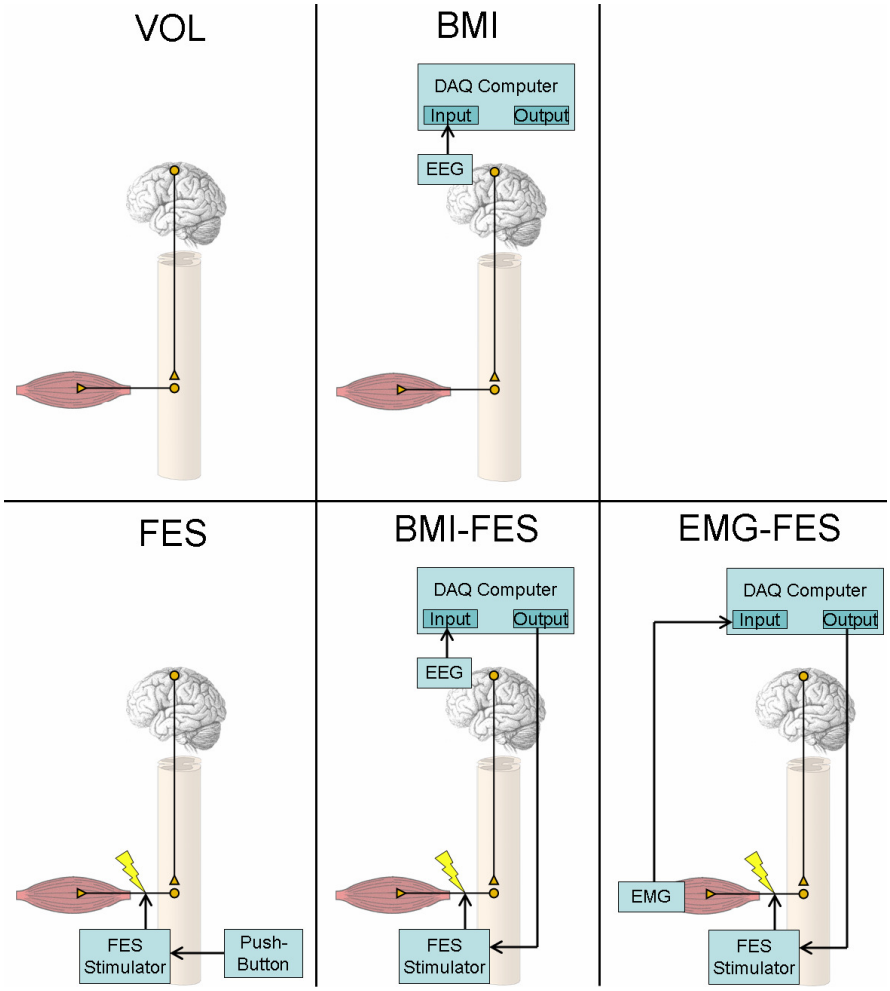

Figure 16. Experimental setups used in each of the five treatments. The groups are "voluntary" (VOL), "functional electrical stimulation only" (FES), "brain-machine interface only" (BMI), "brain-machine interface-controlled functional electrical stimulation" (BMI-FES), and "electromyography-controlled functional electrical stimulation" (EMG-FES). Components include the data acquisition (DAQ) computer (which records physiological signals and provides control signals), the electroencephalography (EEG) electrodes and amplifier (which record brain activity), the electromyography (EMG) electrodes and amplifier (which record muscle activity), the functional electrical stimulation (FES) unit (which provides peripheral nerve stimulation), and a push button (which directly controls the FES stimulator).

In the “voluntary” (VOL) intervention, grasping was performed as normal, without assistance.

In the “functional electrical stimulation only” (FES) condition, the participant was able to begin performing a grasp (for example, squeezing lightly on the block), but could only complete it (for example, manipulating the block or lifting it against gravity) with the aid of FES, which the experimenter activated via a push-button.

In the “brain-machine interface only” (BMI) condition, the participant was able to begin performing a grasp, but was asked to only complete the full grasp when a non-invasive, EEG-based BMI predicted that they were attempting to perform a grasp (as relayed to them with visual feedback on the monitor of the controlling computer).

In the “brain-machine interface-controlled functional electrical stimulation” (BMI-FES) condition, the participant was able to begin performing a grasp, but could only complete it with the aid of FES, which was activated when an EEG-based BMI predicted that they are attempting to perform a grasp.

In the “electromyography-controlled functional electrical stimulation” (EMG-FES) condition, the participant was able to begin performing a grasp, but could only complete it with the aid of FES, which was activated when EMG electrodes detected this preliminary muscle activation.

II.3.5. Electromyogram Recording

EMG recording in this study served two purposes. First, in the EMG-FES condition, it was used to control the onset of FES (as outlined in the "EMG-FES System" section below). Second, it was used in the three of the assessments (motor evoked potential, maximum voluntary contraction, and matched forces, as outlined in their respective sections below).

At the start of each session, recording electrodes (Kendall Meditrace 200 Ag/AgCl foam electrode, Covidien, USA) were attached over the FCR and APB muscles of both arms, in accordance with locations outlined by Zehr (2002). For FCR, two electrodes were placed along a line from the medial epicondyle of the humerus to the radial styloid, with the center point between these two electrodes being approximately one-third of this distance (closer to the medial epicondyle). This location was confirmed by palpating the participant's arm while asking them to voluntarily flex their wrist, and identifying muscle movements. For APB, one electrode was placed on the muscle belly, while the other was placed on the side of the first metacarpal (note that we are not able to properly discriminate between APB and flexor policis brevis due to their small size and near location, and are therefore referring to them collective as APB for the sake of brevity). The ground electrode was placed on the lateral epicondyle of the left humerus. Signals were amplified with a Bortec AMT-8 amplifier (Bortec Biomedical, Canada). Unless otherwise stated, the FCR recordings were amplified at a gain of 5000, and the APB recordings were amplified at a gain of 1000. The amplified signals were then recorded using a data acquisition (DAQ) board (National Instruments, USA) connected to a PC.

The programs used for recording were all custom LabView scripts, and varied from one application to the next.

II.3.6. Functional Electrical Stimulation Therapy

Self-adhesive electrodes (StimTrode ST5050, Axelgaard, USA), coated with a conductive gel (Spectra 360, Parker Laboratories, USA), and a Compex Motion stimulator (Compex, Switzerland) were used to stimulate the following muscles: flexor carpi radialis (FCR, for wrist/finger flexion), abductor pollicis brevis / flexor pollicis brevis (APB, for thumb flexion), and extensor digitorum (for wrist/finger extension). Stimulation pulses were biphasic, and were applied at a frequency of 40 Hz. The pulse width from increased from 0 to 250 µs when stimulation turned on, and decreased back to 0 µs when it turned off. Stimulation amplitudes (that is, currents) varied by session, participant, and muscle, and were determined in such a way as to provide functionally relevant contraction, to the extent that it was considered tolerable by the participants. The amplitude ranges were 8-19 mA for FCR (mean 12.48 ± 2.67), 3-10 mA for APB (mean 6.93 ± 2.09), and 9-19 mA for extensor digitorum (mean 13.86 ± 2.52). The FES was turned on when triggered by whichever control modality was being used (push-button, BMI, or EMG). In the case of BMI- and EMG-controlled FES, this involved passing a 5 ms, 2 V pulse to an input port on the stimulator, through an optical isolator (Q403, Action Instruments, USA), in order to prevent ground loops. In the case of the push-button control, the button (Jelly Bean Twist, Technical Solutions, Australia) was connected directly to the stimulator. Stimulation in the flexors was ramped up over 1 s and held until the experimenter manually sent the next signal. The command signal at

this point was sent manually (rather than through BMI or EMG control), since both EEG and EMG recordings were subject to large artifacts during FES application, caused by the applied current travelling through the user's skin to the recording site. Following this manual signal, the stimulation in the flexors ramped down over 0.5 s. Stimulation in the extensors began ramping up at the same time as the flexors began ramping down. This consisted of a 1 s ramp up, 2 s of constant intensity, and a ramp down over 0.5 s. The system then remained at rest with no stimulation until the next trigger signal was manually sent.

II.3.7. Brain-Machine Interface / Brain-Machine Interface Functional Electrical Stimulation

In sessions using the BMI, the participant's skin was prepared at two EEG locations, as identified with the 10-20 system: either at C_1 and C_5 (for right-arm grasping) or at C_2 and C_6 (for left-arm grasping). The locations were intended to be on either side of the section of M1 responsible for hand and wrist function. Skin preparation involved first abrading the skin with Nuprep skin prep gel (Weaver and Company, USA), then cleaning the skin with an alcohol swab (HealthCare Plus, Canadian Custom Packaging, Canada).Two Grass F-E5GH gold disc electrodes (Astro-Med, Canada) were then affixed to these two sites with Ten20 conductive EEG paste (Weaver and Company, USA). A third, clip-style electrode (Grass F-E34DG, Astro-Med, Canada) was attached to the contralateral ear lobe to act as a ground, again using the Ten20 conductive paste. All three electrodes were connected to a single cable (Grass F-P5IC3/REV1, Astro-Med, Canada), such that the recorded signals represented the difference in potentials between the two recording

electrodes. This signal was conveyed to a Grass IP511 AC amplifier (Astro-Med, Canada), where the signal was bandpass filtered from 10-30 Hz, and amplified with a gain of 20,000. These EEG recordings were sent, in real-time, through a DAQ board into a computer. A Labview program took these recordings, bandpass filtered them within a given frequency band, determined the power of that band (termed the "bandpower"), and took the average of this power and a specified number of immediately previous bandpowers. If this average was on one side of the specified threshold, it was considered a "hit". When only "hits" are consecutively achieved for a specified duration, a short voltage pulse was sent through the DAQ into the stimulator, where it was interpreted as a command to begin the pre-set program of stimulation (as outlined in the "FES Therapy" section above). In order to generate appropriate activity in the signal, subjects were asked to attempt the performance of a grasping motion when they wanted to receive flexion-inducing FES.

II.3.7.1. Initial System Calibration and Parameter Selection

In order to determine what values to use for the various parameters within the Labview program, a calibration routine was implemented in the first session (this is why the randomized order of treatment conditions was not allowed to require BMI control in the first session). The routine obtained two recordings of 10 seconds each, one while the subject was at rest, and one while the subject was attempting a grasping motion. The program then performed an analysis based on receiver operating characteristic (ROC) curves, which were based on a simulation of the same procedure that would be applied in

real-time during the actual training sessions. This analysis tested different values for the following parameters within the system, with possible values indicated in brackets:

- Frequency Range: from which to derive bandpowers (10-12, 11-13, 12-14, 13-15, 14-16, 16-20, 18-22, 20-24, or 24-32 Hz) (only one range is employed at a time)
- Bandpower Window: duration of the time window over which bandpower is determined (0.25, 0.5, 1, or 2 s)
- Mean Window: number of previous bandpowers to include in the final mean value (1, 3, 5, 10, 15, or 20)
- Increase/Decrease: whether the mean value needs to be above or below the threshold to be counted as a "hit" ("increase" or "decrease").
- Required Duration: number of "hits" required to cause a switch in the stimulation pattern (1, 3, 5, 10, 15, or 20)

For each set of parameter values, a range of thresholds was also tested, and the resulting number of true positive, false positive, true negative, and false negative time points for each threshold was determined. However, the results from all threshold values within a given set of parameter values were used to determine a single value for that set of parameter values, which indicated its ability to determine between resting and active states. The set of parameter values that yielded the greatest discriminative ability was then used for the training sessions. These parameters were saved in a text file, which could be reloaded in future sessions. In this way, it was hoped that the same neural function would be trained in each session, allowing for the strongest associative pairing

between the neural activity and the peripheral stimulation, and thereby, the greatest neuroplasticity and functional benefit.

The threshold value of the system was recalibrated for each separate session that involved brain-machine interface. This procedure began with the same ten second recordings of the user's resting and active mental states. The threshold that provided the greatest relative ratio of true results to false results was selected for that session. The chief difference between this and the initial, full calibration was that while the initial calibration required all of the parameter values to be determined, the daily recalibration of the threshold simply loaded the parameter values that were previously save in the text file, and only the range of different thresholds was tested. This recalibration of the thresholds was intended to account for possible differences between days caused by reapplication of the electrodes. Note that while the initial calibration required approximately 16.5 hours to run (to account for all of the different parameter value combinations that must be tested), subsequent recalibrations, in which only the threshold was adjusted, required processing time on the order of seconds.

The program also incorporated an option to test the user's level of volitional control over the BMI. In this mode, the user was given textual cues which alternated between "Relaxed" and "Active". The duration of each command was pseudorandom, on the range of 7-13 s, balanced such that equal time was devoted to both states. During the "Active" periods, subjects were asked to attempt a grasping movement. The program simultaneously analyzed the incoming EEG patterns, classifying them as "Resting"

(corresponding to extension/rest in the FES) or "Active" (corresponding to flexion in the FES). An accuracy was then derived from this, with chance predictions representing an accuracy of 50%. A p-value for this accuracy was also derived based on a binomial distribution. This test was administered at the beginning and end of each session in which BMI control is employed. The purpose of obtaining this data was to be able to determine whether or not there was any correlation between the user's level of volitional BMI control and any changes observed in the main assessments (MEP, grip strength, matched forces ratio, and MVC).

II.3.8. Electromyogram Functional Electrical Stimulation System

The EMG system was designed around the same principle as the BMI – that is, it is a Labview program in which a criterion value must cross a threshold for a given length of time (measured as a number of "hits", although in practice, this value was kept at a constant value of one "hit") in order to begin stimulation. Since EMG recordings were affected by stimulation artifacts in the same way that EEG recordings were, stimulation was again required to be turned off manually by the experimenter. The program contained an optional bandpass filter in order to smooth the obtained signals – this was, however, deemed unnecessary upon investigation of most of the signals. Following this optional filtering, the signal was rectified (squared), after which the criterion value was derived. The potential criterion values employed were different than those used in the BMI system, owing to the much simpler nature of analyzing EMG signals. The program may be guided by the mean, variance, or range of the rectified signal. Preliminary experiments suggested that these three signals are largely interchangeable, with all three

providing highly responsive control when employed by an able-bodied user. The range value was therefore selected as the criterion for all participants. The threshold was be chosen manually by asking the user to perform several voluntary contractions and observing the resultant change in the chosen criterion function. A more sophisticated and reproducible calibration was not considered necessary, due to the relatively clear nature of EMG signals.

II.3.9. Assessments

Each session began with a battery of assessments, consisting of MEPs, grip force, and MVC.

II.3.9.1. Motor Evoked Potential

II.3.9.1.1. Theory

In general, the MEP procedure involved providing a number of TMS pulses to the participant's M1. These pulses created neural signals that were carried to the relevant muscles (in this case, APB), causing a contraction. These contractions were then registered through EMG. An increased EMG response for a given level of TMS stimulation is to be taken as an indication of improved efficiency in the corticospinal tract, and evidence of neuroplasticity. This may, however, happen as a result of increased sensitivity (a lowered threshold or a leftward shift in the recruitment curve, either of which would indicate increased synaptic efficiency), or as a result of an increase in the maximum evoked potential (which would indicate a greater number of axons contributing to the elicited contraction) (Knash et al. 2003). Therefore, a full recruitment

curve was tested, rather than simply one predefined stimulation intensity. The suggestion that changes in MEP may indicate potential for functional recovery is supported by the theoretical nature of the MEP, by the finding that MEP amplitude and grip strength are correlated in stroke patients (Thickbroom et al. 2002), and by the repeated findings that treatments which upregulate MEP, such as FES (Everaert et al. 2010) and paired associative stimulation (Wolters et al. 2003), can also cause long-term functional benefits (Popovic et al. 2011, Uy et al. 2003, respectively). However, some evidence to the contrary exists, such as Stein et al. (2013), in which a variety of treatments which initially caused neuroplasticity in able-bodied participants failed to provide similar results in participants with various neurological conditions. These assumptions will be more thoroughly explored in the Discussion (§II.5.7, §IV.1).

While MEP is typically portrayed as being a direct activation of the UMNs, and therefore a straight-forward representation of the connections within the CST, the underlying physiology is in fact more complicated. A given MEP response, while appearing unitary, is in fact that sum of several component waveforms, which temporally overlap. These include the direct wave (in which UMNs are activated by the stimulation itself), and several indirect waves (in which nearby neurons are activated, which in turn activate UMNs through any number of intracortical synapses) (Rothwell 1997). An increased MEP amplitude can therefore be interpreted either as an upregulation of the CST itself (as intended), an upregulation of the intracortical networks, or a synchronization of the different indirect waves. Caution must therefore be utilized to ensure proper interpretation of the MEP-based results.

II.3.9.1.2. Specific Methodology

MEP was elicited with a Medtronic MagPro R30+M stimulator with a MagVenture MCF-B65 figure-eight coil (Medtronic, USA). The coil was initially placed with its center point over C_4 on the 10-20 grid, which was intended to approximate the hand/arm area of the right M1 (or C_3 in the event of a left-handed participant). The coil was held with the handle at a 45° angle pointing in the posterior-lateral direction. This orientation was intended to produce a posterior-to-anterior current within the M1, while compensating for the angle of the central sulcus. From the initial position, the coil was moved while providing TMS pulses at approximately 70% maximum stimulator output (MSO). The resulting MEPs were visualized, and the location was selected which consistently produced the highest-amplitude MEP response. This was typically found to be anterior and medial to the starting location. Keeping the coil in this location, the stimulation amplitude was then decreased until a level was found where visible MEPs were elicited by approximately half of the stimulations. This intensity was taken to be the participant's motor threshold.

Following this, MEPs were evoked and recorded at a range of stimulation intensities, each separated by 10% of the MSO, with 10 stimulations being applied at each intensity. The order in which the intensities were presented was kept constant, but followed a pre-generated pseudo-random pattern, excluding intensities which were more than approximately 7% below the participant's motor threshold. This was done to prevent hysteresis effects which can skew the response curve (Moller et al 2009). The order in

which intensities were presented was as follows: 60, 70, 40, 90, 50, 80, 30, and 100% of the MSO.

The recorded EMG traces were later analyzed offline in accordance with the methods of Devanne et al. (1997) and Knash et al. (2003). First, recordings were truncated to exclude any time point earlier than 5 ms post-stimulation, in order to remove the stimulation artifact. Then, the peak-to-peak amplitude of the remaining recording was derived, as was the mean amplitude for a given stimulation intensity. The mean response amplitudes were plotted against the stimulation intensity used to elicit them. From this, the value "M" was defined as the maximum mean response obtained. The value "H" was subsequently determined by first defining a half-maximal response as ([M - b] / 2) + b, where b is the background activity in the absence of any stimulation (determined as the lowest mean response obtained)), then finding adjacent stimulation intensities in which the lower intensity produced a mean response of less than the half-maximal response, and the higher intensity produced a mean response of more than the half-maximal response, and lastly performing linear interpolation between these two mean responses in order to find the point at which the interpolated line intersects the half-maximal response. Note that the original methodology, as defined by Devanne et al. (1997), involved fitting a sigmoid curve over the response curve. However, it was determined to be unnecessary for the purposes of this study to derive the entire curve – only "M", "H", and "MEP_H" (the value which will be explained in the remainder of this section) were required. These values are outlined in Figures 17 and 18.

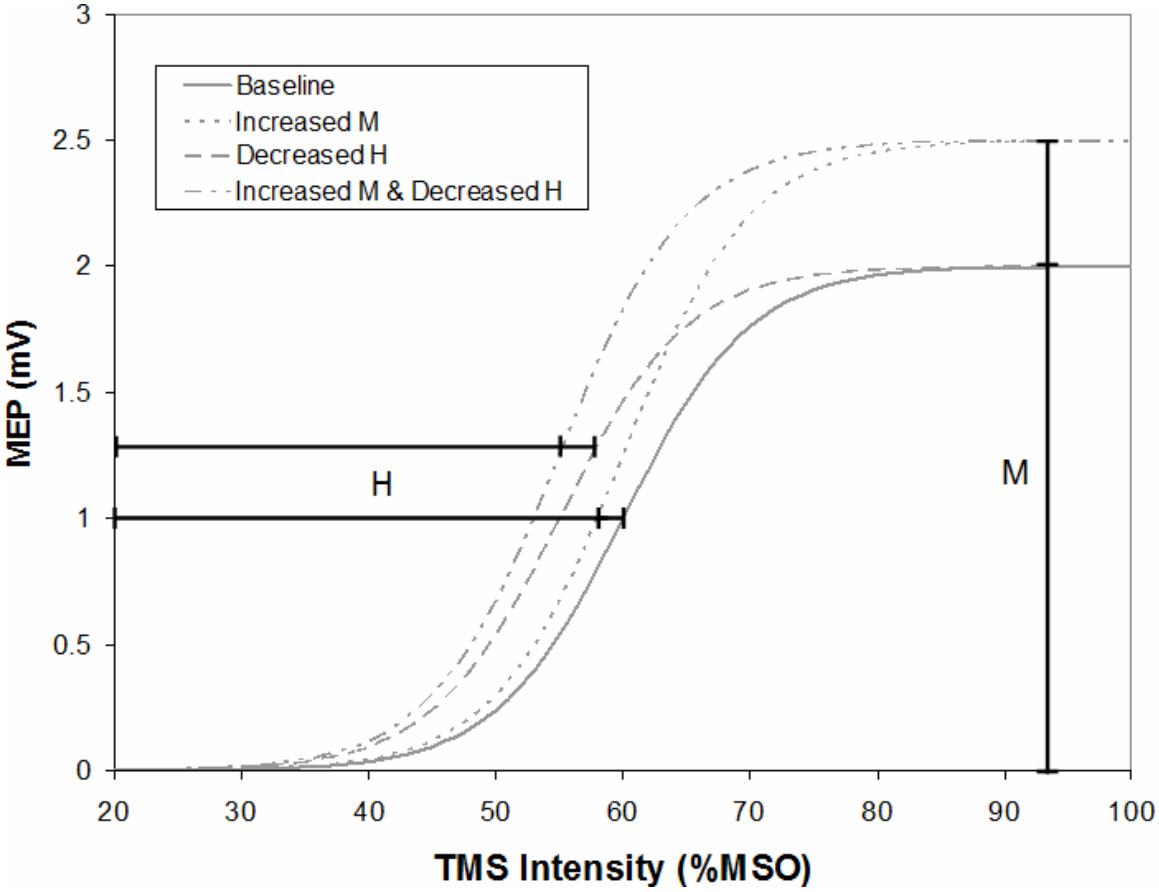

Figure 17. Outline of the "M" and "H" values derived from the MEP recruitment curve, illustrated using simulated data. The solid gray line represents a pre-intervention curve, while the dashed gray lines illustrate hypothetical post-intervention curves in which either the maximum response amplitude ("M") has been increased by 0.5 mV, in which the stimulus intensity which results in a half-maximal response ("H") has been decreased by 5% MSO, or in which both changes have occurred. It can be seen from this representation that changes in M can cause changes in H, but that changes in H need not result in changes in M. Note also that the response level which is defined as "half-maximal" changes with the most current value of "M", rather than being fixed to the pre-intervention values, hence the two separate horizontal lines (one for M = 2.0 mV, and one for M = 2.5 mV).

A third value, termed "MEP_H", was determined only after both pre- and post-intervention values for "H" and "M" had been determined. It is intended to represent the post-intervention amplitude of an MEP elicited by a TMS pulse at the intensity defined as "H" pre-intervention. Since this exact intensity falls between two of the actual intensities tested, linear interpolation was used to estimate the post-intervention response. The pre-intervention response was defined as ([M – b] / 2) + b, as outlined earlier, and did not need to be separately calculated. This value is outlined in Figure 18.

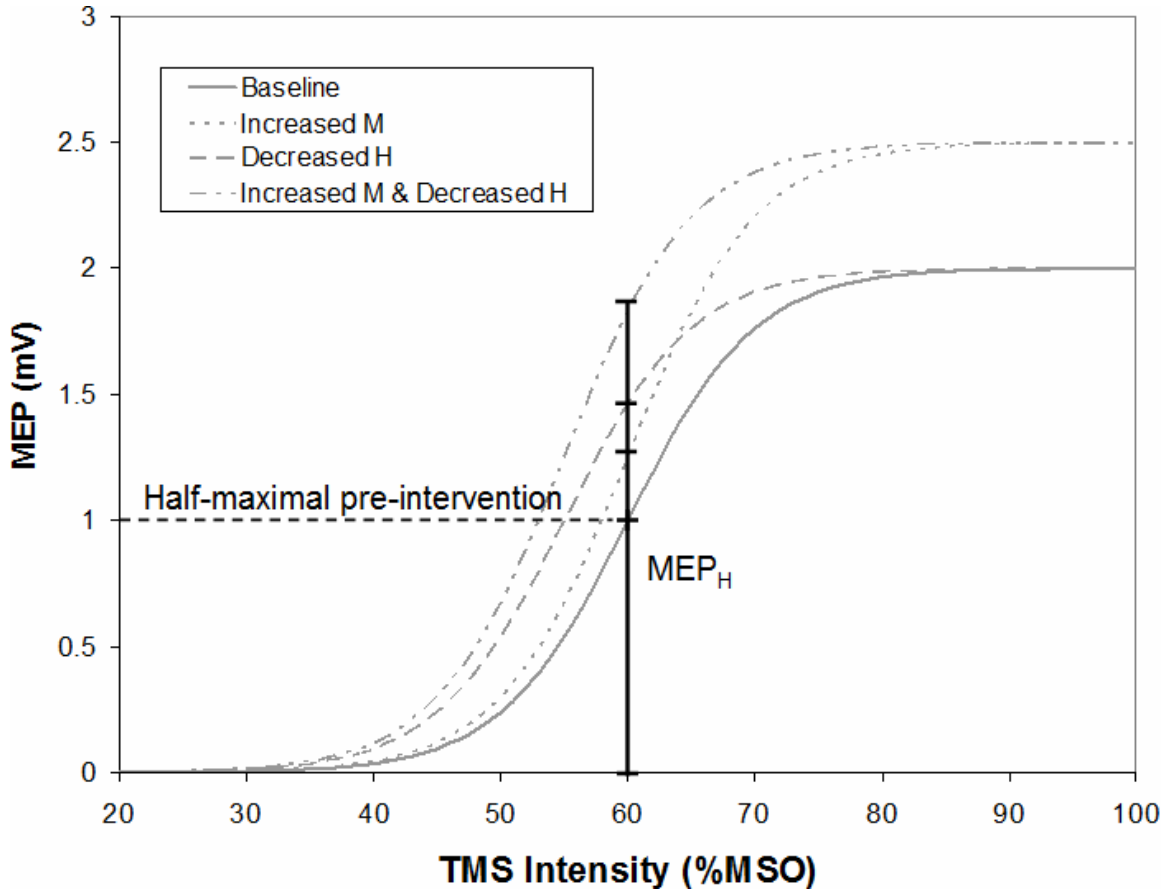

Figure 18. Outline of the "MEP_H" value derived from the MEP recruitment curve, illustrated using simulated data. The solid gray line represents a pre-intervention curve, while the dashed gray lines illustrate hypothetical post-intervention curves in which either the maximum response amplitude ("M") has been increased by 0.5 mV, in which the stimulus intensity which results in a half-maximal response ("H") has been decreased by 5% MSO, or in which both changes have occurred. "MEP_H" is defined as the response to stimulation at the intensity which, pre-intervention, resulted in a half-maximal response (this intensity is represented by the dashed black line). It can be seen from this illustration that changes in either "M" or "H" can result in changes in "MEP_H".

Two similar measures were also calculated. The first, termed "MEP_{120}", was defined as the mean response amplitude at the stimulation intensity which was closest to 120% of the participant's resting motor threshold. The second, termed "MEP_{1mV}", was defined as the mean response amplitude at the stimulation intensity which produced a response with an amplitude of at least 1 mV pre-intervention. These values are outlined in Figures 19 and 20.

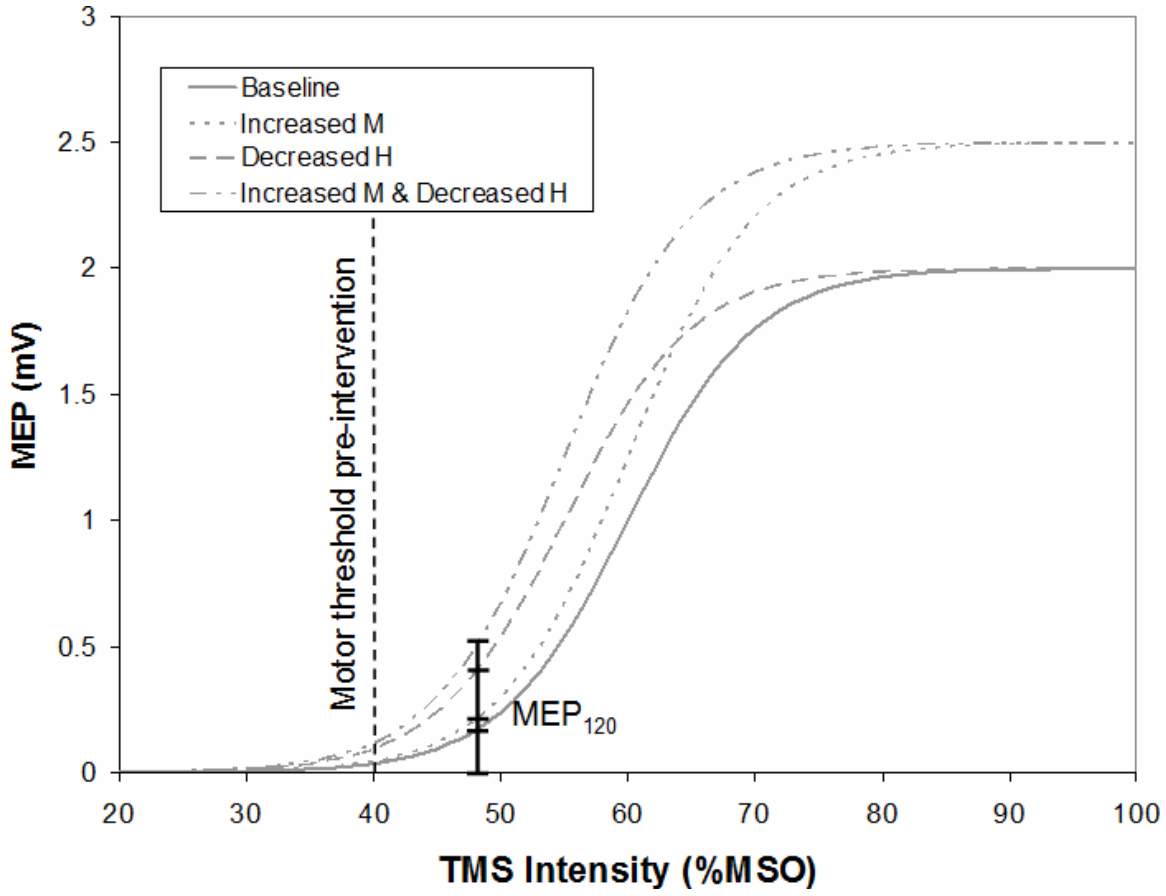

Figure 19. Outline of the "MEP_{120}" value derived from the MEP recruitment curve, illustrated using simulated data. The solid gray line represents a pre-intervention curve, while the dashed gray lines illustrate hypothetical post-intervention curves in which either the maximum response amplitude ("M") has been increased by 0.5 mV, in which the stimulus intensity which results in a half-maximal response ("H") has been decreased by 5% MSO, or in which both changes have occurred. "MEP_{120}" is defined as the response to stimulation at an intensity of 120% of the resting motor threshold pre-intervention (this intensity is represented by the dashed black line). It can be seen from this illustration that changes in either "M" or "H" can result in changes in "MEP_{120}".

