Spinal and Supraspinal Control of Reflexes:
In health, under general anesthesia, and in Parkinson's disease

by

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ABSTRACT

Reflexes have been used extensively for over a century both in the clinic and laboratory as a tool to assess functional connectivity within the spinal cord. In order to support the co-ordinated movement of muscles, the reflex arc is continuously under the influence of numerous peripheral and descending spinal pathways. The Hoffmann (H)-reflex is an electrically induced reflex that is analogous to the mechanically evoked stretch reflex. In this thesis we studied the H-reflex and related pathways under different conditions, such as during contraction, under general anesthesia and in Parkinson's disease, to evaluate the effect of each condition on different spinal circuits.

The thesis begins by systematically characterizing the time-course of post-activation depression in the soleus muscle of healthy participants using paired-pulse reflexes. We compared the recovery of an H-reflex to a reflex root evoked potential (REP) that is elicited following transcutaneous stimulation of the lumbar spine. Each type of response (i.e. H-reflex or REP) was conditioned by either an H-reflex or an REP. Transcutaneous spinal stimulation is a relatively new technique used to augment motor activity following neurological injury. To identify the influence of muscle activation, tests were conducted in both contracted and resting states. While there were many similarities between the H-reflex and REP, transcutaneous spinal stimulation produced more post-activation depression when it was assessed using paired pulse REPs, suggesting that the pathway mediating the spinally-evoked response was more susceptible to being inhibited. Using transcranial magnetic stimulation (TMS), we also demonstrated that descending input can virtually eliminate post-activation depression of the H-reflex and REP.

These studies revealed that the soleus H-reflex and REP recruit an overlapping population of afferents and are similarly modulated by volitional drive and descending input. Evidence here also suggests that the scientific theory describing the mechanism of post-activation depression as a depletion of neurotransmitter is less likely.

This thesis then describes how the removal of post-activation depression of the H-reflex through corticospinal input was adapted for use in the operating room. The technique was used to monitor motor pathways and reduce the risk of injury to the spinal cord in anesthetized patients undergoing spine surgery. The technique could be administered without producing the noticeable patient movement that is typically observed using conventional motor evoked potential (MEP) monitoring techniques.

Finally, we describe a pilot study where the H-reflex and related descending and peripheral pathways were examined in a group of individuals with Parkinson's disease (PD). These series of experiments demonstrated that the transmission of signals within the spinal cord may be abnormal in people with PD and may be normalized, to some degree, through parkinsonian medication and deep brain stimulation (DBS).

In summary, this thesis investigates how the H-reflex is modulated by both peripheral and descending connections within the spinal cord in both healthy individuals and pathological states. The research here aims to contribute to current studies in the clinic and laboratory on human spinal cord circuitry.

PREFACE

This thesis in an original work by Jennifer C. Andrews. The research project, of which this thesis is part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name “Activation of cortico-spinal circuitry with trans-cutaneous spinal stimulation”, Pro000226605, September 20, 2012, Project Name “Reflex conditioning during spinal cord monitoring” Pro00047978, July 17, 2014, and Project Name “Effect of deep brain stimulation on spinal cord circuitry in individuals with Parkinson’s disease”, Pro00056935, April 29, 2015.

All neurophysiology experiments were performed at the University of Alberta in Dr. Richard Stein’s laboratory or the Stollery Hospital. All studies from Chapters 2 through 5 were designed, conducted, analyzed, and drafted by Dr. Richard Stein, Dr. Francois Roy and Jennifer Andrews. Data collection and editing of the manuscript from Chapter 4 was also conducted in collaboration with Drs. Kelvin Jones, Douglas Hedden, James Mahood, Marc Moreau, and Eric Huang. Clinical assessments for Chapter 5 were performed by Dr. Fang Ba. All contributors were employed at the University of Alberta.

Chapters 1 and 6 were original work by Jennifer Andrews with the supervision of Drs. Stein and Roy. Chapter 2 was published March 2015, by Neuroscience Letters doi: 10.1016/ j.neulet.2015.01.041. Chapter 3 was published July 2015, by the Journal of Neurophysiology doi: 10.1152/ jn.01007.2014. Chapter 4 has been accepted for publication by Clinical Neurophysiology August 11, 2016 MS. No. CLINPH-D-16-9422R2.Chapter 5 is a pilot study and with the addition of more participants and the collection of more data may be submitted for publication.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Ia	primary muscle spindle afferents
Ib	primary afferents from golgi tendon organs
αMN	alpha motor neurons
ANOVA	analysis of variance
Cl^-	chloride
cm	centimeter
CPN	common peroneal nerve
CST	corticospinal tract
D-wave	direct wave
DBS	deep brain stimulation
EMG	electromyography
EPSP	excitatory post-synaptic potential
GABA	gamma-aminobutyric acid
$GABA_A$	A-type (ionotropic) GABA receptor
GPe	globus pallidus externus
GPi	globus pallidus internus
H/H-reflex	Hoffmann reflex
H_{max}	maximal H-reflex response
Hz	hertz
L	lumbar
L-DOPA	levodopa
I-wave	indirect wave
IN	interneurons
IPI	interpulse interval
IONM	intraoperative neuromonitoring
IPG	impulse generator
M-wave	motor wave
M_{max}	maximum M-wave
mA	milliamp
MEP	motor evoked potential
MG	medial gastrocnemius
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
ms	millisecond
MVC	maximum voluntary contraction
Na^+	sodium
P-value	level of significance
PAD	primary afferent depolarizing
PD	Parkinson's disease
PTN	posterior tibial nerve
REP	root evoked potential
RM	repeated measures

RMT	resting motor threshold
RST	reticulospinal tract
s	second
S	spinal
SD	standard deviation
SE	standard error
SSEP	somatosensory evoked potential
STN	subthalamic nucleus
t-test	Gosset's Student distribution
TA	tibialis anterior
TES	transcranial electrical stimulation
TMS	transcranial magnetic stimulation
TN	tibial nerve
UPDRS	Unified Parkinson's Disease Rating Scale

CHAPTER 1: INTRODUCTION

1.1 FOREWORD

In order to guide our movements, the nervous system requires incoming sensory information. Co-ordination of muscle movement is dependent upon neural information relating to muscle length, muscle velocity, and external forces or loads acting on the muscle. Specialized sensory receptors located within the skin, joints, and skeletal muscles relay this information to the central nervous system where it is used to control motor behaviour. The stretch reflex is the simplest type of motor behaviour. In 1910, Paul Hoffmann described the Hoffmann (H)-reflex as the electrically evoked analogue of the human stretch reflex. The appeal of the H-reflex is related to the ease with which it can be evoked and studied in many muscles throughout the body. The H-reflex is under the influence of multiple segmental and descending pathways within the spinal cord. An examination of how these pathways modulate the reflex response may provide important information regarding different patterns of connections within the spinal cord of both healthy individuals and pathological states. In this thesis, we investigated how neural inputs from both peripheral and descending pathways act on the soleus H-reflex in resting and contracted muscles. These studies were also conducted using a reflex root evoked potential (REP) which was produced by applying a transcutaneous stimulus to the thoracolumbar spine. By doing so, we could compare the modulation of reflex responses activated along a peripheral nerve versus those activated along the corresponding spinal nerve root. Further to this we examined how these connections are influenced under general anesthesia and described a potential new intraoperative neuromonitoring (IONM) technique used to reduce the risk of injury to the spinal cord during spine surgery. Finally, we studied many of these pathways in a group of individuals with Parkinson's disease. Given that connections from the brain and spinal cord are known to change in people with the disease, we aimed to understand which pathways are affected and to what degree they are normalized through two standard clinical treatments: parkinsonian

medication and deep brain stimulation (DBS). These research projects on spinal cord circuitry are important because in order to create knowledge and ask new questions that can lead to therapies for motor dysfunction, we need to know more about the basic pathways involved and the specific changes that occur under different conditions.

1.2 SPINAL REFLEXES

A spinal reflex is a stereotyped motor response to a specific sensory stimulus. Following the activation of a muscle spindle, a signal is relayed along an afferent fiber to the spinal cord where it is then transmitted transynaptically to an alpha motor neuron (αMN). This pathway is commonly described as a pure monosypantic response, though it has been shown to be consistently under the influence of oligosynaptic connections that may contribute the later portions of the response (Burke et al. 1984). Reflexes, such as the stretch reflex, are designed to induce a rapid reaction to an external stimulus by bypassing the brain and eliminating the additional time required to process and respond to a given stimulus. A reflex pathway can also be activated in a clinical setting, using a patellar tap, whereby a hammer strike to the patellar ligament will cause the activation of stretch receptors in the quadriceps muscle and the corresponding reactionary contraction. Electrical activation of an afferent fiber along this same pathway will bypass the muscle spindle and produce an H-reflex. The study of spinal reflexes provides information about the function of pathways within the spinal cord.

1.2.1 Muscle receptors

Information such as the length of a muscle and the amount of force generated by a given movement is relayed to the central nervous system through muscle receptors. Muscle spindles and Golgi tendon organs are two different types of muscle receptors that are particularly important in the control of motor behaviour through the activation of a reflex arc. Muscle spindle primary endings, located throughout skeletal muscle, respond to length of a muscle as well as the velocity of contraction while Golgi tendon organs, located at the junction between the tendon and the muscle, are activated by changes in muscle tension.

1.3 PERIPHERAL NERVE STIMULATION

Peripheral nerves function to transmit important sensory information about the environment toward the central nervous system and to relay efferent motor commands outward to the body. Compared to the mechanical activation of a reflex, activating a nerve electrically has the advantage of exciting neural tissue consistently at the same location using a controlled stimulus intensity. The electrical activation of a peripheral nerve will engage the reflex arc along with related connections within the spinal cord and ascending connections to the brain.

Given that the reflex arc is influenced by other peripheral and descending sources, peripheral nerve stimulation provides information regarding surrounding circuits and their potential role in various motor behaviours. Reflexes are also a valuable clinical tool used to assess function of the nervous system. The monitoring of electrically evoked responses is important for the evaluation of peripheral nerve injuries and has been used in the operating room for nearly 40 years (Kline and DeJonge 1968). The following sections will discuss the physiology of electrically activating neural tissue. The production of an H-reflex and M-wave through peripheral nerve stimulation and how these responses are changed in active muscle is also described.

1.3.1 Electrophysiology

To understand how electrical stimulation is used to activate neural tissue we must first discuss the flow of ions across a cell membrane and how a signal is transmitted along an axon. The following subsections describe how a neuron is activated, the mechanisms which mediate an action potential, and a technique commonly used to record the electromyographic activity produced by the innervated muscle.

1.3.1.1 Electrical activation of a neuron

Peripheral nerves are commonly activated by placing the cathode on the skin over the nerve and the anode somewhere along the nerve a short distance away (bipolar stimulation) or at distant site such as on the other side of the limb (monopolar stimulation). As the stimulation is applied, electrons will flow from the cathode, through the nerve membrane, toward the anode. This will result in a depolarization of the membrane. Provided the potential difference is large enough, voltage-gated sodium channels will open and trigger the production of an action potential (discussed in the following section). When a mixed peripheral nerve is electrically stimulated directly, the fibers with the largest axon diameter (i.e. lowest resistance) will be activated first (Pierrot-Deseilligny and Burke 2012). Smaller and smaller diameter axons will continue to be activated as the intensity of stimulation is increased.

1.3.1.2 Action Potential

The inside of a neuron is negatively charged with respect to the outside when the cell is at rest. In other words, the potential difference across the membrane (inside with respect to outside) is negative. An action potential is a rapid reversal of this potential difference where the inside of the cell will become temporarily more positive than the outside. Voltage-gated ion channels embedded in the cell membrane will open if the membrane potential reaches the channel's threshold. Once a channel opens, positively charged sodium (Na^+) ions flow into the cell and increase the membrane potential which in turn, causes the opening of more voltage-gated Na^+ channels. The action potential will continue in both directions along the axon when activated electrically. The propagation of an action potential toward the synaptic terminal is known as orthodromic conduction while antidromic conduction is a propagation of the signal in the opposite direction. The cell begins to repolarize once Na^+ channels become inactivated and delayed voltage gated potassium (K^+) channels open. Once K^+ channels open, K^+ will flow out of the cell and return the electrochemical gradient to its resting state. In neurons, the action potential is vital for communication between cells.

1.3.1.3 Electromyography

Electromyography (EMG) is a technique used to record the electrical activity produced by skeletal muscle following the activation of motor units. A motor unit is a single motor neuron and all of the muscle fibers it innervates. When a motor unit is activated, a signal or action potential is carried along the motor neuron toward the muscle. After the action potential is transmitted across the neuromuscular junction, the signal will evoke action potentials in all the muscle fibers that are innervated by the motor neuron. The electrophysiological activity produced by multiple motor units can then be recorded using EMG. To detect surface myographic signals, two recording electrodes are typically placed on the skin above the muscle (bipolar recording). Gentle abrasion of the skin and cleansing using alcohol swabs is done to lower the electrode impedance and increase the signal-to-noise ratio of the EMG signal. The signal is used to evaluate the amount of activity produced by motor units supplying the muscle of interest.

1.3.2 H-reflex and M-wave recruitment curve

The electrically evoked soleus H-reflex (Figure 1.1) is studied in the soleus muscle following transcutaneous stimulation of the posterior tibial nerve (TN) in the popliteal fossa (Schieppati, 1987). A low intensity stimulus to a mixed peripheral nerve such as the TN, will activate sensory Ia nerve fibers first due to the large diameter of their axons relative to the αMN (Latash 1998). As shown in Figure 1.1, the excitation of primary muscle spindle (Ia) afferents will result in the propagation of an action potential toward the αMN in the spinal cord. Here the electrical activity will lead to the release of synaptic vesicles containing a chemical transmitter that can be transmitted from the Ia terminal, across the synapse and then towards the αMN. The transmitters will then bind to post-synaptic receptors and lead to the opening of ion channels and the depolarization of the post-synaptic dendritic membrane. Provided the synaptic inputs to the αMN are sufficient to bring the αMN to firing threshold, an action potential will be initiated at the axon hillock which then propagates along the αMN out from the spinal cord and towards the neuromuscular junction at the muscle. As the stimulus intensity is increased further, the axons of the largest diameter αMNs will be activated. The direct activation of motor axons will result in the production of an M-wave. Due to the shorter path that an M-wave must travel to

reach the neuromuscular junction relative to an H-reflex, the M-wave will appear ~20 ms earlier on the soleus EMG. An increase in stimulus intensity, beyond that required to reach the maximum amplitude of the reflex (H_{max}) will result in the incremental decrease of the H-reflex as the M-wave amplitude increases. This is due to antidromic signals propagating from the αMN, at the site of stimulation, toward the spinal cord to collide with and occlude signals from the H-reflex (Aagaard et al. 2002). Eventually, further increases in stimulus intensity will not increase the amplitude of the motor response. At this point, the M-wave has reached its maximum amplitude (M_{max}).

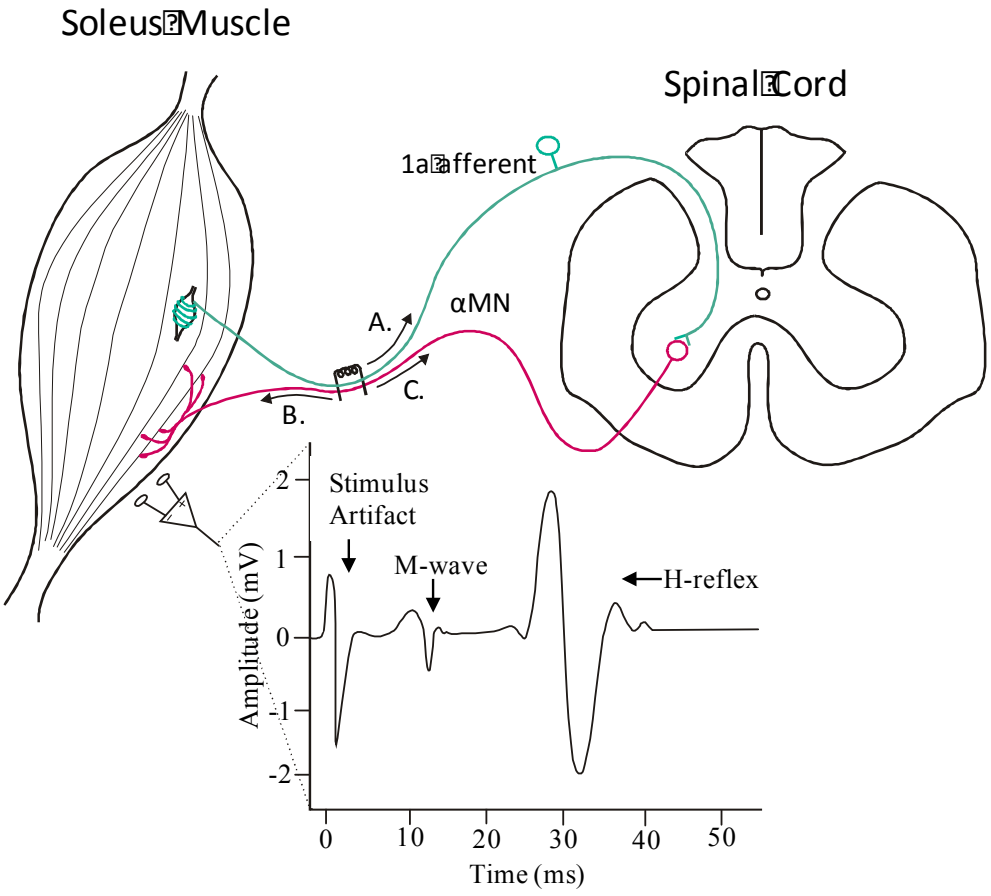

Figure 1.1: Schematic representing the H-reflex pathway and direct muscle response (M-wave). *A:* When a low intensity stimulus is delivered to a mixed peripheral nerve, action potentials are initially produced in the largest diameter Ia afferent fibers. These signals will travel toward the

synapse between the Ia afferent and αMN where they give rise to excitatory post-synaptic potentials (EPSPs), which in-turn, result in the propagation of action potentials along the αMN toward the muscle. Once these signals reach the muscle they are recorded as an H-reflex using EMG. The direct activation of αMN axons will result in the orthodromic propogation of action potentials toward the muscle and the production of an M-wave (B). Compared to the H-reflex, the M-wave will appear ~20 ms earlier on the soleus EMG due to the shorter path that an M-wave must travel to reach the muscle. Action potentials travelling antidromically toward the spine (C) will collide with and occlude action potentials produced by the reflex response travelling orthodromically along the same fibers.

1.3.3 Effect of muscle contraction on the soleus H-reflex

H-reflexes are dramatically changed when measured during contraction compared to rest. In particular, the amplitude of a soleus H-reflex is increased during plantarflexion (extension of the ankle). With voluntary contraction, the excitability of the motor neuron pool is raised close to firing threshold, thereby increasing the likelihood of producing a reflex response. Due to the increased excitability of the motor neuron pool, an H-reflex can be recorded in virtually all accessible limb muscles (Pierrot-Deseilligny and Burke 2012) using a lower stimulus intensity (Burke et al. 1989).

1.4 TRANSCUTANEOUS SPINAL STIMULATION

In 1943, Lloyd demonstrated that direct stimulation of posterior roots in the cat will result in the reflexive activation of adjacent motor neurons. A similar dorsal root-ventral root reflex activation has been studied via EMG recordings in humans following epidural stimulation of the lumbar spinal cord (Jilge et al. 2004). The production of a spinally-evoked reflex response was also possible using transcutaneous electrical stimulation over the low thoracic and lumbar spine in humans (Minassian et al. 2007). These multisegmental responses, known as reflex root-evoked potentials (REP; Roy et al. 2012; 2014), posterior root-muscle reflexes (Minassian et al. 2007) or

multi-segmental monosynaptic responses (Courtine et al. 2007), are reminiscent of the H-reflex (Maertens de Noordhout et al. 1988; Courtine et al. 2007; Minassian et al. 2007). REPs are produced through the activation of sensory axons within the same peripheral nerves as the H-reflex; only the site of activation occurs at the spinal nerve roots. As the stimulating cathode is progressively moved from the lumbar spine rostrally toward the thoracic spine, the latency of the REP decreases, consistent with a shorter reflex arc (Maertens de Noordhout et al. 1988). In contrast, there is an opposite change in latency when motor rather than sensory nerve roots are activated during the stimulation.

REPs exhibit the features of post-activation depression (see section 1.5.1), whereby a second stimulus delivered within 300 ms of the first, will elicit a smaller response (Courtine et al. 2007; Minassian et al. 2007, 2009; Minassian et al. 2011). Paired stimuli, 50 ms apart, can be used as confirmation that the REP was produced reflexively, given that post-activation depression will diminish the second response if produced trans-synaptically (Roy et al. 2012; 2014). Akin to the H-reflex (Burke et al. 1989; Hultborn and Nielsen 1998), the depression is reduced during a voluntary contraction.

An REP can be composed of both reflex and M-wave waveforms but the amount of each is dependent on the intensity of stimulation and the location of the stimulating cathode along the spine. High intensity stimulation was shown to activate motor roots directly when the cathode was placed over the lumbosacral enlargement (Troni et al. 2011). Roy and colleagues (2012) showed that sensory roots are optimally targeted over L1-L3, while motor roots are optimally targeted over the lower thoracic and sacral spine, in seated patients. The preferential activation for sensory fibers over the lumbar spine may be related to the proximity of posterior roots relative to the stimulating electrode, as compared to anterior roots.

Evidence that corticospinal inputs can interact with spinal interneurons and the REP, comes from condition-test paradigms. Anodal stimulation of the thoracolumbar spine produces a long latency facilitation of the MEP and a conditioning MEP was shown to facilitate a trans-spinally evoked

response delivered up to 50 ms later (Knikou 2014). Likewise, TMS delivered 11-25 ms before a spinal stimulus using cathodal stimulation can facilitate the REP (Roy et al. 2014). Such descending input delivered 8 to 13 ms earlier can also decrease the post-activation depression produced using paired-pulse REPs (50 ms apart).

1.5 PATHWAYS ACTING ON THE H-REFLEX

The monosynaptic H-reflex is under the influence of numerous peripheral and descending pathways. When compared under different test conditions (i.e. during contraction, under general anesthesia, pathological states etc.), H-reflex amplitude can be used to evaluate how these conditions effect spinal cord circuitry. Here we review several different peripheral pathways that are known to influence the size of the H-reflex either through a pre- or post-synaptic mechanism. Subsections are further broken down to examine the effect of different test conditions (i.e. volitional drive and/or descending input) on the H-reflex directly or indirectly through related pathways. Given that transmission within the spinal cord is altered in patients with Parkinson's disease and that these changes contribute to the disorders in movement, we also reviewed several studies that have looked at these different pathways and discussed how they are affected.

1.5.1 Pre-synaptic influence

1.5.1.1 Post-activation depression

A previous activation of afferent fibers mediating an H-reflex will result in the depression of a subsequent reflex. This phenomenon, known as post-activation depression, homosynaptic depression, or rate dependent depression, is a form of synaptic plasticity that is said to play an important role in behavioral habituation and in maintaining sensitivity to novel stimuli (Cohen et al. 1997; Hultborn and Nielsen 1998). Post-activation depression may last up to 10 s at rest (Crone and Nielsen 1989; Pierrot-Deseilligny and Burke 2012; Andrews et al. 2015a) but when studied in an active muscle, is greatly reduced during sitting and abolished while standing (Stein et al. 2007).

The attenuated depression observed during a voluntary contraction is said to be due to a background level of post-activation depression, caused by the Ia activity occurring during contraction, that can only be marginally increased with additional activations of the sensory-motor synapse (Pierrot-Deseilligny and Burke 2012).

Post-activation depression is suggested to be mediated by a presynaptic mechanism. In Aplysia, the activation of post-synaptic glutamate receptors was not capable of inducing post-activation depression (Armitage and Siegelbaum 1998). Work in humans using transcranial magnetic stimulation (TMS) have also supported this conclusion (Hultborn et al. 1996). Here, a motor evoked potential (MEP) from TMS was not depressed following a previous activation to the same reflex arc.

The mechanism behind post-activation depression is not entirely agreed upon. Katz and colleagues in 1977 and others (Palmieri et al. 2004; Encyclopedia of Neuroscience, 2009) have suggested that the long lasting inhibition is likely the result of neurotransmitter depletion. This explanation is less likely given that, following repeated stimulation, the decreased number of nearby vesicles in the presynaptic terminals of sensory neurons (Bailey and Chen 1988) is not large enough to account for the post-activation depression of a subsequent response (Armitage and Siegelbaum 1998). Rather than an outright depletion of neurotransmitters, post-activation depression may instead be related to a change to the probability of neurotransmitter release at the sensory-motor synapse (Hultborn et al. 1996; Armitage and Siegelbaum 1998). Other authors suggest that the altered likelihood of neurotransmitter release is related to an increase in presynaptic inhibition (Schieppata and Crenna, 1987; Crone and Nielsen, 1989). While this theory may account for the first few hundred milliseconds of the depression, it does not explain the entire duration of post-activation depression as presynaptic inhibition of this pathway lasts no longer than 400 ms (Nielsen et al. 1995c). Please refer to *section 1.5.1.2* for a more detailed discussion of presynaptic inhibition.

Peripheral nerve afferents have been shown to interact with descending inputs from the corticospinal tract (CST; Deuschl et al. 1991; Nielsen et al. 1993; Poon et al. 2008; Guzman-Lopez et al. 2012). More specifically, transcranial magnetic stimulation (TMS) will attenuate or even eliminate the post-activation depression acting on the H-reflex (Roy et al. 2014; Andrews et al. 2015b). Additional evidence that post-activation depression is under supraspinal control comes from patients with Parkinson's disease. The reduced post-activation depression observed in patients off treatment for parkinsonian symptoms, was restored to normal levels during deep brain stimulation (DBS) of the subthalamic nucleus (STN; Raoul et al. 2012).

1.5.1.2 Presynaptic inhibition

To avoid overwhelming the nervous system with incoming sensory information, signals travelling along Ia afferents can be selectively inhibited by interneuronal connections within the spinal cord (Fig 2). Presynaptic inhibition has been shown to reduce monosynaptic transmission at the Ia-αMN synapse through the activation of $GABA_A$ receptors and primary afferent depolarization (Eccles et al. 1961; Rudomin 1990; Rudomin and Schmidt 1999). Activation of $GABA_A$ receptors located on Ia terminals will trigger an efflux of chloride (Cl^-) ions thereby producing a slight depolarization of the afferent terminal. The depolarization may be attenuated due to an increase in membrane conductance and the Cl^- mediated shunting of Na^+ current. This will decrease the size of an incoming action potential and subsequently reduce the release of excitatory neurotransmitter into the Ia-αMN synaptic cleft (Eccles et al.1961; Rudomin & Schmidt, 1999). An increase in presynaptic inhibition will, in-turn, depress H-reflex amplitude.

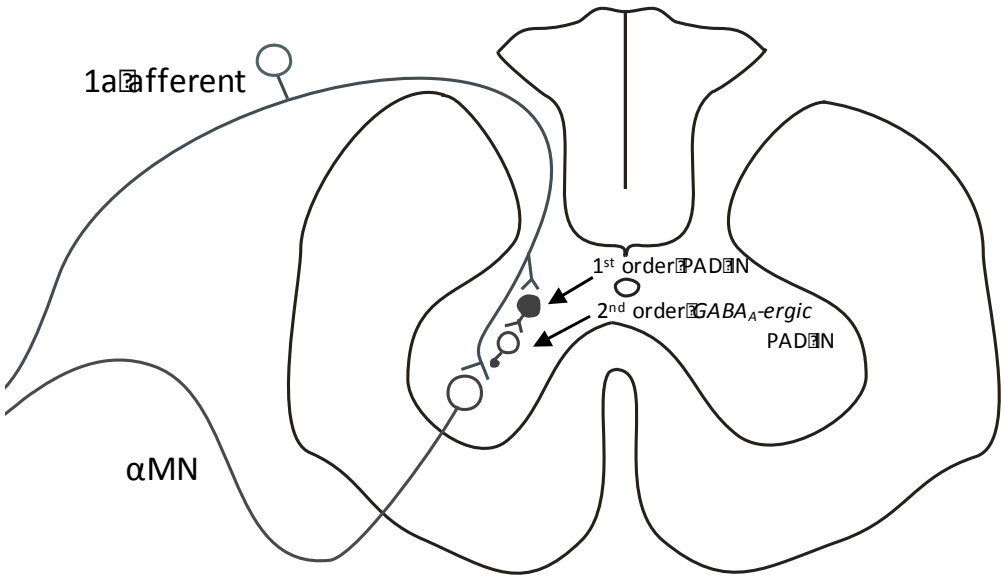

Figure 1.2: Illustration of the pathways mediating presynaptic inhibition. For this figure and the ones that follow, excitatory synapses are denoted by Y-shaped connections and inhibitory synapses by small filled circular connections. The first-order excitatory primary afferent depolarizing (PAD) interneuron (IN) is represented by a filled circle and the last-order $GABA_A$-ergic PAD IN by an open circle. First-order PAD INs receive excitatory input from Ia afferents and relay this input to the 2nd-order PAD IN, which in-turn, activate $GABA_A$ receptors on Ia terminals inducing a depolarization of the afferent due to the outflow of Cl^- ions. This will reduce the amplitude of an incoming action potential and decrease the release of neurotransmitters.

Activity in Ia and Ib afferents will reduce motor neuronal excitability through increased presynaptic inhibition from close agonists, antagonists, and even muscles acting on distant joints. More specifically, a train of stimuli to the common peroneal nerve (CPN) will induce a transient inhibition of the soleus H-reflex (El-Tohamy and Sedgwick 1983). This long lasting inhibition (300-400 ms) of the soleus H-reflex, has been attributed to presynaptic inhibition of the Ia terminals (Faist et al. 1996; Capaday et al. 1995). The long duration of presynaptic inhibition, which is in large part due to the slow release and reuptake of GABA (Rudomin and Schmidt 1999),

differentiates presynaptic inhibition of Ia terminals from a potential postsynaptic influence on the αMN (Pierrot-Deseilligny and Burke 2012). Presynaptic inhibition of Ia afferents projecting to homonymous motor neurons will be reduced during voluntary contraction of the target muscle (Iles 1996). In contrast, the voluntary activation of a muscle will increase the presynaptic inhibition of Ia afferents projecting to motor neurons of non-contracting muscles (Iles and Roberts 1987) potentially to help isolate the contraction.

Inputs from descending sources such as the CST, have been shown to facilitate the H-reflex through a reduction in presynaptic inhibition (Valls-Sole et al. 1994; Iles 1996; Meunier 1999), an effect which is increased during contraction (Iles 1996). Cortical control of presynaptic inhibition is presumed to be mediated through connections from the CST to inhibitory interneurons onto the PAD interneurons involved in presynaptic inhibition (Meunier and Pierrot-Deseilligny 1988; Pierrot-Deseilligny and Burke 2012).

Presynaptic inhibition from the lower limb is shown to decrease in patients off their parkinsonian medication (Roberts et al. 1994; Morita et al. 2000). When treated with L-DOPA medication, presynaptic inhibition was shown to return to control values and these effects were significantly correlated with improvements in bradykinesia and walking speed (Morita et al. 2000).

1.5.2 Post-synaptic Influence

1.5.2.1 Reciprocal inhibition

Reciprocal inhibition describes a physiological process designed to achieve co-ordinated muscle contraction, whereby the activation of one muscle will induce the relaxation of its antagonist (Fig 1.3). This is particularly important during movement where the contraction of one muscle acting at a joint and the subsequent relaxation of its antagonist insures muscles are not competing against one another. Reciprocal inhibition is mediated by a single interneuron known as the Ia inhibitory interneuron (see solid grey interneuron Fig 1.3). This connection will convert and relay the excitation from the Ia afferent into the inhibition of its antagonistic motor neuron (Eccles et

al 1956) through the release of the inhibitory neurotransmitter glycine from the terminals of Ia inhibitory interneurons.

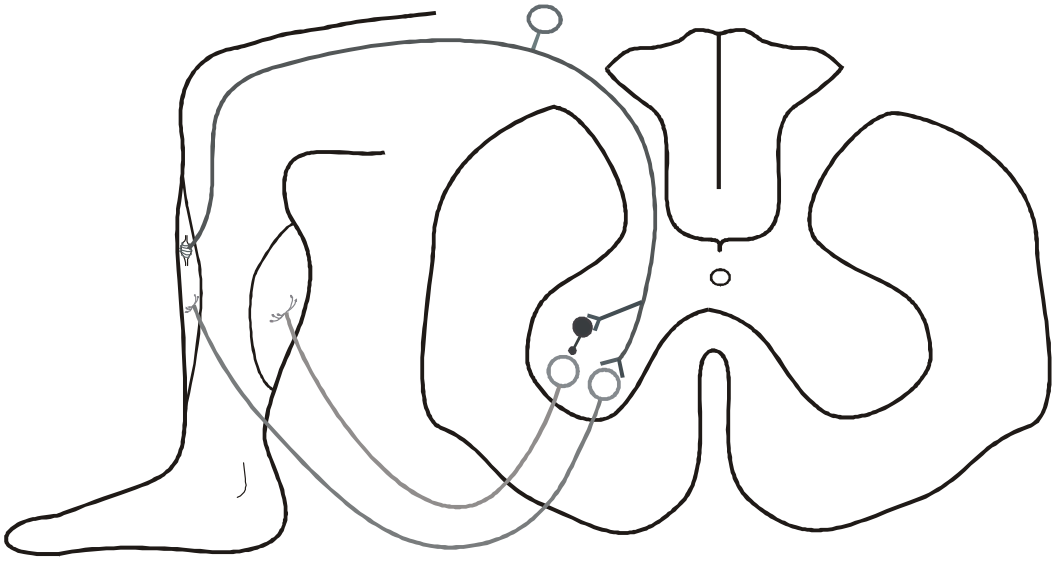

Figure 1.3: Reciprocal inhibition between ankle flexors and extensors. Ia afferents from the tibialis anterior (TA) muscle have monosynaptic connections to homonymous TA alpha motor neurons (αMNs). These Ia afferents also have excitatory connections to Ia inhibitory interneurons (IN), thereby inhibiting antagonistic αMNs of the soleus.

This phenomenon has been extensively studied in ankle flexors and extensors where the electrical stimulation of the common peroneal nerve (CPN) was shown to induce a transient inhibition of the soleus H-reflex (Crone et al. 1986). This inhibition, which has a rapid onset (1 ms) with a brief duration (~2-3 ms), is increased during dorsiflexion (flexion of the ankle; Crone et al. 1987) and decreased during plantarflexion (Tanaka 1974; Crone et al. 1987), relative to rest.

There is strong evidence that Ia inhibitory interneurons receive monosynaptic excitatory and inhibitory input from descending tracts (Jankowska et al. 1976). This means that supraspinal centers can control opposing muscles acting at the same joint using only a single motor

command. In addition, higher centers can control the degree of joint stiffness through these connections by enabling the co-contraction of opposing muscle groups.

Compared to age-matched controls, reciprocal inhibition of the soleus H-reflex at rest was found to be increased in patients with Parkinson's disease. This finding has been attributed to increased activity in extrapyramidal pathways such as the reticulospinal tract (Delwaide et al. 1993). When studied during a voluntary dorsiflexion, however, reciprocal Ia inhibition of the soleus H-reflex was replaced by a facilitation in patients with Parkinson's disease (Hayashi et al. 1988).

1.5.2.1 Autogenic Ib inhibition

Ib afferents originate from Golgi tendon organs and are located at the junction between the muscle and tendon (Pierrot-Deseilligny and Burke 2012). These connections were originally thought to serve as an autogenetic protective reflex by preventing the muscle from overloading during contraction. It is now understood that tendon organs also provide continuous feedback about the strength of the muscle contraction. Studies in humans (Pierrot-Deseilligny et al. 1981) and animals (Eccles et al. 1957) have shown that the dominant role of Ib afferents is inhibition of homonymous and heteronymous agonistic motor neurons, with the excitation of antagonistic motor neurons. Aside from strict antagonists, heteronymous Ib inhibition has been shown to exist between virtually all investigated muscle combinations in both upper and lower limbs (Pierrot-Deseilligny and Burke 2012).

