Neuronal mechanisms underlying retraining of walking after incomplete spinal cord injury

by

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ABSTRACT

Inhibitory feedback from sensory pathways is important for controlling movement. In this thesis we characterize a long-latency inhibitory spinal pathway to ankle flexors that is activated by low-threshold, *homonymous* afferents. In non-injured participants, this pathway was activated by both descending and sensory inputs. A spinal origin of this inhibition was confirmed by reproducing its effect on evoked responses from direct activation of corticospinal pathways. We propose that inhibitory feedback from spinal networks activated by low-threshold homonymous afferents helps regulate the activation of flexor motoneurons by the corticospinal pathways.

In the second part of the thesis we compared changes in corticospinal and spinal pathways in response to endurance and skill locomotor training in participants with incomplete, chronic spinal cord injury. Both forms of training increased the maximum evoked potential (MEP_{max}) elicited by transcranial magnetic stimulation over the motor cortex, but only in tibialis anterior muscles that had smaller MEP_{max} values before training, no matter when the specific type of training was performed. Endurance and precision training also increased the excitability of inhibitory spinal networks, as demonstrated by increases in the long-latency, homonymous spinal inhibition described above and by increases in the inhibitory component of the cutaneomuscular reflex. The increase in the descending activation of the spinal cord and the increase in excitability of inhibitory spinal networks may mediate the improved volitional control of walking and reduction of involuntary muscle spasticity, respectively, that are observed in response to intensive motor training.

PREFACE

The walking training of SCI participants for chapter 3 of this thesis were done in collaboration with in Dr. Jaynie Yang at University of Alberta. All neurophysiological assessments were designed by me. The data analysis and discussion of all chapters as well as the literature review are my original work.

A version of Chapter 2 of this thesis has been published as Zewdie ET, Roy FD, Okuma Y, Yang JF, and Gorassini MA, "Long-latency, Inhibitory Spinal Pathway to Ankle Flexors Activated by Homonymous Group 1 Afferents," *Journal of Neurophysiol, vol* 111, 2544-2553. I was responsible for the data collection and analysis as well as the manuscript composition. Roy FD, Okuma Y and Yang JF assisted with the data collection and contributed to manuscript edits. Gorassini MA was the supervisory author and was involved with concept formation and manuscript composition.

A version of Chapter 3 of this thesis has been published as Zewdie ET, Roy FD, Yang JF, and Gorassini MA, "Facilitation of Descending Excitatory and Spinal Inhibitory Networks from Training of Edurance and Precision Walking in Participatns with Incomplete Spinal Cord Injury," *Progress in Brain Research,* vol.218:127-155. Roy FD and Yang JF assisted with the data collection and contributed to manuscript edits. Gorassini MA was the supervisory author and was involved with concept formation and manuscript composition.

To my beloved grandmother W/o Zerfitu Tsegaw, I know you are proud of me from the heavens.

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LIST OF ABBRIVATIONS USED

10MWT	10 meter walk test
6MWT	6 minute walk test
AMT	active motor threshold
A-P	anterior-posterior
BW	bat wing coil
BWSTT	body-weight support treadmill training
CM	corticomotoneuronal
CMEP	cervicomedullary motor evoked potentials
CMR	cutaneomuscular reflexes
CPG	central pattern generator
CPN	common peroneal nerve
CST	corticospinal tract
CUSUM	cumulative sum
DC	double cone
DPN	deep peroneal nerve
emf	electromotive force
EMG	electromyogram
EPSPs	excitatory post synaptic potentials
FDI	first dorsal interosseous
FN	femoral nerve
FRA	flexor-reflex afferents
GAD	glutamic acid decarboxylase
IPSPs	inhibitory post synaptic potentials
ISI	interstimulus interval
KCC2	potassium-chloride cotransporter
L-M	lateral-medial
LTD	long-term depression
LTP	long-term potentiation
M1	primary motor cortex
MEP	motor evoked potentials
MEP_{max}	maximum motor evoked potential
MLR	mesencephalic locomotor region
MMT	manual muscle test
MT	motor threshold
MVA	motor vehicle accident
MVC	maximum voluntary contraction
NTSCI	non-traumatic spinal cord injury
PAD	primary afferent depolarization
PAS	paired associative stimulation

PET	positron emission tomography
PIC	persistent inward currents
PMRF	ponto-medullary reticular formation
PSTH	post stimulus time histogram
PT	perception threshold
RMT	resting motor threshold
SCI	spinal cord injury
SCI-FAP	spinal cord injury-functional ambulation profile
SICI	short-interval intracortical inhibition
SMEP	spinal motor evoked potentials
STDP	spike timing-dependent plasticity
TA	tibialis anterior
TES	transcranial electrical stimulation
TMS	transcranial magnetic stimulation
TN	tibial nerve
TSCI	traumatic spinal cord injury
WHO	world health organization

CHAPTER 1.INTRODUCTION

1.1. Forward

In this thesis we characterized a spinal inhibitory pathway to an ankle flexor muscle that is activated by both homonymous afferents and corticospinal pathways in non-injured participants and in individuals with incomplete spinal cord injury (SCI). The potential use of training for strengthening transmission in this spinally mediated pathway and in spared corticospinal pathways was also tested in participants with SCI. The type of intensive motor therapy that is best to improve or recover walking function in patients with incomplete spinal cord injury was determined. In addition, how these different forms of intensive motor therapy affected the spared, undamaged nervous system to potentially mediate the observed improvements in walking function was assessed. By knowing what neuronal pathways are associated with walking recovery we can develop further therapies to enhance specific neuronal pathways to better improve walking function.

1.2. Investigating the Human Motor System using Transcranial Electric and Magnetic Stimulation

In this thesis, I used the technique of transcranial stimulation to assess changes in descending motor pathways and spinal circuits in response to locomotor training in participants with incomplete spinal cord injury (SCI). Here, I will briefly summarize the physics and biophysics of Transcranial Electrical Stimulation (TES) and Transcranial Magnetic Stimulation (TMS), as well as overview the neuronal structures that are activated by these stimulation techniques. Also discussed are how multiple descending volleys in fast corticospinal pathways are activated by external stimulation and how TMS is used to prime the human motor cortex for motor rehabilitation.

1.2.1. Physics and Biophysics of Transcranial Electric and Magnetic Stimulation

1.2.1.1. Design of Stimulator Coils

Almost 45 years ago Merton and Morton (1980) built a high voltage electrical stimulator that could be used to activate the motor cortex via surface electrodes placed over the scalp. In TES, electric current flows in a perpendicular direction to the stimulating electrodes. The physical principle used in TES is the transfer of short lasting electric current from its source to the excitable neurons of the brain. However, the skull and the scalp have high resistance and therefore, very high voltage is required in order to stimulate the neuronal tissue of the brain. Merton and Morton (1980) used an electrical stimulator (condenser) which contains 0.1μF capacitors charged up to 2800V. The current was then discharged through electrodes that were placed on the scalp over the motor cortex because they could see the effects of stimulation most readily by observing or recording muscle movements. Since the resistance between electrodes attached to the scalp was very variable (Hill et al. 1980), the use of an external shunt resistance of up to 10Ω was needed to ensure that the time constant of discharge was less than 10μs.The time constant determines the time required to charge the capacitors, therefore, the smaller the time constant, the faster the capacitors get ready to be discharged.

TES was effective but painful so TMS was developed to stimulate the brain. Similar to TES, the main principle of TMS is the induction of short-lasting electric current in the brain. However, unlike TES, which involves direct transfer of current through electrodes, TMS uses electromagnetic induction to deliver current to the brain. For example, a short lasting (150-300μs) electric current is applied to a stimulating coil to produce a rapidly changing magnetic field which, in turn, induces a flow of electric current in nearby conductors – including the human brain. The current that is applied to the brain from TMS is governed by the Faraday-Henry law, which states that if an electric conductor is linked by a time-varying magnetic flux *F*, a current is observed in the circuit. This current is due to

the electromotive force (emf) induced by the time-varying flux, whose magnitude depends on the rate of change of the magnetic flux *dF/dt;* therefore:

$$\varepsilon = -\frac{dF}{dt}$$

where ε = electromotive force (emf) [measured in voltage, V]

F = magnetic flux [measured in weber, Wb]

t = time [measured in seconds, s]

The magnetic flux is created due to the current that is passing through the stimulating inductor coil, and can be defined as $F=LI$, where L is the inductance of the coil, which depends on the material property of the coil, and I is the current that is passing through the coil.

As shown in Figure 1.1, the magnetic stimulator typically consists of two distinct parts: a high current pulse generator (capacitor) producing a discharge current of 5000 amps or more; and a stimulating coil (inductor coil) producing magnetic pulses with a field strength of 1 tesla or more and a pulse duration of 1ms (Hovey and Jalinous 2008).

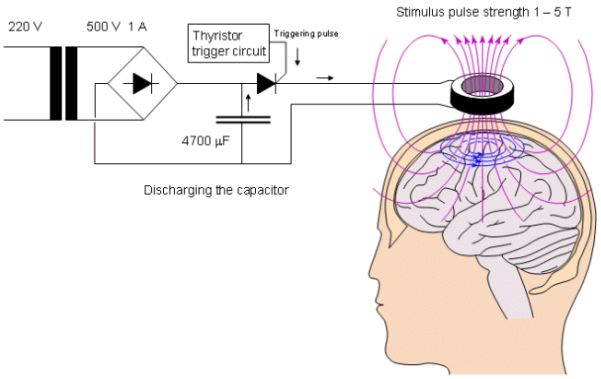

Figure 1.1: The principle of the magnetic stimulator. The current generated, when the charge stored in the capacitors is discharged through the inductor coils, creates a magnetic field that can penetrate through the skull and induce secondary current in the opposite direction. From Malmivuo and Plonsey 1995.

If the induced current is of sufficient amplitude and duration, it will stimulate neural tissue in the same way as TES. Just like electrical stimulators, TMS stimulators also contain capacitors that are charged up to 2.8kV. When the capacitor discharges this energy, apart from that lost in the wiring and the capacitor, it is transferred to the coil and then returned to the instrument to help

reduce coil heating. Typically, 500J of energy has to be transferred from the energy storage capacitor into the stimulating coil in around 100μs, which means during the discharge, energy initially stored in the capacitor in the form of electrostatic charge is converted into magnetic energy in the stimulating coil in approximately 100μs (Hovey and Jalinous 2008). This fast energy transfer leads to a rapid rise of magnetic field (around 30kT/s) which in turn induces current in the brain in the order of 1-20mA/cm^2.

The induced current direction and the waveform of the pulses determine how effective the stimulation can be in activating cortical neurons. The current that is induced by TMS in the brain tissue flows in a direction that is parallel to the stimulating coil but perpendicular to the magnetic field created by the coil. The most effective induced current direction is the one that is in the same direction as the propagation of nerve signals in the axons. Thus, as described below, axons that are orientated parallel (or horizontal) to the stimulating coil placed on the surface over the motor cortex are most effectively activated by TMS. Two types of waveforms are currently used – monophasic and biphasic. Biphasic stimulators are more effective than monophasic stimulators because a biphasic waveform produces a current having a longer duration and therefore, can produce more current to activate neurons (Sommer et al. 2006).

1.2.1.2. Types of TMS Coils

To focus current in different parts of the brain, TMS (inductor) coils are designed in different shapes to vary the spatial field and depth of the induced electric field. A circular coil typically has a diameter of 8-15cm (Figure 1.2A), with a maximum induced current at the outer edge of the circle and a maximum magnetic field directly under the center of the circle (~400V/m). It has a good penetration to the cerebral cortex where, at a depth of 2.0 cm, the electric field strength is 140V/m (Epstein et al. 2008).

The current generated with a circular coil activates the motor cortex asymmetrically, with greater activation on the side where the coil current flows

from posterior to anterior across the central sulcus (Schmid et al. 1990). Hence, with the coil placed centrally on the vertex, the induced current predominantly stimulates the left motor cortex as the current flow will be posterior to anterior. In contrast, coils that have two smaller circular coils side-by-side provide more focal stimulation. The magnetic fields created by currents flowing in opposite directions at the junction of the two circular coils results in a more focal stimulation directly under the junction. However, due to the smaller diameter size of the coils the penetration level is limited. Neurons located at a depth of 10.5mm from the surface of the skull will be stimulated by a circular coil and 11.5mm for a figure-of-eight coil (Epstein et al. 2008). Typically side-by-side coils range from very small flat coils for brain mapping work, such as figure-of-8 coils (Fig. 1.2b), to large contoured versions such as bat-wing coils (Fig. 1.2c) or Double Cone coils (Fig. 1.2d) to stimulate deeper neural structures in the brain such as the leg motor cortex .

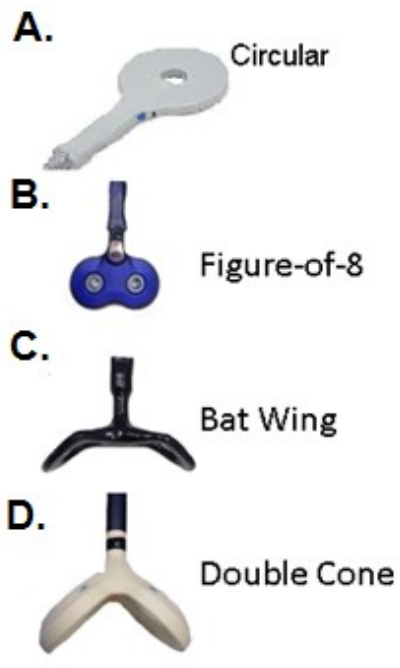

Figure 1.2: TMS Coil types A) Circular coil has only a single winding with superficial current penetration. B) Figure-of-8 coil has a flat shape with two adjacent windings and is used to activate more superficial hand areas of the cerebral cortex. C) Bat-wing coil has a flat center with bent wings and is used for activating leg areas of the cerebral cortex. D) Double-cone coil has two large cup shaped windings positioned side-by-side in an angle and can activate deeper areas of the cortex more powerfully than the bat wing coil.

1.2.2. Neuronal structures activated by Transcranial Magnetic Stimulation (TMS) and Transcranial Electric Stimulation (TES)

1.2.2.1. Stimulation of the Motor Cortex

The cerebral cortex is a complex structure composed of excitable elements (cell bodies and axons) that vary in size, location, orientation and function, all which influence their response to TMS or TES (Di Lazzaro et al. 2008). Multiple techniques of applying TES and TMS have been used to stimulate specific elements in the motor cortex. There are anodal, cathodal, unifocal or bifocal stimulation configurations for TES and lateral-medial (L-M) and antererior-posterior (A-P) configurations for TMS.

When we compare TES and TMS in general, each of them produces very different electric fields. The most localized field is produced by magnetic stimulation. However, a localized field from TMS also decreases the ability to stimulate neurons buried deep in the brain because a magnetically induced field drops off faster than an electrically produced one (Epstein et al. 2008). In the motor cortex, specifically in Area 4, there are neurons on the surface that are oriented vertically and neurons deeper within the precentral gyrus that are oriented horizontally. Rushton (1927) had shown that the electrical field must have a component parallel to the neuron in order to stimulate it. Given that magnetic stimulation produces a current flow that is parallel (in a horizontal direction) to the surface of the cortex, the only neurons that are going to be stimulated directly are the ones that are parallel to the surface of the cortex. These neurons with horizontally aligned axons are typically located in deeper layers of the cortex. Since the strength of an electric field created by magnetic stimulation falls off with depth, these parallel neurons might not be stimulated if they are located very deep. In contrast, the electric field produced during electric stimulation has components both parallel and perpendicular (vertical) to the surface of the head. In the area directly under the anode, where the field has its maximum magnitude, the electric field is directed radially inward. This radially directed electric field can activate axons or axon Hillocks of the corticospinal tract

(CST) neurons. However, further away from the anode the field becomes tangential, running parallel to the surface of the head, away from the anode and toward the cathode.

1.2.2.2. Descending Volleys activated by TMS and TES

Evidence that multiple descending volleys in the CST can be activated by electrical stimulation was first proposed in the mid-1950s by Patton & Amassian (1954). The motor cortex was stimulated in anesthetized cats and monkeys using single, square-wave electrical shocks applied through bipolar electrodes to the exposed cortical surface. Single-unit recordings were taken from the bulbar pyramid and lateral column of the cervical spinal cord (i.e. above and below the pyramidal decussation). A single shock to the cortical surface activated multiple CST neurons. The first wave was produced by direct excitation of CST neurons while later waves originated from indirect activation via cortical interneurons. Accordingly, they coined the terms D- and I- waves to describe the first and the later responses respectively. Several lines of evidence pointed to the D and I wave hypothesis: 1) the latency of D-waves agreed with the conduction time of large corticospinal fibers; 2) I-waves were found to be more susceptible to cortical injury and asphyxia than D-waves; 3) when micro-stimulating deep white matter, the size of D-waves increased and I-waves appeared when the stimulating electrode was pulled through the grey matter. This was an important finding since it provided initial evidence that multiple descending volleys from I-waves were generated trans-synaptically through excitatory corticocortical fibers when stimulating the surface of the motor cortex with a single pulse.

The differences between the orientation of current flow induced by TMS and TES (horizontal and vertical, respectively) were used to investigate what cortical structures were activated in the first papers describing the effects of single-pulse stimulation of the motor cortex in man (Day et al. 1987; Rothwell et al. 1987). The latency of the descending volleys evoked by TES and TMS varied markedly according to the type of stimulation and the stimulation intensity (Day

et al. 1987). When comparing TMS and TES, the latency of the first increase in firing probability of FDI motor units, as shown by the peaks in the Post Stimulus Time Histogram (PSTH, Figure 1.3a top graph), was earlier with anodal TES at 25ms compared to TMS which occurred about 2ms later (bottom graph). Responses evoked by anodal TES have an early latency since they are produced by activating axons of the CST directly, whereas the later TMS responses and multiple peaks in the PSTH are due to transsynaptic activation of CST neurons. In addition, the multiple peaks in the PSTH in response to TMS were postulated to arise from the arrival of several excitatory post synaptic potentials (EPSPs) at the motoneuronal membrane. Alternatively, these multiple peaks could have resulted from a single long duration EPSP that was interrupted by one or more shorter inhibitory post synaptic potentials (IPSPs). The latency of the EPSPs was consistent with arrival of multiple monosynaptic (corticomotoneuronal) inputs from D- and I-volleys (Day et al. 1989).

Epidural recordings of responses to TMS and TES (Di Lazzaro et al. 1998; Di Lazzaro et al. 2008; Kaneko et al. 1996; Nakamura et al. 1996) also confirmed the distinct activation pattern of D- and I-waves. Di Lazzaro and co-workers (1998) used bipolar electrodes implanted into the cervical epidural space of two conscious human subjects and recorded spinal volleys during TMS and TES. At threshold for detection of a surface volley in the lumbar CST (AMT, Figure 1.3b), more D-wave responses were evoked by TES (Anodal Stimulation) than TMS (Magnetic Stimulation), and the reverse was true for I-wave responses where they were more prominent after TMS (see +3 to + 9% intensities). As indicated by the first dotted line, D-waves were evoked by anodal TES but not TMS. Both stimulation techniques could evoke I-waves. With increasing intensity of TES, D-wave responses of shorter latency were evoked, indicating activation of axons at white matter sites lying deeper below the cortex. In contrast, responses to TMS had stable response latencies, since the timing of the trans-synaptic activation of the CST via interneurons is less likely to change as the simulation intensity increases. However, epidural recordings could fail to detect activity seen with

intra-axonal recordings. For instance, using intra-axonal recordings, Edgley and colleagues (1997) demonstrate that both TES and TMS can produce D- and I-waves.

A B

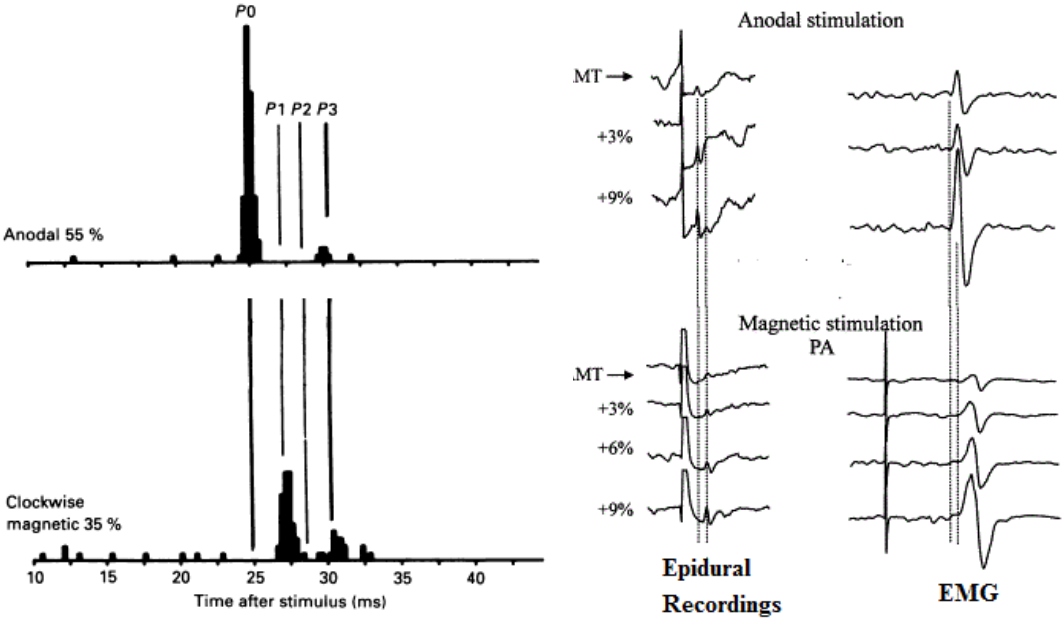

Figure 1.3: TES and TMS stimulations. A) Post-stimulus time histogram from a single motor unit in FDI activated by anodal TES (top) and TMS (bottom). From Day et al. 1989. B) Descending volley evoked by anodal TES (top) and TMS (bottom) using increasingly strong stimulus intensities. Modified from Di Lazzaro et al. 1998.

Furthermore, during voluntary contractions, the amplitude of the D-wave elicited by TES was unaffected by the level of background contraction, but the amplitude of I-waves elicited by TMS were significantly increased (Di Lazzaro et al. 1998). Voluntary contractions can produce synaptic facilitation that could reflect changes in synaptic excitability in the cortex (Nielsen and Petersen 1995). Since D-waves reflect direct activation of CST, it would not be affected by voluntary contraction unlike the trans-synaptic activation of I-waves.

1.2.3. Descending pathways from the cerebral cortex

1.2.3.1. Origin and Projections

The main descending motor pathway from the cerebral cortex to the spinal cord that is strongly activated by TMS is the corticospinal tract (CST). The CST originates from large pyramidal cells in the fifth layer of the cerebral cortex. Over 60% of the CST fibers originate from the primary motor cortex, supplementary motor area and premotor cortex (Ropper and Samuels 2009). A substantial proportion of the fibers (about 40%) originate from the primary somatosensory area and the parietal cortex (Dum and Strick 1991). The corticospinal tract is the only descending motor track that is known to make monosynaptic connections with spinal motoneurons in humans (Lemon 2008; Weber and Eisen 2002). Unlike in cats where there are no functional corticomotoneuronal (CM) connections (Illert et al. 1976), in humans the presence of CM connections on upper limb (Colebatch et al. 1990) and lower limb (Nielsen et al. 1995) motoneurons has been demonstrated. Functionally, muscles that require greater precision, such as the index finger and thumb, have greater CM connections (Courtine et al. 2007). In human lower limbs, the monosynaptic projection to the tibialis anterior (TA) muscle is surprisingly strong, comparable in magnitude to the finger muscles (Nielsen et al. 1993). This may be related to the precision required to clear the toes above the ground during the swing phase of human walking (Capaday et al. 1999).

TMS over the motor cortex can activate projections to other cortical (Siebnet et al. 2001) and subcortical regions (Strafella et al. 2001). For instance, TMS over the primary motor cortex (M1) can trans-synaptically activate corticoreticular (Fisher et al. 2012) and corticovestibular (Kawai & Nagao 1992) pathways, which can produce or influence spinal motor output via reticulospinal and vestibulospinal pathways, respectively. Single unit recordings from ponto-medullary reticular formation (PMRF) in the brainstem of anaesthetized monkeys revealed multiple latency responses evoked by TMS performed over M1 (Fisher

et al. 2012). The multiple latency responses may indicate the existence of multiple pathways from M1 to the PMRF.

1.2.3.2. Activation of Spinal Circuitry

Descending volleys activated by TMS can have both an excitatory and inhibitory effects on lower motoneurons in the spinal cord either by exciting the motoneurons directly via the CM pathway or through polysynaptic pathways that contain interposed excitatory and inhibitory interneurons. For example, a sub-threshold (below MN firing threshold) TMS can increase the amplitude of the TA and FDI H-reflex via the CM pathway given the short-latency of the facilitation (Nielsen et al. 1993). On the other hand, sub-threshold TMS over the SOL motor cortex can inhibit the H-reflex of the SOL muscle at rest (Nielsen et al. 1993). The SOL H-reflex inhibition was enhanced during antagonist muscle contraction (dorsiflexion), and reversed to facilitation during agonist contraction (plantarflexion), which demonstrates a task-related change in the effect of a descending volley on spinal MNs (Nielsen et al. 1993). Based on the short onset latency, it was proposed that the H-reflex facilitation observed during agonist contraction was mediated by the activation of CM cells projecting directly onto the SOL MN pool (Fig. 1.4, green line), whilst the inhibition observed during antagonist contraction was mediated by the CST activating Ia inhibitory interneurons that in turn synapse directly onto the SOL MN pool (Fig. 1.4, site A, Nielsen et al. 1993).

There are other interneuron pathways that descending volleys from TES or TMS can modulate. For instance, TMS can decrease presynaptic inhibition, which is mediated by primary afferent depolarization (PAD) and transmitted by axo-axonal GABA synapses. For example, heteronymous facilitation of the SOL H-reflex by femoral nerve (FN) afferents is increased by TMS when both femoral nerve stimulation and TMS are given together prior to evoking SOL H-reflex (Fig. 1.4, site B) (Meunier and Pierrot-Deseilligny 1998). Interestingly, the facilitation of the SOL H-reflex was more than the algebraic sum of the effect by

the separate stimuli. The extra facilitation of the SOL H-reflex was attributed to the suppression of PAD neurons by more upstream inhibitory interneurons that were activated by TMS (Fig. 1.4, site B). Furthermore, TMS decreases the long lasting inhibition (D1 inhibition) of the SOL H-reflex (Fig. 1.4, site C blue lines) by CPN afferents (Meunier and Pierrot-Deseilligny 1998). Altogether, the increase of heteronymous Ia facilitation and decrease of D1 inhibition by TMS suggest a decrease in presynaptic inhibition of Ia afferents by the descending volley activated by TMS.

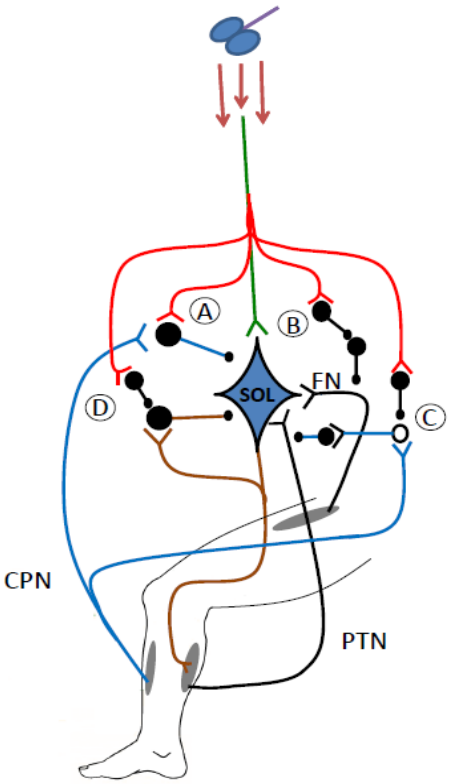

Figure 1.4: Effects of descending volley on spinal interneurons. TMS activated descending volley can: **A)** increase reciprocal inhibition via facilitation of Ia inhibitory INs (Masakado et al. 2001), **B, C)** decrease presynaptic inhibition via suppression of PAD INs (Meunier and Pierrot-Deseilligny 1998), **D)** decrease of recurrent inhibition via suppression of Renshaw cells (Mazzocchio et al. 1994).

The inhibitory interneurons mediating Recurrent Inhibition (Fig. 1.4, site D) can also be modulated by descending pathways activated by TMS as assessed by a paired H-reflex technique (Mazzocchio et al. 1994). As shown in Fig. 1.5A, a focused stimulation to the posterior tibial nerve (PTN) produces an isolated SOL H-reflex (H1) which activates Renshaw cells orthodromically via recurrent collaterals from the motor axon (see brown lines in Fig. 4D). If a high intensity PTN stimulus is given (Fig. 1.5B), a maximum motor response (Mmax) is

produced and H1 is vanished due to collision with the antrodromic volley in the motor axon. In both cases (A & B), sub-threshold TMS did not change any of the responses. However, when the two intensities of PTN stimulus are given together with an ISI of 10 ms (A + B), the H1 reflex collides with the antidromic volley and eliminates it, so that the H' reflex mediated by the supermaximal test stimulus can pass through. As shown in Fig. 1.5C, TMS increases the H' reflex amplitude, which is believed to be through the suppression of the Renshaw Inhibitory interneurons (Fig. 1.4, site D). Since the TMS did not change the reference H reflex (Fig.1.5D), the facilitation of H' reflex is not due to a direct facilitation of SOL MNs by the descending volley.

To expand the characterization of descending activation of spinal inhibitory networks, in the first part of this thesis (Section 2.0) we describe the facilitation of spinal inhibitory networks that are activated by both descending inputs from TMS to the tibialis anterior motor cortex and by low-threshold, homonymous afferents from the common peroneal nerve (CPN).

