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Associative plasticity and afferent regulation of corticospinal excitability in uninjured individuals and after incomplete spinal cord injury

by

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To my dear Faye Wong – “Just write it down”.

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ABSTRACT

Cortical representations are plastic and are allocated based on the proportional *use* or *disuse* of a pathway. A steady stream of sensory input maintains the integrity of cortical networks; while in contrast, alterations in afferent activation promote sensorimotor reorganization. After an incomplete spinal cord injury (SCI), damage to the ascending and/or descending pathways induces widespread modifications to the sensorimotor system. Strengthening these spared sensorimotor pathways may be therapeutic by promoting functional recovery after injury.

Using a technique called transcranial magnetic stimulation (TMS), we show that the leg motor cortex is facilitated by peripheral sensory inputs via disinhibition and potentiation of excitatory intracortical circuits. Hence, in addition to its crucial role in sensory perception, excitation from peripheral sensory afferents can reinforce muscle activity by engaging, and possibly shaping, the activity of the human motor cortex. After SCI, the amount of excitation produced by afferent stimulation reaching the motor cortex is expectantly reduced and delayed. This reduction of sensory inflow to the motor cortex may contribute to our findings that cortical inhibition is down-regulated after SCI, and this compensation may aid in the recruitment of excitatory networks in the motor cortex as a result of the damage to its output neurons. By repeatedly pairing sensory inputs from a peripheral nerve in the leg with direct cortical activation by TMS, in an intervention called paired associative stimulation, we show that the motor system can be potentiated in both uninjured individuals and after SCI. In the uninjured subjects, we show that in order to produce associative facilitation, the time window required for coincident activation of the motor cortex by

TMS and peripheral sensory inputs is not as narrow as previously thought (~100 vs. ~20 ms), likely due to the persistent activation of cortical neurons following activation by TMS. The potential to condition the nervous system with convergent afferent and cortical inputs suggests that paired associative stimulation may serve as a priming tool for motor plasticity and rehabilitation following SCI.

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LIST OF ABBREVIATIONS USED

Ia	primary muscle spindle afferents
Ib	primary afferents from golgi tendon organs
II	secondary muscle spindle afferents
Aβ	cutaneous afferents
Ag-AgCl	silver-silver/chloride
AH	abductor hallucis
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMT	active motor threshold
ANOVA	analysis of variance
ASIA	American Spinal Injury Association
BWS	body-weight support
BWSTT	body-weight-supported treadmill training
Ca^{2+}	calcium
cCPN	contralateral common peroneal nerve
CMEP	cervicomedullary (motor) evoked potential
CMR	cutaneomuscular reflex
CNS	central nervous system
CPN	common peroneal nerve
cPTN	contralateral posterior tibial nerve
CST	corticospinal tract
D-wave	direct descending wave
DC	direct current
EMG	electromyogram
EPSP	excitatory post-synaptic potential
GABA	γ-Aminobutyric acid
GABA-A	A-type (ionotropic) for GABA
GABA-B	B-type (metabotropic) receptor for GABA
HAM	hamstring muscle
H_{max}	maximum H-reflex response
H-reflex	Hoffmann reflex
ICF	intracortical facilitation

iSCI	incomplete spinal cord injury
ISI	interstimulus interval
I-wave	indirect corticospinal wave (may include wave number such as I3)
LAI	long-latency afferent inhibition
LICI	long-interval intracortical inhibition
LTD	long-term depression
LTP	long-term potentiation
MEP	motor evoked potential
MEP_{max}	maximum MEP response
M_{max}	maximum M-wave
MMS	manual muscle strength
MSO	maximum stimulator output
MT	motor threshold
MVC	maximum voluntary contraction
M-wave	motor wave
NMDA	N-methyl-D-aspartic acid
PAS	paired associative stimulation
PPF	paired-pulse facilitation
PTN	posterior tibial nerve
QUAD	quadriceps muscle
RMS	root mean square
RMT	resting motor threshold
rTMS	repetitive transcranial magnetic stimulation
SAI	short-interval afferent inhibition
SCI	spinal cord injury
SD	standard deviation
SEM	standard error (SE) measurement
SICF	short-interval intracortical facilitation
SICI	short interval intracortical inhibition
$SICI_{max}$	maximum SICI
SNR	signal-to-noise ratio
SOL	soleus muscle
TA	tibialis anterior muscle

tDCS	transcranial direct current stimulation
TES	transcranial electric stimulation
TMS	transcranial magnetic stimulation
TN	tibial nerve
TS-ADJ	test stimulus intensity adjusted
VL	vastus lateralis
WISCI II	walking index for spinal cord injury II