Low intensity stimulation of the medial gastrocnemius (MG) nerve will induce an inhibition of the soleus H-reflex that is maximal after ~5-6 ms and lasts for 10 ms following the conditioning stimulus (Pierrot-Deseilligny et al. 1981). This inhibition is explained by connections from Ib afferents within the MG nerve to Ib interneurons with inhibitory connections to soleus αMNs (Figure 4). Ib inhibition is most commonly studied at rest given that the inhibition is greatly reduced in active motor neurons (Fournier et al. 1983; Stevens and Yang 1996; Pierrot-Deseilligny and Burke 2012). The stronger the contraction, the greater the suppression caused by the homonymous Ib pathway (Pierrot-Deseilligny and Fournier, 1986).

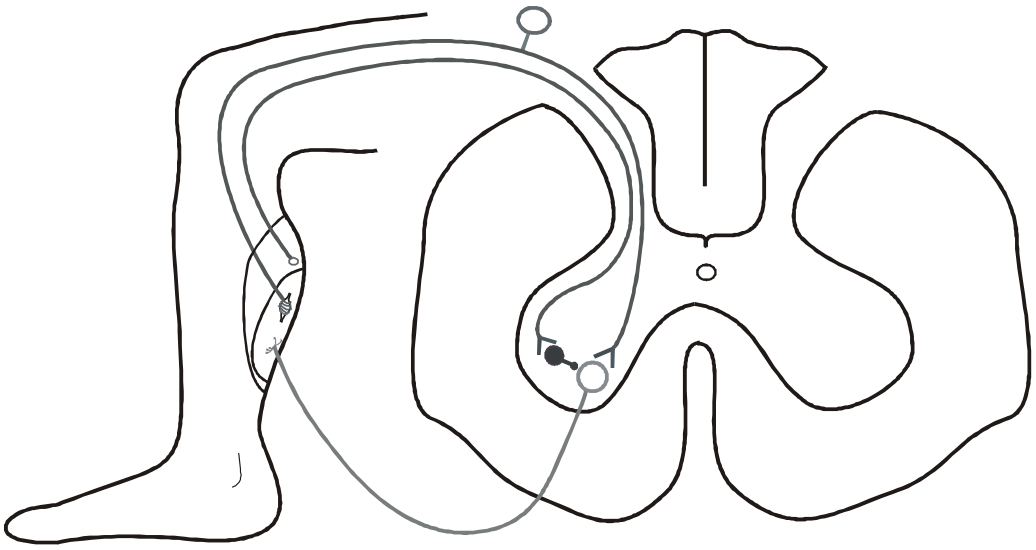

Figure 1.4: Ib inhibitory pathway of the leg. Ib afferents originating from Golgi tendon organs of the medial gastrocnemius (MG) activate Ib inhibitory interneurons (IN) which inhibit heteronymous soleus alpha motor neurons (αMNs).

Descending motor pathways such as the CST and rubrospinal tract have excitatory monosynaptic connections to Ib interneurons (Pierrot-Deseilligny and Burke 2012). By contrast, Ib interneurons receive inhibitory input from the reticulospinal tract (RST). Further evidence that Ib interneurons are under supraspinal control comes from studies on patients with Parkinson's disease. When studied in patients off their parkinsonian medication, MG-induced Ib inhibition of the soleus H-reflex was reduced and possibly replaced by a facilitation (Delwaide et al. 1991). High frequency DBS to the STN was shown to restore Ib inhibition to control levels in conjunction with improvements to rigidity (Potter et al. 2008). Changes in Ib inhibition are likely the result of either decreased activity from tracts with facilitatory connections (i.e. CST) or increased activity from

tracts with inhibitory connections (i.e. RST; Delwaide et al. 1993). Increased deviations of Ib inhibition from normal were correlated with increased severity of rigidity (Delwaide et al. 1993).

1.6 TRANSCRANIAL MOTOR CORTEX STIMULATION

Stimulation of the motor cortex will activate descending pathways and produce an electrophysiological response in the muscle known as an MEP. Famous Canadian neurosurgeon, Wilder Penfield, pioneered MEP research in humans in the 1930s. Through the direct stimulation of the brain in conscious patients undergoing awake craniotomy procedures, he was able to develop a complex map of the body, known as the human motor homunculus, represented along the motor cortex. Studies using MEPs have greatly advanced since then. The introduction of transcranial stimulation in humans has created new possibilities for the non-invasive study of human motor control (Penfield and Jasper 1954). The following sections describe two different techniques developed for the transcutaneous stimulation of the motor cortex; TES and TMS. Descriptions of TMS are further developed to characterize the activation of corticospinal pathways either directly or indirectly by the stimulation.

1.6.1 Transcranial electric stimulation (TES)

Non-invasive stimulation of the human brain was demonstrated in 1980 by Merton and Morton. Through transcranial electrical stimulation (TES), the motor cortex can be stimulated through the skull using surface electrodes placed over the scalp. The result is an EMG response known as an MEP that can be recorded contralaterally from peripheral muscles. Use of TES in laboratories is limited due to the discomfort created as high currents (> 250 mA) pass between the stimulating electrodes. An exception to this limitation; however, is its use in the operating room in patients under general anesthesia where painful stimulation is no longer a concern. In this context, an MEP demonstrates intact motor pathways (Kothbauer et al. 1998; MacDonald et al. 2013) and has been used to replace the Stagnara wake-up test.

1.6.2 Transcranial magnetic stimulation (TMS)

The use of TMS in 1985 by Barker and colleagues enabled the study of motor pathways in awake patients with minimal discomfort. Given that the induced current flows within the brain, this limits the activation of pain fibers located on the scalp (Chen et al 2008). The magnetic stimulator was developed based on the principle of electromagnetic induction, where a rapidly changing magnetic field produced around a wire in response to the flow of current through the wire will induce a secondary current within conductive tissue under the stimulating coil. TMS is commonly used to measure the activity and function of circuits within the brain and spinal cord. Clinically, TMS is used to assess the damage caused by trauma to neural tissue i.e. stroke, multiple sclerosis, spinal cord injury, and other movement disorders such as Parkinson's disease (Groppa et al. 2012).

1.6.2.1 Motor cortex

The primary motor cortex, also known as Brodmann's area 4, is located in the dorsal part of the frontal lobe within the precentral gyrus (Kendel et al. 1991). This region contains neural networks involved in the execution of voluntary movements. Representation of human musculature along the motor strip, or motor homunculus, is organized in an orderly fashion with the muscles of the foot located in the convexity of the medial longitudinal fissure and muscles of the tongue and pharynx dorsal to the lateral sulcus. Stimulation over the motor cortex will produce the activation of contralateral muscles by activating corticospinal axons in layer V of the cerebral cortex.

1.6.2.2 Corticospinal tract (CST)

Corticospinal neurons from pyramidal cells in layer V of the motor cortex descend in the white matter through the internal capsule (Kendel et al. 1991). Many of these fibers will cross the midline in the medulla and send long axons down the opposite side of the spinal cord. Corticospinal neurons from the motor cortex will descend the lateral CST (Hall et al. 2005). Here CST neurons synapse either directly onto lower motor neurons or onto the interneuronal circuitry

of the spinal cord. Damage to the CST can lead to the development of various symptoms such as spasticity, hyperreflexia, and motor weakness (Lemon and Griffiths 2005).

Approximately 80% of CST fibers cross over in the medulla making these connections important in the control of the opposite side of the body. The CST has many different functions including the descending control of: afferent input (Cheema et al. 1984; Wall and Lidierth 1997), spinal reflexes (Pierrot-Deseilligny and Burke 2012), autonomic control (Bacon and Smith 1993), and excitation/inhibition of motor neurons (Alstermark and Lundberg 1992; Porter and Lemon 1993) among others. Studies in humans by Kuypers (1981) have provided evidence of the existence of monosynaptic connections between CST fibers and motor neurons. These projections are a uniquely primate feature (Lemon 2008) and correlated with the species' index of manual dexterity (Heffner and Masterton 1983).

1.6.2.3 Descending influence from the CST

Transcranial stimulation over the human motor cortex produces a short-latency muscle response consistent with a monosynaptic connection from the CST to αMNs (Rothwell et al. 1991). Activation of the motor cortex has been shown to produce an early facilitation of the soleus H-reflex compatible with the activation of direct monosynaptic connections from the motor cortex to the soleus motor neuron pool (Cowan et al. 1986; Brouwer and Ashby, 1992; Nielsen et al. 1993; Nielsen and Petersen, 1995a). Approximately 1-5 ms later, a low intensity stimulus from TMS can also evoke a transient phase of inhibition acting on the soleus H-reflex (Nielsen et al. 1993; Nielsen and Petersen 1995b) likely through the excitation of inhibitory interneurons projecting to soleus αMNs (Cowan et al. 1995; Nielsen et al. 1993). During a voluntary tonic plantarflexion, the early facilitation persists for 20-25 ms and tends to overwhelm the period of early inhibition (Nielsen et al. 1993) making it more difficult to isolate.

Conditioning effects of transcranial stimulation on the soleus H-reflex were also studied in people with Parkinson's disease (Morita et al. 2002; Potter-Nerger et al. 2008). The early period of facilitation of the H-reflex shown in control subjects using subthreshold TMS was not present in

those with Parkinson's disease during contraction (Morita et al. 2002; Potter-Nerger et al. 2008). In some patients, this early period of CST-αMNs facilitation was even replaced with an inhibition. Following a pallidotomy (Morita et al. 2002) and during STN-DBS (Potter-Nerger et al. 2008) in those with Parkinson's disease, this phase of early excitation was restored to control levels.

1.6.3 Corticospinal volleys

TES currents transmitted through the axons of corticospinal neurons, which are orientated perpendicular to the surface of the cortex, are responsible for activating fast conducting neurons and producing a short latency volley known as a direct (D)-wave (Di Lazzaro et al. 2008). In contrast, exciting neurons oriented parallel to the surface of the cortex, within cortical grey matter, will produce an indirect, trans-synaptic activation of the CST and elicit several slightly later responses known as indirect (I)-waves. I–waves are produced at fairly equal time intervals (~1-ms apart) which to a certain extent, reflects the number of synapses between the interneuron being activated and the corticospinal neuron where responses are recorded. Recordings from CST neurons have shown that a single axon may produce both D- and I-waves (Patton and Amassian 1954) suggesting that CST neurons are capable of high frequency firing.

In general, TES and TMS produce unique fields of activation. TMS will excite conductive tissue more superficially thereby limiting the activation of deep brain tissue (Epstein et al. 2008) For this reason, low intensity TMS will preferentially activate CST neurons indirectly (Di Lazzaro et al. 2008). However, studies in monkeys showed that a high intensity stimulus from TMS will also produce a D-wave, along with the more commonly expected I-waves (Edgley et al. 1997; reviewed in Di Lazzaro et al 2008). When threshold for D- and I-waves were tested in the same group of corticospinal neurons, TMS had a higher affinity than TES for producing I-waves, and the opposite was true in the case of D-waves. Coil shape and the targeted motor areas may add to the complexity and the prediction of activation patterns using TMS.

1.7 INTRAOPERATIVE NEUROMONITORING

Intraoperative neurophysiological monitoring (IONM) is used to reduce the risk of injury to the nervous system during surgery. Traditionally, somatosensory evoked potentials (SSEP) were used to monitor both ascending and descending spinal cord pathways on the hope that damage to motor pathways would be reflected in changes to the dorsal column and the SSEP signals. Unfortunately, due to the distinct vasculature of the two systems, cases of motor impairment without concomitant loss of SSEPs were still being reported (Lesser et al. 1986; Newer et al. 1995). This therefore led to the introduction of TES into the operating room to extend the coverage to descending spinal motor pathways (Calancie et al. 1998).

1.7.1 Intraoperative TES and MEPs

The monitoring of the CST dates back to 1870, when Fritsch and Hitzig used direct stimulation of the cortex in dogs to produce movement in muscles on the opposite side of the body (Fritsch and Hitzig 2009). During surgery, TES and MEP monitoring is used to assess functional integrity of descending motor pathways in the human spinal cord (Kothbauer et al. 1998; MacDonald et al. 2013). As mentioned previously, the CST has many different functions such as the control of voluntary movement. Damage to the CST will produce a loss or decrease in the size of the MEP. The amplitude and morphology of an MEP thus provides important information about CST physiology.

1.7.2 General anesthesia

The success of using single pulse TES in the operating room was limited due to the suppressive effects of general anesthesia. In a non-anesthetized patient, a single pulse of TES over the motor cortex produces several activations of the CST in the form of D- and I-waves (Patton and Amassian 1954; Katayama et al. 1988). However, I-waves are suppressed under general anesthesia and the same stimulation from TES will only produce a D-wave (Boyd et al. 1986). Given that multiple excitatory post-synaptic potentials (EPSPs) are required to bring the motor neurons to firing

threshold, several pulses from TES are optimal to produce an MEP in patients under general anesthesia.

In 1993, Taniguchi and colleagues introduced a technique in the operating room of multi-pulse TES which enabled motor neurons to more consistently reach their firing threshold. This technique was paramount in overcoming the suppressive effects of anesthesia on the MEP (Erickson 1949; Wood et al. 1988; Sloan 1998). Multi-pulse TES has since become the preferred technique used in detecting iatrogenic motor tract injury (MacDonald et al. 2013). The overall goal of multi-pulse TES during IONM is to reduce the incidence injury to motor pathways within spinal cord.

1.7.3 Conditioning techniques

MEP monitoring may be restricted in some patients with pre-existing neurological disorders where the production of MEPs is affected (Chen et al. 2007; Master et al. 2008; Sloan et al. 2008). For example, in very young children (Andersson et al. 1999; Erb et al. 2005) and patients with neuromuscular weakness (Langeloo et al. 2001), single-train TES may be insufficient for producing intraoperative MEPs. Double (Journee et al. 2004; Journee et al. 2007) and multiple trains of TES (Tsutui et al. 2015) may be beneficial in these instances. In addition, alternate techniques such as afferent facilitation (Taniguchi and Schramm 1991; Andersson and Ohlin 1999) and post-tetanic facilitation (Hayashi et al. 2008) are used to achieve sufficient depolarization of motor neurons.

1.7.3 Reflex monitoring

Monitoring H-reflexes in conjunction with traditional IONM techniques will not only enable the assessment of CST function but also the complex peripheral systems involved in motor control (Leppanen 2006). H-reflexes (where present) measure the function of a large portion of motor pool output (20-100%; Leis et al 1996). Since they produce little to no patient movement in the surgical field (Leppanen 2006) they can also be applied continuously without interfering with

surgery. A change in reflex amplitude may directly signify a compromise to segmental motor pathways but is limited to only providing indirect information about the integrity of the pyramidal and extrapyramidal systems. To provide a more direct measure of supraspinal function, the H-reflex may be combined with an MEP. The spatio-temporal summation of these responses has been described as the most sensitive means of detecting spinal cord injury (Leppanen 2006). Monitoring using this technique may be particularly useful in spastic patients, where spinal reflexes are exaggerated, likely due to enhanced motor neuron excitability (Calancie et al. 1993). Further to this, monitoring using the composite TES/H-reflex response may also be beneficial in myelopathic patients where the MEP is already more difficult to elicit.

1.8 PARKINSON'S DISEASE

Parkinson's disease (PD) is a progressive disorder that affects as many as 100,000 Canadians (reviewed in Rizek et al. 2016). The average age of diagnosis is 60, with the majority of those diagnosed over the age of 65. The disorder was first described in 1817 by Dr. James Parkinson as "the shaking palsy." Cardinal motor features associated with the disease include tremor, bradykinesia, rigidity, and postural instability. Other symptoms may include difficulties with speech, fatigue, depression, and changes in cognition. Each person with PD is unique and will likely experience different symptoms of the disease.

1.8.1 Pathophysiology

PD affects several regions of the central nervous system. For example, PD involves the death of dopamine-producing cells of the substantia nigra (Figure 1.5). Dopamine deficiency leads to the motor disturbances associated with PD (Penney and Young 1986; DeLong 1987; Crossman 1987). This was discovered when the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was found to produce a selective loss of dopaminergic neurons in the substantia nigra pars compacta. The pathophysiology of parkinsonism is characterized by increased output from the basal ganglia nuclei (i.e. globus pallidus internus (GPi) and STN) which in-turn causes excessive

inhibition of brainstem motor systems (Marsden 1982; Crossman 1987; Obeso et al. 1997). A dopamine deficiency and reduced activation of dopaminergic receptors results in diminished excitation of the direct pathway (i.e. direct projections from the putamen to basal ganglia nuclei) and reduced inhibition of the indirect pathway (i.e. indirect connections through globus pallidus externus (GPe); Penney and Young 1986) leading to a dramatic suppression of the voluntary motor systems and the development of PD motor signs.

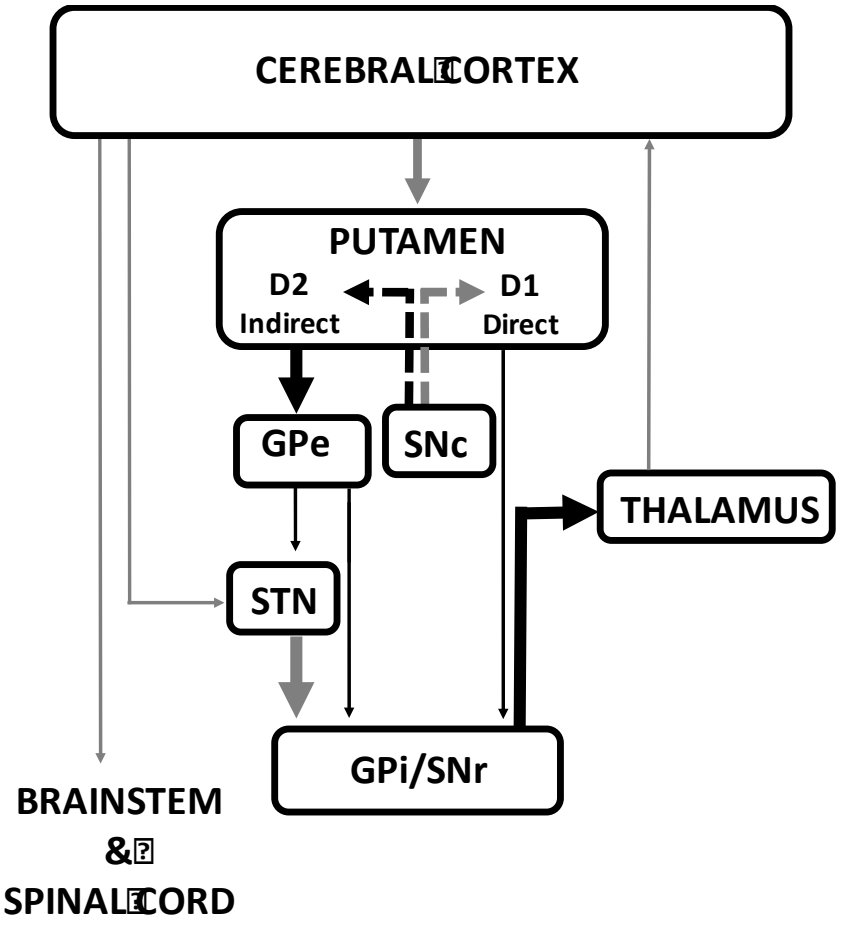

Figure 1.5: Schematic illustration of the basal ganglia-thalamocortical circuit in Parkinson's disease (PD). Excitatory and inhibitory pathways are indicated by grey and black arrows, respectively. The thickness of the arrows indicates an increase (larger arrow) or a decrease (smaller arrow) in firing rate between specific connections. Dashed arrows from the substantia nigra pars compacta (SNc) signify a decrease in input to the putamen due to the death of dopamine producing cells in PD. Through the direct (D1) pathway, a decrease in dopaminergic

input leads to a decrease in inhibition of basal ganglia nuclei (i.e. globus pallidus internus (GPi) and substantia nigra pars reticulate (SNr)). Inhibitory output from the indirect (D2) pathway is increased and so the output from the globus pallidus internus (GPe) is decreased resulting in reduced inhibition of the basal nuclei and the subthalamic nucleus (STN). This increase in excitatory input and decrease in inhibitory input to the basal ganglia nuclei will lead to excessive inhibition of the thalamus which will in turn decrease its excitatory output to the cerebral cortex.

1.8.2 Treatment

Early symptoms of the disorder are mainly treated through dopamine agonists or levodopa (L-DOPA). Once L-DOPA crosses the blood-brain barrier, it is converted to dopamine. Increased concentration of the neurotransmitter and the subsequent activation of dopamine receptors in the brain improves symptoms of PD. While these treatments are sufficient for symptom management during the early stages of the disease, they may eventually become less effective as the disease progresses and dopaminergic neurons continue to be lost. With time, as therapeutic efficacy of L-DOPA decreases and motor symptoms worsen, adverse effects may emerge (i.e. dyskinesia) (Fahn and Calne 1978; Lesser et al. 1979).

When symptoms become inadequately controlled by medication, treatment may involve the implantation of a medical device known as DBS. The two most common targets of DBS electrodes are the STN and the GPi. The electrodes in the brain are connected to an impulse generator (IPG) implanted in the chest wall via wires tunneled under the skin. High frequency stimulation from DBS was originally believed to have a lesion-like effect given that DBS in PD mimics the effects of a lesion of the same structure (Benazzouz and Hallett 2000). However, this may not be the case since ablation of the GPi will produce parkinsonism but DBS to this area will reverse parkinsonism (Vitek et al. 2004). DBS mechanisms are likely more complicated and involve a combination of both excitatory and inhibitory effects on the neural tissue being stimulated (Montgomery 2006), but the specific mechanism of action for DBS is not completely understood. Despite the positive initial outcome following DBS, symptoms may eventually worsen in later stages of PD.

1.8.3 Parkinson's disease and spinal cord circuity

Transmission within spinal pathways is altered in patients with PD and these changes contribute to the movement disorders associated with the disease (Delwaide et a. 1991; Delwaide et al. 1993; Raoul et al 2012). It is important to understand which pathways are affected by PD and the extent to which DBS and/or medication modulates these pathways. Although a favorable response to L-DOPA is positively correlated with outcome following DBS, few studies have investigated changes in motor control following combined DBS and drug therapy. With a better understanding of the changes that occur in spinal cord circuitry following these treatments, future therapies may involve stabilizing or even augmenting these benefits and may, in turn, lead to better control of PD symptoms.

1.9 THESIS OUTLINE

1.9.1 Chapter 2

This thesis focuses on the electrically-evoked reflex response and the surrounding pathways that influence this response. The thesis begins by characterizing the time course of post-activation depression in the soleus muscle and compares the recovery of a peripherally-evoked H-reflex to a spinally-evoked REP in a group of healthy subjects. To induce post-activation depression, each type of response (i.e. H-reflex or REP) was conditioned by either an H-reflex or an REP. The recovery from post-activation depression was evaluated at various interpulse intervals, using three different stimulus intensities both with subjects at rest and during a voluntary contraction. Recovery of the depressed response increased as a function of the interpulse interval and was greatest during voluntary contraction as compared to rest. Post-activation depression acting on the H-reflex was generally similar whether it was conditioned by another H-reflex or by an REP. The same was true for an REP conditioned by the H-reflex, though the REP doublet showed slightly greater depression. This suggests that the pathway mediating the spinally-evoked response was more susceptible to being inhibited. Given that post-activation depression will only

affect a reflex response if that same sensory-motor synapse activated by the reflex was activated previously, this suggests that both the H-reflex and REP are mediated along the same pathway. This study is important firstly, because transcutaneous spinal stimulation and the REP has been used to augment voluntary motor activity and reduce spasticity in those with incomplete spinal cord injury, and secondly, because improving our understanding of the pathways that mediate this response will help with the implementation of these interventions.

1.9.2 Chapter 3

Chapter 3 builds on the previous chapter by investigating the influence of descending input from TMS on post-activation depression produced by double pulse H-reflexes and REPs in healthy individuals. The interaction was first studied by Roy et al. (2014) but is expanded here to compared between: active and resting states, the H-reflex and REP, and different TMS intensities. We began by characterizing the interaction during voluntary contraction given that post-activation depression will already be reduced in an active muscle. To test the strength of the interaction, the influence of an MEP on a depressed H-reflex was further investigated at rest. TMS was shown to significantly reduce and even reverse post-activation depression. The influence of an MEP on a depressed reflex was strong enough to remove post-activation depression produced by a pair of peripheral nerve stimuli delivered 10 ms apart. Further to this, paired pulse TMS could recover a second and third H-reflex when triple pulse peripheral nerve stimulation was applied at ~25 ms intervals. Traditionally, it was believed that post-activation depression may be mediated by a depletion in neurotransmitter at the sensory-motor synapse. Evidence from this chapter suggests that alternate mechanisms may be responsible for post-activation depression given that a second response could be recovered at intervals too short (10-15 ms) to allow the restoration of the neurotransmitter supply.

1.9.3 Chapter 4

Chapter 4 is a direct extension of the previous chapter in that the removal of post-activation depression by a low intensity transcranial stimulus was adapted for use in the operating room.

Here the interaction was used to monitor important pathways within the spinal cord in 20 patients undergoing spine surgery. Multi-pulse TES is routinely used during surgery to monitor function of motor pathways. This technique can only be applied intermittently throughout a procedure as the high intensity stimulus from TES can cause the contraction of trunk and limb muscles which may be disruptive to the ongoing surgery. The present technique proposes to monitor CST pathways, as would be done with traditional MEP monitoring, but by combining a "subthreshold" MEP to the spinal circuits which mediate post-activation depression of the H-reflex. A low intensity stimulus is advantageous because it can be used without increasing patient movement during surgery. In this study, we demonstrated that TES could successfully remove post-activation depression in 20/20 patients under general anesthesia (a state of decreased excitability). The strength of the interaction did not diminish throughout surgery and patient movement was eliminated in 19/20 patients. In one case, there was a significant event during surgery whereby the placement of a stabilizing rod caused the loss of MEPs. This loss was then correlated with the loss of the TES-post-activation depression conditioning effect. This suggests that the interaction may monitor the same essential pathways as traditional monitoring techniques, and may be particularly beneficial during surgeries where patient movement is disruptive to the on-going surgery.

1.9.4 Chapter 5

This last experimental chapter focused on several different circuits within the spinal cord of people with PD receiving both medication and DBS for treatment of parkinsonian symptoms. More specifically, we studied changes in how the H-reflex is modulated by post-activation depression, Ib inhibition, presynaptic inhibition, reciprocal inhibition, and descending input from the CST. These pathways were chosen because they are all well-studied in control groups and have been shown to be altered in people with PD. Further to this, changes to each of these pathways in PD are correlated with the severity of various motor symptoms associated with the disease (i.e. rigidity, bradykinesia, and tremor). This study aims to determine the degree to which each pathway is affected in patients not receiving treatment for their parkinsonian symptoms and then to characterize the influence of parkinsonian medication and DBS both together and in

isolation (note a total of 4 treatment conditions). To determine the degree of change that occurred during these 4 treatment conditions, results from our patient group were compared to a control group of the same age and gender. Given that the mechanism of action for DBS and how it works in conjunction with parkinsonian medication is not completely understood, improving our understanding of the therapeutic mechanism of action may help with the development of more effective therapies.

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CHAPTER 2: POST-ACTIVATION DEPRESSION IN THE HUMAN SOLEUS MUSCLE USING PERIPHERAL NERVE AND TRANSCUTANEOUS SPINAL STIMULATION

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2.1 INTRODUCTION

Transcutaneous electrical stimulation over the mid lumbar spine activates dorsal roots to elicit a reflex root-evoked potential (REP; Roy et al. 2012; Roy et al. 2014). These multisegmental responses display notable similarities to the Hoffmann or H-reflex showing many of the characteristics that suggest the activation of Ia afferents, followed by transynaptic recruitment of motor neurons (Maertens de Noordhout et al. 1988; Courtine et al. 2007; Minassian et al. 2007). The latency of an REP decreases as the cathode is progressively moved rostrally from the lumbar spine to the low thoracic spine consistent with a shorter sensory-motor reflex arc (Maertens de Noordhout et al. 1988).

Post-activation or homosynaptic depression refers to a reduction of reflex amplitude in response to a previously activated sensory-motor synapse. This is associated with a change in the Ia afferent terminal leading to a transient reduction in neurotransmitter release (Katz et al. 1997, Castellucci et al. 1974; Lev-Tov and Pinco 1992; Elliot et al. 1994; Armitage and Siegelbaum 1998; Hultborn et al. 1996). REPs exhibit the hallmark features of post-activation depression such that the second stimulus delivered within the next 40–300 ms will elicit a smaller response (Minassian et al. 2011) and similar to the H-reflex (Burke et al. 1989; Hultborn and Nielsen 1998), the depression is reduced during a voluntary contraction.

The present study compared the rate of recovery of the soleus REP and H-reflex at intervals from 25 to 200 ms using paired pulse stimuli (i.e., two REPs or two H-reflexes). The REP was also conditioned by a size-matched H-reflex, and the H-reflex was in turn, conditioned by an REP. The effect was studied at three different stimulation intensities, both during a voluntary contraction

and at rest. We hypothesized that, in addition to being modulated as a function of the motor state, the rate of recovery would differ between conditions given that a spinal stimulus results in bilateral, multi-segmental activation of lumbosacral roots as opposed to activation of focal ipsilateral afferents within the tibial nerve (Maertens de Noordhout et al. 1988; Courtine et al. 2007; Minassian et al. 2007). Trains of transcutaneous spinal stimulation are being sought for regulating spasticity (Hofstoetter et al. 2014; Nielsen et al. 1996) and driving locomotion after spinal cord injury (Minassian et al. 2012; Hofstoetter et al. 2013) providing motivation towards increasing our understanding of the spinally-evoked REP.

2.2 METHODS

2.2.1 Participants

Eight able-bodied volunteers were recruited to participate in the study. Volunteers were screened for potential contraindications to the stimulation including chronic back pain or prior spinal surgery. Participants provided written consent to the experimental protocol that was approved by the Health Research Ethics Board at the University of Alberta.

2.2.2 Recording and stimulation

Participants were seated with their left leg in a metal brace maintaining a 100-degree angle of the knee and ankle. The electromyogram (EMG) was recorded from the soleus and tibialis anterior muscles using a pair of silver–silver chloride surface electrodes (3.5 × 2.2 cm; Vermont Medical Inc., Bellow Falls VT) placed 3cm below the distal border of the gastrocnemius and on the belly of the tibialis anterior, 2/3 of the distance from the lateral malleolus to the knee. The EMG was band-pass filtered between 10–1000 Hz, amplified 1000× (Octopus, Bortec Technologies, Calgary, Canada) and digitized at 5kHz using Axoscope Hardware (Digidata 1200 series, Axon Instruments, Union City, CA). The EMG was full-wave rectified and low-pass filtered at 3 Hz so that the participants could monitor their level of EMG using an oscilloscope. Maximum voluntary contraction (MVC) was determined at the start of the experiment.

Transcutaneous spinal stimulation was delivered using a constant-current stimulator (Digitimer

DS7A; Digitimer Ltd., Welwyn Garden City, UK) with the pulse width set to 1 ms. Vertebral levels were identified and marked following palpation by a physiotherapist. The anode (7.5 × 13 cm; Axelgaard Manufacturing Co., Fallbrook, California) remained fixed throughout the experiment above the ipsilateral anterior superior iliac spine. A silver–silver chloride cathode (5 × 5 cm; WalkAide premium electrode; Innovative Neurotronics, Austin TX) was placed on the midline over the spinal column. The cathode was initially placed over the 3rd and 4th lumbar intervertebral space and then moved more rostral in 2.5 cm increments until the position that produced the largest soleus REP was identified.

The tibial nerve was stimulated using 1 ms pulses (SD9 Stimulator, Grass Instruments, West Warwick, RI USA) with the cathode in the popliteal fossa and the anode 4 cm more proximal (3.5 × 2.2 cm; Vermont Medical Inc.,). Distance between electrodes was measured from the center of each electrode. The optimal position was determined using a handheld monopolar probe (1 cm tip), as the site that produced the largest H-reflex with minimal encroachment from the antagonist muscle. A silver–silver chloride surface electrode (2.2 × 2.2 cm; Vermont Medical Inc.) was then placed at the optimal site. Constant pressure was applied over the electrodes using an athletic wrap.

2.2.3 Recovery of the H-Reflex and REP

We studied the recovery of the H-reflex and REP from post-activation depression. The soleus, being mainly composed of slow-twitch fibers (Burke et al. 1974) was chosen since large reflexes are produced by both peripheral and transcutaneous spinal stimulation. Recruitment curves were collected for the H-reflex and M-wave by gradually increasing the stimulus intensity from threshold until reaching the maximal H-reflex amplitude and further to a level that elicited the maximal M-wave (Fig. 2.IA). Spinal doublets were delivered 50 ms apart and used to assess the contribution of the reflex within the REP (see Fig. 2.2D); the presence of a first response and the complete attenuation of a second, was indicative of a reflex (Roy et al. 2012; Courtine et al. 2007; Minassian et al. 2007). For the REP recruitment curve (Fig. 2.IB), the stimulation intensity was increased from threshold up to a level where the first REP was maximal or the intensity where the first REP increased at the same rate as the second REP, which was consistent with direct

activation of motor axons.

At a half maximal level of response activation, excitatory and inhibitory influences can either increase or decrease the response amplitude (Zehr and Stein 1999). The recovery was therefore studied using a half maximal control reflex. To characterize the effect of the stimulus intensity on the recovery, near threshold and maximal control responses were also tested. The three levels of response activation were represented as low, medium, and high stimulation intensity and based on the range of H-reflex amplitudes generated in each subject, were typically represented by amplitudes of 1, 2 and 3 mV, respectively.

The recovery from post-activation depression was evaluated using conditioning-test intervals of 25, 50, 75, 100, 150 and 200 ms. The second H-reflex was conditioned by an H-reflex (H → H) or a size matched REP (REP → H). An REP was also conditioned by a size matched H-reflex (H → REP) or the same REP (REP → REP). While post-activation depression is commonly studied at varying stimulus frequencies (Ishikawa et al. 1966; Burke et al. 1989), this experiment utilized paired pulse stimulation to compare the recovery profiles of the H-reflex and REP over intervals showing strong inhibition. Since the H-reflex requires ~12 ms to reach the level of the spinal electrode, a 12 ms delay was added to the H→REP intervals (see Fig. 2.2B). Without the delay, the test REP inputs would otherwise arrive at the spinal cord ~12 ms too early. Conversely, a 12 ms delay was subtracted for the REP → H condition (see Fig. 2.2C). The 24 interactions (4 conditions × 6 intervals) were tested in a pseudo-random order and each condition was repeated 4 times (96 tests per trial). Testing was done at 0.2 Hz, as the depressive effects are sufficiently small at this point to justify a rate of one stimulus every 5 s (Crone and Nielsen 1989; Pierrot-Deseilligny and Burke 2005). Care was taken to ensure that the amplitude of the H-reflex and REP were well matched. If the two responses deviated substantially from the target value, those trials were rerun. The number of repeated trials varied from subject to subject and in some cases, no trials were repeated. The same protocol was used to collect data during an isometric contraction maintaining a plantarflexion of 15–20% of MVC and with the subject at rest.

2.2.4 Analysis

The data were analyzed using Matlab (MathWorks, Natick, MA, USA). Data within a trial were sorted according to the different conditions. The amplitudes were evaluated by measuring the size of each response peak-to-peak. The first reflex served as a control. The second reflex was expressed as a percentage of the control response, and complete recovery equaled 100%. While a sigmoid can be used to approximate a recovery curve having a threshold, a rising phase, and a maximum amplitude (Capaday 1997), the data along the rising phase of two recovery curves were compared using linear regression. This was done over the intervals that exhibited a monotonic increase in amplitude for both curves (25–150 or 25–200 ms). Statistical analysis was completed using SPSS analytics software (SPSS Inc., IL, USA). Data were analyzed using a four-way repeated measures analysis of variance (RM-ANOVA) treating the motor state (rest, voluntary contraction), stimulus intensity (low, medium, high), condition (H→H, REP→H, H→REP, REP→REP) and interval (25, 50, 75, 100, 150, 200 ms) as within-subject factors. Separate three-way RM-ANOVA were applied to the voluntary and resting data. Individual two-way RM ANOVAs, which treated condition and interval as within-subject factors, were applied to the data prior to performing Bonferroni corrected post-hoc tests. Data are presented as mean ± SE. Statistical significance was set at $P < 0.05$.