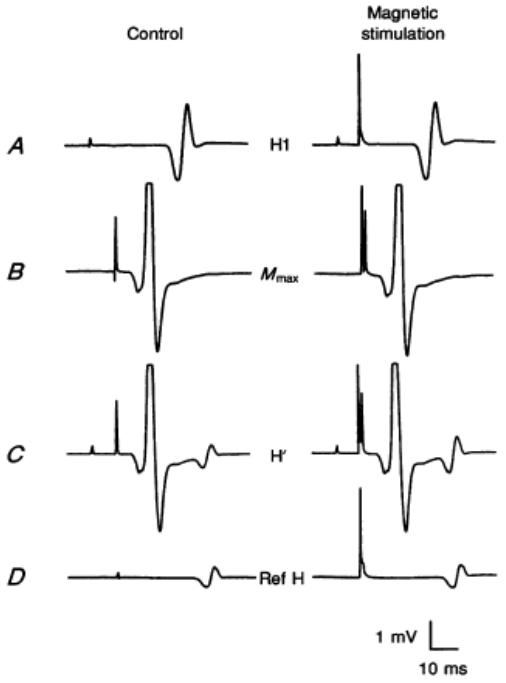

Figure 1.5: Recurrent Inhibition of SOL with and without TMS. **A) A**n isolated PTN stimulus producing H1 reflex. **B)** High intensity PTN stimulus producing maximum motor response (Mmax) and no H-reflex. C) Combined conditioning and test stimulus (A+B) with ISI of 10ms producing H' reflex. D) PTN stimulus producing a reference H-reflex (Ref H) of the same size and latency as the H' reflex. From Mazzocchio et al. 1994.

We show, a long-latency inhibitory spinal pathway to ankle flexors that is activated by low-threshold, muscle and cutaneous afferents.

1.2.4. Sensory Activation of Cortical Networks Studied by TMS

Sensory inputs are integrated with motor control commands at spinal, subcortical and cortical levels. The spatial and temporal characteristics of sensory inputs to the motor cortex can directly or indirectly influence changes in its excitability and reorganization as assessed using TMS. For instance, single-pulse TMS conditioned by afferent inputs allow measurements of corticospinal excitability changes related to specific sensory inputs that are activated artificially through electrical or mechanical stimulation (Bertolasi et al. 1998), or naturally through various motor tasks (Lemon et al. 1995). Because motor training, such as endurance and precision walking, activates sensory inputs to the brain and spinal cord, I discuss below studies examining the influence of peripheral sensory activation on the excitability and plasticity of the motor cortex.

1.2.4.1. Short-latency Afferent Inhibition

A TMS paradigm, called Short-latency Afferent Inhibition (SAI), can be used to investigate sensory-motor integration. In this paradigm, a peripheral nerve that is stimulated prior to the activation of the motor cortex depresses motor evoked potentials (MEPs) elicited by TMS (Tokimura et al. 2000). The afferent volley from an intrinsic muscle of the human hand takes about 20ms to arrive at the motor cortex (Gandevia et al. 1984) via the anterolateral pathway. SAI requires a minimum ISI that is close to the latency of the N20 component of a somatosensory evoked potential, and lasts for about 7-8ms (Tokimura et al. 2000). For instance, median nerve electrical stimulation 19 ms prior to TMS of the motor cortex suppressed responses evoked by TMS but not by TES (Tokimura et al. 2000). In the same study, epidural recordings from the cervical epidural space of five patients during TMS over the motor cortex confirmed that the most prominent effect of SAI was on the I2 and I3 waves, whilst the D and I1-wave were not affected by SAI (Tokimura et al. 2000). Based on this evidence, the pathway mediating SAI is considered to be of cortical origin. The largest effects of MEP modulation by sensory input were seen in muscles that were nearest to

the site of peripheral nerve stimulation, suggesting a somatotopical organization of the SAI effect (Classen et al. 2000).

Sensory input can also facilitate on-going EMG activity through trans-cortical reflex pathways (Christensen et al. 2001). The earliest responses evoked by a muscle stretch or skin stimulation has a pure spinal origin due to the short latency of the responses (Nielsen and Kagamihara 1993). On the other hand, the longer latency stretch responses were shown to involve the trans-cortical pathway. Among the earliest evidence of this long-latency pathway came from the stimulation of peripheral receptors that led to the synchronized descending discharge of the CST in anesthetized cats (Adrian and Moruzzi 1939). More convincing evidence in humans came from studying long-latency responses to the stretch of thumb muscles in patients with Klippel-Feil syndrome (Capaday et al. 1991). Patients who suffer from this syndrome exhibit mirror movements due to a CST that projects to both sides of the body (Christensen et al. 2001). In these patients, stretching of one muscle leads to reflex discharge of the corresponding muscles on the other side, strongly supporting the animal studies that afferent volleys activated by muscle stretch can activate CST pathways (Matthews et al. 1990).

In addition, studies using TMS have also supported this claim. MEPs evoked in the flexor pollicis longus muscle were facilitated if they were evoked during the period of the long-latency stretch reflex and not if they were evoked during the short-latency stretch reflex, even when the size of the two reflex components were approximately equal (Day et al. 1991). Furthermore, TMS at an intensity that was below threshold for evoking MEPs during the short-latency reflex period could produce a response if given within the long-latency period. Similar effects were not present when low-intensity TES was used instead of TMS (Day et al. 1991). Overall the above findings suggest that the long-latency component of the stretch reflex is mediated, in part, through transcortical pathways and can increase the excitability of the motor cortex.

1.2.4.2. Paired Associative Stimulation

Repetitive activation of sensory inputs to the motor cortex can also produce long-term changes in the excitability of the motor cortex. Using an experimental paradigm called Paired Associative Stimulation (PAS), it is possible to induce enduring changes in the excitability of the human motor cortex that can last for several hours (Ridding and Taylor 2001; Stefan et al. 2002; Stefan et al. 2000). The technique uses repetitive pairing of electrical stimulation of a peripheral nerve and TMS of the motor cortex. The TMS pulse is given at the same time the afferent inputs that are activated by the peripheral nerve stimulation arrive at the motor cortex. The effect of PAS on MEP size was found to be dependent on the timing of the TMS pulse with respect to the afferent stimulation, which is consistent with the spike timing-dependent plasticity (STDP) paradigm observed in reduced animal experiments (Florian 2007).

As shown in Figure 1.6, if the presynaptic neuron is activated during the depolarizing phase of the action potential in the postsynaptic neuron ($\Delta t > 0$), long-term potentiation (LTP) between the pre- and postsynaptic neuron is induced due to the high calcium level in the postsynaptic neuron (Markram et al. 2011). On the other hand, if a presynaptic neuron is activated during the afterhyperpolarization of the postsynaptic neuron ($\Delta t < 0$, i.e., during low calcium levels), then LTD is induced. Similarly, during PAS with an ISI of -10ms (PAS10), which ensures the afferent volley arrives at primary motor cortex (M1) after the TMS and likely during the afterhyperpolarization of the CST neuron, LTD-type effects were induced in the motor cortex as reflected in reduced MEPs (Stefan et al. 2000). On the other hand, when an ISI of 25ms (PAS 25) was used, which ensures that afferent inputs arrive at the motor cortex when the CST neurons are depolarized, long term potentiation (LTP-type) effects were induced as evidenced by increases in MEP responses.

When using an excitatory PAS intervention (25 ms ISI) for 30 minutes (90 pairings at 0.05 Hz), MEPs can be enhanced for 60 to 120 minutes after the

intervention (Stefan et al. 2000). This effect can be blocked with the administration of dextromethorphan, an NMDA receptor agonist (Stefan et al. 2000). The site of PAS-induced plasticity is cortical given that MEPs evoked by electrical cervicomedullary stimulation remained unchanged after PAS (Stefan et al. 2002). Furthermore, epidural recording of CST activity evoked by TMS showed that PAS changed the later I-waves (Di Lazzaro et al. 2009).

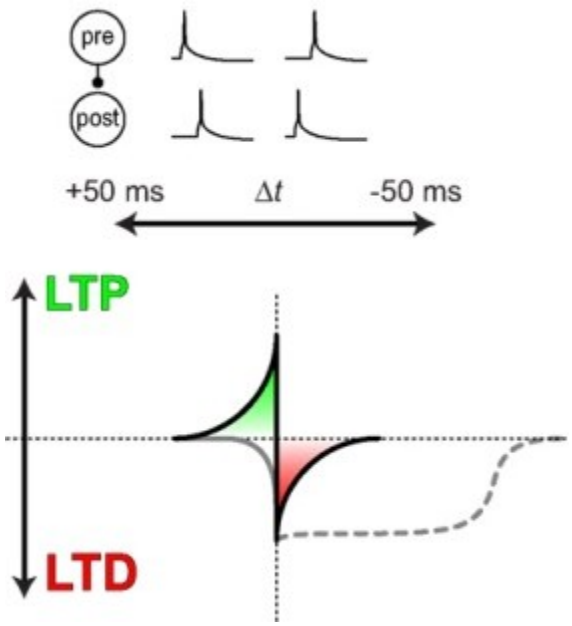

Figure 1.6: Schematic of order-specific STDP function. As illustrated by the inset traces, presynaptic activity followed by postsynaptic activity ($\Delta t > 0$) produces LTP, while post-followed by presynaptic activity ($\Delta t < 0$) produces LTD. Maximal plasticity in both directions is produced at ISIs close to zero (Markram et al. 2011).

1.3. Spinal Cord Injury

1.3.1. Epidemiology of SCI

In *Chapter 3* of this thesis, we examine the electrophysiology of participants with spinal cord injury (SCI). Thus, in this section I briefly describe the epidemiology and characteristics of SCI from provincial, national and global perspectives.

1.3.1.1. Provincial Perspective

SCI is a devastating event that can have a significant effect on the health, economy and social life of the individual, their family and society (Parsons 1991). Based on data collected from 1997 to 2000, the average yearly *incidence* of SCI

in Alberta was 52.5 cases per million persons (Dryden et al. 2003). The incidence rate was consistently higher for males than for females for all age groups, with a male to female ratio of 2.5:1. A motor vehicle accident was the most common cause of SCI (56.4%) followed by falls (19.1%). Among those who had motor vehicle accidents, the highest incidence occurred to those between 15 and 29 years, while falling accidents were common among those older than 60 years. Cervical level injuries accounted for most (61.5%) of the injuries while the rest were thoracic, lumbar and sacral-level injuries. Most of these injuries were incomplete (81.8%). The trend in the mortality rate of people with SCI remained low (7%), similar to that reported in 1993 (Tator et al. 1993).

1.3.1.2. National Perspective

In Canada, the *prevalence* of SCI was estimated to be 85,556 persons [(51% traumatic SCI (TSCI) and 49% non-traumatic (NTSCI)] in 2010 (Noonan et al. 2012). The age-specific prevalence of TSCI (Fig. 1.7) was higher in younger population demographics (age 15-29) while NTSCI was concentrated in older age groups (Noonan et al. 2012). The incidence rate also followed a similar trend, where the highest incident rate of TSCI occurred in people of age 25-34 years (Fig 1.8a), and the highest incident rate of NTSCI occurred in people of age 65-69 years (Fig 1.8b) even though the age profile of Canadians decreased in these ages. The estimated lifetime economic burden per individual with TSCI ranges from $1.5 million for incomplete paraplegia to $3.0 million for complete tetraplegia, resulting in a total of $2.67 billion annual cost associated with 1389 new persons with TSCI surviving their initial hospitalization (Krueger et al. 2013).

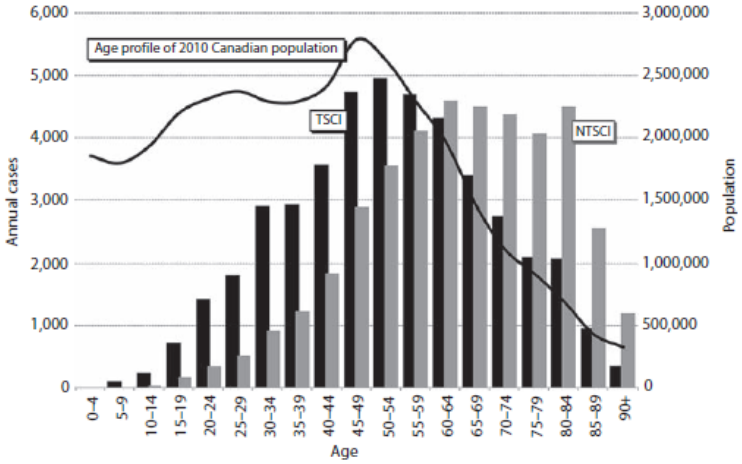

Figure 1.7: Age-specific *prevalence* estimate of SCI in Canada. From Noonan et al. 2012.

A. TSCI **B. NTSCI**

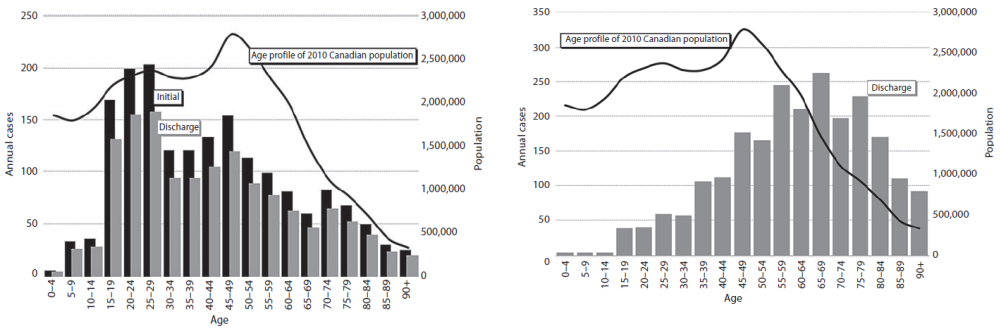

Figure 1.8: Age-specific *incidence* rates of (A) TSCI and (B) NTCSI in Canada. Initial incidence (black bars) refers to the number of SCI at injury scene. Discharge incidence refers to number of SCI patients discharged into the community, and has lower number due to deaths related to SCI. The age profile of the 2010 Canadian population is presented for comparison. From Noonan et al. 2012.

1.3.1.3. Global Perspective

According to the comprehensive 2013 report by the World Health Organization (WHO), the global impact of SCI depends on a range of factors including: the age at which the injury occurs; the extent of the injury; the availability and timing of resources and services, and the environment in which the person lives which includes physical, social, economic and attitudinal factors. According to this report, there were no reliable global or regional estimates of SCI prevalence due to a lack of national SCI data from most countries in the world. When comparing the prevalence rate of SCI in countries which reported national SCI data (Iran, Finland, Norway, Iceland, Canada and Australia), Canada had the highest prevalence of SCI (1298/million population) as well as the highest incidence rate (Fig. 1.9). The global incidence rate to TSCI was reported to be 40 – 80 new cases/million population, meaning that 250 000 to 500 000 succumb to a SCI every year in the world (Bickenbach et al. 2013). Similar to what was shown in Alberta and Canada, the two common age-associated peaks in TSCI incident rate occurred in young adults (male: 20-29; female 15 -19) and in older people (males 70+; females 60+).

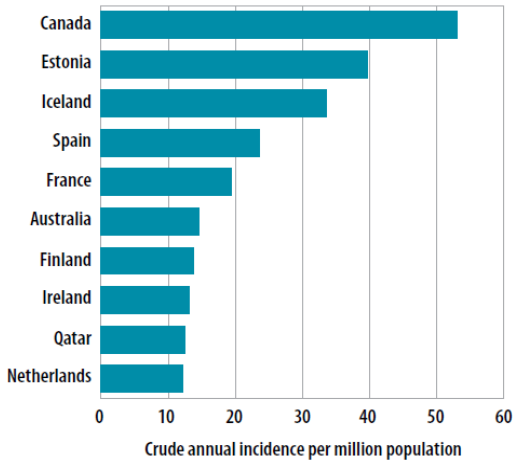

Figure 1.9: Global Variation in country-level estimates of annual incidence of TSCI (Bickenbach et al. 2013).

From the regional data collected from 2 to 3 countries per continent, motor vehicle accidents were the top cause of TSCI in all regions. Compared to other continents, Africa had the highest percent of SCI cases caused by motor vehicle accidents (Fig. 1.10). Due to the large aging population in the Eastern Mediterranean and South-East Asia, the percentage of TSCI cases caused by falls was equivalent to what was caused by motor vehicle accidents elsewhere.

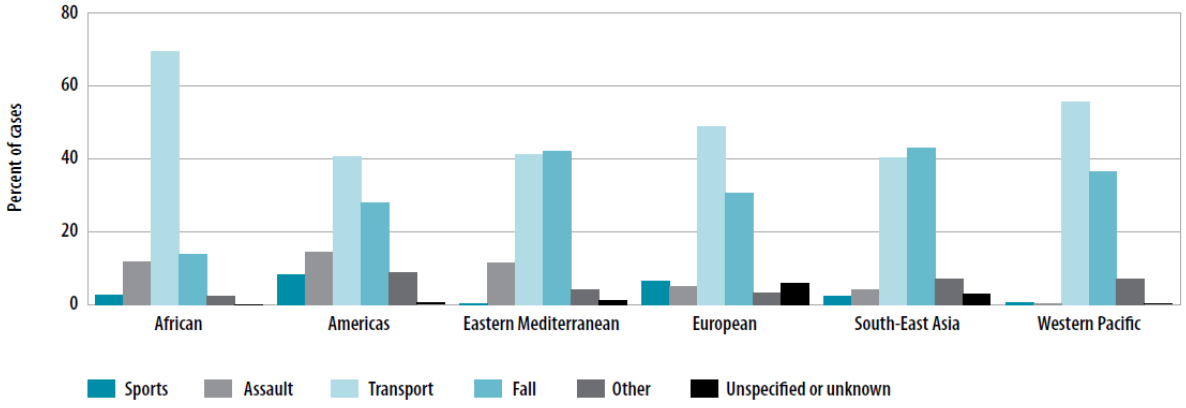

Figure 1.10: Distribution of TSCI in the regions of the world. The number of countries providing data for regional summary are: African 3; American 4; Eastern Mediterranean 5; European 13; South-East Asia 3; and Western Pacific 3 countries. From WHO report 2013.

In terms of mortality rates associated with SCI, people with SCI are 2 to 5 times more likely to die prematurely than people without SCI (Bickenbach et al. 2013). The mortality rate tends to be higher in low-income countries due to

difficulty to prevent secondary conditions, such as urologic complications and pressure sores. For example, Sierra Leone had an average morality rate of 29% and Nigeria 35%, while the rates in Canada and USA were 11.6% and 6.1% respectively.

1.3.2. Characterization of SCI

The spinal cord contains 31 segmental levels where paired spinal nerves, containing sensory and motor fibres, emerge from the vertebral column. The effect of SCI mainly depends on the spinal level at which the injury occurs and the severity of the injury sustained by the spinal cord.

1.3.2.1. Level of Injury

SCI can affect any level of the cervical (C1-C8), thoracic (T1-T12), lumbar (L1-L5), or sacral (S1-S5) spinal cord segments. Damage to the cervical spinal cord results in impairment of both upper and lower extremity functions (termed as quadriplegia) and injury to the thoracic, lumbar or sacral spinal cord results in impairment of lower extremity function (termed as paraplegia). For injuries below the cervical level, there is a difference between the vertebral level injury and spinal cord level injury (Field-Fote 2009). For example, damage to C4 vertebrate results in damage at or near the C4 segment. However, damage to L1 vertebra can affect the roots of the spinal nerves related to L1 through S5 because the lumbar enlargement, the region of the spinal cord containing the motoneurons that innervate the lower extremities, is comprised of spinal segments L1-S3 but located anatomically at vertebral levels T9-T12 (Field-Fote 2009).

1.3.2.2. Severity of Injury

Usually the terms ‘incomplete’ or ‘complete’ are used to suggest whether there is a partial or no, respectively, descending and ascending transmission below the level of injury. The American Spinal Cord Injury Association (ASIA) has developed a more systematic classification of severity of SCI using the ASIA impairment scale (Curt et al. 1998). This scale is a 5 point ordinal scale which

classifies individuals from 'A' (complete SCI) to 'E' (normal sensory and motor function). Pin prick and light touch sensation tests at 28 dermatomes and manual muscle test of 10 key muscles, in combination with anal sensory and motor function determine the ASIA classification (Priebe and Waring 1991).

1.4. Neuronal Plasticity after Spinal Cord Injury

The CNS is able to adapt and re-organize based on experience and use, and this occurs as a result of physiological and structural plasticity (Feldman 2009). Functional, structural and neurochemical changes have been observed in peripheral nerves, spinal cord, brainstem, thalamus and cortex after SCI (for review see Raineteau and Schwab 2001), stroke (Cauraugh and Summers 2005; Pekna et al. 2012) and motor rehabilitation (Fouad and Tetzlaff 2012; Harkema 2001; Knikou 2010; Schaechter 2004). The re-organization might occur in pre-existing circuits through synaptic plasticity, or by the appearance of new circuits through anatomical plasticity, such as growth of axonal branches and dendrites (see Review by Raineteau and Schwab 2001). In the paragraphs below, I outline different types of neural plasticity that can occur after chronic SCI and rehabilitation training, which is important in regards to *Chapter 3* of this thesis examining electrophysiological changes in response to endurance and skill walking training in participants with incomplete SCI.

1.4.1. Spinal Plasticity after SCI

1.4.1.1. Spasticity after SCI

One form of mal-adaptive plasticity after SCI is the development of spasticity which is classically defined as "a velocity dependent increase in the resistance to passive movement of a limb, which is often associated with hyperactive tendon reflexes" (Lance 1980). The more broadened definition describes spasticity as a "disordered sensorimotor control, resulting from an upper motoneuron lesion, presenting as intermittent or sustained involuntary activation of muscles" (Pandyan et al. 2005). Although spasticity develops after damage to the CNS due to stroke (Ohn et al. 2013), cerebral palsy (Benini and Shevell

2012), multiple sclerosis (Balantrapu et al. 2014) and head injury (Cohen et al. 2009), the spasticity that results after SCI has distinct properties, such as spasms that involve both flexors and extensors, and hypereflexia during movement (Adams and Hicks 2005). Muscle hypertonus, hyperreflexia, clonus and long-lasting cutaneous reflexes in response to non-noxious stimuli are also common clinical symptoms of spasticity after chronic spinal cord injury (D'Amico et al. 2014).

1.4.1.2. Changes in Motoneuron Activation

Motoneurons can maintain repetitive firing for several seconds following a brief (1 s) depolarization as a result of the activation of voltage-dependent persistent inward currents (PICs). PICs produce sustained depolarizations (or plateau potentials) even after the original synaptic input has been terminated. In the intact spinal cord, this depolarization can be terminated by inhibitory synaptic inputs from descending and segmental spinal circuits, which can hyperpolarize the membrane potential sufficiently to remove the activation of PICs. In addition, PICs can amplify weak synaptic inputs to MNs and along with the plateau potentials, provide a powerful augmentation of muscle contractions (Grillner et al. 2000; Lee and Heckman 2001).

After chronic SCI, PICs re-emerge and contribute to the MNs plateau potentials that are large in amplitude (Hounsgaard and Mintz 1988). The plateau potentials can be initiated by low threshold excitation of cutaneous and muscle afferents (Knikou 2010). In spinal rats (Li et al. 2004), cats (Conway et al. 1988) and SCI humans (Gorassini et al. 2004), there is a strong correlation between spasms and plateau potentials, leading to a conclusion that spasms are mediated by the underlying plateau potential. These spasms are key features of the spasticity syndrome which develops in 65-78 % of patients after SCI (Maynard et al. 1990).

Over the weeks following SCI, spinal motor neurons recover their excitability. Two mechanisms have been identified that involve the recovery of

neuronal excitability. The first one is the downregulation of potassium-chloride cotransporter (KCC2) after SCI in MN membranes, which depolarizes the Cl- equilibrium potential and results the reduction in strength of postsynaptic inhibition (Boulenguez et al. 2010). The second is the evolvement of spontaneously active serotonergic receptors 5HT2C (Murray et al. 2010). The hyperactivity of motoneurons is also related to decrease in neuromodulatory substances. For instance, serotonin receptors that mediate PIC activation become constitutively (spontaneously) active following chronic complete and incomplete SCI of rats (Murray et al. 2010) and human (D'Amico et al. 2013).

Chronic SCI can also lead to degeneration, apoptosis, atrophy, and transneuronal changes in the dorsal column and other spinal cord regions (Crowe et al. 1997). For example, in rats and monkeys neurons and glia in the spinal cord undergo apoptosis after SCI (Liu et al. 1997).

1.4.1.3. Changes in Sensory Transmission and Spinal Reflexes

Transmission in the sensory pathways to MNs is modulated by activation of serotonin (5-HT) and norepinephrine (NE) receptors on the primary afferent terminals (for Review see D'Amico et al. 2014). Activation of Gi-coupled serotonin (5-HT1) and norepinephrine (α2) receptors located on afferent terminals and excitatory interneurons normally inhibit transmission of sensory pathways (Jordan et al. 2008).

Following spinal cord injury there is a reduction in 5HT and NE activation on the primary afferent terminal due to the loss or reduction of descending sources of 5HT and NE (D'Amico et al. 2014; Jacobs et al. 2002; Jordan et al. 2008; Murray et al. 2011; Rank et al. 2011), resulting in excessive MN activation from sensory pathways. For instance, SCI leads to changes in the activation of short (oligosynaptic) and long (polysynaptic) latency spinal reflexes (Dietz and Sinkjaer 2007). The early component of spinal reflexes successively gets smaller in amplitude while the late spinal reflex component increases in amplitude following months after SCI. For instance, a single pulse of electrical stimulation

of sacral dorsal roots of acutely-injured rats now evokes long-lasting (>5s) reflex responses or spasms (Li et al. 2004) that are capable of triggering plateau potentials and self-sustained firing, which lead to involuntary muscle spasms.

Furthermore, homonymous and heteronymous pre-synaptic inhibition, which modulates afferent transmission to MNs, is reduced after SCI (Hultborn et al. 1987; Mailis and Ashby 1990). This decrease most likely results from a reduction in descending facilitation of primary afferent depolarizing interneurons (PAD) after SCI (Mailis and Ashby 1990). Following spinal lesion, reciprocal inhibition from extensors to flexors is stronger than normal (Ashby and Wiens 1989). However, reciprocal inhibition from flexors to extensors is reduced after SCI (Yanagisawa 1980). The difference between changes in flexor and extensor reciprocal inhibition after SCI arises from the reduced dominant corticospinal facilitatory effect that is directed more towards flexor motoneurons and their associated Ia interneurons (Brouwer and Ashby 1991). Similarly, recurrent inhibition was increased in participants with SCI (Shefner et al. 1992) when using the homonymous, paired H-reflex technique (Pierrot-Deseilligny and Bussel 1975). However, a reduced amount of recurrent inhibition at rest was reported in spastic SCI patients (Mazzocchio and Rossi 1989). Lastly, the cutaneous reflex pathways also undergo a change in latency after SCI. For example, the latency of the long lasting reflex mediated by the flexor reflex afferents, which include the group II, joint and cutaneous afferents, was increased in SCI participants (Roby-Brami and Bussel 1987).

1.4.2. Cortical Plasticity after SCI

Changes in motor maps (motor cortical representations) have been demonstrated after limb immobilization and amputation (Liepert et al. 1995; Ridding and Rothwell 1995). Similarly, longstanding injuries including SCI tend to also cause extensive cortical map changes. Animal studies of somatosensory cortical reorganization after SCI (Green et al. 1998; Humanes-Valera et al. 2013) have shown that the loss of sensory input from any given body part to the

somatosensory cortex can narrow the area of the topographic representation of that specific body part. Topographical re-organization of the primary motor cortex (M1) after SCI has also been demonstrated in SCI rats (Raineteau and Schwab 2001), where motor cortical territories controlling intact body parts tend to enlarge and invade the area of disused body parts.

In humans, cortical re-organization after SCI had been accessed using brain imaging and TMS. Functional magnetic resonance imaging (fMRI) of the primary somatosentory cortex after complete SCI revealed that tactile stimulation of the body part above the injury caused activation of the cortex that normally represents a body part below the injury level (Perani et al. 2001). Furthermore, positron emission tomography (PET) images show chronic SCI was related to regional changes in metabolism in cortical as well as subcortical regions (Roelcke et al. 1997).

The TMS intensity required to evoke a motor-evoked potential (MEP) was lower above and higher below the lesion (Ellaway et al. 2004) in people with SCI compared to age-matched controls. As shown in the summary table 1, most TMS evoked cortical circuits are altered after SCI (for review see Bailey et al. 2014). In general, after SCI, corticospinal excitability is reduced in both upper and lower limbs as indicated by reduction of the size of MEPs (Curt et al. 1998; Edwards et al. 2013), as well as increase of the threshold of evoking an MEP at rest (RMT) and during active (AMT) contraction (Freund et al. 2011). Short-interval intracortical inhibition (SICI) is also reduced after SCI (Roy et al. 2011) indicating a reduction in cortical inhibitory circuits after SCI.

Structural changes in other subcortical regions are also present after SCI, such as in the thalamus (Ding et al. 2005) and brainstem (Jain et al. 2000). In addition, the Red Nucleus also undergoes reorganization after pyramidal tract lesion. For instance, the rubrospinal system has a prominent excitation on extensor muscles (Lawrence and Kuypers 1968a; b). However, after the unilateral

transaction of CST in monkeys, an almost equal activation and facilitation of extensors and flexors was observed (Belhaj-Saif and Cheney 2000).