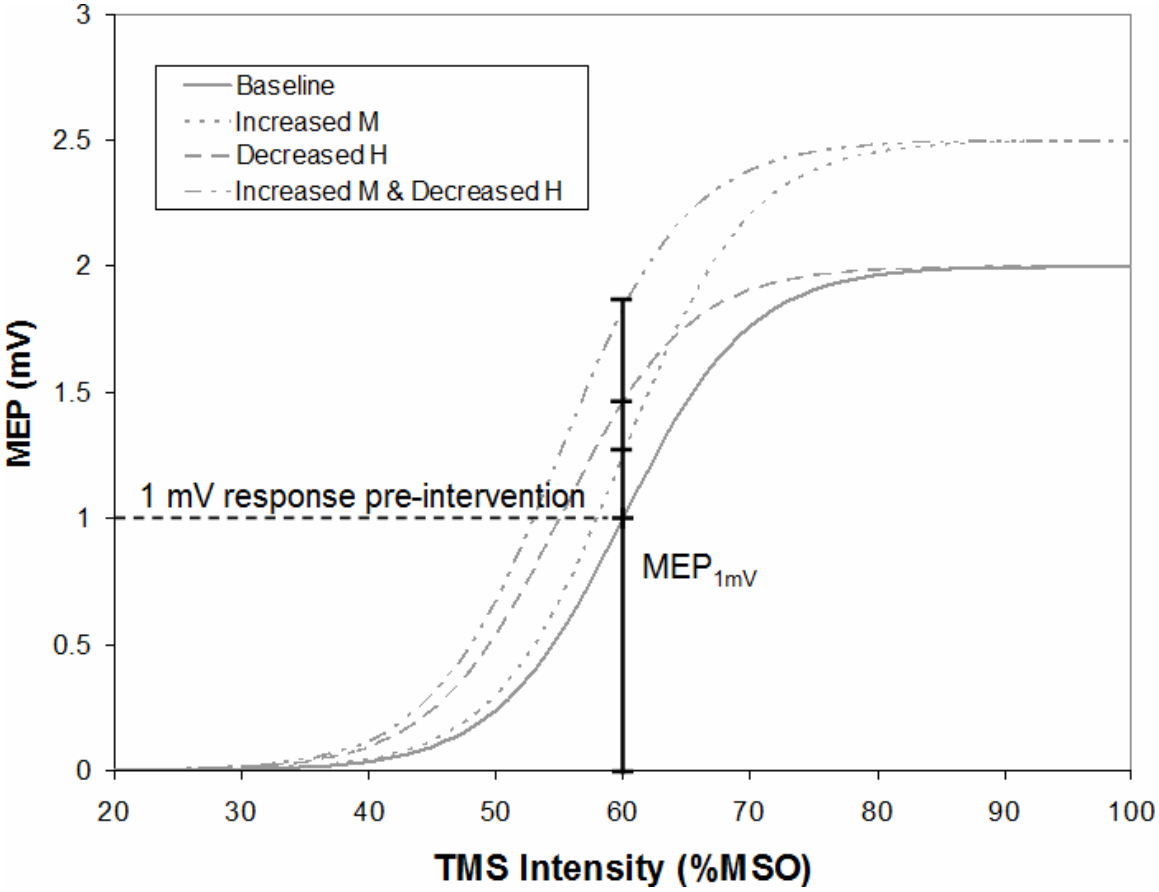

Figure 20. Outline of the "MEP_{1mV}" value derived from the MEP recruitment curve, illustrated using simulated data. The solid gray line represents a pre-intervention curve, while the dashed gray lines illustrate hypothetical post-intervention curves in which either the maximum response amplitude ("M") has been increased by 0.5 mV, in which the stimulus intensity which results in a half-maximal response ("H") has been decreased by 5% MSO, or in which both changes have occurred. "MEP_{1mV}" is defined as the response to stimulation at the lowest intensity which, pre-intervention, resulted in minimum response of 1 mV (this intensity is represented by the dashed black line). It can be seen from this illustration that changes in either "M" or "H" can result in changes in "MEP_{1mV}".

The final MEP-based measure employed was the area under the recruitment curve, termed "MEP_{AUC}". This method was introduced in Carson et al. (2013), and has been conducted accordingly herein. Both changes in "M" and "H" are expected to result in changes in "MEP_{AUC}".

II.3.9.2. Grip Force and Maximum Voluntary Contraction

Grip force was assessed by asking the participant to grip a hand dynamometer (Jamar Plus+, Sammons Preston, USA) as strongly as they could, for 3 seconds. The grip force for each repeat was taken to be the maximum force achieved. While the participant was gripping the dynamometer, EMG activity was being recorded from the FCR and APB muscles. MVC was determined by recording the mean rectified EMG signal for each repeat. Participants were asked to complete 3 repeats for each hand in each round of assessments. The means of these 3 repeats were derived for each hand, and the ratio between the left (treated) and right (untreated) sides was taken as the final outcome measure of these assessments. This analysis process was the same for both MVC and grip force.

II.3.9.3. Matched Forces

This assessment was based on the method presented in Taylor and Martin (2009). Its purpose was to measure changes in the input-output relationship of the spinal synapses on both sides of the body. A general outline of the method is provided in Figure 21.

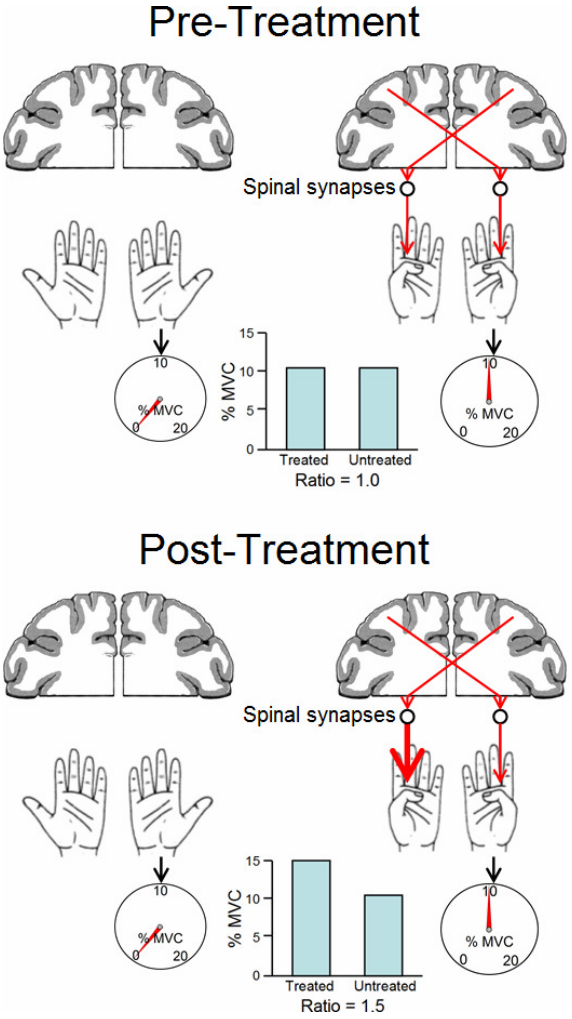

Figure 21. Outline of the matched forces method. Participants are instructed to perform identical ballistic thumb contractions on both sides, which are intended to provide equal descending volitional commands from the motor cortices of both hemispheres. Feedback is provided from the EMG recordings of the untreated (here, right) thumb only, guiding the participants to reach 10% of MVC. However, EMG data from both thumbs is collected, and the relative ratio of their amplitudes is determined. If the intervention is effective, the identical descending volitional commands will still be present, but the treated limb will produce a greater EMG signal in response, due to enhanced efficacy at the spinal synapse, increasing the relative ratio.

Participants were asked to perform weak, ballistic, bilateral contractions of their APB muscles, by adducting their thumbs across their palms. Participants were asked to produce identical contractions in both thumbs simultaneously during the test, timed in accordance with a color-coded cue signal. A red light, which lasted for 3 s, was the cue to relax. A yellow light, which lasted for 1 s, was the cue to be ready to perform a contraction. The start of the final, green light, which lasted 1 s, was taken as the participants' cue to perform the contractions. Visual feedback was presented to the participants in the form of a virtual dial. This dial represented the mean rectified APB EMG activity in their untested side, with an upward position indicating 10% of the pre-intervention MVC. Participants were not informed that this feedback was only coming from their untested side, only that the dial represented their muscle activity. The purpose of this was to require participants to generate the same amount of descending voluntary drive from both sides of the body, both before and after the intervention. Any changes in the resulting EMG could therefore be taken as indicative of sub-cortical mechanisms, such as plasticity at the spinal synapse. Since the participants were asked not to correct their movements after the initial contraction, but rather, to return immediately to a resting position, the involvement of sensory feedback was expected to be minimal.

Participants were allowed to practice these contractions, with feedback, until they felt comfortable in generating approximately 10% MVC. However, participants were told that the accuracy of their contractions was not as important as trying to generate the same movement with both hands. Following practice, they were asked to complete 20

repetitions of the movement. From each EMG trace, the maximum rectified value was taken, and the mean ratio between the maximum values of the two sides was taken as the final outcome measure.

While it was not discussed in the original paper by Taylor and Martin (2009), this test may be confounded by cross-body training effects, wherein training on one side of the body improves performance on the contralateral side, particularly in terms of strength (for a review, see Carroll et al. 2006). Since the mechanisms of cross-body training effects are not presently understood, it cannot be fully determined to what extent these effects may interact with the treatments in question.

II.3.10. Intervention and Post-Intervention Assessments

Each session's intervention, following preliminary assessment, consisted of one hour of continual grasping and manipulating small blocks. Details of the 5 different interventions are provided in §II.3.4 above. Participants were informed that they can take rest breaks if necessary, but they should ideally be able to complete the one hour intervention without any breaks (working continuously). In practice, no participants opted to take this break.

Following each session's intervention, the same battery of assessments was conducted as were conducted at the beginning of the session. Differences between the pre- and post-intervention assessment results were taken as indicative of effects of the intervention, provided that fatigue could be ruled out as a possible explanation. While this was initially assumed to be the case, subsequent analysis has since suggested that fatigue may in fact

have been present in the post-intervention assessments, as will be discussed later (§II.4.13, §IV.2).

II.3.11. Statistics

Descriptive statistics for continuous values, where presented, consist of the mean ± standard deviation of the given set of values. These values are those shown in the graphs of the outcomes measures (Figures 24 through 32). Descriptive statistics for non-continuous values, such as participant genders, are presented as relative frequencies.

The inferential statistical methods employed herein were chosen to make use of the within-subjects design, including the collection of both pre- and post-intervention measurements. The primary question being investigated by these statistical methods, as per the hypotheses outlined in §II.2, was whether or not any of the treatments had different effects on the time-course of any or all of the outcome measures. Thus, the most important factor was the interaction between time (defined categorically as either pre- or post-intervention) and treatment. All inferential statistical methods were applied identically to each of the nine outcome measures outlined in §II.3.9.

To this end, the primary inferential statistical method chosen was a repeated-measures ANOVA. This method was considered ideal since it accounts for multiple measurements being taken across time, as was the case in this study. It was also considered ideal since it is intended for use with within-subjects experimental designs. The method can therefore properly account for baseline variability in the subject population, as was suggested to be

necessary in §II.3.3. The assumption of normal distribution necessary for ANOVA analysis was confirmed by Lilliefors tests ($p \geq 0.0512$). The assumption of equality of variances was not rigorously examined, but visual examination of the data (Figures 24 through 32) suggests that this assumption is valid.

The repeated-measures ANOVA was applied both to the entire data set (that is, including all five treatments), and to narrowed data sets consisting of only the data from two treatment conditions (this resulted in 10 pairings of treatments being investigated). The purpose of testing the entire data set was to determine whether any of the treatments differed from each other in their ability to elicit pre-post changes in the outcome measures. The purpose of testing the narrowed data sets was to directly compare the effects of the two treatments being investigated. For example, if the two treatments being examined were the FES and BMI-FES groups, this test would deteremine whether the different control schemes in these two conditions resulted in different pre-post changes in the outcome measures. In all cases, the test was considered significant if the time x treatment interaction term varied significantly ($p \leq 0.05$). The time x treatment interaction term was taken as the primary statistical measure because, as suggested above, it best addressed the question of whether the time-course of each outcome measure was being altered differently by the various treatments. Single-factor p-values (that is, p-values from either the “time” or “treatment” factor independent of each other) are not reported since their interpretation is not meaningful in the context of the research questions being asked. For example, if only “time” was considered, the different treatment groups would be lumped together, and we could only conclude whether or not

they collectively affected the time-course of the outcome measures. Similarly, if only "treatment" was considered, the pre- and post-intervention measures would be lumped together, and baseline variability in the data would not be distinguishable from treatment effects.

Additional correlation analyses were conducted to determine whether any potential confounding factors could alternately explain the observed results. These potential confounds included:

- Participant age
- Participant gender
- MEP threshold value
- FES intensity (in the FES, BMI-FES, and EMG-FES conditions only)
    - In the FCR channel
    - In the APB channel
    - In the extensor channel
    - The sum of both of the "grasping" channels (FCR and APB)
    - The sum of all three channels
- BMI calibration parameters (in the BMI and BMI-FES conditions only)
    - Frequency band
    - Bandpower window
    - Mean window
        - Note: the required number of "hits" and whether the BMI responded to an increase or decreased in bandpower were not

considered, since these parameters were the same for all participants, as outlined in §II.4.12)

- BMI control test results (in the BMI and BMI-FES conditions only)
    - Percent accuracy before intervention
    - Percent accuracy after intervention
    - Average of pre and post percent accuracy
    - Pre-post difference in percent accuracy
    - Binomial p-value for control before intervention
    - Binomial p-value for control after intervention
- BMI threshold value (in the BMI and BMI-FES conditions only)

Each of the potential confounds above was matched with each the "pre-post change" outcome measures (defined as the post-intervention measure divided by the pre-intervention measure), and both the Pearson correlation coefficient and its relevant p-value were determined. In addition, differences across treatment conditions were tested for each of the above correlates (excluding age and gender, for which no differences were present since each participant completed all 5 conditions), using paired-samples t-tests. For these tests, significance was defined as $p \leq 0.05$.

Additional analysis was conducted on the MVC and grip force data to examine the possible effects of fatigue on the data. Paired-samples t-test were conducted comparing the pre- and post-intervention MVC values (both left and right arms, both FCR and APB), grip forces (both left and right), and the left-to-right ratios of these values (FCR

MVC, APB MVC, and grip force). These analyses were conducted both on data from each separate treatment condition, and from a pooled data set that disregarded treatment condition. Again, significance was defined as $p \leq 0.05$. The rationale for these tests is that fatigue, if present, should be seen as a general decrease in the post-intervention values as compared to the pre-intervention values. Fatigue specific to the intervention should only be visible in the treated limb, while more central fatigue should be visible in both limbs in approximately equal measure. More conventional measures of fatigue, such as mean or median EMG frequency (Hagg 1992), fatigue index, fatigue time, or torque-time integral (Nguyen et al. 2011) were not conducted, since it did not become apparent that fatigue would be a key factor worth measuring until mid-way through data collection. Since the experimental protocol could not be modified at such a time, it was necessary to interpret fatigue from the limited data already being collected. The fatigue data must therefore be interpreted cautiously, since the measures being used are not rigorously validated, and may be subject to varying interpretations.

Chapter II.4: Results

In total, 10 able-bodied participants completed all 5 sessions, 6 male and 4 female. Participants ranged from 24 to 32 years of age (mean ± SD: 28.4 ± 2.3). All participants self-identified as right-handed.

II.4.1. Sample Recordings

Figure 22 presents a single representative MEP recording, while Figure 23 presents a representative MEP recruitment curve.

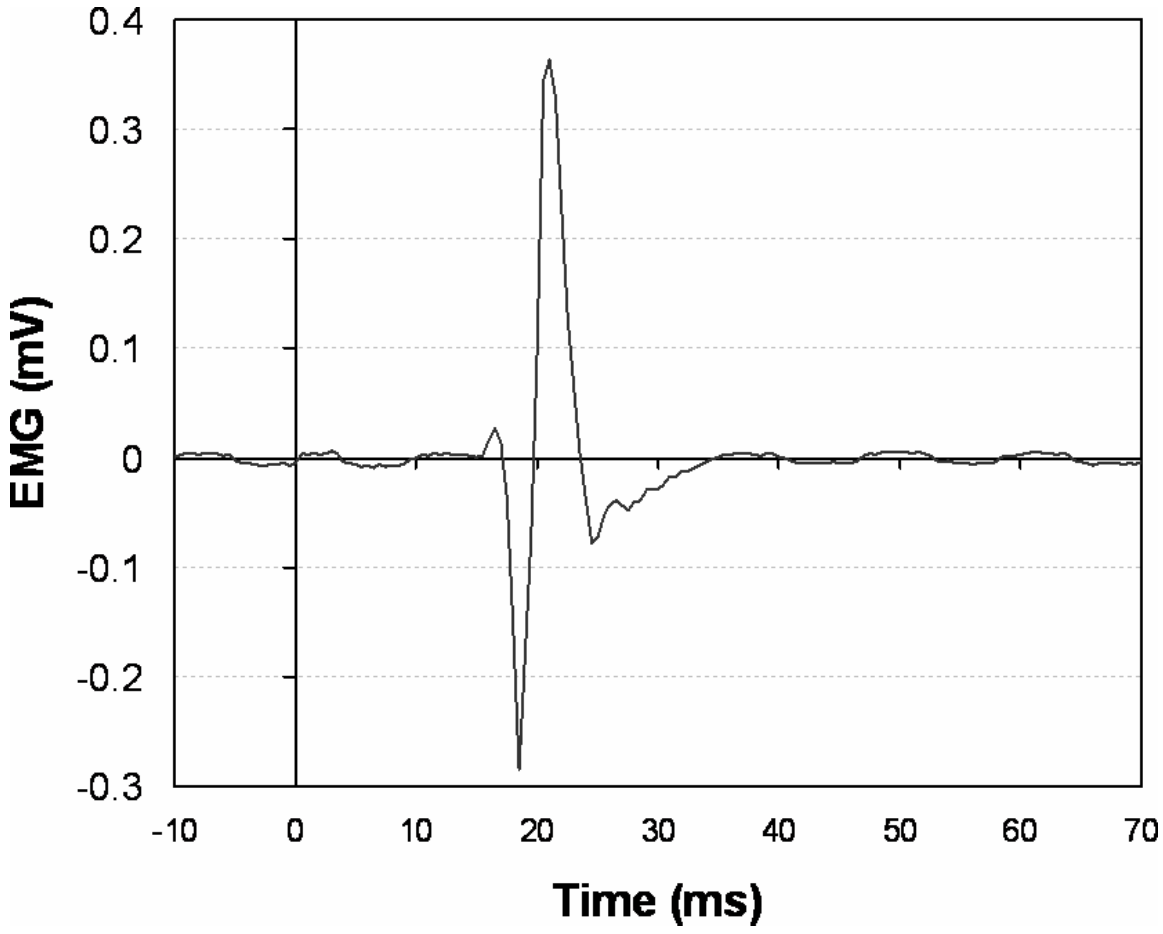

Figure 22. Representative motor evoked potential (MEP) recording, taken pre-intervention. This recording was in response to a TMS stimulus at 90% of the maximal stimulator output (MSO), triggered at time zero. Electromyogram (EMG) recordings were sampled at 2000 Hz.

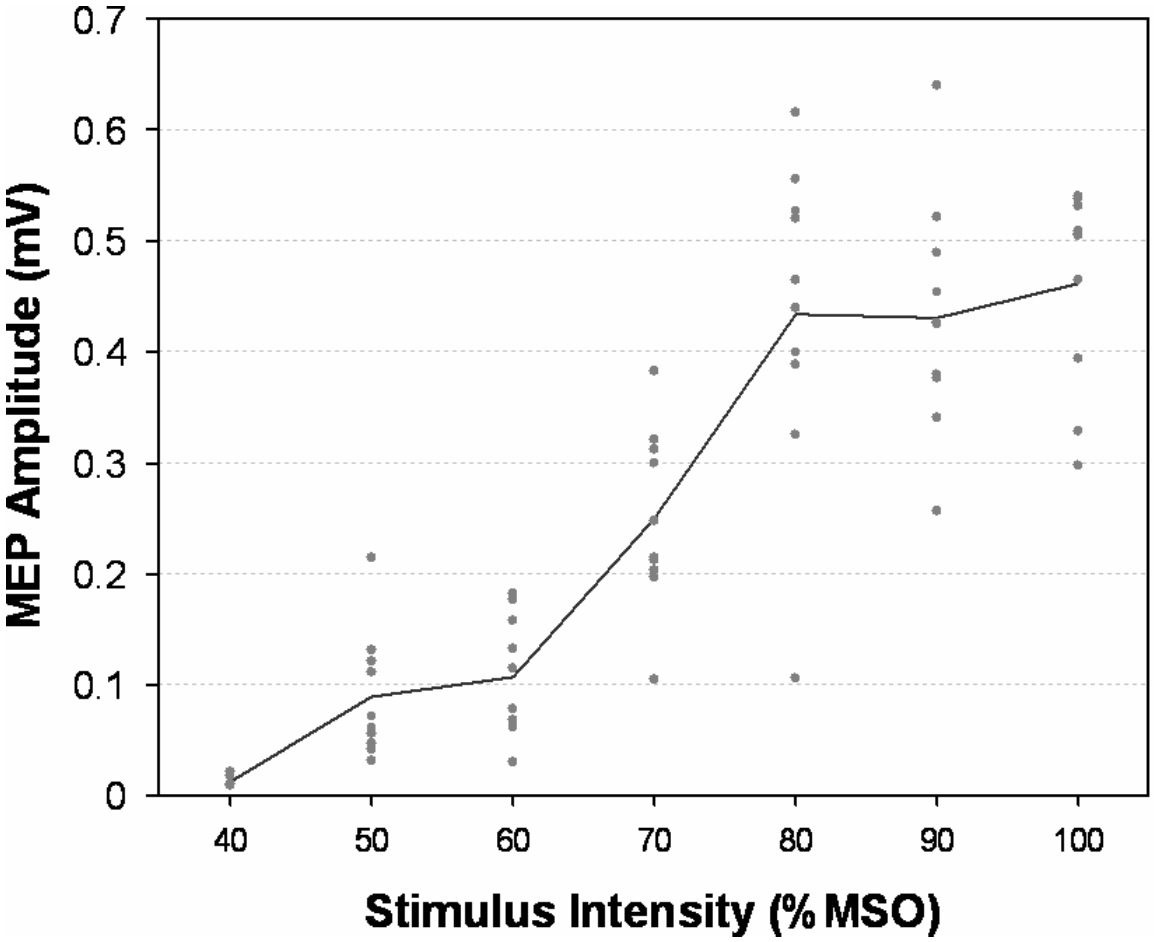

Figure 23. Representative motor evoked potential (MEP) recruitment curve, taken pre-intervention. Filled circles represent individual MEP responses, while the solid line represents the mean at each stimulation intensity. While individual responses show considerable variability, the mean responses fit an approximately sigmoidal curve.

Neurophysiological signals from other assessments were not retained – rather, analysis was conducted at the time of the assessment, and only the derived results were saved. Representative EMG recordings from the MVC and matched forces assessments therefore cannot be presented herein. Similarly, EEG recordings during the BMI control tests and calibration were not retained, and also cannot be presented.

II.4.2. Motor Evoked Potential: Maximum Response

The initial ANOVA showed significant effects on the time/condition interaction ($p = 0.0320$). Pre-post changes in the "M" value were found to be greater in the BMI-FES condition than in the FES condition ($p = 0.0212$), greater in the EMG-FES condition than in the FES condition ($p = 0.0194$), and greater in the EMG-FES condition than in the BMI condition ($p = 0.0499$). Visually, it can be seen that these difference arises both from a decreased maximal MEP in the "FES" and "BMI" conditions and an increased maximal MEP in the "BMI-FES" and "EMG-FES" conditions. These results are summarized in Figure 24.

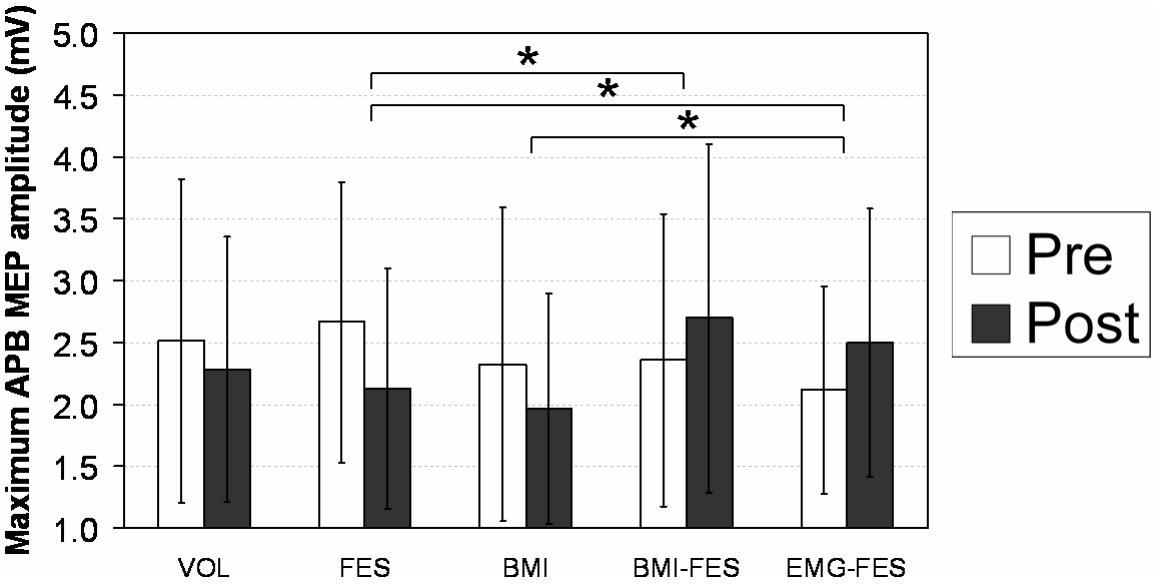

Figure 24. Mean pre and post maximum APB MEP amplitude ("M"). * indicates a significant difference between treatment conditions ($p \leq 0.05$). M was increased to a greater extent in the BMI-FES condition as compared to the FES condition ($p = 0.0212$), in the EMG-FES condition as compared to the FES condition ($p = 0.0194$), and in the EMG-FES condition as compared to the BMI condition ($p = 0.0499$).

II.4.3. Motor Evoked Potential: Estimated Response to Half-Maximal Stimulation

The initial ANOVA showed significant variance in the time/condition interaction ($p = 0.0436$). Pre-post changes in "MEP_H" were found to be significantly greater in the BMI-FES condition than in the VOL condition ($p = 0.0.0476$). They were also found to be greater in the EMG-FES condition than in the VOL condition ($p = 0.0020$). Lastly, they were found to be greater in the EMG-FES condition as compared to the FES condition ($p = 0.0341$). These results are summarized in Figure 25.

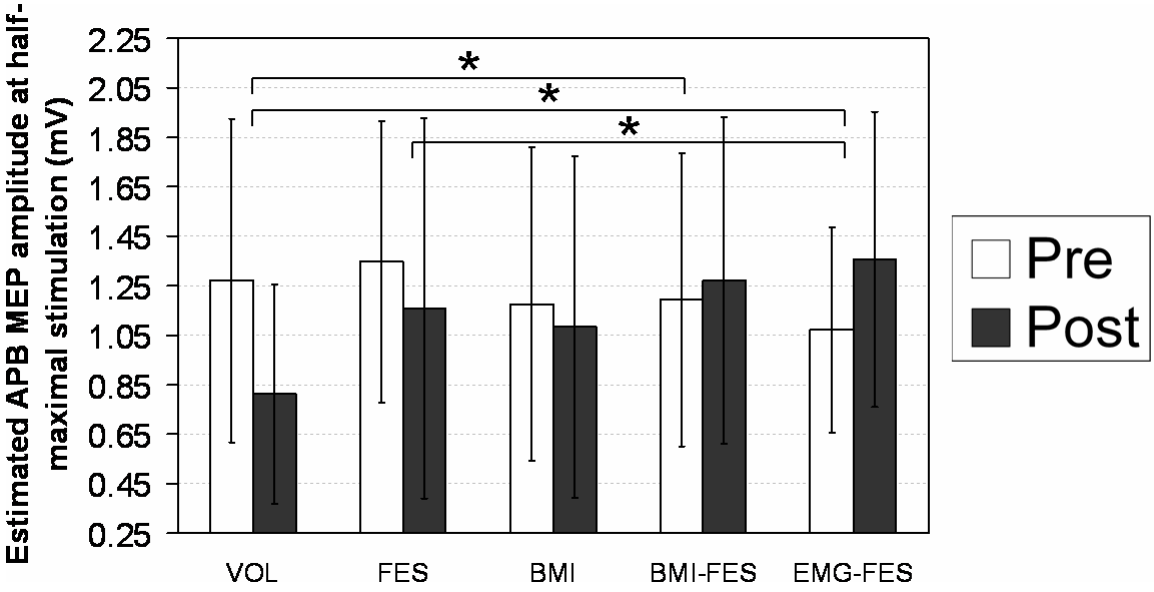

Figure 25. Mean pre and post estimated APB MEP amplitude in response to the stimulus intensity which caused a half-maximal MEP pre-intervention ("MEP_H"). * indicates a significant difference between treatment conditions ($p \leq 0.05$). MEP_H was increased to a greater extent in the BMI-FES condition as compared to the VOL condition ($p = 0.0436$), in the EMG-FES conditions as compared to the VOL condition ($p = 0.0020$), and in the EMG-FES condition as compared to the FES condition ($p = 0.0341$).

II.4.4. Motor Evoked Potential: Response at 120% of Motor Threshold

The initial ANOVA showed significant variance ($p = 0.0207$). Pre-post changes in "MEP_{120}" were found to be greater in the EMG-FES condition as compared to both the VOL condition ($p = 0.0088$), and the BMI condition ($p = 0.0312$). These results are summarized in Figure 26.