2.3 RESULTS

We examined the recovery profile of the REP and H-reflex from post-activation depression. The optimal location for administering spinal stimulation was over the L2/L3 intervertebral space for the majority of subjects (range L1–L3 levels; see Roy et al. 2012). The maximum REP (6.0 ± 0.6 mV; 81% of M-max), measured during a voluntary contraction, was larger than the maximum H-reflex (4.1 ± 0.5 mV; 55% of M-max; $F(1,7) = 14.60$; $P = 0.007$).

2.3.1 Representative subject

Fig. 2.2 shows in an example subject the conditioning effect of a tibial nerve or spinal stimulus on the H-reflex (Fig. 2.2A & C) and REP (Fig. 2.2B & D). In all 4 conditions tested during contraction, the test response was negligible at the 25ms interval, while substantial recovery was evident after 150–200ms. This result was expected given the gradual, long-latency recovery from post-

activation depression. Fig. 2.2D shows that the recovery of the REP → REP was delayed by ~50 ms, as compared to the three other conditions. Data collected at rest showed a similar trend (Fig. 2.2E–H), and the inhibition of the REP doublet was stronger across all intervals (Fig. 2.2H). In this subject, there was almost no recovery of the REP → REP condition, even at 200 ms (Fig. 2.2H), whereas the other conditions showed considerable recovery.

2.3.2 Group data

Three target levels of activation were used to characterize the effect of the stimulus intensity on the recovery profile. The average amplitudes within the group were 18%, 30%, and 47% of M-max during a voluntary contraction and 18%, 33%, and 46% of M-max at rest. The size of the H-reflex, averaged across subjects, was within ± 2.7% of the average REP, for the three levels of response activation. Grouping the data according to motor state, intensity, condition, and interval, a four-way RM ANOVA showed a significant state and ($F(1,7) = 55.43$; $P < 0.001$) state × intensity effect ($F(2,14) = 6.85$; $P = 0.008$). The two motor states (voluntary/rest) were therefore analyzed separately. For data collected during voluntary contraction, a three-way RM ANOVA showed a significant intensity effect ($F(2,14) = 8.63$; $P = 0.004$; Fig. 2.3A, C, and E) indicating that the rate of recovery varied as a function of the stimulus intensity. Subsequent two-way RM ANOVAs showed similar condition effects at the low (Fig. 2.3A; $F(3,21) = 8.50$; $P = 0.001$), medium (Fig. 2.3C; $F(3,21) = 12.56$; $P < 0.001$) and high intensities (Fig. 2.3E; $F(3,21) = 5.96$; $P = 0.004$). Post-hoc testing showed that the REP → REP condition was different from the H→REP, REP→H, and H→H conditions at the low (Fig. 2.3A; all $P < 0.05$) and medium intensities (Fig. 2.3C; all $P < 0.05$), while the REP → REP versus H → REP comparison was different at the high intensity (Fig. 2.3E; $P = 0.001$). Linear regression showed that the REP → REP condition measured over the 25–150 ms intervals for the 8 subjects was 65% compared to the H → H condition (Fig. 2.4A).

In contrast to the voluntary data, the effect of stimulation intensity on the recovery at rest was not significant (three-way RM ANOVA: $F(2,14) = 1.10$; $P = 0.36$; Fig. 2.3B, D, F). A two-way RM ANOVA on the pooled data showed a significant condition effect at rest ($F(3,21) = 7.20$; $P = 0.002$) and post-hoc testing showed that the REP → REP condition was different from the H → REP condition ($P = 0.003$). For the purpose of comparison, the recovery of the REP → REP condition

at rest was 67% as compared to the H → H condition (Fig. 2.4B).

There were significant main effects between voluntary and rest conditions for both the REP → REP ($F(1,7) = 43.61$; $P < 0.001$) and H → H conditions ($F(1,7) = 64.35$; $P < 0.001$). For instance, the REP doublet recovered to 68 ± 6% after 150 ms during a contraction (filled symbols in Fig. 2.4D), while the recovery was 20 ± 6% at rest (open symbols). Across the intervals, the recovery of the REP → REP at rest was only 30% as compared to the recovery during a contraction (Fig. 2.4D), which was similar to the 31% observed for the H → H (Fig. 2.4C).

2.4 DISCUSSION

The present study compared the recovery of the H-reflex and the transcutaneous, spinally-evoked REP from post-activation depression. Recovery of the H-reflex was equivalent whether an H-reflex or an REP served as the conditioning stimulus. This suggests that the peripheral nerve stimulus had equivalent effects on the recovery of the monosynaptic H-reflex as the bilateral, multi-segmental activation produced by the spinal stimulus. In contrast, the REP → REP condition exhibited more inhibition as compared to the H → H, REP → H, and REP → H conditions suggesting that post-activation depression was stronger during paired pulse transcutaneous spinal stimulation.

The maximum REP (6.0 ± 0.6mV) evoked during a tonic contraction was larger than the maximum H-reflex (4.1 ± 0.5 mV). The maximum H-reflex underestimates the total number of motor neurons that are synaptically activated due to occlusion that occurs when the axons of motor neurons become directly activated as the stimulus to the tibial nerve is increased (Palmieri 2004). Occlusion was less apparent for the REP as a low stimulus intensity favors the activation of the dorsally located sensory roots as compared to the anteriorly positioned motor roots (Roy et al. 2012; Courtine et al. 2007; Minassian et al. 2007).

As an active motor state is known to facilitate the recovery of an H-reflex (Burke et al. 1989; Hultborn and Nielsen 1998), the recovery of the REP from post-activation depression was compared between motor states. The present study demonstrated that a voluntary contraction

will facilitate the recovery of both the H-reflex and REP. Peripherally and spinally-evoked responses reached near control values by 150–200 ms. The recovery of the REP was only 30% at rest as compared to during contraction, which was similar to the 31% observed for the H-reflex. Thus, just as with the H-reflex, post-activation depression of the REP is diminished in an active muscle.

An REP produced by cathodal stimulation resulted in a reduction of the soleus H-reflex in a manner that was consistent with the time-course for homosynaptic depression. Magnetic and electric anodal spinal stimulation has also been shown to inhibit the soleus H-reflex (Knikou 2013a; Knikou 2013b), though the interaction and magnitude of the recovery cannot be directly compared to present data due to the marked methodological and potentially neurophysiological differences in the recruitment of post-activation depression using the different types of stimulation. Here, spinal stimulation had the same effect on the H-reflex as electrical stimulation of the tibial nerve when tested 25–200 ms later. Thus, the multi-segmental, bilateral inputs activated by the spinal stimulus may not contribute strongly to the post-activation depression of the H-reflex, so the REP → H is effectively equivalent to the H → H.

The persistent depression of the second response observed for the REP → REP, relative to the other three conditions, provides evidence in support of stronger inhibition during paired pulse spinal stimulation. The disparity was weaker using a high stimulus intensity, potentially because the reflexes tended to be more robust. The difference was also larger in an active muscle, as compared to rest, likely because the responses during voluntary activity were more weakly inhibited.

Heteronymous Ia excitation can directly sum with the homonymous excitatory post-synaptic potentials that evoke the soleus H-reflex (Pierrot-Deseilligny and Burke 2005). For instance, heteronymous input from the femoral nerve has strong Ia projections on soleus motor neurons. Given that a spinal stimulus will recruit a population of spinal elements, an REP likely involves the summation of more heteronymous projections. Only, the REP → REP condition may be distinct, because a wider range of afferents will be activated by the conditioning stimulus and will inhibit its multi-segmental inputs. As the heteronymous projections may not be reduced by a preceding

H-reflex this would enable the H → REP condition to be equivalent to the H → H. Overall, the REP → REP findings may be explained by increases in the amount of inhibition to the heteronymous pathways.

As spinal stimulation may cause the paraspinal muscles to contract, a second REP may instead be smaller because of anatomical changes around the spinal column caused by contraction. More specifically, magnetic stimulation of the spine has been shown to reduce the effective strength of a second stimulus due to contraction of the paraspinal muscles (Darabant et al. 2011). This explanation is however less likely because the spine will be maximally displaced after 90 ms (Darabant et al. 2011) and would have caused the amount of H → H and REP → REP recovery to be most different at the 100ms IPI, which was not observed. Further, the disparity between the conditions was less apparent at the highest stimulus intensity when movement caused by the paraspinal muscle contraction would have been the greatest.

Transcutaneous spinal stimulation has been shown to reduce spinal excitability (Hofstoetter et al. 2014; Nielsen et al. 1996) and may be used to augment motor activity during locomotion (Minassian et al. 2012; Hofstoetter et al. 2013). The present study showed that the REP doublets were the most strongly inhibited, suggesting that the increased suppression of the REP may be relevant in the context of repetitive spinal stimulation when administered using a transcutaneous approach.

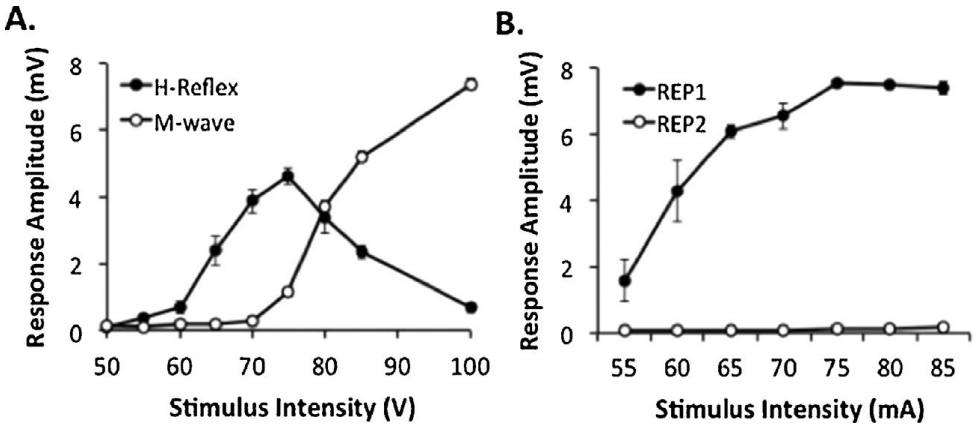

Figure 2.1: Soleus H-reflex and REP recruitment curves in a representative subject. Amplitudes are plotted as a function of the stimulus intensity using tibial nerve (A) and spinal stimulation (B). Spinal stimulation in (B) was delivered over the L2/L3 intervertebral space. REP1 and REP2 were elicited 50 ms apart.

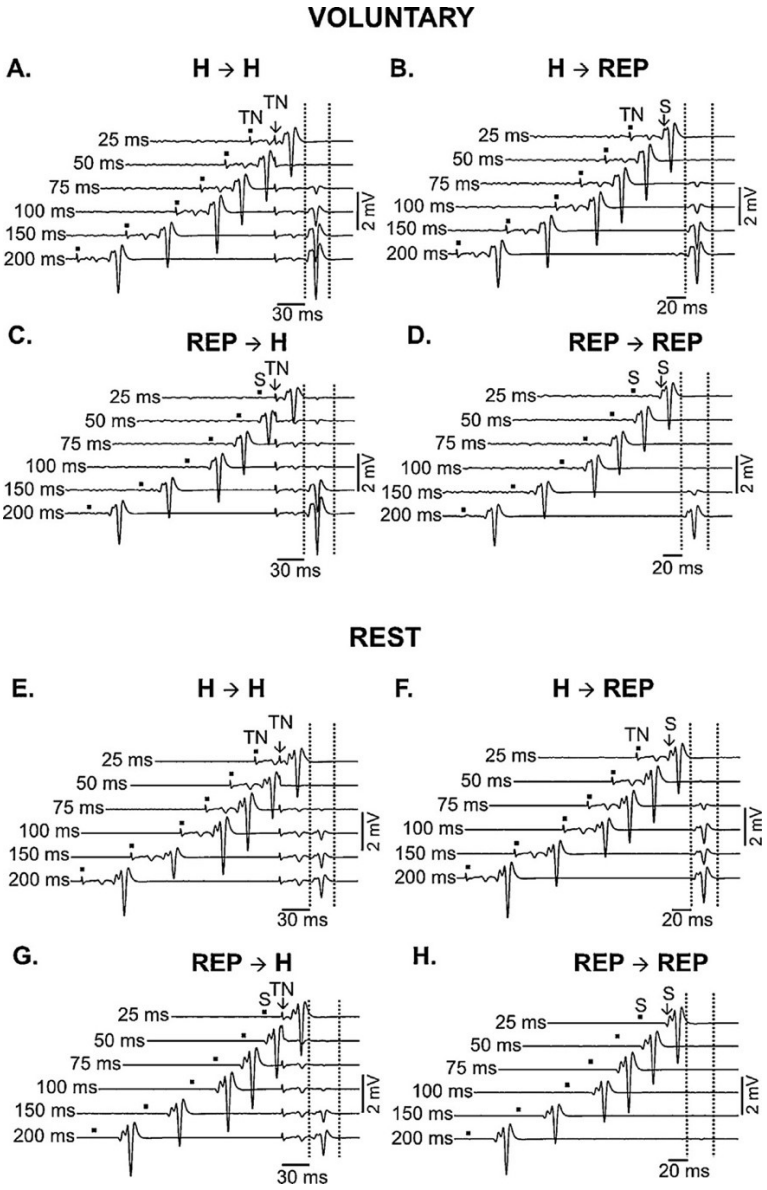

Figure 2.2: Traces in a representative subject showing the effect of paired pulse stimuli (A, D, E, and H) and the effect of a conditioning stimulus (H-reflex or REP) on the alternate reflex type (B, C, F, and G). Four conditions were tested during a voluntary contraction: H → H (A), H → REP (B), REP → H (C), REP → REP (D). The six lines in each graph represent the different conditioning-test intervals ranging from 25 ms (top trace) to 200 ms (lowest trace). Each trace depicts the averaged response produced by four stimuli. The REP was matched to the half maximal H-reflex. Test responses are shown within the vertical dotted lines. Time of the tibial nerve (TN) and spinal (S) stimuli are indicated. First and second stimuli are shown using squares and arrows, respectively. Data at rest are shown in (E–H).

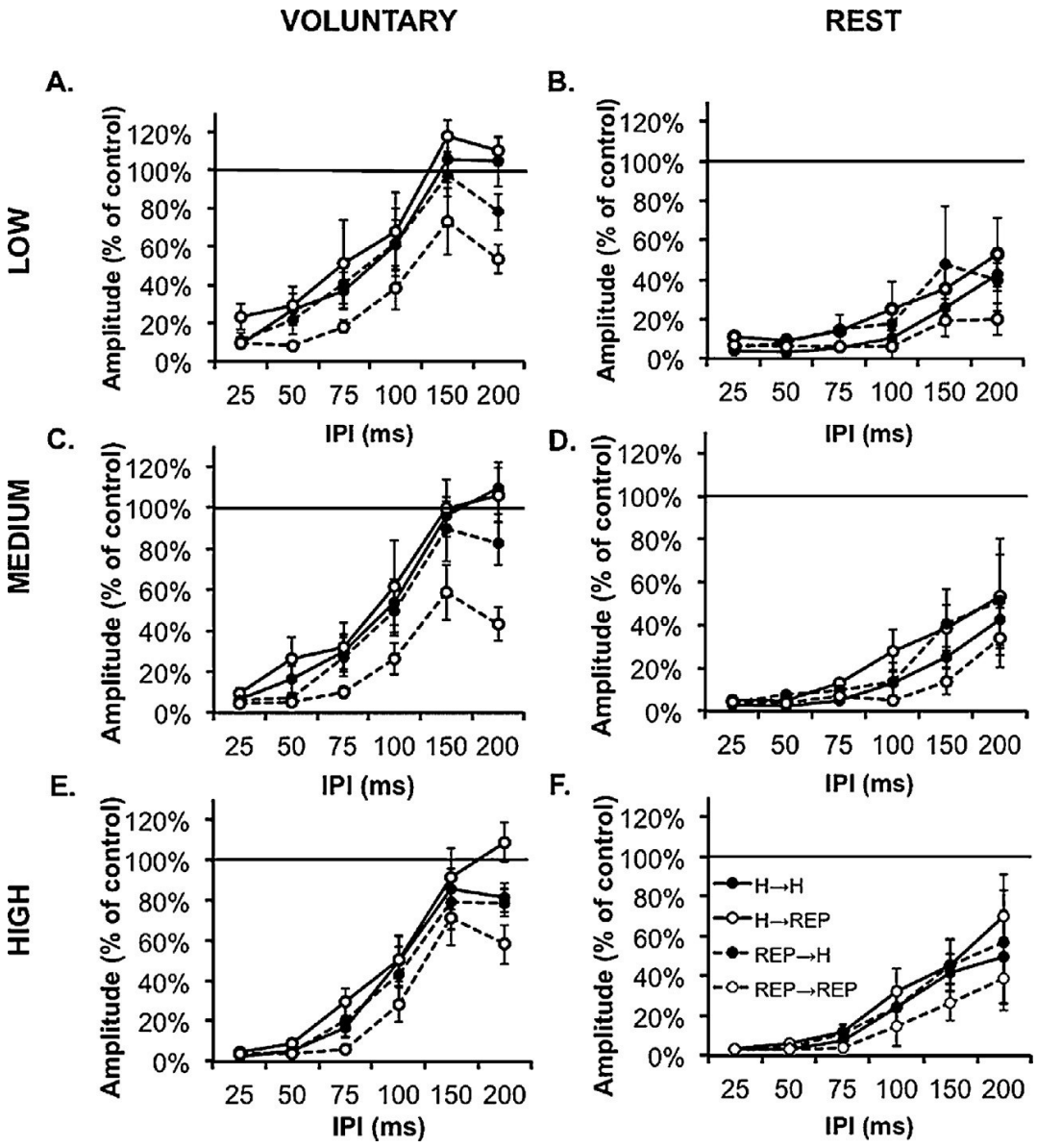

Figure 2.3: Recovery of the H-reflex (filled circle) and REP (open circle) when preceded by the first H-reflex (solid line) or REP (dashed line). Data during a contraction were collected at three activation levels: low (A), medium (C), and high (E) and expressed as a percent of the control response. Complete recovery from depression is represented by the solid line at 100%. The corresponding data are shown at rest in (B,D,F).

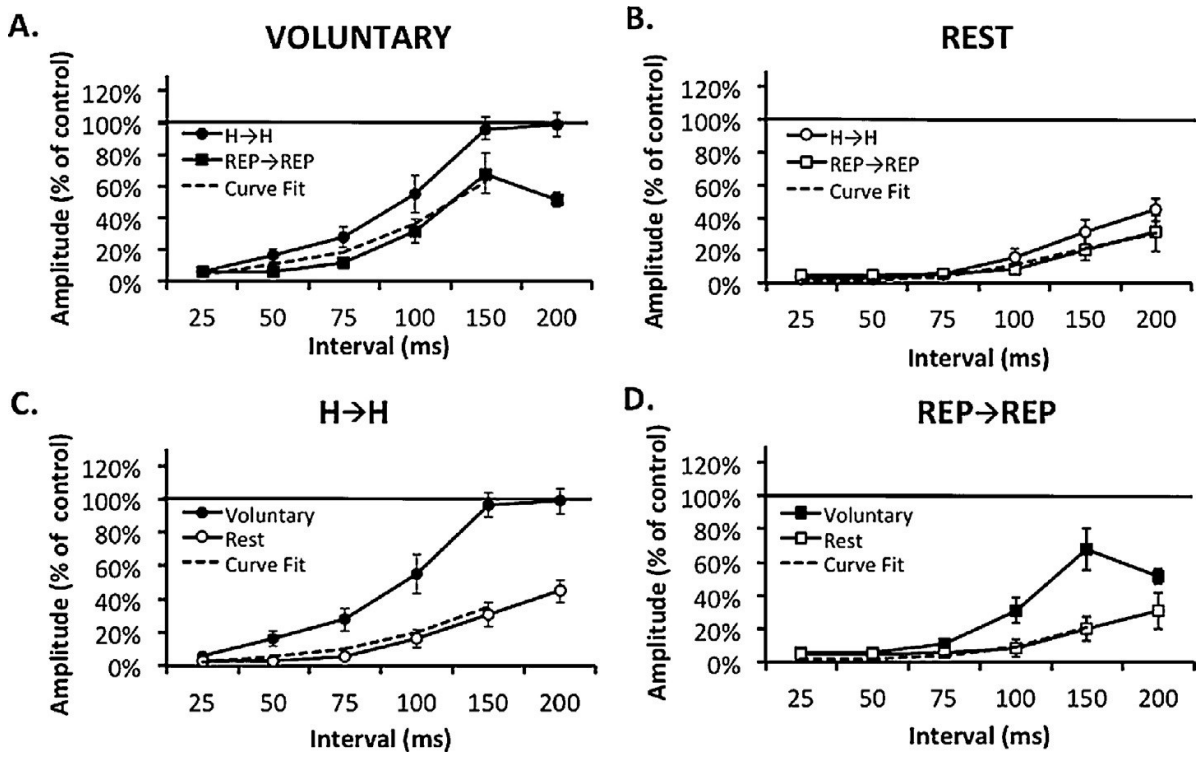

Figure 2.4: Comparison of post-activation depression using paired pulse conditions (H → H and REP → REP). Data are shown during a voluntary contraction (A; filled symbols) and at rest (B; open symbols). The same data are used to illustrate the effect of motor state for the H → H (C; circles) and REP → REP (D; squares). Upper recovery curve was scaled using linear regression to fit the lower curve ('Curve Fit'; dashed line). Linear regression was applied over the portion of the curves showing a monotonic increase in response amplitude.

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CHAPTER 3: REDUCED POST-ACTIVATION DEPRESSION OF THE SOLEUS H-REFLEX AND ROOT EVOKED POTENTIAL FOLLOWING TRANSCRANIAL MAGNETIC STIMULATION

A version of this chapter has been published.

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3.1 INTRODUCTION

In the period following the activation of a sensory-motor synapse, a monosynaptic reflex induced along the same pathway may be inhibited for 100–200 ms during contraction (Andrews et al. 2015; Stein et al. 2007) and up to 10 s at rest (Crone and Nielsen 1989; Pierrot-Deseilligny and Burke 2005). This phenomenon is known as either post-activation depression or homosynaptic depression and has been characterized in humans (Crone and Nielsen 1989; Magladery et al. 1952; Paillard 1955; Rothwell et al. 1986) and animals (Eccles et al. 1961; Frank and Fuortes 1957) after peripheral nerve stimulation. Post-activation depression of the Hoffmann (H)-reflex is thought to be presynaptic to the α-motor neuron and has been associated with an increase in presynaptic inhibition (Crone and Nielsen 1989; Schieppati 1987) and changes in the presynaptic terminal leading to a temporary reduction of neurotransmitter release (Armitage and Siegelbaum 1998; Castellucci and Kandel 1974; Elliot et al. 1994; Lev-Tov and Pinco 1992; reviewed in Hultborn et al. 1996). A similar suppression has been observed with transcutaneous stimulation over the thoracolumbar spine whereby the excitability of a reflex root evoked potential (REP) will change for up to 10 s after an earlier stimulus (Andrews et al. 2015; Courtine et al. 2007; Minassian et al. 2007, 2009). After a first response, a soleus reflex REP evoked 150 ms later will recover to 68% of the control response during a contraction and 20% at rest. This corresponds to ~66% of the recovery observed using two H-reflexes (Andrews et al. 2015). The difference may relate to the fact that the transcutaneous spinal stimulus activates multiple, bilateral lumbosacral roots (Courtine et al. 2007; Maertens de Noordhout et al. 1988; Minassian et al. 2007) in addition

to the fibers located within the tibial nerve (TN).

Corticospinal input can interact with spinal interneurons, as transcranial magnetic stimulation (TMS) decreases post-activation depression of REPs during double-pulse (50 ms) stimulation (Roy et al. 2014) and, similarly, reduces presynaptic inhibition of the H-reflex (Iles 1996; Meunier 1999; Valls-Solé et al. 1994). The effect of TMS on post-activation depression is transient, lasting ~25 ms, and is maximal when descending inputs arrive at the motor neuron pool 8–13 ms before the afferent inputs produced by the transcutaneous spinal stimulus (Roy et al. 2014). Such a delay shares similarities to the period of facilitation measured with a single soleus REP (Knikou 2014; Roy et al. 2014) or H-reflex (Nielsen and Petersen 1995; Serranova et al. 2008; Valls-Solé et al. 1994) conditioned by TMS. Post-activation depression of the soleus H-reflex is reduced in Parkinson's disease, although the strength of the pathway can be enhanced by using subthalamic nucleus stimulation, potentially, through a reticulospinal pathway (Raoul et al. 2012). Thus descending projections from both pyramidal and extrapyramidal systems likely modulate post-activation depression of monosynaptic reflexes.

We previously reported that TMS can reduce post-activation depression of an REP in the hamstrings using transcutaneous spinal stimulation, and a similar effect was observed in the soleus muscle of most subjects (Roy et al. 2014). The present study builds on previous work by *1*) directly comparing the recovery time course of the H-reflex with the REP from post-activation depression in the presence of TMS and *2*) characterizing the role of TMS intensity on the recovery. We employed double-pulse stimulation to elicit two soleus H-reflexes (or REPs) with the TMS pulse time-locked to the second reflex response. To provide maximal recovery, the study was initially conducted during an isometric plantarflexion, given that voluntary drive increases motor neuron excitability (Pierrot-Deseilligny and Burke 2005) and reduces post-activation depression (Andrews et al. 2015; Burke et al. 1989; Hultborn and Nielsen 1998; Rothwell et al. 1986). The interaction on the H-reflex was further characterized in a relaxed motor state, as the time course of recovery differs substantially between resting and active states.

3.2 METHODS

3.2.1 Participants

Ten able-bodied volunteers (5 women, 5 men; age 23–59 yr) were recruited to participate in the study. Volunteers were screened for potential contraindications to the stimulation including a history of seizures, implanted devices, chronic back pain, or prior spinal surgery. Participants provided written consent to the experimental protocol approved by the Health Research Ethics Board at the University of Alberta.

3.2.2 Recording and stimulation

Participants were seated with their left leg in a metal brace maintaining a 100° angle of both knee and ankle joints. EMG responses were recorded from the soleus and tibialis anterior muscles with a pair of silver-silver chloride surface electrodes (3.5 × 2.2 cm; Vermont Medical, Bellows Falls, VT) placed 3 cm below the distal border of the gastrocnemius and along the belly of the tibialis anterior, two-thirds of the distance from the lateral malleolus to the knee. The EMG was band-pass filtered between 10 and 1,000 Hz, amplified 1,000× (Octopus; Bortec Technologies, Calgary, AB, Canada), and digitized at 5 kHz with Axoscope Hardware (Digidata 1200 series; Axon Instruments, Union City, CA). The EMG was also full-wave rectified and low-pass filtered at 3 Hz so that the participants could monitor their level of EMG on an oscilloscope. At the start of the experiment, each participant's maximum voluntary contraction (MVC) was determined during an isometric plantarflexion.

The TN was stimulated with 1-ms pulses (SD9 Stimulator; Grass Instruments, West Warwick, RI) with the cathode on the popliteal fossa and the anode 4 cm more proximal (measured from the center of each electrode). The optimal position was determined with a handheld monopolar probe (1-cm tip) as the site that produced the largest soleus H-reflex with minimal encroachment from the antagonistic tibialis anterior muscle. Threshold was determined as the minimum intensity capable of producing a visible EMG response.

Transcutaneous spinal (S) stimulation was performed with a constant-current stimulator

(Digitimer DS7A; Digitimer, Welwyn Garden City, UK) with the pulse width set to 1 ms. Vertebral levels were identified and marked after palpation by a physiotherapist. The anode (7.5 × 13 cm; Axelgaard Manufacturing, Fallbrook, CA) was placed above the ipsilateral anterior superior iliac spine. A silver-silver chloride cathode (5 × 5 cm; WalkAide premium electrode; Innovative Neurotronics, Austin, TX) was initially placed on the back, midline over the 3rd and 4th lumbar intervertebral space. The electrode was then moved progressively more rostral in 2.5 cm increments until the position that produced the largest soleus REP was identified.

TMS was delivered over the motor cortex to elicit motor evoked potentials (MEPs) in soleus. Monophasic pulses were delivered with a MagStim 200^2 stimulator (Magstim, Whitland, UK). Paired pulses were delivered with a BiStim^2 module (Magstim). The double-cone coil (P/N 9902-00: external wing diameter 110 mm) was orientated to produce posterior-to-anterior currents in the brain. The coil was initially positioned over the right motor cortex (1 cm lateral and 1 cm posterior of the vertex) and then moved in 1-cm increments until the optimal position for producing an MEP in the left soleus muscle was determined (range: 0–2 cm posterior and 1–2 cm lateral).

Recruitment curves were collected for the H-reflex by gradually increasing the stimulus intensity from threshold and onward until reaching H_{max} amplitude. For the M wave, the stimulus intensity was further increased beyond the level required to elicit H_{max} to the intensity that produced M_{max}. Separate recruitment curves were also collected for the MEP and REP. Double pulses of spinal stimulation were administered 50 ms apart to distinguish the reflex REP from the direct co-activation of motor axons in the ventral roots. The presence of a first response and the reduction of a second because of post-activation depression was indicative of a reflex REP (Courtine et al. 2007; Minassian et al. 2007; Roy et al. 2012). The stimulation intensity was increased from threshold up to REP_{max} or the intensity at which the first REP increased at the same rate as the second REP, which was consistent with the direct activation of motor axons.

3.2.3 Optimizing the TMS-H reflex and TMS-REP delay

The delay of the TMS pulse relative to a second TN stimulus (TMS-TN) was optimized prior to

testing the effect of TMS on post-activation depression. TMS was set to 10–15% of the maximal stimulator output (MSO) above threshold. The stimulus intensity for the H-reflex was adjusted to produce half of its maximal soleus EMG response, as both excitatory and inhibitory influences can be observed at this level of response activation (Zehr and Stein 1999). Post-activation depression was induced with two TN stimuli 100 ms apart, as this interval typically permitted a small amount of recovery of the second H-reflex. In some subjects, the interpulse interval (IPI) was increased (150– 275 ms) in order to produce a visible second reflex response. TMS was then delivered 5, 7, 10, 13, 15, 17, 20, and 25 ms before the second TN stimulus. The optimal delay was defined as the interval that produced maximum facilitation of the second reflex and was fixed for the duration of testing. As the latency of the REP was ~12 ms shorter than the H-reflex, the TMS-S delay was 12 ms longer than the TMS-TN delay to ensure that the timing of the inputs reaching the motor neuron pool was equivalent.

3.2.4 TMS on spinal reflexes during contraction

The effect of TMS on the recovery from post-activation depression produced by double-pulse H-reflexes was studied with a half maximum reflex response amplitude during an isometric plantarflexion at 15–20% of MVC. Eight subjects were tested. To study the interaction across time, the IPI between the pairs of TN stimuli was systematically increased from 5 to 100 ms (i.e., 5, 10, 15, 25, 50, 75, and 100 ms). TMS was timed in relation to the second TN (or S) stimulus using the optimal delay described above, which remained fixed throughout the experiment. TMS was administered at five intensities (threshold, 5%, 10%, 15%, and 20% MSO above threshold) to test the effect of stimulus intensity on the recovery curves. Conditions without TMS were collected for comparison. Each condition was repeated four times. Testing was done at 0.2 Hz, as the effect of post-activation depression is sufficiently small after 5 s (Crone and Nielsen 1989; Pierrot-Deseilligny and Burke 2005). The above protocol was repeated with REPs to compare the effect of activating additional afferents on the recovery curve.

3.2.5 TMS on the H-reflex at rest

We investigated the interaction (i.e. TMS-TN) at rest to characterize the impact of motor state.

Eight subjects were tested (2 new subjects; done ~10–12 wks after the first experiment) with the same protocol described above for the H-reflex. Given that the recovery curve at rest exhibited a bimodal shape, the time course was reassessed with a finer resolution of IPIs (5, 8, 10, 12, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, and 200 ms). TMS was delivered at 15% MSO above threshold. Each IPI was tested four times, and eight stimuli were applied with TMS alone. The recovery of the H-reflex from post-activation depression without TMS was also measured.

3.2.6 TMS on triple-pulse H-reflexes at rest

Post-activation depression during multipulse stimulation was evaluated with H-reflexes produced by a short train of electrical stimuli (3 pulses, typically ~40 Hz). We tested the same eight subjects involved in the previous study at rest. The chosen IPI was determined as the interval that produced the greatest recovery for IPIs < 50 ms (see above). The H-reflex was adjusted to elicit a half maximal amplitude. As preliminary experiments had shown that the excitation produced by one TMS pulse was too brief (see also Roy et al. 2014) to simultaneously facilitate both the second (H2) and third (H3) H-reflexes, H2 and H3 were each conditioned by a TMS pulse. The interaction was tested at four TMS intensities (threshold, 5%, 10%, and 15% MSO above threshold), as well as without TMS. Each condition was repeated four times. On the basis that paired-pulse TMS will facilitate the MEP (Poon et al. 2008; Valls-Solé et al. 1992; Wassermann et al. 1996), the MEP doublet was also evaluated without TN stimulation.

3.2.7 Analysis

The data were analyzed with MATLAB (MathWorks, Natick, MA). The amplitudes of the H-reflex, REP, and MEP were measured peak to peak. H2 and REP2 were expressed as percentages of their unconditioned control responses (H1 and REP1), and full recovery was represented by 100%. At IPIs ≤ 15 ms, H1 (or REP1) could alter the shape of H2 (or REP2) since the peaks could overlap. This was particularly evident when TMS would also facilitate the first reflex because of spatiotemporal facilitation (Poon et al. 2008; Roy et al. 2014). To remove the contribution of H1 (or REP1) at IPIs ≤ 15 ms, the control reflex was *1*) aligned with H1 (or REP1), *2*) scaled with linear regression, and then *3*) subtracted from the experimental data. Traces not having a visible H2 (or

REP2) were given a value of zero. Latencies were determined by visual inspection of the waveforms. Statistical analysis was performed with SPSS analytics software (SPSS, Chicago, IL). H-reflexes and REPs collected during a contraction were analyzed with a three-way repeated-measures analysis of variance (RM-ANOVA) treating "condition" (H reflex and REP), stimulus "intensity" (5 levels plus "without TMS" condition), and "interval" (7 levels) as within-subject factors. Two-way RM-ANOVAs were applied to the H-reflex and REP conditions separately. A two-way RM-ANOVA was used across subjects for intervals > 25 ms to determine M-wave consistency. IPIs ≤ 25 ms were excluded, as waveforms tended to overlap at short intervals. A histogram was created with the rest data to illustrate the IPIs that most frequently showed an early effect by counting the number of times H2 recovered to within 50% of each subject's maximal early recovery. During multi-pulse stimulation, H-reflexes were analyzed with a two-way RM-ANOVA treating the reflex amplitude (H2 and H3) and TMS stimulus "intensity" (4 levels plus "without TMS" condition) as within-subject factors. Data are presented as means ± SE. Statistical significance was set at $P < 0.05$.

3.3 RESULTS

3.3.1 TMS on spinal reflexes during contraction

Figure 3.IA shows representative subject data where pairs of TN stimuli were applied 100 ms apart. The second H-reflex (H2; Fig. 3.IA, *top*) was reduced to 71% of the first (H1) because of post-activation depression. Administering a TMS pulse 15 ms before the second TN stimulus (Fig. 3.IA, *middle*) resulted in the facilitation of H2 (233% of control; post-activation facilitation), which was also considerably greater than the size of the conditioning MEP (Fig. 3.IC, *bottom*). The interaction was modulated as a function of the TMS-TN delay (Fig. 3.IB).

Figure 3.2 shows the recovery of the H-reflex and the REP from post-activation depression in an example subject. Both the H-reflex and REP were almost entirely suppressed at IPIs ≤ 50 ms (Fig. 3.2, A and B). When the depressed reflexes were conditioned with TMS, both reflexes showed marked recovery at all IPIs ≥ 10 ms (Fig. 3.2, C and D).