The mechanisms of cortical plasticity include "unmasking" of the horizontal connections through reactivation of previously silent synapses. One way this can be achieved is through a decrease of the neurotransmitter GABA (γ-amninobutyric acid) mediated inhibition (Jacobs and Donoghue 1991). Furthermore, changes in synaptic strength through LTP and LTD at the synapses of horizontal collaterals that ends on pyramidal cells can also contribute (Hess and Donoghue 1994; Hess et al. 1994).

Circuit	Mechanism Probed	Conditioning Stimulus (CS)	Test Stimulus (TS)	Interval Between CS and TS (ISI)	Changes in SCI
MEPs	Corticospinal Excitability	N/A	Supra threshold TMS	N/A	Reduced in upper and lower limbs
RMT	Neuron membrane excitability	N/A	MEP>50μV in 5/10 trials from TMS	N/A	Increased in upper and lower limbs
AMT	Number of neurons near threshold	N/A	MEP> 00μV in 5/10 trials from TMS	N/A	Increased in upper and lower limbs
SAI	Afferent regulation of cortex	Supra threshold nerve stimulation	Supra threshold TMS	N20 + ~2-8ms	Unknown at rest Reduced in TA during active
LAI	Afferent regulation of cortex	Supra threshold nerve stimulation	Supra threshold TMS	N20 + ~200ms	Unknown
SP	Intracortical inhibition, transcollosal connectivity	N/A	Subthreshold or Supra threshold TMS	N/A	Contra lateral is reduced, Ipsilateral is unknown
IHI	Interhemispheric inhibition, transcollosal connectivity	Supra threshold TMS	Supra threshold TMS	~10ms (Short) or ~40ms (Long)	Short is normal at rest, altered during active Long is unknown
SICI	Intracortical inhibition	Subthreshold TMS	Supra threshold TMS	~1 - 6ms	Reduced in TA Normal for FDI
LICI	Intracortical inhibition	Supra threshold TMS	Supra threshold TMS	~50 - 200ms	Normal in FDI at rest Increased in FDI during active
ICF	Intracortical facilitation	Subthreshold TMS	Supra threshold TMS	~10 - 25ms	Unknown

Table 1: Summary of TMS evoked motor circuitry in SCI (From Bailey et al. 2014) MEP: Motor Evoked Potential; RMT: Resting Motor Threshold; AMT: Active Motor Threshold; SAI: Short-latency Afferent Inhibition; LAI: Long-latency Afferent Inhibition; SP: Silent Period; IHI: Inter Hemispheric Inhibition; SICI: Short-interval Intracortical Inhibition; LICI: Long-interval Intracortical Inhibition; ICF: Intracortical Facilitation; TMS: Transcranial Magnetic Stimulation; N20: Time for Nerve Pulse to Travel to Cortex; TA: Tibialis Anterior; FDI: First Dorsal Interosseous

1.4.3. Plasticity Associated with Walking Training

After SCI, the appropriate combination of neurons that are activated in a functional sequence is altered. The reorganization following SCI is influenced by the frequency with which the sensorimotor circuits experience the specific

patterns of activity. For instance the repetitive activity of a motor task, such as stepping, over a period of weeks increases the probability of completing a successful step (Barbeau et al. 1987; de Leon et al. 1998b).

Improved motor performance after SCI is highly dependent on motor-training induced biochemical and electrophysiological changes in the spinal cord and in the supraspinal centers of motor control (Cote and Gossard 2004; Cote et al. 2003; Tillakaratne et al. 2002). In Chapter 3 of this thesis, we examine the electrophysiological and clinical changes following two types of gait training of participants with spinal cord injury (SCI). Thus, in this section I briefly outlined animal and human evidence of biochemical and electrophysiological changes following motor training after SCI.

1.4.3.1. Biochemical changes

Glycine and γ-aminobutyric acid (GABA), the two major inhibitory neurotransmitters in the spinal cord, play a role in modulating the spinal circuitry that generates stepping in chronic spinal cats (Edgerton et al. 2001). In a complete preparation, where any EMG pattern was absent from the hind limbs of spinal transected cats, the administration of the glycine antagonist strychnine resulted in a rhythmic EMG bursting pattern (de Leon et al. 1999). Similarly, in adult cats whose pre-drug performance was poor, treadmill locomotion and weight support were improved dramatically by the GABA antagonist bicuculline (Robinson and Goldberger 1986). These results reflect that there is an upregulation of both glycinergic and GABAergic neurotransmitter systems within the spinal cord. However, an increase in GABA may result not in an increase in inhibition but increase in excitation due to reduction in KCC2 activity after SCI in MN membranes. Down regulation of KCC2 results in a positive shift in membrane potential of chloride leading to a change in neuronal function from inhibitory to excitatory (Edgerton and Roy 2010). Training can upregulate KCC2 expression through BDNF-activating Trk receptors, and thus restore endogenous inhibition (Boulenguez et al. 2010).

Similarly, increase in the level of GABAergic neurotransmitter in the spinal cord after SCI can be encountered by motor training (Edgerton et al. 2001; Tillakaratne et al. 2002). The level of persistent inhibition in the neural networks can be reduced by repetition of a specific motor task. These effects have been demonstrated in spinal animals that have been trained to step (de Leon et al. 1998b) or stand (de Leon et al. 1998a). Repetitive use of the extensor musculature may down-regulate the glutamic acid decarboxylase (GAD_{67}) associated with extensor motor neurons. GAD is an enzyme in GABAergic neurons that is used to synthesize GABA. Tillakarantne and colleagues (2000) reported elevated levels of GAD67 protein after a complete transection in cats. Compared to control cats, GAD67 immunoreactivity was higher in the lumbar cords of transected cats, suggesting increased GABA synthesis. However, after 30 minutes/day for 5 days/week bipedal step training, the GAD67 level was reduced significantly. Similarly, Ichiyama and colleagues (2011) demonstrated appreciable injury-induced increase in the ratio of inhibitory to excitatory synapses (F/S boutons) in apposition with both α -MNs and γ -MNs, but this ratio returns to normal in rats that successfully recover stepping in response to locomotor training.

1.4.3.2. Change in reflex pathways: Animal Studies

Chronic SCI can also result in electrophysiological changes which can be modified by repetitive training of a motor task. There was improved coordination of motor pools controlling the hindlimb musculature following step training in spinal animals, as shown by EMG bursting patterns (Lovely et al. 1990). Likewise, step training greatly improved the transmission in polysynaptic excitatory group I load pathways (Cote et al. 2003). Overground walking or stepping on a treadmill consists of swing and stance phases. The majority of the time per step is spent in the stance phase, covering more than 60% of the gait cycle. During this phase, weight support is crucial for successful stepping, and it is reinforced by intriguing mechanisms, such as reflex reversal. This occurs when Ib inhibition (negative feedback) in extensors is replaced by excitation (positive feedback) (Gossard et al. 1994). The reflex reversal only happens during

locomotion (Stephens and Yang 1996) or after injection of drugs such as L-dopa (Gossard et al. 1994) or clonidine (McCrea et al. 1995). Therefore, sensory feedback from load receptors in the legs, the crucial source of input that the transected spinal cord can use to trigger recovery, has an influential effect on the activity of the CPG for locomotion (Cote et al. 2003).

Cote and her colleagues (2003) investigated the change in transmission of spinal proprioceptive pathways, more specifically group Ia and Ib pathways, from extensors of spinalized cats after weight-supported treadmill training with and without injection of clonidine. Clonidine is an alpha-2 (α2) adrenergic receptor agonist, which is a G-protein coupled receptor associated with the Gi subunit that inhibits the production of cAMP from ATP (Birnbaumer 2007), thereby resulting in reduced transmitter release. Clonidine has also been reported to depress activity of both Ia- and Ia/Ib-inhibitory interneurons (Hammar and Jankowska 2003). Three spinal proprioceptive pathways were studied: the monosynaptic pathway from group Ia afferents originating in muscle spindles of extensors, the disynaptic inhibitory pathway from group Ib afferents of extensors originating in Golgi-tendon organs, and the polysynaptic excitatory pathway from group Ib and Ia afferents of extensors (Cote et al. 2003). Clonidine injection significantly decreased transmission in disynaptic pathways without affecting monosynaptic transmission. Since α2 receptors have been reported to be found on some spinal interneurons and on some primary afferent terminals (Hammar and Jankowska, 2003), the reduction of disynaptic inhibition by clonidine might be mediated by actions on primary afferents as well as on neurons contacted by them, and the resulting depression may reflect the sum of the modulatory actions at both of these locations (Hammar and Jankowska, 2003). However, in comparison, treadmill training resulted in a reduction in both monosynaptic excitation and disynaptic inhibition. The former is due to training increasing presynaptic inhibition onto Ia terminals in the ventral horn (Cote et al. 2003). Training enhances the decrease in Ib inhibition after clonidine injection. This

disappearance of disynaptic inhibition precedes the appearance of polysynaptic excitation and its enhancement with training.

1.4.3.3. Change in reflex pathways: Human Studies

Locomotor training by using body-weight support treadmill training (BWSTT), in which lower extremities are partially unloaded by a harness, results an improvement in walking function in individuals with incomplete SCI (Field-Fote 2001; Field-Fote and Tepavac 2002; Musselman et al. 2009). Task specific sensory experience, control of limb coordination and speed, as well as, postural and weight bearing conditions are advantages of using BWSTT (Field-Fote 2009). Unlike overground walking with the use of braces and load bearing assistive devices, BWSTT minimizes compensatory gait strategies (Hicks and Ginis 2008). Overground walking training differs considerably from BWSTT because gravity has its full effect without speed and repetitive sensory contact. Both training types can be modified to adjust the involvement and engagement of peripheral and central nervous system, resulting in plasticity in all (spinal, superspinal and cortical) levels. Furthermore, rehabilitation training, in combination with pharmacological treatments, can promote the repair of injured spinal cord (reviewed in Fouad and Tetzlaff 2012).

The recovery of walking in individuals with SCI from motor training is associated with the strengthening of the CST (Thomas and Gorassini 2005) and activity dependent plasticity of spinal (Edgerton and Roy 2009) and cortical networks (reviewed in Sanes and Donoghue 2000). Most of the neural plasticity induced by training is thought to be mediated by the sensory stimulation that is generated during gait training (Fouad and Pearson 2004; Harkema 2001; Wernig et al. 1995; Yang et al. 1991). Sensory information that matches the kinematics and kinetics properties associated with speed of walking, loading-unloading patterns, change in joint angles, as well as trunk and head positions is generated during different phases of stepping (Harkema 2001). This information can be integrated at the spinal and cortical level and induce activity-dependent plasticity.

Extensive sensory information processing occurs at the spinal cord level, which can facilitate locomotion. However, unlike other mammals, following human complete SCI, recovery of locomotion has been unattainable (Manella et al. 2010).

1.5. Control of Walking

Locomotion is a complex process that requires continuous interaction between central and peripheral nervous system circuits. Animal and human studies have advanced our knowledge of the control mechanisms of movement at spinal, supraspinal and cortical levels. In the paragraphs below, I outlined mechanisms of sensory and cortical influence on spinal locomotion circuits which are important in regards to Chapter 2 and 3 examining changes in sensory and descending activation of spinal cord after walking training.

1.5.1. Central Pattern Generator (CPG)

The role of spinal circuits can be studied by transecting the central nervous system at two selected points. To examine the influence of the spinal circuits in isolation from the brainstem, the thoracic level is transected (spinal preparation). To isolate the spinal circuits from the cerebral cortex, the midbrain level may be transected (decerebration preparation). After eliminating the influence of higher brain centres through spinalization and decerebration, Sherrington (1898) found that the monkey's hindlimbs continued to exhibit alternating movements. Similarly, Graham Brown (1911) showed that deafferented (sensory input to the spinal cord removed) spinal animals could exhibit rhythmic walking movements, and proposed the existence of 'half centres'. He described half centres as flexor and extensor spinal circuits that were wired to mutually inhibit each other. Later on, two historic experiments done by Jankowska & Lundberg (1967a) and (Shik and Orlovsky 1965) also used spinal and decerebration preparations to demonstrate the possibility of generating rhythmic stepping movement without rhythmic command signals from supraspinal structures or sensoryafferents. Jankowska & Lundberg (1967) showed that locomotor-like rhythm could be

evoked in spinal cats by administering a monoaminergic agent, such as L-Dopa (levodopa, 3, 4-dihydroxy-L-phenylalanine), to mimic the activity of neurotransmitters from the supraspinal center. Similarly, Shik and Orlovsky (1965) showed that it was possible to make decerebrated cats walk on a treadmill by electrical stimulation of their mesencephalic locomotor region (MLR). Altogether, the above mentioned studies lead to the conclusion that hindlimb rhythmic activities were controlled by spinal 'intrinsic factors', which later were named 'central pattern generators (CPGs)' by Grillner (1985). CPGs are neural circuits that generate periodic motor commands for rhythmic motor pattern production without rhythmic sensory or descending input. To be classified as a rhythmic generator, a CPG requires two or more processes to interact such that each process sequentially increases and decreases. As a result of this interaction, the system repeatedly returns to its starting condition (Hooper 2000).

Many experiments have been performed in different species (lamprey, birds, fish, quadrupeds, and humans) to localize and characterize the basic neuronal machinery of locomotor CPGs (reviewed in Guertin 2012). Some studies have proved that the vertebrate CPG is fairly localized (Cazalets et al. 1995). On the contrary, other studies claimed a more distributed system where a series of semi-autonomous oscillators were dynamically connected by propriospinal pathways (Yakovenko et al. 2002). Overall, most studies seem to agree that rhythmogenesis (the cellular and ionic mechanisms that generate the rhythm in the CPG) is stronger in one or two spinal cord segments just rostral to the lumbosacral enlargement than in more caudal segments. (Deliagina et al. 1983; Kiehn and Kjaerulff 1998; Marcoux and Rossignol 2000). These rostral segments may lead the other segments in generating the locomotor rhythm. Despite significant evidence of the CPG in other animals and primates, only indirect evidence is used to explain the existence CPGs in humans. In contrast to animal studies, it is difficult to isolate the effect of CPG (with no descending and sensory inputs) *in vivo* in humans (Guertin 2014). Indirect evidence for the presence of CPGs includes spontaneous rhythmic patterns of EMG activity resembling

bipedal stepping in patients with incomplete (Calancie et al. 1994) and complete (Calancie 2006; Nadeau et al. 2010) spinal cord injury (SCI). For instance, in people with a motor complete SCI, electrical epidural stimulation (5 -50Hz frequency) induced rhythmic activity of limbs, and initiated step-like movements (Jilge et al. 2004; Minassian et al. 2004).

1.5.2. Sensory Influence on CPG

Sensory inputs are important for modulating and adapting motor output generated through CPG circuits. Proprioceptors, which are located in muscles and joints, are involved in the automatic regulation of stepping, such as duration of the stance phase, whereas exteroreceptors, which are located in the skin can adjust stepping to external stimuli (even with only the spinal cord left). During locomotion or fictive locomotion (motoneurons firing that could result in the movement pattern if the motoneurons were still attached to the muscles), group I afferents (inputs from Ia and Ib) from leg muscles can increase extensor activity as well as reset CPG-mediated step cycle to a new extensor phase (Guertin et al. 1995). Strengthening of extension is also mediated by group II inputs from flexors (Perreault et al. 1995). Similarly, Jankowska and coworkers (1967a; b) demonstrated that flexor-reflex afferents (FRA) can reset the step cycle to a new flexion. Stimulation of some specific afferents, such as the sacral nerve, is capable of triggering CPG-mediated activity (see review Guertin 2012).

Sensory inputs can also alter locomotor rhythm in a phase dependent manner. In a characteristic phenomenon known as reflex reversal (Yang and Stein 1990; Zehr and Duysens 2004) the same stimulus excites one group of motoneurons during one phase of locomotion and excite the antagonist motor neurons during another phase. A specific example of this phenomenon is seen in cats. When electrical stimulation was given at the dorsal paw in cars during swing phase, it produced flexion and inhibited the extension (Forssberg et al. 1975). Interestingly, when the same stimulus was given at the same location during stance, extensors are reinforced. In human, similar phase dependent modulation

was observed, in which the sign of the reflex changed from excitation of muscles in one phase of walking to inhibition of the same muscles in another phase (Duysens et al. 1990; Yang and Stein 1990). Moreover, sensory input can influence the walking cycle through a transcortical loop. The cerebellum receives information from afferents in order to modulate the step cycle. The two ascending tracts, the dorsal and ventral spinocerebellar, send information concerning CPG output from muscle afferents and spinal neurons to the cerebellum (Arshavsky et al. 1972a; b).

1.5.3. Descending Influence on CPG

Descending signals from supraspinal centers can activate, refine and guide locomotor activity. Compared to other vertebrates, CPG networks controlling human gait have more extensive supraspinal influence. In the spinal preparation of animals, electrical or pharmacological stimuli are needed to produce locomotor behaviours (Pearson and Gordon 2000). Descending signals that originate from the brainstem and travel via reticulospinal pathway can initiate locomotion (Douglas et al. 1993). Decerebrated cats, whose cerebellum, brainstem and spinal cord were intact, could not normally walk on a treadmill, but walked normally after tonic electrical activation of the MLR (Shik et al. 1966). The MLR activated the medial reticular formation. Neurons in the medullary reticular formation, whose axons descend in the ventrolateral region of the spinal cord, can be excited by stimulation of the MLR. Transection of axons of these neurons (ventrolateral funiculus of spinal cord) prevented MLR stimulation from initiating movement (Mori et al. 1992). In addition, in decerebrate cats intrathecal administration of glutamate receptor agonists in the spinal cord prevented initiation of locomotion when MLR was simulated (Douglas et al. 1993). Therefore, to activate locomotion and control its speed, the signals are transmitted to the spinal cord by glutaminergic neurons from the MLR.

About half of the one million CST axons originate from the motor cortex (Latash 2008). TMS studies demonstrated the direct role of motor cortex for

locomotion by examining the ankle muscles during treadmill walking (Capaday et al. 1999). The SOL MEP during stance phase of walking was 26% less compared to the SOL MEP during voluntary ankle plantarflexion. On the contrary, during stance phase, MEPs in the inactive TA were enhanced compared to the TA MEPs during voluntary ankle plantarflexion. These results suggest a relative involvement of supraspinal mechanisms to the motor pattern of human leg movement (Capaday et al. 1999; Petersen et al. 1998). Subthreshold TMS during walking also provided evidence that the motor cortex actively drives the motoneurones of the leg muscles examined during human locomotion (Iglesias et al. 2012).

In addition to CST, the rubrospinal tract, which originates in the red nucleus of the midbrain, can influence MNs of lower limb, mainly flexor, muscles (Knierim 1996). The overlap between CST and rubrospinal pathways provides potential compensation following locomotion after SCI (Lawrence and Kuypers 1968b). Furthermore, descending tracts of the vestibulospinal pathway that originate in the vestibular nucleus, pons and medulla play a role in antigravity balance and postural control during locomotion (Knierim et al. 1996). Furthermore, damage to the cerebellum alters the normal pattern of locomotor movements including the speed and range of movements (Topka et al. 1998), implying that descending signals from cerebellum are crucial for functional locomotion.

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CHAPTER 2. SLONG-LATENCY, INHIBITORY SPINAL PATHWAY TO ANKLE FLEXORS ACTIVATED BY HOMONYMOUS GROUP 1 AFFERENTS

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2.1. Introduction

Inhibitory networks in the spinal cord are important for controlling the force, timing and ultimately, the precision of movement (reviewed in Pierrot-Deseilligny and Burke 2005, Chpt. 11). Feed-forward control of spinal inhibitory interneurons comes from descending motor commands whereas feedback control comes from the afferents activated during movement. The interaction between descending and peripheral inputs onto spinal inhibitory networks can be studied by examining the suppression of spinal reflexes by transcranial magnetic stimulation (TMS) to the primary motor cortex or alternatively, the suppression of cortically-evoked responses by afferent nerve stimulation. For example, the short-latency, short-lasting suppression of the soleus H-reflex by TMS is thought to be mediated by pre-motoneuronal, Ia reciprocal inhibitory interneurons that are activated by the corticospinal tract innervating the antagonist, tibialis anterior (TA) (Kudina et al. 1993; Nielsen et al. 1993).

Inhibitory interneurons that are located more upstream from the motoneuron have also been examined. For example, group I afferents from the common peroneal nerve (CPN) supplying the TA muscle converge, along with corticospinal inputs, onto inhibitory interneurons that synapse onto lumbar propriospinal neurons, the latter with projections onto the quadriceps motoneuron pool (Marchand-Pauvert et al. 1999). This was evidenced by the strong suppression of quadriceps motor evoked potentials (MEPs) by a prior low-intensity stimulation of the CPN at interstimulus intervals of 4 to 11 ms, latencies consistent with the activation of propriospinal pathways. Suppression of MEPs by heteronymous afferents can also occur at much longer intervals between TMS and peripheral nerve stimulation. For example, suppression of the TA MEP by stimulation of the tibial nerve at the ankle (Roy and Gorassini 2008) or suppression of the soleus MEP by CPN stimulation (Geertsen et al. 2011) occur at interstimulus intervals near 35-45 ms. This long-latency suppression is thought to be mediated by spinal inhibitory interneurons synapsing onto lumbar

propriospinal neurons that receive convergent inputs from heteronymous group 1 sensory and feedforward corticospinal inputs (Geertsen et al. 2011).

The long-latency inhibition of motoneurons from these upstream, inhibitory interneurons has only been shown for heteronymous pathways (Geertsen et al. 2011; Iglesias et al. 2008; Roy and Gorassini 2008;). Here we refer to "homonymous" as afferents originating from the tested muscle and "heteronymous" as afferents originating from surrounding or antagonist muscles (cf Pierrot-Deseilligny and Burke 2005). In this paper we show properties of a long-latency, spinal inhibitory pathway to the TA motoneuron pool that is activated by low-threshold afferents from the homonymous CPN, which may be involved in regulating or damping the activation of ankle flexor muscles by the corticospinal tract. The CPN was activated using a train of 3 pulses delivered at various intervals before the TMS pulse and at very low stimulation intensities ($\leq$0.7 x motor threshold). This triple-pulse of stimulation was below the threshold of recruiting either the M-wave or H-reflex so we were able to examine inhibition of the TA MEP without effects from motoneuron refractoriness or recurrent inhibition. The purpose of these experiments was to: 1) characterize the effect of stimulating the CPN afferents over a range of intensities on the suppression of the TA MEP and voluntarily-activated electromyogram (EMG), 2) determine if cutaneous afferents contribute to the TA MEP suppression and 3) determine the site of origin (e.g., cortical, sub-cortical and/or spinal) of the MEP suppression.

2.2. Methods

All experiments were carried out with the approval of the Human Research Ethics Board at the University of Alberta and with informed consent of the participants. Our sample comprised of 14 neurologically intact control participants (9 male) ranging in age from 19 to 58 years (30.5 ± 10.4, mean ± SD).

2.2.1. EMG Recordings and Peripheral Nerve/Skin Stimulation

A pair of Ag-AgCl electrodes (Kendall; Chicopee, MA, USA, 3.8 cm by 2.2 cm) was used to record surface EMG from the TA and soleus muscles with a ground electrode placed on the patella. The EMG signals were amplified by 1000 and band-pass filtered from 10 to 1000 Hz (Octopus, Bortec Technologies; Calgary, AB, Canada) and then digitized at a rate of 5000 Hz using Axoscope 10 hardware and software (Digidata 1400 Series, Axon Instruments, Union City, CA). The full-wave rectified and smoothed EMG (100 ms time constant, Neurolog NL703 - EMG Integrator, Digitimer UK) from the TA muscle was displayed on an oscilloscope screen. To measure the EMG from a maximum voluntary contraction (MVC), participants were seated in a chair with their ankle and knee joints positioned at 90º. The foot was strapped to a metal plate for isometric dorsiflexion and the knee was held stable by strapping. Participants were given verbal encouragement and a visual display of their rectified and smoothed TA EMG to help them contract as hard as possible. Contraction trials were repeated until at least 2 consistent peak EMG levels, varying less than 10%, were held for at least 1 s. Unless otherwise stated, all responses were collected during a tonic dorsiflexion of 10% MVC.

Peripheral nerves and skin were electrically stimulated using a constant current stimulator (1-ms rectangular pulse; DS7A, Digitimer, Welwyn Garden City, UK) in a bipolar arrangement with Ag-AgCl electrodes (Kendall; Chicopee, MA, USA, 2.2 cm by 2.2 cm).

2.2.2. Conditioning of TA MEPs by incrementing intensities of CPN stimulation

The effect of conditioning the TA MEP by a prior stimulation of the homonymous CPN was examined in 12 participants. MEP responses were evoked in the right TA muscle which was the dominant leg in all participants. Transcranial magnetic stimulation (TMS), inducing posterior-anterior currents in the motor cortex, was delivered to the contralateral motor cortex using a custom-

made figure-of-eight batwing coil (P/N 15857; 90mm diameter) that was connected to a Magstim 200 stimulator (Magstim; Dyfed, UK). First, the hotspot for the TA muscle was determined while evoking MEPs that were ≈300μV in size while the participant maintained a background contraction of 10% MVC. Typically, the hotspot for the TA muscle was 1 to 2 cm lateral and 0 to 2 cm posterior or anterior to vertex. A TA MEP amplitude of ≈500μV was used as the unconditioned, test response which is near half of the maximum TA MEP in uninjured, healthy controls (Roy and Gorassini 2008). The TA MEP was then conditioned by stimulating the CPN at interstimulus intervals (ISIs) of 40, 50 and 60 ms (triple-pulse stimulation) before the TMS. The CPN was stimulated in increasing order at intensities of 0.1, 0.2, 0.3, 0.5, 0.7, 0.9 and 1.1 x's motor threshold (xMT) for a total of 7 intensities of stimulation. Motor threshold was determined with single pulse stimulation and determined as the current intensity producing a discernible and reproducible direct motor response (M-wave) at rest. Bipolar stimulation was used to stimulate the CPN with the cathode placed below the neck of the fibula after the optimal site to produce small isolated contractions of the TA muscle, with minimal to no peroneal muscle activation, was identified. The anode was placed 2 cm proximal to the cathode near the outer edge of the popliteal fossa. At each of the 7 intensities of CPN stimulation, 2 unconditioned TA MEPs (14 in total) and 6 conditioned TA MEPs (42 in total) were evoked in random order. In 9 of the 12 participants, single-pulse CPN stimulation was also tested at an ISI of 40 ms at the same intensities used for the triple-pulse stimulation. All conditioned MEPs were measured peak-to-peak and expressed as a percentage of the unconditioned, test MEP. The amount of MEP suppression at a given intensity was averaged across all participants.

We measured the onset latency when the conditioned MEP became smaller than the unconditioned test MEP. This was done to determine if the inhibition occurred directly onto the TA motoneurons (i.e., conditioned MEP was suppressed immediately at its onset) or if the inhibition occurred a few synapses upstream from the TA motoneurons (i.e., suppression of MEP was delayed by a

few ms, as per Chaix et al. 1997). To better reveal the differences in the conditioned and unconditioned MEP from the background EMG, the cumulative sum (CUSUM) of the rectified MEP was first calculated (Brinkworth and Turker 2003) using the following equation:

$$CUSUM\ (t) = \sum_{0}^{t} |EMG\ (t)| - mean(|bkdEMG\ |)$$

where EMG(t) is the EMG at the time t in milliseconds, and mean(|bkdEMG|) is the average of the rectified EMG measured 100 - 200 ms prior to TMS or CPN stimulation. The TMS pulse was delivered at $t = 0$. Because the data was sampled at 5000 Hz, time t was increased in 0.2 ms increments. A CUSUM was calculated for the test MEP (average of 14 trials) and the average of the conditioned MEP at 0.5 and 0.7 x MT (average of 12 trials) by applying the above equation to the EMG at 10 to 70 ms post TMS, blanking out the TMS stimulation artifact within the first 10 ms. The 0.5 and 0.7 x MT trials were both used because they had the most consistent MEP suppression. To compare the latency of MEP suppression when antidromic activation of the motoneuron and recurrent inhibition were strongly activated (Meuneir et al. 1994), in 5 subjects we also stimulated the CPN at 1.5 x MT (average of 6 trials). Here we predicted that the latency of MEP suppression would be earlier given that the motoneuron would be inhibited by shorter pathways. The difference between the CUSUMs of the test and conditioned MEPs (Δ CUSUM) was also calculated. The onset of the MEP was best determined visually from the unrectified EMG. The latency when the conditioned MEP began to deviate from the test MEP was measured at the start of the steep slope in the Δ CUSUM line (see Figure 2.2) and also compared to when the unrectified MEP and CUSUM traces began to deviate from one another.

2.2.3. Time course of TA EMG and MEP suppression by CPN stimulation

The effect of a triple-pulse CPN stimulation on voluntary EMG activity generated in the TA muscle was examined in 8 participants, all of whom participated in Experiment 1. The CPN was stimulated with 3 pulses (10 ms ISI) every 3 seconds for 40 trials while the participant maintained a steady, dorsiflexion of 10% MVC using visual feedback as described above. The stimulation intensity to the CPN was adjusted so that there was a clear suppression of the voluntary TA EMG that was not preceded by a prior excitatory H-reflex or M-wave. On average, the stimulation intensities used were between 0.2 to 0.7 xMT (0.44 ± 0.16 xMT). To determine the duration of the EMG suppression, the onset and offset of the suppression was measured as the time points where the rectified and smoothed EMG (2nd order butterworth filter at 200 Hz low-pass) fell below (for onset) and then crossed above (for offset) a level that was 3x's the standard deviation of the EMG measured -200 to -10 ms before the CPN stimulation. To measure the average background EMG suppression across the 8 participants, the rectified and smoothed EMG data was binned every 5 ms and the average value for each bin was then averaged across the 8 participants. For each participant, either the 0.5 xMT or 0.7 xMT trial was used, whichever intensity resulted in the largest EMG suppression.