CHAPTER 1: Introduction

1.1 PREFACE

Sensory afferents play an important role in modulating corticospinal tract (CST) excitability (Rothwell and Rosenkranz 2005). After injury to the central nervous system (CNS), the human brain and spinal cord are modified in part due to the lack of sensory afferent input (Chen et al. 2002; Pierrot-Desseilligny and Burke 2005). Altering sensory input induces reorganization of the neuronal circuitry associated with movement as evidenced by changes in the motor evoked potential (MEP) elicited using transcranial magnetic stimulation (TMS). In this thesis, our aim is to examine the role of afferent regulation of corticospinal excitability to leg muscles in healthy individuals and after an incomplete spinal cord injury (SCI). These studies investigate the networks engaged in voluntary movement and their interaction with sensory inputs from the lower leg. Our findings add to the body of literature showing that the strength of CST projections can be modified by pairing electrical nerve and cortical stimulation in an intervention called paired associative stimulation (PAS). In addition, we examine the recruitment of intracortical inhibitory circuits in the intact and injured CNS since their connections are altered by afferent information (Aimonetti and Nielsen 2001) and play a pivotal role in the induction of cortical plasticity (Ziemann et al. 2001).

1.2 SPINAL CORD INJURY

A lesion to the spinal cord results in sensorimotor dysfunction and can have a devastating impact on an individual's quality of life. In Canada the annual incidence is 1,100/year (Rick Hansen Foundation), and there are presently over 41,000 Canadians living with a SCI. Care requirements alone can vary from $1.25 million for an incomplete thoracic injury to $5 million for a complete cervical injury affecting both arms and legs. Men are generally 4 times more likely to be afflicted by SCI and motor vehicle accidents continue to be the leading cause of SCI in Canada (55%) followed by sporting accidents and medical conditions (27%) and falls (18%). As the corticospinal system is crucial for functional motor recovery after subcortical insult (Thomas and Gorassini 2005; Ward et al. 2006), the potential to increase the strength of spared CST connections may be useful for improving motor recovery after injury.

1.3 MOTOR CORTEX STIMULATION

In 1870, Fritsch and Hitzig (new translation 2009) discovered that motor areas of the cerebral cortex could be identified using electrical stimulation. Since then, motor cortex stimulation has greatly advanced our knowledge of the physiological basis of

motor control. The primary motor cortex (Brodmann's area 4) contains a variety of neuronal elements which form a complex map of the body and together these neuronal networks are crucial for generating voluntary movement (Kandel et al. 2000). Merton and Morton (1980) introduced the technique of transcranial electrical stimulation (TES) to stimulate the intact human motor cortex. Although the stimuli can be applied from the surface of the head, the overall procedure can be quite painful as TES requires high intensity electrical currents to activate cortical neurons. To circumvent this problem, Barker (1985) developed a technique called transcranial magnetic stimulation (TMS) whereby rapidly changing magnetic fields can excite populations of cortical neurons via the process of electromagnetic induction. Because magnetic fields are readily transmitted though the skull and can be applied with minimal discomfort, TMS has been increasingly popular for studying the motor system in intact humans.