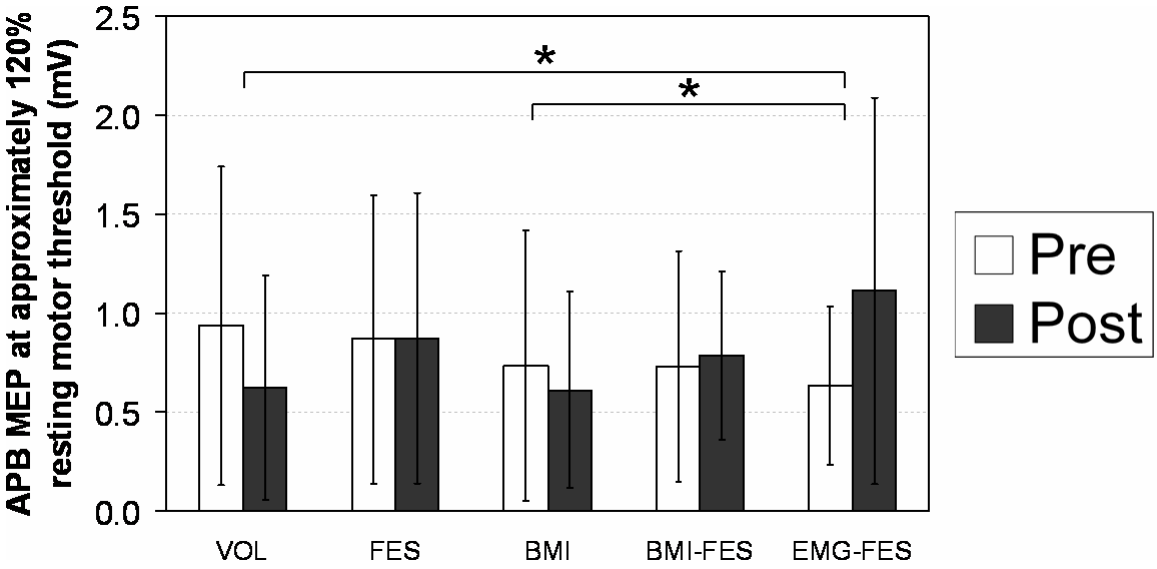

Figure 26. Mean pre and post APB MEP amplitude in response to the stimulus intensity which most closely approximated 120% of the participant's resting motor threshold ("MEP_{120}"). * indicates a significant difference between treatment conditions ($p \leq 0.05$). MEP_{120} was increased to a greater extent in the EMG-FES condition as compared to both the VOL condition ($p = 0.0088$), and the BMI condition ($p = 0.0312$).

II.4.5. Motor Evoked Potential: Response at Intensity Which Previously Provided 1 mV Response

One participant had a maximum MEP amplitude of lower than 1 mV in all conditions, while another participant had a maximum MEP amplitude of lower than 1 mV in only the VOL and BMI conditions. Thus, the results presented herein have a lower sample size than the other measures (n = 8 for VOL and BMI, n = 9 for FES, BMI-FES, and EMG-FES, as compared to n = 10 for all other outcome measures). The initial ANOVA showed significant variance ($p = 0.0097$). Pre-post changes in the "MEP_{1mV}" measure were found

to be greater in the EMG-FES condition as compared to the VOL ($p = 0.0042$), FES ($p = 0.0054$), and BMI ($p = 0.0285$) conditions. These results are summarized in Figure 27.

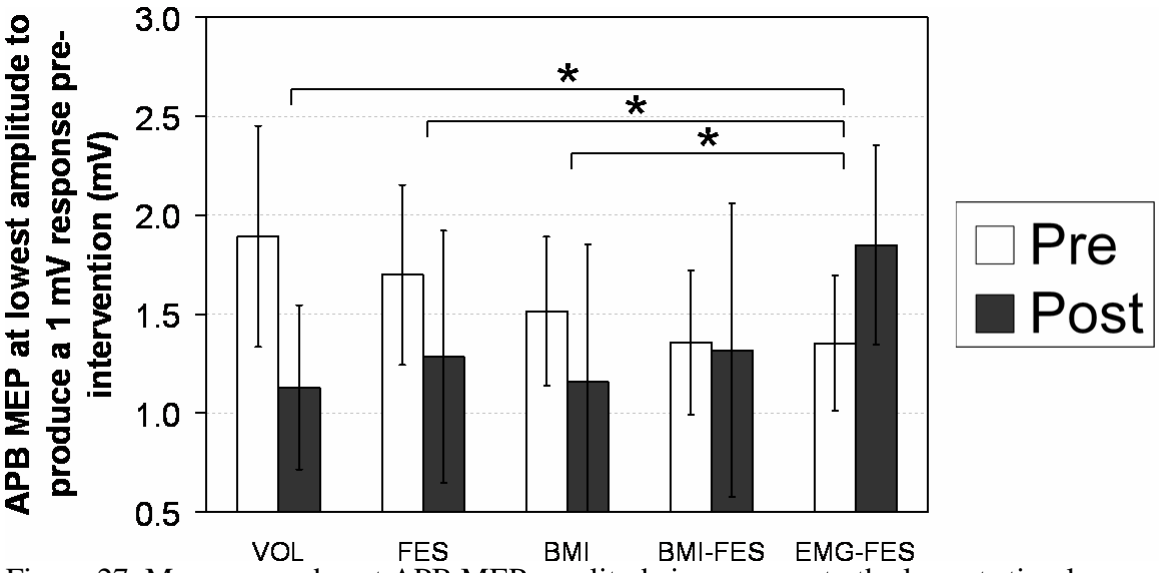

Figure 27. Mean pre and post APB MEP amplitude in response to the lowest stimulus intensity which caused an MEP response of at least 1 mV pre-intervention ("MEP_{1mV}"). * indicates a significant difference between treatment conditions ($p \leq 0.05$). MEP_{1mV} was significantly increased to a greater extent in the EMG-FES condition as compared to the VOL ($p = 0.0042$), FES ($p = 0.0054$), and BMI ($p = 0.0285$) conditions.

II.4.6. Motor Evoked Potential: Intensity Required for Half-Maximal Response

The initial ANOVA failed to show significant variance in the time/condition interaction ($p = 0.3301$). Pre-post changes in the "H" value were not found to significantly differ across conditions ($p \geq 0.1305$). These results are summarized in Figure 28.

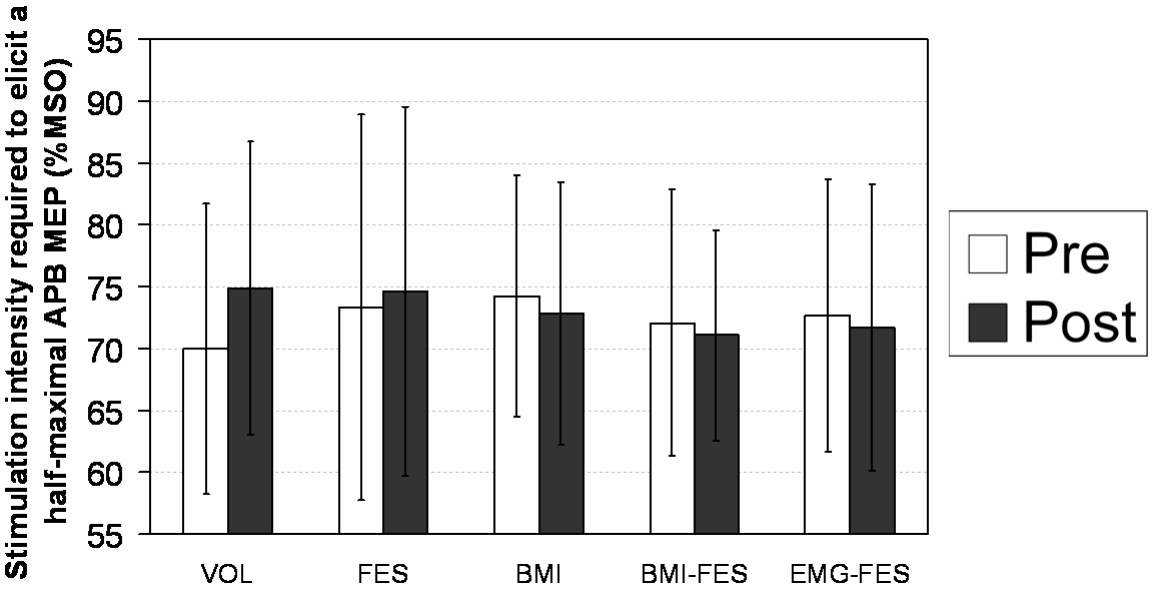

Figure 28. Mean pre and post TMS amplitude required for a half-maximal APB MEP ("H"). No significant differences between treatment conditions were noted ($p \geq 0.1305$).

II.4.7. Motor Evoked Potential: Area Under Recruitment Curve

The initial ANOVA showed significant variance ($p = 0.0368$). Pre-post changes in the "MEP_{AUC}" measure were found to be greater in the EMG-FES condition as compared to the VOL ($p = 0.0116$), FES ($p = 0.0229$), and BMI ($p = 0.0465$) conditions. These results are summarized in Figure 29.

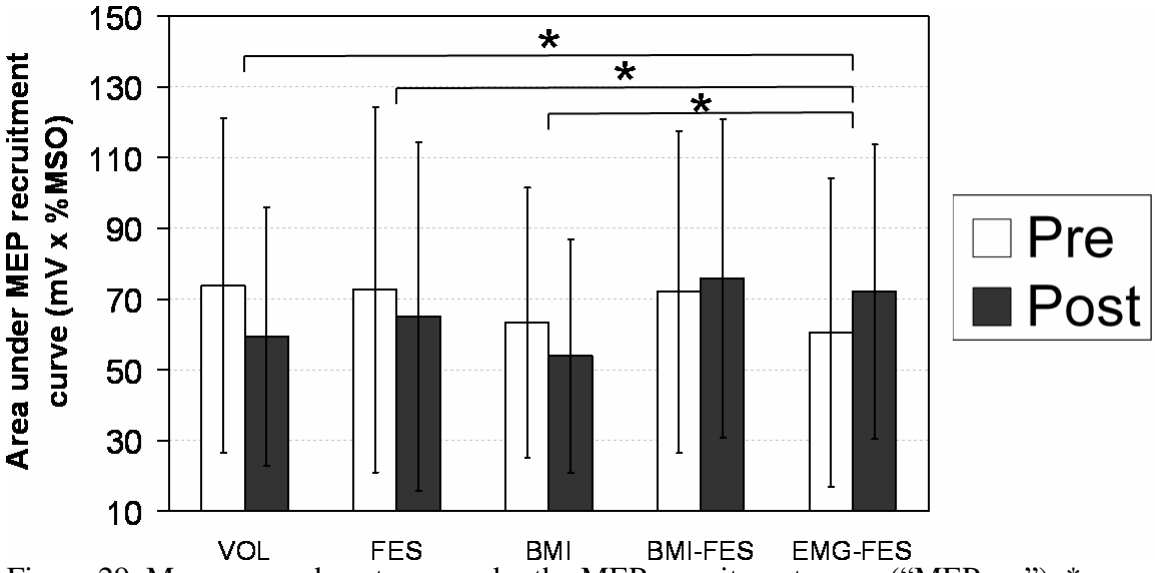

Figure 29. Mean pre and post area under the MEP recruitment curve ("MEP_{AUC}"). * indicates a significant difference between treatment conditions ($p \leq 0.05$). MEP_{AUC} was significantly increased to a greater extent in the EMG-FES condition as compared to the VOL ($p = 0.0116$), FES ($p = 0.0229$), and BMI ($p = 0.0465$) conditions.

II.4.8. Maximum Voluntary Contraction

The initial ANOVA failed to show significant variance ($p = 0.1330$). Pre-post changes in the ratio between left and right APB MVCs were not found to significantly differ across conditions ($p \geq 0.0880$). These results are summarized in Figure 30.

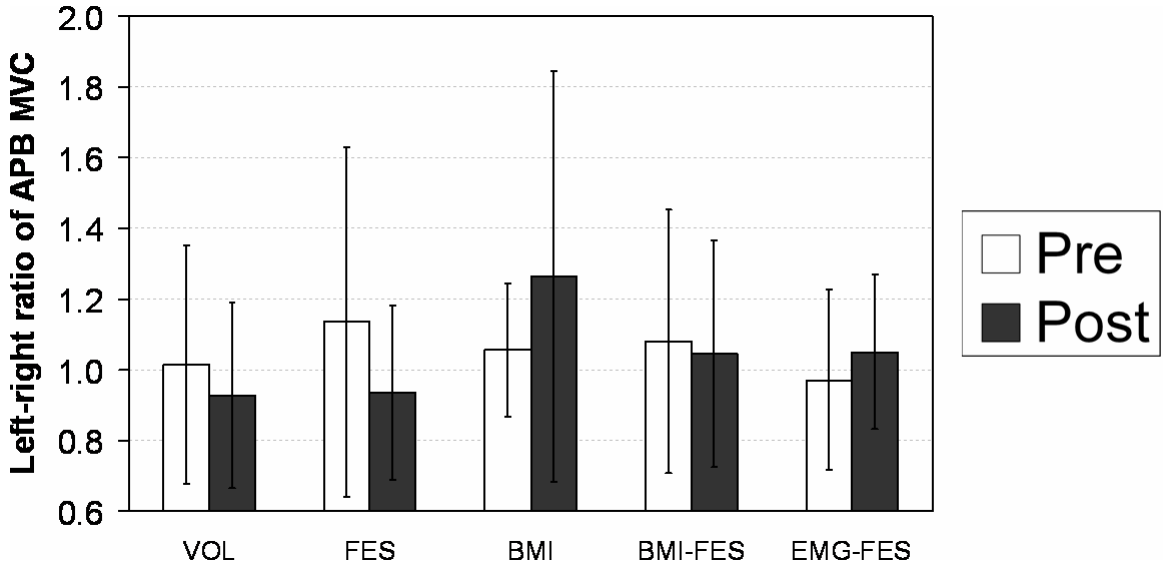

Figure 30. Mean pre and post left-to-right ratios of APB MVCs (defined as left ÷ right). No significant differences between treatment conditions were noted ($p \geq 0.0880$).

II.4.9. Grip Force

The initial ANOVA failed to show significant variance in the time/condition interaction ($p = 0.9690$). Pre-post changes in the ratio between left and right grip forces were not found to significantly differ across conditions ($p \geq 0.5288$). These results are summarized in Figure 31.

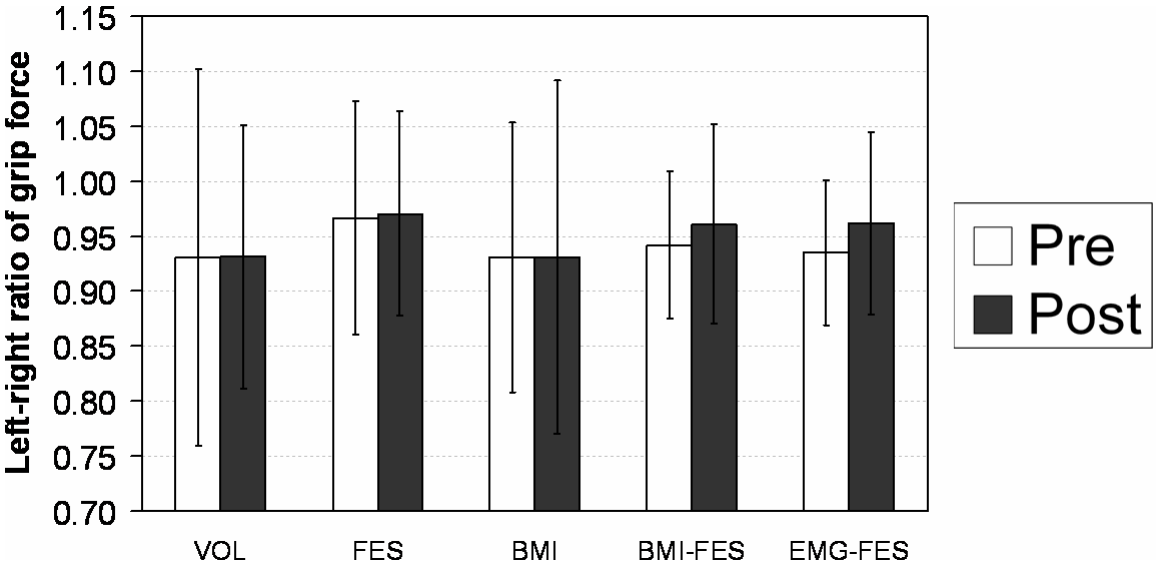

Figure 31. Mean pre and post left-to-right ratios of grip forces (defined as left ÷ right). No significant differences between treatment conditions were noted ($p \geq 0.5288$).

II.4.10. Matched Forces

The initial ANOVA failed to show significant variance in the time/condition interaction ($p = 0.4215$). Pre-post changes in the ratio between left and right muscle activity during the matched forces task were found to be significantly greater in the VOL condition as compared to FES ($p = 0.0226$), although this does not retain significance following Bonferroni correction. These results are summarized in Figure 32.

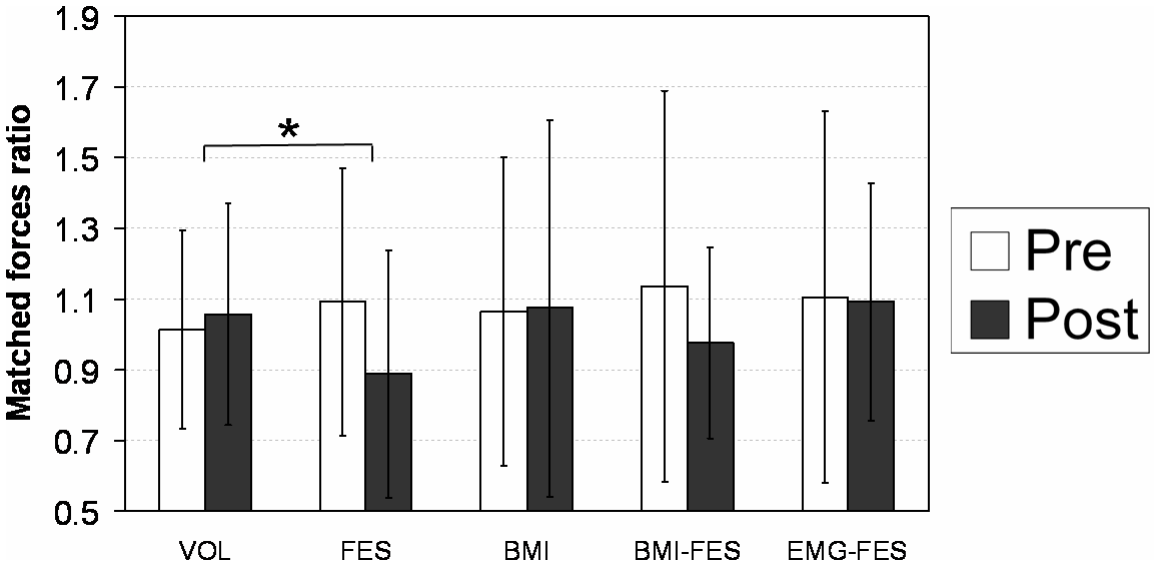

Figure 32. Mean pre and post left-to-right ratios of EMG activities during the matched forces test (defined as left ÷ right). * indicates a significant difference between treatment conditions ($p \leq 0.05$). The left-to-right ratio was significantly increased to a greater extent in the VOL condition as compared to the FES condition ($p = 0.0226$).

II.4.11. Brain-Machine Interface Control

The mean accuracy obtained during the BMI control test was 49.4±1.9% (range: 43.5-58.1%), with 50% representing chance. Accuracy was significantly greater in the pre-training control test (mean 50.4%) as compared to the post-training control test (mean 48.4%) ($p = 0.0201$). Accuracy was also found to be significantly greater in the BMI-FES condition (mean 50.8%) than in the BMI condition (mean 48.0%) ($p = 0.0068$). These results are summarized in Figure 33. Accuracy was not found to correlate significantly with any of the BMI calibration parameters ($p \geq 0.5501$). Pre-intervention and post-intervention accuracies were not found to significantly correlate to each other (p

= 0.1921), nor were pre-intervention and post-intervention binomial p-values (p = 0.1223).

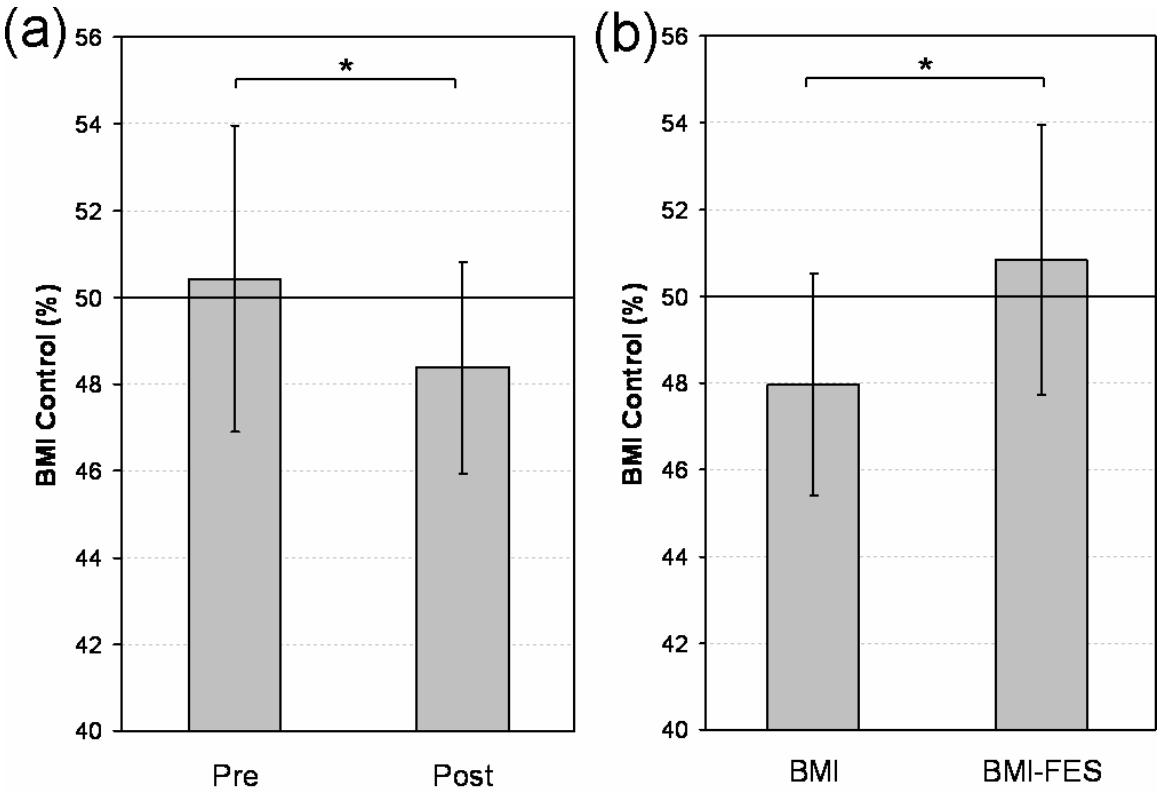

Figure 33. Percent accuracy during the BMI control task, group either by time point (pre- or post-intervention (a)) or by treatment condition (BMI or BMI-FES (b)). 50% indicates random chance, while positive values indicate better-than-chance control and negative values indicate worse-than-chance control. Control was found to be significantly better pre-intervention as compared to post-intervention (p = 0.0201), and in the BMI-FES condition as compared to the BMI condition (p = 0.0068).

II.4.12. Brain-Machine Interface Calibrations

Following the BMI calibration procedure outlined in §II.3.7.1, all 10 able-bodied participants were assigned calibrations which triggered stimulation as a result of bandpower decreases, rather than increases. These calibrations are outlined in Table 2. All of the assigned bandpowers were 2 Hz in width, ranging from 10-12 Hz to 13-15 Hz, despite possible bandpowers of up to 24-32 Hz being considered in the calibration procedure. All participants were assigned one of the two longest possible "mean window" lengths (15 or 20 bandpower windows), with the exception of participant #1, who used the shortest possible length (1 bandpower window). Assigned "bandpower window" lengths varied considerably throughout the possible range of 0.25-2 s. All participants were assigned the lowest possible "required hits" (1 "hit").

Table 2. Outline of the calibration parameters determined for each participant.

Participant	Increase/ Decrease	Range (Hz)	Bandpower Window (s)	Mean Window (indices)	Required Duration (indices)
1	Decrease	13-15	0.25	1	1
2	Decrease	12-14	0.25	20	1
3	Decrease	13-15	0.5	5	1
4	Decrease	12-14	2	15	1
5	Decrease	13-15	0.25	15	1
6	Decrease	13-15	2	15	1
7	Decrease	10-12	0.25	15	1
8	Decrease	10-12	2	20	1
9	Decrease	13-15	0.5	15	1
10	Decrease	11-13	0.5	15	1

A full description of possible parameter values is outlined in §II.3.7.1.

II.4.13. Possible Confounding Factors

The pre-post change in grip force was found to have a significant positive correlation with the total FES intensity from all three channels ($p = 0.0280$). This is visualized in Figure 34.

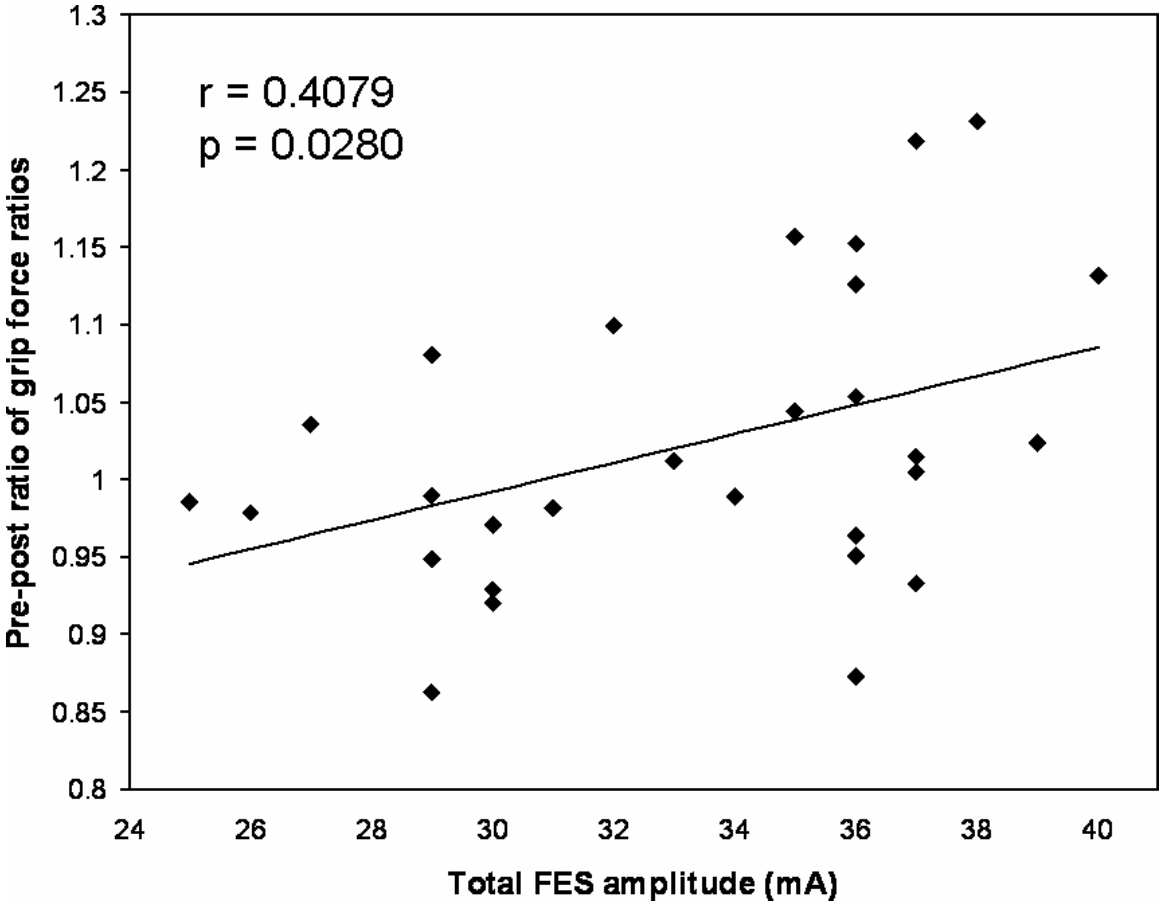

Figure 34. Positive correlation between the pre-post ratios of left-to-right grip force ratios and the FES amplitudes across all three channels ($r = 0.4079$, $p = 0.0280$).

A number of additional, non-significant trends were noticed, with p-values between 0.05 and 0.10. Despite their non-significance, these results are being reported in order to provide a more thorough picture of the potential factors involved in producing the

observed changes in outcome measures. The pre-post change in grip force had a slightly positive correlation with the FES intensity in the APB channel ($p = 0.0729$), similar to the significant correlation mentioned above with the total FES intensity. By contrast, the pre-post change in the maximum APB MEP had a slightly negative correlation with the APB FES intensity ($p = 0.0584$), but positively correlated with the mean of the frequency range used in the BMI calibration ($p = 0.0786$). Pre-post changes in the maximum APB MEP were also found to have a slightly positive correlation with post-intervention BMI control ($p = 0.0949$). Lastly, pre-post changes in the half-maximal APB MEP intensity were found to have a slightly positive correlation with pre-intervention BMI control ($p = 0.0790$).

The FES intensity in the APB channel was found to be significantly greater in the BMI-FES condition as compared to the EMG-FES condition ($p = 0.0145$, with the mean intensities being 7.7 mA in the BMI-FES condition and 6.3 mA in the EMG-FES condition). These results are summarized in Figure 35.