Within the group, H_{max}, REP_{max}, and MEP_{max} measured during a voluntary contraction were 3.6 ±

0.4 mV, 5.2 ± 0.7 mV, and 1.2 ± 0.2 mV (46%, 67%, and 15% of M_{max}), respectively. Whenever present, M-waves were maintained at a low level of response activation and did not vary significantly throughout experimentation [2-way RM ANOVA: $F(2,14) = 2.159$; $P = 0.152$]. As REP_{max} was larger than H_{max} ($P < 0.001$), the control REP (REP1; 3.2 ± 0.6 mV) was also larger than the control H-reflex (H1; 2.2 ± 0.3 mV, $P < 0.001$). Reflex responses were well matched at 61–62% of their maximum amplitude and were elicited at 43.7 ± 3.8 V (H-reflex) and 45.0 ± 3.8 mA (REP). As the latencies of the MEP (32.2 ± 1.3 ms) and the H-reflex (31.9 ± 1.1 ms) were similar, a TMS pulse administered 14.4 ± 0.9 ms before the TN stimulus caused the MEP waveform to overlap during the first part of the conditioned reflex. The overlap was comparable for the REP, as TMS was delivered ~26 ms before the spinal stimulus because the latency of the REP (20.5 ± 0.6 ms) was ~12 ms shorter than the H-reflex.

Grouping the experimental data into three factors ("condition," "interval," and "intensity"), a three-way RM-ANOVA showed a significant "condition" effect [$F(1,7) = 7.27$; $P = 0.031$], indicating that the facilitatory effect of the corticospinal input was greater on the H-reflex compared with the REP. Separate two-way RM-ANOVAs applied to the H-reflex and REP data resulted in significant "interval" [H reflex: $F(6,42) = 12.11$, $P < 0.001$; REP: $F(6,42) = 11.19$, $P < 0.001$] and "intensity" [H reflex: $F(5,35) = 16.77$, $P < 0.001$; REP: $F(5,35) = 36.51$, $P < 0.001$] effects, suggesting that the recovery was affected by both interval and intensity. With TMS set to 20% MSO above threshold (i.e., 40.6 + 20% MSO; Fig. 3.3B), both H-reflexes and REPs reached control values at IPIs as short as 10 and 15 ms, respectively (Fig. 3.3, C and D). Beyond the 50-ms IPI, reflex responses exceeded control values. In particular, post-activation depression was replaced by post-activation facilitation, as the recovered H-reflex was 243 ± 51% of its control value at the 75-ms IPI ($P < 0.05$). This was also 667 ± 79% of the conditioning MEP amplitude (Fig. 3.3B). In the absence of TMS, post-activation depression of the H-reflex was consistently less than the REP [2-way RM-ANOVA: $F(1,7) = 10.44$, $P = 0.014$; compare open circles in Fig. 3.3].

3.3.2 TMS on the H-reflex at rest

Figure 3.4 shows the effect of TMS on post-activation depression of the H-reflex at rest in two

example subjects. The profile of the first subject demonstrated a narrow peak at the 10-ms IPI, which was superimposed on a gradual later recovery starting at 50 ms (Fig. 3.4A). The second subject exhibited a peak at 15 ms, which was followed by a period of depression from 25 to 75 ms and then an increase to 176% at 100 ms (Fig. 3.4B). In both examples the MEP was <5% of the control H-reflex, so its direct contribution was negligible. Within the group, all subjects showed evidence of bimodal recovery. The values of H_{max} and MEP_{max} within the group were 3.6 ± 0.4 and 0.4 ± 0.2 mV (51% and 6% of M_{max}), respectively, while the control H-reflex was 2.3 ± 0.3 mV (64% of H_{max}). The MEP (Fig. 3.4C) threshold at rest was 48.1 ± 3.9% MSO. A two-way RM-ANOVA revealed a significant main effect for "intensity" [$F(5,35) = 15.13$, $P < 0.001$] and an "intensity" × "interval" interaction effect [$F(30,210) = 1.84$, $P = 0.007$]. Averaged across subjects, the H-reflex recovered to 72 ± 23% of control values at the 25-ms IPI and decreased to 40 ± 10% at the 50-ms interval (Fig. 3.4D). In the six subjects who participated in both parts of the study, a three-way RM-ANOVA showed a significant difference between the voluntary and rest conditions [$F(1,5) = 24.31$, $P = 0.002$; data not shown], which is indicative of state-dependent differences.

The recovery profile of the resting H-reflex was further examined with a finer range of IPIs. Two example subjects are shown in Fig. 3.5, A and B, to highlight some of the differences in the time course. The first subject had a narrow peak from 15 to 25 ms (Fig. 3.5A), while the second subject had a broader peak from 10 to 35 ms superimposed on a gradual later recovery (Fig. 3.5B). Within the group, there was a significant "interval" effect [$F(14,98) = 1.93$, $P = 0.032$; Fig. 5C] and the IPIs that most frequently produced the short-latency increase were from 15 and 30 ms (Fig. 3.5D).

3.3.3 TMS on triple-pulse H-reflexes at rest

Figure 3.6B shows the recovery of two depressed H-reflexes with double-pulse TMS in an example subject. TN triplets were delivered with a 25-ms IPI, and two TMS pulses were used to condition the second and third H-reflexes (Fig. 3.6A). While both H2 and H3 were completely suppressed (see Fig. 3.6B, *top*), corticospinal input caused the partial recovery of both depressed reflexes (see Fig. 3.6B, *middle*). Figure 6*D* illustrates the group recovery of H2 and H3 at the different TMS intensities. The H-reflexes were significantly facilitated by TMS [$F(1,15) = 12.71$, P

= 0.003], and the increases to H2 and H3 were not different [$F(1,7) = 0.18$, $P = 0.9$]. There were also no differences between the facilitatory effects of the four TMS intensities [$F(3,21) = 28$, $P = 0.84$], presumably since the amplitudes of the corresponding MEP doublets were similar (Fig. 3.6C). Averaged across all TMS intensities, H2 and H3 recovered to 42 ± 17% of control values. This was three times larger than the MEP doublet, which on its own was only 14 ± 11% of the control H-reflex.

3.4 DISCUSSION

This series of experiments demonstrated that TMS can reduce and even reverse post-activation depression of the H-reflex and REP in soleus. In the presence of a suprathreshold TMS pulse, full recovery of H2 and REP2 was possible in as little as 10–15 ms during voluntary contraction. To characterize the effect of motor state, we further explored the strength of this interaction using the H-reflex at rest. While the recovery was weaker at rest, the profile exhibited a more distinct bimodal recovery pattern, having an early peak of excitation (~25 ms) superimposed on a later, more gradual recovery. The early excitation was also present with triple-pulse stimulation at ~25 ms IPIs, given that two consecutively depressed H-reflexes could be facilitated by two time-locked MEPs.

3.4.1 Transient TMS-induced corticospinal excitation on spinal neurons

TMS caused a reduction in post-activation depression of the H-reflex and REP at all IPIs ≥ 10 ms. The strength of the interaction progressively increased from the threshold TMS intensity (see Fig. 3.3C) and was typically maximal when TMS was 15% MSO above threshold. While the effect of TMS-induced corticospinal excitation on post-activation depression of the REP can last for ~25 ms (see Roy et al. 2014), the strength of the interaction was maximal when the first corticospinal volley reached the motor neuron pool ~14 ms before the segmental input. This timing agrees with previous reports using TMS and single H-reflexes (Nielsen and Petersen 1995; Serranova et al. 2008; Valls-Solé et al. 1994). A similar profile has also been observed after subthalamic nucleus stimulation, presumably through the activation of corticospinal tract fibers within the internal capsule (Costa et al. 2011). Several mechanisms have been proposed to explain the facilitatory

time course in soleus, including a decrease in the amount of presynaptic inhibition acting on Ia afferents (see Costa et al. 2011) along with the temporal summation of excitatory postsynaptic potentials from slow corticospinal fibers and/or indirectly from polysynaptic pathways (Nielsen and Petersen 1995).

3.4.2 Comparing motor state and reflex type

With TMS, recovery of the H-reflex was greater during a contraction relative to rest, which is similar to the trend observed for the soleus REP (Roy et al. 2014). The effect of motor state is likely attributed to the well-known reduction in post-activation depression that occurs during voluntary contraction (Burke et al. 1989; Hultborn and Nielsen 1998; Rothwell et al. 1986). In general, the recovery time courses of the H-reflex and REP with TMS were comparable, suggesting that the pathways underwent similar modulation. However, the recovery was less for the REP, even though both reflexes were matched as a percentage of their maximum amplitude. Weaker recovery of the REP by TMS can likely be explained by findings that REPs (compared to H-reflexes) are more susceptible to suppression during double-pulse spinal stimulation (Andrews et al. 2015).

3.4.3 Potential mechanism of recovery

Without TMS, post-activation depression was remarkably robust at IPIs < 50 ms and was partly caused by a combination of presynaptic (Crone and Nielsen 1989) and postsynaptic (Poon et al. 2008; Roy et al. 2014) inhibition. Motor neurons are inhibited after an H-reflex (Poon et al. 2008) or an REP (Knikou et al. 2014; Roy et al. 2014), likely because of the afterhyperpolarization of motor neurons (Matthews 1996) and recurrent inhibition (Windhorst 1996). With TMS, the second reflexes (REP2 and H2) could overcome the afterhyperpolarization at IPIs ≥ 10 ms even though the period of afterhyperpolarization in soleus motor neurons can last for 100 ms (Matthews 1996).

Post-activation depression is thought to be associated with a temporary reduction of neurotransmitter release (Armitage and Siegelbaum 1998; Castellucci and Kandel 1974; Elliot et al. 1994; Lev-Tov and Pinco 1992; reviewed in Hultborn et al. 1996) and/or changes in the

presynaptic terminal leading to increased presynaptic inhibition (Crone and Nielsen 1989; Schieppati 1987). Studies in animals have suggested an interaction between presynaptic inhibition and post-activation depression. For example, Davies et al. (1985) demonstrated that the administration of benzodiazepines will produce a prolongation of presynaptic inhibition that in turn will alter post-activation depression. In addition, repetitive activation of peripheral afferents has been shown to decrease the efficacy of presynaptic inhibition (Enriquez-Denton et al. 2002). As descending drive can decrease the amount of presynaptic inhibition of Ia fibers acting on soleus motor neurons with TMS (Iles 1996; Valls-Solé et al. 1994), the marked recovery of the H-reflex and the REP may, in part, be attributed to the modulation of neurotransmitter release from Ia fibers to soleus motor neurons. This mechanism is consistent with the finding that the control and recovered (second) reflexes had similar morphologies.

3.4.4 Short-latency recovery of the H-reflex

The recovery profile of the H-reflex at rest (see Fig. 3.4C) exhibited a bimodal pattern that consisted of a short-latency period of recovery at ~25 ms superimposed on a gradual later recovery. The two distinct phases were less apparent during a contraction, likely because volitional drive both reduced post-activation depression and increased the size of the soleus MEP, which may accelerate the second phase of recovery. While a reduction in presynaptic inhibition by TMS may have induced the recovery of the H-reflex and REP over all intervals ≥ 10 ms, the existence of a short-latency contribution, which tended to subside by 50 ms, suggests involvement of another mechanism.

After an initial activation, motor neurons are relatively refractory for 3–4 ms, after which time the threshold for a second response will be decreased for a period of ~20 ms (Burke et al. 2001). This can be explained by the existence of supernormal excitability that occurs after direct depolarization of motor axons (Burke et al. 2001). This period of increased excitability may allow more axons to reach threshold, thereby producing the early peak in excitability observed in the present study. The profile of supernormality is dynamic and shifts to longer IPIs after a particularly strong initial depolarization (Burke et al. 1998) and may explain why the optimal IPI, which was typically clustered from 15 to 30 ms, varied between subjects. In line with the time

course of supernormality, motor units are known to fire twice in close succession (2–20 ms) (Stein and Parmiggiani 1979) because of excitatory properties within the soma (Jones et al. 1995). Motor unit doublets occur at the onset of movement (Piotrkiewicz et al. 2013) and are responsible for maximizing twitch force during rapid volitional contraction. The presence of double (or even triple) reflexes in this experimental paradigm may therefore relate to the physiological adaptation for improving musculoskeletal contractions.

As many spinal inhibitory circuits are also under supraspinal influence (Baldissera et al. 1981), changes in the strength of spinal inhibitory pathways may also contribute to the early excitation. For instance, corticospinal excitation can cause Renshaw cells to be temporarily inhibited for ~25 ms (Mazzocchio et al. 1994), leading to an increase in motor neuron excitability. Collateral activation of Renshaw cells during the first reflex will produce recurrent inhibition on the motor neuron. This extra inhibition normally lasts for ~40 ms (Pierrot-Deseilligny and Burke 2005) and leads to a strong reduction in excitability of motor neurons. Removal of recurrent inhibition by TMS may therefore cause motor neurons to remain depolarized for longer. As Renshaw cells are only active for ~40 ms, the effect will naturally diminish at longer intervals.

The purpose of this study was to characterize the effect of TMS-induced corticospinal excitation on post-activation depression of the H-reflex and REP. The recovery profile exhibited a distinct bimodal curve characterized by an early peak superimposed on a later, more gradual recovery. It is likely that the two phases of recovery are mediated by distinct mechanisms and may be explained by a combination of pre- and postsynaptic mechanisms. Still, further investigation is required to distinguish between them.

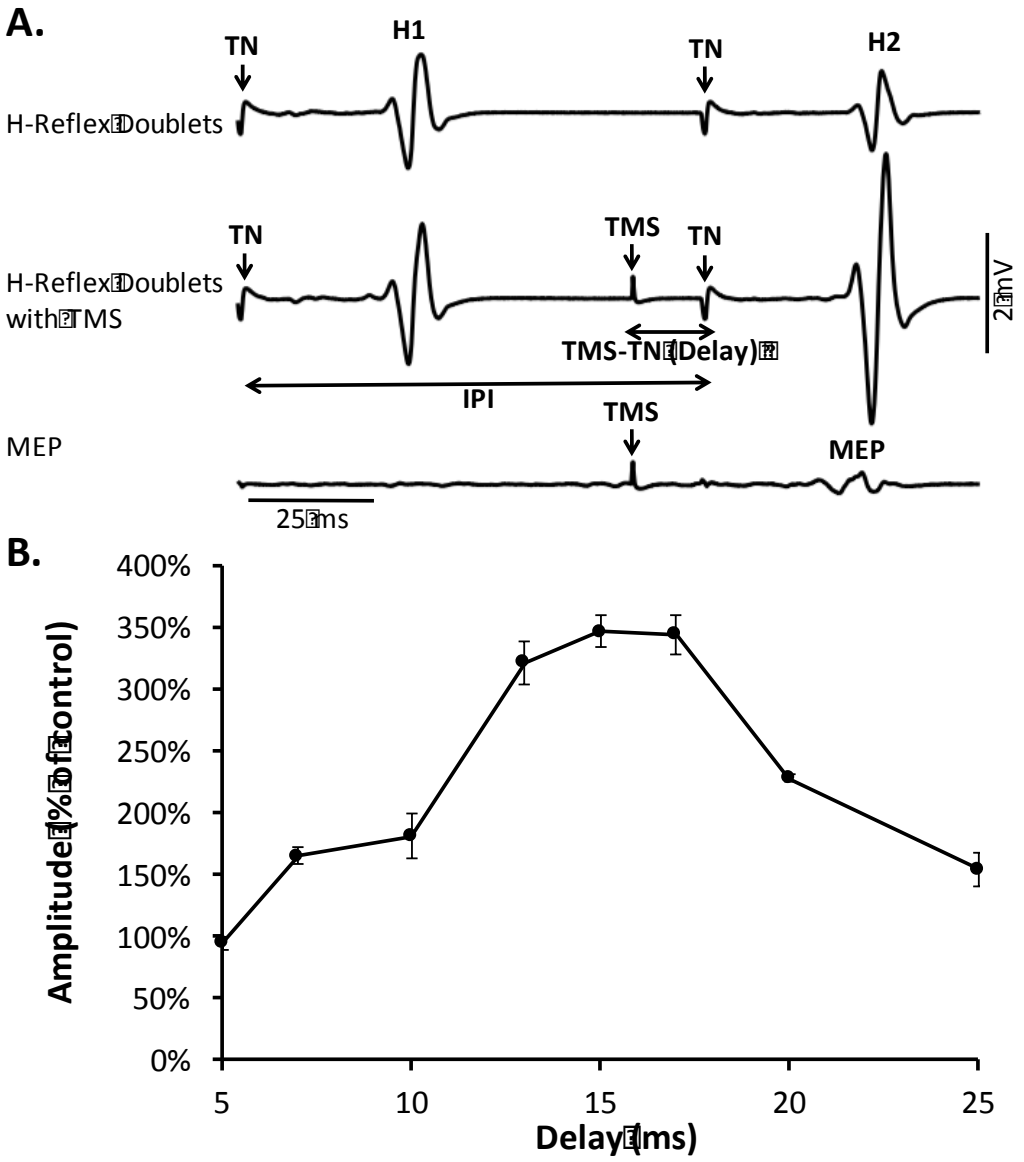

Figure 3.1: Representative data showing the timing of the different stimuli. *A*: post-activation depression was produced with 2 tibial nerve (TN) stimuli delivered with an interpulse interval (IPI) of 100 ms. The second H-reflex (H2) was reduced compared with the first (H1) (*top*). Transcranial magnetic stimulation (TMS) delivered before the second TN stimulus (TMS-TN delay) caused facilitation of H2 (*middle*). MEP, motor evoked potential. *B*: varying the TMS-TN delay resulted in changes in the size of H2. The curve was used to determine the optimal TMS-TN delay.

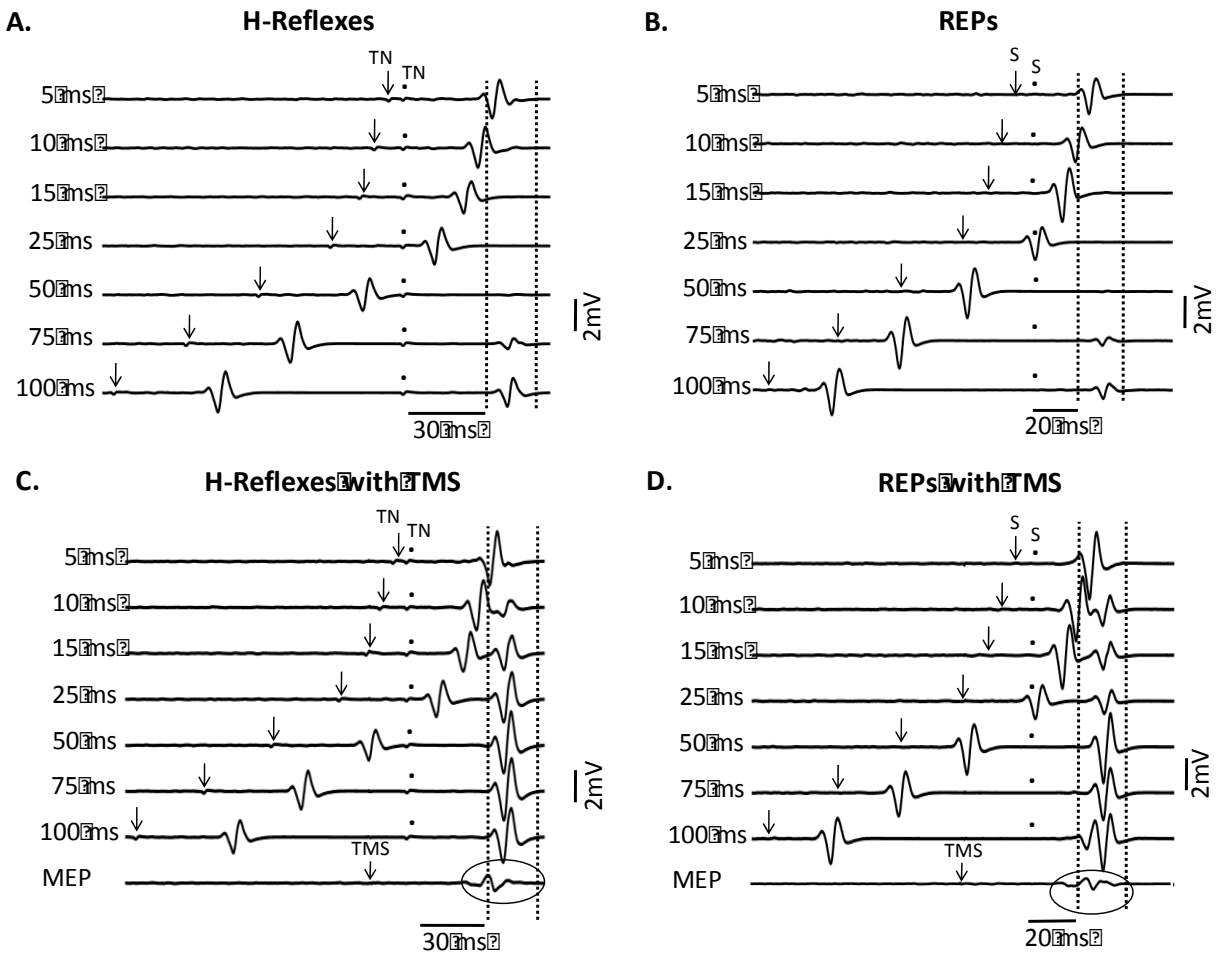

Figure 3.2: Single-subject traces showing the recovery of the H-reflex (*A* and *C*) and root evoked potential (REP) (*B* and *D*) from post-activation depression with and without TMS. Consecutive traces show the recovery of the reflexes at IPIs ranging from 5 (*top*) to 100 ms (*bottom*). Effect of TMS on the recovery is shown in *C* and *D*. TMS was 15% maximal stimulator output (MSO) above threshold. Traces represent the average of 4 sweeps, and ovals highlight the MEP. Arrows and dots represent the times of 1st and 2nd TN and spinal (S) stimuli, respectively. Time of the TMS pulse is indicated with an arrow in MEP trace. H2 and REP2 were measured within the time window between the vertical lines.

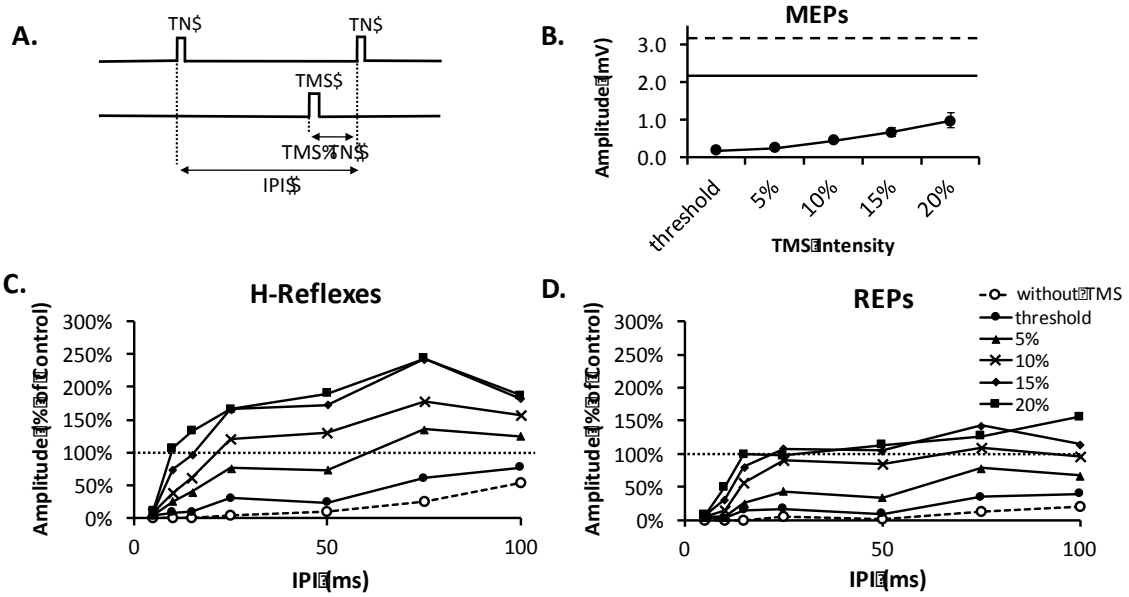

Figure 3.3: A: schematic of the stimulation protocol. TMS was delivered prior to the 2nd TN stimulus (TMS-TN delay). The TMS-S delay was 12 ms longer when testing the interaction with the REP. *B*: average MEP amplitude plotted as a function of intensity. Control H-reflex (solid line) and REP (dashed line) amplitudes are shown in *B* for reference. *C* and *D*: effect of corticospinal input on the recovery of the H-reflex (*C*) or REP (*D*) from post-activation depression during a voluntary contraction. TMS was delivered at 5 different intensities (filled symbols). Data are from 8 subjects. Conditioned reflexes are represented as % of their control (H1 or REP1). Error bars in *C* and *D* have been omitted for clarity.

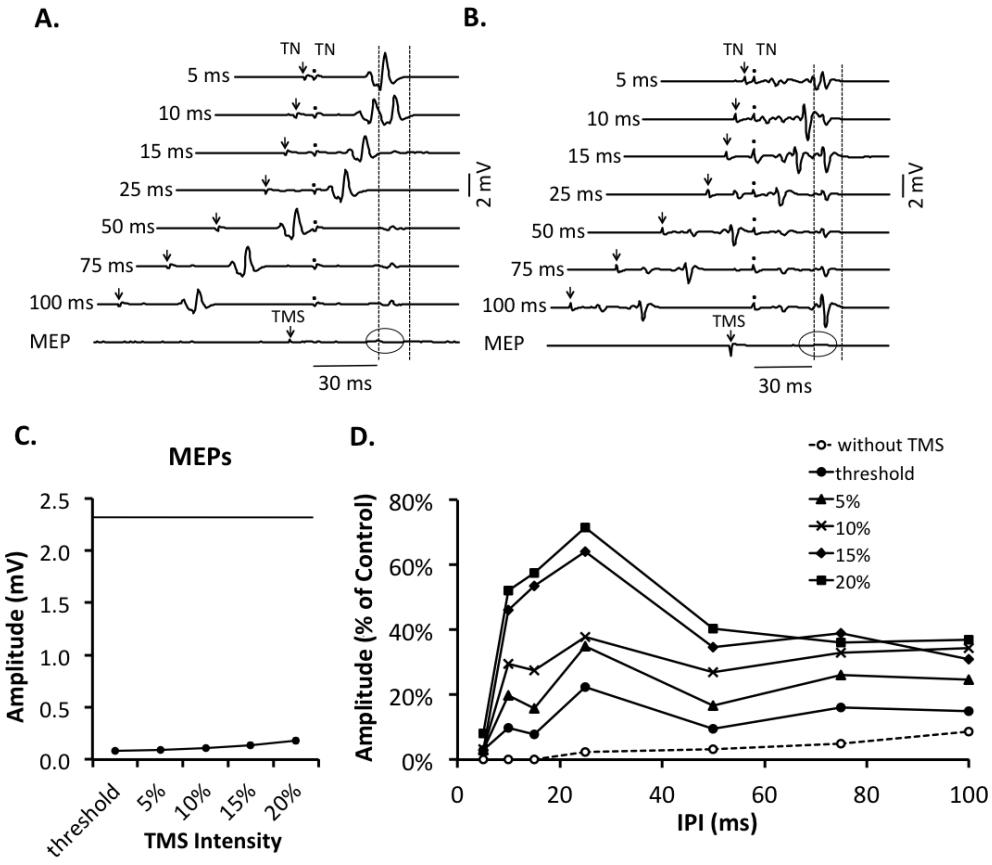

Figure 3.4: Example traces in 2 subjects showing the effect of corticospinal input on post-activation depression of the H-reflex at rest. Figs. 3*A* and *B* are structured in the same manner as Fig. 3.2, *C* and *D*. *C*: average MEP amplitudes plotted as a function of intensity. Control H-reflex (solid line) is shown for reference. *D*: group data from 8 subjects showing the effect of TMS intensity (filled symbols) on the size of the depressed H-reflex (open circles). Error bars in *D* have been omitted for clarity.

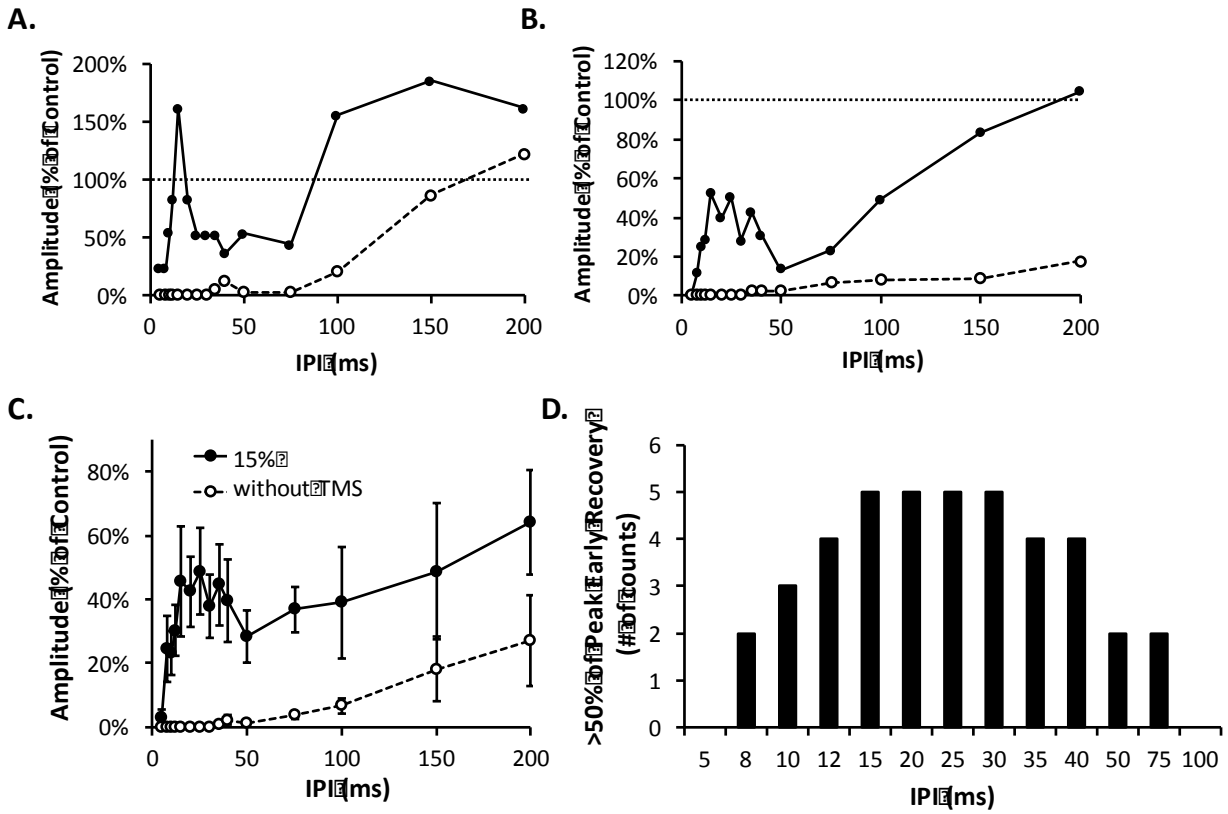

Figure 3.5: *A* and *B*: single-subject data showing the effect of corticospinal input on post-activation depression of the resting H-reflex. Recovery of H2 is shown with (filled circles) and without (open circles) TMS. *C*: group average. *D*: no. of times the 2nd H-reflex recovered to within 50% of the peak early recovery. Data are from 8 subjects.

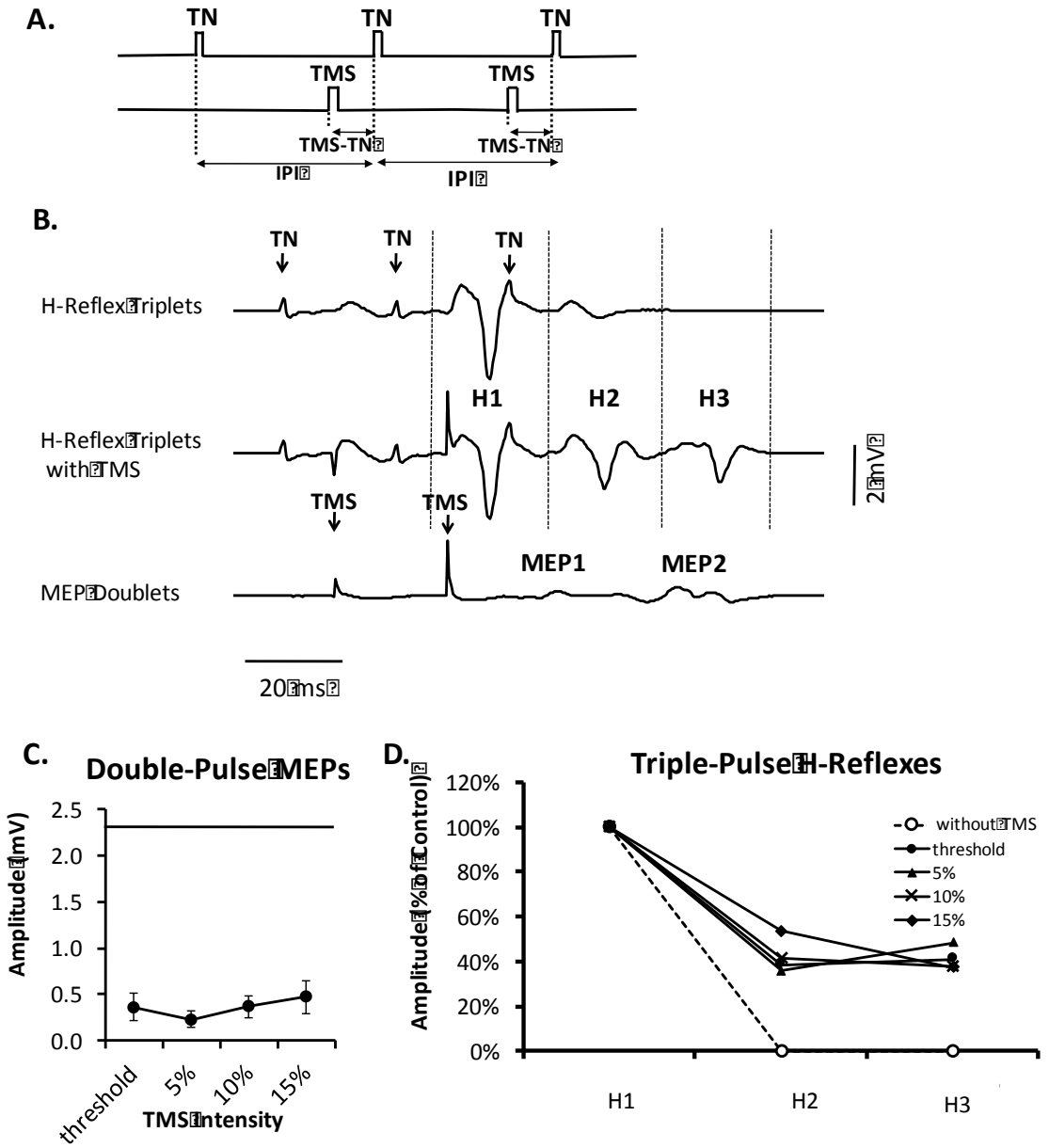

Figure 3.6: H-reflex triplets paired with double-pulse TMS. *A*: schematic of the stimulation protocol. Two time-locked TMS pulses were paired with H2 and H3. *B*: traces show the effect of stimulating the TN 3 times (*top*). Only H1 is evident while the M waves are present. H2 and H3 are evident when conditioned by TMS (*middle*). The 3 H-reflexes fall within the windows separated by vertical dashed lines. Double-pulse TMS produced two MEPs (i.e., MEP1 and MEP2; *bottom*). *C*: amplitude of double-pulse MEP is presented in the same manner as in Fig. 3.4*C*. *D*: group data from 8 subjects showing the effect of the different TMS intensities on H2 and H3.