The time course of the suppression of the TA MEP was also examined in more detail by systematically varying the ISI of the triple-pulse CPN stimulation in these same participants. Triple-pulses with ISIs starting at 10, 20, 30 ms were gradually increased to triple-pulses with ISIs of 45, 55, 65 ms in steps of 5 ms (e.g., 10/20/30 ms, 15/25/35 ms ... to 45/55/65 ms) for a total of 8 different ISIs. Again, stimulation intensities were used that did not produce an early, short latency excitatory reflex or M-wave with an average stimulation intensity of 0.54 ± 0.1 xMT. At each ISI, 2 unconditioned MEPs (16 in total) and 8 conditioned MEPs (64 in total) were evoked in random order.

2.2.4. Conditioning of TA MEPs evoked by Cervicomedullary and Spinal Cord Stimulation

To examine if the site of MEP suppression by the prior CPN stimulation occurred at a spinal site, we tested if MEP suppression still occurred when the axons of the corticospinal tract were activated directly at the level of the pyramidal decussation or at the level of the thoracic spinal cord. Cervicomedullary motor evoked potentials (CMEPs) were elicited in 3 participants (two of whom also participated in Experiments 1 and 2), with a double cone coil placed behind the inion in two participants (as per Taylor and Gandevia 2004) and with electrical transmastoid stimulation in the third participant because TMS at the inion did not produce CMEPS in this participant. Because of the shorter conduction time from the site of brainstem stimulation to the lumbar spinal cord (≈ 5ms), the CMEP was conditioned with a prior triple-pulse CPN stimulation at ISIs of 45, 55 and 65 ms, again using intensities that were below excitatory reflex threshold in these participants. Ten unconditioned and 10 conditioned CMEPs were evoked every 8s. To activate the axons of the corticospinal tract at the level of the thoracic spinal cord, in 2 of these 3 participants on a separate experiment day, a cathode comprising of a gel electrode （Kendall; Chicopee, MA, USA, 3.8 cm x 2.2 cm) was placed between the T3 and T4 vertebrae and an anode was placed 10 cm above the cathode at approximately C7 (as per Martin et al. 2008). The spinal cord was then electrically stimulated using a high-voltage, multipulse-current stimulator (Digitimer, D185-Mark IIa; UK) with currents of 1.3 and 1.6 amps. The SMEP was then conditioned by a prior triple-pulse CPN stimulation at ISIs of 50, 60 and 70 ms at intensities below excitatory reflex threshold (≤ 0.9 xMT) in these 2 participants. Again, longer ISIs were used to account for the shorter conduction time from spinal stimulation to the lumbar spinal cord (≈10 ms). Five unconditioned and 5 conditioned CMEPs were evoked every 8s. In the same respective experiment, suppression of the

CMEP or SMEP was compared to suppression of the MEP evoked by TMS over the motor cortex.

2.2.5. Conditioning TA MEPs by skin stimulation

Suppression of the TA MEP could occur at very low intensities of CPN stimulation, sometimes as low as 0.2-0.3 xMT. To determine if activation of afferents innervating the skin just underneath the stimulating electrode contributed to the MEP suppression or if sensory axons activated within the CPN were responsible, the anode was moved 4 cm away from its position over the CPN closer to the midline of the shin but within the sensory dermatome of the CPN (see 'OFF Nerve' location in Fig. 5). Six of the 12 participants from Experiment 1 participated in these experiments. Similar to Experiment 1, stimulation intensities of 0.1, 0.2, 0.3, 0.5, 0.7, 0.9 and 1.1 x MT were used with triple-pulses at ISIs of 40, 50 and 60 ms with 2 unconditioned (14 in total) and 6 conditioned (42 in total) MEPs per stimulation intensity. In the same experiment, suppression of the TA MEP was also confirmed by placing the cathode over the CPN ('ON Nerve' location).

The skin between the first and second toes, which is innervated by the deep peroneal nerve (DPN), the cutaneous branch of the CPN, was also stimulated to further determine if cutaneous afferents contributed to the MEP suppression. In 6 of the 12 participants from Experiment 1, the cathode was placed on the lateral side of the first toe and the anode was placed at the base of the first and second toe (see Fig. 5 for location of stimulating electrodes, 2.2 cm x 2.2 cm). The skin was stimulated with a triple-pulse (1-ms wide) train at ISIs of 45, 55 and 65 ms to compensate for the longer time it takes the afferent signal to reach the spinal cord when travelling from the foot compared to the knee. In this way, the afferent signal reached the spinal cord at the same time relative to the arrival of the descending activation of the spinal cord from TMS as in the CPN experiment. Stimulation intensities at 0.1, 0.3, 0.5, 1.0, 1.5, 2.0 and 2.5 x sensory perception threshold (PT) were used to determine maximum suppression of the TA MEP.

2.2.6. Statistical Analysis

To compare the effect of CPN stimulation on the TA MEP at the various stimulation intensities (Experiment 1) or ISIs (Experiment 2), a one-way ANOVA for repeated measures was used with post hoc Student's t-tests (Bonferroni-corrected) to determine the stimulation intensities or ISIs where the conditioned MEP was smaller than the unconditioned, test MEP. All statistical analyses were performed using SPSS20 software. Single and triple-pulse CPN stimulation (Experiment 1) and ON and OFF nerve stimulation (Experiment 4) were compared using two-way repeated measures ANOVA, treating the stimulation type (single or triple pulse, ON and OFF nerve) and stimulation intensity (0.1, 0.2 xMT, etc) as within subject factors. A paired Student's t-test was used to compare the effect of CPN stimulation on the MEP and CMEP (Experiment 3) and on the effect of DPN and CPN stimulation on the unconditioned, test TA MEP (Experiment 4) because the data were normally distributed. Data are presented in figures as mean ± SE and in the text as mean ± SD unless otherwise stated. Significance was set at $P < 0.05$.

2.3. Results

2.3.1. Conditioning of TA MEP by incrementing intensities of CPN stimulation

Conditioning of the test TA MEP by a prior triple-pulse stimulation to the homonymous CPN at ISIs of 40/50/60 ms decreased the amplitude the MEP starting at a stimulation intensity of 0.2 xMT and peaked at an intensity of 0.7 xMT, as shown for a single participant in Figure 2.1A. Here, an M-wave or H-reflex from triple-pulse CPN stimulation was not seen until 1.1 xMT. In the 12 participants examined there was a significant main effect on the conditioned MEP, expressed as a percentage of the test MEP, at the various CPN stimulation intensities used (ANOVA: $F(7,11) = 9.56$, $P<0.001$, Fig. 2.1B). Post-hoc analysis revealed that the conditioned MEP was significantly reduced in size compared to the test MEP at stimulation intensities between 0.5 to 1.1 xMT (all $P \leq 0.001$).

A visible M-wave or H-reflex appeared only at stimulation intensities between 0.9 to 1.1 xMT. As evidence of this the mean amplitude of the rectified EMG, measured during the time period where an expected M or H-wave should be, remained within ± 5% of the pre-stimulus mean EMG for stimulation intensities ≤ 0.7 xMT, but not at the 0.9 or 1.1 xMT intensities. Thus, we only considered MEP suppression at stimulation intensities of 0.7 xMT or less to rule out effects from recurrent inhibition or motoneuron refractoriness. When averaged across the 0.5 and 0.7 xMT stimulation intensities (Fig. 2.1B), the TA MEP was reduced by 32.7 ± 14.7%. In contrast to the triple-pulse stimulation, single-pulse stimulation of the CPN, at an ISI of 40 ms (open circles, Fig. 2.1C), produced little to no effect on the TA MEP at low intensities of stimulation (≤ 0.7 xMT), as tested in 9 of the 12 participants on a different experiment day. There was a significant interaction with the number of pulses used (single vs. triple) and stimulation intensity (two-way repeated measures ANOVA: $F(7,8) = 2.665$, $P = 0.036$), with post-hoc analysis revealing larger TA MEP suppression at 0.5 and 0.7 xMT with the triple-pulse stimulation. Similar results were found when using single-pulse stimulation at ISIs of 50 and 60 ms (data not shown). The onset latency when the conditioned MEP became smaller than the test MEP was measured as demonstrated for a single uninjured control participant in Figure 2.2 (unconditioned MEP: gray traces, conditioned MEP: black traces). To better visualize the differences between the unconditioned and conditioned MEP, the CUSUM of the rectified MEPs were calculated (middle panel, see Experiment 1 in Methods for details) and subtracted from one another to give a Δ CUSUM (bottom panel). The onset of the MEP was first determined visually from the unrectified EMG (top graphs, marked by solid vertical line) and the latency when the test and conditioned MEPs began to deviate from one another was determined as the start of the steep slope in the Δ CUSUM line (marked by dotted grey line).

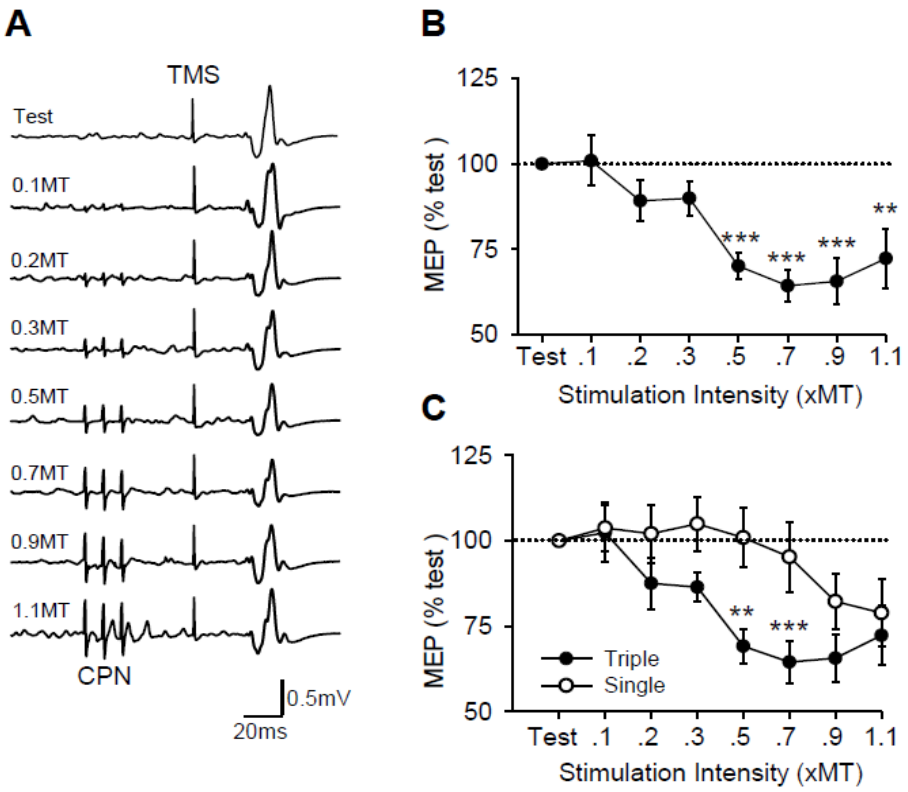

Figure 2.1. Suppression of TA MEPs by CPN nerve stimulation: uninjured controls **A)** Average of 14 unconditioned (Test) MEPs and 6 conditioned MEPs at each incrementing intensity of CPN stimulation from a single uninjured control participant. Three pulses of CPN stimulation were given at 40/50/60 ms prior to the TMS. **B)** Average peak-to-peak amplitude of the conditioned MEP expressed as a percentage of the test MEP (average test MEP amplitude: 538.6 ± 159.3μV) at each of the stimulation intensities: data from 12 uninjured controls. **C)** Comparison of MEP suppression from single-pulse (at ISI = 40 ms, open circles) and triple-pulse (ISI = 40/50/60ms, solid circles) stimulation of CPN in 9 of the 12 control participants (single-pulse test MEP: 511.32 ± 184.3μV; triple-pulse test MEP: 550.90 ± 176.7μV). Error bars represent mean ± SE. Asterisks in **(B)** indicate significant difference between conditioned MEP and unconditioned MEP (100%) and in **(C)** between triple and single-pulse stimulation. ** $P < 0.01$ and *** $P < 0.001$.

The latency between the onset of the TA MEP and the start of the steep slope in the Δ CUSUM was 3.7 ms for this participant when the conditioning CPN stimulation was sub-motor threshold (average of 0.5 & 0.7 xMT, Fig. 2.2A). When the stimulation intensity in this same participant was increased to supra-threshold levels to evoke direct inhibition of the TA motoneurons by recurrent inhibition and from motoneuron refractoriness, the conditioned MEP was suppressed at its onset with a 0 ms delay (Fig. 2.2B). In the 12 participants from Experiment 1, the latency from the start of the MEP to when the conditioned MEP began to deviate from the test MEP was 4.2 ± 3.1 ms on average for the 0.5 and 0.7 xMT stimulation intensities. This is in contrast to the conditioned MEP at 1.5 xMT (supra-threshold) where a large M-wave and H-reflex were present and where the conditioned MEP was suppressed earlier at 0.2 ± 0.3 ms after the onset of the MEP (Mann-Whitney Rank Sum Test, $P = 0.008$, $n = 5$).

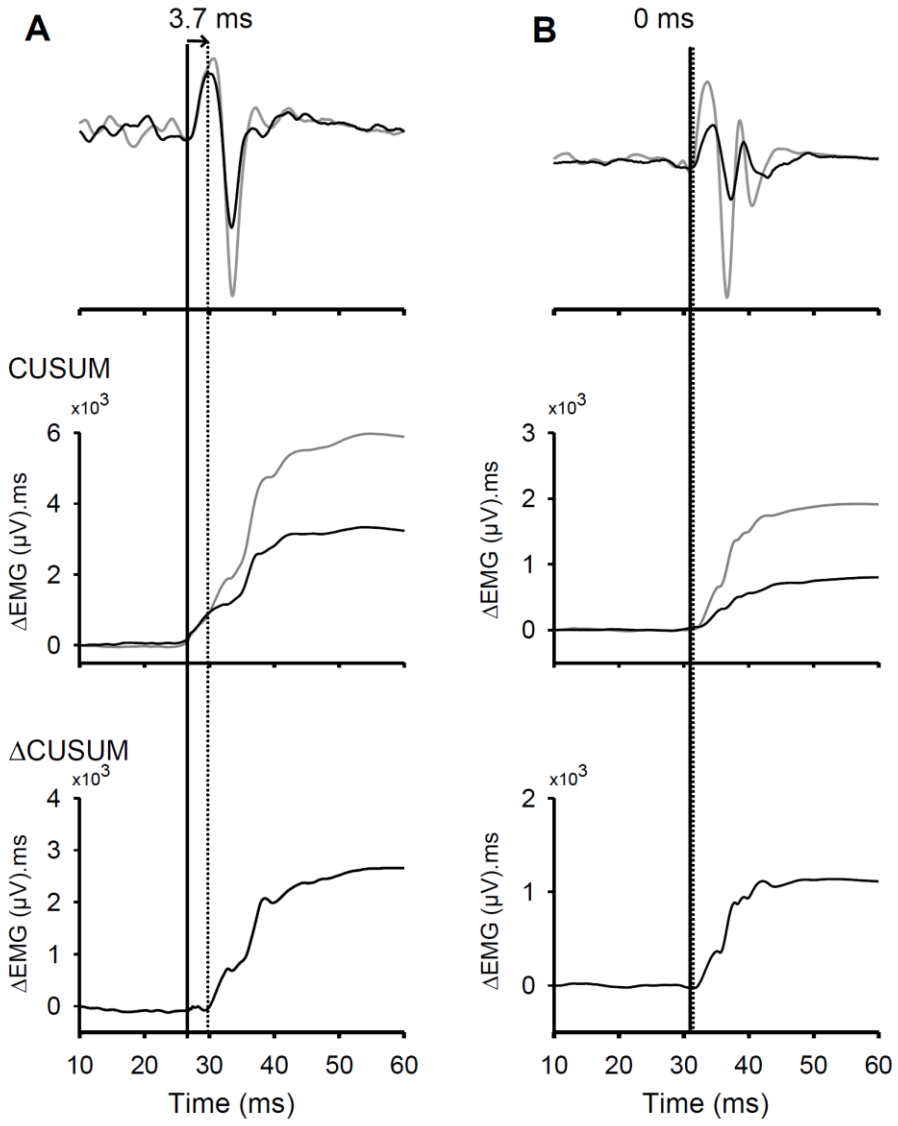

Figure 2.2. Latency of TA MEP Suppression by CPN stimulation
A) *Top graph***:** Average of 14 unconditioned test MEPs (gray trace) and 12 CPN-conditioned MEPs (average of 0.5 & 0.7 xMT stimulation trials; black trace) in a single participant. Onset of MEP is indicated by black vertical line and dotted vertical line indicates when the conditioned MEP begins to deviate from the test MEP. *Middle graph:* CUSUM of the unconditioned test MEP (gray trace) and CPN-conditioned MEP (black trace). *Bottom graph:* Difference between the test and conditioned MEP CUSUMs (Δ CUSUM). Note the dotted vertical line marks the start of the steep slope of the Δ CUSUM when the test and conditioned MEPs begin to deviate from one another. **B**) Same as in (**A**) for the same control participant but using a CPN-conditioning stimulation intensity of 1.5 xMT to activate recurrent inhibition of the TA motoneurons. The TMS pulse occurred at time 0 (not shown).

2.3.2. Time course of TA EMG and MEP suppression by CPN stimulation

Triple-pulses of low-intensity stimulation to the CPN also suppressed voluntary EMG activity in the TA muscle. As shown for 3 participants, suppression of TA EMG activity below the mean background (grey shaded areas in Fig. 2.3A, average of 40 trials) occurred without a prior excitatory reflex response at stimulation intensities ≤ 0.7 xMT. The average onset of the EMG suppression occurred at 65.9 ± 7.1 ms after the first pulse of CPN stimulation and ended at 112.9 ± 11.6 ms (see Experiment 2 in Methods for defining the period of EMG suppression). In Figure 2.3B the duration of the TA EMG suppression is displayed for each of the 8 participants tested with time 0 ms starting at the first pulse of CPN stimulation. Also shown on the same time axis is a single trial where a TA MEP was conditioned by a prior triple-pulse CPN stimulation at the standard ISIs of 40/50/60 ms. Note that at these ISIs, the TA MEP occurred during the same time period as when the voluntary EMG was suppressed. That is, the average onset and offset time of the TA MEP following the first pulse of CPN stimulation was 88 ± 2 and 104 ± 10 ms respectively (i.e., MEP duration of ~16 ms), and occurred within the average window of time that the voluntary TA EMG was suppressed (between ~65and 115 ms), the latter marked by dark circles and a thick line in Figure 3B. Note that the average amount of EMG suppression over this entire window was 15.4 ± 11.7% of the pre-stimulation EMG at the 0.5 and 0.7 xMT intensities.

In these same participants (plus one), we also examined in more detail the time course of TA MEP suppression by using different ISIs of triple-pulse CPN stimulation, starting at ISIs of 10/20/30 ms before TMS and increasing to ISIs of 45/55/65 ms in steps of 5 ms (see x-axis labels in Fig. 2.3C for ISIs used). Overall, there was a significant main effect of ISIs on the TA MEP [ANOVA: $F(7,8) = 3.623$, $P = 0.003$, Fig. 2.3C]. Similar to Experiment 1, the TA MEP was significantly suppressed (by 23.65 ± 16.16%, $P = 0.003$) when using a triple-pulse conditioning stimulation at the 40/50/60 ms ISI. In addition, the TA MEP was

also significantly suppressed (by 28.06 ± 23.85%, P = 0.009) at an earlier triple-pulse ISI of 25/35/45ms.

To examine if the suppression of background EMG activity by low-intensity CPN stimulation was the main contributor to the suppression of the TA MEP, we examined if the average profile of the EMG suppression for all participants matched the averaged profile of MEP suppression across the various ISIs measured in Figure 2.3C. Unlike the MEP suppression profile which increased, decreased and then increased again across the different ISIs (Fig. 2.3C), the average background EMG suppression displayed a single U-shaped profile (Fig. 2.3D), peaking at 85 ms after the first pulse of CPN stimulation. The location of the 16 ms-duration MEP for 3 example ISIs is also indicated in Figure 2.3D and demonstrates that although the conditioned MEP at the 35/45/55 ms ISI occurred near the peak of background EMG suppression, it was not suppressed compared to the test MEP (see arrow in Fig. 2.3C). The same holds true for other ISIs (e.g., 20/30/40, 30/40/50 ms) where the conditioned MEP was not suppressed but occurred during similar levels of background EMG suppression as the MEPs evoked at the 25/35/45 and 40/50/60 ms ISIs which were suppressed (stars in Fig. 2.3C). Thus, there was not a 1:1 relationship between the amount of MEP and background EMG suppression produced by the low-intensity CPN stimulation. Likewise, MEPs evoked at rest, i.e., with no background EMG, could still be suppressed by CPN stimulation although the inhibition was more variable and prominent at 0.3 xMT. When tested in 4 of the participants from this experiment, the average MEP suppression at rest was 27.5 ± 12.6% (P = 0.02) across the 0.3 and 0.7 xMT stimulation intensities (test MEP size: 364.7 ± 109.5 μV).

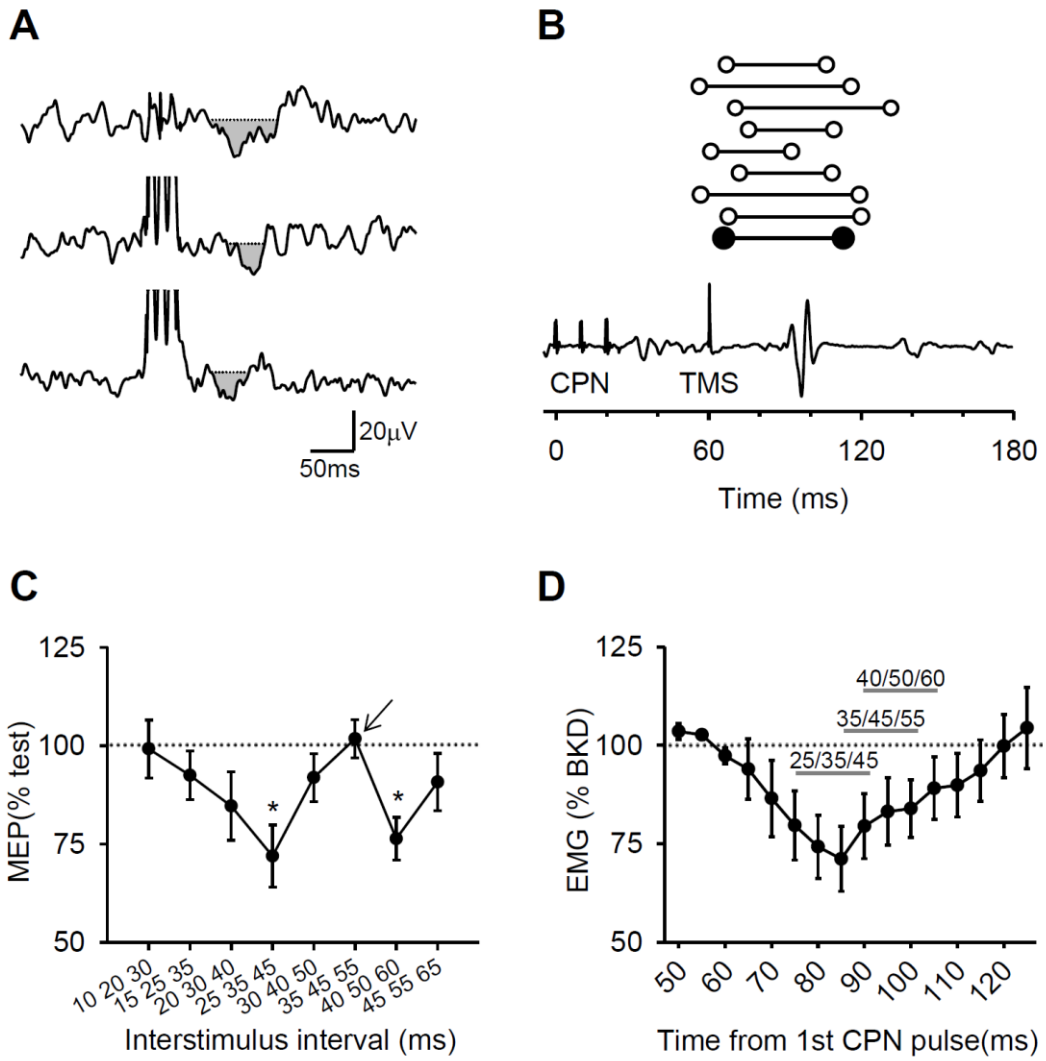

Figure 2.3. Time course of triple-pulse CPN stimulation on tonic TA EMG and MEP

A) Average of 40 rectified EMG traces evoked during 10% maximum dorsiflexion and following triple-pulse CPN stimulation (10 ms ISIs): data from 3 uninjured control participants. The mean background EMG is marked by the dotted line and the grey shaded area indicates EMG below the mean background. **B)** *Top traces*: onset and offset times of EMG suppression for 8 control participants (open circles) with group average indicated by large solid circles. Time 0 aligned to the first pulse of CPN stimulation. Stimulation intensities ranged from 0.2 to 0.7 xMT (average: 0.44 ± 0.16 xMT). *Bottom trace*: Example TA MEP conditioned by CPN stimulation 40/50/60 ms before TMS with the first pulse of CPN stimulation aligned to time 0 ms. **C)** Conditioning of TA MEP by triple-pulse CPN stimulation at different ISIs in 9 uninjured controls (average test MEP: 551.68 ± 261.3 μV). Error bars represent mean ± SE. Asterisks in **(C)** indicate significant difference from unconditioned MEP (100%). * $P < 0.05$.

2.3.3. Conditioning of TA MEPs evoked by Cervicomedullary or Spinal Cord Stimulation

To determine if the suppression of the TA MEP by a prior CPN stimulation was mediated at sub-cortical sites, we compared the conditioning effect of CPN stimulation on evoked potentials produced by stimulating the corticospinal tract directly, either at the cervicomedullary junction (CMEP) or at the level of the thoracic spinal cord (SMEP). As shown for a single participant in Figure 3.4A, a prior triple-pulse CPN stimulation (see figure legend for ISIs used) reduced the peak-to-peak amplitude of the cortical MEP, brainstem CMEP and spinal SMEP (unconditioned MEP gray line, conditioned MEP black line). The amount of MEP and CMEP suppression was similar in the 3 participants tested (Fig. 2.4B: black and dark gray bars respectively, $P = 0.77$). In the 2 participants tested with spinal cord stimulation (who were also tested with the brainstem stimulation on a different day), the amount of SMEP suppression was also similar to the amount of MEP suppression (Fig. 2.4B: light gray bar). This indicates that the MEP suppression from TMS was likely mediated at sub-cortical and/or spinal sites. Similar to the MEP, the conditioned CMEP and SMEP began to deviate from the test (unconditioned) responses at 3.0 ± 0.6 and 3.3 ± 0.8 ms, respectively, as demonstrated for the single participant in Figure 3.4A.

The evoked responses recorded in the TA muscle were similar when using TMS over the motor cortex and when using electrical stimulation over the thoracic spinal cord (compare MEP vs SMEP in Fig. 2.4C), suggesting that the corticospinal tract was activated in a similar manner, either at rest (left traces) or during a background contraction (right traces). On average, the onset latency of the SMEP was 21.2 ± 0.1 ms, the CMEP 24.2 ± 1.2 ms and the MEP 30.1 ± 1.2 ms.

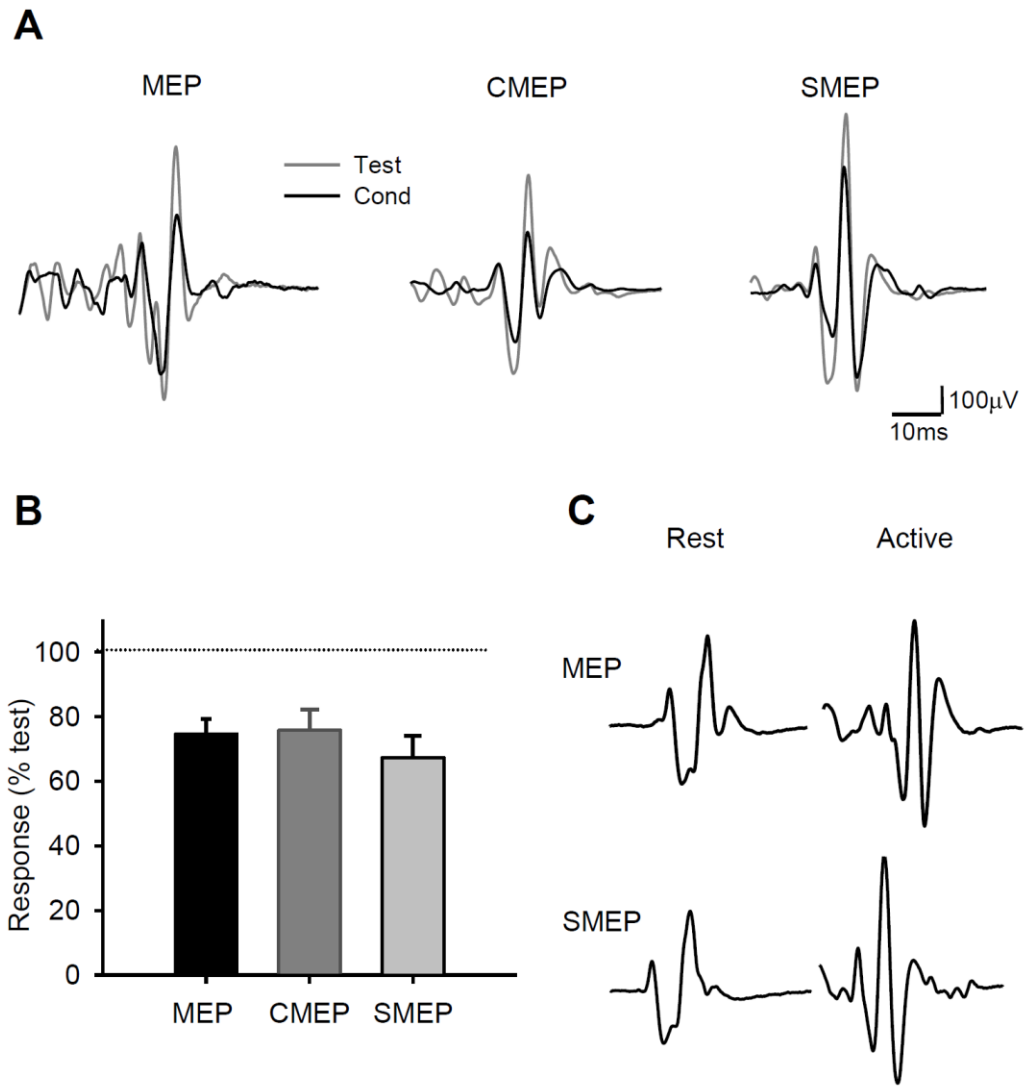

Figure 2.4. Suppression of TA MEP, CMEP and SMEP by CPN stimulation **A)** Average of 5 unconditioned test (gray trace) and 5 CPN-conditioned (black trace) TA MEPs, CMEPs and SMEPs recorded from an uninjured control participant at 40/50/60 ms, 45/55/65 ms and 50/60/70 ms ISIs respectively. **B)** The peak-to-peak amplitude of the conditioned MEP and CMEP and SMEP is expressed as a percentage of the corresponding unconditioned test response. The average unconditioned test MEP and CMEP were 429.24 ± 106.5µV and 819.88 ± 1071.0µV respectively (n = 3) and the unconditioned test MEP and SMEP (n = 2, recorded on a separate day) were 532.35 ± 108.1 µV and 919.00 ± 284.3µV, respectively (conditioned MEP on this day was 69.5% of test MEP, not shown). **C)** Average of 5 unconditioned test MEPs and SMEPs from a single control participant recorded at rest (left traces) and during dorsiflexion (right traces). All traces are aligned to the start of the TMS or spinal electrical stimulation pulse. Error bars represent mean ± SE. Calibration lines are for both **A** and **C**.