1.3.1 Corticospinal volleys

1.3.1.1 Animal studies

Initial studies in animals have revealed that motor cortex stimulation evokes descending corticospinal volleys that elicit twitches throughout the body (for review see Boling et al. 2002). When combined with direct recordings from the bulbar pyramid or the dorsolateral surface of the cervical spinal cord, these cortical stimuli evoke a series of high frequency descending waves (Patton and Amassian 1954). The first of these waves, which is termed "D" wave, persists after cortical injury and was thought to originate from "direct" activation of the pyramidal tract neurons. The later waves, termed "I" waves, depend on the integrity of the cortical grey matter and likely originate via "indirect" trans-synaptic activation of pyramidal tract neurons.

Insight into the action of TMS on corticospinal neurons were obtained from a series of experiments where both TMS and TES were applied to the motor cortex of anesthetized monkeys (Edgley et al. 1997). Descending waves were recorded from the axons of corticospinal neurons at the lumbar level and TMS was applied to the leg motor cortex using a large round coil (90 mm in diameter). D- and I-waves were elicited in most pyramidal tract neurons having a wide range of conduction velocities (25 to 95 m/s). Descending responses consisted of up to 3 or 4 consecutive waves and had a periodicity of approximately ~1.6 ms. The relative TMS threshold for evoking I-waves was lower than D-waves. Following a TMS pulse, inputs from fast corticospinal axons (>60 m/s) are more strongly reflected in the MEP despite only constituting a small fraction of the CST spectrum (only 8% with diameter >4 µm). Although motor cortex stimulation elicits a train of high frequency descending waves, similar responses have yet to be reported in freely behaving animals (see Di Lazzaro et al. 2008) stressing a salient difference between artificial cortical stimulation and natural voluntary movement.

1.3.1.2 Human studies

Similar to animal studies, descending volleys from TMS in human subjects are typically delayed and more complex than the volleys evoked from TES (Day et al. 1989). In agreement with animal experiments, TES applied to the human motor cortex predominantly activates the axons of pyramidal tract neurons (i.e. D-waves) when done at low intensities. Dispersed trans-synaptic activation of pyramidal neurons is likely responsible for the delayed and more numerous motor volleys observed using TMS (see Di Lazzaro et al. 2008). Since an incomplete SCI preferentially impairs the large diameter fast corticospinal neurons (Curt and Dietz 1999), the relative contribution of slowly conducting axons in the MEP may be altered on the basis that the MEP is delayed in latency and reduced in amplitude.

1.3.2 Single pulse stimulation

The MEP response evoked using TMS is widely used in the study of human physiology and corticospinal conduction in healthy individuals and in patients with CNS dysfunction (for reviews see Di Lazzaro et al. 2008; Chen et al. 2008). Stimulus recruitment curves and cortical maps are two popular techniques that have emerged for examining changes in cortical reorganization and excitability (Devanne et al. 1997; Ridding and Rothwell 1997). The size of an MEP is related to the strength of the corticospinal projections and is influenced by the excitability of both the cerebral cortex and the motoneuron pool. When stimulating motor axons, the size of the motor response is reflected in the compound muscle action potential (see Rösler and Magistris 2008). The compound muscle action potential is roughly proportional to the number of motor axons that were activated and increases sigmoidally for increasing intensities. For motor cortex stimulation, the size of the peak-to-peak MEP increases in a similar manner, but always remains smaller than the compound muscle action potential elicited at supramaximal intensity. At the motoneuron level, there are various factors affecting the size of the maximum MEP including the number of motoneurons recruited by the TMS pulse, the number of motoneurons that fire more than once to the descending volleys and the relative synchronization of the TMS-induced discharge.

1.3.2.1 Voluntary contraction

Rothwell et al. (1987) and Hess et al. (1987) were among the first to show that MEPs are increased during a voluntary contraction. A muscle contraction reduces the MEP threshold and increases the size and duration of the descending volleys, but has no effect on the D-wave activated using TES (Di Lazzaro et al. 1998a). In general, voluntary contraction facilitates the recruitment of both motor cortex neurons and spinal cord circuits and can reduce the amount of variability in the MEP (Nielsen 1996). MEPs are increased during strengthening contractions (Hess et al. 1987), though individuals with an incomplete SCI show a recruitment pattern that is less steep and may be altered to regulate the descending and segmental excitation to the motoneuron pool after the injury (Davey et al. 1999a).