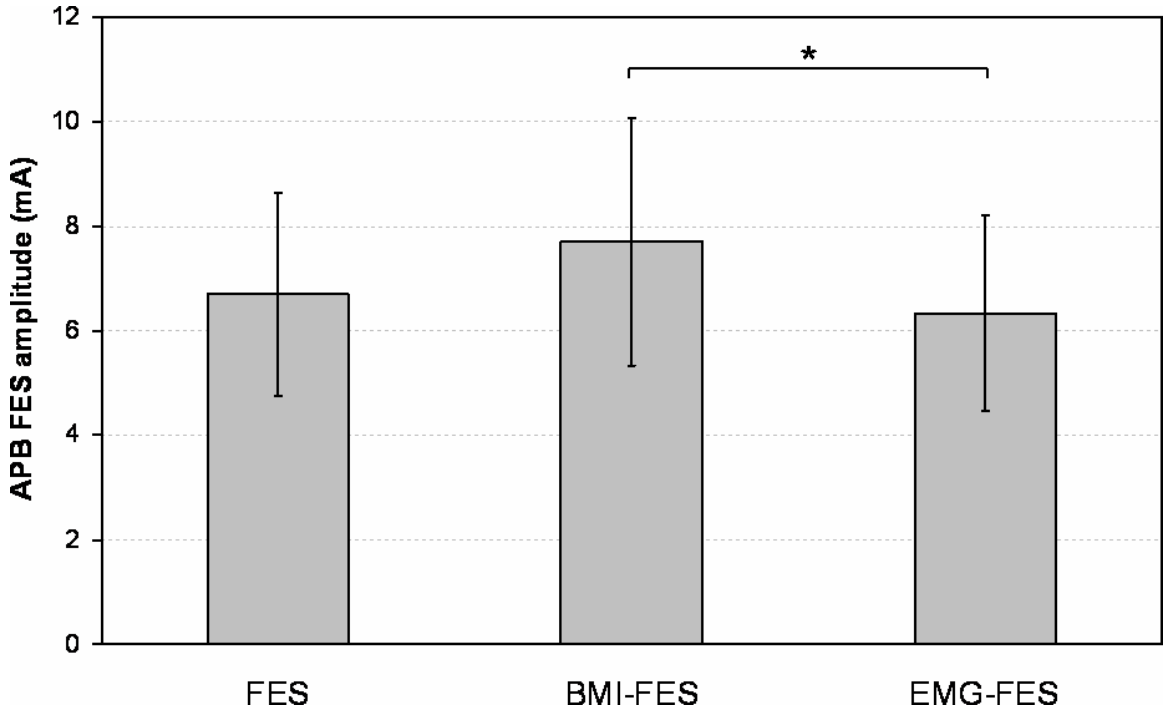

Figure 35. Differences between the APB FES amplitudes across the three conditions employing FES. * indicates a significant difference ($p \leq 0.05$). The intensity in the BMI-FES condition was found to be significantly greater than that in the EMG-FES condition ($p = 0.0145$).

The additional analysis to determine the potential effects of fatigue showed that the intervention often resulted in decreased MVC and grip force. In a pooled data set that disregarded treatment condition, this effect was significant for both MVC and grip force, for both arms, and for both muscles (in MVC) ($p \leq 0.0010$), with the exception of the right FCR MVC ($p = 0.0644$). However, the left-to-right ratios were not significant, for MVC in either muscle or for grip force ($p \geq 0.4795$). These results are summarized in Figures 36 and 37. Taken together, these results suggest that while fatigue significantly decreased muscle activity and force, both arms were equally affected.

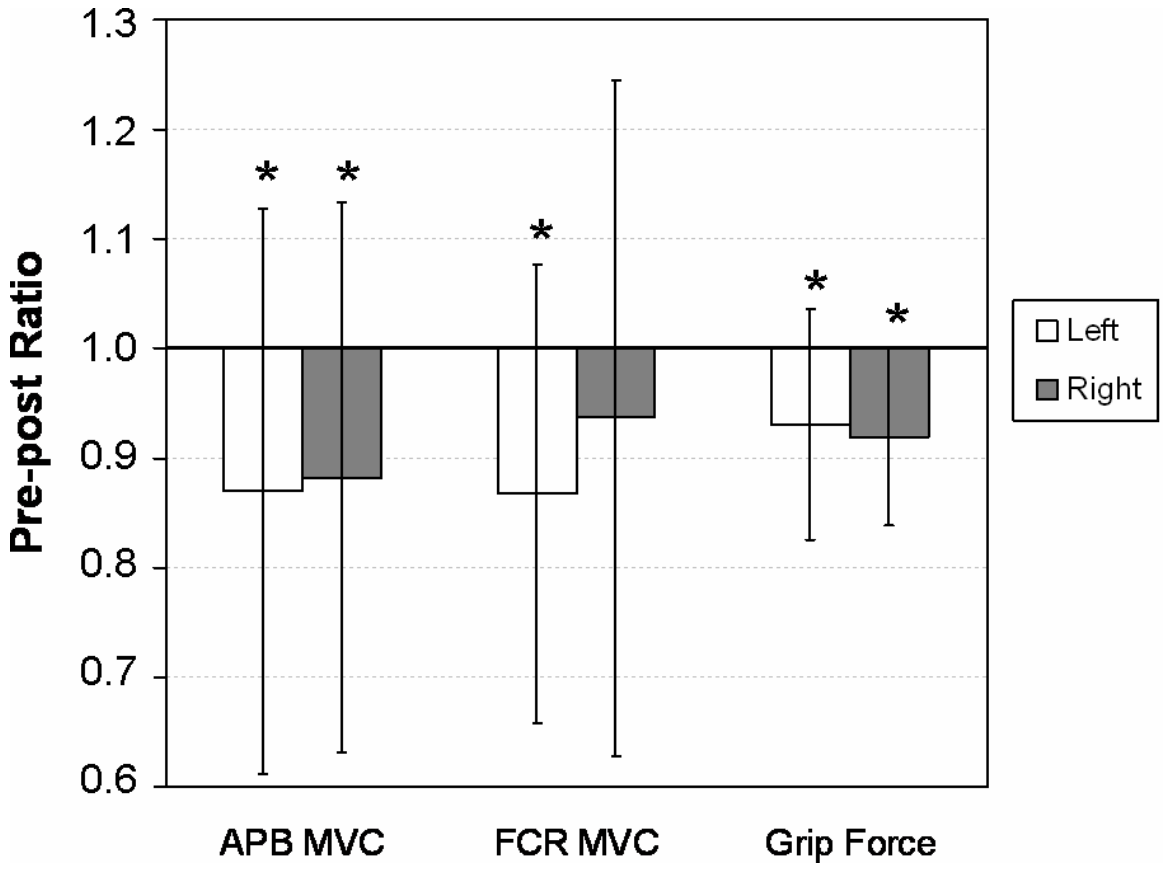

Figure 36. Pre-post differences in MVC and grip force suggesting bilateral fatigue. Data in the fatigue analysis is pooled, irrespective of participant or treatment condition. * indicates a significant decrease ($p \leq 0.0010$). The only measure which did not reach significance, although it approached it, is the right FCR MVC ($p = 0.0644$).

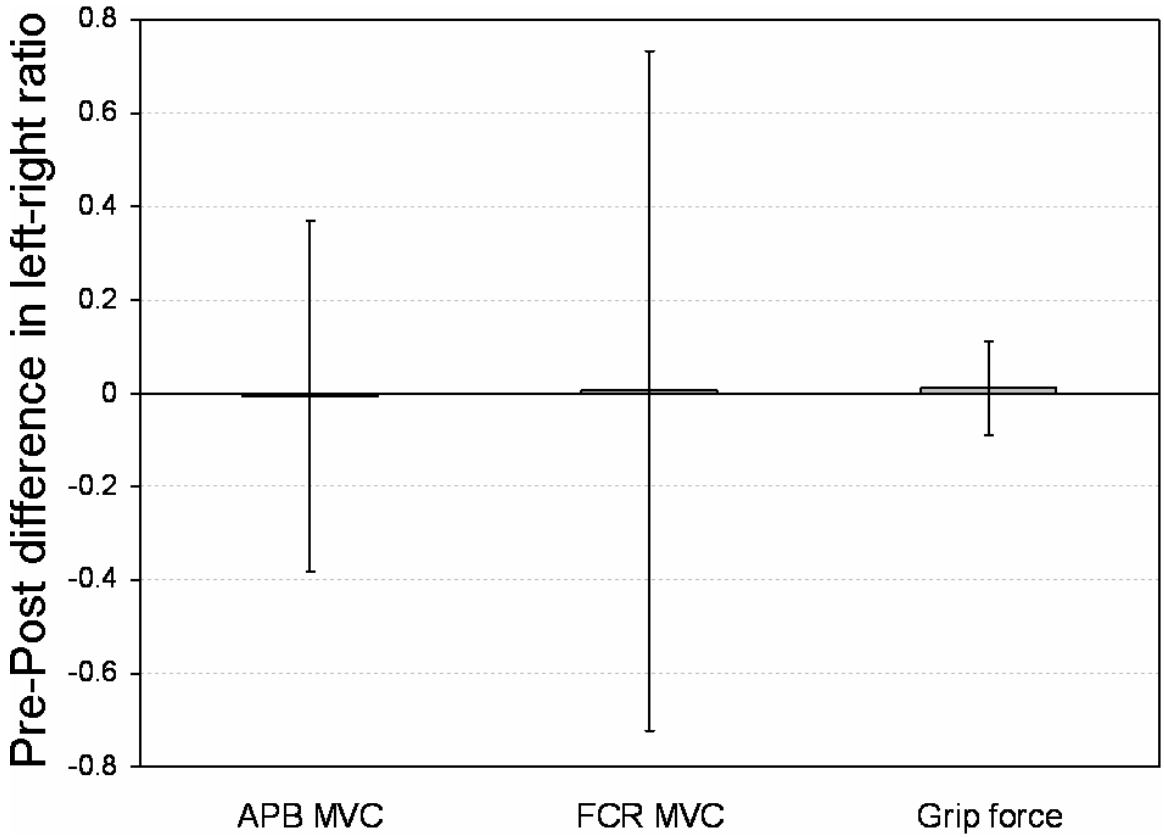

Figure 37. Pre-post difference in the left-right ratios (defined as left ÷ right) of MVC and grip force measures. Data in the fatigue analysis is pooled, irrespective of participant or treatment condition. No such measures are significant ($p \geq 0.4795$), suggesting that the fatigue observed in Figure 36 is equal in both arms.

These results are largely mirrored when the data for each treatment condition is analyzed separately. In the left APB, significant MVC decreases are only seen in the FES and BMI-FES conditions ($p = 0.0035$ and $p = 0.0220$, respectively; $p \geq 0.0684$ in other conditions). In the right APB, significant MVC decreases are only seen in the BMI and EMG-FES conditions ($p = 0.0400$ and $p = 0.0500$, respectively; $p \geq 0.0951$ in other conditions). In the left FCR, significant MVC decreases are only seen in the VOL and EMG-FES conditions ($p = 0.0011$ and $p = 0.0304$, respectively; $p \geq 0.1491$ in other

conditions). In the right FCR, no significant MVC changes were seen in any condition ($p \geq 0.0696$). Significant decreases in the left-hand grip force were only seen in the BMI and BMI-FES condition ($p = 0.0037$ and $p = 0.0421$, respectively; $p \geq 0.0678$ in other conditions). Significant decreases in the right-hand grip force were seen in all conditions ($p \leq 0.0355$). No significant changes were seen in any of the left-to-right ratio measures (FCR MVC, APB MVC, or grip force), in any condition ($p \geq 0.2022$). In spite of these results, when pairs of conditions were directly compared to each other on these pre-post change measures, very few significant results were noticed (with the exceptions being the left FCR MVC when comparing BMI-FES to EMG-FES and considering the pre-post changes as a difference ($p = 0.0341$), the right APB MVC when comparing VOL to BMI and considering the pre-post changes as a ratio ($p = 0.0384$), and the right FCR MVC when comparing VOL and EMG-FES and considering the pre-post changes as a ratio ($p = 0.0218$); in all other cases, $p \geq 0.0503$).

Chapter II.5: Discussion

II.5.1 General Interpretation of Results

MEP-based measures tended to show decreases in the VOL and FES group and increases in the BMI-FES and EMG-FES groups, providing support for the hypothesis of greater MEP upregulation in these groups (as outlined in §II.2). In many cases, these changes were statistically significant. However, the high variability meant that these trends were not always able to generate significant comparisons. These results provide the strongest evidence to suggest that BMI-FES and EMG-FES are able to generate neuroplasticity to a greater extent than conventional control strategies. Comparisons between the BMI and EMG-FES groups were often significant, but those between the BMI and BMI-FES groups were not, leaving open the question of whether the BMI and FES components were acting synergistically in this treatment condition.

More functional measures, such as grip force and MVC, were not affected. While this is a refutation of the hypotheses of visible effects of the treatments on these outcomes (as outlined in §II.2), it is not surprising given that these assessments test for larger, systems-level plasticity, and since the treatment was relatively brief.

Taken together, these results constitute weak proof-of-concept evidence to suggest that BMI-FES, and especially EMG-FES, are able to produce greater neuroplasticity than conventional FES or voluntary grasping. These provide confirmation of some of the original hypotheses (namely, that MEP would be upregulated to a greater extent in the BMI-FES and EMG-FES groups), while refuting other hypotheses (namely, that

significant changes would also be visible in MVC and grip force, and that conventional FES would also be successful in producing MEP upregulation). Further discussion (the remainder of §II.5 and the entirety of §IV) will seek to further explain these findings.

II.5.2. Effect of Lack of Volitional Brain-Machine Interface Control

Prior to these experiments, it was unclear whether or not a user who does not obtain voluntary BMI control or the perception thereof would be able to induce neuroplasticity with the BMI, as this might have prevented the pairing between the descending commands and the peripheral stimulation. It was not expected that users would obtain volitional control over their BMIs over such briefs periods, based both on the relevant literature, which indicates that successful learning of BMI control is a skill that can take months of regular practice to develop (Birbaumer et al. 1999, Wolpaw and McFarland 1994), and our personal experience (McGie 2009). It should be noted, however, that this is not universally true, and that other groups investigating non-invasive BMIs are able to obtain considerable control with minimal training (Niazi et al. 2012). The cause of this divide in the literature, with some groups showing a lengthy learning process, and some groups showing immediate volitional control, has not yet been elucidated.

One perspective, based on the neurophysiological theory behind these experiences, suggests that no volitional control should be necessary, as the connection between volitional control areas of the brain (mostly within the prefrontal cortex (Goldman-Rakic et al. 1996, Ramsey et al. 2006)) are not involved in the critical pathway (the CST beginning in the M1). The source of the M1 activity, whether it be in the prefrontal

volitional areas or the somatosensory cortex or in subcortical areas such as the cerebellum or the basal ganglia, does not matter for the purposes of LTP-like plasticity induction, only that M1 activity detected by the BMI eventually reaches the spinal synapse. In fact, it could even be conceived that the loss of volition may promote neuroplasticity, by preventing cognitive control from overriding subconscious processing, as was observed in Kaplan et al. 2005.

More empirically, however, it has been suggested that volitional BMI control may be necessary. Niazi et al. (2012) found that the extent to which brief brain-controlled stimulation increased users' maximum MEP was correlated with the ratio of true-positive to false-positive predictions generated by the BMI. The original figure is reproduced in Figure 38. While the correlation would appear strong ($r = 0.8$), no p-value is presented for the correlation, suggesting that the sample size may not have been sufficient to allow the correlation to reach significance. The high variability found throughout the Niazi et al. study may have been responsible for this.

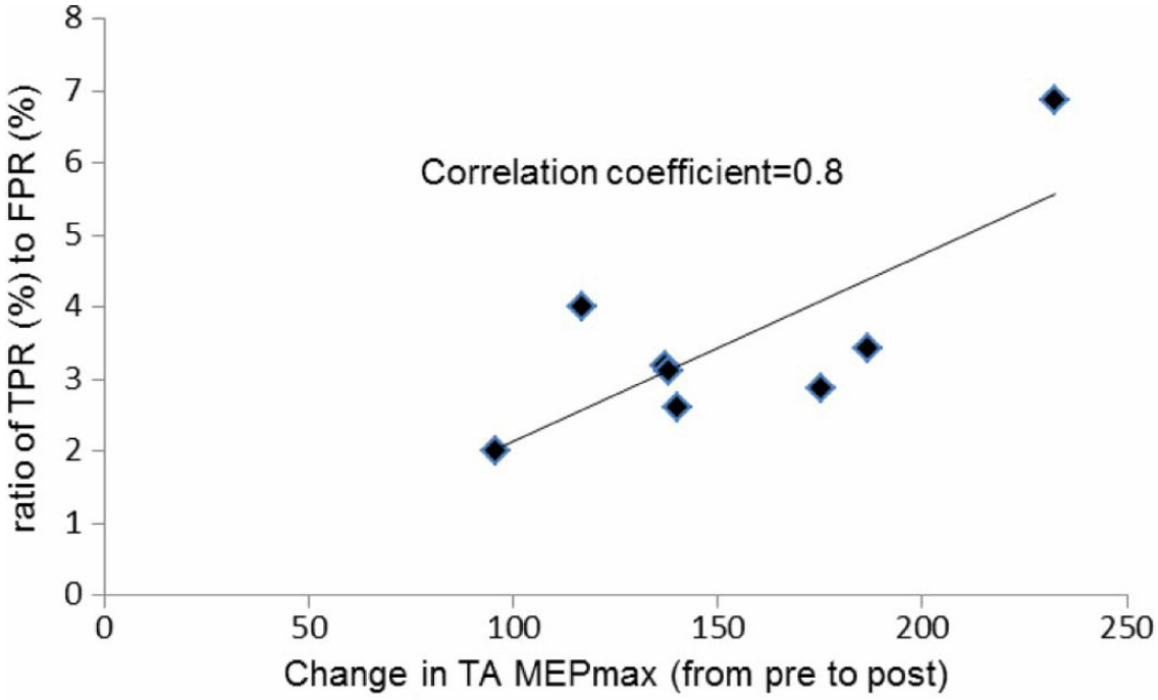

Figure 38. Reproduced from Niazi et al. (2012). The relative increase in maximum tibialis anterior motor evoked potential is correlated with the accuracy of the user's BMI prediction. No p-value is presented for this correlation.

In the present experiments, as predicted, very little volitional control over the BMI was observed, as outlined in §II.4.11. Despite this, the BMI-FES condition showed significant MEP upregulation on some measures. It could be suggested that the success of the BMI-FES group, as compared to the BMI group, is due to the fact that the BMI-FES group showed significantly higher BMI control than the BMI group. However, while statistically significant, this difference is in fact extremely small, amounting to a 2.8% increase to a level only 0.8% better than chance. Furthermore, the post-intervention BMI control was found to be significantly lower than the pre-intervention control, suggesting that if small changes in BMI control could greatly affect MEP-based outcomes, then there should be a trend towards MEP downregulation when one considers

the change scores. In truth, the narrow range of BMI control accuracies obtained (between 43.5-58.1%) is not a broad enough sample for us to state whether or not superior BMI control could lead to superior outcomes. The results do, however, suggest strongly that BMI control is not an absolute prerequisite for inducing neuroplasticity with a BMI-FES system.

II.5.3. Limitations of Switch-Based Functional Electrical Stimulation Paradigms

All three FES paradigms applied in the BMI-FES study can be described as "switch-based" – that is, the stimulation switches between different modes in response to a trigger command, from resting, to flexion, to extension, and returning to rest. By contrast, one can envision non-switch-based systems, which respond to command signals in a graded fashion (for example, an EMG-FES system in which a smaller voluntary contraction results in a smaller level of stimulation, and a greater level of contraction results in a greater level of stimulation). The "switch-based" approach was chosen for two reasons. The first is that previous studies which have shown functional benefits from FES have employed switch-based control (i.e., Kapadia et al. 2011). Since our primary purpose was to compare across the three FES conditions (thus elucidating the effect of the control strategy), using switch-based control allowed us to directly compare the novel treatments (BMI-FES and EMG-FES) to the current "gold standard" (FES). The second reason is that current from the FES travels along the user's skin, reaching the EEG or EMG electrodes and severely contaminating the signals. Thus, accurate EEG or EMG recordings can only be obtained while the FES is fully off. Any non-switch-based control strategy would require continued monitoring of the control signals during this

contamination. Many approaches for removing stimulation artefacts have been suggested and implemented successfully in the literature, the details of which are beyond the scope of this discussion. However, in the present study, no approach was found successful enough to maintain reliable control signals during FES. A switch-based strategy was therefore a necessity.

Unfortunately, switch-based control strategies, as implemented herein, are highly artificial, and are not ideal from the perspective of neuroplasticity induction, since they cause discrepancies between the user's volition and the stimulation. For example, an EMG-FES or BMI-FES user may intend to perform a small movement, and send a small amount of volition command from their brain to their muscles. But the magnitude of the neurological signals generated by this intention may not be sufficient to cross the necessary threshold for stimulation, and the descending commands are therefore not met by antidromic firing, resulting in no LTP-like plasticity, or even possibly LTD-like plasticity.

Another example would be a user who successfully starts their FES with a volitional command, but then relies fully on the FES to perform the movement, thereby removing the descending commands for much of the FES duration. The FES system, unable to recognize this change due to stimulation artifacts, continues to stimulate, producing antidromic firing that is not met by descending commands. Again, this may cause a lack of LTP-like plasticity, or even possibly LTD-like plasticity. Although participants are

instructed to continue to actively engage their volition for the duration of the stimulation, such discrepancies may still occur.

Many more such scenarios can be envisioned, in which a switch-based FES system can cause a mismatch between volitional descending commands and antidromic firing, and may therefore impair synaptic upregulation. It is, in fact, these exact kinds of mismatches which the BMI-FES and EMG-FES systems were intended to avoid, since they can easily occur in conventional, manually-controlled FES. While a more direct and natural system should theoretically be able to produce better potentiation, such a system was not found to be technically feasible in the current application. Despite this limitation, the results of the present study suggest that at least some level of positive neuroplasticity can be produced by switch-based BMI-FES and EMG-FES systems, leaving open the suggestion that more natural systems may be able to produce even greater effects.

II.5.4. Potential Confounding Effects of Functional Electrical Stimulation Intensity

The analysis of potentially confounding factors (as reported in §II.4.13), by and large, found a lack of correlation between the assessment results and the factors under investigation. The key exceptions to this lack of correlation primarily concerned the intensity of FES applied. The BMI-FES condition was found, on average, to use higher APB stimulation intensities than the EMG-FES and FES conditions (Figure 35). While the APB stimulation intensities were found by themselves to only slightly correlate with grip force, and not for any other outcome measures, the overall FES stimulation intensity was found to have a significant positive correlation with changes in grip force (Figure

34). Taken together, these results would seem to provide slight evidence to suggest that the increased APB FES intensity in the BMI-FES condition may have slightly skewed results in its favour, particularly on the grip force measure, but with the insinuation that this effect may spill over into other assessments. However, a contrary argument comes from the finding that APB FES intensity in fact trended towards having a negative correlation with changes in the maximum MEP. Given the full body of this evidence, it seems unlikely that the variation in stimulation intensities would have skewed the outcomes of the study to any considerable extent.

II.5.5. Potential Confounding Effects of Participant Engagement

One confounding factor not accounted for in the study design is participant engagement in the grasping training. Of the five treatment conditions, three (BMI, BMI-FES, EMG-FES) involve what is effectively biofeedback, since the user is able to see visualizations of their EEG or EMG activity. Users were intentionally allowed to see this feedback in order for them to obtain the best possible control over their stimulation. Using these control schemes may require the participants to be aware of their own physiological states, and may cause them to be more attentive to the grasping practice. This attentiveness could cause increased descending drive from the M1, which could in turn enhance spinal plasticity. Similar lines of reason suggest that attention, by increasing cortical activity, could increase the effectiveness of cortical plasticity. Attention has shown to be an essential part of other plasticity-inducing stimulation protocols, such as PAS (Stefan et al. 2004) and repetitive TMS (Conte et al. 2007). The suggestion that similar effects could be seen in FES protocols, while not specifically established in the

literature, would be consistent with the finding that passive electrical stimulation did not induce as much neuroplasticity as electrical stimulation activated concurrently with voluntary activity (Barsi et al. 2008). The attention-based explanation of the present findings therefore suggests that the BMI-FES and EMG-FES conditions have their enhanced neuroplastic effects due to increased descending drive, rather than a more consistent pairing of descending drive with antidromic firing.

In truth, the two hypotheses are not mutually exclusive, and both converge on the eventual explanation of neuroplasticity through synaptic LTP. While no participants self-reported a sense that their attention or engagement was different across conditions, no direct empirical measure was employed, since engagement was not considered as a factor in the initial experimental design. Engagement and attention therefore cannot be ruled out as confounding factors.

The BMI condition should theoretically involve the same level of engagement as the BMI-FES condition. However, without the antidromic firing created by the FES, the increased descending drive caused by increased attention would not be able to fulfill the necessary conditions for LTP-like plasticity, and the overall neuroplasticity may not be enhanced. The inclusion of this group in the experimental protocol, and the fact that the BMI group tended to have less MEP upregulation than the BMI-FES group, therefore does not conclusively resolve the question of the importance of attention in inducing neuroplasticity.

The practical ramification of this debate is that if attention and engagement are primarily responsible for the neuroplastic effects, then similar results should be achievable through simpler, lower-cost modifications to FES training. A change in protocol as simple as reminding the participant to focus on the task at hand, or conducting training sessions only when the participant is alert and unfatigued, could hypothetically have the same effects. Elucidating whether or not this could be the case would, however, require an additional study, or at very least, additional treatment conditions added on to the present study.

II.5.6. Brain-Machine Interface Calibration Parameters

The BMI parameters selected by the calibration procedure shed light on the exact nature of the BMI used in the present experiment. The bandpowers used collectively covered the narrow frequency range from 10-15 Hz, and in all cases, a decrease in bandpower was taken as indicative of cortical activity. This is consistent with established BMI systems which primarily search for bandpower decreases (termed "event-related desynchronization", or "ERD"), in lower frequency bands such as alpha (8-13 Hz), beta (15-25 Hz), and mu (10-12 Hz) (Crone et al. 1998a, Pfurtscheller and da Silva 1999), and bandpower increases (termed "event-related synchronization", or "ERS") in higher frequency bands such as gamma (> 30 Hz) (Crone et al. 1998b).

The finding that longer mean window lengths were generally preferred is in strong agreement with previous work in our lab (McGie 2009), with the suggestion being that averaging over longer windows allows for a smoothing of the criterion function, which

may help to remove or minimize the effects of noise or contamination. It is worth noting, however, that the one participant who had the smallest possible mean window length selected (Participant 1) was also the only participant in which FES did not appear to considerably contaminate the EEG signal. It is reasonable that a shorter window would decrease the duration of contamination following the offset of FES, since the contaminated data would be included in the criterion signal for a shorter period of time. During an extended period of FES, however, this effect should not occur. At present, no satisfactory explanation for this observation can be suggested. The possibility still remains that this is purely coincidental. A more thorough investigation of this possible phenomenon was not within the scope of the present study, but could be beneficial to future applications of BMI-FES.

Every participant was noted to have a "required duration" of 1 "hit", the lowest possible value. While the reasons for this remain unclear, one suggestion comes from the observation that most participants did not obtain measurable volition control over the BMI. In such an instance, the criterion signals from the resting and active periods during calibration may not have differed greatly from each other. The ideal threshold will therefore be at approximately the mean value of the criterion signal. Since the signal oscillates rapidly around this mean, the threshold would be crossed many times. As such, a parameter set which required a long duration before switching modes would be very unlikely to reach that requirement before the threshold was crossed again, and would result in a constant prediction of rest, resulting in a 'chance' prediction accuracy of 50%. A system with a short required duration, however, would be able to generate some

predictions of activity, and would therefore have the potential to rise above 50% prediction accuracy.

II.5.7. Barriers to Clinical Application

While the MEP-based results suggest the potential for BMI-FES and EMG-FES to further improve hand function in individuals with SCI, a number of barriers stand between these findings and successful clinical application. The most obvious concerns pertain to the design of this study, specifically the short time-frame and the use of able-bodied participants. The assumption that short-term, MEP-based outcomes are indicative of a treatment's potential for functional benefit was supported by existing literature in the Introduction (II.1.3.2), but the observed decreases in MEP in response to a treatment that is known to provide functional benefit (conventional FES) highlight the potential limitations of this approach. However, this specific concern will be more thoroughly discussed in the "Limitations of Short-Term Methodology" section (§IV.1), and is primarily mentioned here in order to illustrate a specific example of the limitation.

Another set of concerns pertains more to the practical logistics of employing these control strategies in individuals with SCI. Both BMI-FES and EMG-FES require the application of recording electrodes, which increases the necessary setup time for each FES treatment session. Physiotherapists or other individuals conducting these sessions may find this extra time cumbersome, especially the application of EEG electrodes, which require careful measurement of the user's scalp. They may also find the need to monitor the control system bothersome and distracting. In particular, technical issues

such as dealing with the stimulation artifacts would require them to have a fairly thorough understanding of the control system, which would likely be well outside of the experience of most physiotherapists or other potential users, and it is not yet clear to what extent these processes could be simplified, automated, or streamlined in future applications. It was frequently observed during the current study that the novel control systems, particularly the BMI, required frequent maintenance and attention, as the quality of signals shifted over the session and various parameters needed on-the-fly modification. Practitioners may also find that by having to focus some of their attention on the control system itself, they may not be able to attend as thoroughly to the recipient and their grasping, which may be seen as a negative. Therapists or other practitioners who found this overly troublesome may instead wish to resort to the simpler push-button controlled FES, in spite of any potential for functional benefit. Successful adoption of EMG-FES and especially BMI-FES in a clinical setting may therefore be prevented due to their greater complexity.

Additionally, not all users may be able to properly employ these novel control strategies. The ability to use a BMI is fairly universal, but may be marred in cases where the user has a traumatic brain injury comorbid to their SCI. This has been suggested to be the case in as many as 60% of SCI cases (Macciocchi et al. 2008), though the majority of these cases are quite mild. More concerning is the use of EMG-FES, since it requires users to retain at least some degree of voluntary function in the affecting muscles. This suggests greater potential for use among individuals with incomplete, rather than complete SCIs, although this was already the proposed application based on neurophysiological

principles (§I.2). Additionally, at least 50% of individuals with tetraplegia caused by SCI develop spasticity (Noreau et al. 2000), in which muscles develop a certain rigid tone. Having constant ongoing muscle activity may greatly degrade the signal-to-noise ratio of the employed EMG signals, and therefore result in less accurate FES control. The extent to which this may be an issue cannot be determined in the present study.

Part III: Spinal Associative Stimulation Study

Chapter III.1: Introduction

III.1.1. Spinal Associative Stimulation

One challenge of trying to induce spinal LTP-like plasticity with FES is that it requires the pre-synaptic activity to come from the user's own volition. If this activity were instead under external control, then the pairing between it and the post-synaptic activity could be assured by precise timing.

Researchers hoping to devise novel rehabilitative treatments for individuals who have had a stroke or strokes have developed a technique called "paired associative stimulation" (PAS) (Stefan et al. 2000), which functions on the same principle as BMI-FES and EMG-FES, yet targets the motor cortex for plasticity rather than the spinal synapse. In PAS, ascending sensory fibers in the limb of interest are stimulated. Unlike FES, this typically involves single or low-frequency pulses, and is therefore termed "peripheral nerve stimulation" (PNS) rather than FES. Signals from PNS ascend through normal sensory pathways until they reach the primary somatosensory cortex, which in turn connects to M1 through various networks. At the moment that these signals are expected to reach M1, the brain is non-invasively stimulated through TMS. If the timing is correctly predicted, then the motor cortex becomes transiently upregulated. This upregulation has been shown to be dependent on NMDA channels (Stefan et al. 2002) and Ca^{2+} influx (Wolters et al. 2003), and is therefore proposed to act through LTP. That PAS is highly dependent upon the delay between the two stimuli (termed the inter-

stimulus interval, or ISI) (Wolters et al. 2003) further suggests that it is acting through LTP.