2.3.4. Conditioning TA MEPs by skin stimulation

Because suppression of TA MEPs by CPN stimulation could occur at very low intensities (< 0.7 xMT), we examined if activating cutaneous receptors directly underneath the cathode contributed to the MEP suppression or if activation of low-threshold afferent fibres within the CPN was necessary. When the cathode was moved medially away from the CPN (OFF Nerve, Fig. 2.5A, open circles), the TA MEP was not suppressed at low stimulation intensities compared to when the cathode was located over the CPN (ON Nerve, solid circles). In the 6 participants tested, conditioning the TA MEP with OFF Nerve stimulation did not suppress the TA MEP at low stimulation intensities (Fig. 2.5A, bottom graph). There was a significant interaction between 'stimulation site' (ON and OFF Nerve) and 'stimulation intensity' ($F(5, 6) = 3.356$, $P=0.03$) with larger MEP suppression occurring with ON Nerve stimulation at the 0.5 and 0.7 xMT intensities, indicating that low-threshold afferents activated within the CPN contributed to the TA MEP suppression.

Based on the results above, low-threshold muscle or cutaneous afferents within the CPN likely contributed to the TA MEP suppression. To determine if low-threshold cutaneous afferents played a role, we selectively activated the cutaneous branch of the CPN, the deep peroneal nerve (DPN), which innervates the skin between the first and second toe of the foot (see shaded area in Fig. 2.5B). In the 6 participants tested (on a separate experiment day to the ON and OFF nerve experiment), the perception threshold when stimulating the skin of the toe with triple-pulse stimulation was 0.68 ± 0.1 mA. The peak suppression of the TA MEP when testing a range of stimulation intensities above and below perception threshold was 27.0± 5.9% of the test MEP across all 6 participants (gray bar in Fig. 2.5B, $P < 0.001$) and occurred at an average stimulation intensity of 0.72 ± 0.5 x perception threshold. In comparison, the peak suppression of the TA MEP from triple-pulse CPN stimulation recorded on the same day was 40.66 ± 14.6% of the test MEP (black bar in Fig. 2.5B, $P < 0.01$) and occurred at an average stimulation intensity of 0.50 ± 0.25 xMT.

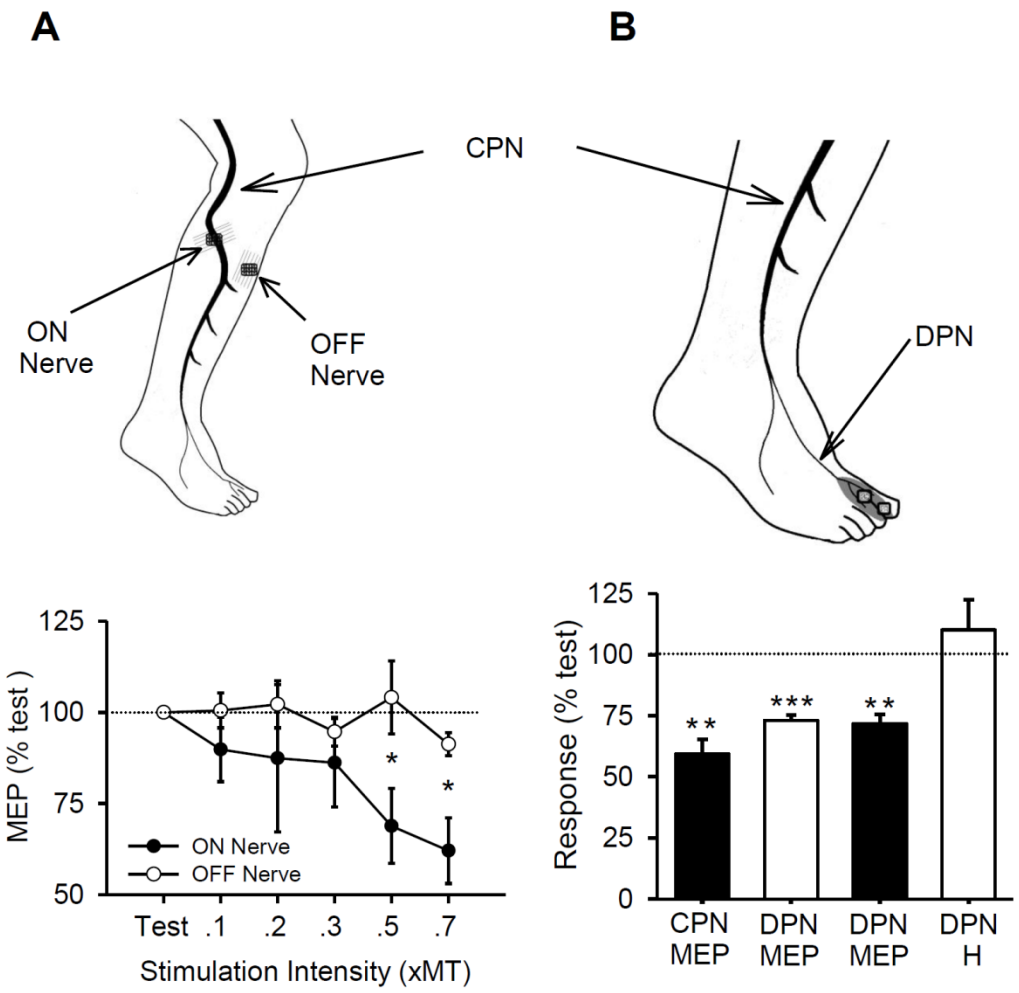

Figure 2.5. Contribution of cutaneous afferents to suppression of the TA MEP

A) *Top*: Schematic of cathode placement over the CPN (ON Nerve) and on the skin in the same dermatome but away from the CPN (OFF Nerve). Anode was placed 2 cm proximal to ON nerve stimulation site (not shown). *Bottom graph*: comparison of ON Nerve (solid circles) and OFF Nerve (open circles) stimulation on the TA MEP in 6 uninjured controls (ON Nerve test MEP: 421.06 ± 117.4 µV; OFF Nerve test MEP: 476.72 ±184.0 µV). The average motor threshold for the CPN was 3.19 ± 1.4 mA (single pulse stimulation). **B)** *Top:* Schematic of anode and cathode placement between the first and the second toe to stimulate the area of skin innervated by the DPN (gray shaded area). *Bottom graph:* Peak CPN and DPN suppression of TA MEP in 6 uninjured controls (CPN test MEP: 597.2 ±106.5; DPN test MEP: 643.4 ± 111.5µV). Bars represent mean ± SE. Asterisks in **(A)** indicate significant difference between ON and OFF Nerve stimulation and in **(B)** indicate significant difference from unconditioned MEP (100%). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

In contrast to the TA MEP, there was no suppression of the TA H-reflex by DPN stimulation. In 4 participants tested, DPN stimulation (0.8 ± 0.5 x perception threshold) produced a peak MEP suppression of 28.2 ± 7.4% ($P < 0.01$) whereas the H-reflex was facilitated, but not significantly, by 10.1 ± 24.5% ($P = 0.47$).

2.4. Discussion

2.4.1. Summary of findings

In uninjured, healthy participants, TA MEPs produced from TMS to the motor cortex were consistently suppressed by a prior sub-threshold triple-pulse, but not single-pulse, stimulation to the CPN applied 40, 50 and 60 ms earlier (peak MEP suppression of 41%). In fact, in 5 participants, conditioning of the TA MEP by a prior CPN stimulation was tested on 3 separate experiment days and the absolute amount of MEP suppression differed each day by only 7.3% on average at the 0.5 and 0.7xMT stimulation intensities. Muscle activity generated by a weak voluntary contraction was also suppressed by low-intensity, triple-pulse CPN stimulation and occurred in the absence of a prior excitatory reflex response. Evoked responses from direct activation of the corticospinal tract at the level of the brainstem and spinal cord were also suppressed by appropriately timed, low-threshold CPN stimulation. Moreover, stimulation of the skin innervated by the deep peroneal nerve (DPN), which contains the cutaneous branch of the CPN, also suppressed the TA MEP. We discuss below that the suppression of TA MEPs and voluntary EMG likely resulted from the activation of spinal inhibitory networks by low-threshold homonymous muscle and cutaneous afferents and provides a negative feedback system to help regulate the activation of flexor motoneurons by the corticospinal tract.

2.4.2. Origin of TA MEP and voluntary EMG suppression by triple-pulse CPN stimulation

2.4.2.1. Spinal location

The suppression of the TA MEP and voluntarily activated EMG was likely mediated by low-threshold CPN afferents activating inhibitory networks in the spinal cord. This is supported by the finding that responses evoked by direct stimulation to the corticospinal tract, both at the level of the brainstem (at pyramidal decussation, Gandevia et al. 1999; McNeil et al. 2009; Ugawa et al. 1991) and the spinal cord (C7-T4, Martin et al. 2008) were also suppressed by appropriately timed CPN stimulation. The activation of the corticospinal tract by direct stimulation to the spinal cord likely activated similar fast-conducting corticospinal tract axons as were activated with TMS over the motor cortex given the very similar shapes of the evoked potentials (SMEPs) recorded in the TA muscle (e.g., Fig. 2.4C). A selective contribution by the corticospinal to the SMEP may result from its strong monosynaptic connection to the TA motoneuron pool compared to other descending tracts in the spinal cord (Lemon 2008). Thus, the preserved suppression of the similarly shaped SMEPs by low-threshold CPN stimulation strongly suggests that the TA MEPs were suppressed via processes occurring in the lumbar spinal cord. This is in agreement with other studies showing that afferent inputs from the leg, unlike that from the upper limb, do not inhibit cortical circuits but mainly activate inhibitory circuitry in the spinal cord (Geertsen et al. 2010, 2011; Roy and Gorassini 2008).

2.4.2.2. Spinal interneurons

The fact that the TA MEP/voluntary EMG could be suppressed by CPN stimulation at intensities that were below M-wave or H-reflex threshold indicates that processes like recurrent inhibition or motoneuron refractoriness were not involved (Meunier et al. 1994; Tucker et al. 2005). Rather, activation of spinal inhibitory interneurons interposed between the CPN afferents and the TA corticospinal tract likely mediated this inhibition. One likely candidate is inhibitory interneurons that synapse onto propriospinal-like interneurons

innervating the TA motoneuron pool given the long (40 - 60 ms) conditioning intervals needed to suppress the TA MEPs by homonymous CPN stimulation. A similar propriospinal site of action has recently been suggested for inhibitory *heteronymous* afferent pathways from the CPN to the soleus motoneuron pool (Geertsen et al. 2011) and from tibial nerve afferents to the TA motoneuron pool (Roy and Gorassini 2008), both of which also require long (≈ 35-45 ms) conditioning-test stimulation intervals. The propriospinal-like interneurons inhibited by the CPN afferents may form part of an indirect corticospinal pathway to the soleus motoneuron pool (Geertsen et al. 2011). The conditioning-test intervals (35-45 ms) that suppress the soleus and TA MEP are longer than that required for the activation of inhibitory, CPN-activated interneurons that synapse directly onto quadriceps propriospinal neurons, the latter a tri-synaptic pathway that requires conditioning-test intervals of only 4 to11 ms (Marchand-Pauvert et al. 1999). Thus, the homonymous afferent pathway studied here may involve more than 3 synapses and is likely different than the di- and trisynaptic, non-reciprocal Ib and Ia inhibitory pathways studied previously (Cavallari and Katz 1989; Forget et al. 1989a,b; Malmgren and Pierrot-Deseilligny 1988; Pierrot-Deseilligny et al. 1981). The inhibition of the TA MEP at ISIs of 25/35/45 ms, which had overlapping ISIs with 40/50/60 ms ISIs, could have recruited similar pathways or other inhibitory pathways with fewer synapses.

Alternatively, inhibition of the TA MEP could involve a spinal inhibitory mechanism with a long time course of activation to reach functional inhibition. For example, pre-synaptic inhibition occurs within the time scale of the homonymous suppression of the TA MEP (e.g., 40 to 200 ms, El-Tohamy and Sedgwick 1983; Hultborn et al. 1987) and is also enhanced by spatial facilitation, i.e., 3 pulses of stimulation produce larger inhibition than one pulse (Burke et al. 1994). Thus, pre-synaptic inhibition onto terminal axons of propriospinal or other excitatory interneurons interposed between the CPN and TA corticospinal tract could have mediated the long-latency spinal inhibition. Moreover, inhibition likely did not occur directly onto the TA motoneurons (Iglesias et al. 2008).

Unlike stimulation intensities above motor threshold (at 1.5xMT), where there was likely direct recurrent inhibition onto the TA motoneurons (Meunier et al. 1994), only the later portions of the TA MEP were suppressed by low-intensity stimulation of the CPN (from 4.2 ms onwards), suggesting that inhibitory inputs a few synapses upstream from the TA motoneurons were involved (as per Chaix et al. 1997). However, the lack of early MEP inhibition could have resulted from only the later recruited motor units, having either higher thresholds and/or activated by later I-waves, being inhibited by the CPN stimulation. Interestingly, the responses evoked by cervicomedullary or spinal cord stimulation, which produce more of a single descending volley, were also suppressed at later latencies (~3 ms) from the onset of the unconditioned CMEP or SMEP, providing supportive evidence for a site of action upstream from the motoneuron. In addition, stimulation of the DPN, the cutaneous branch of the CPN, only suppressed the TA MEP and not TA H-reflex. This is in agreement with findings from Roy and Gorassini (2008) and Geertsen et al. (2011) where heteronymous nerve stimulation (tibial nerve to TA and CPN to soleus, respectively) produced a similar long-latency (35-45 ms) suppression of the MEP but not the H-reflex. As the inhibitory profile of these two pathways is quite similar to the homonymous CPN-TA MEP pathway studied here, it is likely that they share similar upstream inhibitory interneurons.

2.4.2.3. Effects from the background contraction

Given that TA MEPs were evoked during a background contraction and that triple-pulse CPN stimulation could also suppress low-levels of voluntary EMG, the CPN stimulation could have suppressed the TA MEP by inhibiting upstream interneurons in corticospinal pathways activated by the voluntary background contraction. As demonstrated in Figures 3C and D not all of the MEP suppression can be accounted for by a decrease in background EMG because many MEPs at the various ISIs tested, which were evoked during similar levels of background EMG suppression, were not suppressed by the CPN stimulation. Likewise, MEPs could still be suppressed when evoked at rest when there was no

background EMG activity. Taken together, suppression of background EMG by the sub-threshold CPN stimulation was likely not the only contributor to MEP suppression. Activation of spinal inhibitory interneurons distinct from the ones producing the decrease in background EMG likely also contributed to the suppression of the MEP at the 25/35/45 and 40/50/60 ms ISIs.

2.4.2.4. Afferents

Suppression of the TA MEP from triple-pulse CPN stimulation occurred at very low intensities (< 0.7 x MT) in the control participants. We ruled out that activation of afferents innervating the skin directly below the cathode contributed to the MEP suppression because only when the cathode was over the CPN did MEP suppression occur and thus, was likely activating low-threshold muscle (Ia and Ib) and cutaneous afferents in the CPN. In addition, activation of low-threshold cutaneous afferents likely also contributed given that stimulation of the skin innervated by the deep peroneal nerve (the cutaneous branch of the CPN) also suppressed the TA MEP but to a lesser extent. Thus, as with many spinal inhibitory interneurons and propriospinal neurons, there may be a convergence of afferents conveying different sensory modalities onto the homonymous, long-latency spinal inhibitory pathway.

2.5. Future directions

In this study we have characterized the activation of spinal inhibitory circuitry by low-threshold homonymous afferents. Future studies are required to directly examine if this pathway regulates the activation of the TA motoneuron pool by the corticospinal tract, to help control the force, timing and ultimately the precision by which ankle flexors are activated during movement and if the excitability of this pathway is affected by injury to the central nervous system.

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CHAPTER 3. FACILITATION OF DESCENDING EXCITATORY AND SPINAL INHIBITORY NETWORKS FROM TRAINING OF ENDURANCE AND PRECISION WALKING IN PARTICIPANTS WITH INCOMPLETE SPINAL CORD INJURY

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3.1. Introduction

Sensorimotor training is one of the essential interventions for restoring control over hand and leg movements after injury to the nervous system (Adkins et al. 2006; Fouad and Pearson 2004; Wolpaw and Tennissen 2001). For many individuals living with spinal cord injury (SCI), restoration of functional walking is an important goal (Anderson 2004), particularly since 60 to 80% of individuals with motor-incomplete SCI (iSCI) will regain some walking function (Dobkin et al. 2006). One form of walking training is body-weight supported treadmill training (BWSTT) where participants are given assistance to move their legs if needed. Improvements in walking from BWSTT are associated with strengthening of spared connections from the motor cortex to the spinal cord (Benito Penalva et al. 2010; Hajela et al. 2013; Norton and Gorassini 2006; Thomas and Gorassini 2005). Thus, training protocols which engage the motor cortex may provide the best outcomes for the recovery of walking after iSCI.

Animal studies demonstrate that training of skillful movements induces enlargement of motor maps in the primary motor cortex (Carmel et al. 2014; Nudo 2006). Likewise, partial injury to the corticospinal tract produces sprouting, the amount of which is correlated to functional improvements in skilled walking on a horizontal ladder (Carmel et al. 2010). Furthermore, in cats the motor cortex contributes to precise control of gait by driving muscle activity during obstacle avoidance, especially in the flexor muscles (Drew et al. 2008). In a pilot study, when individuals with iSCI were trained in complex walking skills, walking improved beyond that with BWSTT (Musselman et al. 2009). Thus, training of precise walking skills may induce larger increases in descending control of walking, and improved function, compared to mass practice on the treadmill.

We report here the changes in descending and peripheral activation of the spinal cord in response to the two forms of walking training: endurance training on the treadmill vs precision training over ground (clinical findings published in Yang et al. 2014). First, we compared changes in the strength and excitability of

responses evoked in the ankle flexor muscle, the tibialis anterior (TA), by transcranial magnetic stimulation (TMS) before and after precision and endurance training, (see Yang et al. 2014 for training details). The TA muscle was chosen because corticospinal pathways activate this muscle during walking (Petersen et al. 2001), weakness in this muscle produces a common clinical problem of foot-drop (Stewart 2008) and the TA is most likely to show changes when stepping over obstacles (McFadyen et al. 1994) and on targets (Schubert et al. 1999). Second, we examined the extent to which low-threshold afferents from the nerve supplying the TA muscle (the common peroneal nerve, CPN) inhibit the descending activation of TA motoneurons in response to TMS over the motor cortex. Activation of homonymous afferents from the CPN at very low intensities of stimulation (0.5 to 0.7 x motor threshold) activates inhibitory circuitry in the spinal cord through a long-latency pathway (Zewdie et al. 2014). Given that spinal inhibition is reduced after SCI (Calancie et al. 1993; Knikou 2005; Roby-Brami and Bussel 1987; Schindler-Ivens and Shields 2000) and that spastic muscle activation can be reduced by motor training (Gorassini et al. 2009; Knikou and Mummidisetty 2014; Manella and Field-Fote 2013), we measured if endurance or precision training could enhance this long-latency, spinal inhibitory pathway. Third, we examined the effects of walking training on the cutaneomuscular reflex (CMR) which comprises part of the flexor-reflex afferent pathway, the latter considered to be activated by excitatory and inhibitory interneurons within the central pattern generator for walking (Jankowska et al. 1967). Specifically, we examined if endurance or precision training strengthened the inhibitory component of the CMR evoked in the TA muscle which reflects activation of inhibitory, post-synaptic potentials in the TA motoneuron (Norton et al. 2008).

3.2. Methods

All experiments were approved by the Human Research Ethics Board at the University of Alberta and conducted with informed consent after appropriate screening by a physical therapist. Our sample comprised of 16 participants with

incomplete spinal cord injury (iSCI), see Table 1 for participant details. Inclusion criteria for the iSCI participants were: injuries sustained no sooner than 7 months prior to the start of the training given that lower leg muscle strength can plateau 6 months after injury (Petersen et al. 2012), injury levels between C1-L1 and the ability to walk independently for at least 5 m with a walking aid and/or braces. The exclusion criteria were: head injury, cognitive or musculoskeletal impairments that could make walking training difficult, epileptic history or head implants that preclude TMS.

3.2.1. Study Design

A randomized, single-blinded, cross-over design was used. Participants were block-randomized (block size 4, Fig. 3.1A) to start with either Endurance (End) or Precision (Prec) training by picking a label out of a box. Each type of training was ~1 hour/day, 5x/week, for 2 months. After the first phase of training (P1), participants rested for 2 months with no training (R1), then crossed over to the other form of training for 2 months (P2), followed by another 2 months of rest (R2). Baseline clinical and electrophysiological measures were taken twice before training (B1 and B2), separated by at least 1 week to confirm reliability of the measurements and to ensure no spontaneous motor recovery, and once after each training phase or rest period (Fig 3.1A). By using a cross-over design, we maximized the number of participants receiving each type of training, and counterbalanced the order of training.

3.2.2. Intervention

3.2.2.1. Precision Training

Participants had to step over obstacles of different heights and onto targets of different sizes. Obstacles were 3 cm high Styrofoam blocks (stacked to increase height), varying in width from 4 - 20 cm in increments of 4 cm. Targets were fabric circles, with diameters ranging from 7 - 10.5 cm. The maximum ability to clear obstacles and cover targets was determined by trial-and-error prior to the beginning of Precision Training.

A different course was designed each day, consisting of a straight track (15 m) with ~15 obstacles and targets (i.e., ~1 m apart), which the participants would traverse in both directions. Based on the error recorded each day, defined as any touch to the obstacle or incomplete covering of a target, the course was altered for the next day to aim for 80% success. The emphasis was on accuracy, not speed. Extra steps between obstacles and targets were allowed at the beginning of training, and rest breaks were taken as needed. As participants improved, course difficulty was increased by requiring that each obstacle/target was executed without taking extra steps in between. Reducing gait aids also challenged walking balance.

3.2.2.2. Endurance Training

Participants walked on the treadmill, with body-weight support and manual assistance only if needed. The treadmill speed was initially set to be faster than their over ground, self-selected walking speed. Walking as far and as fast as possible with minimal rests was encouraged. Progression involved reducing manual assistance and body-weight support, increasing walking speed and distance, while reducing rest breaks. Further details of the training protocols can be found in Yang et al. 2014.

3.2.3. Clinical Outcome Measures

The following clinical outcomes were measured by a physical therapist blinded to the type of training received.

The 6 minute walk test (6 MWT): The 6 MWT was used to measure the distance travelled in 6 min (Guyatt et al. 1985) as an assessment of walking endurance.

The 10 meter walk test [(10 MWT(ss)]: The 10 MWT(ss) was used to measure the time it took a subject to walk 10 meters (Dittuno and Ditunno 2001; Ditunno et al. 2000) at a self-selected (ss) speed.

	Age (Yr)	Yrs Post Injury	Injury Level	Cause	Coil Type	MMT (max 80)	6 MWT (m)	10 MWT (m/s)	SCI FAP (max 2100)
End 1st									
P1(F)	49	2.5	C6	MVA	BW	51.5	17.4	0.05	649.6
P2(M)*	24	1.0	T6	MVA	DC	40.0	114.0	0.37	118.9
P3(M)	25	1.1	T4	MVA	BW	48.0	47.5	0.13	235.0
P4(M)	57	34.9	C5	MVA	DC	43.0	298.0	0.90	59.6
P5(M)	48	0.7	T12	Fall	DC	43.5	118.7	0.23	140.6
P6(M)	65	1.2	C3	MVA	DC	39.0	19.3	0.05	864.1
P7(M)	60	0.6	C3	Bull attack	DC	65.5	172.0	0.47	286.0
P8(M)*	46	7.3	C6	MVA	DC	36.0	43.0	0.14	307.7
P9(F)	63	6.0	C4	MVA	DC	51.5	278.3	1.13	10.1
Mean ± SD	**49 ±15**	**6.1 ±11.1**				**46.4 ± 8.9**	**123.1 ±106.8**	**0.39 ±0.39**	**296.8 ±283.9**
Prec 1st									
P11(F)	43	1.1	T1	Sport	DC	51.0	46.0	0.15	454.0
P13(F)	21	1.0	C6	MVA	DC	36.5	67.3	0.25	262.3
P16(M)	34	1.2	C4	Gun Shot	BW	56.0	337.0	1.10	8.2
P17(M)	32	0.8	T2	Fall	DC	53.5	53.3	0.16	230.9
P18(F)	41	3.5	T12	Infection	DC	48.5	38.3	0.17	601.9
P19(F)*	50	1.8	T6	Tumor	DC	50.0	64.0	0.20	166.3
P20(M)	52	1.4	T10	Surg. bleed	DC	48.0	120.0	0.37	117.4
Mean ± SD	**39 ±11**	**1.5 ±0.9**				**49.0 ±6.2**	**103.7 ±106.3**	**0.34 ±0.34**	**263.0 ±203.2**

Table 3.1 Demographic, injury and clinical measures before training.

Participants are divided to 2 groups: those who started with endurance (top half) and those who started with precision (bottom half) as the first phase (P1) of training. The age of the participants and the number of years after the participant sustained a spinal cord injury were measured at the start of the training. MVA = motor vehicle accident. DC = double cone and BW = bat wing coil used for TMS experiments. The manual muscle test (MMT) includes scores from 1 to 5 for flexion/extension of the hip, knee and ankle joints and for hip abduction/adduction: 8 scores per leg for a maximum score of 80 for both legs (5 x 8 x 2). The 6 minute walk test (6MWT), the 10 m walk test (10MWT) and the Spinal Cord Injury-Functional Ambulation Profile (SCI-FAP) scores are the average of 2 baseline measures before training. The group average (mean) and the standard deviation (SD) of age, years post injury, MMT, 6MWT, 10MWT and SCIFAP scores are presented in the bottom rows of each group. * = missed the R2 experiment. Participant numbering corresponds to those listed in Yang et al. 2014.

Spinal Cord Injury-Functional Ambulation Profile (SCI-FAP): The SCI-FAP, a combined score of 7 items, (Musselman et al. 2011), was used to assess walking skill in tasks encountered in daily life. The highest total score on the SCI-FAP is 2100 with a maximum of 300 for each of the 7 tasks.

Manual muscle test: The manual muscle test (MMT), a 5 point scale, was used to measure the voluntary muscle strength of the lower extremities (Kendall 2005). The MMT score from eight muscle groups, including flexors and extensors of the hip, knee and ankle, and abductors and adductors of hip, for both the right and left leg, were summed to give a total score out of 80 (Table 1).

3.2.4. Electrophysiological Measures

3.2.4.1. EMG Recordings and Peripheral Nerve Stimulation

A pair of Ag-AgCl electrodes (Kendall; Chicopee, MA, USA, 3.8 cm by 2.2 cm) was used to record surface EMG from the tibialis anterior (TA) and soleus muscles on both legs with a ground electrode placed on the patella. The EMG signals were amplified by 1000 and band-pass filtered from 10 to 1000 Hz (Octopus, Bortec Technologies; Calgary, AB, Canada) and then digitized at a rate of 5000 Hz using Axoscope 10 hardware and software (Digidata 1400 Series, Axon Instruments, Union City, CA).

The common peroneal nerve (CPN) and the tibial nerve at the ankle (TN) were electrically stimulated using a constant current stimulator (1-ms rectangular pulse; DS7A, Digitimer, Welwyn Garden City, UK) in a bipolar arrangement with Ag-AgCl electrodes (Kendall; Chicopee, MA, USA, 2.2 cm by 2.2 cm). Bipolar stimulation was used to stimulate the CPN with the cathode placed below the neck of the fibula after identifying an optimal site that produced a small isolated contraction of the TA muscle, with minimal to no peroneal muscle activation. The anode was placed 2 cm proximal to the cathode near the outer edge of the popliteal fossa. Similarly, a bipolar arrangement was used to stimulate the TN with the cathode placed posterior to the medial malleolus and the anode 2 cm

distal to produce a small isolated contraction of the abductor hallucis brevis (AHB) muscle.