1.3.2.2 TMS Coil

The size of an MEP depends on the location and orientation of the coil on the surface of the head (see Rösler and Magistris 2008). Early studies using a circular coil centred over the vertex showed that clockwise currents in the brain preferentially activate cortical neurons in the left hemisphere. Figure-of-eight coils were later developed to improve the focus of the magnetic stimulus. To stimulate the deeper structures of the leg motor cortex, larger coils including a double cone coil or variants of the 90 mm figure-of-eight coil are frequently used.

1.3.2.3 Monosynaptic and polysynaptic CST connections

Motor unit experiments have shown that corticospinal neurons activated using TMS can project to motoneurons via a monosynaptic connection (Palmer and Ashby 1992; Bawa et al. 2002). Direct corticomotoneuronal connections likely exist for all muscles (de Noordhout et al. 1999) and may be important for motor tasks that require skilled or precise movements. In animals, direct corticomotoneuronal connections have only been reported in the upper limb of primates, while cats and other quadrupeds only have polysynaptic connections (see Schieber 2007). In combination with the direct monosynaptic inputs, polysynaptic excitatory and inhibitory connections also exist in the human CST system (Iles and Pisini 1992; Nielsen and Petersen 1995; Morita et al. 2000).

1.3.2.4 TMS-induced EMG suppression

Cortical stimulation is generally used to activate the motor pathways, however despite its apparent facilitatory effect, TMS can also suppress or interfere with motor activity. Davey et al. (1994) where the first to show that very-low TMS can inhibit the voluntary background EMG activity. The suppression of voluntary EMG activity is thought to occur through the activation of intracortical GABAergic interneurons (Classen and Benecke 1995) and acts to reduce motor output to the target muscle. Low-intensity subthreshold TMS likely inhibits the ongoing activity of fast-conducting corticospinal cells; the pathway that is considered to provide descending drive to motoneurons during voluntary movements (Butler et al. 2007)

1.3.2.5 Brainstem stimulation

Corticospinal tracts can be directly activated with electrical and magnetic pulses in awake human subjects (for review see Taylor and Gandevia 2004). Transmastoid stimulation can be used to safely exclude a cortical component when its responses are compared to TMS. This cannot always be done using TES since it can elicit a mixture of D- and I-waves (Edgley et al. 1997). Non-invasive corticospinal tract stimulation is done by passing a high-voltage electrical stimulus between the mastoid processes or by magnetic stimulation over the back of head. Both approaches likely activate the corticospinal tract at the cervicomedullary junction (i.e. pyramidal decussation) and evoke short-latency responses in both upper and lower limbs. Unlike TMS, this

method only evokes a single descending corticospinal volley, and in the upper limb, responses are predominantly evoked via a monosynaptic corticomotoneuronal connection.

1.3.3 Paired-pulse stimulation

Paired pulse experiments are intended to give insight into the human motor cortex and the cortical mechanisms involved in motor control. Various approaches have been developed to examine the interaction within the motor cortex itself and between cortical areas on the same or the opposite hemisphere (for review see Chen 2004). In the following section, we consider the technique of delivering two successive pulses to the motor cortex.