More recently, it has been suggested that the spinal synapse, rather than the cortex, could be a potential target for this kind of paired stimulation. In this case, the PNS is intended not to stimulate sensory fibers, but rather, to send antidromic firing through the αMNs, back to the spinal cord, much as has been suggested to happen during FES (Rushton 2003). Meanwhile, the TMS, rather than being intended to stimulate the cortex directly, is intended to send a descending signal down through the corticospinal tract. If properly timed, the two signals should meet at the spinal synapse and induce LTP-like plasticity there. Cortes et al. (2011) coined the term "spinal associative stimulation" (SAS) to distinguish this process from PAS.

III.1.1.1. Critical Review

The field of SAS is much less developed than that of PAS, and can currently be said to consist of only 4 studies, which are summarized in Table 3.

Table 3. Summary of the literature concerning spinal associative stimulation.

Authors	Year	Target muscle	Population	Stimulation frequency (Hz)	Treatment duration (min.)	Number of pulses
Taylor & Martin	2009	Biceps	Able-bodied	0.1	8.3	50
Leukel et al.	2012	Soleus	Able-bodied	0.2	30	360
Cortes et al.	2011	Soleus	Able-bodied	0.1	15	90
Bunday & Perez	2012	FDI	Able-bodied & SCI	0.1	16.6	100

Authors	Year	Interstimulus interval (ms)	PNS intensity	TMS intensity	Outcome measures
Taylor & Martin	2009	-3	M_{max}	"slightly above" RMT	CMEP, matched forces
Leukel et al.	2012	1	H = 15-25% M_{max}	1x RMT	TMS-facilitated H-reflex
Cortes et al.	2011	-20	H = 0.5-1 mV	0.8 x RMT	H-reflex
Bunday & Perez	2012	4 to 6	1.2 x M_{max}	100% MSO	MEP, CMEP, F-wave (during SAS only), pegboard

"H" refers to the H-reflex, while "M_{max}" refers to the maximum M-wave evoked through peripheral nerve stimulation. "RMT" refers to the resting motor threshold for evoking MEPs, while "MSO" refers to the maximum TMS stimulator output. A positive interstimulus interval is one in which the PNS precedes the TMS, while a negative interstimulus interval is one in which the TMS precedes the PNS.

The first such study (Taylor and Martin 2009) involved 15 able-bodied participants, who received 50 paired pulses targeting the biceps, at a frequency of 0.1 Hz. Both PNS and TMS were suprathreshold, with the PNS being set to evoke a maximal M-wave, and the TMS being set "just above" the threshold for observing motor evoked potentials (MEP). Corticospinal transmission was determined by cervicomedullary evoked potentials (CMEP), which are similar to MEPs, except that the stimulating coil is held over the base of the participant's neck, in order to evoke activity in the corticospinal tract without involving the cortex. When the ISI was chosen appropriately, the bicep CMEP amplitude was upregulated for approximately 30 minutes, as indicated in Figure 39. In this case, the optimal ISI was one in which the TMS preceded the PNS by 3 ms.

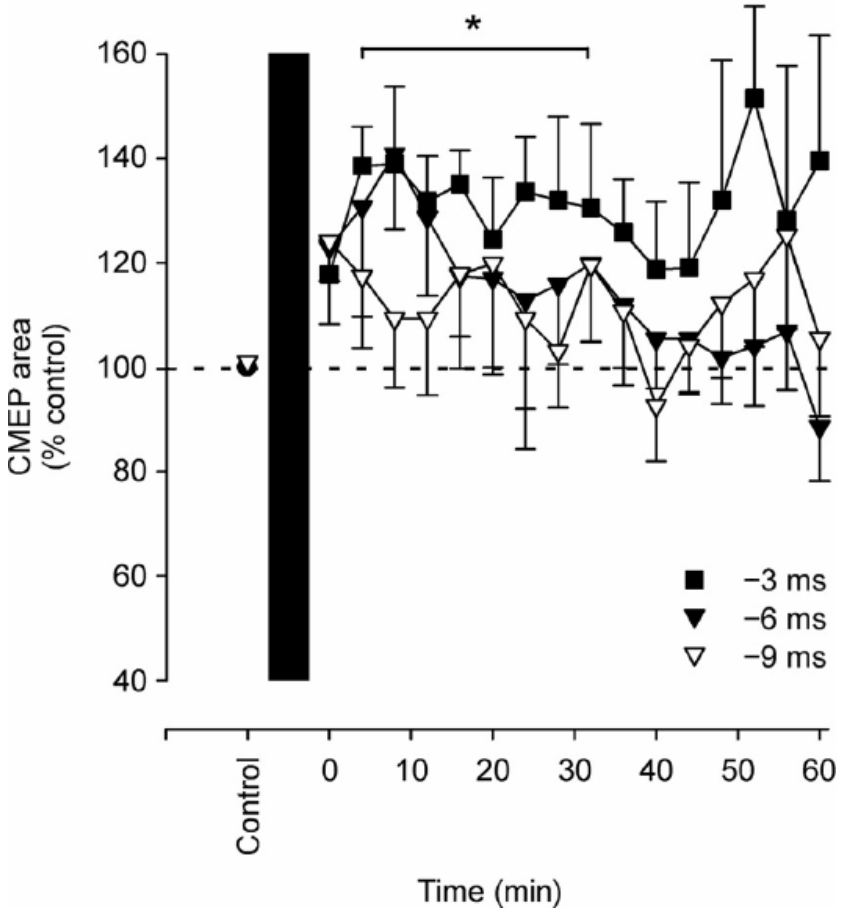

Figure 39. Reproduced from Taylor and Martin (2009). Biceps cervicomedullary evoked potential (CMEP) amplitude is shown to increase for 30 minutes following 50 pairs of 0.1 Hz SAS in able-bodied participants. The different symbols indicate different interstimulus intervals (ISIs) between the TMS and PNS. Note that the figure has been modified, in order to harmonize the ISI values presented with the definitions used in a broader scope of literature, in particular the literature focussing on PAS. While the original paper defined a positive ISI value as one in which TMS precedes PNS, the typical definition of a positive ISI value is one in which PNS precedes TMS. The original figure therefore had the ISIs listed as +3 ms, +6 ms, and +9 ms, respectively. * indicates a significant difference ($p < 0.05$) between the -3 ms group and controls.

A novel assessment was also employed, termed the “matched forces” test. Participants were asked to make small ballistic movements with the same force on both sides of the body. The participants were unaware that the visual feedback that they were basing their movements pertained only to the side of their body that was not trained by the SAS. Since the untrained side should not have been affected by the intervention, the descending command that they send to both sides of the body is expected to be equal. A functionally-relevant upregulation of the corticospinal tract on the trained side was therefore expected to be visible as an increased force or EMG activity in response to this identical descending command. The same ISI which showed maximal CMEP upregulation was shown to also maximally upregulate both force and EMG activity during the matched forces test, as shown in Figure 40.

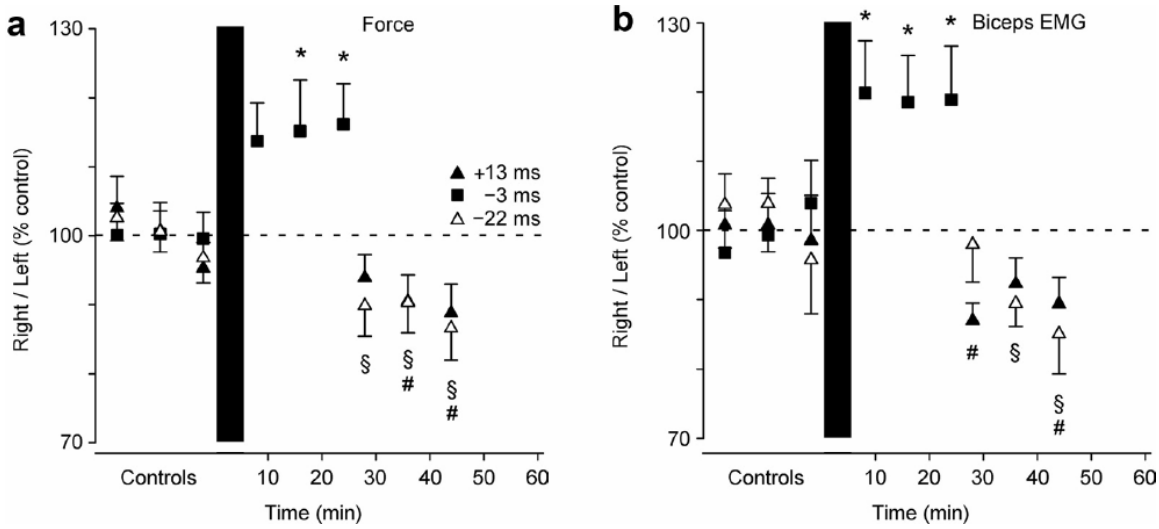

Figure 40. Reproduced from Taylor and Martin (2009). The amount of force and the level of EMG activity generated by the trained (right) bicep were increased for at least 25 minutes following 50 pairs of 0.1 Hz SAS in able-bodied participants. The thick black bar represents the period in which the SAS was applied. The different symbols indicate different interstimulus intervals (ISIs) between the TMS and PNS. Note that, as with Figure 39, the figure has been modified in order to harmonize the ISI values presented with the definitions used in a broader scope of literature, in particular the literature focussing on PAS. The original figure therefore had the ISIs listed as -13 ms, +3 ms, and +22 ms, respectively. # indicates a significant difference ($p < 0.05$) between the +13 ms group and controls. * indicates a significant difference ($p < 0.05$) between the -3 ms group and controls. § indicates a significant difference ($p < 0.05$) between the -22 ms group and controls.

On the whole, this study provides strong evidence to suggest the potential of SAS, but it is limited by its use of able-bodied participants, short-term effects, and a single stimulation frequency.

The second SAS study (Cortes et al. 2011), enrolled 13 able-bodied participants, who received 15 minutes of 0.1 Hz paired stimulation, targeting their soleus muscle. The PNS intensity was set to evoke a small (0.5-1 mV) H-reflex, which is likely to be sub-threshold for direct motor activation (M-wave). Similarly, the TMS was set to a sub-threshold level, approximately 80% of the motor threshold for MEP. In both modalities, this represents the only instance in the SAS literature where exclusively subthreshold stimulation is used. Only one ISI was tested, in which TMS preceded PNS by 20 ms (in accordance with the definitions we will use, this is therefore an ISI of -20 ms). The outcome measures for this study all pertained to the H-reflex recruitment curve. The threshold, amplitude, and width of the recruitment curve were all shown to decrease following SAS, as indicated in Figure 41. While these results certainly show some degree of neuroplasticity, the exact nature of this plasticity and how it may contribute to future therapies remain unclear.

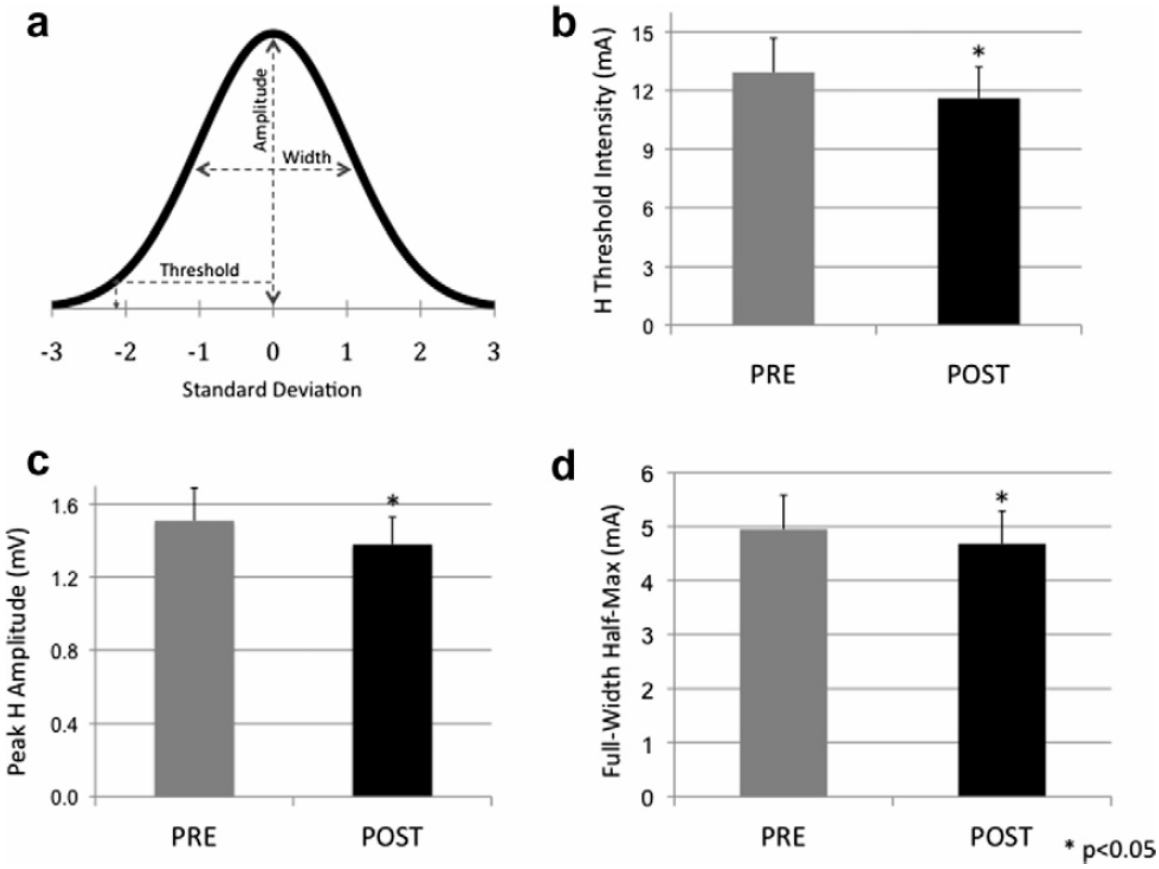

Figure 41. Reproduced from Cortes et al. (2011). (a) shows an example of a fitted Gaussian curve meant to approximate the recorded H-reflex recruitment curves, with stimulation intensity on the horizontal axis and reflex amplitude on the vertical. Threshold intensity (b), peak amplitude (c), and Gaussian width (d) are all shown to significantly decrease following the SAS intervention.

This study shares the same limitations as Taylor and Martin (2009), in that it focuses on short-term effects with able-bodied individuals, and uses only a single stimulation frequency. Furthermore, its choice of outcome measure greatly limits its utility, since H reflexes do not indicate actual motor function or even corticospinal connections. Rather, they indicate the specific synaptic strength between Ia afferent fibers and αMNs, and also

the general excitability of the αMNs. The use of only a single ISI further limits the utility of the study.

The third SAS paper (Leukel et al. 2012), recruited three groups of able-bodied volunteers. Group 1 (n=9) and Group 2 (n=8) received an SAS intervention, which consisted of 30 minutes of 0.2 Hz paired stimulation, targeting the soleus, with an ISI of +1 ms. PNS intensity was set to induce an H-reflex that was approximately 15-25% the size of the maximum M-wave (it is not clear whether this intensity was generally sub-threshold or supra-threshold with regards to the M-wave). TMS intensity was set to the approximate motor threshold for MEP. Group 3 (n=9) received only 0.2 Hz repetitive TMS at the same intensity. In Groups 1 and 3, the outcome measure was the amplitude of the soleus H-reflex when it was facilitated by appropriately timed TMS to M1. In Group 2, the outcome measure was the same facilitated H-reflex amplitude, but with TMS facilitation coming from the cervicomedullary junction. The conditioned H-reflex amplitude was seen to increase after SAS for certain ISIs tested, but not for others, with results being less clear, but still occasionally significant, when the conditioning happened through the cervicomedullary junction. The conditioned H-reflex amplitude was seen to either be unaffected or to decrease slightly following repetitive TMS. The results are summarized in Figures 42 and 43. As with the Cortes et al. (2011) study, these results may show some degree of neuroplasticity, but the exact location of this plasticity (that is, exactly which synaptic connections are affected) and how it may contribute to future therapies remain unclear. It is important to note that while different ISIs were used for H-reflex facilitation, only one ISI (+1 ms) was used for the SAS intervention.

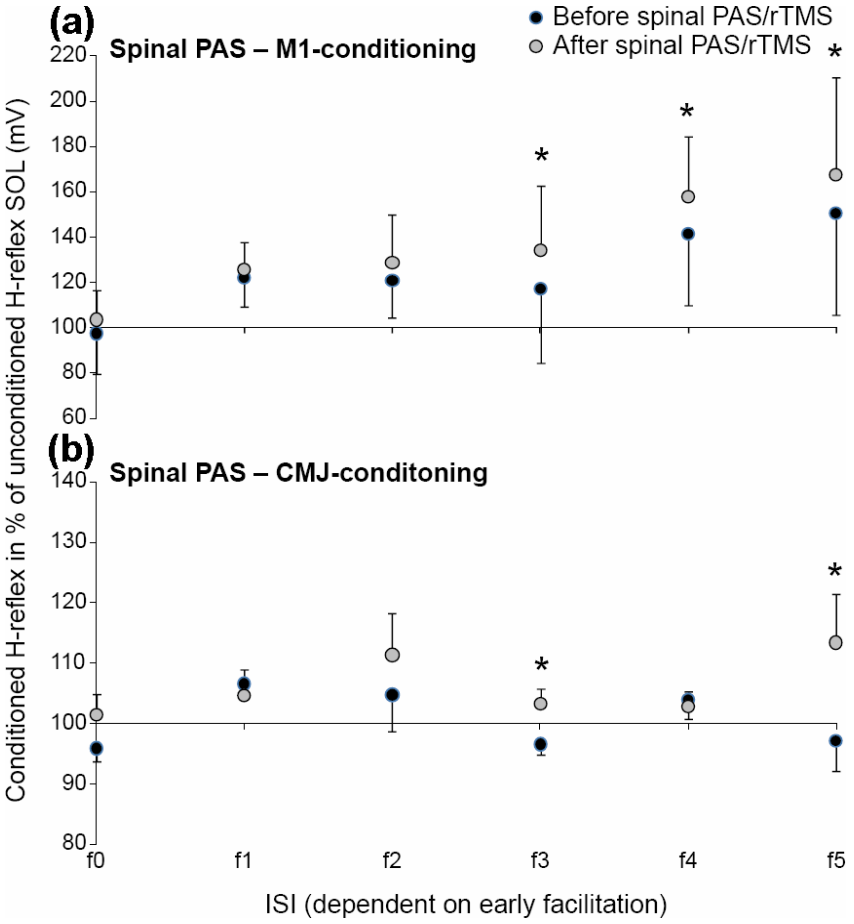

Figure 42. Reproduced from Leukel et al. (2012). Section (a) represents Group 1, in which the effects of SAS were examined with H-reflexes facilitated by TMS delivered to M1. Section (b) represents Group 2, in which the effects of SAS were examined with H-reflexes facilitated by TMS delivered to the cervicomedullary junction. The bottommost figure represents Group 1, in which the effects of repetitive TMS were examined with H-reflexes facilitated by TMS delivered to M1. * indicates a significant difference between pre- and post-intervention assessments ($p < 0.05$). The ISIs referred to on the horizontal axis are those used for the H-reflex facilitation, not those used in the SAS intervention. The exact ISIs varied among conditions, but are all separated by 1 ms difference. In the section (a), the ISIs range from +5 ms (f0) to -1 ms (f5). In section (b), the ISIs range from +9 ms (f0) to +4 ms (f5).

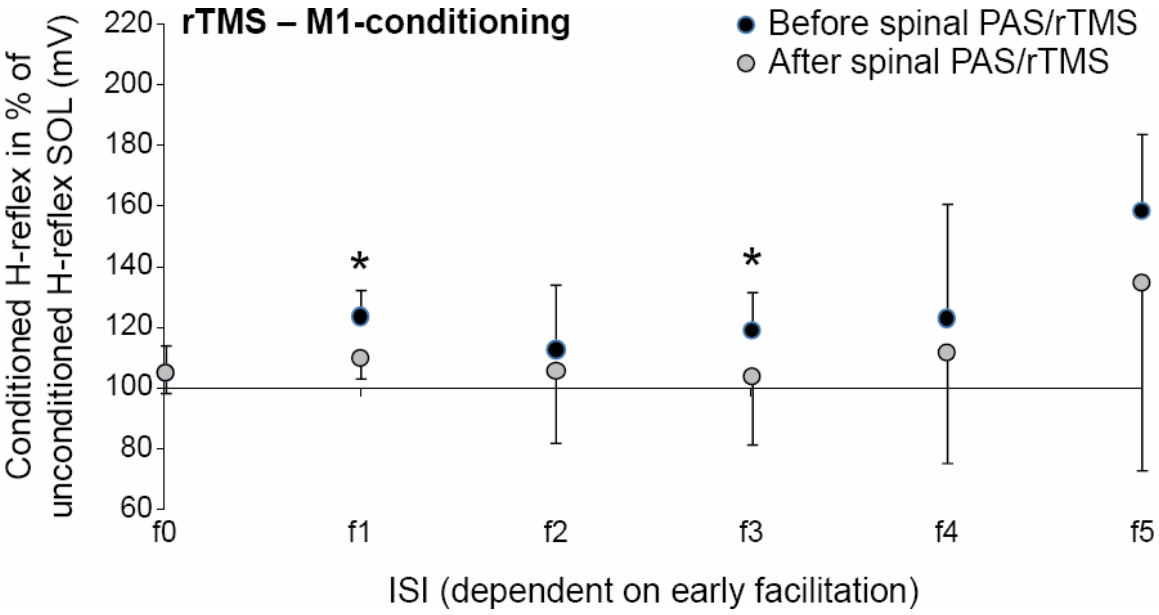

Figure 43. Reproduced from Leukel et al. (2012). This figure represents Group 1, in which the effects of repetitive TMS were examined with H-reflexes facilitated by TMS delivered to M1. * indicates a significant difference between pre- and post-intervention assessments ($p < 0.05$). The ISIs referred to on the horizontal axis are those used for the H-reflex facilitation, not those used in the SAS intervention. The exact ISIs varied among conditions, but are all separated by 1 ms difference. In the this figure, the ISIs range from +5 ms (f0) to -1 ms (f5).

This study, in addition to sharing the common limitations of the two aforementioned studies (able-bodied participants, short-term effects, and a single stimulation frequency), solely employs an unconventional and poorly-established outcome measure, whose interpretation is open to debate. The high variability in the results makes it even more difficult to derive meaningful conclusions. It furthermore explored only a single ISI value in its intervention, and one which disagrees strongly with the suggested ISI proposed by Cortes et al. (2011).

The final SAS study (Bunday and Perez et al. 2012) was novel in that, in addition to 14 able-bodied individuals, it recruited 19 individuals with chronic, incomplete SCI at levels C4-C8. The SAS protocol consisted of 100 paired pulses delivered at 0.1 Hz, targeting the first dorsal interosseus (FDI) muscle. For participants with SCI, the TMS amplitude was set to 100% of the maximum stimulator output (MSO), to compensate for the fact that the descending signal was expected to be greatly diminished at the injury site. The TMS amplitude for the able-bodied participants is not stated,. The PNS amplitude was set to 120% of the minimum intensity required to evoke a maximal M-wave. In both modalities, then, this represents the strongest stimulation employed in any of the SAS papers. The ISI used for plasticity induction was approximately 6.5 ms, which the ISI used for "control" stimulation was approximately 13 ms. The study employed a wide variety of assessments. MEPs, in response to both TMS and transcranial electrical stimulation, were shown to increase for at least 30 minutes following SAS, in both able-bodied and spinally-injured participants, as indicated in Figure 44.

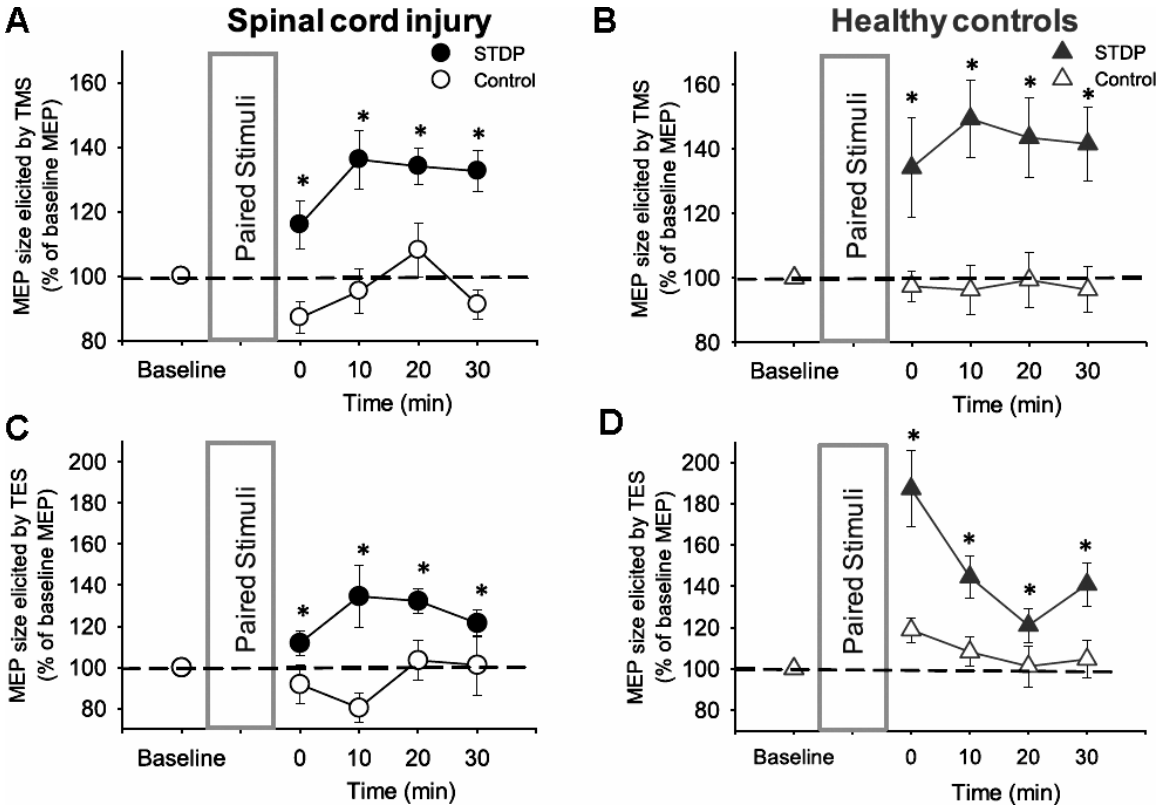

Figure 44. Reproduced from Bunday and Perez (2012). MEP is shown to increase for at least 30 minutes following SAS. Parts (A) and (B) indicate MEP evoked by TMS, while parts (C) and (D) indicate MEP evoked by transcranial electrical stimulation. Parts (A) and (C) indicate participants with SCI, while parts (B) and (D) indicate able-bodied participants. "STDP" refers to "spike-timing-dependent plasticity", which the authors use to refer to their SAS protocol. * indicates a significant difference between the STDP/SAS group (ISI = 6.5 ms) and controls (ISI = 13 ms) ($p < 0.05$).

Voluntary motor output was tested by asking participants to produce contractions of approximately 10% MVC. While visual feedback was provided, it was varied slightly in an effort to allow participants to rely on their own internal calibrations (that is, their motor cortex's preconception about how much descending drive is necessary to create a

certain level of muscle activity). This therefore serves the same purpose as the "matched forces" method in Taylor and Martin (2009), albeit seemingly less controlled. In this test, both force and EMG activity were shown to increase for at least 30 minutes following SAS, in both able-bodied and spinally-injured participants, as indicated in Figure 45.

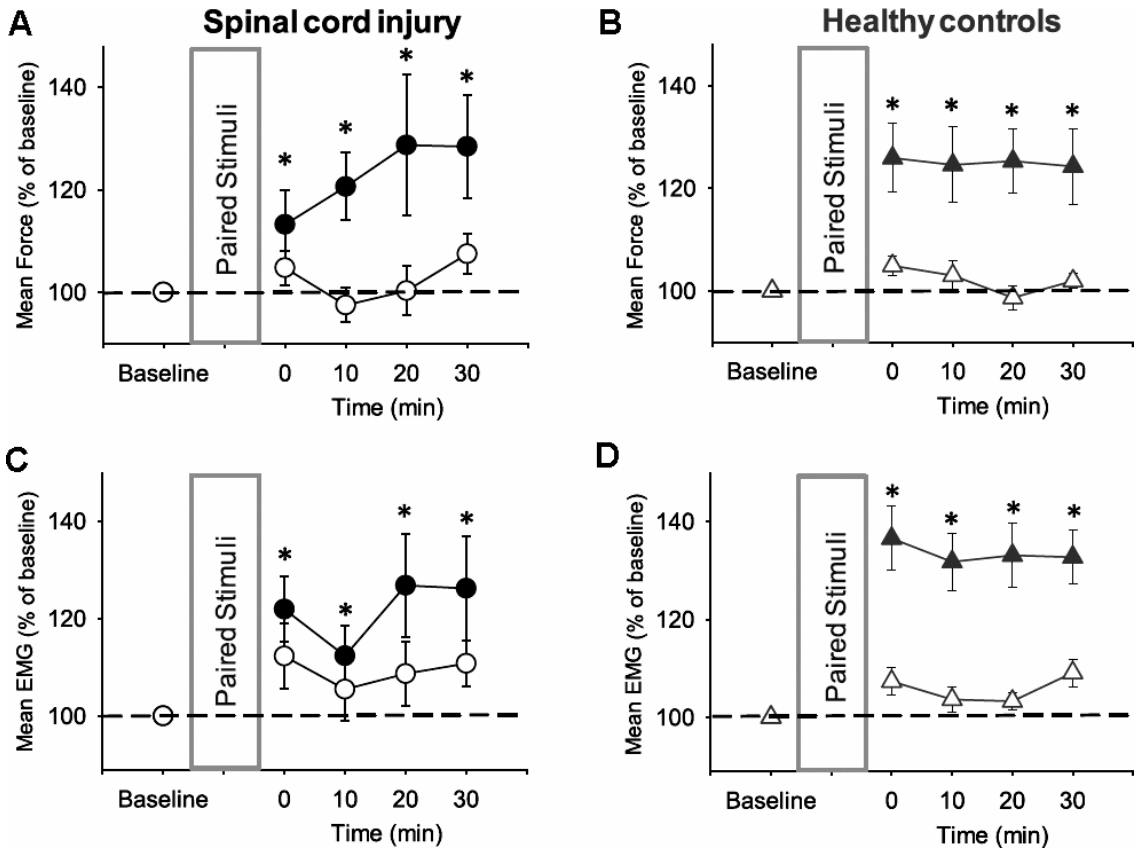

Figure 45. Reproduced from Bunday and Perez (2012). Force and EMG activity during voluntary motor output are shown to increase for at least 30 minutes following SAS. Parts (A) and (B) indicate force, while parts (C) and (D) indicate EMG activity. Parts (A) and (C) indicate participants with SCI, while parts (B) and (D) indicate able-bodied participants. Filled circles represent the SAS protocol, while open circles represent the controls. * indicates a significant difference between the STDP/SAS group (ISI = 6.5 ms) and controls (ISI = 13 ms) ($p < 0.05$).