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CHAPTER 4: INTRAOPERATIVE SPINAL CORD MONITORING USING LOW INTENSITY TRANSCRANIAL STIMULATION TO REMOVE POST-ACTIVATION DEPRESSION OF THE H-REFLEX

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4.1 INTRODUCTION

Monitoring of transcranial motor evoked potentials (MEPs) is currently the preferred technique for assessing the integrity of motor pathways during spine surgery (Kothbauer et al. 1998; MacDonald et al. 2013). The presence of an MEP represents intact motor pathways (Kothbauer et al. 1998). In 1993, Taniguchi and colleagues introduced the technique of high-frequency (300-500 Hz) multi-pulse transcranial electrical stimulation (TES) to more consistently bring lower motor neurons (LMN) to firing threshold and produce a muscle MEP. This approach helps overcome the suppressive effects of anesthesia (Erickson 1949; Wood et al. 1988; Sloan 1998; MacDonald et al. 2003) and has been essential to current intraoperative neurophysiological monitoring (IONM) practices. Multi-pulse TES has been used to detect iatrogenic motor tract injury with the overall goal of reducing the incidence of post-operative neurological deficits. However, pre-existing neurological conditions (Langeloo et al. 2001) and the immature central nervous system of young children (Andersson and Ohlin 1999; Erb et al. 2005) can affect the production of an MEP (Chen et al. 2007; Master et al. 2008; Sloan et al. 2008). Double trains are often used to enhance low amplitude responses (Journée et al. 2004; Journée et al. 2007). Likewise, complementary techniques including the use of multiple trains of TES (MacDonald et al. 2003; Tsutsui et al. 2015), afferent facilitation (Taniguchi 1991; Andersson and Ohlin 1999) and post-tetanic facilitation (Hayashi et al. 2008) have been shown to enhance the depolarization of LMN thereby augmenting leg MEPs.

As an adjunct to multi-pulse TES, intraoperative recording of the Hoffmann (H)-reflex can be used to monitor a complete sensorimotor pathway (Leppanen et al. 1993; Leppanen et al. 1995). Reflex monitoring not only enables the assessment of a large portion of the output from the motor pool (20-100%; Leis et al. 1996), but can also be applied continuously throughout surgery with little to no disruptive patient movement (Leppanen 2006). A change in reflex amplitude may directly signify a compromise to segmental motor pathways (reviewed in Leppanen 2006). Reflex monitoring remains limited, however, as it only provides indirect information about pyramidal and extrapyramidal connectivity, such as during spinal shock (Calancie et al. 1993). To provide a more direct measure of descending motor function, the H-reflex can be paired with an MEP (Cowan et al. 1983, 1986; Taniguchi et al. 1991). While the spatio-temporal summation of these responses has been described as an effective way of enhancing low amplitude MEPs without increasing patient movement (Journée et al. 2007), accurate assessment of the interaction requires baseline values of the H-reflex and MEP as well as continuous knowledge of the combined, composite response. This added complexity may have limited its clinical acceptance.

Following the production of an H-reflex, a subsequent reflex induced along the same pathway can be reduced for up to 10 s at rest (Andrews et al. 2015a; Crone and Nielsen 1989; Pierrot-Deseilligny and Burke 2005). This phenomenon, known as post-activation depression or homosynaptic depression, has been characterized in humans following peripheral nerve stimulation (Magladery et al. 1952; Paillard 1955; Rothwell et al. 1986) and has been associated with changes in presynaptic inhibition (Crone and Nielsen 1989; Schieppati 1987) and/or changes in the amount of neurotransmitter release from the Ia terminal (Hultborn et al. 1996). Transcranial magnetic stimulation (TMS) can remove post-activation depression in awake individuals (Roy et al. 2014; Andrews et al. 2015b) and the strength of the interaction is graded as a function of the stimulus intensity. The presence of an interaction likely confirms the preservation of descending corticospinal pathways in the spinal cord. It has not yet been shown whether the interaction is preserved under general anesthesia; a condition of increased spinal inhibition. The aim of this study is first to investigate the effect of corticospinal excitation on post-

activation depression under general anesthesia, and secondly, to examine the stability of this interaction during routine IONM taking place during spinal deformity correction surgery.

4.2 METHODS

4.2.1 Patients

Twenty pediatric patients (three male) aged 10-17 years (mean age, 14.1 ± 0.4 years) undergoing corrective surgery for spinal deformity were recruited from a single clinical site to participate in this study. Inclusion criteria were pediatric patients with spinal deformity, requiring surgical correction with routine IONM. In 17 patients, the primary diagnosis was adolescent idiopathic scoliosis; 2 had a congenital form of scoliosis, and one was diagnosed with kyphosis. One patient with congenital scoliosis was also diagnosed with Sotos syndrome (i.e. cerebral gigantism). The rostral and caudal end vertebrae targeted for instrumentation were respectively between T2-T9 and L1-S2. Patients and/or their guardians assented/consented to the experimental protocol as approved by the Human Health Research Ethics Board at the University of Alberta.

4.2.2 Anesthetic regime

General anesthesia was maintained with propofol, ketamine, and remifentanil in 18 patients. Sevoflurane (0.2 to 0.5 MAC or minimum alveolar concentration), propofol and sufentanil was used in 2 of the patients. No muscle relaxants were used during the procedure.

4.2.3 Recording and Stimulation

Data were collected during multimodal IONM using a Cadwell Elite neuromonitoring system (Kennewick, WA USA). For the study, MEP recordings were evaluated in the right medial gastrocnemius (MG), tibialis anterior (TA) and abductor hallucis (AH) using a pair of subdermal

needle electrodes. The H-reflex was elicited in the MG following stimulation of the tibial nerve (TN) using a constant-current stimulator (Digitimer DS7A; Digitimer Ltd., Welwyn Garden City, UK) with the cathode on the popliteal fossa and the anode 4 cm more proximal (see Andrews et al. 2015b). The conditioning MEP was delivered using a constant-voltage stimulator (Digitimer D185) with a pair of bent needle electrodes placed at C1-C2. Anodal TES was provided using a train of 1-5 monophasic pulses (50 μs duration) using an interstimulus interval of 1.1 ms. The peripheral and central stimulators were triggered using custom-written MATLAB (MathWorks, Natick, MA) software. Experimental testing was performed intermittently throughout the surgical procedure.

4.2.4. Parameter Optimization

The TES intensity was initially adjusted to elicit a muscle MEP that was conducive for routine IONM (i.e. ~275 V; 5 pulses; 1.1 ms ISI). The H-reflex intensity was adjusted to produce a response that was 50-100% of its maximum. The intensity was periodically adjusted to maximize the H-reflex/M-wave ratio. Post-activation depression was induced by two TN stimuli 100 ms apart, as this interval typically resulted in a small amount of recovery of the second H-reflex (H2; see top trace Fig 4.1A; see Andrews et al. 2015b). Three steps were taken to maximize the influence of the MEP on post-activation depression. First, the delay between TES and the second TN stimulus (i.e. TES/TN delay) was varied from 0 to 20 ms (see Roy et al. 2014). Once, the optimal delay was determined, the interpulse interval (IPI) of the two TN stimuli was systematically increased from 10 to 150 ms. Each condition was repeated without TES for comparison. The IPI that produced the greatest increase in H2 with TES (conditioned) relative to H2 without TES (unconditioned) was chosen and fixed for the duration of the procedure. Lastly, the number of TES pulses was varied from 0 to 5 to evaluate the strength of the effect as a function of the TES intensity. The number of TES pulses was chosen based on the presence of appreciable recovery of H2 while minimizing patient movement caused by the MEP. Note that while the H-reflex generated plantarflexion, this movement was far away from the surgical field and caused minimal interference. Patient movement refers to visible activity within or around the surgical field, which is known to interfere with surgery (Owen 1999; MacDonald et al. 2013). Using the three parameters optimized for each patient, the interaction was tested both with and without TES for the duration of the procedure.

4.2.5 Statistics

H-reflex and MEP amplitudes were measured peak-to-peak. Post-activation depression was measured by expressing the second H-reflex (H2) as a percentage of the first H-reflex (H1; control). In half the trials, H2 was conditioned by an MEP. Complete recovery of H2 equaled 100%. Given that low intensity TES was used, MEPs were typically absent or were small when present. To identify an MEP, each response was required to meet three criteria: 1) occur in a predictable time window following the stimulus artifact, 2) have a morphology similar to a suprathreshold MEP, and 3) be ≥ 3X the amplitude of the peak-to-peak background activity assessed within a 25 ms window, 50 ms prior to the stimulus. To average the data across patients, the duration of each surgery was normalized based on the last IONM data point. The data were then grouped into 10 identical bins of time. If no data were collected during a given period of time, the bin was left empty. In one procedure, there was a transient loss to the lower extremity MEP, as detected during routine IONM, and the experimental data collection ceased shortly thereafter. The final experimental data point was used to mark the end of the procedure. Statistical analysis was performed using SPSS analytics software (SPSS, Chicago, IL). The removal of post-activation depression (or degree of facilitation) as induced by TES, was demonstrated by calculating the quotient of the conditioned H2 divided by unconditioned H2. In other words, a conditioned H2 twice the size of its unconditioned counterpart was represented as 2X. Conditioned and unconditioned H2 values were compared using a paired student's t test. Linear regression analysis was used to measure the stability of H1 and H2 across time. Data are presented as mean ± standard error (SE). Statistical significance was set at $P \leq 0.05$.

4.3 RESULTS

4.3.1 Single Patient Data

Figure 4.1A-C shows the optimization protocol in a representative patient. Pairs of TN stimuli were delivered 100 ms apart and the TES/TN delay was systematically increased from 0 to 20 ms

(Fig. 4.1A). While the facilitation of H2 was not widely different between the 8 different TES/TN delays, 10 ms was marginally superior based on real-time inspection of the data (see the fifth trace in Fig. 4.1A). The optimal TES/TN delay was then fixed while the IPI was increased from 10 to 150 ms (traces from 25 to 150 ms in 25 ms increments shown in Fig. 4.1B; see grey traces). This sequence was repeated without TES (Fig. 4.1B; see black traces). A 100 ms IPI was chosen as this interval had the greatest difference between the conditioned and unconditioned H2 amplitudes. The third step examined the effect of the number of TES pulses on the amount of H2 facilitation (Fig. 4.1C). Two pulses of TES were chosen based on the recovery of the conditioned H2 relative to its unconditioned counterpart and the absence of patient movement within the surgical field.

4.3.2 Group Data

The average duration of a given surgery was 295.5 ± 26.0 minutes. In the majority of patients, the optimal TES/TN delay and IPI was 7.5 ms (range: 0 ms to 10 ms; Fig. 4.1D) and 100 ms (range: 50 to 125 ms; Fig. 4.1E) respectively. Care was taken to minimize patient movement from TES while still maintaining the strength of the interaction. The number of TES pulses used to facilitate H2 (range: 0 to 5; Fig 4.1F) was typically 2. On occasion, 3 or more pulses were required to overcome the suppressive effects of general anesthesia, particularly in the presence of sevoflurane. On average, the TES intensity was delivered at 304.6 ± 23.0 V. Average MEP amplitudes measured in TA, MG, and AH were 17.1 ± 2.7 μV, 5.1 ± 0.2 μV, and 11.9 ± 3.1 μV, respectively. Note that due to small changes in background activity, values that were less than ~10 μV showed no visible response and were likely subthreshold. Using the optimal TES intensity determined earlier, lower limb MEPs that met the aforementioned criteria, were not at any time produced in 13/20 of the cases (Table 4.1). In the 7 cases where lower limb MEPs were sporadically produced (typically < 50% of the time), they were not detected in all muscle groups. MEPs were most common in TA. In fact, only 3 of these patients ever produced an MEP > 100 μV. No patient movement was observed within the surgical field in 19/20 cases. In the one instance, when there was mild shoulder and neck movement from TES, general anesthesia was being

maintained with 0.5 MAC of sevoflurane, 100 mcg/kg/min of propofol, and 0.04 mcg/kg/min of sufentanil. Three TES pulses at 400 V were being used to overcome the suppressive effects of the anesthetic agents.

Measured across time and in all 20 patients, H-reflex data were recorded in 177 out of the 200 bins (88.5%) as 23 of the time-points (11.5%) were missing due to the concurrent IONM demands and/or long periods of electrosurgery. The average amplitude of H1 measured across all patients was 1.84 ± 0.30 mV at baseline and decreased to 1.21 ± 0.22 mV by the end of the surgical procedure (linear regression: $P < 0.05$). At the start of the procedure, post-activation depression reduced the size of H2 to 9.5 ± 1.6% of control values. When conditioned with a weak TES stimulus, the H2 responses increased to 51.1 ± 7.1% of control (paired t-test: $P < 0.0001$). Measured across time, these unconditioned and conditioned H2 ratios slightly increased to 12.5 ± 2.5% and 76.2 ±12.1% of control by the end of the surgical procedure (linear regression: both $P < 0.05$; see Fig. 4.2A). When comparing the size of the conditioned and unconditioned H2 responses at all individual time-points, the conditioned H2 was at least 2X greater than the unconditioned H2 ≥ 96.6% of the time (see dotted lines Fig. 4.2B). In fact, 48.6 % of the time, a conditioned H2 was at least 5.0X greater than an unconditioned H2 (see dashed lines in Fig. 4.2B).

In 19/20 surgical procedures, there were no IONM changes. In one patient, the concurrent IONM revealed a loss to the lower limb MEPs shortly after the second rod was placed (Fig. 4.3A). The MEPs promptly recovered once the rod was removed. The conditioned/unconditioned H2 ratio was 3.1X prior to the change, decreased to 0.8X within minutes after the MEP loss, and then increased to 3.8X once the rod was removed (raw amplitudes shown in Fig. 4.3B). At this point, the experimental testing ceased in order to focus on IONM, and the procedure was associated with two more transient MEP changes. The patient suffered no post-operative deficit.

4.4 DISCUSSION

TES was used to remove post-activation depression of the H-reflex during spinal deformity correction surgery. Despite the suppressive effects of general anesthesia on neuronal excitability, the conditioning effect was observed in all patients and the recovery of H2 did not diminish throughout the surgical period. In most cases, TES was delivered at an intensity that produced no leg MEP, and in-turn, no patient movement within the surgical field.

To optimize the effect of a conditioning MEP on the depressed H-reflex, various stimulation parameters were adjusted and the values were relatively consistent across patients. In > 50% of the cases we used a 7.5 ms delay between the MEP and H2 stimuli, but this parameter may be less critical as delays from 0 to 10 ms exhibited comparable degrees of facilitation. Note that a TES/TN delay of 0 ms would at times appear slightly superior as this was always the first delay delivered in the sequence, and would be less susceptible to post-activation depression produced by the previous stimuli. Previous findings using TMS in awake individuals to remove post-activation depression of the soleus H-reflex had shown that a ~14 ms delay produced the largest effect (Andrews et al. 2015b). Journée and colleagues (2007) described a similar early peak of facilitation when TES was applied ~10 ms prior to the H-reflex. Given the considerable inhibition of LMNs during general anesthesia (MacDonald et al. 2013), the spatiotemporal summation of descending CST volleys onto LMNs are more likely to occur at short delays. The timing or IPI between the two successive H-reflexes may have been a more sensitive parameter and was chosen based on the greatest amplitude difference between the conditioned and unconditioned H2 responses. IPIs greater than 50 ms and up to 125 ms were often selected as these intervals typically permitted a small amount of recovery without TES (see Andrews et al. 2015b).

The suppression of indirect (I)-waves and LMN excitability by general anesthesia dictates that several excitatory post-synaptic potentials from the CST are necessary to bring LMNs to firing threshold (Amassian 2002; MacDonald 2006). As the number of TES pulses increased, so did the size of the conditioned H2. In other words, higher TES intensities produce a greater reduction in post-activation depression. This relationship is likely related to the incremental increase in the MEP amplitude observed when increasing the length of the TES train (Taniguchi et al. 1993). A

similar relationship was observed between post-activation depression and the size of the MEP using TMS (Andrews et al. 2015b). The present study aimed to maximize the influence of TES on post-activation depression while limiting MEP generation and the associated patient movement. To meet these criteria, the number of TES pulses used was typically 2 (90% of the time) or 3. Evoked response amplitude has been shown to gradually fade throughout surgery, likely a result of declining LMN excitability (MacDonald 2006; MacDonald et al. 2013). While this phenomenon may have caused a decrease in the size the control H1 response over time, it did not alter the present interactions. In fact, there was even a slight increase in the recovery of H2 (both unconditioned and conditioned) throughout the surgical period, given that it was measured as a percentage of the declining control H1 response. This suggests that while fade may influence the size of the H-reflex, it may not affect the production of post-activation depression nor its subsequent removal by TES.

Post-activation depression was preserved under general anesthesia. Following a weak TES stimulus, the depressed H-reflex was facilitated to a least 2X the size of the unconditioned H2 > 96% of the time. Using low intensity TES, the effect was quite stable throughout surgery and uniform across patients, despite the use of 0.2 to 0.5 MAC of sevoflurane in two patients. These patients required a stronger transcranial stimulus, as would be expected under these conditions (Pajewski et al. 2007). Facilitation techniques using subthreshold MEPs to condition an H-reflex allow continuous monitoring without patient movement (Journée 2007). MEPs in the legs were not visible in over half of the patients (13/20), and when present, were typically small and produced no movement within the surgical field in 19/20 cases.

The monitoring of MEPs during spine surgery is important for assessing motor function (Kothbauer et al. 1998; MacDonald et al. 2013), but can fail in some patients with pre-existing conditions where the MEP is small or absent. Since the present interaction is preserved using a subthreshold MEP, an H-reflex/MEP combination may help monitor the integrity of CST fibers projecting to sacral LMNs. This approach may be relevant in cases where MEPs are diminished, such as in myelopathic patients (Lyon et al. 2004), very young children (Andersson and Ohlin

1999; Erb et al. 2005) and patients with neuromuscular disorders (Langeloo et al. 2001). Upper motor neuron lesions are linked to increased in spasticity and exaggerated spinal reflexes (Ashby et al. 1974; Taylor et al. 1984; Little and Halar 1985; Calancie et al. 1993), which in-turn may increase the success of H-reflex testing and conditioning.

As mentioned previously, monitoring CST function using a low intensity MEP to condition a single H-reflex or F-wave (Lepannen 2006) can be used continuously throughout surgery given that interference produced by patient movement is minimal (Journée et al 2007). H-reflexes can be variable so intraoperative events may be misinterpreted as variability of the reflex. The benefit of the proposed technique is that the first H-reflex (H1) delivered in a pair will reduce the amplitude of the second (H2) and provide a stable standard with which to compare an H2 conditioned by TES. Any change in the size of a conditioned H2, caused by a reduced ability for descending input from the CST to remove post-activation depression, should be apparent upon comparison with an unconditioned H2.

The criteria for detecting a meaningful change in CST function from this approach remains to be established. Results from one patient showed a total loss of facilitation during a significant MEP change. This result suggests that the two events were linked, though the data remain limited. A change in the degree of facilitation of the depressed H-reflex by TES, in the absence of another physiological change, could indicate a significant event, which would require further evaluation using routine MEP testing.

Evidence that descending corticospinal inputs can remove post-activation depression has been shown in adults using TMS (Roy et al. 2014; Andrews et al. 2015b), but can now be extended to the pediatric population using TES. TMS was shown to reduce and even reverse post-activation depression of the H-reflex. The interaction, as tested here (i.e. IPIs from 50-125 ms), may be caused by a combination of pre- and post-synaptic mechanisms, namely changes to the circuitry mediating post-activation depression and post-synaptic facilitation, respectively. Given that the activation of CST fibers can inhibit the circuitry producing presynaptic inhibition of Ia afferents

(Iles 1996), the interaction may be explained by the removal of presynaptic inhibition by TES. However, the recovery was less when the unconditioned H2 was absent, suggesting that the interneuronal interaction may be strongly suppressed under general anesthesia. A more likely mechanism involved the summation of excitatory post-synaptic potentials from TES and the afferent input at the LMN. This is supported by evidence that greater facilitation from TES was produced when the unconditioned H2 was small but present (~10% of H1). Moreover, the effect increased in magnitude as the size of the unconditioned H2 increased, as was achieved when using longer IPIs (see Fig. 4.1E).

The removal of post-activation depression using low intensity TES enabled the CST to be monitored with little to no patient movement in the surgical field. The approach may be reminiscent of D-wave monitoring given that the interaction provides a global assessment of spinal motor function. The approach is non-invasive and can access CST function at a level that is caudal to most D-wave recordings (i.e sacral cord). The requirement to administer three timed stimuli for H-reflexes and TES however adds to the complexity of routine IONM. Presently, the technique has been tested in a group of pediatric patients, a majority of whom had idiopathic scoliosis. Future work may involve patients with pre-existing neurological deficits where conventional MEP monitoring is hampered and H-reflexes may be exaggerated.

Table 4.1

Group data from 20 patients depicting the percentage of trials when MEPs were observed in leg muscles, following low intensity TES. A value of '-' indicates that MEPs were absent.

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
TA	64%	83%	56%	-	-	-	43%	-	-	-	28%	-	-	17%	-	-	42%	-	-	-
MG	-	-	-	-	-	-	-	-	-	-	5%	-	-	-	-	-	-	-	-	-
AH	-	-	-	-	-	-	-	-	-	-	-	-	-	17%	-	-	-	-	-	-

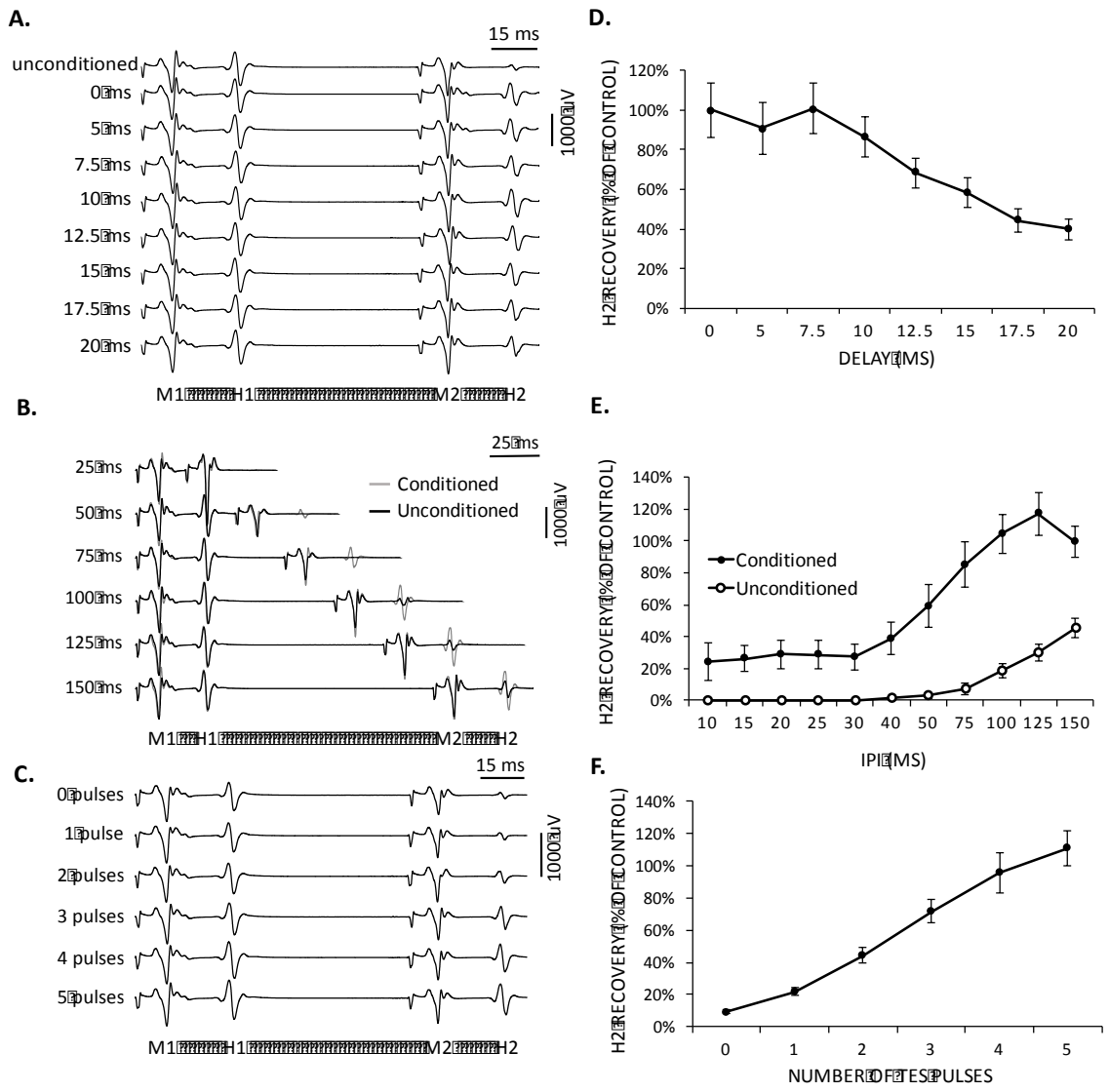

Figure 4.1: Optimization protocol recorded in MG in a representative patient (A-C) and averaged across the group (D-F). (A) Single-patient traces show post-activation depression of the H-reflex (i.e. H2) when stimuli are delivered 100 ms apart (top trace). TES was then given 0 ms (second trace) to 20 ms (bottom trace) before the second TN stimulus. TES stimulus artifacts were not visible on this scale. (B) With the TES/TN delay set to 10 ms, the effect of the interpulse interval (IPI) was tested. IPIs from 25 to 150 ms (25 ms increments) are represented with TES (conditioned; grey traces) and without TES (unconditioned; black traces). (C) Using a 100 ms IPI, the number of TES pulses was varied from 0 to 5. Labels in A-C show the first and second M-wave (M1 and M2) and H-reflex (H1 and H2). Group data for A, B, and C are represented in D, E, and F, respectively. Error bars indicate standard errors.

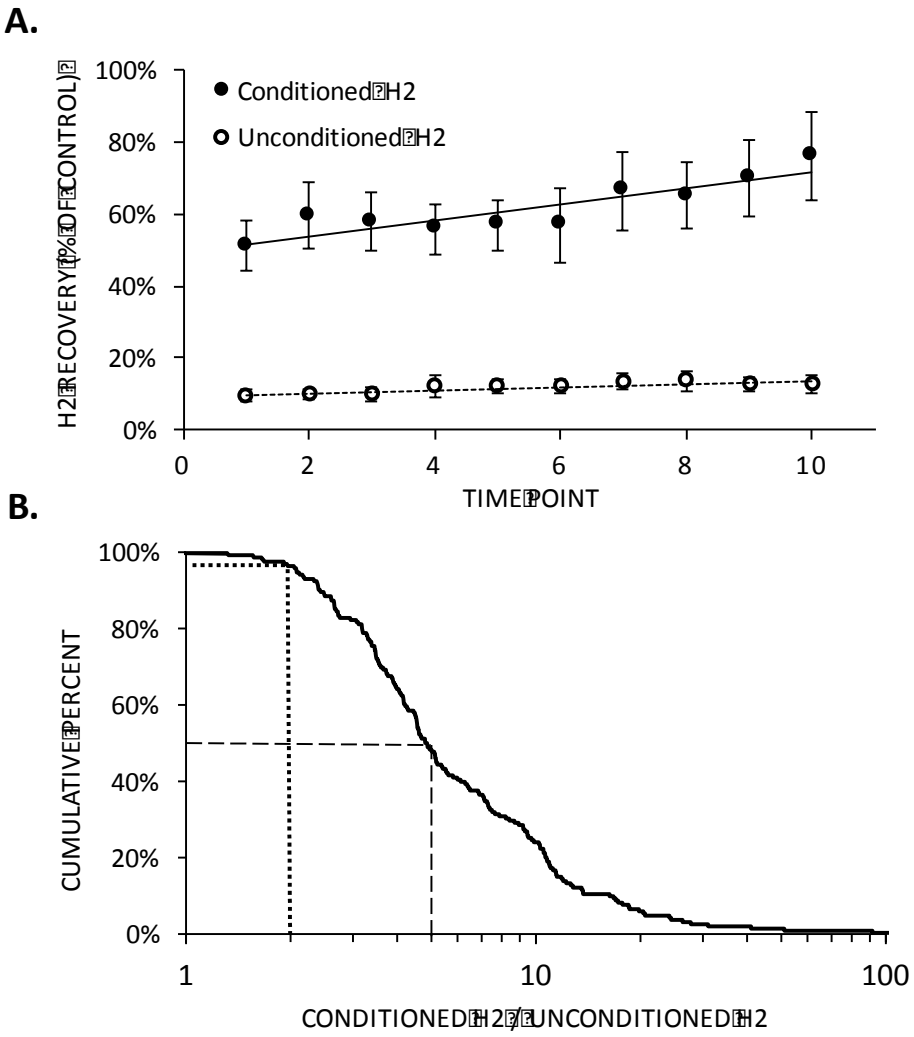

Figure 4.2: (A) Graph shows the recovery from post-activation depression of a conditioned (filled circles) and unconditioned (open circles) H2 response plotted as a function of time and averaged across patients. The duration of each procedure was normalized and then subdivided into ten bins of time. Linear regression lines show increases to the conditioned (solid line) and unconditioned (dashed line) H2 ratios as a function of time. (B) Graph shows the percentage of data points where the conditioned H2 was greater or equal to a given multiple of the unconditioned H2. The incidence where a conditioned H2 exceeded an unconditioned H2 by 2X (dotted line) and 5X (dashed line) is shown.

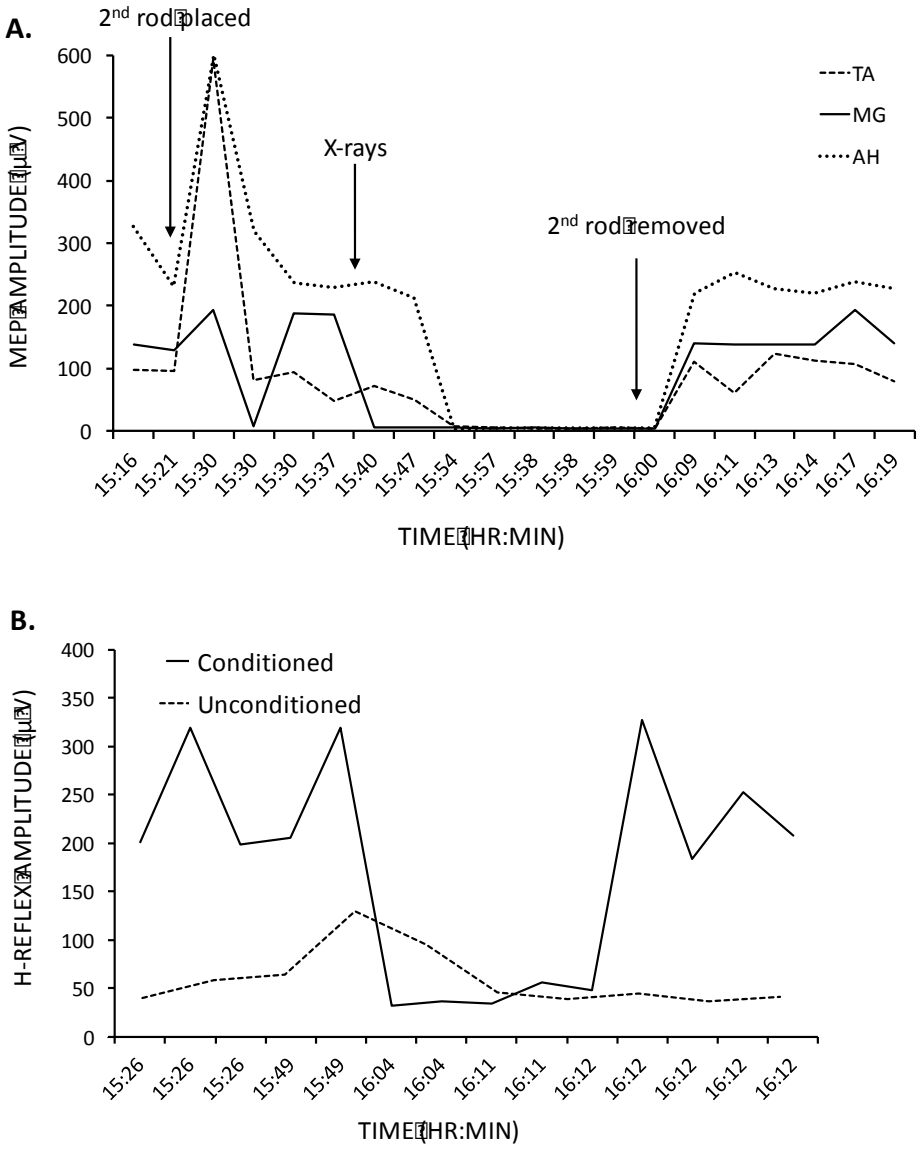

Figure 4.3: (A) Graph depicts the loss of MEPs recorded in the right TA (dashed line), MG (solid line), and AH (dotted line) in one patient. Amplitudes are shown peak-to-peak. The disappearance occurred ~34 minutes after the 2nd rod (right side) was placed and immediately after the anterior-posterior and lateral x-rays. MEPs recovered following the removal of the right rod. MEP changes in the left leg were similar (data not shown). Each timestamp on the x-axis represents one stimulus, and occasionally, more than one stimulus was delivered within a minute. (B) Experimental conditioned H-reflex data (right leg) leading up to, during and after the event. Conditioned (solid line) and unconditioned (dashed line) H2 responses are expressed as their peak-to-peak amplitude.

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CHAPTER 5: PILOT STUDY SHOWING THE EFFECT OF DEEP BRAIN STIMULATION ON SPINAL CORD CIRCUITRY IN PARKINSON'S DISEASE

5.1 INTRODUCTION

Parkinson's disease (PD) is a progressive disorder of the central nervous system that affects motor control. Cardinal motor symptoms of PD include: tremor, rigidity, bradykinesia, and postural instability (Jankovic et al. 1998). The severity of these symptoms correlates with changes to spinal cord circuity. Many groups have investigated changes in cortical motor areas while relatively few have focused their attention on pathways within the spinal cord, a major area of output from these supraspinal centers. Abnormal transmission of signals within spinal pathways is known to contribute to the disorders of movement that develop following damage to the basal ganglia (Pierrot-Deseilligny and Burke 2012).

Connections from the brainstem to spinal interneurons allow spinal reflexes to be used as an indirect measure of brainstem activity (Delwaide et al. 2000). The Hoffmann (H)-reflex is an electrically evoked analogue of the stretch reflex and is commonly used to assess functional connectivity within the spinal cord. Following a conditioning stimulus, the modulation of an H-reflex can also assess signal transmission in various spinal pathways in both healthy individuals and pathological states.

5.1.1 Post-activation depression in PD

Post-activation depression induced by a paired-pulse paradigm of the H-reflex has been used for decades to evaluate spinal excitability in patients with PD (McLeod and Walsh 1972). Decreases in post-activation depression in PD have been shown to be linked to increased rigidity. However, in the presence of high frequency deep brain stimulation (DBS) to the subthalamic nucleus (STN), the rigidity is diminished and post-activation depression is restored (Raoul et al. 2012). The H-

reflex recovery curve has been used to predict early onset of PD (Sabbahi et al. 2002) and even determine the likelihood of success following treatment using dopamine agonists in the later stages of the disease (Fujita and Cooper, 1971). While H-reflex recovery curves reveal increased motor neuron excitability in patients with PD at rest (McLeod and Walsh 1972; Sabbahi et al. 2002; Raoul et al 2012), to our knowledge, it is unknown how the recovery curve is affected in PD during contraction; a condition of increased motor neuron excitability (Pierrot-Deseilligny and Burke 2012).

5.1.2 Ib interneurons in PD

Ib inhibition is said to linearize the relationship between muscle length and tension (Nichols and Houk 1976). In PD, Ib inhibition is reduced and may be linked to the increases in rigidity in PD (Delwaide et al. 1991). While treatment with DBS (Potter et al. 2004) and an orally administered precursor of dopamine named levodopa (L-DOPA; Delwaide et al. 1991), have been shown to restore the abnormally reduced Ib inhibition, their effects on this pathway (whether combined or in isolation) have yet to be studied in the same patient.

5.1.3 Presynaptic inhibition in PD

Changes in presynaptic inhibition are also thought to contribute to the development of motor signs in PD (Roberts et al. 1994). More specifically, decreased presynaptic inhibition of the lower limb observed in patients with PD was seen in conjunction with increases in rigidity (Delwaide et al. 1993), bradykinesia and slowed walking speed (Morita et al. 2000). How this pathway is influenced by L-DOPA and/or DBS; however, remains unknown.