1.3.3.1 Short-interval intracortical inhibition (SICI)

Kujirai et al. (1993) were the first to report that conditioning the motor cortex using a subthreshold TMS pulse can considerably reduce an MEP (i.e. test MEP) when evoked 1 to 5 ms later. This inhibitory phenomenon is called short-interval intracortical inhibition (SICI) and likely involves cortical circuits given that TES responses or H-reflexes are not inhibited by the conditioning stimulus. Direct recordings of descending corticospinal volleys have supported a cortical mechanism on the basis that the subthreshold stimulus suppresses the late I-waves produced by the second stimulus (Di Lazzaro et al. 1998b). There exists a “U” shaped relationship between the intensity of the conditioning stimulus and the amount of inhibition which in the upper limb is centered on 0.7 to 0.8 of the resting motor threshold (Kujirai et al. 1993). GABAergic neurons, which constitute 25-30% of the motor cortex (Jones 1993), and their activation was initially proposed by Kujirai et al. (1993) to explain SICI. The inhibition is likely mediated by intracortical GABAergic circuits and administering positive allosteric modulators of the GABA-A receptor (i.e. lorazepam or diazepam) enhances SICI (Ziemann et al. 1996; Ilic et al. 2002). In agreement with human corticospinal recordings, GABA-A agonist muscimol suppresses later I-waves in the hand motor cortex of macaque monkeys (Shimazu et al. 2004). Afferent inputs from peripheral nerve stimulation or muscle vibration in the upper limb can interact with neuronal circuits that regulate SICI (Aimonetti and Nielsen 2001; Rosenkranz and Rothwell 2003). After an incomplete SCI, there is evidence that the early GABAergic component of the inhibition is reduced potentially due to a reduction in sensory input (Davey et al. 1999b; Shimizu et al. 2000; Saturno et al. 2008). Presently a systematic evaluation of SICI in the SCI population has yet to be conducted.

1.3.3.2 Intracortical facilitation (ICF)

Paired-pulse TMS can induce robust MEP facilitation if the interval between the pulses is 10 to 25 ms (Kujirai et al. 1993). A cortical origin for ICF has been partially confirmed, particularly at a 25 ms interval due to a prominent increase in the later I-waves (Nakamura et al. 1997). However, descending corticospinal volleys are

unchanged in 6 patients implanted with epidural electrodes at intervals that induce strong MEP facilitation (i.e. 10-15 ms; Di Lazzaro et al. 2006). ICF is potentiated by N-methyl-D-aspartate (NMDA)-receptor agonists and is reduced by NMDA-receptor antagonists (Ziemann et al. 1996, 1998a). Similar to SICI, populations of neurons that mediate ICF are modifiable by afferent stimulation (Aimonetti and Nielsen 2001; Rosenkranz et al. 2003) and may be involved in regulating motor cortex excitability.

1.3.3.3 Short-interval intracortical facilitation (SICF)

Short-interval intracortical facilitation (SICF), also referred to as I-wave facilitation, is a striking phenomenon where the amplitude of the MEP is modulated in the same fashion as the high frequency descending corticospinal volleys (~600 Hz). Ziemann et al. (1998b) showed that the MEP is facilitated at discrete intervals of approximately 1.0-1.5, 2.5-3.0 and ~4.5 ms analogous to the periodicity of descending I-waves if the two stimuli are delivered at or above the motor threshold. Such facilitation is not produced using TES suggesting that the effect occurs at a cortical level (Ziemann et al. 1998b; Chen and Garg 2000). A cortical site has also been supported by epidural recordings showing that I2 and I3 waves are enhanced at ISIs of 1-1.4 ms (Di Lazzaro et al. 1999). Facilitation of the motor unit at an I3 latency (and sometimes I2 latency) similarly corroborates these findings (Hanajima et al. 2002; Ilic et al. 2002). The physiological origin of SICF likely involves the interaction of I-wave inputs in the periodic bombardment of pyramidal neurons.

1.3.3.4 Long-interval intracortical inhibition (LICI)

Valls-Sole et al. (1992) and Claus et al. (1992) were the first to show that two suprathreshold TMS pulses delivered 50-200 ms apart strongly suppressed the second MEP. Such inhibition resembles the cortical silent period although the two phenomenons are not identical. Although spinal circuitry is refractory and inhibited following a TMS pulse delivered 50 ms earlier, epidural recordings have suggested that LICI (occurring at the later latencies) is cortical in origin (Nakamura et al. 1997; Chen et al. 1999). LICI is enhanced by baclofen, a GABA-B agonist, suggesting that it involves GABA-B receptors (McDonnell 2006). Interestingly, SICI is also reduced in the presence of LICI suggesting that GABA-B receptors are involved in the regulation of SICI (Sanger et al 2001).