The final test was the nine-hole peg test, which was only employed with participants with SCI. This test requires the participant to pull 9 small wooden pegs out of the holes in which they are standing, place each in a small cup, and then replace each peg into the original holes. It is used as a standard test of manual dexterity for individuals with SCI (Kalsi-Ryan et al. 2012). The time required to complete this task was shown to significantly decrease 20 and 30 minutes after SAS, but not immediately or 10 minutes after, which is shown in Figure 46.

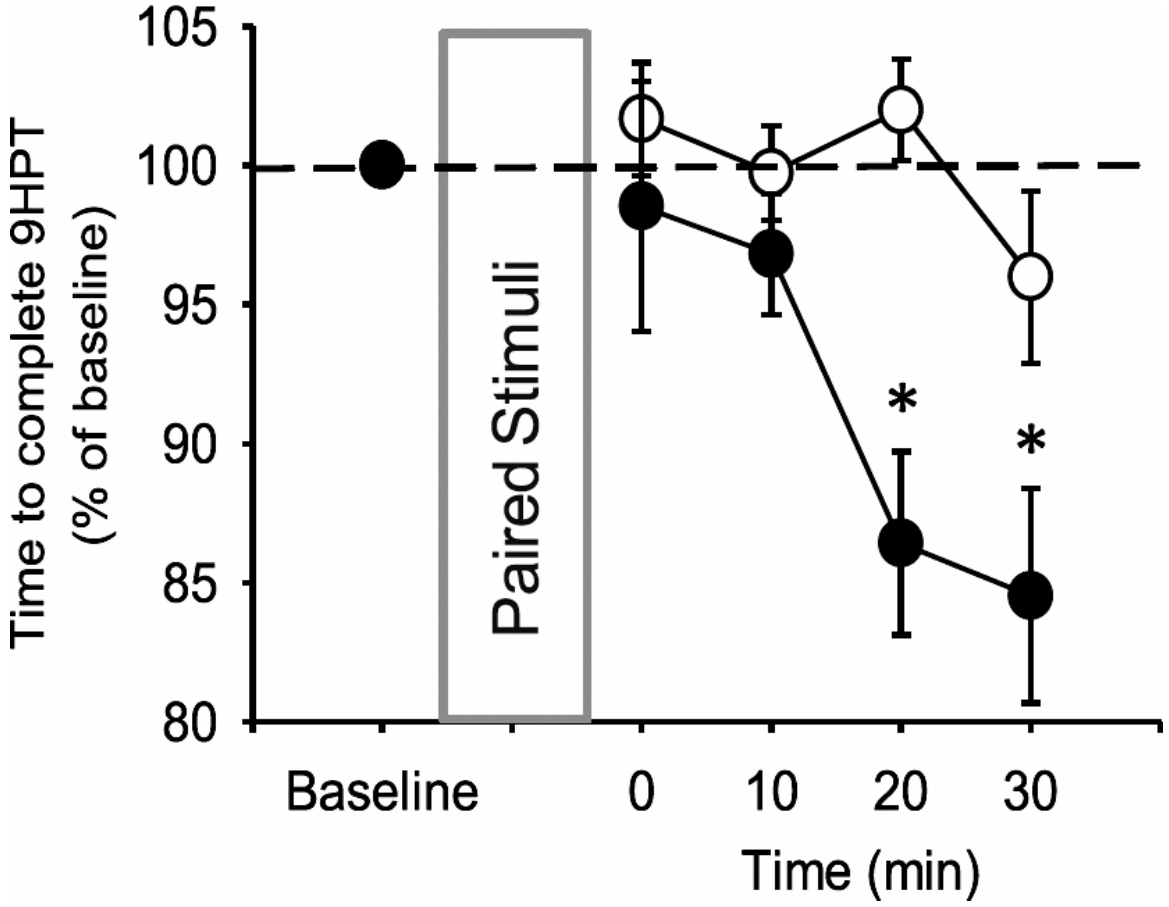

Figure 46. Reproduced from Bunday and Perez (2012). The time required for participants with SCI to complete the nine-hole-peg test is decreased 20 and 30 minutes after SAS, but not immediately or 10 minutes after. Filled circles represent the SAS protocol, while open circles represent the controls. * indicates a significant difference between the STDP/SAS group (ISI = 6.5 ms) and controls (ISI = 13 ms) ($p < 0.05$).

This final study provides the strongest evidence to date of the potential utility of SAS, by including a wide range of assessments, as well as participants with SCI. Its primary limitation is its use of a single stimulation frequency.

III.1.1.2. Summary

Taken as a whole, the SAS literature to date can be considered insufficient. The differences in assessment methods make it difficult to compare results from one study to the others, as do differences in the stimulation protocols, particularly in terms of ISI. Despite this, the stimulation frequency has gone relatively unexplored, with every study but one (Leukel et al. 2012) using 0.1 Hz stimulation. Therefore, two key goals of the SAS studies conducted herein will be to employ a wide range of outcome measures and to investigate a range of stimulation frequencies.

The issue of stimulation frequency is considered vital since it has been shown to be a key factor in modulating LTP-like plasticity in other stimulation protocols. The BCM curve (as discussed in §I.2), based on hippocampal slices, suggests that repetitive stimulation at low frequencies causes LTD (with the most LTD occurring with 1 Hz), and that stimulation frequencies of at least 20 Hz are necessary to induce LTP-like plasticity (Bear 1996). Studies investigating the potential therapeutic uses of TMS typically use high frequencies for this reason, such as 5 Hz (Kuppuswamy et al. 2011) or 20 Hz (Kumru et al. 2013). The extreme of this is theta burst stimulation, in which 50 Hz stimulation is applied in short bursts (Teo et al. 2010). While the majority of PAS studies employ low frequencies (on the range of 0.05-0.25 Hz (Ziemann et al. 2008)), some studies have been able to show positive results by pairing single pulses of TMS with high-frequency trains of PNS, such as 20 Hz (Uy et al. 2003) or 30 Hz (Pitcher et al. 2003). Pitcher et al. (2003) were additionally able to show LTD induction when 3 Hz stimulation was used rather than 30 Hz stimulation, lending credence to the suggestion

that the frequencies involved in the BCM curve may be applicable to more systems-level stimulation protocols. Based on this background and theory, the investigation of a range of stimulation frequencies will be considered as the primary objective of this study.

The SAS literature, similar to the PAS literature, is largely focussed on short-term effects. While a long-term study involving repeated sessions of SAS would be highly beneficial, it is unfortunately not considered practical to run such a large study within the scope of the present thesis. The SAS studies conducted herein will therefore continue to investigate short-term effects.

III.1.2. Objectives

The primary objective of the investigations into spinal associative stimulation is to test the effect of stimulation frequency on the outcome of the treatment. A secondary objective is to employ a wide range of outcome measures in order to more thoroughly evaluate the effects of the treatments. This study will be termed the “spinal associative stimulation” (SAS) study.

Chapter III.2: Hypotheses

- Participants are hypothesized to have improved grip force and MVC following 15 minutes of specifically-timed paired transcranial magnetic stimulation (delivered to the area of primary motor cortex controlling the APB muscle) and peripheral nerve stimulation (delivered to the recurrent branch of the median nerve, which innervates APB).

- These improvements are hypothesized to coincide with alterations in the APB MEP, either in terms of a lowered motor threshold, lowered stimulation intensity required to elicit a half-maximal contraction, or an increased maximal contraction.

- The changes are hypothesized to be short-lasting, and the return to baseline results should be visible within 60 minutes of the end of treatment.

- It is hypothesized that changing the frequency of paired stimulation should have an effect on these outcome measures, although the exact nature of this change cannot be hypothesized from the existing literature.

Chapter III.3: Methods

III.3.1. General

All procedures presented herein were approved by the Research Ethics Boards of the University of Toronto and the University Health Network, Toronto Rehabilitation Institute. All procedures were conducted in an electromagnetically shielded room (Raymond EMC, Canada).

III.3.2. Participant Populations

This study sought to recruit able-bodied volunteers. Participants were subject to the following criteria:

Inclusion criteria:

- Participants must be 19 to 65 years of age.
- Participants must have a clear understanding of the goal of the study, which is to develop and investigate the "spinal associative stimulation" technique so that it may later be tested as an intervention for individuals with SCI or other motor disorders.
- Participants must be able to understand English.

Exclusion criteria:

- Participants must not have a brain injury or brain-related neurological illness.
- Participants must not have any known motor disorders (for example, SCI, stroke, Parkinson's disease, amyotrophic lateral sclerosis, or muscular dystrophy).

- Participants must not have a previous seizure, epilepsy, or a history of seizure or epilepsy in any first-degree relatives.
- Participants must not be taking any medications which lower the seizure threshold (these include: antiasthmatics, antibiotics, antidepressants, insulin, estrogen, stimulants, neuroleptics, antihistamines, baclofen, lithium, and oxytocin).
- Participants must not have an allergic reaction to alcohol.
- Participants must not have a pacemaker.
- Participants must not have aneurysm clips.
- Participants must not have heart or vascular clips.
- Participants must not have prosthetic valves.
- Participants must not have any intracranial metal prostheses or metal in the head.
- Participants must not be pregnant or be unsure of whether or not they may be pregnant.
- Participants must not have tinnitus.
- Participants must not have bipolar disorder.
- Participants must not have increased intracranial pressure or a history thereof.

Two groups of able-bodied participants were recruited, one for experiments using the synchronous ISI and one using the asynchronous ISI (the details of these conditions are described below in §III.3.4.1).

In the “synchronous ISI” group, 9 participants completed the study, 4 male and 5 female. Two participants in this group declined to complete one session in the “5 Hz” condition (described below in §III.3.5) due to excessive discomfort from the TMS coil during the SAS intervention. Participants in this group ranged from 20 to 45 years of age (mean SD: 29.1 + 6.679). One participant in this group self-identified as left-handed – for this participant, all sidedness in the study was reversed (thus, TMS was applied to the left side of their head in order to affect their right hand), and the reported left-to-right ratio measures for MVC, grip force, and matched forces tests are in fact the right-to-left ratios.

In the “asynchronous ISI” group, 12 participants completed the study, 7 male and 5 female. One participant in this group declined to complete one session in the “5 Hz” condition (described below in §III.3.5), again due to excessive discomfort from the TMS coil during the SAS intervention. Participants in this group ranged from 25 to 45 years of age (mean ± SD: 30.0 ± 5.3). All participants in this group self-identified as right-handed.

Note that 7 participants (3 male and 4 female) participated in both the “synchronous ISI” and “asynchronous ISI” groups.

III.3.3. Overall Study Design

The overall study design is outlined in Figure 47 below.

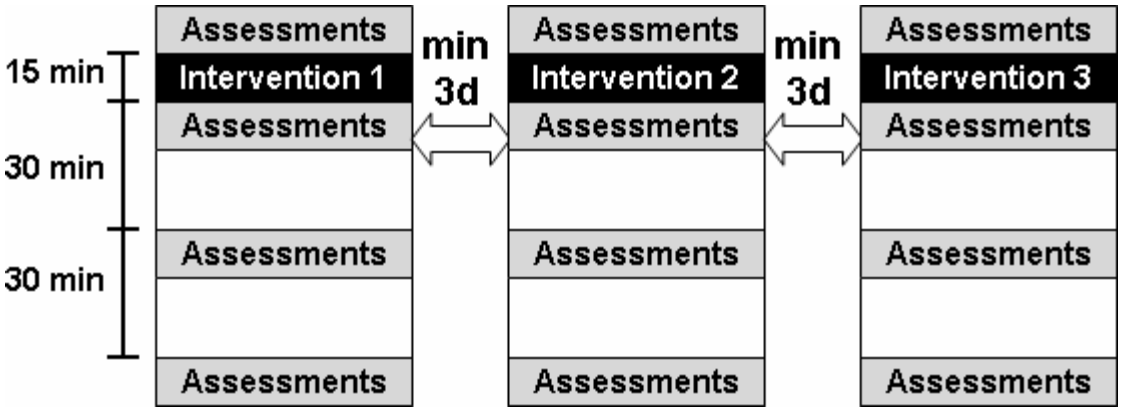

Figure 47. Overall design of the SAS study for each participant. 3 interventions were applied, each for 15 minutes, with assessments pre-intervention, immediately post-intervention, 30 minutes post-intervention, and 60 minutes post-intervention. Each session was separated from the others by a minimum of 3 days.

Each participant took part in 3 sessions (or 6 in total if they were in both the "synchronous ISI" and "asynchronous ISI" groups), each to test one of the 3 conditions (which are described in the "Intervention/Treatment" section below). Since each participant was undergoing each of the 3 interventions, this was a within-subjects design. Each session was separated from the others by a minimum of 3 days in order to minimize carryover effects from one session to the next. In all cases, both interventions and assessments pertained to the non-dominant hand (in able-bodied individuals) or the more-impaired hand (in individuals with SCI).

A given session consisted of a pre-intervention battery of assessments, and intervention, and 3 rounds of post-intervention assessments.

III.3.4. Initial Setup

EMG recording was set up exactly as described for the BMI-FES study (§II.3.5). The proper location and threshold for TMS stimulation was also established as per the BMI-FES study (§II.3.9.1.2). The proper location and threshold for PNS stimulation were not determined until either the F-wave latency determination (for the participant's first session, see section (§III.3.4.1.1)) or the beginning of the SAS treatment (for all subsequent sessions, see section (§III.3.5)).

III.3.4.1. Interstimulus Interval Determination

In each participant's first session of the SAS study, prior to the pre-intervention assessments, the ideal delay between the delivery of the TMS and peripheral nerve stimulation (PNS) pulses (termed the interstimulus interval, or ISI) with which to stagger PNS and TMS pulses during the SAS intervention was determined by measuring the latencies of two EMG response: the F-wave and the MEP. These latencies were taken as indicators of the approximate conduction times involved as neural responses to TMS and PNS travelled through the corticospinal tract (CST). The intention, as described in the Background section (§III.1.1) was to have the two signals meet at the spinal synapse, to facilitate long-term potentiation (LTP) induction. This step was considered necessary since different participants may have different conduction times, and may therefore require different ISIs in order for the two signals to effectively meet at the spinal synapse.

Participants in the “synchronous ISI” group received treatment with the ISI determined as below. Participants in the “asynchronous ISI” group received treatment with the actual ISI of each individual paired pulse being randomly distributed within the range of 15-20 ms greater than the ideal ISI.

III.3.4.1.1. F-Wave Latency

F waves (with “F” standing for “foot”, where they were first discovered) (Renshaw 1941) are EMG responses seen after high-intensity, low-duration pulses of PNS. The F wave is thought to result from antidromic firing along the nerve (that is, a wave of depolarization going back up the nerve to the cell body). When particularly strong antidromic firing reaches the spike initiation zone (the axon hillock), it may be capable of exciting neighbouring axons, sending orthodromic firing back down towards the muscle (Renshaw 1941). However, most of the neighbouring axons will likely be in a refractory state, since they are also undergoing antidromic firing, and will therefore unable to fire orthodromically. Only the small proportion of axons which were not involved in the original activation therefore contribute to the final EMG response (Renshaw 1941). The result is that the F-wave is generally of a very low amplitude. As the F-wave travels first antidromically back to the cell body, and then along the entire length of the axon to the muscle, it appears in EMG recordings much later than the direct orthodromic muscle activation (the M-wave). In some muscles, its latency roughly overlaps with that of the Hoffman reflex (the H-reflex), which indicates activation of the α motor neurons (αMNs) by Ia sensory afferents that are also activated by the PNS. However, the muscle of

interest for this study (APB) shows H-reflex in only 0-21% of individuals (Zehr 2002), and the F-wave is therefore generally visible.

F-wave was elicited using a custom bipolar probe electrode, which consisted of two flattened screw heads embedded in a plastic block, with the centers of the screws separated by approximately 2.2 cm. The electrode was attached to a Compex Motion stimulator (Compex, Switzerland), and was measured using the same Bortec EMG amplifier previously described (§II.3.5) (Bortec AMT-8, Bortec Biomedical, Canada). For this section, the left APB was recorded from, and the ground electrode was on the left APB channel, rather than the left FCR as it typically is. The amplified EMG was recorded using a Powerlab 16 SP system (AD Instruments, USA), running the LabChart software (AD Instruments, USA). The software was set to seek out the stimulation artifact (defined as the first moment in which the amplified EMG response exceeded 200 mV), and to record a period starting 10 ms prior to the artifact, and ending 90 ms after it. The Compex Motion stimulator was set to provide monopolar pulses with a pulse width of 100 μs every 2 s. The intensity of this stimulation was manually increased by 1 mA after each pulse, up to a maximum of either 70 mA, or when the participant asked to cease stimulation. Following this, the response was identified as a small deviation from the stimulation artifact pattern occurring approximately 20-30 ms after the stimulation artifact. The latency of the F-wave was determined by visual inspection, and was defined as the time between the first moment at which the stimulation artifact was visible and the first moment at which the F-wave was visible. Not all responses were analyzed in this manner, since the latency of the response, once it appeared, was relatively robust.

Approximately 5-10 responses were analyzed in this way, with preference given to those responses elicited at higher stimulation amplitudes. This method of latency determination, though crude and approximate, was deemed necessary since the large stimulation artifact and the small response would have made automated latency determination prohibitively difficult.

III.3.4.1.2. Motor Evoked Potential Latency

The TMS used to evoked MEP was delivered using the same stimulator used for the MEP assessment above (see §II.3.9.1) (Medtronic MagPro R30+M stimulator with a MagVenture MCF-B65 figure-eight coil, Medtronic, USA). EMG recording was and was measured using the same Bortec EMG amplifier previously described (§II.3.5) (Bortec AMT-8, Bortec Biomedical, Canada). As with the F-wave, recordings were taken from the left APB, and the ground electrode was on the left APB channel, rather than the left FCR as it typically has been in these experiments. Similarly, rather than being recorded through a custom LabView software as it is when determining the stimulus-response curve, the amplified EMG was recorded using a Powerlab 16 SP system (AD Instruments, USA), running the LabChart software (AD Instruments, USA). This was considered necessary since the custom LabView software involved significant processing delays, which were found in preliminary experiments to produce inaccurate estimations of MEP latency. The software was set to seek out the first moment in which the amplified EMG response exceeded 200 mV (corresponding to a pre-amplification response of at least 40 μV), and to record a period starting 50 ms prior to the artifact, and ending 50 ms after it. Whereas this trigger generally represented the stimulation artifact

in the F-wave calculation, it herein instead tended to represent the start of the MEP itself. Since only the latency was being measured in this section, rather than the entire stimulus-response curve, the stimulation intensity was kept constant at 70% MSO. 10 stimuli were provided, and the latency was determined from these by visual inspection, defined as the time between the first moment at which the stimulation artifact was visible and the first moment at which the MEP was visible. Participants remained at rest during the latency determination. This was considered vital since voluntary contractions can alter MEP latency (Weber and Eisen 2002), and was chosen in order to be consistent with the manner in which TMS was to be applied during the SAS intervention. Coil orientation can also affect MEP latency (Rothwell 1997), and was therefore also kept consistent between the latency determination procedure and the SAS intervention.

III.3.4.1.3. Calculation

Figure 48 outlines the various conduction times involved in the MEP and F-wave latencies.

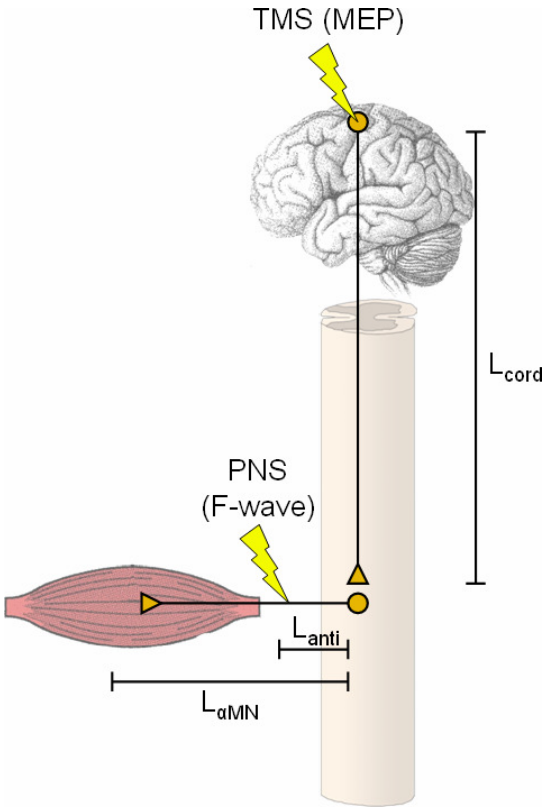

Figure 48. Outline of the various conduction times involved in the determination of the ideal inter-stimulus interval (ISI) for induction of spinal long-term plasticity (LTP) in spinal associative stimulation (SAS). “L” in this figure stands for “latency”, and “anti” is short for “antidromic firing”. TMS (transcranial magnetic stimulation) and PNS (peripheral nerve stimulation) are the two stimuli being paired, while MEP (motor evoked potential) and F-wave are the two observable muscle responses resulting from them.

The ideal ISI is defined as that in which the antidromic response to PNS and the orthodromic response to TMS meet at the spinal synapse. If we defined a positive ISI as meaning the PNS precedes TMS, and a negative ISI as meaning that TMS precedes PNS, then the ideal ISI is the time it takes for the antidromic PNS signal to reach the spinal

synapse, minus the time it takes for the orthodromic TMS signal to reach the spinal synapse. Therefore, with "L" standing for "latency":

$$ISI = L_{anti} - L_{cord} \quad (1)$$

The F-wave must travel antidromically from the point of stimulation back to the spinal synapse, and then orthodromically from the spinal synapse to the muscle. Its latency is therefore defined as:

$$L_F = L_{anti} + L_{\alpha MN} \quad (2)$$

This can be rearranged as follows:

$$L_{anti} = L_F - L_{\alpha MN} \quad (3)$$

The MEP must travel from the cortex, across the spinal synapse, and out to the muscle. Its latency is therefore defined as:

$$L_{MEP} = L_{cord} + L_{\alpha MN} \quad (4)$$

This can be rearranged as follows:

$$L_{cord} = L_{MEP} - L_{\alpha MN} \quad (5)$$

If (3) and (5) are substituted into (1), we derive:

$$ISI = (L_F - L_{\alpha MN}) - (L_{MEP} - L_{\alpha MN}) \quad (6)$$

The two $L_{\alpha MN}$ values cancel out (since this latency contributes equally to both L_F and L_{MEP}), and we are left with:

$$ISI = L_F - L_{MEP} \quad (7)$$

It is from (7) that the ideal ISI is calculated for each participant, following the determination of L_F and L_{MEP} in their first session of SAS. Note that the delay in response due to the neuromuscular junction (that is, the delay between acetylcholine release and muscle contraction) is not included, since it is present equally in both L_F and

L_{MEP}. The delay in L_{MEP} due to synaptic transmission at the spinal synapse is considered negligible.

III.3.5. Intervention/Treatment

The experimental setup for the SAS study is outlined in Figure 49.

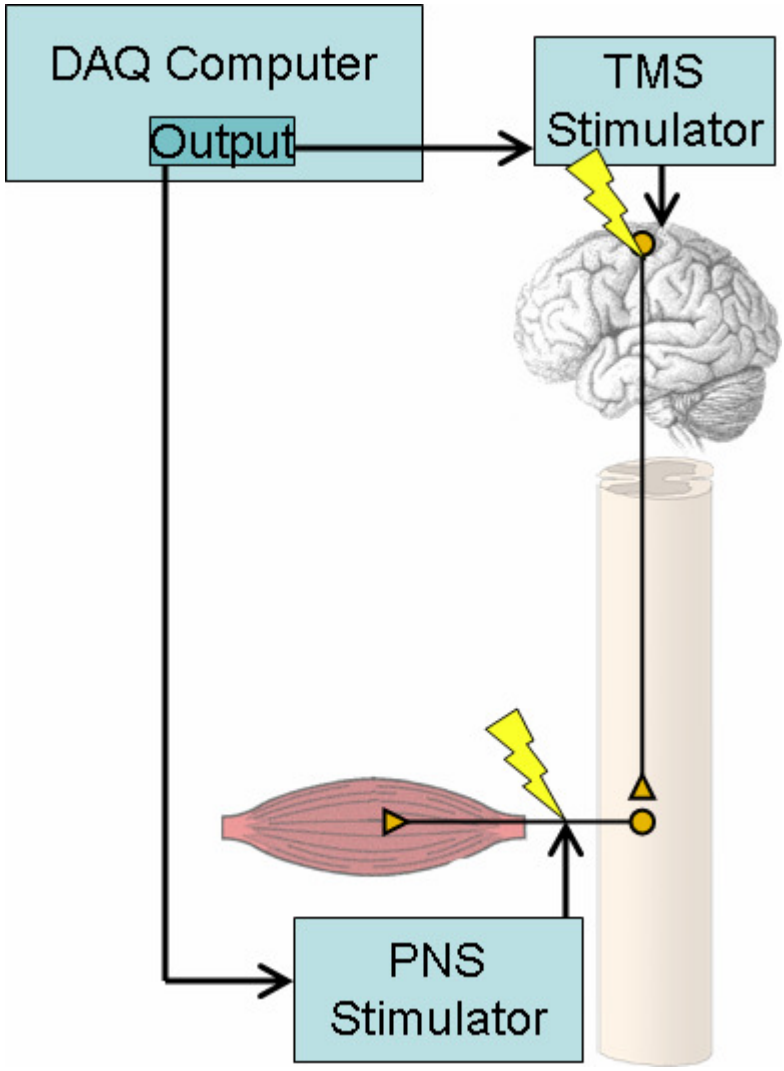

Figure 49. The experimental setup for the SAS study. The setup includes a data acquisition (DAQ) computer, which controls both the transcranial magnetic stimulation (TMS) unit (for stimulating the brain) and the peripheral nerve stimulation (PNS) unit.

Each of the three interventions consisted of 15 minutes of paired TMS and PNS. This duration was considered sufficient since it is comparable to the treatment durations employed in the SAS studies outlined in §III.1.1.1. The TMS was delivered using the same stimulator used for the MEP assessment above (see §II.3.9.1) (Medtronic MagPro R30+M stimulator with a MagVenture MCF-B65 figure-eight coil, Medtronic, USA). The TMS coil was positioned over the area that provided the optimal APB MEP during the pre-intervention assessments. The intensity of TMS stimulation was set to 1.2 times the threshold intensity for visible APB MEP. The PNS was delivered through the same custom-built bipolar probe used to elicit F-waves, connected to a Master8 stimulator (A.M.P.I., Israel) with two Iso-Flex isolator units (A.M.P.I., Israel). The PNS stimulator was placed one the underside of the wrist with a conductive gel (Spectra 360, Parker Laboratories, USA). Pulses of approximately 4 mA (adjusted for each participant) were delivered at 0.5 Hz. The optimal location was determined as that which provided the strongest visible contraction of the thumb. The electrode was then fixed in place with tape and surgical wrap. The intensity of the PNS stimulation was set to 1.2 times the threshold intensity for visible APB M-waves. The pulse-width of the PNS stimulation was kept constant at 1 ms. Both the TMS and PNS were controlled by a custom LabView script, with control signals being sent through an analog output of the computer's DAQ board (for the TMS) or through a USB connection (for the PNS). Participants were asked to remain relaxed for the duration of the stimulation. The ISI was set to the value previously determination as ideal (see §III.3.4.1).

The three treatment conditions differed primarily in the frequency of the paired stimulation provided. They were as follows:

1. Continuous paired stimulation at 0.1 Hz.
2. Continuous paired stimulation at 1 Hz.
3. Pulsed paired stimulation at 5 Hz, consisting of a 2 s train of 10 pulses followed by 8 s without stimulation.

These three protocols are graphically represented in Figure 50. Note that these protocols are independent of the "synchronous ISI" and "asynchronous ISI" groups – thus, if a given participant chose to enrol in both the "synchronous ISI" and "asynchronous ISI" groups, they would complete 6 sessions in total, one for each pairing of ISI group and treatment condition. It can be noted that some of these three conditions differ in other ways than the intended investigational variable (stimulation frequency), namely, in terms of the total number of pulses delivered, and the overall pattern of stimulation. This is was, however, a necessary limitation in order to maintain a constant duration of treatment across all three conditions. The ramifications of this limitation are more thoroughly discussed in §III.5.4. The rationale for choosing these stimulation protocols was based on LTP literature and theory (particularly the BCM curve and the therapeutic uses of high-frequency TMS, as discussed in §III.1.1.2), and issues of participant safety and comfort (Wassermann 1998). The "bursting" pattern used in the 5 Hz condition was validated by the finding that short breaks are necessary for proper induction of neuroplasticity when using a 5 Hz repetitive TMS protocol (Rothkegel et al. 2010). The 0.1 Hz condition was chosen in order to provide a point of comparison with the existing SAS literature, while the 1 Hz condition was chosen as a mid-point between the 0.1 Hz and 5 Hz conditions.

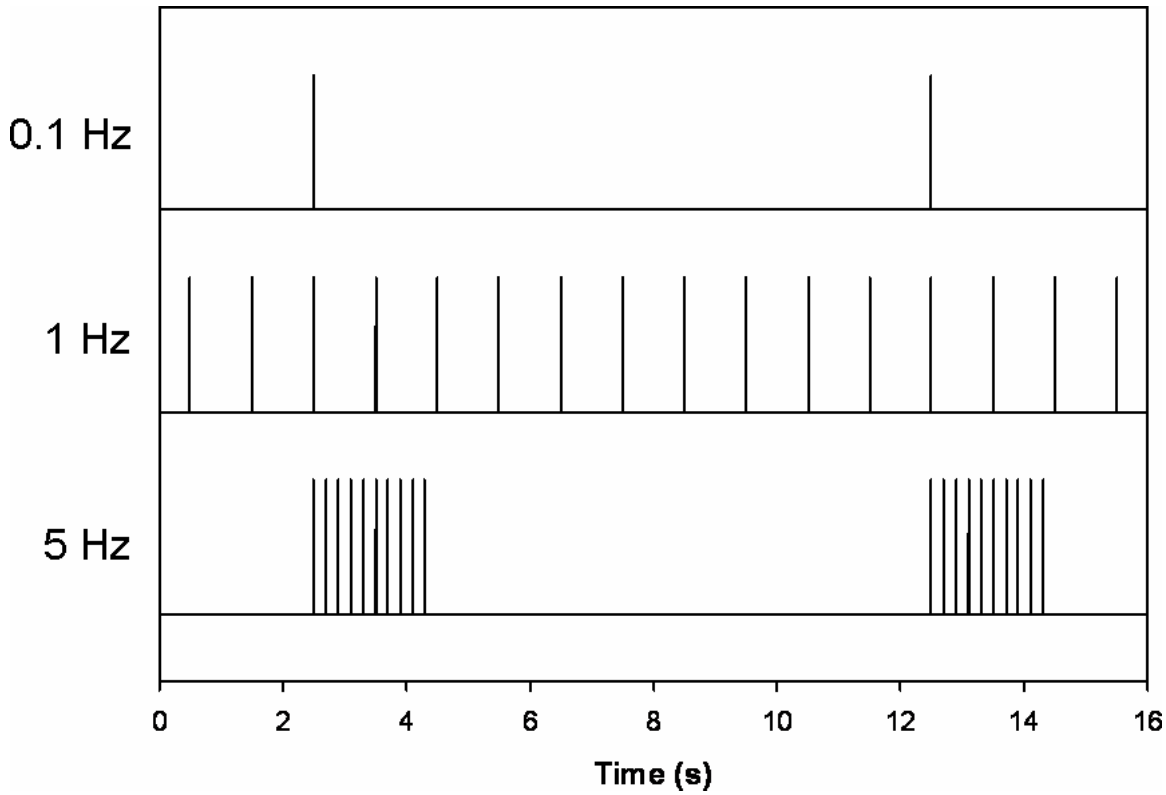

Figure 50. The three stimulation protocols employed in the spinal associative stimulation (SAS) study. Spikes represent both transcranial magnetic stimulation (TMS) and peripheral nerve stimulation (PNS) pulses, since the delays between the two stimulation types are too small to be seen at this time scale. While the 0.1 Hz and 1 Hz conditions provide continuous stimulation, the 5 Hz condition provides stimulation in 2 s bursts, with 8 s of rest in between.