5.1.4 Ia interneurons in PD

While an increase in reciprocal Ia inhibition of the soleus is observed in patients with PD at rest (Delwaide et al. 1993) the opposite effect occurs during a voluntary dorsiflexion (Hayashi et al. 1988). When parkinsonian symptoms are treated with L-DOPA, reciprocal Ia inhibition was

restored. However, the impact of DBS on this pathway at rest or during contraction has yet to be shown.

5.1.5 Corticospinal tract (CST) input in PD

The motor cortex is a major target of the output from the basal ganglia and the corticospinal tract (CST) is in turn a major target of the output from the motor cortex (Porter and Lemon 1993). Studies of corticospinal physiology in humans (Merton and Morton 1980) were also conducted in people with PD to trace the disrupted output from the basal ganglia, to cortical motor neuronal connections (Dick et al. 1984; Caramia et al. 1988). These studies concluded that signal transmission along the CST was normal. Studies using transcranial magnetic stimulation (TMS) to condition the H-reflex; however, can be used to measure both pyramidal and extrapyramidal drive to motor neurons (Nielsen et al 1993; Nielsen and Petersen, 1995). Interestingly, abnormal cortical motor control over the H-reflex was observed in those with PD (Morita et al. 2002; Potter-Nerger et al. 2008). The early facilitation of the H-reflex compatible with direct monosynaptic connections from the motor cortex to the soleus motor neuron pool (Cowan et al. 1986), that was previously reported in control subjects (Nielsen et al. 1993; Nielsen and Petersen, 1995a) was not present in those with PD during contraction (Morita et al. 2002) or at rest (Potter-Nerger et al. 2008). In some patients the early facilitation was even replaced with an inhibition. The modulation of the H-reflex by descending motor input was restored to control levels following a pallidotomy (Morita et al. 2002) and during STN-DBS (Potter-Nerger et al. 2008).

While many of the aforementioned pathways have been studied in patients with PD off parkinsonian medication, to our knowledge, the same cannot be said for patients receiving both medication and DBS. The goals of this study are threefold. First, we aim to determine which pathways within the spinal cord are altered in PD. Secondly, we want to evaluate the extent by which DBS and/or parkinsonian medication normalizes these pathways and the effect that these changes have on the severity of motor symptoms. Lastly, we will explore whether both forms of treatment work in parallel or have distinct effects. Since DBS and medication are thought to work differently in improving motor symptoms of PD, we predict they will have disparate effects on

normalizing spinal cord circuitry. The results of these series of tests may help identify potential physiological markers of PD and establish the sensitivity of each circuit to parkinsonian treatment.

5.2 METHODS

5.2.1 Participants

For this experiment, we aim to recruit 10 patients with PD and 10 control subjects. Control subjects will be sex and age-matched to our patient population to avoid any gender bias or aging effects (Kido et al. 2004). To date, we have recruited 4 patients with PD (age: 65 ± 3) and 10 age-matched controls (age: 60 ± 3). The average number of years since diagnosis for the patient group was 19 ± 1 year. For the control of motor symptoms associated with the disease, two patients had DBS electrodes implanted bilaterally in the GPi, one had a bilateral STN implant, and one had a unilateral left STN implant. All patients were operated on using MRI targeting and intraoperative electrode recording and stimulation. The average time since surgery ranged from 3 months to 7 years (mean 3 ± 1 year). Table 5.1 details various clinical features for the control and patient groups. Participants were screened for potential contraindications to the stimulation including history of seizures, chronic back pain or prior spinal surgery. All participants provided written consent to the experimental protocol approved by the Health Research Ethics Board at the University of Alberta.

5.2.2 Recording and stimulation

Participants were seated with their leg in a metal brace maintaining a 100-degree angle of both knee and ankle joints. In our patient group, we selected the leg on the side of the body that was most affected by PD. EMG responses were recorded from the soleus muscles using a pair of silver-silver chloride surface recording electrodes (3.5 x 2.2cm; Vermont Medical Inc., Bellow Falls VT) placed 3 cm below the distal border of the gastrocnemius. EMG from the tibialis anterior (TA) was recorded by placing two recording electrodes along the belly of the tibialis anterior, 2/3 of

the distance from the lateral malleolus to the knee. Muscle activity was recorded from the medial gastrocnemius (MG) by placing two recording electrodes along the belly of the MG 10-15 cm above the distal border of the muscle. The EMG was band-pass filtered between 10-1000 Hz, amplified 1000x (Octopus, Bortec Technologies, Calgary, Canada) and digitized at 5 kHz using Axoscope Hardware (Digidata 1200 series, Axon Instruments, Union City CA). The EMG was also full-wave rectified and low-pass filtered at 3 Hz so that the participants could monitor their level of EMG on an oscilloscope. Each participant's maximum voluntary contraction (MVC) for the soleus and TA was determined during an isometric plantarflexion and dorsiflexion, respectively. The MVC of each muscle was recorded as the average of three contractions.

The tibial nerve (TN) was stimulated using 1 ms pulses (Digitimer DS7A; Digitimer, Welwyn Garden City, UK) with the cathode on the popliteal fossa and the anode 4 cm more proximal. The optimal position of the nerve was determined using a handheld monopolar probe (1 cm tip) as the site that produced the largest soleus H-reflex with minimal encroachment from the antagonist, TA muscle. To stimulate the common peroneal nerve (CPN) the cathode was placed at the lowest threshold point near the neck of the fibula and the anode was placed 4 cm more distal. The MG nerve was stimulated at the lower part of the popliteal fossa, ~ 10 cm distal and ~5 cm lateral to the TN stimulating electrodes. Threshold was identified as the minimum intensity capable of producing a visible EMG response.

TMS was delivered over the motor cortex to elicit a soleus MEP. Monophasic pulses were delivered using a MagStim 200^2 stimulator (Magstim, Dyfed, UK). The double-cone coil (P/N 9902-00: external wing diameter 110 mm) was orientated to produce posterior-to-anterior currents in the brain. The coil was initially positioned over the right motor cortex (1 cm lateral and 1 cm posterior of the vertex) and then moved in 1 cm increments until the optimal position for producing an MEP in the left soleus muscle was determined.

5.2.3 Stimulation protocols

We employed several different electrophysiological techniques to test spinal cord function in control subjects and people with PD. For a majority of experiments, modulation of the soleus H-reflex following a conditioning stimulus was used to evaluate the effect of the different testing conditions on spinal cord circuitry. The remaining two experiments involved assessing the average rectified EMG activity measured during the contraction of ankle flexors and extensors.

5.2.3.1 Post-activation depression

We investigated the recovery of a soleus H-reflex from post-activation depression. Recruitment curves were collected for the soleus H-reflex by gradually increasing the stimulus intensity from threshold and onward until reaching the maximum H-reflex amplitude (H_{max}). For the M-wave, the stimulus intensity was further increased to elicit the maximal M-wave amplitude (M_{max}). A half maximal level of activation ($H_{max}/2$) was selected on the basis that excitatory and inhibitory influences can either increase or decrease a half-maximal response amplitude (Crone et al. 1990; Zehr and Stein 1999). To examine the time-course of recovery, we evaluated post-activation depression using paired pulses to the TN. The conditioning pulse (H1) was separated from the test pulse (H2) by intervals of 25, 50, 75, 100, 200, and 300 ms. Each pair of pulses was delivered 5 s apart as the depressive effects from a previous activation are sufficiently small at this point to allow testing at this frequency (Crone and Nielsen 1989; Pierrot-Deseilligny and Burke 2012). All data were collected at rest and during a voluntary isometric plantarflexion, 15-20% of MVC (Roy et al. 2014).

5.2.3.2 Ib inhibition

Ib inhibition was studied in the soleus muscle at rest following conditioning input from the MG nerve. The procedure for studying Ib inhibition was adapted from a protocol developed by Pierrot-Deseilligny and colleagues (1981). Resting threshold for the motor response produced by MG nerve stimulation was determined at the start of the experiment. An $H_{max}/2$ was conditioned by a single pulse to the MG nerve adjusted to 98% of motor threshold. The MG nerve stimulus (conditioning stimulus) always preceded the TN stimulus (test stimulus) by either 4, 5, or 6 ms. These intervals were studied as they typically permit the greatest reduction in H-reflex amplitude

from Ib inhibition (Pierrot-Deseilligny et al. 1981; Delwaide et al. 1991; Delwadie et al. 1993). Twenty unconditioned H-reflexes were averaged to establish a control value and 20 conditioned H-reflexes were collected for each of the three different condition-test intervals (80 total stimuli). Each condition-test pair was administered 3 s apart (Delwaide et al. 1991).

5.2.3.3 Presynaptic inhibition

Presynaptic inhibition of soleus Ia afferents was assessed following a conditioning stimulus to the CPN innervating the TA. CPN stimulation was delivered at a stimulus intensity 1.4 X motor threshold (MT). An $H_{max}/2$ was conditioned by a train of 5 pulses at 300 Hz every 3 s. The start of the conditioning train was delivered 29 ms prior to delivery of the SOL H-reflex (the interval between the last conditioning shock and the test stimulus was 16 ms). This protocol was adapted from one developed by El-Tohamy and Sedgwick (1983). The mean amplitude of 20 conditioned reflexes were normalized to the mean amplitude of 20 unconditioned reflexes.

5.2.3.4 Reciprocal Ia Inhibition at rest

Reciprocal Ia inhibition was evaluated using a protocol adapted from Crone and colleagues (1987). An $H_{max}/2$ was conditioned by CPN stimulation delivered at 1.0 X MT. Reciprocal Ia inhibition was tested at 2, 3, 4 ms. Conditioned (20 repeats at each interval; 60 total) and unconditioned (20 total) soleus H-reflexes were presented in pseudorandom order every 3 s.

5.2.3.5 Reciprocal Ia inhibition during contraction

The protocol used here was developed by Kido and colleagues (2004). Reciprocal inhibition of soleus EMG activity during planarflexion was evoked following stimulation of the CPN 1.5 X MT. CPN motor threshold was determined while patients maintained a voluntary plantarflexion (15-20 % of MVC). Participants held and maintained a plantarflexion while 50 stimuli were delivered to the CPN every 2 s (i.e. 100 s total). Reciprocal inhibition was also assessed in TA during a dorsiflexion following stimulation of the TN.

5.2.3.6 TMS on the H-reflex

TMS was used to condition a soleus H-reflex and the interaction was studied at rest. Once the optimal location for producing a soleus MEP was determined and threshold identified, a single TMS pulse 95% of resting motor threshold (see appendix A), was used to condition an $H_{max}/2$ at condition-test intervals ranging from -5 ms to 20 ms (i.e. -5, -4, -3, -2.5, -2, -1.5, -1.0, -0.5, 0, 1, 2, 3, 4, 5, 10, 15, and 20 ms). Negative intervals indicate that TMS was applied after the peripheral nerve stimulus. Twenty unconditioned H-reflexes were averaged to establish a control value and compared with 10 conditioned-test pairs. Stimuli were delivered in pseudorandom order every 5 s.

5.2.4 Treatment Protocol

All experiments were tested in age-matched controls and in people with PD. Severity of motor symptoms in PD was assessed using the Unified Parkinson's Disease Rating Scale (UPDRS) at the beginning of each treatment session. The UPDRS is made up of several parts but only the motor examination (part III) was included in this study. Each subscale has five possible clinical scores with 0 = normal, 1 = slight, 2 = mild, 3 = moderate, and 4 = severe. In the PD group, the UPDRS and aforementioned experiments were repeated 4 times under the following conditions: 1) OFF meds/OFF stim (without L-DOPA and off DBS), 2) ON meds/OFF stim (with L-DOPA and off DBS), 3) OFF meds/ON stim (without L-DOPA and on DBS), and 4) ON meds/ON stim (with L-DOPA and on DBS). Experiments took place over two contiguous days with morning and afternoon sessions. Patients were randomly placed in one of four different experimental schedules (Table 5.2). In the OFF conditions, L-DOPA was stopped and/or DBS was turned off at least 12 hours before the experiment. Patients were defined as clinically ON, >60 mins after L-DOPA was administered or DBS was turned on.

5.2.5 Analysis

As mentioned previously, subscales for the UPDRS motor examination had 5 possible clinical scores and each subscale had between 1 and 5 measures (depending on the number of areas

examined). For example, 'rigidity' had a total of 5 measures (20 points total): face, left arm, right arm, left leg and right leg. For a given subscale, the clinical scores for each measure were added and averaged across patients. The data were analyzed using Matlab (MathWorks, Natick, MA, USA). The amplitude of the H-reflex was measured peak-to-peak. Paired t-tests were used to compare between treatment groups and unpaired t-tests were used to compare between the control and patient groups.

For post-activation depression, H2 was expressed as a percentage of its unconditioned control response (H1) and full recovery was represented by 100%. The normalized H2 amplitude was compared between rest and during voluntary contraction. Using a mixed-design or 'split-plot' analysis of variance (ANOVA) we compared post-activation depression between the control and 4 treatment groups. Here we conducted a three-way mixed-design ANOVA treating muscle state (rest and voluntary contraction), treatment (4 levels), and interval (6 levels) as within-subject factors. This enabled a within-subjects analysis (muscle state and interval) to be completed simultaneously with a between-subjects analysis (control group vs patient groups). To compare between control subjects and patients OFF meds/OFF stim we used a one-way between-subjects ANOVA.

The degree of inhibition (or facilitation) for the other experiments was calculated by expressing the conditioned H-reflex as a percent of the unconditioned H-reflex amplitude. ANOVA testing and individual paired and unpaired t-tests assessed changes between the 5 testing conditions for the remaining experiments. To evaluate the degree of reciprocal inhibition of the averaged rectified EMG in soleus and TA, we measured the activity within a 10 ms window that included the peak inhibition (see Kido et al. 2004). Peak inhibition was expressed as a percent of the background EMG activity measured during contraction within a 40 ms pre-stimulus window. To calculate the maximum early phase of facilitation when using TMS to condition the H-reflex, the peak amplitude of a conditioned H-reflex (normalized to control) was selected within a pre-determined window (-5.0 to -3.0 ms) for each patient and averaged across the group. The same protocol was used to calculate the maximum 1st inhibition (-2.0 to -1.0 ms), 2nd inhibition (1.0 to

3.0 ms) and late facilitation (10.0 to 20.0 ms). Statistical analysis was performed using SPSS analytics software (SPSS Inc., Chicago IL, USA). Data are represented as means ± SE. Statistical significance was set at $P < 0.05$.

5.3 RESULTS

5.3.1 Clinical Evaluation

Table 5.2 depicts the severity of motor symptoms in the 4 patients with PD, quantified by the motor examination portion of the UPDRS. DBS and L-DOPA medication combined (i.e. ON meds/ON stim), reduced motor symptoms by 62.5% (paired t-test: $P < 0.05$). On average, L-DOPA medication alone (i.e. ON meds/OFF stim) reduced motor symptoms by 34.7% (paired t-test: $P > 0.05$) compared to an OFF meds/OFF stim state and motor symptoms were reduced by 45.8% of control (paired t-test: $P < 0.05$) with DBS alone (OFF meds/ON stim).

5.3.2 H-reflexes

In patients with PD OFF meds/OFF stim, the H_{max}/M_{max} ratio mean value (23.5 ± 7.9%) was not different from ON meds/ON stim (25.1 ± 8.5%), ON meds/OFF stim (22.4 ± 10.5%) or OFF meds/ON stim (24.6 ± 6.1%; $P > 0.05$). Further to this, the value of H_{max}/M_{max} ratio for the control group (25.3 ± 4.6%) was not different from the patient group ($P > 0.05$).

5.3.1 Post-activation depression

The recovery of an H-reflex from post-activation depression was studied at rest and during contraction in people with PD and compared to controls. Figure 5.1 shows the recovery from post-activation depression in a representative control subject (Fig. 5.1A) and in PD OFF meds/OFF stim (Fig. 5.1B) at rest. The overall recovery of H2 for the control group (Fig. 5.2A & B; dotted line) was visually less than the PD group (Fig 5.2A&B; solid lines). A three-way mixed-design ANOVA revealed that the difference was not significant between muscle state [$F(1,3) = 1.761$, $P = 0.276$] but the difference between intervals [$F(5,15) = 34.670$, $P < 0.001$] was significant.

Figures 5.2C & D show the data collected for the 5 different testing conditions for a representative interval where pairs of TN stimuli were delivered 200 ms apart at rest (Fig. 5.2C) and during contraction (Fig. 5.2D). In controls, the H2 was reduced to 23.7 ± 4.8% and 65.8 ± 11.1% of control at rest and during contraction, respectively. When OFF meds/OFF stim; however, the H2 was only reduced to 81.6 ± 20.8 and 96.8 ± 9.0% of control at rest and during contraction, respectively. At rest, the degree of post-activation depression was significantly different between control and patients OFF meds/OFF stim [$F(1,12) = 12.466$, $P = 0.004$].

5.3.2 Ib inhibition

The inhibition of a soleus H-reflex when conditioned by a single stimulus to the MG nerve in a representative control subject and in PD OFF meds/OFF stim is illustrated in Figures 5.3A and B, respectively. The average Ib inhibition for all five testing conditions is represented in Figure 5.3C. Given that the inhibition produced at intervals 4, 5 and 6 was not significantly different between the 5 testing conditions [$F(2,6) = 0.799$, $P = 0.492$], the three values were pooled together. In controls, the degree of Ib inhibition induced in control subjects (92.9 ± 2.6%) was significantly greater than the inhibition induced in patients OFF meds/OFF stim (99.2 ± 2.1%; unpaired t-test $P = 0.008$). The difference between controls and the 4 treatment groups was significant [$F(1,11) = 1438.255$, $P < 0.001$]. An unpaired t-test revealed that the control group was not only different from OFF meds/OFF stim but also from ON meds/ON stim ($P < 0.05$). OFF meds/OFF stim was significantly different from the ON meds/ON stim condition alone (paired t-test: $P < 0.05$) and ON meds/ON stim was different from all conditions (paired t-test: all $P < 0.05$).

5.3.3 Presynaptic inhibition

Figure 5.4 shows the average presynaptic inhibition produced on a soleus H-reflex for the control group and four treatment conditions at rest. In the control group, a conditioning train from the CPN reduced the soleus H-reflex to 81.4 ± 10.4% of control. Interestingly, in the different treatment conditions, presynaptic inhibition appeared to increase and the H-reflex decreased to

77.0 ± 9.4%, 63.1 ± 15.5%, 73.2 ± 15.6%, and 60.0 ± 22.8% of control in the OFF meds/OFF stim, ON meds/ON stim, ON meds/OFF stim, and OFF meds/ON stim treatment groups, respectively. Though a slight increase in presynaptic inhibition (and the consequent decrease in H-reflex amplitude) was apparent in people with PD in all treatment groups, the difference was not statistically significant [$F(1,3) = 1.535$, $P = 0.271$].

5.3.4 Reciprocal Ia inhibition at rest

Figure 5.5 depicts reciprocal Ia inhibition of the soleus H-reflex at rest when a single conditioning pulse to the CPN was applied 2 to 4 ms earlier in a group of control subjects and people with PD. The data collected at the 2, 3 and 4 ms intervals were not statistically different [$F(2,6) = 0.184$, $P = 0.836$] and so were averaged together to increase statistical strength. In controls, a soleus H-reflex was reduced to 93.4 ± 1.4% of control. Reciprocal Ia inhibition appeared to be slightly less in the four treatment conditions: OFF meds/OFF stim (95.0 ± 4.3%), ON meds/ON stim (96.6 ± 4.8%), ON meds/OFF stim (99.1 ± 2.4%), and OFF meds/ON stim (93.3 ± 3.1%). However, the difference between controls and the 4 treatment conditions was not significant [$F(1,11) = 1.284$, $P > 0.05$].

5.3.5 Reciprocal Ia inhibition during contraction

We investigated the influence of reciprocal Ia inhibition from a conditioning stimulus onto the voluntary activity of its antagonist. Figure 5.6A illustrates the results in a representative control subject and Figure 5.6C represents the average reciprocal Ia inhibition produced in the 5 testing conditions. A conditioning pulse from the antagonist CPN reduced the averaged rectified soleus EMG activity produced during plantarflexion to 70.0 ± 4.7% of the pre-stimulus background EMG in controls. This value was relatively unchanged in patients with PD OFF meds/OFF stim (72.4 ± 5.6%), ON meds/ON stim (68.8 ± 7.3%), and ON meds/OFF stim (76.9 ± 10.2%). The only unique measure recorded during plantarflexion was observed in patients OFF meds/ON stim (53.6 ± 8.5%). The difference between controls and the 4 treatment groups was significant [$F(1,3) = 125.082$, $P = 0.002$]. When the reverse interaction was studied (Figure 5.6B &D), on-going TA

EMG activity was reduced to 64.5 ± 6.3% of background following a conditioning TN stimulus in control subjects. While reciprocal inhibition during voluntary dorsiflexion appeared to be reduced in PD for all treatment groups (OFF meds/OFF stim (86.9 ± 6.2%), ON meds/ON stim (83.4 ± 11.7%), ON meds/OFF stim (80.4 ± 6.3%), and OFF meds/ON stim (70.2 ± 8.3%)), the difference was not significant [$F(1,3) = 192.793$, $P > 0.05$].

5.3.6 TMS on the H-reflex

The trend of facilitation and inhibition of a soleus H-reflex by subthreshold TMS (95% of RMT), was similar between our pilot study (see appendix A) and our aged-matched control group (Figure 5.7A). The early phase of excitation peaked at the -4.0 ms interval (i.e. TMS pulse applied after TN stimulus) and resulted in an increase in the H-reflex to 112.2 ± 5.6% of control. The first (-2.0 ms) and second phases (2.0 ms) of inhibition reduced the H-reflex to 78.3 ± 5.0% and 87.5 ± 6.2% of control, respectively. A later, long-latency facilitation immediately followed the second inhibitory phase and continued to increase to 176.7 ± 14.5% of control at the 15.0 ms interval. At this point the degree of excitation appeared to plateau as the H-reflex at the 15.0 ms interval was facilitated to a similar degree at the 20.0 ms interval (175.0 ± 19.7%).

The interaction was also studied in patients with PD (Figure 5.7B-E). In OFF meds/OFF stim, the early phase of excitation was shown to remain strong at 122.1 ± 19.7% of control. Interestingly, the early excitatory phase was not as large in ON meds/ON stim (96.6 ± 9.4%), ON meds/OFF stim (101.1 ± 15.1%), or OFF meds/ON stim (112.1 ± 12.4%). While the first phase of inhibition was completely attenuated in OFF meds/OFF stim to 102.7 ± 24.6% of control, it was recovered in ON meds/ON stim (78.6 ± 10.7%), ON meds/OFF stim (87.6 ± 6.0%), and OFF meds/ON stim (87.0 ± 7.6%). The second inhibitory phase was not different in OFF meds/OFF stim (88.5 ± 9.1%) compared to controls though it was less apparent in ON meds/ON stim (96.7 ± 14.0%) and OFF meds/ON stim (102.5.1 ± 17.7%). In the ON meds/OFF stim the inhibition appeared to reverse to facilitation (133.2 ± 15.0%). While the later, long-latency facilitation was still apparent in the OFF meds/OFF stim treatment condition, it only reached 137.3 ± 12.0% of control at the 15.0 ms interval. In fact, in no treatment condition did the late phase of excitation return to within 30%

of control. The greatest late recovery occurred in the ON meds/ON stim treatment condition, where the H-reflex was facilitated to 141.2 ± 28.6% at the 5.0 ms interval. Figure 5.8 illustrates the maximum (early and late) inhibition (Fig 5.8A) and (1^{st} and 2^{nd}) facilitation (Fig 5.8B) produced by controls and the 4 treatment groups. The early phase of excitation was always between the -5.0 ms and -3.0 ms interval and peaked at 134.0 ± 15.5% of control in PD OFF meds/OFF stim. The first phase of inhibition (-2.0 ms to -1.0 ms) reduced the H-reflex to a maximum of 62.8 ± 7.4% of control in PD ON meds/ON stim while the second phase of inhibition (1.0 ms to 3.0 ms) reduced the H-reflex to a maximum of 78.6 ± 5.9% of control in control subjects. The later, long-latency facilitation was greatest in control subjects and reached 173.4 ± 20.5% of control values.

5.4 DISCUSSION

The present series of pilot experiments describe the electrophysiological assessment of various pathways within the spinal cord of people with PD. Spinal cord circuitry was explored in people with PD in the following four treatment conditions: 1) OFF meds/OFF stim (without L-DOPA and off DBS), 2) ON meds/OFF stim (with L-DOPA and off DBS), 3) OFF meds/ON stim (without L-DOPA and on DBS), and 4) ON meds/ON stim (with L-DOPA and on DBS). The four treatment conditions were also compared to a group of age-matched control subjects.

5.4.1 H_{max}/M_{max} ratio

For a majority of the tests performed in the present study, a modulation of the H-reflex amplitude following a conditioning stimulus was used to assess the integrity of various peripheral and descending pathways in PD. There is some controversy in the literature as to how the H_{max}/M_{max} ratio is affected in PD. Studies have shown the ratio to increase (Delwaide 1984), decrease (Raoul et al. 2012) or remain unchanged (Angel and Hoffmann 1963; Dietrichson and Sorbye 1971, Kushnir et al. 2001). The present results demonstrated no difference in the H_{max}/M_{max} ratio between control subjects versus people with PD and between the 4 different treatment groups. Deviations from control values observed for the different experiments can therefore be attributed to influences outside of the monosynaptic reflex arc.

5.4.2 Post-activation depression

A previous activation of a reflex pathway will result in the attenuation of a subsequent H-reflex induced to the same pathway for up to 10 s at rest (Crone and Nielsen 1989; Pierrot-Deseilligny and Burke 2012; Andrews et al. 2015). A breakdown of this mechanism, following spinal cord injury (Schindler-Ivens and Shield 2000) and stroke (Lamy et al. 2009), was shown to lead to the development of spasticity. Similarly, reduced post-activation depression in PD is correlated with increased rigidity (Raoul et al. 2012).

Post-activation depression recovered faster in those with PD OFF meds/OFF stim as compared to controls. Consistent with work by other groups (Olsen and Diamantopulos 1967; Sabbahi et al. 2002; Raoul et al. 2012), the present results suggest that post-activation depression may be reduced in those with PD. McLeod and Walsh (1972) and Sax et al. (1976) showed that the reduced period of inhibition following a preceding activation of the reflex pathway was restored to control after L-DOPA treatment. The present results involving our first 4 patients, agree with the literature given that post-activation depression tended to increase toward control levels in the ON meds/ON stim condition.

A possible explanation for the reduced post-activation depression observed at rest in patients with PD may involve the involuntary background activity produced from tremor given that in controls, post-activation depression is reduced during voluntary contraction (Rothwell et al. 1986; Hultborn and Nieslen 1998). However, this explanation is unlikely since the post-activation depression was also reduced in patients asked to perform a voluntary contraction. Another possible explanation for the altered post-activation depression in PD is the disuse of the Ia-alpha motor neuron synapse, an explanation used to describe the development of spasticity following spinal cord injury that involves a reduction in post-activation depression that occurs over time (Schindler-Ivens and Shield 2000). This explanation is also unlikely given that the decreased post-activation depression in OFF meds/OFF stim was reversed once DBS was turned on. Rather than being attributed to changes within the monosynaptic reflex arc, the abnormal reduction in post-

activation depression may originate from altered input from descending pathways. DBS probably influences descending input to spinal connections involved in motor control (Chen and Lemon 2004) and in-turn restores facilitation of inhibitory circuits (Potter-Nerger et al. 2008).

5.4.3 Ib inhibition

In our control group, stimulation of the MG nerve induced an inhibition of the soleus H-reflex at 4-6 ms. These results are in agreement to earlier reports using a similar protocol (Pierrot-Deseilligny et al. 1981). This pathway was chosen on the basis that the connections from the MG nerve to soleus motor neurons are primarily (if not entirely) group Ib (Pierrot-Deseilligny et al. 1981). Ib inhibition provides a linear relationship between muscle length and tension (Nichols and Houk 1976). A breakdown of this mechanism is thought to lead to increased muscle stiffness. At present, the results reveal a loss of Ib inhibition in PD OFF meds/OFF stim. These findings are similar to earlier studies that also showed diminished Ib inhibition in PD (Delwaide et al. 1991). The reduced Ib inhibition is likely related to enhanced input from the reticulospinal tract (Delwaide et al. 1991; Potter et al. 2004). The increased activity of the STN in PD could lead to enhanced activity of the pedunculopontine nucleus which would in turn increase reticulospinal tract activity. Given that the reticulospinal tract has inhibitory connections with Ib interneurons, an increase in activity from this descending pathway would cause a decrease in the facilitation of Ib interneurons (Delwaide et al. 1991). This is supported by evidence that when DBS was turned on, Ib inhibition was restored to control levels (Potter et al. 2004) likely through a reduction in the abnormal activity of basal ganglia nuclei in PD with DBS (Limousin et al. 1997).

5.4.4 Presynaptic inhibition

At this time, we have not observed a difference between presynaptic inhibition in PD compared to age-matched controls. These results may change with the recruitment of more patients since other authors have described a decrease in presynaptic inhibition from the TA to soleus Ia terminals in patients with PD (Roberts et al. 1994; Pierrot-Deseilligny and Burke 2012).

5.4.5 Reciprocal Ia inhibition at rest

Short-latency reciprocal Ia inhibition was examined in people with PD and compared to controls. Reciprocal inhibition is vital for coordinated contraction. Through the activation of inhibitory interneurons, the contraction of a muscle will induce the relaxation of its antagonist. Reciprocal Ia inhibition in humans has been well studied between ankle flexors and extensors in both uninjured individuals (Mizuno et al. 1971; Tanaka et al. 1974; Crone et al. 1987) and in people with PD (Delwaide et al. 1993). In PD, reciprocal inhibition from the TA to the soleus muscle was found to be significantly increased (Delwaide et al. 1993). In fact, the disruption of this spinal inhibitory mechanism was found to be correlated with increased rigidity by the same group. In contrast to these results we did not observe a significant difference between control and any of our PD treatment groups. If anything, reciprocal Ia inhibition seemed to be reduced in PD. These results may become clearer with the recruitment of additional patients with PD.

5.4.6 Reciprocal Ia inhibition during contraction

Given that the assessment of H-reflex amplitude produced during contraction is more relevant to understanding the role of reflexes in movement, relative to rest (Stein and Thompson 2006), we also investigated the influence of reciprocal inhibition onto voluntary activity of its antagonist. A conditioning pulse to the CPN reduced the averaged soleus EMG activity produced during plantarflexion similarly between control and PD groups, aside from the ON stim alone treatment group. In OFF meds/ON stim, the inhibition of soleus EMG activity was dramatically stronger. These results were unexpected as one would predict a similar increase in inhibition in patients ON meds/ON stim. Further investigation is necessary to understand these changes. Reciprocal Ia inhibition during dorsiflexion was unique from the results obtained during plantarflexion. Compared to control subjects, reciprocal Ia inhibition of the TA during dorsiflexion appeared to be reduced in PD for all 4 treatment conditions, though the difference was not significant at this point. A study by Hayashi et al. (1988) investigated reciprocal inhibition of the soleus H-reflex following voluntary dorsiflexion in people with PD. The reciprocal inhibition observed in controls was replaced with a facilitation of soleus motor neurons and attributed to dysfunction in

descending control over reciprocal inhibitory interneurons. While this protocol does not directly correspond to the present one, the results may be explained by a similar mechanism.

5.4.7 TMS on the H-reflex

We studied the effect of a conditioning pulse from TMS over the motor cortex on the excitability of soleus motor neurons by measuring changes in H-reflex amplitude in healthy controls and patients with PD. Previous work (see appendix A) revealed that a conditioning pulse from TMS (95% of RMT) was optimal for evoking an early phase of excitation, followed by two distinct inhibitory phases and a later, long-latency phase of excitation.

Studies in humans have described the existence of monosynaptic connections between the CST and motor neurons (Kuypers 1981). Given the fast conduction speed of the CST and the single synaptic delay at the motor neuron, a volley transmitted along the CST triggered simultaneously with a TN stimulus would likely arrive at the motor neuron pool first (Nielsen et al. 1993). Therefore, the early facilitation that occurred at the -4.0 ms interval is likely to be monosynaptic in origin. Other reports have demonstrated a reduction in early facilitation in PD during plantarflexion that was later restored following pallidotomy (Morita et al. 2002). These results may suggest abnormal corticospinal drive to leg motor neurons. A similar study in people with PD, during contraction, also showed a marked reduction of early facilitation that returned to control levels with STN-DBS (Potter-Nerger et al. 2008). For the present study, abnormal corticospinal facilitation of the leg motor neuron pool was only apparent in patients receiving some form of treatment. In patients OFF meds/OFF stim; however, the early monosynaptic facilitation appeared unchanged. These findings were unexpected and may be attributed to the low number of patients recruited.

The first phase of inhibition induced by the conditioning TMS pulse onto the H-reflex is likely due to the activation of Ia inhibitory interneurons that mediate reciprocal inhibition between the TA and soleus (Nielsen et al. 1993). While this phase of inhibition falls within the time window of intervals studied by others (Potter-Nerger et al. 2008), it was not described in their control group

or patients with PD. The present results showed a complete loss of the early inhibition in patients OFF meds/OFF stim. This may be attributed to the same mechanism that altered the early facilitatory phase only here, the abnormally reduced descending input was no longer producing an inhibitory influence via Ia inhibitory interneurons. When parkinsonian treatment was present in any format, the excitatory input to inhibitory interneurons was restored and the early inhibition recovered.

The origin of the second inhibitory phase is less well understood. Nielsen and Peterson (1995) described a similar inhibition of the soleus H-reflex present in several subjects that may result from the summation of multiple inhibitory post-synaptic potentials from the CST at the motor neuron. Evidence that this phase of inhibition was not affected OFF meds/OFF stim but was reduced in the remaining 3 treatment conditions was puzzling. Given the large standard error and small subject pool, additional subjects are necessary to make conclusions.

The later, long-latency facilitation can be distinguished from the early monosynaptic facilitation based on threshold, timing, and amplitude (Nielsen and Peterson 1995). The pathway responsible for this phase of facilitation likely involves indirect polysynaptic connections from the reticulospinal tract (Nielsen and Peterson 1995). Studies that have looked at Ib inhibition in PD have attributed the altered transmission along this pathway in patients OFF meds/OFF stim to altered excitatory activity from the reticulospinal tract (Potter et al. 2004). Present results suggest a similar change in facilitation from the reticulospinal tract. This is supported by evidence that the later, long-latency facilitation was diminished to a greater degree than the early monosynaptic excitation when considering patients with PD for all treatment conditions. In addition, studies using spinal reflexes and TMS provide evidence that CST excitability is not altered by DBS during the medication OFF state (Angel & Hoffmann, 1963; Cunic et al. 2002; Dauper et al. 2002; Delwaide 2000; Delwaide et al. 2001), also suggesting that observed changes are due to extrapyramidal pathways such at the reticoluspinal tract.

5.4.8 Conclusions

This study was designed to measure the functional connectivity within the spinal cord of people with PD. We show that transmission of signals within spinal circuits may be affected. The results presented here, in conjunction with research by other groups, demonstrate that while parkinsonian treatment reduces motor symptom severity, DBS and L-DOPA may also restore signal transmission within the spinal cord. Since changes to different pathways correlate with the severity of motor symptoms, restoring these pathways to normal, using any tool, may further reduce symptom severity. In general, while circuits within the spinal cord may be affected in patients not receiving treatment for PD and that these different treatments likely play a role in normalizing these pathways, the data are still limited and a greater number of patients are required in order to make more definite conclusions.

Table 5.1

Control subject and patient statistics.

Control Subjects			
	No.	Sex	Age (yr)
	1	M	50
	2	F	62
	3	M	59
	4	M	53
	5	M	54
	6	M	58
	7	M	73
	8	M	70
	9	F	74
	10	F	50
Average ± SE	-	-	60 ± 3

Patients						
	No.	Sex	Age (yr)	Duration (yr)	Implant Site	Years Implanted
	1	M	67	20	Bilateral STN	7
	2	M	69	20	Left STN	3
	3	F	68	15	Bilateral Gpi	1.5
	4	M	54	21	Bilateral Gpi	2
Average ± SE	-	-	65 ± 3	19 ± 1	-	3 ± 1

Table 5.2

Patients were randomly assigned to one of 4 groups (A-D). OFF medication (meds) or deep brain stimulation (stim) is indicated by a 0, while ON either treatment is indicated by a 1.