1.4 PERIPHERAL NERVE STIMULATION

Stimulating peripheral nerves can be used to engage the circuitry of the human spinal cord and to probe excitability of the sensorimotor cortex. Peripheral nerves are generally activated by placing the cathode over the nerve and the anode distally or on the opposite side of the limb. For a comprehensive depiction of the human spinal cord circuitry activated using peripheral nerve stimulation, readers are encouraged to read *The circuitry of the human spinal cord: its role on motor control and movement disorders* by Pierrot-Desseilligny and Burke (2005). In addition, the following four sections (1.4.1 to 1.4.4) have been summarized from this textbook.

1.4.1 Sensory afferents and electrical threshold

The threshold for activation of sensory afferents using electrical stimulation is related to the axon diameter. In humans, the relationship between the stimulus intensity and the fibre type has been well characterized for the posterior tibial nerve supplying the soleus muscle. Myelinated afferents with a large axon diameter have the fastest conduction velocity and the lowest threshold for electrical stimulation. Group Ia fibres emerge from muscle spindles and fire according to the length and velocity of the muscle. They have a low electrical threshold, which is slightly below the threshold of Ib afferents. For instance, the axons of Ia fibres in the posterior tibial nerve are activated at 0.5-0.6 × motor threshold. Ib afferents originate from golgi tendon organs and provide information about the strength of the contraction. Ib afferents generally provide an inhibitory input onto the motoneuron pool and their inputs are delayed by approximately 1 ms since their inputs reach the motoneurons via a disynaptic connection. In muscle nerves, group II afferents arise from secondary spindle endings and provide information about muscle length. Group II afferents have an electrical threshold that is approximately twice that of Ia afferents (i.e. 1.2-1.3 times the motor threshold in the posterior tibial nerve) and are 65-67% slower. Although their monosynaptic inputs onto motoneurons are weak, they strongly affect propriospinal neurons and play a central role in modulating motoneuron excitability during rhythmic movements such as gait. Low-threshold cutaneous afferents originating from mechanoreceptors (Aβ fibres) also participate in modulating motoneuron excitability with latencies that are compatible with both spinal and transcortical mechanisms (see 1.4.4 Cutaneomuscular reflex). When stimulating a peripheral nerve it is important to understand the contribution of the different fiber types in order to properly tease out the overall mechanism of the response.

1.4.2 Monosynaptic excitation

The Hoffmann reflex (H-reflex) was the first of the human reflexes studied using electrical peripheral nerve stimulation. The earliest part of the response is consistent with the discharge of motoneurons activated by monosynaptic connections. Primary muscle afferents provide the dominant excitatory drive to the motoneuron pool during the H-reflex, though polysynaptic connections are also involved. The sensitivity of the H-reflex depends on the last motoneurons that were recruited and the size of the response provides insight about the strength of the monosynaptic Ia connection and the excitability of the motoneuron pool. Due to its non-invasive nature and its methodological ease, the H-reflex has been an important technique in motor control experiments and for diagnostic studies performed in human subjects.

Although H-reflexes are generally studied in the stimulated limb, the primary afferents from a single limb can project to various motoneuron pools. In the upper limb, inputs from distal muscles project onto proximal muscles, while proximal-to-distal connections are absent. Such heteronymous projections are likely useful for increasing the arm's rigidity during grasping. In the leg, heteronymous connections are weak or absent in many synergist muscles acting on the same joint (e.g. soleus and gastrocnemius), but many transjoint connections exist (e.g. quadriceps onto tibialis anterior and soleus). Presently, the role of heteronymous inputs in the human

leg is still unclear as its distribution differs widely from animals. As humans are the only mammals that stand on two legs, such connections to the soleus muscle may have evolved to stabilize the ankle during stance and heteronymous projections to the gastrocnemius muscle may contribute to forward propulsion during walking.