III.3.6. Assessments

The assessments conducted in the SAS study were identical to those in the BMI-FES study (MEP, grip force, MVC, and matched forces), except that the BMI control test was not conducted in this study. Details of these methods can be found in §II.3.9. The key

difference is that while post-intervention assessment in the BMI-FES study was only conducted at a single time point (that being immediately following the end of the intervention), post-intervention assessment in the SAS study was conducted at 3 time points: immediately following the end of intervention, 30 minutes post-intervention, and 60-minutes post-intervention.

III.3.7. Statistics

The statistical methods applied in the SAS study were identical to those applied in the BMI-FES study (§II.3.11), since both studies employ a within-subjects design in which repeated measures are taken across time, and all participants receive all three treatments in separate sessions. That is, an initial repeated-measures ANOVA was applied to test for overall differences between treatment conditions. Following this, additional repeated-measures ANOVAs were conducted on data from pairs of treatment conditions. In all cases, the p-values reported in the Results section (§III.4) are those from the time x treatment interaction term, for the same reason that this term was chosen in the BMI-FES study (§II.3.11). . The assumption of normal distribution necessary for ANOVA analysis was confirmed by Lilliefors tests ($p \geq 0.0567$). The assumption of equality of variances was not rigorously examined, but visual examination of the data (Figures 51 through 59) suggests that this assumption is valid. These analyses were conducted separately for the "synchronous ISI" and "asynchronous ISI" groups. The key reason for this was the inter-individual variability. Comparisons between the two ISI groups could only be considered valid if they directly paired measurements for each subject, rather than pooling across

subjects prior to applying the comparisons. Since the two groups involved different participant populations, this condition could not readily be met.

Comparisons between the “synchronous ISI” and “asynchronous ISI” groups were made using two methods. In the first method, only the 7 participants who had participated in both groups were analyzed. The analyzed variables were the ratios of the values in the “synchronous ISI” condition to the values in the “asynchronous ISI” condition within a given participant’s session for a given treatment condition. These were then group both by treatment condition and by pairs of treatment conditions (in the latter case, ratios of values between the two treatment conditions were used as the input values), and analyzed with one-sample t-tests. In the second method, all participants were considered, regardless of whether or not they had participated in both ISI groups. Results were grouped by condition, disregarding which participant each data point came from, and were analyzed with an independent-samples t-test.

Additional correlation analysis was also conducted, as per the BMI-FES study, with the only difference being the reduced set of potential confounding factors tested:

- Participant age
- Participant gender
- TMS intensity
- PNS intensity

These analyses were conducted separately for the “synchronous ISI” and “asynchronous ISI” groups.

Note that the MEP threshold and the M-wave threshold were not included as potential confounding factors, since they, being proportional to the TMS intensity and PNS intensity, respectively (TMS intensity = MEP threshold x 1.2; PNS intensity = M threshold x 1.2), would produce exactly the same correlation values.

The additional analysis to determine possible fatigue effects was conducted in the same manner as in the BMI-FES study. However, only the differences between adjacent time points were compared (that is, pre-intervention values were compared to immediate post-intervention values, immediate post-intervention values were compared to 30 minutes post-intervention values, and 30 minutes post-intervention values were compared to 60 minutes post-intervention values). For this analysis, data from both the “synchronous ISI” and “asynchronous ISI” groups were pooled.

Chapter III.4: Results

Representative MEP recordings and recruitment curves in the SAS study were similar to those observed in the BMI-FES study (as presented in Figures 22 and 23).

III.4.1. Interstimulus Interval Determination and Stimulation Parameters

The ISI, defined as defined as ISI = $L_F - L_{MEP}$, was highly consistent across participants, ranging from 4 to 7 ms (mean 5.43 ± 0.98 ms). F-wave latency ranged from 22 to 29 ms (mean 26.667 ± 1.775 ms), while MEP latency ranged from 18 to 23 ms (mean 21.417 ± 1.311 ms). F-wave and MEP latencies were highly correlated ($r = 0.8070$, $p = 0.0015$), which is consistent with the suggestion that larger individuals will have increased conduction times in both the UMNs and αMNs. ISI was highly correlated with F-wave latency ($r = 0.6794$, $p = 0.0151$), but not with MEP latency ($r = 0.1150$, $p = 0.7220$), suggesting that the αMNs played a larger part in determining the ISI than the UMNs.

MEP thresholds were found to vary from 33 to 70% MSO (mean ± SD: 52.75 ± 7.85% MSO). Accordingly, TMS intensities during the intervention ranged from 40 to 84 %MSO (mean ± SD: 63.3 ± 9.4 %MSO). M-wave thresholds were found to vary from 0.70 to 4.10 mA (mean ± SD: 1.88 ± 0.68 mA), resulting in stimulation intensities during the intervention ranging from 0.84 to 4.92 mA (mean ± SD: 2.26 ± 0.82 mA).

III.4.2. Motor Evoked Potential: Maximum Response

The initial ANOVA tests failed to show significant variance in either group ($p = 0.9603$ for "synchronous ISI" and 0.7431 for "asynchronous ISI"). Pre-post changes in the "M"

value were not found to significantly differ across conditions in either ISI group ($p \geq 0.3411$). These results are summarized in Figure 51.

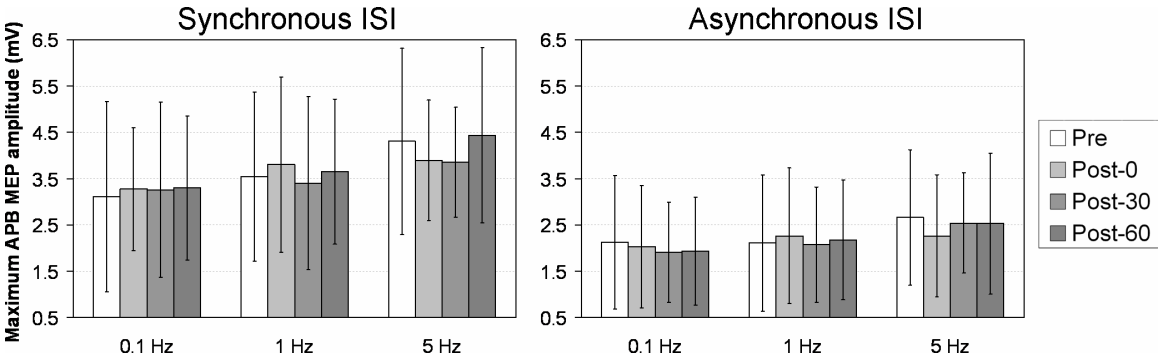

Figure 51. Mean maximum APB MEP amplitudes ("M"). No significant differences between treatment conditions were noted ($p \geq 0.3411$).

III.4.3. Motor Evoked Potential: Estimated Response to Half-Maximal Stimulation

The initial ANOVA tests failed to show significant variance in either group ($p = 0.9688$ for "synchronous ISI" and 0.9841 for "asynchronous ISI"). Pre-post changes in "MEP_H" were not found to significantly differ across conditions in either ISI group ($p \geq 0.7683$). These results are summarized in Figure 52.

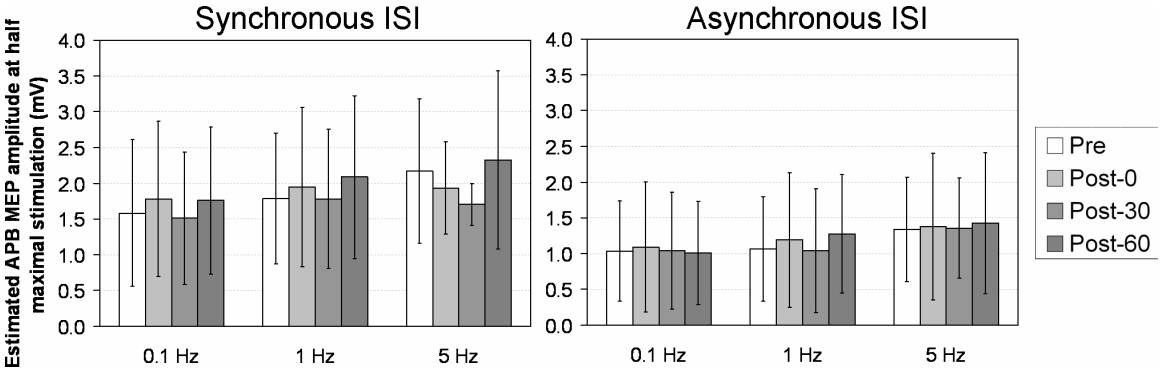

Figure 52. Mean estimated APB MEP amplitudes in response to the stimulus intensity which caused a half-maximal MEP pre-intervention ("MEP_H"). No significant differences between treatment conditions were noted ($p \geq 0.7683$).

III.4.4. Motor Evoked Potential: Response at 120% of Motor Threshold

The initial ANOVA tests failed to show significant variance in either group ($p = 0.6179$ for "synchronous ISI" and 0.4138 for "asynchronous ISI"). Pre-post changes in "MEP_{120}" were not found to significantly differ across conditions in either ISI group ($p \geq 0.4605$). These results are summarized in Figure 53.

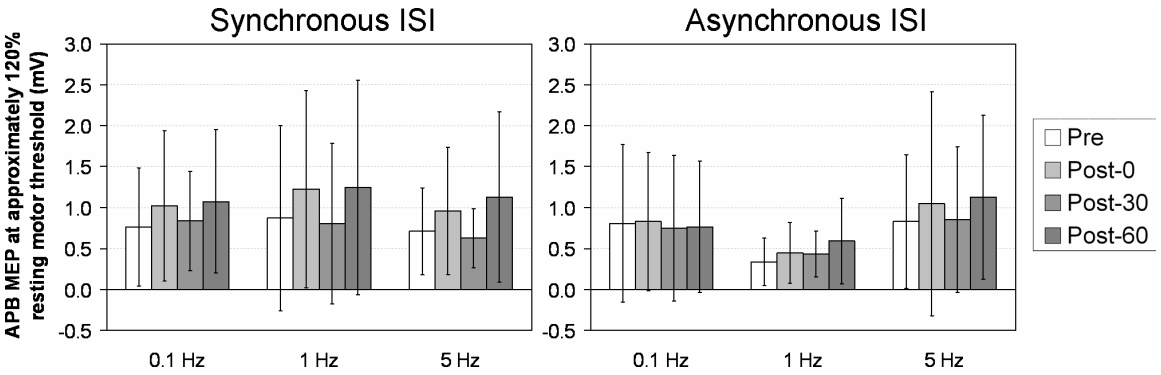

Figure 53. Mean APB MEP amplitudes at approximately 120% motor threshold. No significant differences between treatment conditions were noted ($p \geq 0.4605$).

III.4.5. Motor Evoked Potential: Response at Intensity Which Previously Provided 1mV Response

The initial ANOVA tests failed to show significant variance in either group ($p = 0.9618$ for "synchronous ISI" and 0.6641 for "asynchronous ISI"). Pre-post changes in "MEP_{1mV}" were not found to significantly differ across conditions in either ISI group ($p \geq 0.3079$). These results are summarized in Figure 54.

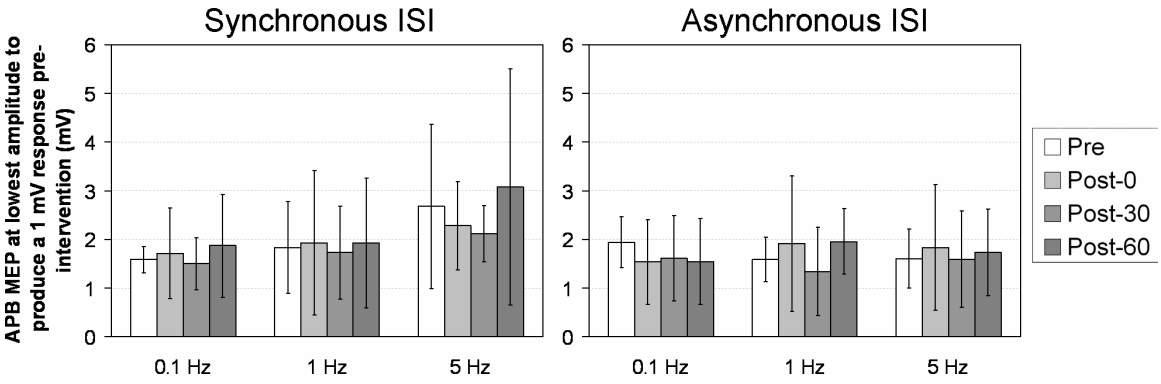

Figure 54. Mean APB MEP amplitudes at the stimulation amplitude which produced a minimum MEP response of 1 mV pre-intervention. No significant differences between treatment conditions were noted ($p \geq 0.3079$).

III.4.6. Motor Evoked Potential: Intensity Required for Half-Maximal Response

The initial ANOVA tests failed to show significant variance in the "synchronous ISI" group ($p = 0.7257$), but did show variance in the "asynchronous ISI" group ($p = 0.0255$). Pre-post changes in "H" were not found to significantly differ across conditions in the "synchronous ISI" group ($p \geq 0.4838$), but were found to differ between the 0.1 Hz and 5 Hz conditions in the "asynchronous ISI" group ($p = 0.0292$). This result seems come primarily from an higher baseline value in the 5 Hz condition, but also from higher

values in the 0.1 Hz condition at all post-intervention time points. These results are summarized in Figure 55.

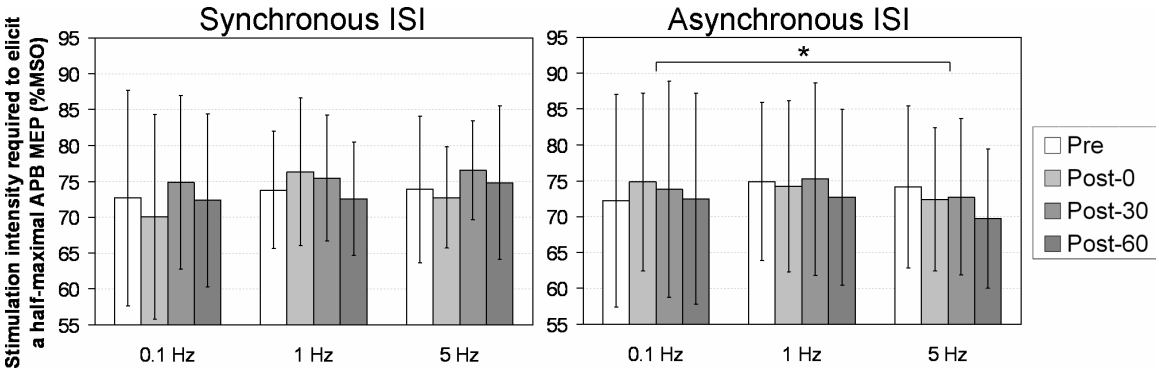

Figure 55. Mean TMS amplitudes required for a half-maximal APB MEP ("H"). * indicates a significant difference between treatment conditions ($p \leq 0.05$).

III.4.7. Motor Evoked Potential: Area Under Recruitment Curve

The initial ANOVA tests failed to show significant variance in either group ($p = 0.7773$ for "synchronous ISI" and 0.6848 for "asynchronous ISI"). Pre-post changes in "MEP_{AUC}" were not found to significantly differ across conditions in either ISI group ($p \geq 0.4414$). These results are summarized in Figure 56.

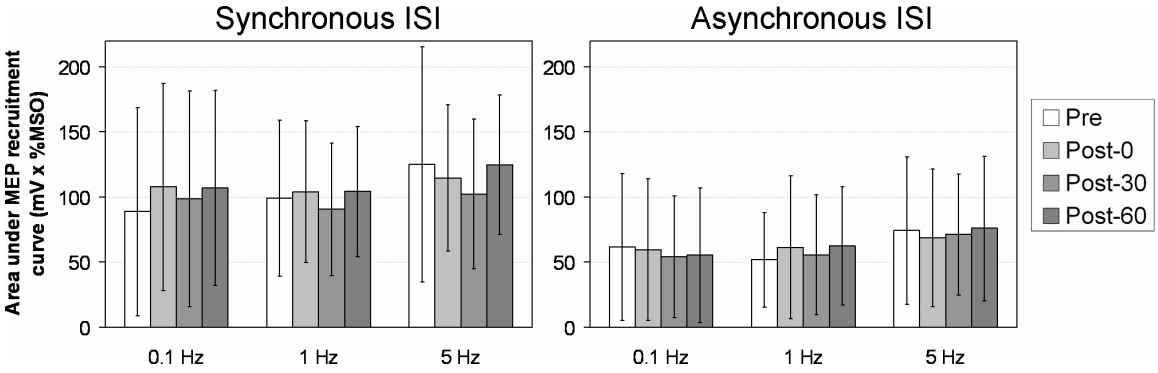

Figure 56. Mean area under the MEP recruitment curve. No significant differences between treatment conditions were noted ($p \geq 0.4414$).

III.4.8. Maximum Voluntary Contraction

The initial ANOVA tests failed to show significant variance in either group ($p = 0.1199$ for "synchronous ISI" and 0.1730 for "asynchronous ISI"). Pre-post changes in APB MVC were not found to significantly differ across conditions in the "synchronous ISI" group ($p \geq 0.1246$), but were found to differ between the 1 Hz and 5 Hz conditions in the "asynchronous ISI" group ($p = 0.0340$). This result seems primarily to come from increased values in the 1 Hz condition immediately post-intervention, and increased values in the 5 Hz condition 60 minutes post-intervention. These results are summarized in Figure 57.

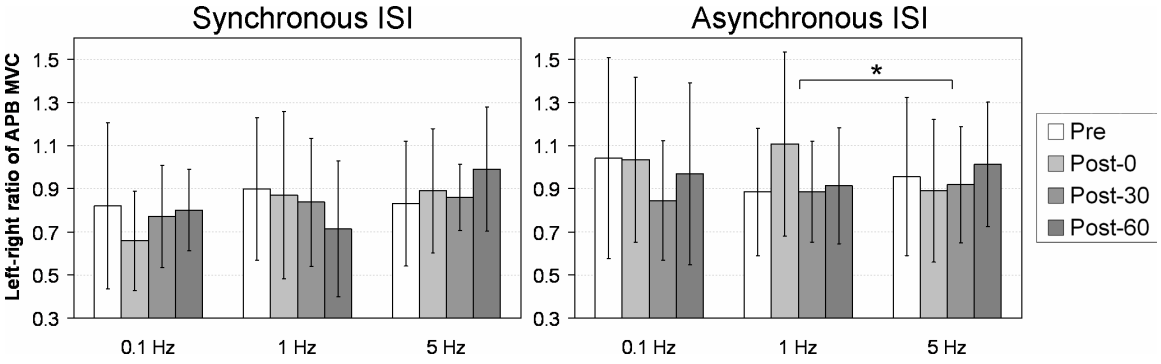

Figure 57. Mean left-to-right ratios of APB MVCs (defined as left ÷ right). * indicates a significant difference between treatment conditions ($p \leq 0.05$).

III.4.9. Grip Force

The initial ANOVA tests failed to show significant variance in either group ($p = 0.8345$ for "synchronous ISI" and 0.2739 for "asynchronous ISI"). Pre-post changes in the grip force ratios were not found to significantly differ across conditions in either ISI group ($p \geq 0.2286$). These results are summarized in Figure 58.

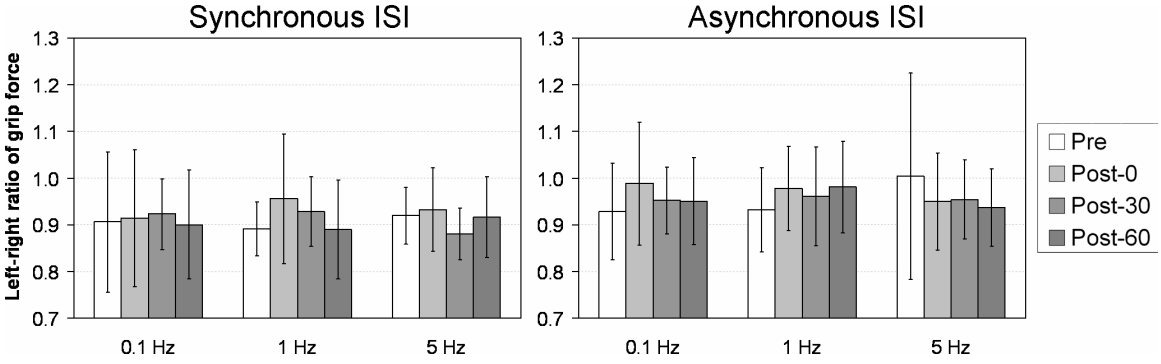

Figure 58. Mean left-to-right ratios of grip forces (defined as left ÷ right). No significant differences between treatment conditions were noted ($p \geq 0.2286$).

III.4.10. Matched Forces

The initial ANOVA tests failed to show significant variance in the "synchronous ISI" group ($p = 0.2604$), but did show variance in the "asynchronous ISI" group ($p = 0.0040$). Pre-post changes in the matched forces ratios were not found to be significant in the "synchronous ISI" group ($p \geq 0.1474$), but significant differences were noted between the 0.1 Hz and 1 Hz groups ($p = 0.0180$) and between the 0.1 Hz and 5 Hz groups ($p = 0.0049$). The differences between the 0.1 Hz and 1 Hz conditions seem primarily to come from decreased values in the 0.1 Hz condition 30 minutes post-intervention. The differences between the 0.1 Hz and 5 Hz conditions come from this same decrease in the 0.1 Hz condition 30 minutes post-intervention, but also from the decrease in the 5 Hz condition immediately post-intervention. These results are summarized in Figure 59.

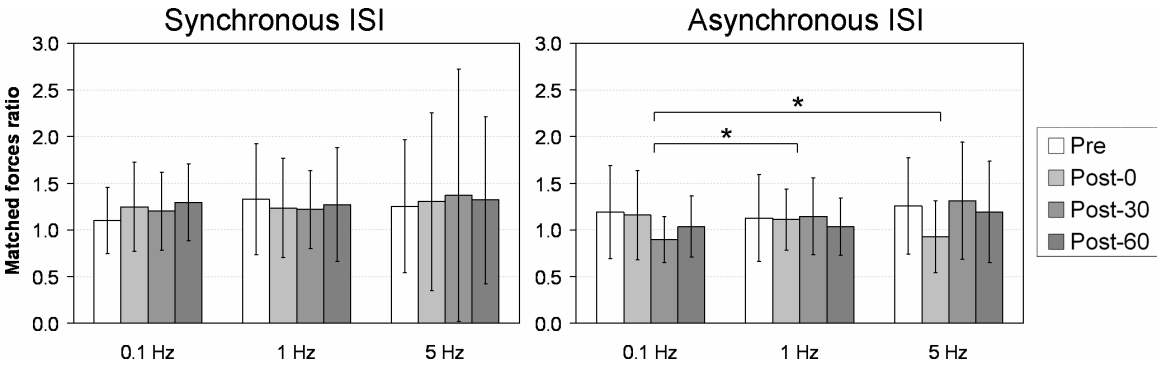

Figure 59. Mean left-to-right ratios of EMG activities during the matched forces test (defined as left ÷ right). * indicates a significant difference between treatment conditions ($p \leq 0.05$).

III.4.11. Comparisons Between Interstimulus Interval Groups

When only participants common to both of the ISI groups were considered, only two comparisons were significant: the pre-post ratio of the left-right MVC ratio was greater in the "asynchronous ISI" group, both when comparing the rate of change from pre-intervention to 30 minutes post-intervention ($p = 0.0111$), and when comparing the rate of change from pre-intervention to 60 minutes post-intervention ($p = 0.0083$). When all participants were included, only three comparisons were significant: the pre-post ratio of the left-right matched forces ratio was greater in the "synchronous ISI" group, both when comparing the rate of change from pre-intervention to 30 minutes post-intervention ($p = 0.0287$), and the rate of change from pre-intervention to 60 minutes post-intervention ($p = 0.0089$), and the pre-post ratio of the left-right MVC ratio was greater in the "synchronous ISI" group, when comparing the rate of change from immediately post-intervention to 60 minutes post-intervention ($p = 0.0081$).

III.4.12. Possible Confounding Factors

Neither TMS nor PNS intensities were found to differ significantly across treatment conditions, in either the "synchronous ISI" condition ($p \geq 0.5985$ and $p \geq 0.3353$, respectively) or the "asynchronous ISI" condition ($p \geq 0.3023$ and $p \geq 0.1022$, respectively).

In the "synchronous ISI" condition, age was found to have a significantly positive correlation with changes in the maximum APB MEP from pre-intervention to immediately post-intervention ($p = 0.0069$) and from pre-intervention to 60 minutes post-

intervention ($p = 0.0153$), with the stimulation intensity which produces a half-maximal response from pre-intervention to immediately post-intervention ($p = 0.044$), and with the estimated APB MEP amplitude at the half-maximal stimulation intensity from pre-intervention to 60 minutes post-intervention ($p = 0.0069$). Note, however, that while increased APB MEP amplitude is a sign of MEP upregulation, increasing stimulation intensity required to produce a half-maximal response indicates a decrease in sensitivity. While the results generally suggest that older participants had more MEP upregulation (contrary to the existing literature, such as Muller-Dahlhaus et al. (2008)), evidence to the contrary is also present.

Also in the "synchronous ISI" condition, gender was found to significantly correlate with the change in stimulation intensity which produces a half-maximal response from pre-intervention to 30 minutes post-intervention ($p = 0.0036$), such that male participants had a greater increase in the necessary stimulation intensity, which suggests that women may be slightly more receptive to the treatment.

In the "asynchronous ISI group", no change-scores were found to significantly correlate with the participant's age or gender, or the TMS or PNS intensities used in each session ($p \geq 0.0649$).

On the whole, this analysis provided very few results for the number of comparisons run. What results there are should furthermore be taken with a note of caution, given the overall lack of significant treatment effects.

The additional analysis to determine potential effects of fatigue generally showed fatigue in many cases, particularly in APB, although the effect seemed to plateau after 30 minutes post-intervention. If data were pooled across treatment conditions, then the APB MVCs of both arms decreased at from pre-intervention to immediately post-intervention ($p = 0.0079$ for the left, 0.0139 for the right), from immediately post-intervention to 30 minutes post-intervention ($p = 0.0020$ for the left, 0.0029), but not from 30 minutes post-intervention to 60 minutes post-intervention ($p = 0.8779$ for the left, 0.9514 for the right). The FCR MVCs only decreased from immediately post-intervention to 30 minutes post-intervention ($p = 0.0370$). Grip force decreased in the right arm, but not the left, from pre-intervention to immediately post-intervention ($p = 0.3712$ for the left, 0.0045 for the right), and in both arms from immediately post-intervention to 30 minutes post-intervention ($p = 0.0005$ for the left, 0.0025 for the right), but in neither arm from 30 minutes post-intervention to 60 minutes post-intervention ($p = 0.3885$ for the left, 0.4524 for the right). These results are outlined in Figure 60.

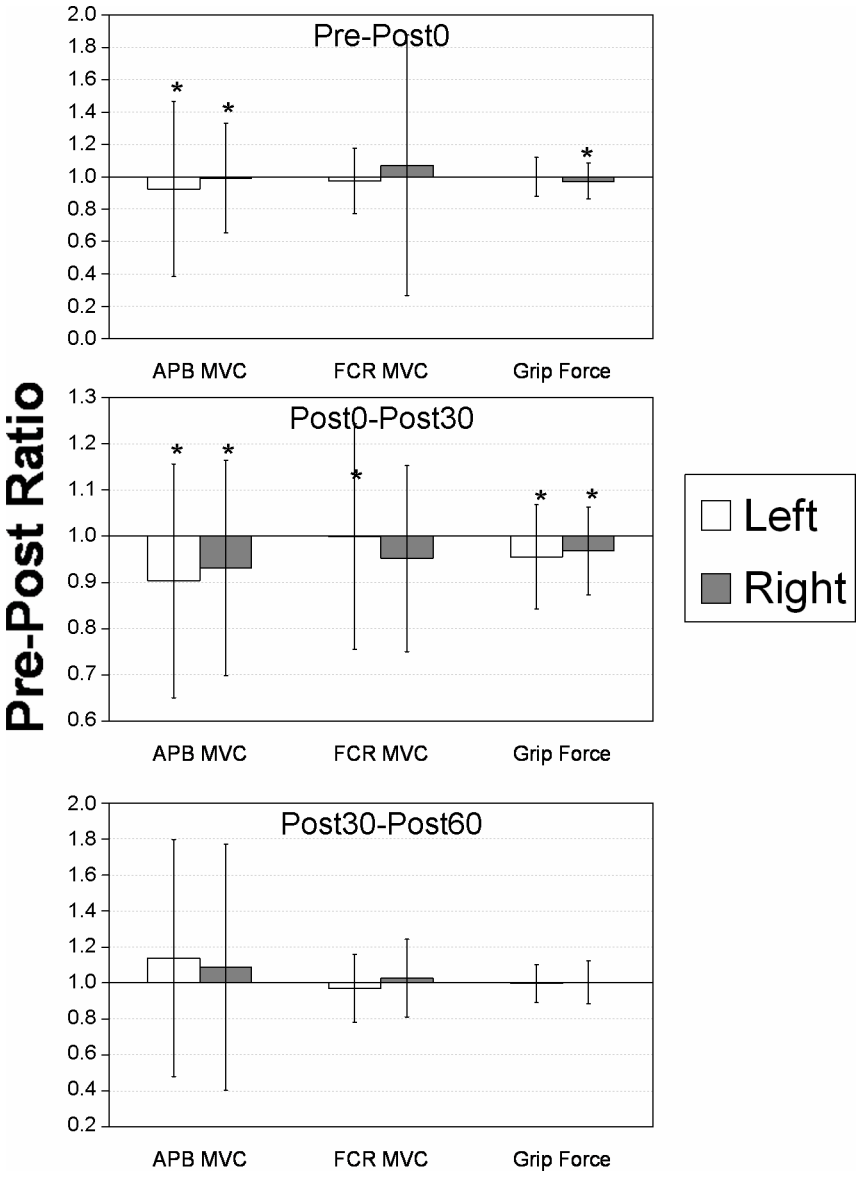

Figure 60. Mean pre-post changes in MVC and grip force measures, when comparing adjacent time-points. * indicates a significant difference from a baseline ratio of 1 ($p \leq 0.05$). Significant decreases are seen up to 30 minutes post-intervention in both arms, suggesting bilateral fatigue independent of the applied treatment.