3.2.4.2. Maximum Voluntary Contraction

A participant's maximum voluntary contraction (MVC) was determined at the start of the experiment by recording the peak rectified and smoothed EMG obtained during three dorsiflexion trials. To obtain an MVC, participants were seated in their wheelchair or in a padded chair with their ankle and knee positioned at 90 degrees. The foot and knee was strapped to a foot plate and the chair respectively for isometric dorsiflexion or held stable by the experimenter for participants seated in their wheelchairs. Participants were given verbal encouragement and a visual display of their rectified and smoothed TA EMG to help them contract as hard as possible. Contraction trials were repeated until at least two consistent MVC recordings were obtained, with peak EMG varying less than 10%.

3.2.4.3. Maximum compound action potential

In 7 participants only, Mmax was evoked in the TA muscle with a 1-ms rectangular pulse to the CPN. The stimulation intensity was increased until the amplitude of the M-wave reached its maximum and stayed constant. Mmax values were compared at baseline and after each phase of training or rest.

3.2.5. Experiment 1: TMS Recruitment Curve

To estimate the strength and excitability of the spared descending pathways activated by TMS over the motor cortex, we measured motor evoked potentials (MEPs) in the TA muscle with single-pulse, transcranial magnetic stimulation (TMS). A double-cone (DC) coil (110mm diameter) connected to a Magstim 200 stimulator (Magstim; Dyfed, UK) was used to induce posterior-anterior currents in the contralateral motor cortex in 13 of the 16 participants tested. In 3 of the participants (P1, P3 and P16, Table 1) who had more readily evoked responses, a figure-of-eight batwing coil (P/N 15857; 90mm diameter)

was used. As described in Results, the legs of the participants were grouped according to the size of their maximum MEP before the start of training (e.g, weak or strong legs). Thus, in the 3 participants where we used the weaker batwing coil, we also tested each leg at baseline with the double cone coil at maximum TMS intensities to make sure they were placed in the correct group. The location over the primary motor cortex that produced the largest TA MEP at intensities near 1.2 times active motor threshold (i.e., hotspot) was first determined. Typically, the hotspot for the TA muscle was 1-2 cm lateral and 0-2 cm posterior or anterior to vertex and a similar hotspot for each participant was used across all experiments. MEPs were elicited in both legs of each participant except for participants P2 and P4 where only one leg was tested because it was difficult to evoke MEPs in the TA muscle of the opposite leg.

In the first baseline experiment (B1), background contraction levels were set to 10% MVC. Because MVCs may increase with training (Thomas and Gorassini 2005), in all subsequent recording days (B2, P1, R1, P2 and R2), the absolute EMG (in μVs) obtained from the first baseline experiment was used for the target background contraction level. The participant had to align their full-wave rectified and smoothed EMG (100 ms time constant, Neurolog NL703 - EMG Integrator, Digitimer UK) over a target line displayed on an oscilloscope screen. To produce a TMS recruitment curve, TMS intensities were increased from 30% to 80% MSO for the double cone coil and from 30% to 100% MSO for the batwing coil, both in steps of 10%. Four stimuli were given at each intensity.

Data analysis: Axoscope files were imported into Matlab R2011b (The Mathworks, Inc., Natick MA) for offline analysis. A Matlab program was used to calculate the peak-to-peak values of the MEP from un-rectified EMG by setting a time window of 15 to 80 ms from the onset of the TMS pulse. For each leg, peak-to-peak amplitudes of four MEPs were averaged at each stimulus intensity to produce a TMS recruitment curve. A four-parameter Boltzman sigmoid (Fig. 1B) accurately fit 138 out of the 173 recruitment curves with a median correlation coefficient (r) of 0.88 (range 0.64 to 0.98). However, in 35 of the 173 recruitment

curves (Fig. 1C), the amplitude of the MEPs did not plateau at the high stimulation intensities so a four-parameter logistic curve was used to better fit the data [median r of fit 0.78 (0.45 to 0.94)]. Three parameters were measured from the TMS recruitment curve. 1) The maximum MEP (MEP_{max}) was measured as the largest response on the recruitment curve, typically at 70 or 80% MSO for the double-cone coil (76.8 ±10.1 % MSO on average) and 90% or 100% MSO for the batwing coil (92.1± 4.3 % MSO). Each leg of a participant was treated independently because for a given participant, changes in MEP_{max} occurring in the right leg did not depend on changes occurring in the left leg when comparing baseline to the last phase (P2) of training] ($r = 0.05$, $p = 0.857$). Thus, we did not average MEP_{max} values from the right and left leg together to give a single value per participant but kept them as independent measures. 2) The stimulation intensity producing half of the MEP_{max} ($SMEP_{½}$) was measured by first identifying the y-value on the fitted line of the TMS recruitment curve (Boltzman or logistic) that was half way between the maximum and minimum MEP ($MEP_{½}$). The TMS intensity (x value) that corresponded to $MEP_{½}$ was then used as $SMEP_{½}$. 3) The stimulation intensity producing MEP threshold (MEP_{thr}) was measured as the intensity (x value) corresponding to the y-value on the fitted line that was 5% of MEP_{max} (after subtracting the background EMG).

3.2.6. Experiment 2: Conditioning of TA MEPs by CPN stimulation

The effect of conditioning TA MEPs by a prior stimulation to the homonymous CPN was examined in 14 of the 16 participants (as per Zewdie et al. 2014), all of whom participated in Experiment 1 on the same day. MEP responses were evoked in the TA muscle of the stronger leg during a 10% MVC dorsiflexion, to evoke a target, unconditioned test MEP amplitude of ≈ 300μV (average test MEP: 358.1 ± 150.2 μV). The motor threshold of the CPN was determined with single pulse stimulation and defined as the amount of current producing a discernible and reproducible direct motor response (M-wave) at rest. The TA MEP was then conditioned by stimulating the CPN at interstimulus

intervals (ISIs) of 40, 50 and 60 ms (triple-pulse stimulation) before the TMS. The CPN was stimulated in increasing order at intensities of 0.1, 0.2, 0.3, 0.5, 0.7, 0.9 and 1.1 x's motor threshold (x MT) for a total of 7 intensities of stimulation. At each of the 7 intensities of CPN stimulation, 2 unconditioned TA MEPs (14 in total) and 6 conditioned TA MEPs (42 in total) were evoked in pseudorandom order.

Data analysis: The conditioned MEPs at all 7 intensities were expressed as percentage of the unconditioned, test MEP. Only stimulation intensities that did not produce an M-wave or H-reflex were used (≤ 0.7 x MT) to measure MEP suppression by low-threshold CPN afferents without effects from motoneuron refractoriness and/or recurrent inhibition (as in Zewdie et al. 2014). The average threshold of the TA M-wave (single pulse stimulation) for the 2 baseline measures was 1.0 ± 0.2 x MT whereas the average H-reflex threshold (triple pulse stimulation) was 1.1 ± 0.1 x MT, both being well above the 0.7 x MT cut off for the conditioning CPN stimulation. Because the stimulation intensity that produced a suppression of the MEP could vary between participants, the maximum amount of MEP suppression (Peak Inhibition) was determined for a single stimulation intensity in each participant (average 0.5±0.2 x MT) and then averaged across the group for each of the 6 different time points (B1, B2, P1, R1, P2 and R2).

3.2.7. Experiment 3: Cutaneomuscular reflexes (CMR)

On a different day from *Experiment 1 and 2*, CMRs were evoked in all 16 participants by stimulating the tibial nerve (TN) just posterior to the medial malleolus, which supplies muscle and cutaneous afferents to the sole of the foot. CMRs were recorded from the same TA muscle as in *Experiment 2* while participants maintained a tonic dorsiflexion of 10% MVC by leaning backward while standing. Visual feedback was provided with a target marked on an oscilloscope and the same absolute contraction level was kept constant between recording days. Responses were recorded from the TA and abductor hallucis brevis (AHB) muscles. The M-wave from the AHB muscle was used to match the

stimulus intensity from day-to-day. The motor threshold (MT) for the AHB muscle was determined as the current intensity producing a discernible M-wave 50% of the time at rest. Three pulses (0.3 ms duration, 5 ms apart) at intensities of 1.0, 1.5, 2.0 and 2.5 x MT were used. At each of the four intensities of PTN stimulation, 15 CMRs were evoked at the 6 different recording days (B1, B2, P1, R1, P2 and R2) in all but 3 participants where only 10 CMRs were evoked due to reduced tolerance for the stimulation or from evoked spasms.

Data analysis: In each participant, CMRs were analysed at the intensity where the background EMG (measured 200 to 10 ms before PTN stimulation) was most constant between the different recording days, which was 1.94 ± 0.40 xMT on average across the 16 participants. To measure the amplitude of the CMR, the EMG was first low-pass filtered at 200 Hz (zero-phase digital filter), then rectified and smoothed using a low-pass butterworth filter at 100 Hz. The mean background EMG was then subtracted from every data point and the average EMG from 30 to 300 ms after PTN stimulation was used as a measure of the total CMR amplitude. To examine the CMR in more detail, we measured the area of the EMG that was above (excitatory component) or below (inhibitory component) the mean background EMG by adding all the positive and negative values, respectively, in the 30 to 300-ms time period.

3.2.8. Statistical Analysis

All statistical analyses were performed using SPSS 20.0 software. Differences in age, years post injury and initial MMT, 6 MWT, 10 MWT and SCI-FAP scores between the groups that started with endurance or precision training were tested with unpaired Student's t-tests (not corrected for multiple comparisons to better reveal differences if any existed). All data were expressed as a percentage of the first baseline measure (B1), including the second baseline measure (B2). For example, data were plotted according to the different *phases* of training chronologically, collapsed across training type (e.g., B2, P1, R1, P2, R2). Data were also plotted according to the different *types* of training, collapsed

across phases [(e.g., B2, End, Rest after Endurance (RE), Prec, Rest after Precision (RP)]. To compare data across the different phases or types of training, a one-way ANOVA for repeated measures (termed One-way ANOVA) was used with post hoc Paired Student's t-tests (Bonferroni-corrected). Normality was confirmed using the Shaprio-Wilk test. Whenever normality test was not passed, natural logarithmic transformation was applied to the data. Mauchly's test was used to confirm the sphericity assumption of the data, and when the assumption was violated, a Greenhouse-Geisser correction factor was used. An unpaired Student's t-test was used to compare the percentage increase in MEP_{max} and MVC between the weak and strong legs which had unequal numbers in the two groups. A paired Student's t-test was used to compare data before and after training, both for data collapsed across *phases* or *types* of training. Data was also grouped chronologically according to whether endurance or precision training was given first (e.g., B2, End, RE, Prec, RP or B2, Prec, RP, End, RE). The effect of training order was compared using a Mixed factor ANOVA, treating the training order (End 1^{st} or Prec 1^{st}) as between subject factors and training phase (P1, R1, P2, R2) as within subject factors. Finally, the percentage change in MEP_{max} between baseline and at the end of the second phase of training [(P2-baseline/baseline) x 100%] was plotted against the corresponding change in MVC for each leg. In addition, the percentage change in MEP_{max} before and after endurance or precision training was plotted against the corresponding change in 6MWT, 10MWT and SCI-FAP scores for the leg with the largest percentage change in MEP_{max}. A Pearson's correlation coefficient (r) was calculated for each of these plots. Data are presented in the figures as mean ± SE and in the text as mean ± SD unless otherwise stated. Significance was set at $P < 0.05$.

3.3. Results

3.3.1. Participants

Seventy potential participants were screened to take part in the study and only 20 received training (see Yang et al. 2014). *Experiments 1* and *3* were performed on

16 of these participants and *Experiment 2* on 14 of them. All 16 participants completed endurance (End) and precision (Prec) training, as well as the post clinical and neurophysiological assessments. Three participants (P2, P8 and P19) did not return for the second post-rest (R2) experiment. There were no significant differences in age, years post injury and initial MMT, 6 MWT, 10 MWT and SCI-FAP scores (all $P > 0.2$) between the groups that started with endurance or precision training (Table 1).

3.3.2. Experiment 1: TMS Recruitment Curves: MEPmax

Responses evoked in the TA muscle to incrementing intensities of TMS followed a sigmoidal pattern in 12 of the 16 participants, as shown in Figures 3.2A&B for the TMS recruitment curves measured at the two baseline recordings (B1, B2), and after the endurance (End) and precision (Prec) training. In the remaining participants (4/16), TMS recruitment curves were better fit with a logistic curve (e.g., Fig. 3.1C, see Methods *Experiment 1* for goodness of fit). As noted for the participant in Figure 2A, MEP amplitudes increased after both endurance and precision training compared to baseline (upper graph), even though background contraction levels remained constant (lower graph). Such increases in MEP amplitudes were readily apparent in recruitment curves where the maximum evoked response (MEP_{max}) was low at baseline (Fig. 3.2A) compared to when the MEP_{max} in the baseline recruitment curve was larger (Fig. 3.2B). When MEP_{max} at baseline for each recruitment curve was plotted in decreasing order of amplitude (Fig. 3.2C), there was a natural division between responses above and below 0.5 mV. Thus, the TMS recruitment curves were divided into strong (n = 17) and weak (n = 13) if the initial MEP_{max} at baseline was above and below 0.5 mV respectively. Moreover, TMS values from the right and left leg of a single participant were treated as independent variables because changes in TMS parameters in one leg did not predict changes in the opposite leg (see *Data analysis* in *Experiment 1* of Methods). Unlike MEP amplitudes, the TMS intensity at which MEPs were evoked (MEP_{max}, $MEP_{½}$ and MEP_{thr}, see Methods and Figs

3.1B&C for definition) did not change over time (Fig. 3.2D, One-way ANOVAs, P all > 0.60).

MEP_{max} was compared across the different phases of training and rest periods chronologically (P1, R1, P2 and R2), irrespective of training type (End or Prec). As with all TMS parameters, MEP_{max} was similar between the two baseline experiments performed on different days (B1 = 0.60 ± 0.33 mV and B2 = 0.61 ± 0.33 mV, paired t-test, P = 0.92). Thus, MEP_{max} for all different phases, including B2, was expressed as a percentage of MEP_{max} values for B1. In the 30 TMS recruitment curves examined from all legs, there was a significant main effect on MEP_{max} across all phases (Fig. 3.3A, One-way ANOVA: $F(4,29) = 2.98$, $P = 0.02$) with post-hoc tests revealing a significant increase in MEP_{max} after the first training phase (P1: 119.30 ± 34.30%, P=0.05), first rest period (R1: 119.30 ± 48.40 %, P = 0.05) and second rest period (R2: 127.80± 54.90 %, P=0.01, significance indicated by shading of bars), despite the type of training received.

When regrouping the data according to the *type* of training received (End or Prec) and thus, collapsing the different phases together, a similar trend emerged with a significant main effect on MEP_{max} (Fig. 3.3B, One-way ANOVA: $F(4,29) = 4.21$, $P = 0.003$) and a significant increase in MEP_{max} after precision training (Prec:120.20 ± 44.0%, P=0.04) and the rest period immediately after precision training (RP: 132.10 ± 50.60%, P <0.001), despite the *order* of training.

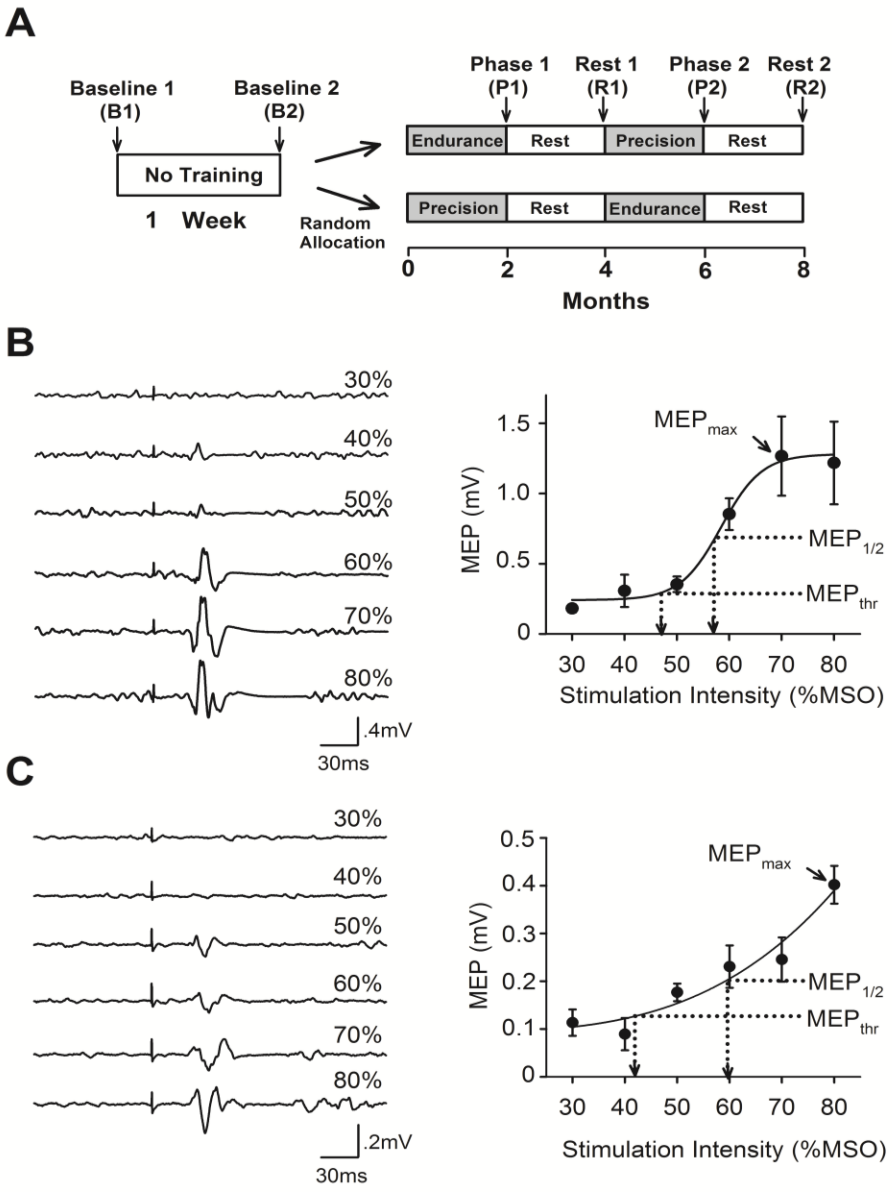

Figure 3.1. Experimental timeline and MEP measures

A) Time-line of electrophysiological and clinical assessments (marked by arrows) at baseline (B1 and B2), after the first phase of training (P1) and rest (R1) and after crossing over to the second phase of training (P2) and rest (R2). Each training or rest block was 2 months. **B&C)** Left Panels: average of 4 TA MEPs evoked from incrementing intensities of TMS. Right Panels: mean peak-to-peak amplitude of MEP from data in left panels to produce TMS recruitment curves. Data in B from participant P11 is well fit by a 4-parameter sigmoid function (to account for offset in background EMG) whereas data in **C** from participant P5 is well fit with a 4--parameter logistic curve. MEPmax, $MEP_{1/2}$ and MEP_{thr} are shown for illustration (see Methods for definition). Error bars represent ± standard error (SE).

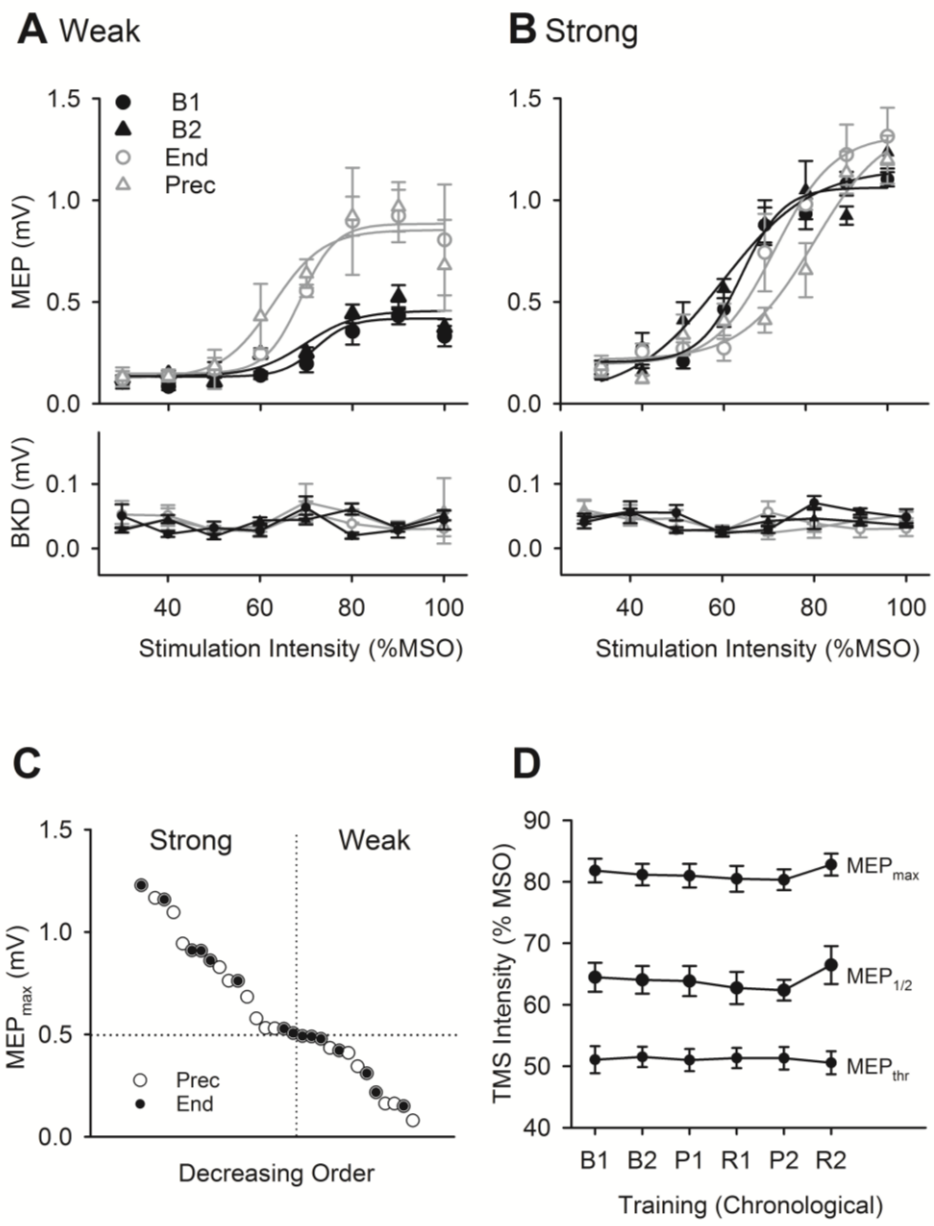

Figure 3.2. TMS recruitment curves

A) *Top:* TMS recruitment curves from a participant having an initial MEP_{max} < 0.5 mV at the 2 baseline (B1, B2) measures (weak), after endurance (End) and after precision (Prec) training. *Bottom:* Mean background EMG for all experiments. **B)** Same as in A but TMS recruitment curve with initial MEP_{max} > 0.5 mV (strong). **C)** Initial MEP_{max} values for each leg in all participants averaged across the 2 baseline measures plotted in decreasing order of amplitude. **D)** Average TMS intensities where MEP_{max}, $MEP_{1/2}$ and MEP_{thr} occurred the on the TMS recruitment curve for each experiment (baseline 1: B1; baseline 2: B2; phase 1: P1; rest 1: R1; phase 2: P2; rest 2: R2). Error bars represent ± SE.

Overall, the average increase in MEP_{max} compared to first baseline was modest (≈ 21%) when grouping data from all legs together. When separating the TMS recruitment curves into weak (< 0.5 mV MEP_{max}) and strong (> 0.5 mV MEP_{max}) based on initial average baseline measures, recruitment curves from weaker legs demonstrated significant increases in MEP_{max} across all experiments (phases) compared to baseline (Fig. 3.3C, 43.30%, One-way ANOVA $F(4,12)=6.41$, $P < 0.001$) compared to the stronger legs which showed very little change (Fig. 3.3D, 3.2%, One-way ANOVA $F(4,16) = 1.04$, $P = 0.40$). Such increases in MEP_{max} occurred after training even though the absolute level of background EMG was maintained at a constant level across all experiments (data not shown, One-way ANOVA: $F(4,29)=1.35$, $P=0.26$).

Given that the increases in MEP_{max} were similar when plotting the data from both legs according to training order (Fig. 3.3A) and training type (Fig. 3.3B), we further verified that increases in MEP_{max} compared to baseline were similar when the data was grouped according to those participants who started endurance first compared to those starting precision first (Fig. 3.3E) and indeed, there were no differences between the two groups (Mixed factor ANOVA: $F(4,28) = 0.45$, $P = 0.77$). Furthermore, we compared increases in MEP_{max} for the different types of training to the immediately preceding phase (Fig. 3.3F) in contrast to comparing everything to baseline as was done previously. For example, if endurance training occurred first, it was compared to baseline (B1/2) and if it occurred second, it was compared to the first rest phase (R1). As with the comparisons to baseline only, MEP_{max} increased with respect to the immediately preceding phase for both endurance and precision training but only in the initially weaker legs, especially for endurance training where the percentage increase in MEP_{max} was greater in the weak legs compared to the strong legs (Fig. 3.3F, $P = 0.03$).

In summary, MEP_{max} increased in response to endurance or precision training in legs that had initially smaller MEP_{max} values, no matter when the specific type of training was performed.

3.3.3. Maximum Voluntary Contractions

Similar to MEP_{max}, when data from both legs were grouped together there was a moderate increase in MVC after training (19.0%) and a significant main effect over time (Fig. 3.4A, One-way ANOVA; $F(4, 29) = 2.80$, $P = 0.03$) and across training type (Fig. 3.4B, $F(4,29) = 3.06$, $P=0.021$). When separating the data into weak and strong legs, the increase in MVC compared to baseline was also greater in the initially weaker legs (Fig. 3.4C, 36.0%, $F(4,12) = 4.70$, $P =0.003$) compared to the stronger legs (Fig. 3.4D, 10.50%, $F(4,16) = 0.96$). Similarly, there was no difference in the profile of MVC increases compared to baseline when the groups were separated into those starting with endurance or precision training (Fig. 3.4E, Mixed ANOVA: $F(4,28) = 1.75$, $P = 0.15$). Finally, increases in MVC, when compared to the immediately preceding phase (B1/2 or R1), were greater in the weaker legs, again especially for endurance training (Fig. 3.4F, 30.90%, $P = 0.04$). Because the increases in MEP_{max} appeared to follow the increases in MVC, we plotted the overall percentage change in these two measures against one another, i.e., baseline compared to the last phase of training, P2. Indeed, there was a significant correlation between the change in MVC and the change in MEP_{max} (Fig. 3.5A, see values in figure). No one form of training seemed to weight the correlation when comparing the scatter of black (End) and open (Prec) symbols. We also examined if there was a relationship between the increases in MEP_{max} (for the leg having the largest change) and the changes in the clinical measures of walking function. There was no relationship of clinical measures to MEP_{max} after precision training (data not shown). However, when plotting changes in MEP_{max} to the changes in the 6MWT (Fig. 5B), 10MWT (Fig. 3.5C) and SCI-FAP (Fig. 3.5D) after endurance training, all correlations were significant except for the SCI-FAP which showed a strong trend (a decrease in SCI-FAP scores indicates improvement).

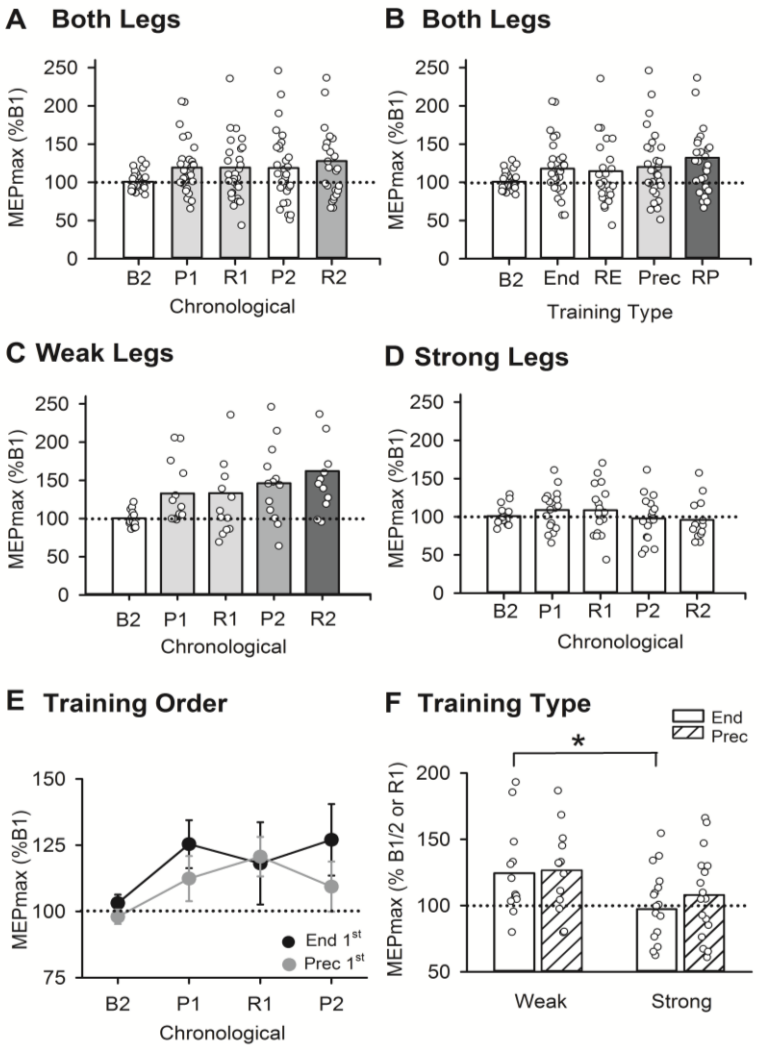

Figure 3.3. Effect of training on MEP_{max}.
A) MEP_{max}, expressed as a percentage of the first baseline (B1) measure, plotted chronologically for each training and rest phase. Values for each leg (both weak and strong) are marked by open circles and group mean by height of bars. Shaded bars indicate mean values significantly different from baseline. **B)** Same as in *A* but with data plotted according to the type of training: End = post endurance, RE = rest after endurance, Prec = post skill, RP = rest after precision. **C&D)** MEP_{max} plotted chronologically as in A for initially weak (C) and strong (D) legs. **E)** MEP_{max} plotted as a function of whether endurance (End) or precision (Prec) was the first phase (P1) of training. For this and subsequent training order graphs, R2 data was not included due to missing points. **F)** $MEP_{max,}$ expressed as a percentage of the immediately preceding phase (B1/2 for P1 training; R1 for P2 training), for endurance and precision training in both weak and strong legs. Asterisk in F indicates significant difference between weak and strong legs after endurance training (*$p < 0.05$). Shading of bars: light gray = $P < 0.05$; medium gray = $P < 0.01$; dark gray = $P < 0.001$). Error bars represent ± SE.