1.4.3 Mechanisms that affect reflex size

Several mechanisms affect the size of the motor response elicited using electrical nerve stimulation. Recurrent inhibition was the first spinal pathway identified and involves inhibitory interneurons called Renshaw cells. Renshaw cells have inhibitory connections to homonymous and synergist motoneurons and provide negative feedback to the motoneuron pool. Recurrent inhibition has a short central delay (i.e. 1-2 ms longer than monosynaptic Ia excitation) and has a long duration of more than 15 ms. Presynaptic inhibition regulates the information flowing through Ia afferents and modifies their inputs before reaching the motoneuron pool. Presynaptic inhibition is a potent mechanism that has a long central delay of ~5ms and a long duration of 300-400 ms. These mechanisms along with activity-dependent hyperpolarization, autogenic inhibition, and post-activation depression can strongly modify the spinal circuitry and should be considered when evaluating responses in muscles that were recently stimulated.

1.4.4 Cutaneomuscular reflex

The method of modulating the surface EMG using tactile cutaneous stimulation was introduced by Gassel and Ott (1970) and has been used to the examine the role of low-threshold cutaneous afferents in human movement. As compared to the upper limb, the cutaneomuscular reflex in the lower limb has a less stereotyped pattern. Electrical stimulation of the tibial nerve at the ankle generally produces excitation at a spinal latency followed by a longer-latency excitation. The longer-latency response is compatible with a transcortical loop. The cutaneomuscular reflex is useful to evaluate the control of normal movement since its connections are diffuse and engage a large number of neuronal networks. Likewise, such reflexes are not only restricted to the stimulated limb, but stimulating cutaneomuscular afferents at the foot can also elicit responses in the contralateral leg (Burke et al. 1991; see 1.4.6 Contralateral afferent inputs).

1.4.5 Long-latency reflex

Activating sensory afferents can increase corticospinal transmission to muscles supplied by the nerve and/or neighbouring muscles (see Chritensen et al. 2000). Following peripheral nerve stimulation, muscle stretch or muscle vibrations, the MEP is facilitated at a latency that is consistent with a transcortical loop (Deletis et al. 1992; Petersen et al. 1998; Rosenkranz & Rothwell, 2003). In the upper limb, such sensory-induced facilitation is associated with decreases in SICI and often involves increases in ICF (Aimonetti and Nielsen 2001; Rosenkranz et al. 2003). In ankle muscles, there is additional evidence that part of this facilitation is mediated at a

cortical level on the basis that cortical MEPs and not subcortical responses are potentiated by the afferent input (Nielsen et al. 1997). Afferent inputs arrive at the hand motor cortex approximately ~20 ms after median nerve stimulation (Tokimura et al. 2000; Stefan et al. 2000); however there is no definitive evidence demonstrating when common peroneal inputs excite the leg motor cortex (see Stinear and Hornby 2005; Mrachacz-Kersting et al. 2007) since the afferent inputs can access the motor cortex directly through the thalamus or indirectly via sequential connections through the somatosensory cortices.

1.4.6 Contralateral afferent inputs

Muscle and cutaneous afferents can modulate motor activity in the contralateral limb particularly during rhythmic movements such as walking and cycling (for review see Brooke et al. 1997; Zehr and Duysens 2004; Frigon and Rossignol 2008). For instance, passive cycling movements can actively inhibit the H-reflex in the contralateral soleus muscle (Collins et al. 1993). While sitting, peripheral nerve stimulation in the contralateral leg can also modulate the H-reflex tested in the ipsilateral leg (Robinson et al. 1979). Although the nature and direction of the effect (i.e. excitation or inhibition) can vary based on the conditioning-test interval, the dominant effect is facilitation of the soleus H-reflexes at latencies longer than 50 ms following contralateral cutaneous and mixed-nerve stimulation (Delwaide et al. 1981; Koceja and Kamen 1992). Presently, relatively little is known about the effect of sensory stimulation on corticospinal connections in the opposite leg, especially with inhibitory connections as shown in animal experiments (Aggelopoulos et al. 1996; Edgley et al. 2006; Frigon and Rossignol 2008).

1.5 MOTOR CORTEX PLASTICITY

Cortical representations are plastic and are continuously modified by experience (Buonomano and Merzenich 1998). Short-term changes in the excitability of the motor cortex in humans and animals can be induced using a variety of methods involving (but not limited to) the addition or removal of sensory input, brain stimulation and motor training. Here we discuss sensorimotor changes following injury and motor recovery, along with transient changes that can be artificially induced using peripheral nerve and brain stimulation.