The left-right ratios of either MVC (in either muscle), or grip force, did not significantly decrease at any time point ($p \geq 0.0990$). Taken together, these results suggest that while

fatigue significantly decreased muscle activity and force, both arms were equally affected, and that the effects of fatigue had reached a plateau by approximately 30 minutes post-intervention. These results are outlined in Figure 61.

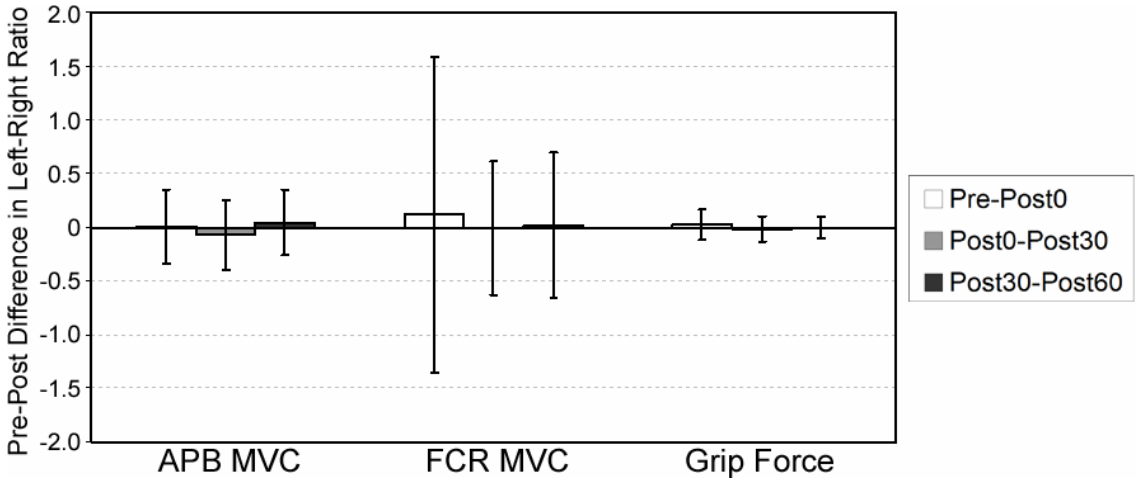

Figure 61. Mean pre-post changes in the left-right ratios of MVC and grip force measures, when comparing adjacent time-points. No results were found to significantly differ from the baseline value of 0 ($p \geq 0.0990$), suggesting that any observed fatigue effects are occurring equally in the left and right limbs.

If data were grouped by condition, six significant results were found: one in the 0.1 Hz condition, four in the 1 Hz condition, and one in the 5 Hz condition. APB MVC was only significantly decreased in the 1 Hz group, in the right arm, from immediately post-intervention to 30 minutes post-intervention ($p = 0.0230$). FCR MVC was only significantly decreased in the 1 Hz group, in the left arm, from immediately post-intervention to 30 minutes post-intervention ($p = 0.0482$). Grip force was found to decreased in the 0.1 Hz group, in the left arm, from pre-intervention to immediately post-intervention ($p = 0.0347$), in the 5 Hz group, in the right arm, from pre-intervention to

immediately post-intervention ($p = 0.0295$), and in the 1 Hz group, in both arms, from immediately post-intervention to 30 minutes post-intervention ($p = 0.0129$ for the left, 0.0082 for the right). Taken together, these provide considerably less evidence towards the presence of fatigue itself, but may suggest slightly greater fatigue in the 1 Hz condition as compared to the other two conditions.

III.4.13. Summary of Results

Very few statistics in the SAS study showed significant treatment effects. These results will be interpreted in the relevant Discussion section (§III.5.1).

Chapter III.5: Discussion

III.5.1. General Interpretation of Results

As previously mentioned (§III.4.13), the spinal associative stimulation is generally considered to have given negative results, refuting all of the hypotheses outlined in §III.2 – that is, the treatments do not seem to have affected the outcome in a statistically significant way. The few statistically significant post-hoc comparisons which were apparent in the results may be by chance – an assertion validated by the fact that none of the post-hoc comparisons would retain significance if they were subjected to Bonferroni correction for multiple comparisons (which would require a maximum p-value of 0.0167 ($0.05 \div 3$, since 3 comparisons are being done within each data set)). These results constitute a refutation of the original hypotheses, which suggested that at least some form of SAS treatment would generate neuroplastic effects, and that these effects could be modified by changing the stimulation frequency. However, the mean effect sizes in the MEP-based measures in some cases (exclusively in the 0.1 Hz and 1 Hz conditions) are roughly comparable to those seen in the BMI-FES study, albeit slightly smaller than those observed by Taylor and Martin (2009) and Bunday and Perez (2012). The chief cause of the lack of statistical significance instead appears to be the variability, despite the many measures employed in the experimental design in order to control for variability. That the same outcome measures were conducted in the same manner in both the BMI-FES and SAS studies suggests that the source of this variability is not simply error in the measurements, but a genuine product of the treatments being applied. If this is the case, it allows us to still make inferences about trends in the data, albeit tentatively and with great caution.

The high variability itself may point to a potential limitation of the SAS method, in that individuals may have widely different responses to the same treatment. In the literature pertaining to similar protocols, and to TMS in general, there is strong evidence to show such variability across participants. Fratello et al. (2006) showed that while mean group effects in response to PAS were consistent across repeated sessions, large variability existed across participants. Muller-Dahlhaus et al. (2008) showed similar variability across participants, going so far as to classify 14 of their 27 participants as "responders" (who showed LTP-like plasticity), and the rest as "non-responders" (who showed LTD-like plasticity), somewhat analogous to the way in which individuals can often be termed as "responders" or "non-responders" with respect to many different medications. However, they suggested that this variability was largely explainable by correlations with participant age (with younger participants more likely to be responders), and by baseline MEP threshold (with lower thresholds correlating with greater responsiveness). This was not observed in the correlation analysis in the present study. Note that while these two studies pertain to PAS, it is reasonable based on the underlying neurophysiological theory to apply the same principles to SAS, in which the limited scope of the literature prevents us from more fully understanding inter-individual variability. Wassermann (2002) provided evidence which, in light of Muller-Dahlhaus (2008) and Fratello's (2006) studies, suggests that variable response to PAS may result from varying response to TMS in general. Both single and paired TMS pulses showed a wide range of responses, and it was highlighted that among the paired-pulse stimulation, both facilitation and inhibition of the second response was seen in at least some proportion of

participants. No correlation was found between these measures and age or gender Siblings were found to have highly correlated MEP threshold values.

Many factors which may affect the capacity of the corticospinal tract to undergo LTP-like plasticity were controlled for in the study. Most notably, a wide range of neuroactive medications which have been shown to modulate neuroplasticity were included in the exclusion criteria, such as baclofen (Dario and Tomei 2004), and antidepressants (Bastos et al. 1999). Similarly, the placement and orientation of the TMS coil were tightly controlled for, as outlined in §II.3.9.1.2, since these are known to be considerable sources of variability in TMS-based experiments (Rothwell 1997). A number of other factors, however, were not controlled for. Sale et al. (2007) have demonstrated that circadian rhythms can modulate an individual's response to PAS, with optimal facilitation occurring in the afternoon and evening. Time of day was not controlled for in the present experiments, in order to allow participants to conduct their sessions when convenient. The effect of circadian rhythms on neuroplasticity is thought to be mediated primarily by the hormone cortisol (Sale et al. 2008). Since cortisol is one of the primary hormones known to mediate stress response, it can be suggested that a participant's mood may also have served to modulate their response to SAS. However, during these experiments, it was not possible for us to measure blood cortisol levels, meaning that this possible source of variability remains unaccounted for. Lastly, genetic factors are known to play a role in controlling the induction of LTP-like plasticity. Chief among these is the common brain-derived neurotrophic factor (BDNF) val66met mutation (Cheeran et al. 2008). This modulatory role is intuitive given BDNF's significant role in guiding neurological

development. It was not possible for us to genotype the participants in these studies, providing another possible source of inter-subject variability in the response to SAS.

This literature supports the finding in the present study that paired-stimulation protocols can have widely different responses in different individuals. It would not be surprising to find that this variability is further increased across individuals with SCI, owing to the heterogeneity of the population (that is, in addition to the regular inter-individual variability, there would also be variability based on the wide range of different injuries). This finding suggests a potential limitation of the clinical utility of PAS and SAS.

One possible reason why this treatment may not have been effective may have come from a mismatch between the muscle fibers targeted by the TMS and by the PNS. It has long been suspected that PNS and FES tend to recruit muscle fibers in the opposite order that volitional signals from the brain do. While natural stimulation from the brain first recruits smaller-diameter fibers, external stimulation tends to first recruit larger-diameter fibers (Knaflitz et al. 1990, Mesin et al. 2010). By contrast, TMS activates muscle fibers in the same order that voluntary muscle activation does (Bawa and Lemon 1993). With increasing stimulation amplitudes, more fibers get recruited until the majority of the muscle is contracting, in both TMS and PNS. Thus, at low stimulation amplitudes (such as those employed in the SAS protocol), TMS and PNS may recruit different muscle fibers. This may prevent the signals from the TMS and the PNS from meeting, since they are both directed towards different synapses. Higher stimulation amplitudes, if they are tolerated by participants, may provide a better pairing of the two induced signals. This

suggestion, however, cannot be validated within the current data, and has not been raised elsewhere in the existing SAS literature. It must therefore remain speculation at the present time.

The negative results in this study are not readily explained by comparisons to the stimulation protocols employed in other SAS studies (as previously outlined in Table 3). In particular, the 0.1 Hz condition is in line with existing protocols, and the outcome measures and stimulation parameters used in our study are very similar to those used in the Bunday and Perez (2012) study (with the key difference being the lower TMS amplitude in our study). A possible explanation as to why increased stimulation amplitude may provide better neuroplasticity was outlined in the preceding paragraph, but as previously stated, this possibility cannot be verified or disproven with the current data.

Ideally, this study would allow us to make statements about the effects of the stimulation frequency and the ISI, as well as about the time-course of any treatment effects induced. Unfortunately, the high variability precludes this possibility, as no clear patterns are observed in the results.

III.5.2. Concerns Pertaining to the Interstimulus Interval

An obvious source for the variability in the results would be the ISI, which many PAS and SAS studies suggest needs to be customized for each individual (for example, Muller-Dahlhaus et al. 2008, Bunday and Perez 2012, respectively). The ISI used in the

"synchronous ISI" group accounted for this, and our calculation suggested a very narrow range of ideal ISIs (4-7 ms), which agreed well with the ISIs used in Bunday and Perez (2012) for similar purposes. However, the calculation used to determine this was somewhat novel, however similar in theory to that employed by Bunday and Perez. Since even differences as slight as 5 ms can significantly alter the effects of PAS protocols (Mrachacz-Kersting et al. 2007), these discrepancies may have been enough to considerably alter either the effect size of the SAS protocol or the variability thereof. By contrast, the "asynchronous ISI" condition is set to an ISI that is, based on the literature, almost certain to provide no treatment effect. It is possible that another ISI would be more ideal, but repeating the entire testing procedure with many ISIs would prove extremely time-intensive, and is well outside the scope of this study.

An additional concern pertaining to the ISI is that the day-to-day consistency of the ideal ISI within a given participant has not been empirically examined within the literature. While it seems intuitive that conduction velocities within the CST should be greatly affected, it cannot be guaranteed due to a lack of information in the literature. In our experimental design, the ideal ISI was calculated during the first session, and was not recalculated thereafter. If day-to-day variations in the ideal ISI did occur, our experimental design would therefore have been unable to account for it.

III.5.3. Potential Confounding Effects of Participant Engagement

While the effects of attention and participant engagement have not been investigated specifically in SAS, it has been investigated in similar stimulation protocols, such as PAS

(Stefan et al. 2004) and repetitive TMS (Conte et al. 2007). In both cases, it was found that the neuroplastic effects of the stimulation protocols, as assessed through MEP, were decreased or lost entirely when the participant did not attend to the hand being stimulated. The suggestion of these studies is that focussing attention on the hand being stimulated increases the excitability of the relevant section of M1, facilitating activation, and thereby, LTP-like plasticity.

In the present study, participants were not specifically asked to attend to their hand. None of the other SAS papers discussed in the Introduction (Taylor and Martin 2009, Cortes et al. 2011, Leukel et al, 2012, Bunday and Perez 2012) explicitly state whether or not they require participants to attend to the site of their stimulation. It therefore cannot be concluded whether or not this factor is primarily responsible for the discrepancies between the current findings and the existing literature, but it is reasonable to suggest that it may have been a confounding factor.

III.5.4. Inability to Implement True Single-Factor Design

The ideal design of any scientific study involves manipulating one factor while all other factors are held constant, such that any changes in the results can be attributed to the one manipulated factor. The key factor under investigation in the SAS study is the stimulation frequency (0.1, 1, or 5 Hz). However, it was impossible to completely isolate this factor given its relationship to other stimulation factors. A summary of the actual stimulation parameters in each group is outlined in Table 4.

Table 4. Outline of the stimulation parameters in each of the three SAS conditions.

Frequency (Hz)	Pattern	# of Stimuli	Duration (min)	TMS Intensity	PNS Intensity	ISI (ms)
0.1	Continuous	90	15	1.2 x MT	1.2 x MT	L_F - L_{MEP}
1	Continuous	900	15	1.2 x MT	1.2 x MT	L_F - L_{MEP}
5	Bursting	900	15	1.2 x MT	1.2 x MT	L_F - L_{MEP}

Note that frequency is the only parameter that was intended to be under investigation.

As an example of why some of these factors are entangled, consider a case in which we wished to hold the number of stimuli constant. In the case of 0.1 Hz stimulation, this would require the duration to increase to 150 minutes. Similarly, if 5 Hz stimulation were applied continuously like the other two conditions, it would either need to involve 4500 stimuli over 15 minutes, or 900 stimuli over 3 minutes. Thus, one factor cannot be held constant without another being manipulated.

With 5 Hz stimulation, there are two additional concerns which necessitate the use of a bursting pattern. One is participant safety. Wassermann (1998), in a comprehensive review of safety guidelines for repetitive TMS, suggested that 5 Hz TMS at 120% of resting motor threshold should not be applied in train longer than approximately 10 s. The primary cause of this recommendation is the increasing risk of seizure induction with greater stimulation frequency and intensity. Even without seizure risks, participants would certainly find 15 minutes of continuous 5 Hz stimulation excessively uncomfortable, due to the sensory stimulation and involuntary muscle contraction that it causes. The other reason for the bursting pattern was the potential for the magnetic coil to overheat. In most participants, coil temperature was noted to increase by approximately

7-12 °C (depending on stimulation intensity) during the 1 Hz and 5 Hz conditions. After an increase of approximately 15 °C, the coil automatically disables itself to prevent damage. If 5 Hz stimulation were applied continuously, the coil would almost certainly reach this point within approximately 4-6 minutes, preventing us from applying the stimulation for a consistent 15 minute duration.

Part IV: General Discussion

IV.1. Limitations of Short-Term Methodology

The present studies use short-term outcomes as a "proof-of-principle" to try to suggest the larger therapeutic potential of the interventions. This approach was chosen for the sake of efficiency, for facilitating participant recruitment, and to allow for a broader range of experimental factors to be investigated. If a design more like a randomized control trial had been used, for example, only one of the five interventions in the BMI-FES study could be investigated per participant, which would require a much larger sample size. However, this approach places its own limitations on the study and its results.

Throughout both studies, experimental sessions were separated by a minimum period of three days. While this was considered sufficient to ensure washout of an physiologically observable experimental effects (as validated in §II.3.3 and §III.3.3), the possibility that certain cellular- or molecular-level changes may have occurred can neither be proven or disproven within the current methodology.

One such limitation is the development of fatigue over the course of each experimental session. In a more long-term application of the experimental interventions, the treatment effects would be expected to persist for a considerable length after the last application of the treatment (were this not the case, the interventions would be of little clinical use). Thus, treatment and assessment could take place on separate days. By contrast, in the current short-term design, participants do not have a chance to recover from the fatiguing

effects of the intervention. Fatigue is therefore present in the post-treatment results, which would not have been present in the pre-treatment results. The implications of this are widespread, and are discussed more thoroughly in the following section (§IV.2).

IV.2. Effects of Fatigue

As previously mentioned (§IV.1), the effects of fatigue (as were shown to be occurring in §II.4.13 and §III.4.12) are a limitation imposed upon this study by its short-term design, and must be carefully considered in the interpretation of the results. In truth, several different forms of fatigue are simultaneously occurring over the course of the experiment. Fatigue would be expected in the treated arm, due to the extensive use of that arm during grasping practice. In theory, this should be exacerbated in FES-based conditions, since FES, unlike voluntary movement, preferentially recruits fast-fatigable muscle fibers (Levy et al. 1990). Rapid onset of fatigue is one of the key barriers of the use of FES as an orthosis in activities of daily living (Thrasher et al. 2005). Pitcher and Miles (2002) have suggested that fatigue induced by voluntary effort and fatigue induced by FES may involve entirely different mechanisms and follow entirely different time courses. This effect, however, is not readily observed in the present results, since fatigue was found to be roughly equivalent across all treatment conditions.

In addition, a more "central" form of fatigue is also present, as evidenced by the fact that the non-treated arm also showed considerable decreases in MVC and grip force, despite not being used at all during the one-hour training period. The most likely cause for this fatigue was the elevated temperature within the electromagnetically shielded room in

which the experiments took place, since the room lacked proper ventilation. That increased ambient temperature hastens fatigue in both exercised and unexercised muscles is established with sports medicine literature, with a wide range of central and peripheral mechanisms being proposed (for example, Periard et al. 2011, Coulange et al. 2008, Cheung and Sleivert et al. 2004, Nybo and Nielsen 2001).

MEPs have been found to decrease following fatiguing tasks (Brasil-Neto et al. 1993). If this is the case, then the apparent decrease in MEP following push-button-controlled FES may not indicate a true decrease in corticospinal connectivity, but rather, fatigue. This would explain the severe discrepancy between our results and those of other studies investigating MEP following FES (Thompson and Stein 2004), as well as those showing the therapeutic potential of FES (Kapadia et al. 2011). If this is true, it would also mean that the beneficial effects of BMI-FES and EMG-FES may be underestimated when compared to the VOL and BMI conditions, since these two treatments were evidently able to overcome the same fatigue that caused MEPs in the FES group to decrease.

The above suggestions, however, remain speculation. Additional information on this matter may have been gained by conducting MEP on the non-treated hand as well as the treated hand. This test was not conducted in an effort to minimize the duration of the experimental sessions, and since fatigue effects were not discerned until the experiments were mostly completed, they could not be added to the protocol mid-way through. In addition, the MEPs from the non-treated hand may still not have been sufficient to elucidate the effects of fatigue, since the non-treated hand will undergo fatigue only as a

result of the ambient temperature at which experiments were conducted, and not fatigue resulting from the grasping training.

Given the wealth of complications brought into the interpretation of the present results by fatigue, it can be concluded that it causes a disconnection between the MEP results and the actual therapeutic potential of the tested interventions, and weakens the overall significance of the results of this study. Caution should especially be implied when considering comparisons between conditions which do and do not involve FES, since the forms of fatigue in these two categories may be very different. However, comparisons within each of these categories should be relatively valid, since fatigue should be the same in each. For example, we can be confident in comparing FES to BMI-FES or FES to EMG-FES, but not in comparing VOL to FES, or BMI to BMI-FES.

IV.3. Interpretations of Motor Evoked Potential-Based Outcome Measures

Knash et al. (2003), in outlining the MEP analysis methods applied in this study, suggest that changes in different parts of the MEP response curve may indicate different types of changes in the nervous system. A leftward shift, indicated by a decrease in H and/or an increase in MEP_H, in the absence of changes in M (Figures 17 and 18), should indicate increased excitability, consistent with the hypothesis of LTP-like plasticity occurring at the spinal synapse. By contrast, an increase in M in the absence of changes to H and MEP_H (Figure 17), should indicate greater overall connectivity and the recruitment of additional muscle fibers, unlike the hypothesis. $MEP_{120,}$ MEP_{1mV}, and MEP_{AUC} will all have similar interpretations to MEP_H.

The present results do not cleanly fit into either of these two categories. No significant change in H was observed, while M was shown to change in certain treatment conditions (Figure 24 and 28). However, the most numerous and significant changes were observed in the MEP_H results, which can be explained by a leftward shift in the input-output curve, and/or by an increase in the maximum MEP amplitude (Figure 18). Given the ambiguity in the interpretations of MEP_H, it is difficult to draw definitive conclusions about the nature of any neuroplasticity that occurs as a result of our interventions.

This difficulty in interpreting the data is compounded by the possibility of increased MEP amplitudes being caused by increased temporally synchrony of the various indirect waves comprising the MEP (as described in §II.3.9.1.1). This interpretation provides an additional mechanism by which the observed results may in fact be caused by cortical, rather than spinal plasticity. However, as stated, the current experimental design does not allow these conflicting interpretations to be resolved, as this was not the primary purpose of these studies. This ambiguity will be further discussed in the following section (§IV.4).

IV.4. Location of Neuroplastic Changes

The purpose of the matched forces test was to elucidate whether observed neuroplastic changes were occurring at the spinal level or at the cortical level. It was for this purpose that Taylor and Martin (2009) originally proposed the method. While MEP amplitude can be affected by changes in the cortex, spinal cord, αMN, or muscle, the matched forces method used bilateral activation to effectively hold cortical involvement constant,

limiting interpretations of change to the other three levels. That changes were observed in the MEP results, but not in the matched forces results, seems to suggest that neuroplasticity was occurring primarily at the cortical level (it should be emphasized that this does not necessarily mean that large-scale cortical reorganization was occurring, but rather, synapse-level changes as outlined in §I.2). This is contrary to the original hypothesis that LTP-like plasticity would occur primarily at the spinal synapse.

While caution must be advised in interpreting these results, since the matched forces method is a relatively novel and untested one, with considerable room for variability in its results (including the possible presence of cross-body training effects (Carroll et al. 2006)), the possibility of cortical plasticity is not unreasonable. FES pulses activate Ia sensory afferents, as well as the αMNs that they were intended to activate, sending signals back up through the corticospinal tract to the primary somatosensory cortex (S1). This area in turn connects to the M1. Should the timing work out such that the signals from S1 reached M1 at the same time that another descending command was being generated by M1, the conditions would be established for inducing LTP-like plasticity at these synapses. Given the complex and interconnected nature of the intracortical connections, this is only one example of how signals induced by FES might result in plasticity involving the M1. However, changes in synaptic connections onto M1 should theoretically not affect MEP amplitudes, since the TMS pulse is intended to activate the UMNs directly, rather than through intracortical connections. This may not always be the case, however, and indirect, intracortical connections have been suggested to contribute to MEP amplitudes in ways that are not yet fully understood (Devanne et al. 1997).

A number of additional assessment techniques, which are outlined below, could shed light on the exact location of the neuroplastic changes. The chief reason why these assessments were not implemented was that many assessment techniques were already being used to investigate whether neuroplastic changes were occurring anywhere in the CST at all, which was the primary research question. Adding more assessments would therefore be burdensome to the participants and time-consuming. Furthermore, since most of the techniques involved applying some form of electrical or magnetic stimulation, their use could interact with the neuroplastic created by the stimulation protocols under investigation, thereby confounding our results (this is also true of the MEP assessments, but this was considered an acceptable risk since it was the primary outcome measure).

One such technique is the cervicomedullary evoked potential (CMEP). This technique, previously discussed in reference to an SAS paper (Taylor and Martin 2009) (§III.1.1.1), involves performing MEPs with the TMS coil held to the back to the participant's neck, rather than the top of their head. The purpose of CMEP is to activate the CST directly, bypassing the cortex entirely. If CMEP changes are absent while MEP changes are present, it can be concluded that the MEP changes are caused by cortical factors. In addition to the aforementioned reasons why assessments for elucidating specific mechanisms were not implemented, CMEP was also not implemented because we were unable to evoke them with our current TMS equipment (the details of which are outlined in section §II.3.9.1).

Another technique for discerning the root cause of observed neuroplastic changes is paired-pulse TMS. The first TMS pulse, in addition to evoking an MEP, sets in motion certain intracortical circuits, which eventually feed back into the M1. If the second pulse is correctly timed, the effect of these mechanisms can be observed as changes in the MEP induced by the second pulse. Depending on the exact interval between the two pulses, the second MEP can be either inhibited (termed intracortical inhibition (ICI), found at delays of 5 ms or less) or facilitated (termed intracortical facilitation (ICF), found at delays of 10-15 ms) (Kujirai et al. 1993). These measures are widely taken as indicators of corticocortical connections, and changes in them following treatments are considered suggestive of cortical neuroplasticity. These methods were not conducted in the present studies so as not to overburden participants with excessive amounts of assessment, and to minimize the participants' exposure to TMS outside of the interventions, since repetitive TMS can cause neuroplastic changes itself (Rothkegel et al. 2010, Funke and Benali 2011). Had they been conducted, however, MEP changes in the absence of ICI or ICF could be taken as indicative of changes occurring solely at the spinal or peripheral level, whereas MEP changes accompanied by ICI or ICF changes may suggest that cortical plasticity is primarily responsible for the observed MEP changes. Such inferences would have to be made with caution, however, since the exact cortical circuitry involved in ICI and ICF is currently unknown.

Finally, a number of measures could have been used to determine whether or not changes were occurring in the periphery (which includes the αMNs, neuromuscular junctions, and

muscles). These measures typically involve providing PNS, as was done in the SAS study. The H-reflex, M-wave, and F-wave would all provide an indication of the αMN excitability, synaptic strength of the neuromuscular junction, and the strength of the muscle itself, with the H-reflex additionally indicating the synaptic strength between the Ia sensory afferents and the αMNs.

IV.5. Future Directions

It was stated in the Introductions for both studies that a major limitation of many current studies on the topic of rehabilitative neuroplasticity is the use of able-bodied participants. This approach brings with it many limitations, as outlined in and §II.5.7. It was hoped that the present studies would be able to include testing on individuals with SCI, in order to circumvent these issues and provide results that were more relevant to clinical application. However, no individuals with SCI were successfully recruited for these studies. The most evident direction for future studies therefore, would be to apply BMI-FES and EMG-FES in individuals with SCI. Applying SAS in such individuals is not supported by the current results, since this treatment did not successfully demonstrate the ability to induce neuroplasticity in able-bodied participants.

Similarly, the existing literature was criticized for relying on short-term methodology, which again creates barriers to clinical application, as outlined in §IV.1. However, practical necessities require the current studies to also be conducted using short-term methodology. Another future direction would therefore be to employ these treatments on a repeated, long-term basis, to observe whether or not they are able to provide more

persistent, functional benefits. As with the use of individuals with SCI, this is a direction intended more for the BMI-FES and EMG-FES treatments than SAS, since SAS was not successful in demonstrating neuroplasticity induction herein.

The preceding discussion has also raised a number of questions about the exact nature and mechanisms of the plasticity induced by BMI-FES and EMG-FES (§IV.3 and §IV.4). In truth, this remains an open question even in the broader FES literature (regardless of control strategy). Future studies which seek to examine these mechanisms could therefore employ methods outlined in the aforementioned sections (such as CMEP, ICI, and ICF) to further determine whether plasticity may be occurring in the motor cortex, spinal cord, or both.

Future studies could also seek to control for some of the unexpected confounding factors that arose in the course of these investigations, such as user engagement (§II.5.5) and fatigue (§IV.2).

Future studies on SAS protocols can expand upon the existing findings by examining a broad range of ISIs and stimulation amplitudes (both PNS and TMS). These factors had been considered to be more well-explored in the current literature than stimulation frequency, and were therefore held constant for the sake of maintaining a limited, manageable scope within the present study. These manipulations may allow us to find a set of stimulation parameters which is more effective at inducing LTP-like plasticity, which can in turn be used as a starting point from which to examine stimulation

frequency. While the importance of ISI in terms of inducing LTP-like plasticity is apparent (as per the LTP theory outlined in §I.2), the potential importance of stimulation amplitude may only become apparent after further experimentation. One possibility, originally discussed in §III.5.1, is that increased stimulation amplitudes may provide more robust pairing between the synapses and muscle fibers targeted by the PNS and TMS, due to broader muscle fiber recruitment at higher intensities.

Part V: Conclusions

BMI-controlled and EMG-controlled FES have been shown to be more effective than conventional FES or voluntary activity at inducing changes in MEP in at least some cases. However, high variability in the outcome measures and inconsistent results necessitate cautious interpretation of these results, and the limited, "proof-of-concept" design and many potential complications in translation prevent us from clearly stating that these treatments will be of benefit to individuals with incomplete SCI. The present study should therefore be considered as weak evidence which provides the initial suggestion that these treatments warrant more detailed investigation. While the plasticity is theoretically intended to occur in the spinal synapse as a direct result of the stimulation, insufficient evidence is available from these results to conclusively state what the exact mechanism of these changes are, and if this differs from the proposed mechanism and location.

Contrary to existing literature, the SAS treatments tested herein have not been effective consistently modulating the nervous system. However, the root cause of this seems to primarily have been excessive variability in the outcome measures. Since the outcome measures applied herein were applied successfully and with much less variability in the BMI-FES study, this variability cannot be fully explained simply as inherent variability in the measures themselves. Rather, the variability is thought to be a genuine response to the treatment itself. That responses to SAS treatment can be so highly individual, and in such unpredictable ways, suggests that SAS may be of limited utility as a therapeutic intervention. Even if some individuals were able to derive benefit from the treatment,

some may also have detrimental responses, with more still having no response. Furthermore, there may not be a clear method by which to determine which will be the case prior to applying the treatment itself. The original question being investigated (the effect of different stimulation frequencies upon the therapeutic effects of SAS) cannot properly be addressed with the current data, and therefore remains open for further research to elucidate.

While it was originally intended that these studies would be conducted both with able-bodied individuals and with individuals with SCI, no injured participants were successfully recruited. The results from able-bodied participants suggest that BMI-FES and EMG-FES, but not SAS, may be of benefit to injured individuals, when compared with interventions currently being applied in clinical trials. However, a wide range of potential complications prevent us from having confidence in this conclusion. Further investigation on short-term effects should therefore be conducted in injured individuals, before testing more long-term applications or clinical trials of BMI-FES and EMG-FES. With proper, thorough investigation, it is hoped that BMI-FES and EMG-FES can be used to further the rehabilitation of individuals with SCI and improve their motor function, functional independence, and quality of life, extending the benefits already offered by standard FES therapy.

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