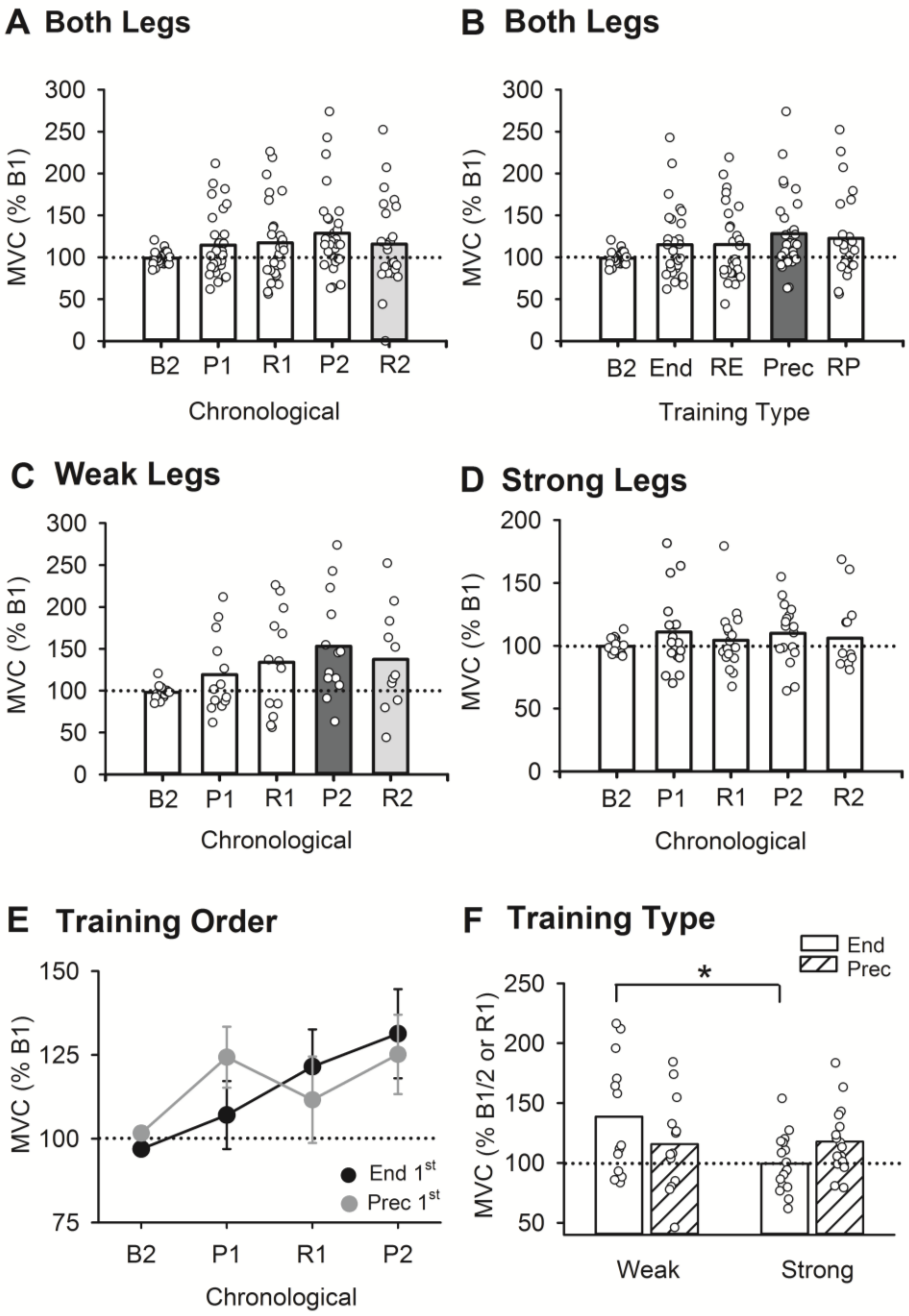

Figure 3.4. Effect of training on Maximum Voluntary Contraction (MVC).

Same format as in Figure 3 but for Maximum Voluntary Contraction (MVC) values.

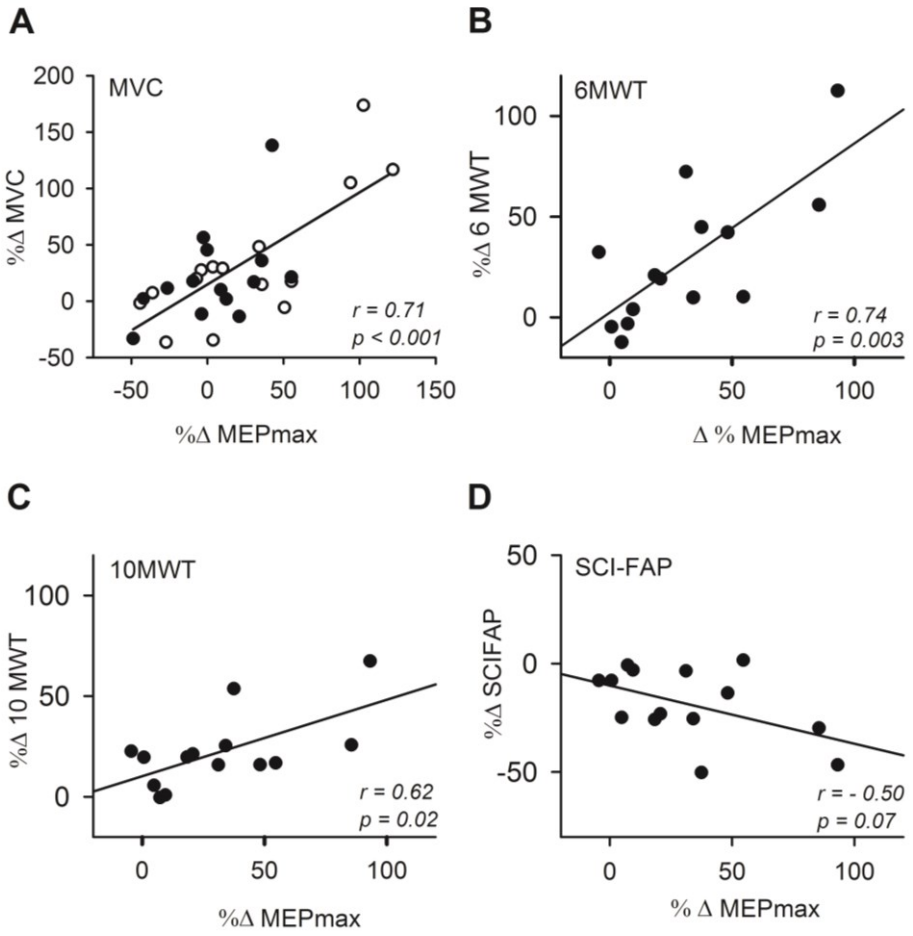

Figure 3.5. Correlation of MEP_{max} with MVC and clinical walking measures

A) Plot of percentage change in MEP_{max} against the corresponding change in MVC for each leg. MEP_{max} and MVC are measured after P2 and expressed as a percentage change from baseline (B1/2). Solid circles represent data where P1 was endurance training and open circles from data where P1 was precision training. **B, C, D)** Same as in A but for changes after endurance training to the immediately preceding phase for 6 min walk test (6MWT, **B)**, self-selected speed for 10m walking test (10MWT, **C**) and Spinal Cord Injury-Functional Ambulation Profile scores (SCI-FAP, **D**). r = correlation coefficient.

3.3.4. Experiment 2: CPN conditioning of TA MEP

Recently we have described a long-latency, spinal inhibitory pathway to TA motoneurons that is activated by both homonymous, low-threshold afferents and descending pathways activated by TMS (Zewdie et al. 2014). Because walking training is associated with decreases in excessive muscle activity during walking (Gorassini et al. 2009; Knikou 2013; Manella and Field-Fote 2013), we examined if endurance and/or precision training could enhance this spinal inhibitory network as a possible mechanism for spasticity reduction. To examine this pathway, a test MEP evoked in the TA muscle of a single leg was conditioned by a prior triple-pulse stimulation to the homonymous CPN at ISIs of 40/50/60 ms and at increasing intensities of stimulation relative to motor threshold (x MT). As shown for a single participant at baseline (Fig. 3.6A), the CPN conditioning stimulation suppressed the TA MEP moderately at 0.5 x MT. However, after the first phase of training (Fig. 3.6B), in this case after precision training, there was a larger suppression of the TA MEP, especially at 0.5 and 0.7 x MT.

Because the stimulation intensity that produced a suppression of the MEP was variable between the different SCI participants and on different days, we measured the maximum amount of MEP suppression that occurred over the entire range of stimulation intensities in each participant and on each day to give a *peak* MEP suppression value (average stimulation intensity across all experiments was 0.5 ± 0.2 x MT). When plotting the amount of peak MEP inhibition across all experiment days (Fig. 3.7A), a significant main effect was present (One-way ANOVA: $F(4,13) = 2.78$, $P = 0.04$), with a significant suppression of the MEP after the first phase (P1) of training of 73.10 ± 8.8% ($P = 0.009$) compared to the average MEP suppression across the two baseline measures of 86.30± 7.20%. When the data was plotted according to the *type* of training received (Fig. 3.7B), a significant main effect was also present (One-way ANOVA: $F(4,13)= 2.65$, $P = 0.04$), with a significant suppression of the MEP after precision (Prec) training ($P = 0.01$).

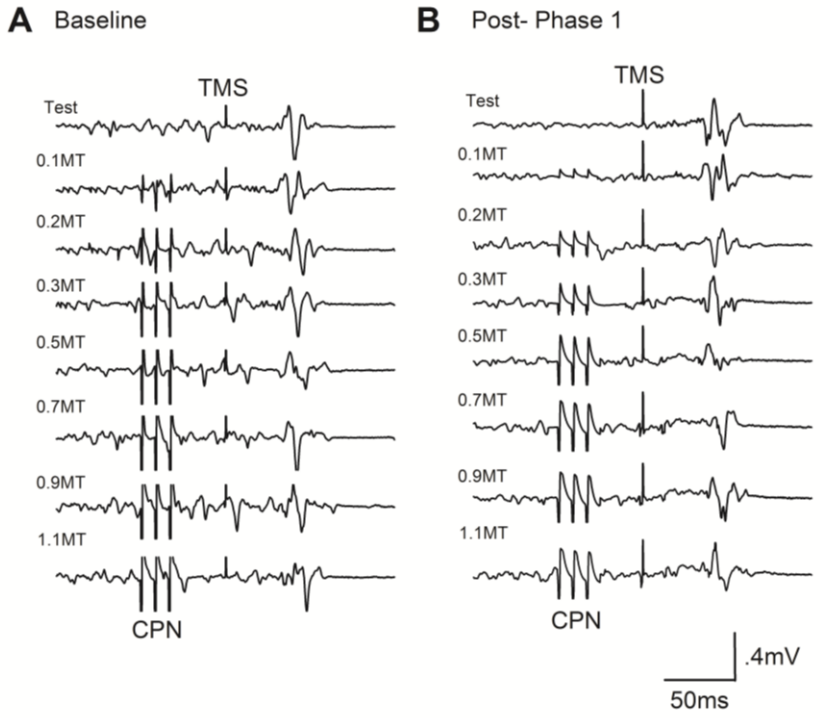

Figure 3.6. Suppression of TA MEPs by low-threshold, homonymous CPN stimulation
Average of 14 unconditioned (test) MEPs and 6 conditioned MEPs at each incrementing intensity of CPN stimulation from a single participant (P6) at baseline **(A)** and after the first phase of (precision) training **(B).** Three pulses of CPN stimulation from 0.1 to 1.1 x motor threshold (MT) were given at 40/50/60 ms prior to the TMS.

Likewise, when the groups were separated into those who started endurance or precision training first (Fig. 3.7C), there was no difference between the two profiles of increased MEP suppression over time (Mixed factor ANOVA: $F(4,12) = 0.30$, $P = 0.87$), although the MEP suppression appeared more stable after endurance training.

The average test MEP size was compared between recording days in order to assess if the amplitude of the unconditioned MEP affected the amount of MEP inhibition. Unlike the amount of MEP inhibition, the amplitude of the average test MEP size did not change across the different experiment days (Fig. 3.7D, One-way ANOVA, $F(13,5) = 0.75$, $P = 0.59$), likely because we purposely controlled

for its size across the different experiments. Likewise, the threshold to evoke an M-wave in the TA muscle was also stable across the different experiment days (Fig. 3.7E, One-way ANOVA, $F(13,5) = 0.24$, $P= 0.94$). In 9 of the 14 participants, the maximum M-wave in the TA muscle (Mmax) was measured and this too did not change (Fig. 3.7F, One-way ANOVA, $F(8,5) = 0.85$, $P = 0.52$).

3.3.5. Experiment 3: Cutaneomuscular Reflexes (CMR)

The mean amplitude of the CMR evoked in the TA muscle from TN stimulation at the ankle (Fig. 3.8A, see *Experiment 3* in Methods), was also examined after the different training and associated rest periods. As shown for a single participant in Fig. 3.8B, the inhibitory component of the CMR, defined as the amount of EMG below the mean pre-stimulus EMG, increased after the first bout of training (precision training in this case). This produced a decrease in the *total* CMR, measured 30 to 300 ms post-stimulus, from 76.0 μV at baseline to 38.6 μV after P1 in this participant. When plotted chronologically, the magnitude of the total CMR, expressed as a percentage of first baseline (B1), was reduced by 52.31 ± 23.60% on average in the 16 participants, with a significant main effect over time (Fig. 3.8C, One-way ANOVA :$F(4,15)= 4.15$, $P = 0.005$). A pair wise comparison was significant after P1 ($P = 0.004$) , P2 ($P=0.007$) and R2 ($P=0.04$) when compared with B1. When the data was plotted according to the type of training received (Fig. 3.8D), a significant main effect was also present (One-way ANOVA: $F(4,15)= 4.46$, $P = 0.003$), with a significant suppression of the mean CMR after endurance (End, $P=0.002$), precision (Prec, $P=0.008$) and rest after precision training ($P=0.02$). When the groups were separated into those who started endurance or precision training first (Fig. 3.9E), there was no difference between the two groups over time (Mixed ANOVA: $F(4,15) = 1.54$, $P = 0.36$), although inhibition appeared more stable when precision training was initiated first.

To examine the CMR in more detail, we compared changes in the total sum (i.e., area) of CMR EMG that was above (excitatory component) and below

(inhibitory component) the mean, pre-stimulus EMG. The magnitude of the excitatory component of the CMR remained close to B1 (100%) across all experiments compared to baseline (sum of all positive y-values, Fig. 3.8F), whereas the inhibitory component (sum of all negative y-values) increased by 148.7% on average compared to baseline. This indicated that the decrease in the total CMR was mainly due to an increase in the inhibitory component of the CMR. The inhibitory component of the CMR started on average 107 ± 35 ms after the first stimulation pulse. The average excitatory component of the rectified CMR EMG (from 30 to 91.2 ms) just before the inhibitory component was 31.71±11.80 μV at baseline and did not change between experiments (One-way ANOVA: $F(4,15)= 0.24$, $P = 0.92$). In addition, there was no significant change in the M-wave threshold of the AHB muscle (not shown), which was used to set the intensity of the TN stimulation (One-way ANOVA; $F(4,15) = 0.53$, $P=0.72$), and was 15.82 ± 6.21 mA, on average, at baseline. Likewise, there was no change in the background contraction levels during the TN stimulation across the different experiments with an average amplitude of 67.71 ± 40.91μV at baseline (data not shown, One-way ANOVA: $F(4,15)=0.37$, $P=0.82$).

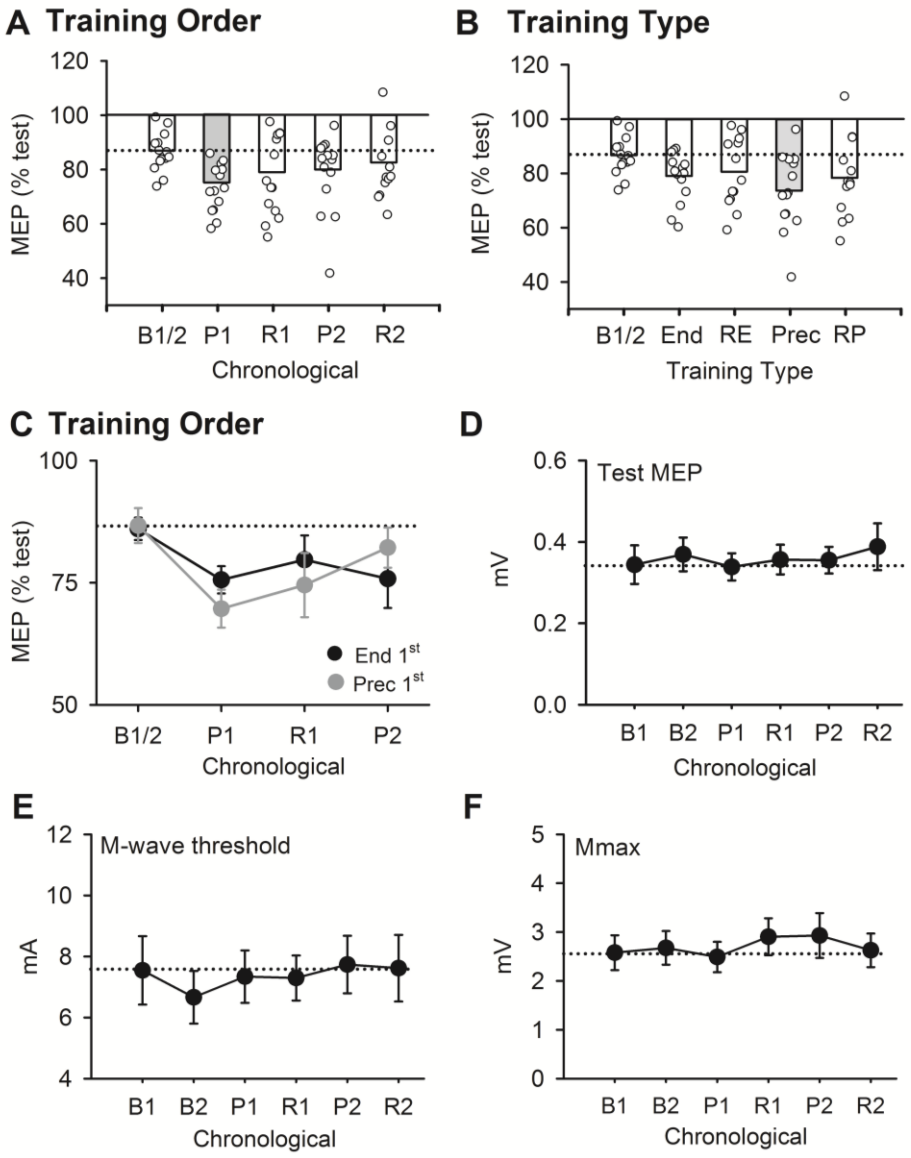

Figure 3.7. Effect of training on suppression of TA MEPs by conditioning CPN stimulation

A) Suppression of the conditioned TA MEP expressed as a percentage of unconditioned test MEP for the averaged baseline (B1/2), the two training (P1 and P2) and rest (R1 and R2) blocks in 14 participants. Data from each leg are marked by open circles and bars represent group means. **B)** Same as in *A* but data is plotted according to the type of training. **C)** Amount of MEP suppression grouped according to whether endurance (solid circles) or precision (gray circles) was the first phase of training. The peak-to-peak amplitude of the test MEP (**D**), the threshold of the TA M-wave (**E**) and the peak-to-peak amplitude of the maximum M-wave (M_{max}) (**F**) for all experiments. Error bars represent ± SE. Shaded bars indicate significant difference from baseline (light gray = $P < 0.05$; medium gray = $P < 0.01$).

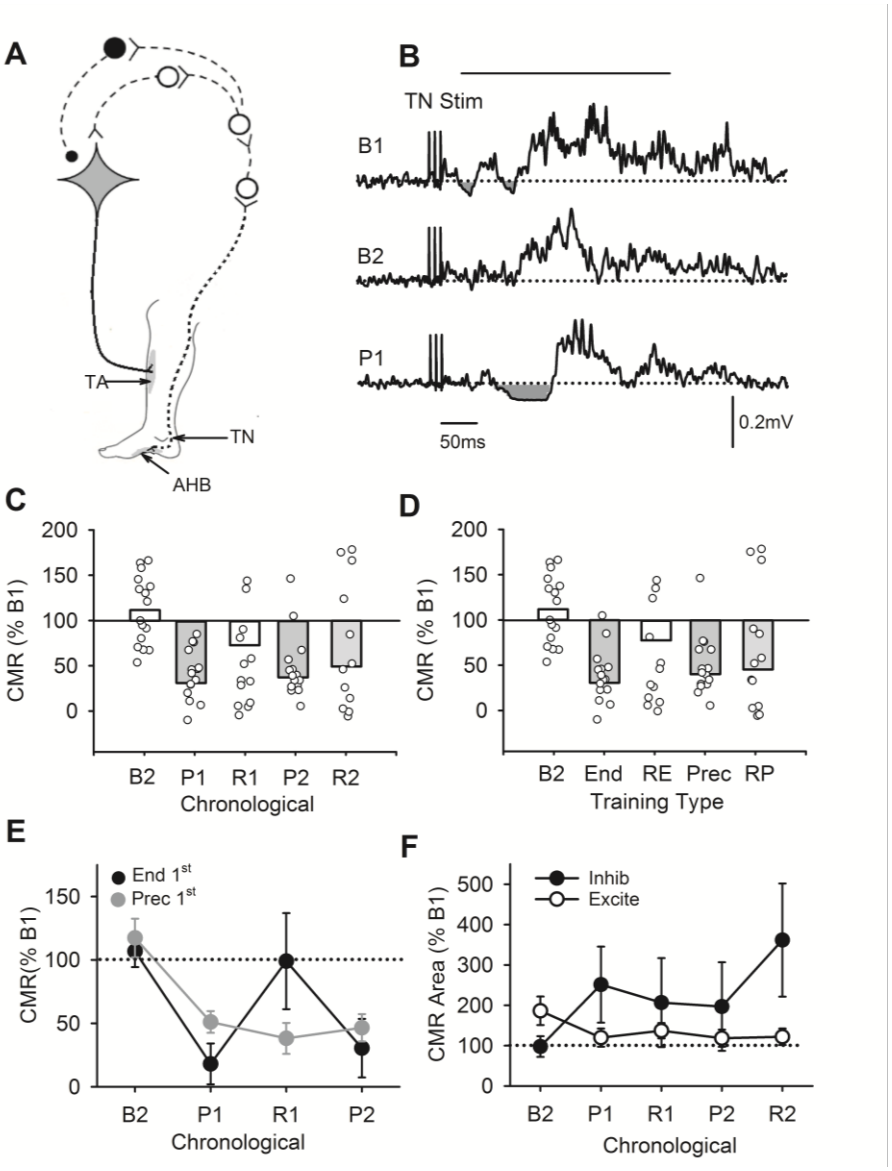

Figure 3.8. Effect of training on cutaneomuscular reflexes (CMR)
A) Schematic of proposed spinal network in the cutaneomuscular reflex (CMR). **B)** Rectified and smoothed TA EMG after TN stimulation from participant P1 at the two baseline experiments (top two traces) and after precision training (bottom trace). Mean background EMG is marked by dashed horizontal line. EMG falling below background (inhibitory component) is marked by shading. CMR was measured from 30 to 300 ms after TN stimulation as marked by horizontal line. **C)** Mean CMR EMG expressed as a percentage of the B1. Open circles represent data from all legs and bars represent group mean. **D)** Same as in C but data is plotted according to the type of training as before. **E)** Data grouped according to whether endurance (solid circles) or precision (gray circles) was the first phase of training. **F)** Area of CMR, expressed as a percentage of B1, plotted separately for the excitatory (open circles) and inhibitory (solid circles) components of the CMR. Error bars represent ± SE. Shaded bars indicate significant difference compare to baseline (light gray = $P < 0.05$; medium gray = $P < 0.01$).

3.4. Discussion

The maximum evoked response from TMS of the motor cortex (MEP_{max}) and the ability to evoke a maximum voluntary isometric contraction in dorsiflexion (MVC) was increased following both endurance and precision walking training, irrespective of when the training was introduced. These results are contrary to what we initially hypothesized where we predicted precision training to show larger increases in responses evoked or initiated from the motor cortex. Likewise, both forms of training produced an increase in the recruitment of inhibitory spinal circuitry as evidenced by greater long-latency inhibition of the TA MEP and the inhibitory component of the CMR. Thus, endurance training, which focused on walking for as long and fast as possible on a treadmill, and precision training, which focused on stepping over obstacles and precise placement of the foot during over ground walking, were both capable of inducing similar changes in these neurophysiological measures despite the differences in motor tasks between the two (Yang et al. 2014).

3.4.1. Increase in the descending activation of spinal cord after training

The persistent increase in MEP_{max} and MVC after training was likely not a result of the day-to-day variability of these responses, but rather, as a consequence of the training given that the two baseline measures were consistently lower than those measured after training. Likewise, the background level of CNS excitability during the TMS experiments was well matched on the different experiment days, as measured by consistent levels of background EMG activity, and likely did not contribute to the increases in MEP_{max} after training. An unexpected finding was that MEP_{max} and MVC (and even walking function, Yang et al., 2014), did not decrease to baseline levels after each two-month period of rest. The fact that many participants continued to walk at home or in the community during these washout periods could have maintained these gains.

As mentioned above, the only notable change in the TMS recruitment curves was the increase in MEP_{max} that was evoked at high intensities of stimulation, compared to $MEP_{1/2}$ and MEP_{thres} which did not change. It may be that only the higher-threshold, descending pathways activated by TMS over the motor cortex were modified by training. Alternatively, new areas of motor cortex that expanded radially from the initial TA hotspot, and thus requiring larger current spread from the higher TMS intensities to activate, were recruited by training. This would have produced TMS recruitment curves that only shifted vertically at the higher stimulation intensities rather than producing a leftward shift indicative of decreases in recruitment thresholds (Ridding and Rothwell 1997). It was interesting that only descending pathways that produced a smaller-amplitude MEP_{max} before training increased in strength in response to training in comparison to the larger MEPs, the latter which were still about half the size typically recorded in non-injured control participants (Capaday et al. 1999; Roy et al. 2010). It may be that a critical amount of damage and/or disuse to a descending pathway is needed in order for intensive training to increase its strength or connectivity, as shown for spontaneous recovery from lesions of different size in the corticospinal tract (Weidner et al. 2001). This also agrees with clinical findings where humans (Yang et al. 2014) and rats (Hurd et al. 2013; Schucht et al. 2002) with moderate motor function benefit more from training compared to those with high motor function.

The descending pathways activated by TMS over the motor cortex could include the direct corticospinal tract with both mono and polysynaptic motoneuron connections (Geertsen et al. 2011) and the indirect cortico-reticular ((Fisher et al. 2012) and cortico-vestibular (Guzman-Lopez et al. 2011) pathways. How each of these pathways contributes to improvements of specific components of walking in the human is unknown. In cat studies, the corticospinal tract is involved in visuomotor coordination needed for obstacle avoidance during walking (Drew et al. 2004) whereas the corticoreticular pathway combines goal-directed walking with other motor actions such as maintenance of posture

(Matsuyama et al. 2004). Given that voluntary walking speed, duration, skill and balance all improved with both forms of training (Yang et al. 2014), it is likely that all of these descending pathways contributed. Because improvements in MEP_{max} were only correlated to changes in walking function after endurance training (Fig. 5), descending pathways activated by TMS over the motor cortex may be more affected by mass practice during endurance training compared to precision training, the latter which involves more visually guided walking tasks but fewer steps per session. Interestingly, endurance training induced bigger improvements in walking distance than precision training whereas walking skill was greater after precision training (Yang et al. 2014).