	Group A		Group B		Group C		Group D	
Day 1	Meds	Stim	Meds	Stim	Meds	Stim	Meds	Stim
Session 1	0	0	0	1	0	0	1	0
Session 2	1	0	1	1	0	1	1	1
Day 2	Meds	Stim	Meds	Stim	Meds	Stim	Meds	Stim
Session 1	0	1	0	0	1	0	0	0
Session 2	1	1	1	0	1	1	0	1

Table 5.3

UPDRS motor examination clinical scores for each patient in the 4 treatment groups. Individual values represent the average clinical score (averaged between the 4 patients) for the 15 different subscales. Numbers in parentheses represent the highest possible clinical score for each subscale when values for the different measures were added together.

UPDRS: Motor Examination	**meds**	**OFF**	**ON**	**OFF**	**ON**
	stim	**OFF**	**OFF**	**ON**	**ON**
Total (out of 108)		36	23.5	19.5	13.5
Tremor at rest (20)		3.25	1.5	2	0
Rigidity (20)		4.75	3.25	3	1.75
Body bradykinesia and hypokinesia (4)		36	23.5	19.5	13.5
Postural Stability (4)		0.5	0	0	0
Speech (4)		1.5	1	1	1
Facial expression (4)		1	1	0.75	0.75
Action tremor of hands (8)		2.25	1.5	1.25	0.5
Finger Taps (8)		4.75	3.75	3.25	2.75
Hand movement (8)		3.25	4	2	2.75
Rapid alternating hand movment (8)		3.75	3.25	1.75	1.75
Leg agility (8)		4.25	2.5	2	1
Arising from chair (4)		0.75	0	0	0
Posture (4)		1	1	1.25	0.75
Gait (4)		0.75	0.25	0.5	0.25
Body bradykinesia and hypokinesia (4)		1.25	0.75	0.75	0.25

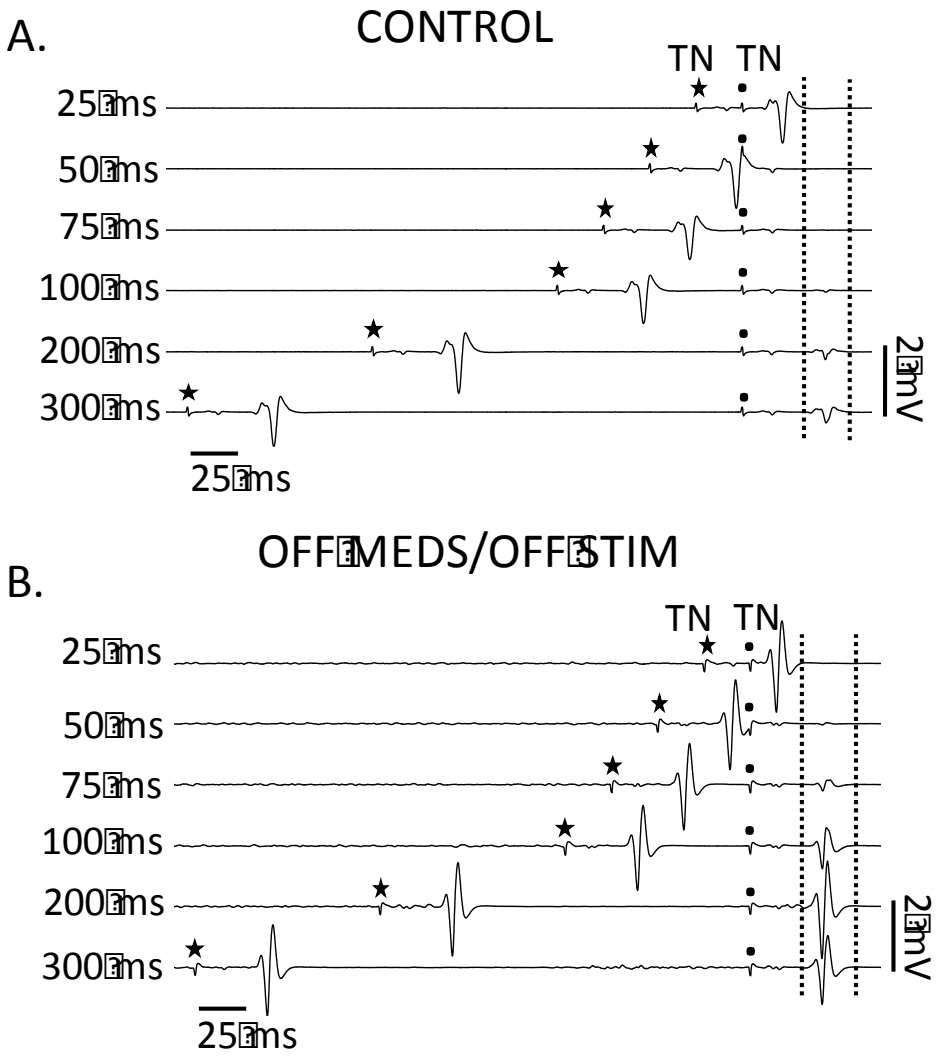

Figure 5.1: Single subject traces showing the recovery of an H-reflex from post-activation depression in a control subject (A) and in PD OFF meds/OFF stim (B) at rest. The six lines in each graph represent the different interpulse intervals ranging from 25 ms (*top trace*) to 300 ms (*lowest trace*). Each trace depicts the averaged response produced by ten stimuli. Time of the conditioning and test TN stimuli are indicated by stars and bullets, respectively. Depressed H-reflex responses are shown within the vertical dotted lines.

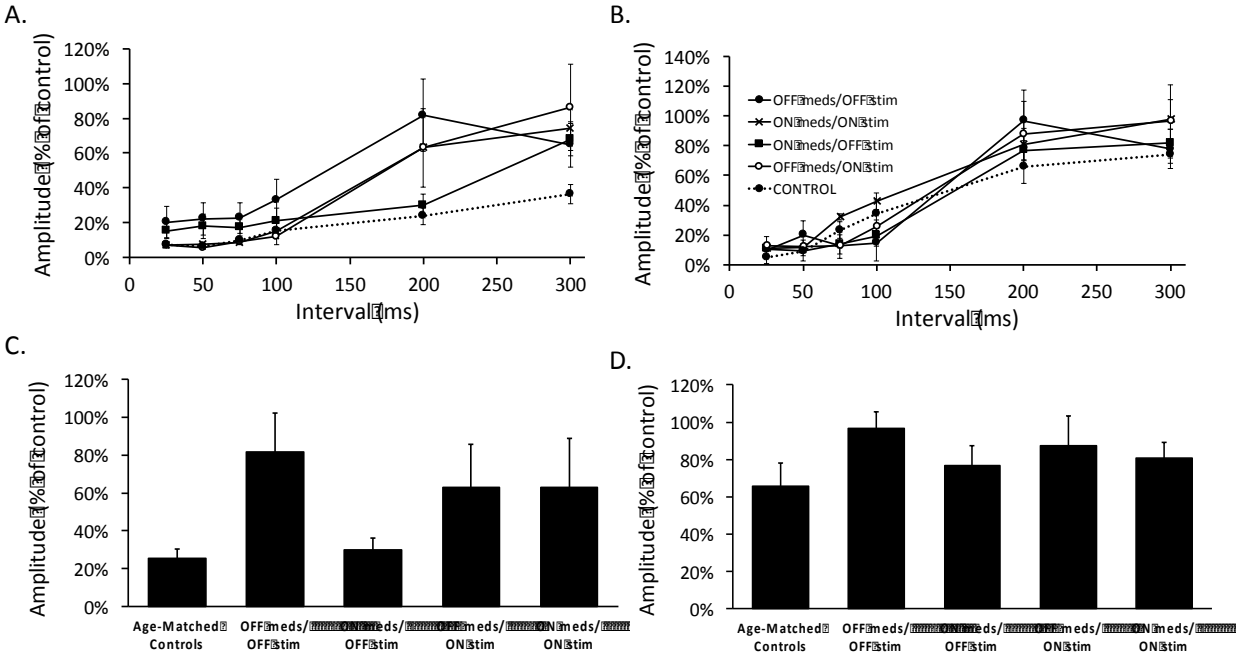

Figure 5.2: H-reflex recovery curves at rest (A) and during contraction (B) representing the amplitude of H2 expressed as a percent of control (H1) for the four different treatment groups (solid lines) and age-matched controls (dotted line). Error bars represent standard error. Bar graphs represent the post-activation depression at rest (C) and during contraction (D) produced at the 200 ms interpulse interval. For this figure and the ones that follow, each column represents the mean and standard error for the control group and in PD for the 4 different treatment conditions.

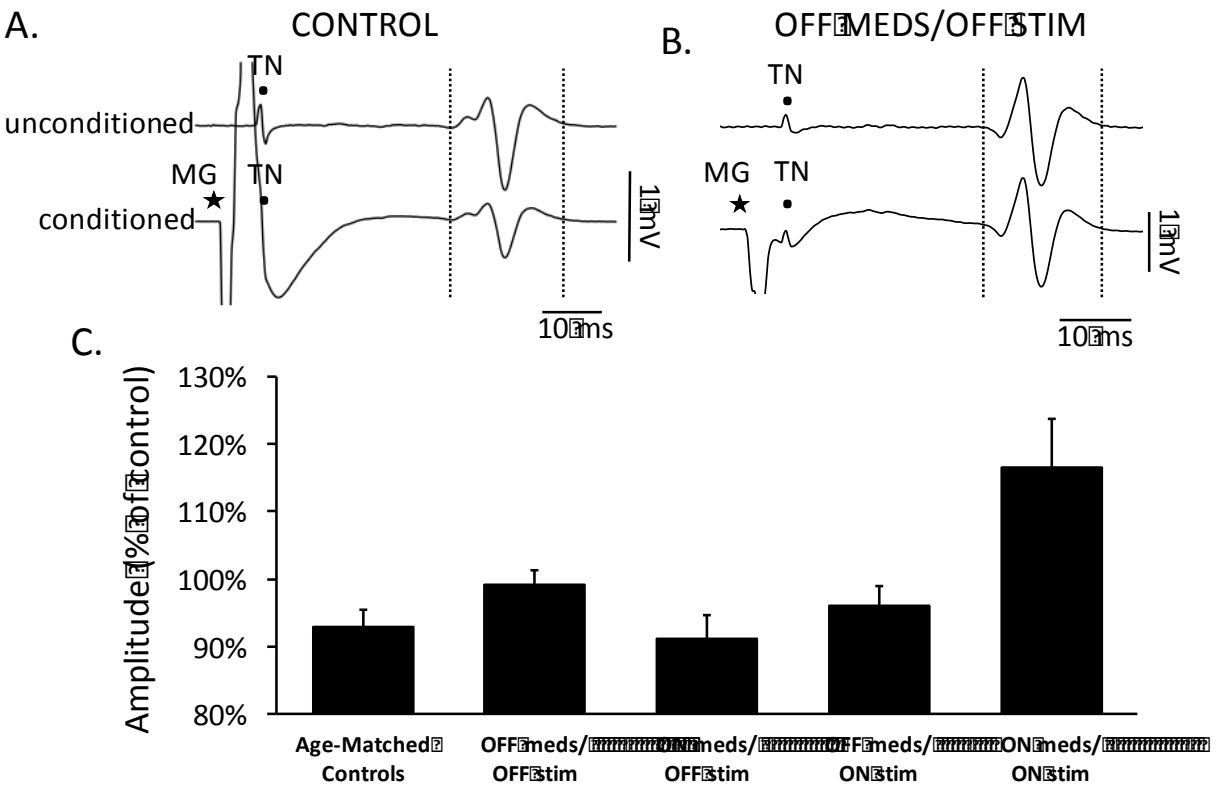

Figure 5.3: Single subject traces illustrating the Ib inhibition of a soleus H-reflex in a control subject (A) and in PD OFF meds/OFF stim (B) for the 5 ms condition-test interval. Unconditioned H-reflexes (*top*) and conditioned H-reflexes (*bottom*) are represented. Conditioning and test stimuli are indicated by stars and bullets, respectively. *C*: Bar graph represents the mean inhibition of the H-reflex produced at condition-test intervals 4 to 6 for controls and in PD for the 4 treatment groups.

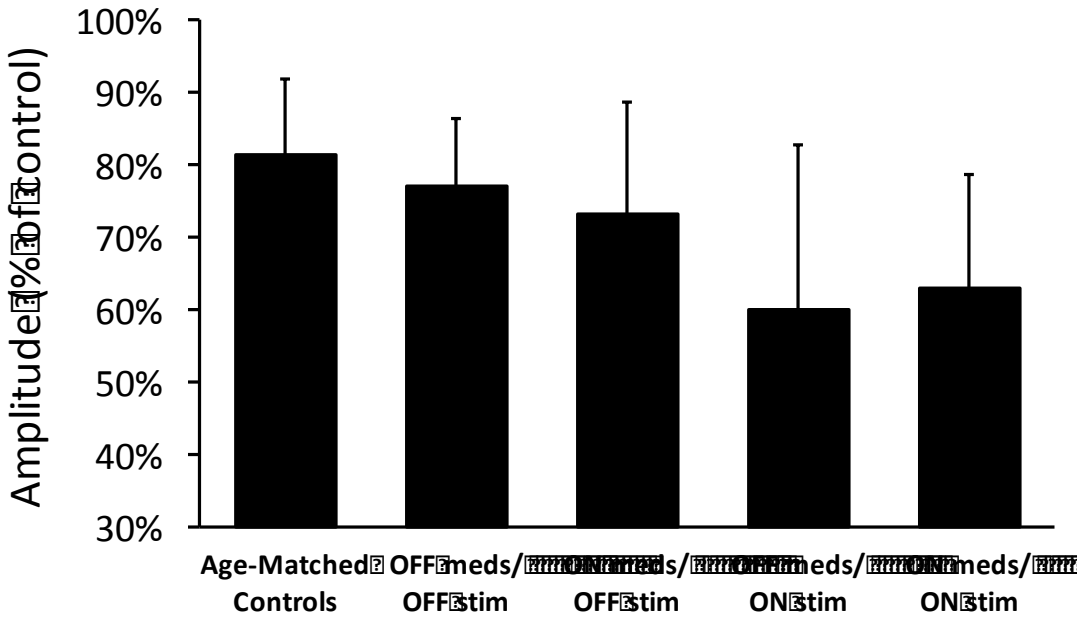

Figure 5.4: Presynaptic inhibition induced on the TN following a conditioning train to the CPN in controls and the 4 patient treatment groups.

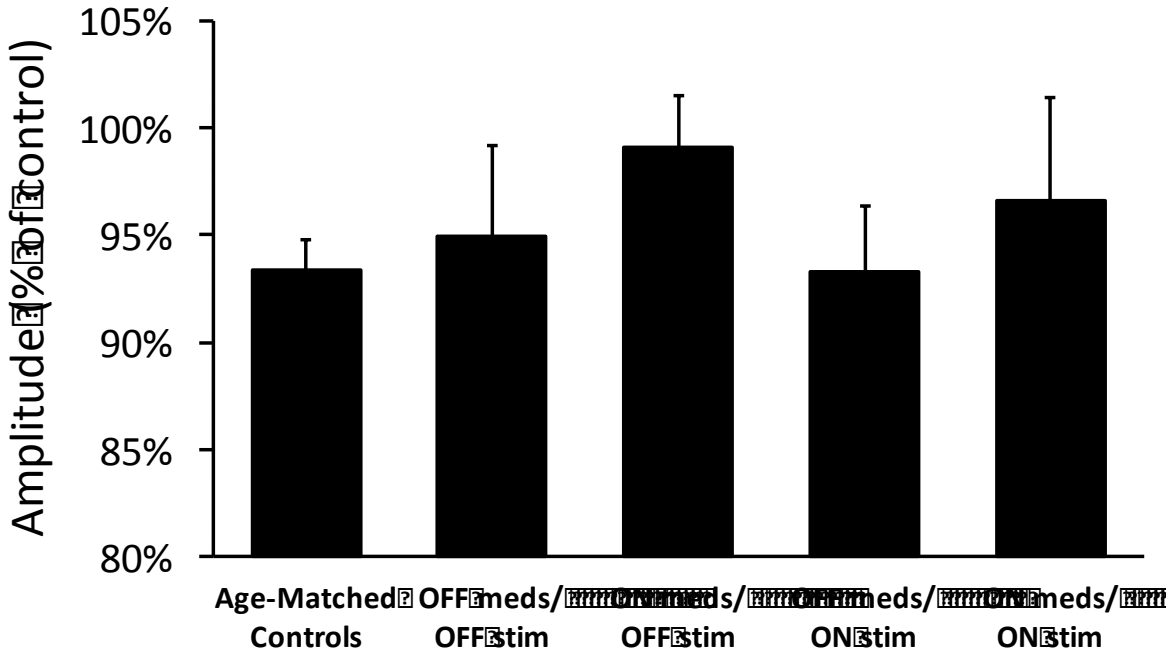

Figure 5.5: Reciprocal Ia inhibition produced when a pulse to the CPN was used to condition the soleus H-reflex. Each bar represents the average inhibition produced at condition-test intervals from 2 to 4 ms in the control group and the 4 patient treatment groups.

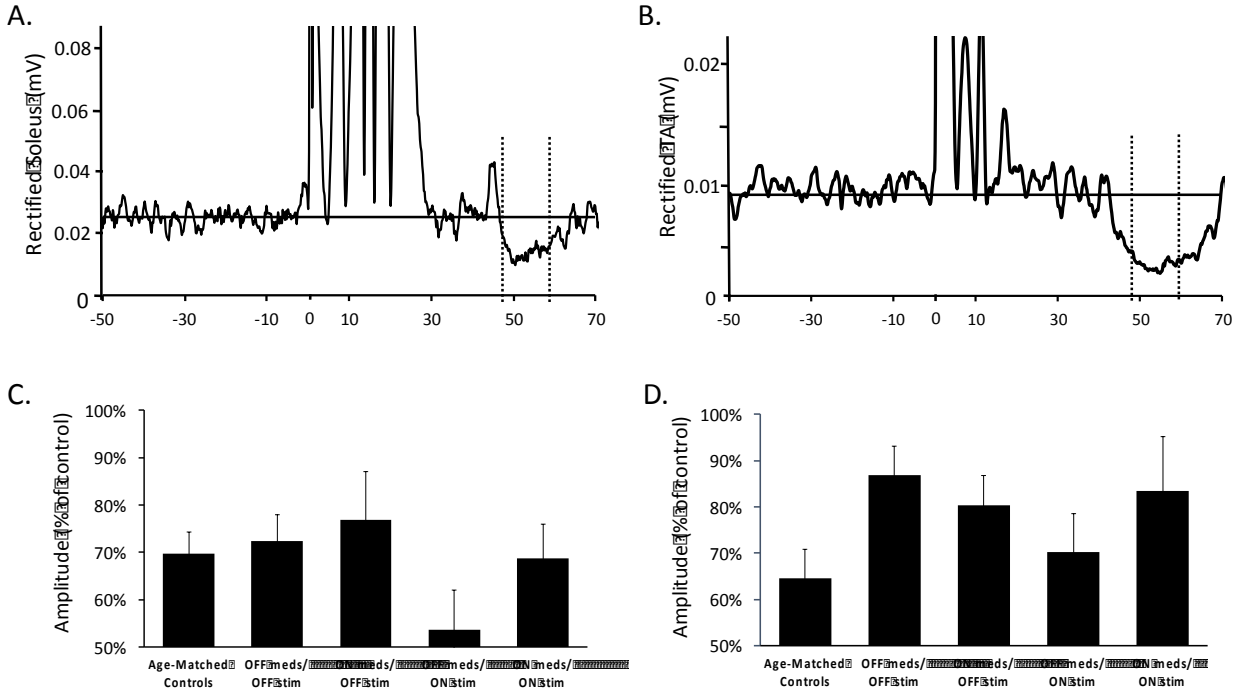

Figure 5.6: Representative control data showing reciprocal Ia inhibition of soleus (A) and TA (B) voluntary EMG activity following a condition stimulus to the CPN and TN, respectively. EMG activity measured for a 10 ms window that included the peak inhibition in the rectified EMG is represented within the vertical dotted lines. Solid horizontal lines indicate background EMG levels. Time 0 refers to the onset of nerve stimulation. Bar graphs represent the reciprocal Ia inhibition measured during plantarflexion (C) and dorsiflexion (D) for the five testing conditions. Each bar illustrates the peak inhibition expressed as a ratio of the prestimulus activity for the 5 testing conditions.

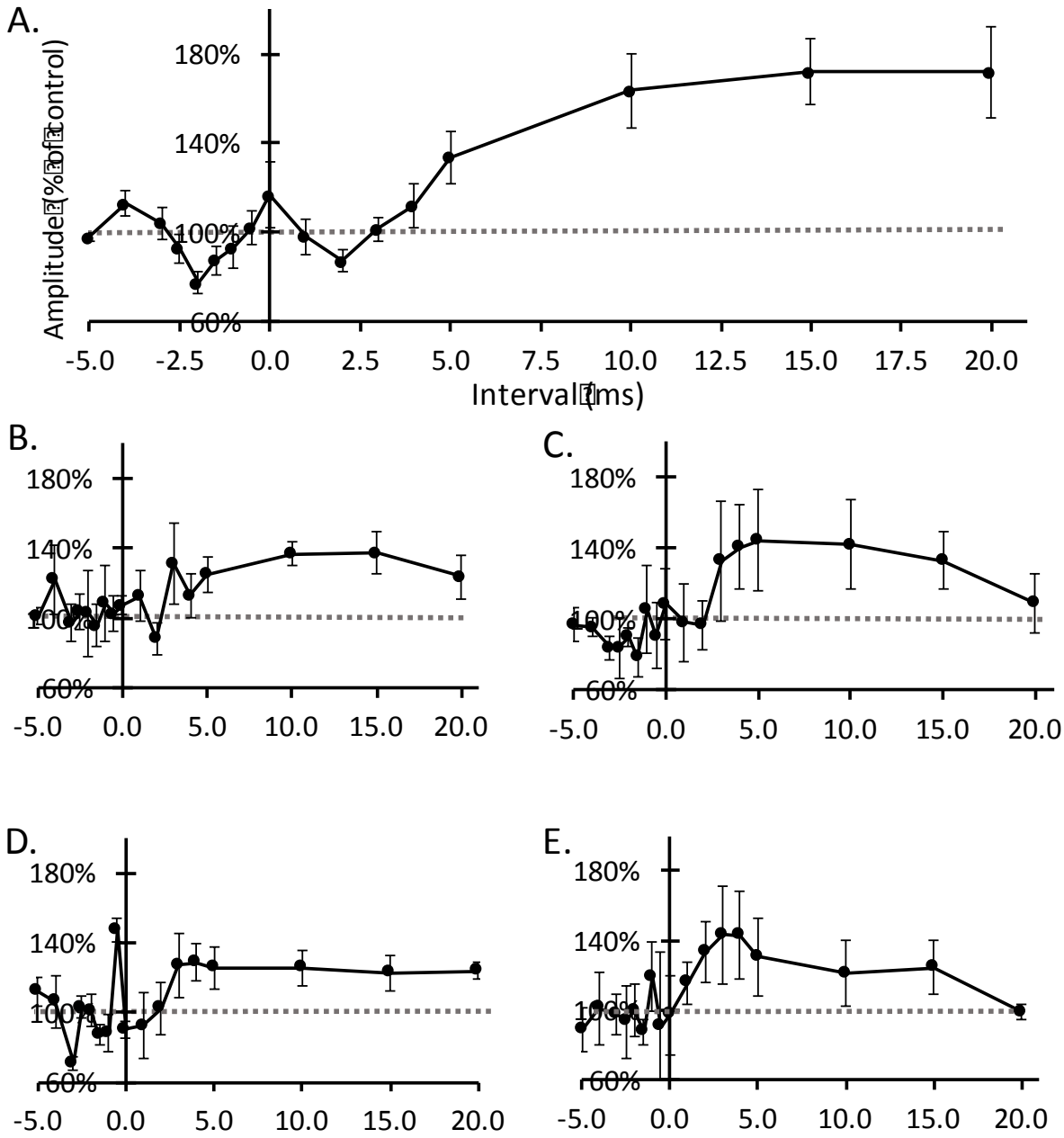

Figure 5.7: Time course of the effect of TMS on the H-reflex in (A) control subjects and patients with PD: (B) OFF meds/OFF stim, (C) ON meds/ON stim, (D) ON meds/OFF stim, and (E) OFF meds/ON stim. Horizontal grey dotted lines depict the amplitude of the control H-reflex prior to conditioning (i.e. no effect of the conditioning stimulus). Vertical and horizontal axis labels in Figs 5.8B-E have been removed to improve clarity but are the same as shown in Fig. 5.8A.

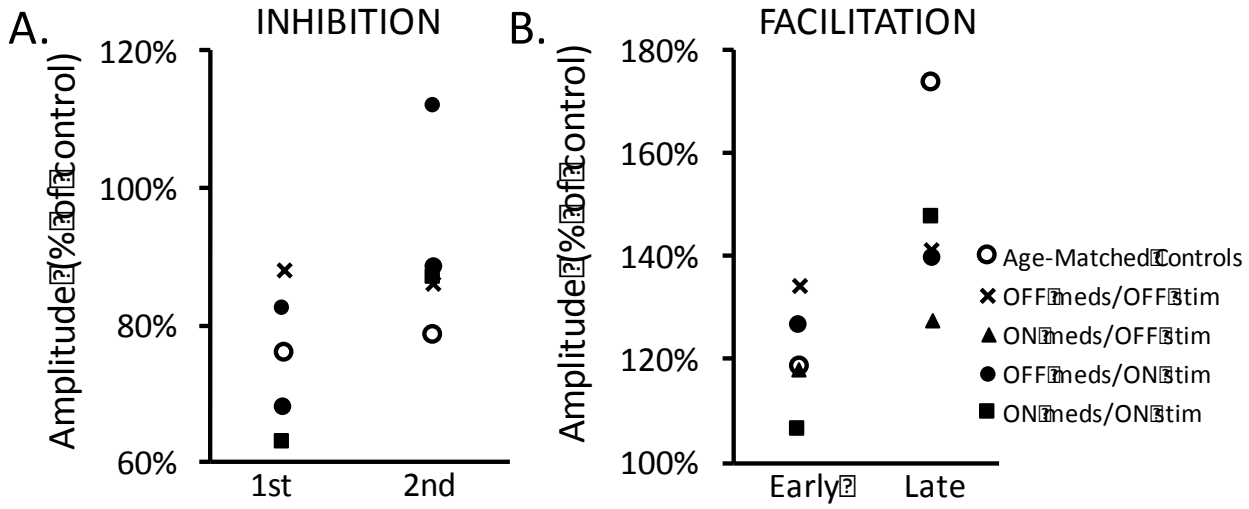

Figure 5.8: Maximum (1st and 2nd) inhibition (A) and (early and late) facilitation (B) produced by the 5 treatment conditions are represented as a percent of control. The 5 testing groups are depicted by 5 different symbols.

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CHAPTER 6: GENERAL DISCUSSION

6.1 THESIS SUMMARY

Reflexes are not only vital for the co-ordination of muscle movement but are also used to provide important information regarding the function of both the peripheral and central nervous systems. In this thesis, we examined circuits within the human spinal cord by means of the Hoffmann (H)-reflex. Studying how the H-reflex is influenced by adjacent pathways both in healthy and pathological states further provides vital information regarding functional connectivity. This chapter begins with a brief overview of our results. We then discuss how each study contributes to previous work on relevant topics, the limitations of each study, and potential future directions for the research.

6.1.1 Changes in spinal excitability to peripherally and trans-spinally evoked reflex responses *(Chapter 2)*

In *Chapter 2* of this thesis, we compared post-activation depression of the well-studied Hoffmann (H)-reflex to the more recently introduced root evoked potential (REP), elicited though transcutaneous spinal stimulation in healthy controls. Recovery of the soleus H-reflex and REP was studied in able-bodied controls following a conditioning REP or H-reflex. Two pulses were delivered 25-200 ms apart and the recovery was characterized using three levels of stimulation both at rest and during contraction. The results revealed that a second response, whether an H-reflex or REP, was depressed following a conditioning REP or H-reflex. Our findings suggest that transcutaneous spinal stimulation will recruit a population of afferents that overlaps with the afferents activated at the popliteal fossa by peripheral nerve stimulation.

6.1.2 Augmenting spinal cord excitability with descending input *(Chapter 3)*

In *Chapter 3,* we systematically characterized the effect of transcranial magnetic stimulation (TMS) on post-activation depression of the H-reflex and REP both at rest and during contraction. This work builds on previous research by Roy and colleagues (2014) where TMS was reported to

reduce post-activation depression produced by paired-pulse REPs. Here we compared the recovery of an H-reflex with an REP from post-activation depression with TMS and systematically characterized the role of TMS intensity on the interaction. A single TMS pulse was able to dramatically reduce the period of depression that follows the activation of a sensory-motor synapse to 10-15 ms for both the H-reflex and REP. Post-activation depression could even be reversed to post-activation facilitation (rather than inhibition) at intervals of 10 to 100 ms. Theories describing the mechanism of post-activation depression in the context of neurotransmitter depletion may be less plausible. Evidence that TMS-induced corticospinal excitation can rapidly influence the recovery of the reflex-arc and essentially 'turn-off' post-activation depression, suggests that an alternate mechanism such as presynaptic inhibition, may be a stronger contributor.

6.1.3 A new technique for monitoring the spinal cord during spinal deformity correction surgery *(Chapter 4)*

Here we investigated a new approach of intraoperative neurophysiological monitoring (IONM) that was used to confirm the preservation of motor pathways within the spinal cord. Using the technique developed in *Chapter 3* for removing post-activation depression with corticospinal excitation, we were able to monitor the integrity of the spinal cord with stimuli that were subthreshold for patient movement. The interaction was apparent in all 20 patients and remained stable across time. Relative to traditional monitoring techniques that utilize multi-pulse transcranial electrical stimulation (TES) to generate a positive motor response, this technique involves monitoring the removal of inhibition. It may also be less disruptive to on-going surgery as it does not cause patient movement within the significant field. This study also supports the theory that a recovered second response *(Chapter 3)* was not just the MEP, given that the interaction was present using a subthreshold TES stimulus.

6.1.4 Pilot study on Parkinson's disease and changes to spinal cord circuitry *(Chapter 5)*

In *Chapter 5* we described the electrophysiological assessment of a number of different circuits within the spinal cord of people with Parkinson's disease (PD). We studied changes to the soleus H-reflex, post-activation depression (at rest and during contraction), Ib inhibition, presynaptic inhibition, reciprocal Ia inhibition (at rest and during contraction) and corticospinal input to motor neurons. Motor symptoms were evaluated using the Unified Parkinson Disease Rating Scale (UPDRS). Experiments were conducted in patients off treatment for parkinsonian symptoms (i.e. meds OFF/stim OFF). Given that deep brain stimulation (DBS) and levodopa (L-DOPA) are believed to have different mechanisms of action, experiments were also conducted in patients receiving each treatment (ON meds/OFF stim; OFF meds/ON stim) and as well when both treatments were combined (ON meds/ON stim). Motor symptoms were the most severe OFF meds/OFF stim. In addition, several experiments revealed changes to spinal cord circuitry in PD off both treatment types. Motor symptoms and spinal cord circuitry appeared to normalize with treatment and ON meds/ON stim made the greatest improvement. However, these results were not yet apparent in all experiments and more patients are needed in order to make definite conclusions.

6.2 CHANGES IN SPINAL EXCITABILITY TO PERIPHERALLY AND TRANS-SPINALLY EVOKED REFLEX RESPONSES

Studies in spinalized animals have identified circuits within the spinal cord that are vital for the control of movement (Sherrington 1910). These studies have improved our understanding of the role of reflexes in generating movement. Similar circuits have been identified in humans with a complete spinal cord injury (Calancie et al. 1994). Regulating the transmission of signals within these circuits, following changes to descending input, may aid in the development of therapies for the restoration of function. In order to do this, we must improve our understanding of how these circuits function in uninjured individuals. In *Chapters 2 & 3* we use a relatively new technique known as transcutaneous spinal stimulation to probe spinal circuits. Transcutaneous spinal stimulation is used to assess connectivity within the human spinal cord non-invasively (Courtine et al. 2007; Minassian et al. 2007, Minassian et al. 2009). We utilized a commonly

studied spinal inhibitory mechanism, known as post-activation depression, to compare between the H-reflex and the transcutaneously elicited root evoked potential (REP).

There is controversy in the literature relating to the origin of the REP. Many authors agree that over the lumbar cord, transcutaneous spinal cord stimulation will preferentially activate afferents from within dorsal roots (Courtine et al. 2007; Minassian et al. 2007; Roy et al. 2012) due to their proximity relative to the stimulating electrodes and the lower threshold of posterior roots as they bend upon entry into the spinal cord at the root entry zone (Minassian et al. 2011). Others argue that a pure motor response is evoked from the stimulation (Knikou 2013a; Knikou 2013b). The results from *Chapter 2* support findings that the REP, when evoked following transcutaneous stimulation over the lumbar spine, is mainly reflexive in origin given that paired-pulse stimulation will not induce post-activation depression and diminish the second response if produced through direct activation of motor fibers.

Results from *Chapter 2* revealed differences and similarities between the REP and H-reflex, some of which have not been reported in the literature. The two reflex responses differed in the maximal amplitude that each stimulus type could evoke. The maximal REP produced during voluntary contraction was larger than the maximal H-reflex likely because transcutaneous spinal stimulation activates dorsal roots selectively (Courtine et al. 2007; Minassian et al. 2007; Roy et al. 2012) and in-turn produces less occlusion that occurs when motor axons are activated directly. Similar to the H-reflex (Burke et al. 1989; Hultborn and Nielsen, 1998), post-activation depression of the REP is reduced during voluntary contraction. In fact, the REP recovered to only 30% at rest compared to during contraction, which was remarkably similar to the 31% recovery observed for the H-reflex.

Not only could a conditioning REP induce post-activation depression on another REP (i.e. REP → REP), but it could also depress an H-reflex (i.e. REP → H) and visa versa when using the H-reflex as the conditioning stimulus (i.e. H → REP and H → H). The time-course of the depression and subsequent recovery was consistent with that of post-activation depression and so suggests that

an overlapping population of afferents were activated by the two distinct forms of stimulation. The multi-segmental, bilateral spinal elements activated by transcutaneous spinal stimulation did not inhibit the H-reflex more strongly than would be observed by paired-pulse H-reflexes. The only unique condition was the paired-pulse REPs. A second REP induced in a pair was depressed to a greater extent than the other three stimulus combinations. This was likely due to the recruitment of additional spinal elements from heteronymous pathways by a conditioning REP that have an increased inhibitory influence on a subsequent REP. The additional inhibition that is apparent for the REP may be relevant to studies that intend to regulate signal transmission within these circuits for improving the motor activity produced during locomotion (Minassian et al. 2012; Hofstoetter et al. 2013).

6.2.1 Technical limitations

The H-reflex will bypass the muscle spindle and so does not occur in the body naturally. Bypassing the muscle spindle (Schieppati et al. 1987), may limit applicability of the H-reflex and REP to human movement. Muscle spindles are important for making on-going adjustments to reflex output during movement (Hicks et al. 1989). With that being said, inducing a stretch reflex via the tendon tap has its challenges especially when force and rate of stimulation must be controlled. Bypassing the muscle spindle has the advantage of attributing changes in H-reflex amplitude to changes in reflex activity within the spinal cord (Palmieri et al. 2004).

6.2.2 Future directions

The sensory-motor synapse involved in the production of the H-reflex is subject to modification by adjacent pathways such as presynaptic inhibition (Rudomin 1990; Rudomin and Schmidt 1999), reciprocal inhibition (Crone et al. 1986) and Ib inhibition (Pierrot-Deseilligny et al. 1981) among others. While we anticipate the H-reflex and REP are modulated in a similar fashion from other spinal pathways, there may still be noteworthy differences given that the REP is a multi-segmental reflex response. In *Chapter 2,* the REP doublet demonstrated a significantly higher degree of depression suggesting the trans-spinally evoked response had a greater affinity for

inhibition. We predict that the spinal stimulus will recruit more inhibitory heteronymous projections. Future work with transcutaneous spinal stimulation could be to apply various condition-test paradigms to test the impact of adjacent pathways and potentially identify the source of the additional inhibition. A full understanding of the pathways involved in modulation of the REP may help improve its use for the restoration of movement after neurological injury.

6.3 AUGMENTING SPINAL CORD EXCITABILITY WITH DESCENDING INPUT

The technique developed in *Chapter 3* demonstrated that TMS can virtually eliminate post-activation depression produced by paired-pulse H-reflexes and REPs delivered at least 10-15 ms apart. In other words, input from the motor cortex to the corticospinal tract (CST) could dramatically reduce the period of decreased excitability that occurs following the activation of a sensory-motor synapse from 10 s (Crone and Nielsen 1989; Pierrot-Deseilligny and Burke 2012) to several milliseconds. The interaction was present both at rest and during contraction. TMS could even recover a second and third H-reflex when triple-pulse peripheral nerve stimulation was used.

As mentioned previously, regulating the activity produced by spinal circuits involved in motor control (when descending input is abnormal or lost) may improve therapies for restoring motor function. Given that TMS was able to recover multiple reflex responses within a short period, one could predict this technique will aid in increasing trans-synaptically evoked motor output where post-activation depression is typically the rate-limiting factor. This may be beneficial for the development of motor therapies following changes to descending input, particularly for interventions involving repetitive spinal stimulation (Minassian et al. 2004; Moshonkina et al. 2012; Hofstoeter et al. 2014; Angeli et al. 2014).

The mechanism of post-activation depression is still a topic of debate. Many authors suggest that the long lasting inhibition is related to an increase in presynaptic inhibition (Schieppata and Crenna, 1987; Crone and Nielsen, 1989; Binder et al. 2009), but others believe that the inhibition is caused by a depletion of neurotransmitters at the sensory-motor synapse (Katz et al. 1997;

Encyclopedia of Neuroscience, 2009). While the present results could not ascertain the true mechanism responsible for the reduction in post-activation depression, the corticospinal excitation likely disinhibited some of the Ia afferent terminals through reduced presynaptic inhibition which in-turn increases Ia excitation to the motor neuron. The rapid recovery observed here may be too short to allow neurotransmitters to be replenished, a process that typically requires a few hundreds of milliseconds to occur. Figure 6.1 depicts a proposed pathway by which post-activation depression and its subsequent removal by TMS, may be transmitted. An obvious pitfall to this proposed mechanism relates to the duration of post-activation depression (up to 10 s at rest; Crone and Nielsen 1989; Pierrot-Deseilligny and Burke 2012) compared to presynaptic inhibition (<400 ms; Pierrot-Deseilligny and Burke 2012). Increased presynaptic inhibition may be responsible for the first ~400 ms of depression and the duration of post-activation depression may be explained by an alternate mechanism.

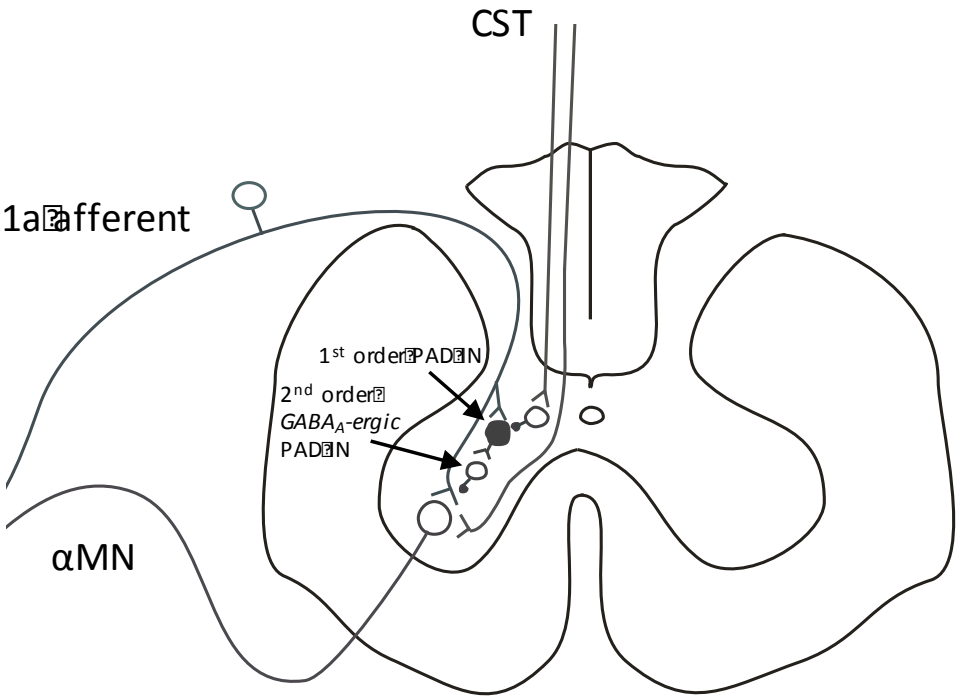

Figure 6.1: Illustration of the proposed pathways involved in producing and removing the early part of post-activation depression. First-order primary afferent depolarizing (PAD) interneurons (INs) receive excitatory input from Ia afferents after eliciting a first H-reflex. This input is then relayed to the last-order PAD IN which will, in-turn, reduce the amplitude of an incoming action

potential produced by a second H-reflex for up to 400 ms. Descending input from the CST will activate a single IN which will essentially 'inactivate' the PAD IN, thus allowing the second H-reflex to transmit across the synapse uninhibited.

6.3.1 Technical limitations

The limitations that applied to *Chapter 2* also apply to this study. But again, an electrically-evoked reflex response allows us to make conclusions about connections within the spinal cord without confounding results from changes to spindle sensitivity. Another limitation of *Chapter 3* involved the use of a suprathreshold motor evoked potential (MEP) from TMS to condition post-activation depression. At this point, the question still remained whether the recovered second response was in-fact an H-reflex and not the MEP itself. This was unlikely for several reasons: 1) motor neurons are inhibited after an H-reflex (Poon et al. 2008), likely due to afterhyperpolarization (Matthews 1996) and recurrent inhibition (Windhorst 1996) and would therefore suppress the MEP at intervals <100 ms, 2) both the control H-reflex and recovered H-reflex had similar morphologies, and 3) the use of a subthreshold MEP in *Chapter 4*, strongly indicated that the recovered response was truly not an MEP.

6.3.2 Future directions

Descending input from TMS was shown to remove post-activation depression. At this point, we are uncertain which pathway(s) are mediating this interaction. Future work will likely involve characterizing this interaction. We theorize that descending input modulates post-activation depression through connections between the CST and presynaptic inhibitory interneurons. To test this, we could first induce presynaptic inhibition of the soleus H-reflex from a heteronymous pathway. The condition-test paradigm used in *Chapter 5* could be applied. Here, a train of stimuli to the common peroneal nerve (CPN), was applied ~15 ms prior to a tibial nerve stimulus (used to evoke the soleus H-reflex). The resultant inhibition of the H-reflex has been attributed to presynaptic inhibition of the soleus Ia terminals (Faist et al. 1996; Capaday et al. 1995). A pre-conditioning pulse from TMS could then be delivered ~14 ms before the test stimulus (tibial nerve

stimulus). If TMS can in-fact remove presynaptic inhibition one would expect the amplitude of the conditioned H-reflex to resemble that of an unconditioned H-reflex. Work by another group demonstrated that presynaptic inhibition of TN afferent terminals decreases following activation of the CST (Iles 1996), but this author used TMS to pre-condition the common peroneal nerve stimulus which was in-turn delivered 100 ms before the soleus H-reflex. To make conclusions about the results from *Chapter 3* we will modify this technique so that TMS conditions the tibial nerve (TN) stimulus.

6.4 A NEW TECHNIQUE FOR MONITORING THE SPINAL CORD DURING SPINAL DEFORMITY CORRECTION SURGERY

Reflex testing is used to assess functional connectivity within the spinal cord (Magladery et al. 1952; Paillard 1955). When combined with a conditioning MEP as in *Chapter 3*, the interaction could be used to simultaneously assess function of both descending and segmental pathways within the spinal cord. In *Chapter 4*, TES was shown to remove post-activation depression of a second reflex delivered in a pair and used to monitor the CST during surgery. The interaction was present in all 20 patients recruited for this study. In one patient, a complete loss of MEPs (the gold standard for CST monitoring), occurred concurrently with the loss of the interaction. The results indicated that the presence of the interaction was linked to the preservation of descending pathways within the spinal cord.

The strength of the interaction developed in *Chapter 3* was demonstrated by the ability for subthreshold TES to remove post-activation depression under general anesthesia (a state of decreased excitability). TES, below the threshold necessary to evoke a motor response, could permit complete recovery of spinal excitability at intervals > 75 ms (see Figure 4.1). Theories that suggest a depletion of neurotransmitter as the underlying mechanism of post-activation depression are further disputed by these results.

The interaction did not diminish with time even though evoked response amplitude has been shown to decline throughout surgery. This is known as ‘fade’ and has been attributed to a gradual

decline in the excitability of the lower motor neuron (MacDonald 2006; MacDonald et al. 2013). While the control response (i.e. first H-reflex delivered in a pair) gradually diminished with time, the interaction itself was unaffected and so the ratio of the second H-reflex relative to control appeared to increase. These results suggest that the effects of anesthetic fade may be less apparent in the production of post-activation depression or in its subsequent removal with descending input.

Traditionally, MEPs are used to monitor pathways during surgery (Kothbauer et al. 1998; MacDonald et al. 2013). However, due to the suppressive effects of general anesthesia, multi-pulse TES is required to bring alpha motor neurons (αMNs) to threshold. Increasing the stimulus intensity to reach threshold under general anesthesia causes strong muscle contractions within the patient. Bite injuries to the lips or tongue are the most common complication associated with multi-pulse TES (MacDonald 2002; MacDonald and Deletis, 2008). Disruption to the on-going surgery by movement within the surgical field is also of great concern. Therefore, traditional MEP monitoring may be limited to intermittent use in order to minimize interference. Given that ischemia can cause damage to neural tissue within several minutes if undetected, there is obvious need for a monitoring technique that can not only provide information about CST function but also be applied continuously throughout surgery. The present study aimed to produce the greatest influence on post-activation depression using TES while limiting MEP generation and the associated patient movement. In fact, low intensity TES (40-60% less than usual) was capable of complete recovery of a depressed H-reflex without producing visible movement of a patient's shoulders or trunk. This provides a new opportunity for continuous spinal cord monitoring without interfering with on-going surgery.

6.4.1 Limitations and future directions

All patients recruited for this study were of adolescent age (range: 10-17) undergoing corrective surgery for spinal deformity. Generalization to an adult population of uninjured individuals may be difficult as spinal excitability changes with age (Kido et al. 2004). As well, functional connectivity may be altered in the clinical group. On the other hand, *Chapter 3* and earlier work

by Roy and colleagues (2014) provided evidence that a suprathreshold TMS pulse can remove post-activation depression in adults. Under general anesthesia, indirect (I)-waves and lower motor neurons are strongly inhibited (MacDonald et al. 2013). Given that a cortical stimulus, subthreshold for producing an MEP, could sustain the interaction despite the suppressive effects of general anesthesia, one could assume the interaction would carry over to a group of adults who are not undergoing surgery.

In the present study, a loss of the interaction was detected in a single patient. Future work could involve a continuation of this study in a larger subject pool to establish the clinical criteria necessary to suggest a meaningful change in H-reflex facilitation. For example, warning criteria for MEP monitoring involves a reduction in amplitude, increase in threshold, and a simplification of signal morphology (MacDonald et al. 2013). As well, a larger subject pool with a greater number of true positive events correlating in time with a loss of the interaction will allow definitive conclusions to be made about functional connectivity when using the present, conditioning technique.

A benefit of this technique as discussed in *Chapter 4* is the ability to produce an interaction with little to no MEP. We proposed that this technique may be particularly beneficial in instances where the MEP may be lost or absent such as in pre-existing neurological conditions (Langeloo et al. 2001) and the immature central nervous system of young children (Andersson and Ohlin 1999; Erb et al. 2005). To confirm this statement, we must first recruit patients with no monitorable MEP and test for the presence of the interaction.

Another potential follow-up study could involve characterizing the recovery of triple-pulse H-reflexes in the operating room. The monitoring technique developed in *Chapter 4* involved comparing a conditioned H-reflex to an unconditioned H-reflex. Differences between conditioned and unconditioned reflex amplitude were used to provide information about CST function. This required two distinct combinations of electrical stimulation to be delivered separately. On-going assessment of the interaction throughout surgery required the monitorist to continually switch

between a conditioned and unconditioned pair of H-reflexes. If TES was used to condition only the third H-reflex delivered in a set of three, one could compare the amplitude of the third H-reflex (conditioned) to the second (unconditioned) to make conclusions about CST integrity. This would require the delivery of only one combination of stimuli (i.e. triple pulse peripheral nerve stimulation with TES timed to condition only the third H-reflex) and so would eliminate the need to switch back and forth between a conditioned and unconditioned H-reflex stimulus paradigm.

6.5 PILOT STUDY ON PARKINSON'S DISEASE AND CHANGES TO SPINAL CORD CIRCUITRY

Parkinson's disease (PD) is a neurodegenerative disorder affecting motor control. Given that the motor symptoms of PD are a major source of discomfort associated with the disease and various motor symptoms have been shown to correlate with changes to spinal cord circuitry, the primary motivation behind this study was to identify pathways within the spinal cord that are altered in people with PD. Secondly, we aimed to characterize the effect of DBS and L-DOPA on these changes. Since DBS and L-DOPA are believed to work differently in improving PD motor symptoms, we hypothesized that they will also differ in how they influence spinal cord circuitry.

Studies by other groups in people with PD have demonstrated changes to various spinal pathways. In fact, a breakdown of post-activation depression (Raoul et al. 2012), Ib inhibition and reciprocal Ia inhibition (Delwaide et al. 1993) has been shown to correlate with the severity of motor symptoms. While it is possible that changes may be occurring at the segmental level, perhaps even at the synapse, this explanation is unlikely because changes to post-activation depression (for example) that were present in the OFF meds/OFF stim state, were immediately reverse once DBS was turned on. Also preliminary results from *Chapter 5* demonstrated no difference in the ratio of the maximum H-reflex amplitude over the maximal M-mave amplitude between people with PD and controls. Instead, the altered circuitry may be the result of disrupted signals from descending pathways that receive input either directly or indirectly from basal ganglia nuclei. DBS likely alters the descending input to circuits that are involved in motor control (Chen and Lemon 2004), resulting in the restoration of inhibitory circuits (Potter-Nerger et al.

2008). The reticulospinal tract is a possible source of this disrupted input. The benefits of DBS in PD are thought to relate to altering the activity of the hyperactive subthalamic nucleus (STN; Limousin et al. 1997). The increased activity of the STN off DBS, could lead to increased activity within the reticulospinal tract (Delwaide et al. 1991; Potter et al. 2008). Input from the reticulospinal tract has an inhibitory influence on Ib interneurons and an excitatory influence on Ia interneurons (Delwaide et al. 1991; Pierrot-Deseilligny and Burke 2012). Changes to reticulospinal tract excitability may; therefore, explain the decrease in Ib inhibition (Delwaide et al. 1991) and increase in reciprocal Ia inhibition (Delwaide et al. 1993) that is observed in people with PD. Presynaptic inhibition also receives inhibitory input from the reticulospinal tract (Pierrot-Deseilligny and Burke 2012) and so this may also explain the decrease in post-activation depression that is observed OFF meds/OFF stim given that we believe post-activation depression to be mediated, at least in part, by increases in presynaptic inhibition.

6.5.1 Limitations

In general, the lack of significance and consistent findings between patients in *Chapter 5* may be due to the heterogeneity of our patient group. Given the lack of patient availability we were unable to control for the following criteria: symptoms experienced by each individual, years since disease onset, lifestyle (i.e. whether active or sedentary), and whether each patient has an inherited or idiopathic form of the disease. As well, for this study we recruited patients with an implanted DBS with no preference for implant site (STN or globus pallidus internus (GPi)) or whether the patient was receiving uni- or bilateral stimulation. While there is no appreciable difference between the stimulation site with regards to the primary outcome (reviewed in Williams et al. 2014), there may be differences in how various motor symptoms such as rigidity are managed (Okun et al. 2009). With only four patients recruited so far, we cannot make conclusions regarding stimulation site but with more patients, future work could involve comparing the results between the two groups. Achieving significance for a diverse group of patients, such as is typically the case of patients with Parkinson's disease, will obviously require a larger subject pool.

6.5.2 Future direction

The obvious next step for this study is to continue with patient recruitment. While the results from several of the experiments conducted in *Chapter 5* are beginning to show predictable trends, the data are still limited and a larger subject pool is necessary to reach statistically significant conclusions. Even though medication and DBS dramatically reduced the severity of motor symptoms, the improvements were not complete and several motor symptoms persisted in our patient group ON meds/ON stim. Changes to various spinal pathways, such as post-activation depression, are shown to correlate with the severity of motor symptoms (Raoul et al. 2012). Given that ON meds/ON stim did not induce complete normalization of spinal excitability one could predict that further restoring these pathways to control levels may have a greater impact on reducing rigidity. Studies in spastic patients have used cycling (Kiser et al. 2005), treadmill walking (Trimble et al. 1998) and gait assisted training (Trompetto et al. 2013) to normalize post-activation depression. Applying these similar techniques in PD could help further reduce the severity of motor symptoms. Future work could involve testing the effect of training and treatment on both motor symptom severity and spinal cord circuitry.

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APPENDIX A: EFFECT OF TRANSCRANIAL MAGNETIC STIMULATION ON SPINAL CIRCUITRY

A.1 INTRODUCTION

Descending pathways in the corticospinal tract (CST) can be engaged through non-invasive transcranial magnetic stimulation (TMS) over the motor cortex. TMS is commonly used to study motor control in humans and when combined with H-reflex testing, the value of TMS in this context is greatly increased (Petersen et al. 2003). A pulse of TMS results in a short latency MEP response which is partly mediated by cortico-motoneuronal connections (Brouwer and Ashby 1990; Rothwell et al. 1991). This excitation will also cause an increase in the amplitude of the H-reflex as shown in healthy controls (Nielsen et al. 1993; Nielsen and Petersen, 1995a; Nielsen and Petersen 1995b) and people with Parkinson's disease (Morita et al. 2002; Potter-Nerger et al. 2008). Approximately 1-5 ms following the early excitation, a low intensity stimulus from TMS will evoke a transient phase of inhibition of the soleus H-reflex (Nielsen et al. 1993; Nielsen and Petersen 1995b) likely through the excitation of inhibitory interneurons projecting to soleus alpha motor neurons (Nielsen et al. 1993). A second phase of inhibition was observed in several subjects by Nielsen and Peterson (1995). This inhibition proceeded the first by a few milliseconds and is thought to result from the summation of multiple inhibitory post-synaptic potentials from the CST at the motor neuron. A later, longer latency facilitation of the H-reflex was also observed by the same group using subthreshold TMS. This facilitation is distinct from the early monosynaptic facilitation and likely mediated through indirect connections from the reticulospinal tract.

In this pilot experiment, we used TMS to condition the soleus H-reflex. The goal of this experiment was to characterize how a weak TMS stimulus modulates the excitability of spinal reflex pathways in soleus at rest. This condition-test study design was conducted in a small group of controls to determine the optimal stimulus parameters required to evoke a strong interaction between the CST and soleus spinal motor neurons. The interaction was later studied a group of

patients with Parkinson's disease (*Chapter 5*) to assess how parkinsonian treatment (i.e. deep brain stimulation (DBS) and Levodopa (L-DOPA)) affects motor cortical control over spinal pathways.

A.2 METHODS

A.2.1 Participants

Five healthy volunteers were recruited to participate in this study. Volunteers were screened for potential contraindications to the stimulation including a history of seizures. Participants provided written consent to the experimental protocol that was approved by the Health Research Ethics Board at the University of Alberta.

A.2.2 Recording and Stimulation

Please refer to *Chapter 5 (section 5.2.2)* for a complete description of the recording and stimulation techniques used here.

A.2.3 Stimulation Protocol

Recruitment curves were collected for the H-reflex and M-wave by gradually increasing the stimulus intensity from threshold until reaching the maximal H-reflex amplitude and further to a level that elicited the maximal M-wave. H-reflex recovery was studied using a half maximal reflex amplitude. The optimal location for producing a soleus MEP was determined and threshold identified. A single TMS pulse was used to condition the soleus H-reflex at condition-test intervals ranging from -5 ms to 20 ms (i.e. -5, -4, -3, -2.5, -2, -1.5, -1.0, -0.5, 0, 1, 2, 3, 4, 5, 10, 15, and 20 ms). Negative intervals indicate that TMS was applied after the peripheral nerve stimulus. This sequence of condition-test intervals was repeated four times using four different TMS intensities that were adjusted to 65%, 80%, 95% and 110% of resting motor threshold (RMT). For several subjects, up to seven TMS intensities were applied but only the four aforementioned intensities will be discussed here. Twenty unconditioned H-reflexes were averaged to establish a control

value. Each condition-test interval was tested 10 times and the amplitude of the conditioned H-reflex averaged. Stimuli were delivered in pseudorandom order every 5 s.

A.2.4 Analysis

The data were analyzed with MATLAB (MathWorks, Natick, MA). The amplitude of the H reflex was measured peak to peak. A conditioned H-reflex was expressed as a percentage of its unconditioned control response, and full recovery was represented by 100%. Statistical analysis was performed with SPSS analytics software (SPSS, Chicago, IL). To calculate the maximum early phase of facilitation, the peak amplitude of a conditioned H-reflex was selected within a pre-determined window (-5.0 to -3.0 ms) for each patient and averaged across the group. The same protocol was used to calculate the maximum 1st inhibition (-2.0 to -1.0 ms) 2nd inhibition (1.0 to 3.0 ms) and late facilitation (10.0 to 20.0 ms). Data are presented as means ± standard error (SE). Statistical significance was set at $P < 0.05$.

A.3 RESULTS

The effect of a conditioning pulse from TMS on the soleus H-reflex was investigated in a small group of controls to establish the optimal parameters necessary to evoke the interaction. Optimal parameters were later applied to a group of people with Parkinson's disease and age-matched controls. Preliminary results in a single subject (Figure A.1) revealed a short facilitation followed by two distinct phases of inhibition truncated by a later, long-latency facilitation of the H-reflex at rest. In this case, a TMS intensity 95% of RMT appeared optimal for evoking the early (109.0± 4.1% at -4.0 ms) and late (122.7 ± 7.2% at 15.0 ms) phases of facilitation without overwhelming either the first (57.2 ± 6.3% at -2.0 ms) or second phase (68.1 ± 8.3% at 2.0 ms) of inhibition. Figure A.1 is illustrated here to demonstrate the persistence of the interaction before averaging across the group.

The predictable time course of facilitation and inhibition persisted in all subjects tested and the group average is represented in Figure A.2. Figure A.3 illustrates the maximum facilitation (Figure

A.3A) and inhibition (Figure A.3B) produced by the group for each TMS intensity. The early phase of excitation was always between the -5.0 ms and -3.0 ms interval and it peaked at 122.5 ± 6.7% of control when a TMS intensity 110% of RMT was used. The first phase of inhibition (-2.0 ms to -1.0 ms) reduced the H-reflex to a maximum of 70.7 ± 8.3% of control at 80% of RMT while the second phase of inhibition (1.0 ms to 3.0 ms) reduced the H-reflex to a maximum of 80.3 ± 5.2% of control at 95% of RMT. The later, long-latency facilitation immediately followed the second inhibitory phase and continued to increase to a peak between 10.0 ms and 20.0 ms. At 110% of RMT the late phase of facilitation reached 188.7 ± 29.3% of control. A TMS intensity of 95% of RMT was optimal for producing all four phases of the interaction. Neither the first (74.3 ± 5.3%) or the second (80.3 ± 5.2%) phases of inhibition were overwhelmed by the surrounding facilitation. At 95% of RMT, the four phases of facilitation and inhibition were all at least 2 SE away from control.

A.4 DISCUSSION

We studied the effect of a conditioning pulse from TMS over the motor cortex on the excitability of soleus motor neurons by measuring changes in H-reflex amplitude in group of healthy controls. While a version of this interaction has been described in the literature (Nielsen et al. 1993), the inhibition has not been demonstrated to a level which showed statistical significance. In other words, the inhibition was only present in a few of the patients studied. Given the novelty of this interaction, the time-course was studied here in a group of five control subjects to determine the optimal TMS intensity and timing necessary to evoke the clearest trend. A TMS intensity of 95% of RMT produced the clearest profile of facilitation and inhibition and so was chosen for subsequent testing in *Chapter 5.* Please refer to *Chapter 5 (section 5.4.7)* for a full discussion of the origin of each phase.

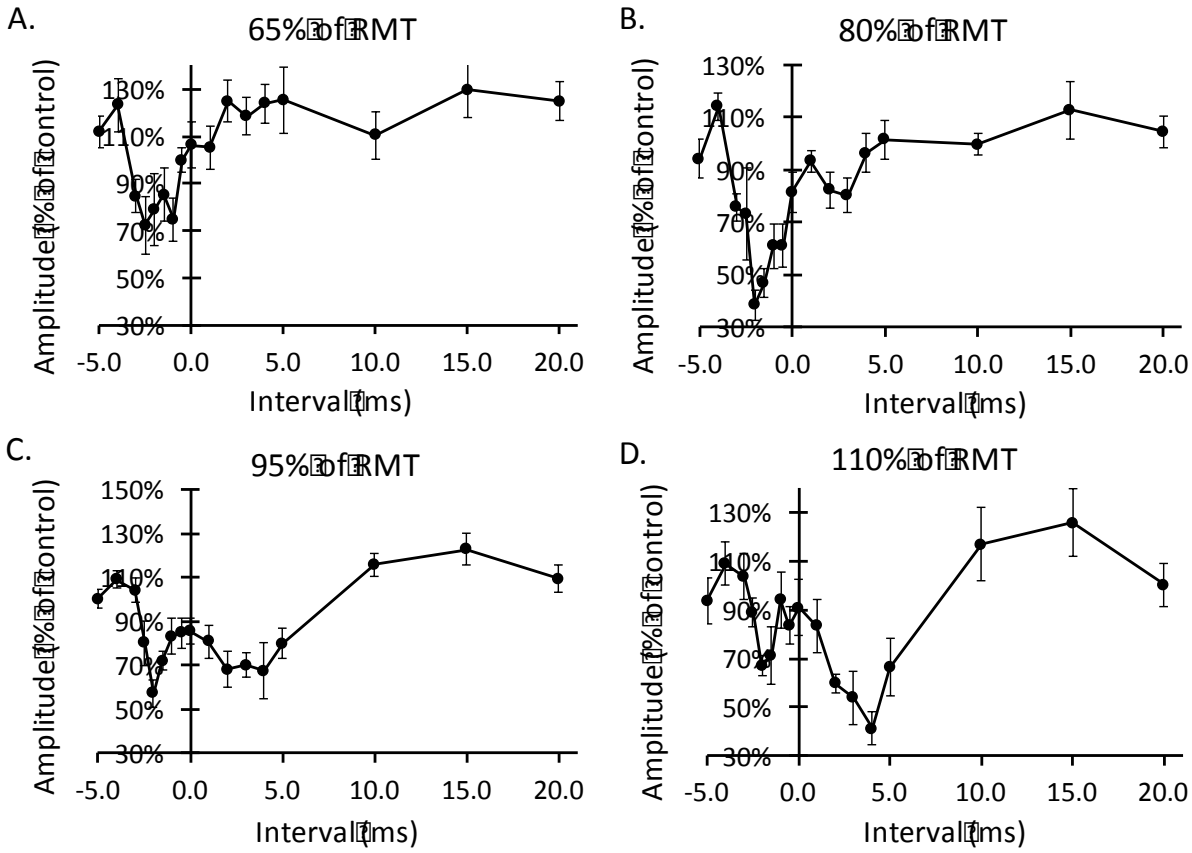

Figure A.1: Time course of the effect of a conditioning pulse from TMS on the soleus H-reflex at rest in a single subject. To identify the optimal parameters for this condition-test paradigm, four different TMS intensities were used: (A) 65% of resting motor threshold (RMT), (B) 80% of RMT, (C) 95% of RMT, and (D) 110% of RMT.

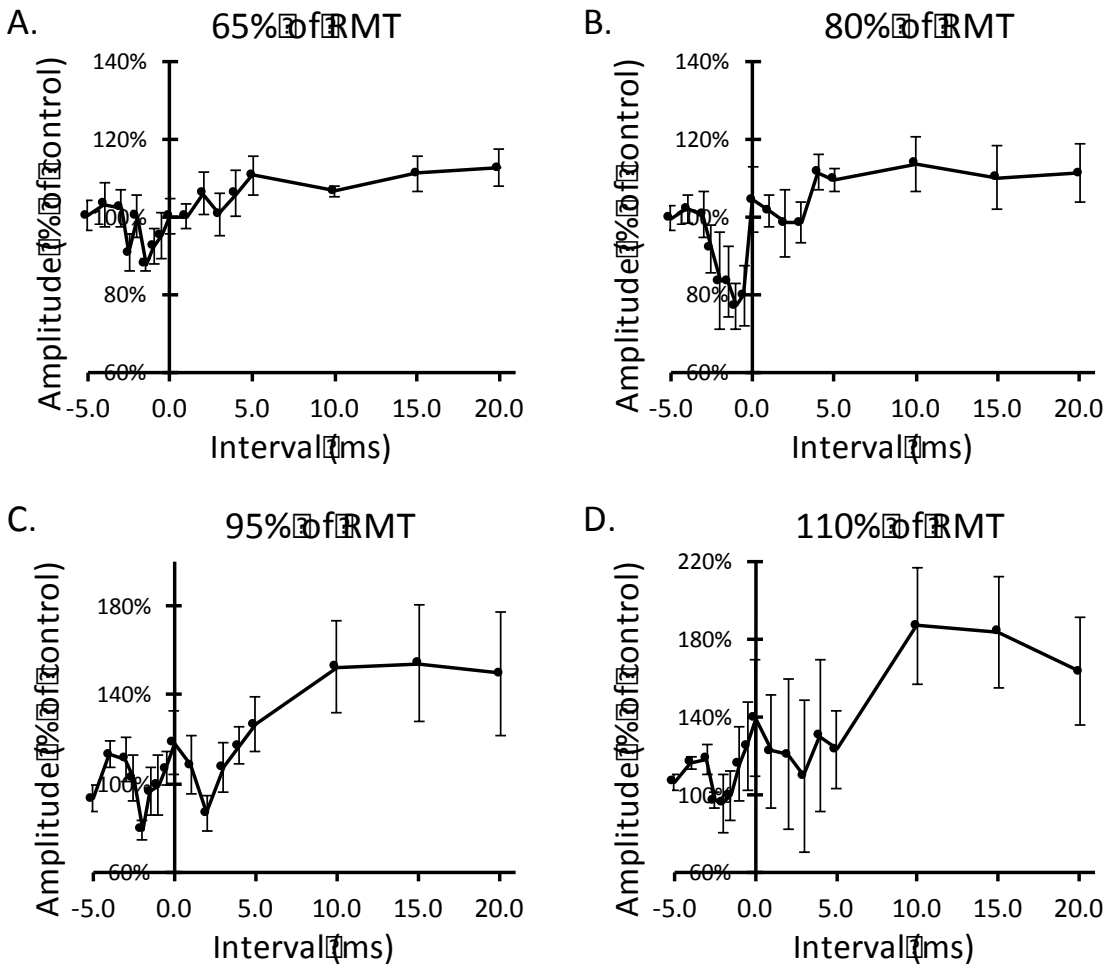

Figure A.2: Time course of the TMS/H-reflex interaction averaged across the group. Different TMS intensities are depicted in the four different graphs. (A) 65% of RMT, (B) 80% of RMT, (C) 95% of RMT, and (D) 110% of RMT.

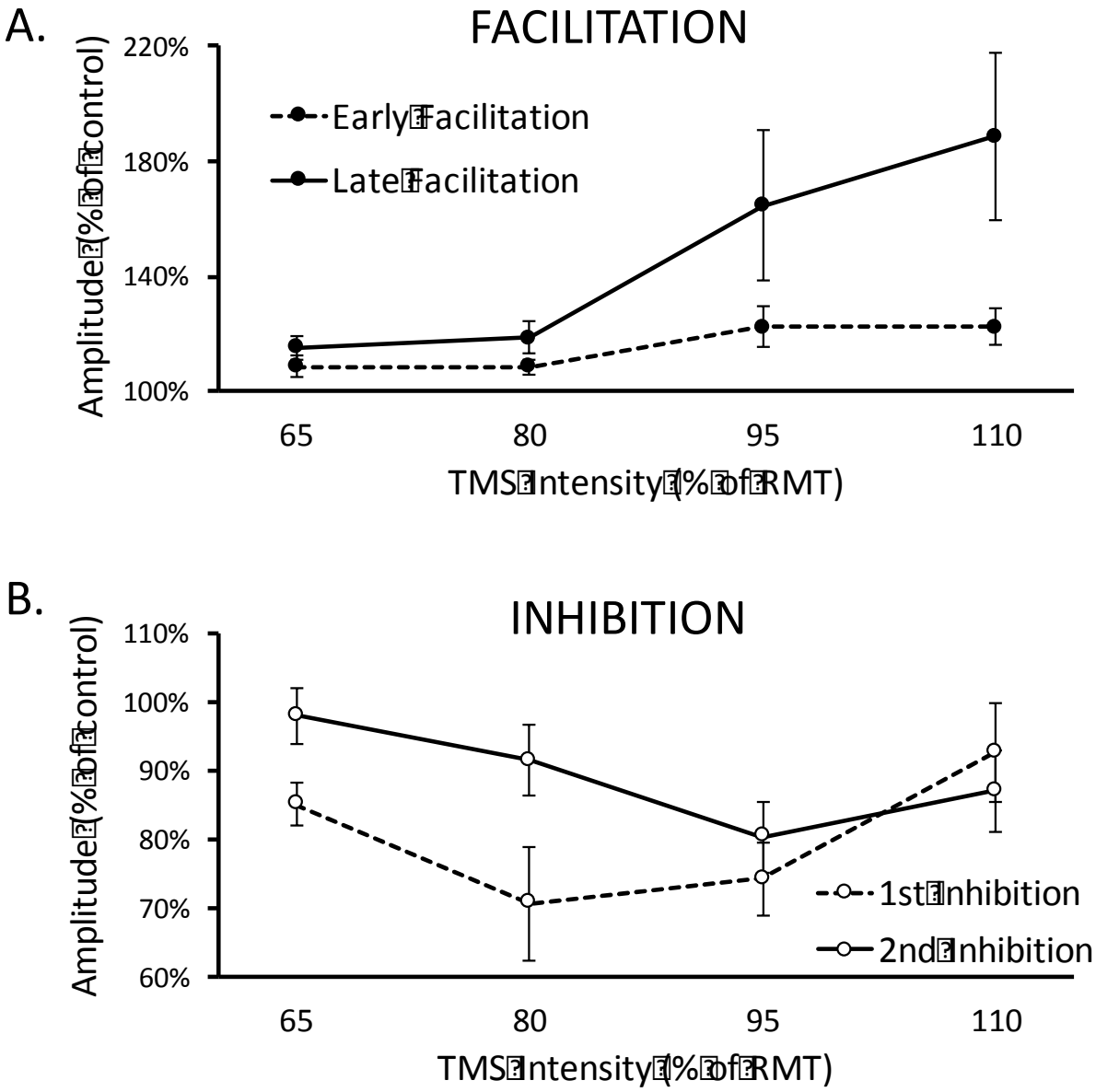

Figure A.3: Maximum facilitation (Figure A.3A) and inhibition (Figure A.3B) produced by the group for each TMS intensity. A TMS intensity 95% of RMT demonstrated all four phases of facilitation and inhibition.

A.5 BIBLIOGRAPHY FOR APPENDIX A

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