3.4.2. Spinal inhibition after SCI and its strengthening by motor training

In addition to strengthening the descending excitation of the spinal cord, both endurance and precision training appeared to increase the strength of inhibitory spinal networks activated by both descending and peripheral afferent pathways. We have recently shown that the long-latency suppression of TA MEPs by low-threshold CPN afferents is likely mediated by spinal mechanisms given that evoked responses in the TA from activation of descending pathways at the level of the brainstem and spinal cord are also inhibited by these same afferent inputs (Zewdie et al. 2014). Likewise, the inhibitory component of the CMR is likely due to activation of spinal inhibitory interneurons given that a similar profile of inhibition can occur in completely transected animals (Edgley and Jankowska 1987; Murray et al. 2011) and because afferent inputs from the leg tend not to suppress cortical circuits (Roy and Gorassini 2008). Increases in the inhibitory component of the CMR were likely due to increases in the excitability of spinal inhibitory interneurons given that the preceding excitatory component of the CMR (i.e., E1) was not modified after training and thus, mechanisms such as increases in post-excitation refractoriness of motoneurons likely did not contribute.

Several mechanisms may have produced the increase in spinal inhibition. First, as with increasing activation of excitatory interneurons/motoneurons by descending inputs (Thomas and Gorassini 2005), intensive motor training may increase the descending activation of inhibitory interneurons by spared descending pathways after iSCI. The participants in this study who demonstrated enhanced suppression of TA MEPs from a conditioning CPN stimulation also showed significant increases in maximum TA MEPs after training, although the two were not correlated. This suggests that spared descending pathways originating from the motor cortex were strengthened by training and may have increased the activation of both excitatory and inhibitory elements in the spinal cord.

Second, training may have increased the ability of motoneurons to produce inhibitory post-synaptic potentials (IPSP) from afferent activation via an increase in the insertion of potassium-chloride co-transporters (KCC2) into the motoneuron membrane. KCC2 maintains the Cl^- equilibrium potential in neurons, and thus, the strength of IPSPs activated in motoneurons and interneurons by sensory inputs (Boulenguez et al. 2010). Following spinal cord injury, insertion of KCC2 is reduced, producing a depolarizing shift in the Cl^- equilibrium potential and a decrease in IPSP activation by sensory inputs (Boulenguez et al. 2010; Murray et al. 2011; Norton et al. 2008). Increases in neuronal activity associated with treadmill walking are known to increase levels of BDNF within the spinal cord, even after spinal cord injury (Macias et al. 2009; Ying et al. 2005). Thus, increases in the BDNF-facilitation of KCC2 and subsequent reduction of the Cl^- concentration in motoneurons and interneurons may increase the magnitude of IPSPs activated by low-threshold, homonymous afferents to facilitate the suppression of TA MEP and inhibitory component of the CMR. In fact, following a complete transection in adult rats, passive cycling increases the insertion of KCC2 into the motoneuron membrane and restores rate-dependent depression (Cote et al. 2014).

Lastly, the transmission of sensory inputs is also reduced by the activation of 5HT1 receptors, likely on afferents and excitatory interneurons (D'Amico et al. 2013; Murray et al. 2011). As with the descending pathways from the motor cortex, intensive exercise and motor training may increase the descending activation of the spinal cord by residual serotonergic pathways from the brainstem (Steinberg et al. 1998) and subsequently, reduce excitatory sensory transmission to help uncover the activation of IPSPs in motoneurons and interneurons (Murray et al. 2010).

Further studies in animal models of spinal cord injury and motor training are needed to resolve these possibilities.

3.4.3. Functional implications

Improvements in walking function from daily, intensive endurance or precision training are associated with increases in descending activation of the spinal cord and in increases in the excitability of inhibitory spinal networks. These neurophysiological changes may lead to improved volitional control of movement as well as reductions in involuntary muscle spasticity as reflected in the reduced spasm-like CMRs recorded in this study. Although both forms of training were challenging in different ways, each provided benefits. For example, during endurance training participants walked on average ~1200 steps per session at 0.6 m/s and improvements in over ground walking distance over 6 min were greater compared to precision training (Yang et al. 2014). In precision training, participants walked on average only ~400 steps per session at 0.17 m/s, and showed comparable improvements in walking skill as for endurance training. Thus, increased walking activity in these two forms of training can modify descending and peripheral activation of the spinal cord and improve different aspects of walking function after chronic, iSCI. Mechanisms of how walking training mediates these changes in neurophysiological and functional outcomes in the human needs to be examined in order to provide an even greater impact.

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CHAPTER 4. GENERAL DISCUSSION

4.1. Thesis Summary

Inhibitory feedback from sensory pathways is important for controlling movement. In *Chapter 2* of this thesis, we characterize, for the first time, a long-latency inhibitory spinal pathway to ankle flexors that is activated by low-threshold, *homonymous* afferents. To examine this inhibitory pathway in uninjured participants we suppressed motor evoked potentials (MEPs) produced in the tibialis anterior by a prior stimulation to the homonymous common peroneal nerve (CPN). The tibialis anterior MEP was suppressed by a triple-pulse stimulation to the CPN applied 40, 50 and 60 ms earlier and at intensities of 0.5 to 0.7 times motor threshold (average suppression of test MEP was 33%). While the triple-pulse stimulation was below M-wave and H-reflex threshold, it produced a long-latency inhibition of background muscle activity ~65 to 115 ms after the CPN stimulation, a time period that overlapped with the test MEP. However, not all of the MEP suppression could be accounted for by this decrease in background muscle activity. Evoked responses from direct activation of the corticospinal tract, at the level of the brainstem or thoracic spinal cord, were also suppressed by low-threshold CPN stimulation. Our findings suggest that low-threshold, muscle and cutaneous afferents from the CPN activate a long-latency, homonymous *spinal* inhibitory pathway to tibialis anterior motoneurons. We propose that inhibitory feedback from spinal networks that are activated by low-threshold, homonymous afferents helps regulate the activation of flexor motoneurons by corticospinal pathways.

In *Chapter 3* of this thesis, we examined if this long-latency, spinal inhibitory pathway could be facilitated in participants with incomplete spinal cord injury in response to two different forms of walking training. One form of training (body weight supported treadmill training) emphasized endurance and speed whereas the other (precision) emphasized walking over obstacles and precise placement of the foot. Sixteen participants with incomplete spinal cord injury received 2 months of endurance or precision training, in random order, with 2 months of rest before crossing over to the other type of training. Both

endurance and precision training increased the suppression of MEPs by a prior low-threshold, triple-pulse stimulation to the CPN, reflecting an increased excitability of the long-latency, inhibitory spinal pathway described in Chapter 2. The inhibitory component of the cutaneomuscular reflex, at a spinal latency, was also increased after endurance and precision training, indicating a general increase in the excitability of inhibitory spinal circuits. In addition, both forms of training increased the maximum motor-evoked potential (MEP_{max}) elicited by transcranial magnetic stimulation over the motor cortex, but only in muscles that had small (<0.5mV) MEP_{max} values before training, no matter when the specific type of training was performed. A similar pattern of training-induced increases in maximum voluntary contractions was also observed. Although walking function was improved by both forms of training, a positive correlation between MEP_{max} and clinical measures of walking function only occurred after endurance training. The increase in the descending excitation of the spinal cord and the increase in excitability of inhibitory spinal networks may mediate the improved volitional control of walking and reduction of involuntary muscle spasticity, respectively, that are observed in response to intensive motor training in participants with incomplete spinal cord injury.

I discuss below the potential pathways that might mediate the long-latency, inhibitory pathways described in *Chapter 2 and 3.* I also discuss how changes observed in this inhibitory spinal pathway, together with changes in descending motor pathways can be related to improvements in functional walking outcomes as a result of motor rehabilitation.

4.2. Mechanisms producing homonymous spinal inhibition

In both SCI and non-injured participants, low intensity triple-pulse stimulation to the CPN resulted in inhibition of the tibialis anterior (TA) MEP,

most effectively at a long ISI of 40/50/60ms. The use of three pulses instead of one pulse when given below H-reflex or M-wave threshold enhanced this inhibitory effect. The fact that the stimulation pulses were below M-wave or H-reflex threshold suggests that motoneuron refractoriness or recurrent inhibition did not mediate this long-lasting, long-latency inhibition (Meunier et al. 1994; Tucker et al. 2005). Given that similar shaped evoked potentials from direct stimulation of descending tracts at the level of the cervical (C7) spinal cord were also suppressed by low-threshold CPN stimulation strongly suggests that the TA MEPs were suppressed via processes occurring in the thoracic or lumbar spinal cord. As discussed in Chapter 2, this is in agreement with other studies showing that low-threshold afferents from the leg, unlike that from the upper limb, do not inhibit cortical circuits but mainly activate inhibitory circuitry in the spinal cord (Geertsen et al. 2011; Roy and Gorassini 2008). Below I describe possible circuits that might mediate this long-latency, inhibitory spinal pathway to the TA motoneuron pool that is activated by homonymous afferents.

4.2.1. Propriospinal Relay Neurons

Group Ia afferents are the largest and most rapidly conducting (55-68m/s, Chaix et al. 1997) peripheral nerve fibers. Cutaneous fibers, such as those contained in the deep peroneal nerve (DPN), can also be fast conducting (40-48m/s, Bayramoglu et al. 2004). Group Ia and cutaneous afferents bifurcate upon entering the spinal cord and travel several segments in both rostral and caudal directions (Meunier et al. 1994). As such, the collaterals of group I and large cutaneous afferents can activate inhibitory interneurons several spinal cord segments rostral to their entry point (Forget et al. 1989). These inhibitory interneurons can synapse onto spinal interneurons, called propriospinal neurons (PSNs), which are located several segments rostral to their target neurons (Marque et al. 2001). In cats, PSNs in the cervical spinal cord receive both monosynaptic excitation (Illert et al. 1981) and prominent disynaptic inhibition (Alstermark et al. 1987) from forelimb afferents. A similar system of PSNs have

been suggested for the human upper limb (Malmgren 1988; Malmgren and Pierrotdeseilligny 1988) and lower limb (Forget et al. 1989) afferents.

A propriospinal site of action has recently been suggested for inhibitory *heteronymous* afferent pathways from the CPN to the soleus motoneuron pool (Geertsen et al. 2011) and from the tibial nerve at the ankle to the TA motoneuron pool (Roy and Gorassini 2008). Both pathways also require long (≈ 35-45 ms) conditioning-test stimulation intervals to suppress the soleus and TA MEPs and contain afferents that enter a similar spinal segment as their target motoneuron pools. Here, we speculate a similar propriospinal pathway that could mediate the homonymous MEP suppression observed in our study (see Fig. 4.1).

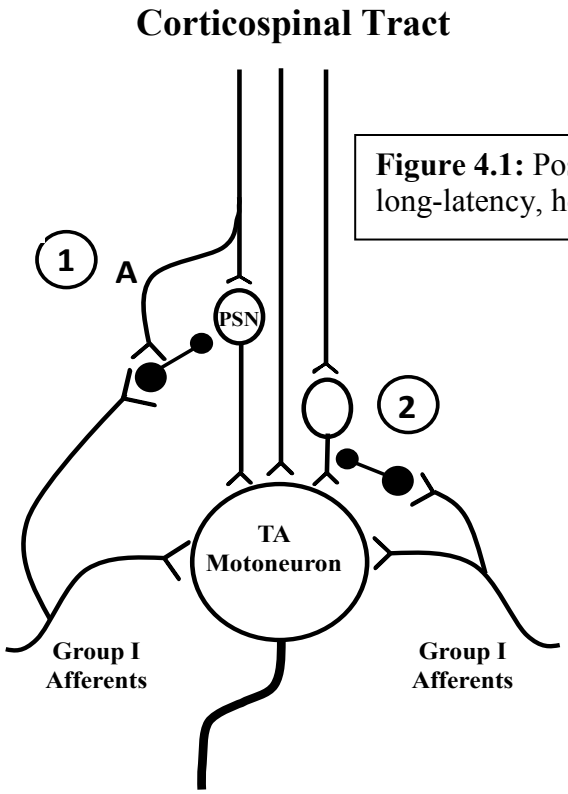

Figure 4.1: Possible pathways mediating long-latency, homonymous inhibition.

Side 1A (disfacilitation of TA motoneurons) depicts pathways previously suggested for heteronymous and/or antagonist reflex pathways in the upper (Burke et al. 1992) and lower limbs (Geertsen et al. 2011; Iglesias et al. 2008; Marchand-Pauvert et al. 1999) where CST and group 1 afferents converge onto an

inhibitory interneuron which then synapses onto an excitatory propriospinal interneuron (PSN), the latter forming an indirect, excitatory CST pathway to the TA motoneuron pool. In this pathway, if we consider the conduction velocity of the fastest afferents in CPN to be 68m/s (Chaix et al. 1997), it would take close to 13ms for the afferent signal to reach the spinal cord from the knee (site of CPN stimulation). If the inhibitory interneurons that synapse onto the PSNs are located in L2-L3 of the spinal cord (Pierrot-Deseilligny and Burke 2005), it will take 5 ms for the afferent signal to travel from the L4-L5 entry point given that interspinal conduction velocities are slow (Forget et al. 1989). For example, PSNs add a central delay of 3.9-5.2 ms (Chaix et al. 1997). Following this, it would take another 5 ms in conduction time to go from the PSNs to the TA motoneuron pool and 17 ms for the action potential to propagate from the TA motoneuron pool to the TA muscle. In total, the activation of the entire pathway could take 13+5+5+17 = 40 ms. The duration of this reflex pathway is about 20 ms shorter than the onset time of EMG suppression that we observed in Chapter 2 which occurred closer to 65 ms after the triple pulse CPN stimulation. The slower onset time of EMG suppression may have resulted from a long duration of time ($\geq$ 20 ms) to build up inhibition of the PSN by the inhibitory interneuron, especially if it was mediated by the activation of GABAb-receptors which have a slower time course of activation (Rogasch et al. 2013).

The pathway in Figure 4.1A could also explain the time course of TA MEP suppression which commenced at ISI intervals as early as 25/35/45 ms. As described above, it could take the afferent volley 13ms from the knee level to the spinal cord and additional 5ms to ascend to the spinal cord level where PSNs are located. The descending volley that is activated by TMS could take about 5-7ms to activate PSN. In total, it could take 13+5+7 $\approx$ 25ms for afferent volley to inhibit the descending volley at the PSN level. This timing matched with the ISI that produced the first significant MEP suppression at the 25/35/45 ms ISI. As shown in Figure 2.2, the onset of the suppressed MEP was 4.2 ± 3.1 ms later than the onset of test MEP. This additional time could be the time it takes for the PSN inhibition to manifest at the MN level.

Although we cannot prove definitively that the homonymous, low threshold inhibitory pathway is mediated by pathways involving PSNs, there are a couple of characteristics of this pathway that suggest PSN involvement. For example, PSNs are activated by low-threshold afferents (Burke et al. 1991), they receive convergent inputs from muscle and cutaneous afferents (Marchand-Pauvert et al. 1999) and they are facilitated by multi-pulse stimulation (Pierrot-Deseilligny 1996). The utility of having a pathway where sensory inputs enter one level of spinal cord and influence PSNs at different level of the spinal cord could be that it allows other inputs, such as group I and cutaneous fibers of different muscles, as well as the CST to converge onto this PSN and result in the optimized modulation of the TA motoneuron pool.

4.2.2. Presynaptic inhibition onto spinal interneurons

As an alternative mechanism, the homonymous inhibitory pathway could also be mediated by a *local* spinal inhibitory mechanism with a long time course of activation. One possibility is pre-synaptic inhibition of excitatory interneurons that are located near the TA motoneuron pool (Fig 4, site 2). Presynaptic inhibition onto Quad and soleus group Ia afferents activated by brief vibration of the TA tendon (three cycles of vibration at 200Hz) induced inhibition of the Quad and soleus H-reflexes within the range of 40-200 ms (Hultborn et al. 1987). Animal experiments demonstrating such inhibition occurred without any observable change in MN membrane potential and conductance (Fuortes et al. 1957) providing a direct evidence of presynaptic inhibition. The suppression of the TA MEP by CPN afferents occurred within this time scale. Although many human studies of presynaptic inhibition (see review by Rudomin and Schmidt 1999) were focused on its effect onto Ia afferent terminals, animal studies demonstrated that presynaptic inhibition could also occur onto other spinal segmental interneurons (Motsnyi et al. 1972). Therefore, pre-synaptic inhibition onto terminal axons of local excitatory interneurons interposed between the CPN

afferents and TA corticospinal tract could have mediated the long-latency, long-lasting spinal inhibition observed in Chapter 2.

4.2.3. Afferents that contributed to homonymous inhibition

We cannot completely prove based only on their low-threshold, that Ia and Ib afferents activated by the 0.3-0.7xMT electrical stimulation of the CPN contributed to the MEP and EMG suppression in Chapters 2 and 3 of this thesis. However, it is fair to conclude that cutaneous afferents activated in the CPN likely contributed to part of the MEP suppression because stimulating the area of skin between first and second toe, which is innervated by a branch of the CPN containing only cutaneous afferents (the DPN), also suppressed the TA MEP, but to a lesser extent than stimulating the CPN. Low-threshold cutaneous afferents activated in the skin immediately under the stimulating electrodes do not contribute in a large way to the MEP suppression because stimulation of the skin in the same dermatome but at a distance away from the CPN did not produce MEP suppression. However, Zehr and colleagues (2001) showed that electrical stimulation of the superficial peroneal nerve (SPN), which is a pure cutaneous nerve, resulted in suppression of voluntary TA EMG activity 50 - 120ms post stimulation, which is similar to the latency of inhibition produced by stimulation of the CPN demonstrated in Chapter 2. Therefore, since stimulation of the CPN includes all afferents in the cutaneous branch of the nerve, as well as additional afferents originating more superficially, we cannot prove that the observed inhibition is mediated partly by muscle afferents. Other techniques should be used to exclusively confirm the contribution of muscle afferents. For example, vibration of the muscle belly or tendon of the TA muscle can be vibrated to more selectively activate Ia fibers. Cutaneous receptors in the skin underneath the vibration have to be inactivated using nerve blockers such as lidocaine, although there are some cutaneous receptors like pacinian corpusles that are sensitive to vibration (Biswas et al. 2015). Thus, there remains the possibility that suppression of the MEP and EMG activity observed in Chapters 2 and 3 of this thesis is solely mediated by low-threshold cutaneous afferents and warrants further study.

4.2.4. Future directions

Further studies in animal and human are needed to determine the neuronal pathways mediating long-lasting, homonymous inhibition observed in Chapters 2 and 3. For instance, the central delay of the homonymous inhibition can be better estimated by investigating the post-stimulus time histograms (PSTHs) of single motor units. These histograms can be used to visualize the rate and timing of MN discharge in relation to an external stimulus. PSTHs can also be used to assess changes in MN discharging probability as a result of post synaptic potential. The effect of a conditioning CPN stimulus on the firing probability change of the TA motor units can be detected by comparing the peaks that occur during monosynaptic CST volley and smaller second peaks that result from volley that passes through the inhibited PSNs (e.g. see Malmgren and Pierrot Deseilligny 1988). Using PSTH, CPN induced inhibition can be investigated in more caudal MN pool than the TA, such as the Biceps Femoris (BF) MN pool located at L5-S2. If CPN activated BF inhibition has a longer central delay, it will suggest that the inhibition is mediated through premotorneurones located rostral to the MNs, such as the PSNs.

4.3. Walking Training after SCI

Motor training induces plasticity at many loci in the nervous system. The specific changes are a function of the type of training and if it is applied in the intact or injured nervous system (reviewed in Edgerton et al. 1997; Markham & Greenough 2004; Koceja et al. 2004; Vaynman & Gomez-Pinilla 2005; Adkins et al. 2006; Rossignol 2006). Interestingly, there has been a greater focus on plasticity induced in the cerebral cortex with skill training and in the spinal cord with endurance training (Adkins et al. 2006). In Chapter 3, we found that both forms of training (skill and endurance) increased the strength of corticospinal pathways in equivalent amounts as evidenced by similar increases in MEPmax. Likewise, both forms of training also affected spinal inhibitory circuity. I discuss

below changes in corticopinal and spinal pathways and how these changes relate to the improvements in functional walking outcomes.

4.3.1. Spontaneous recovery of walking function

As demonstrated in animals, spontaneous neuronal plasticity after SCI occurs through various mechanisms, such as recovery of nerve roots beside the lesion level, reorganization of spared connections, as well as changes in grey matter of the spinal cord near the lesion (Kern et al. 2005; Bradbury & McMahon 2006; Ramer et al. 2005). These mechanisms of neuroplasticity could lead to spontaneous recovery depending on the severity, initial level of injury and strength of muscles below the level of injury (Dittuno et al. 2005). For example, 70-80% of individuals with motor-complete quadrapelgia will recover some motor function in muscles supplied by the spinal cord near the level of the injury within 3 to 6 months if they have some residual motor strength in this area. For those with complete paraplegia, there was no change in neurological level at one year post-injury (Waters et al. 1992). On the other hand, in most incomplete paraplegia, the recovery of volitional contractions was associated with very long (up to 1 year) delays (Calancie et al. 2004). Other similar studies (Marino 1995; Steeves et al. 2011) also concur that prolonged (>1 year) spontaneous recovery is highly probable in persons with incomplete SCI. In general, as summarized by Fawcett and colleagues (2007), “the vast majority of recovery (in motor incomplete SCI) occurs in the first 3 months, but a small amount can persist for up to 18 months or longer”.

In this thesis, 3 of the participants started training less than one year post injury. Although we have used multiple baseline measures to detect if there was any spontaneous change, we cannot exclude the possibility that the spontaneous recovery could have contributed to the neurophysiological and clinical changes observed after training, especially since we only performed our two baseline measures 1 to 2 weeks apart. We did not have a control group in this thesis since we used a cross-over design, where each participant served as their own control.

However, having a separate control group who will not get walking training but get assessed (clinical and neurophysiological measures) at the same time as the experimental group can help identify the time course of spontaneous walking recovery in the population of SCI who show benefit from endurance and precision training. However, such studies require large number of participants which is not feasible in Alberta.

4.3.2. Changes in corticospinal pathways as measured by TMS

In this thesis, the change in the strength of corticospinal inputs was estimated using TMS under stationary conditions before and after two types (precision and endurance) of walking training of people with SCI. Since the output of the primary motor cortex can be assessed in the form of MEP, TMS was used most extensively for studying the integrity and plasticity of the spared CST. A study used TMS during locomotion in incomplete SCI reported that MEP amplitude correlated with the degree of foot drop (Barthelemy et al. 2010), implying transmission in the CST has an important functional role of lifting the foot during the early swing phase of the gait cycle (Perez et al. 2012). TMS output measures, such as MEPmax and MEP1/2 were also used to reveal that locomotor training can enhance spared CST functions (Thomas and Gorassini 2005).

In Chapter 3, TMS outcomes including MEP amplitude, threshold and modulation were compared before and after endurance and precision training. In SCI patients, relatively higher stimulation intensities were needed to produce a complete stimulus recruitment curve. In some SCI patients, it was impossible to achieve MEP_{max} at the maximum possible TMS output (100%MSO) implying the difficulty of activating the CST. Other studies also reported that active motor thresholds (AMT) are increased in SCI patients (Davey et al, 1998, 1999; Bunday and Perez 2012). SCI participants who took part in studies of this thesis also had higher AMTs, possibly as a result of a fewer number of CST axons reaching the TA motorneuron pool. The AMT did not change after both types of walking

training. Although not assessed in this thesis, the majority of studies that used TMS in SCI have reported delays in MEP latencies (for example see Curt et al. 1998).

The MEP_{max} of weak legs increased after training more than strong legs implying that CST pathways with greater damage show greater plasticity in response to training as seen in animal models of SCI (Weidner et al. 2001). In this thesis, MEP measures were done while sitting but training and functional outcome measures were done while walking overground, and training effects might not be generalizable from function to function. For example, stepping and standing are not generalizable to each other (De Leon et al. 1998). The training effects are specific to muscles and neuronal circuits involved in the training and are unlikely to be mediated by general mechanisms such as exercise-induced hormonal changes (Yang et al. 2011). Most functional outcome measures can be influenced by improvements in individual muscles involved in the walking function. Therefore, precision and endurance training induced improvements in the neurophysiological measures of the just the TA muscle might not be generalized to all muscles that are involved during walking. Precision training likely included some aspects of endurance (i.e., walking for >20 minutes/day), and endurance training included some aspects of precision (i.e., foot clearance of the ground still necessary). Therefore, endurance and precision locomotor training were equally effective in expanding the cortical representation to the TA muscle, although endurance produced larger changes in 6 min walk test compare to skill training, likely due to larger number of steps taken. The difference perhaps arises from the number of strides per session, which averaged to 1188 during endurance training but only 388 during precision training.

Overall, eliciting MEPs from TMS over the motor cortex is a reliable tool for predicting motor recovery as well as functional outcome. Results shown in this thesis altogether with the above mentioned studies demonstrate the linkage between TMS measurements of CST function and behavioral deficit observed during locomotion in SCI patients.

4.3.3. Change in Spinal Pathways

4.3.3.1. Homonymous inhibitory spinal pathways

Suppression of the TA MEP by the spinal inhibitory pathway described in Chapter 2 above was strengthened following intensive walking training in participants with SCI. As discussed in the above section, PSNs could be one possibility of a pathway that mediated the homonymous inhibition. PSNs could play an important role in mediating walking recovery from intensive motor training after spinal cord injury in animal models (Courtine et al. 2008; Fischer and Orth 2011). Evidence for the existence of PSNs in humans comes from an interesting study by (Marchand-Pauvert et al. 2001) of a SCI patient who had a lesion at the junction between the C6 and C7 spinal segments and revealed that cutaneous inhibition of the propriospinal neurons was no longer able to modify the MEP of triceps motorneurons, which are located below the lesion. The same cutaneous inputs were able to still inhibit MEP of biceps motorneurons, which are located above the lesion. Thus, PSNs in humans may also play a major role in recovery of walking function after spinal lesion. Similarly, an increase in activation of inhibitory interneuron that synapse onto PSN by spared corticospinal tract pathways could also be a possible mechanism for an increase in homonymous inhibition after training given that training also increases the maximum MEP in these subjects (Thomas and Gorassini 2005).

As discussed in Chapter 3, the increases in the excitability of spinal inhibitory interneurons could likely be mediated by a global change in motoneuron inhibition, possibly due to an up regulation of the potassium-chloride co-transporter (KCC2) as a result of exercise-induced increase in BDNF. Boulenguez and co-workers (2010) showed that increase in BDNF leads to increases in the BDNF-facilitation of KCC2 and subsequently reduction of the Cl- concentration in motoneurons and interneurons. As a result training could increase the ability of motoneurons and interneurons to produce inhibitory post-synaptic potentials (IPSP). Training could also increase descending activation of the spinal cord by serotonergic pathways that subsequently reduce excitatory

sensory transmission to help uncover the activation of IPSPs in motoneurons and interneurons (Murray et al. 2010).

4.3.3.2. CMRs

Similar to the MEP suppression results, the amount of spinal inhibition activated by the cutaneomuscular afferents was increased after both precision and endurance training. The increased spinal inhibition activated by the cutaneomuscular afferents from walking training could have been due to increases in the excitability of spinal inhibitory interneurons given that the preceding excitatory component of the CMR (see Figure 3.8F) was not modified after training.

Studies of spinal injured animals and humans had demonstrated that cutaneous afferents activated during walking are crucial for proper foot placement and successful stepping (Lovely et al., 1986; Barbeau and Rossignol, 1987; Harkema, 2001). Furthermore, cutaneous afferents together with group I afferents activated during walking training mediate recovery from SCI (Fung and Barbeau 1994). As such, motor training could modify transmission in cutaneous pathways (Cote and Gossard 2004) and can be detected by assessing the changes in properties of the cutaneous reflexes before and after training.

4.3.4. Training to improve spasticity

Intensive walking training in participants with incomplete SCI reduces clonus, co-contraction and spasms (Adams and Hicks 2011; Gorassini et al. 2009; Manella and Field-Fote 2013) which may be mediated, in part, by increasing the efficacy of afferent and/or corticospinal activation of inhibitory circuitry in the spinal cord, such as the low-threshold homonymous afferent pathway to the TA motoneuron pool and inhibitory CMR pathways. Thus, intensive motor training may provide another avenue besides suppressive pharmacotherapy, such as baclofen (a GABAb agonist, (Li et al. 2004), to improve residual motor control

while facilitating spinal inhibition and reducing spasticity after incomplete SCI (see D'Amico et al. 2014 for review).

The reduction in the CMR observed in Chapter 3 implies a reduction in spasticity given that inducing a CMR in SCI participants is equivalent to evoking an involuntary spasm, an important feature of spasticity after SCI. However, spasticity can be directly measured using the modified-Ashworth scale (MAS), which was tested to have good inter-rated reliability in patients with spinal cord injury (Mirsha and Genesh 2014). However, due to its low sensitivity to changes in spasticity brought about by motor training in a pilot study, MAS was not used in this thesis.

4.3.5. Future directions

Neural mechanisms underlying improvements in walking as a result of two contrasting forms of waking training were revealed. To benefit from each type of training it may require a certain level of damage to the spinal cord as seen by better improvements of the weaker legs. The neural mechanisms underlying improvements in walking will drive future exploration of better methods for retraining. The information in this thesis can be used to assist future allocation of training methods, so that the training can be tailored to the individual. Methods that prime the motor cortex, such as paired-associative stimulation or repetitive TMS (rTMS) to induce facilitation, may be fruitful to explore. For example, rTMS after SCI can alter cortical inhibition (Belci et al 2004) and can be used to improve clinical and functional outcomes. rTMS can also be used to decrease spasticity (Centonze et al. 2007) and result in better training effect. Additional TMS protocols can also be used to further study cortical and corticospinal reorganization after SCI and walking training. For example, paired-pulse TMS protocols can be used to assess changes in intracortical inhibition and facilitation after SCI (Shimizu et al. 2000; Saturno et al. 2008; Roy et al. 2011) as well as after precision and endurance training.

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