1.5.1 Cortical plasticity with injury

Neural connections are not hard wired and can be modified after injury (for review see Chen et al. 2002). In the following section, we describe the evidence supporting plasticity in the mammalian sensory and motor cortices.

1.5.1.1 *Peripheral nerve injury*

A peripheral nerve lesion or digit amputation in the adult monkey promotes cortical reorganization in the sensory cortex (see Kandel et al. 2000). Following the removal of the sensory input, parts of the somatosensory cortex that were originally supplied by the nerve become responsive to neighboring body parts (Kelahan et al. 1981; Merzenich et al. 1983). With extensive long-term deafferentation in adult primates, parts of the cortex which are 14 mm away from the affected region can be altered (Pons et al. 1991; Manger et al. 1996). Analogous to the removal of sensory input, the skin from two fingers can project to overlapping cortical areas when the fingers are sutured together. In the human somatosensory cortex, phantom limb sensation can be elicited by touching the face or upper body of upper-limb amputees since the sensory areas from the face and upper body are adjacent to those affected by the injury (Ramachandran et al. 1992).

By briefly blocking a nerve, comparable but short-term changes have been induced in the somatosensory cortex of cats (Metzler and Marks 1979). These changes are rapidly evolving and revert back to normal within 2-4 hours after the removal of the nerve block. Ischemic nerve block induces similar changes in the human motor cortex. Biceps MEPs are several-fold larger during forearm deafferentation and return to normal 20 minutes after its termination (Brasil-Neto et al. 1992). Reorganization of the motor system also occurs following amputation, as MEPs are facilitated due to changes in cortical excitability (Ridding and Rothwell 1995; Cohen et al. 1991; Chen et al. 1998).

1.5.1.2 *Central lesion*

Spinalization at the T12 segment of cats promotes the appearance of a second sensory map of trunk and forelimb areas (McKinley et al. 1987). After an incomplete SCI, imaging studies in humans have revealed that neuronal damage to ascending and descending connections has an impact on the excitability of the whole sensorimotor system (Curt et al. 2002). The threshold for a MEP is increased after SCI due to damage of descending corticospinal neurons (Davey et al. 1998; Smith et al. 2000). The MEP is generally produced by the activation of fast corticospinal axons (see Di Lazzaro et al. 2008) and damage to these fast axons results in MEPs that are delayed in latency and reduced in amplitude (Alexeeva et al. 1998; Davey et al. 1998; Calancie et al. 1999). In the subacute phase after SCI, motor cortex activity to muscles directly affected by the injury are depressed (Jurkiewicz et al. 2007). This activity progressively reappears during functional recovery (Puri et al. 1998) in combination with decreased activity in the associated motor areas, which were overactive following the injury (Jurkiewicz et al. 2007). An incomplete SCI can similarly result in either an expansion or a reduction in corticospinal pathways rostral to the lesion as assessed using TMS (Brouwer and Hopkins-Rosseel 1997; Levy et al. 1990; Topka et al. 1991). Such findings indicate that cortical plasticity takes place after SCI (see Chapters 4.1 and 6.1).

1.5.2 Mechanisms of short-term changes

Short-latency cortical reorganization is likely associated with the unmasking of latent synapses. Persistent changes likely involve long-term potentiation and depression (LTP and LTD) of cortical synapses and synaptogenesis. For changes in cortical maps occurring in the span of minutes to hours, several mechanisms have been proposed to explain the unmasking of latent synapses. These include: 1) changes in membrane conductance that can enhance the effect of weak or distant inputs, 2) increased excitatory neurotransmitter release, 3) increased neurotransmitter sensitivity at the post-synaptic terminal, and 4) decreased inhibitory inputs or removal of inhibition from excitatory inputs (see Chen et al. 2002). GABA is the most important inhibitory neurotransmitter in the brain, and presently, the evidence is strongest for removal of inhibition of excitatory synapses through decreases in GABAergic inhibition. GABA is crucial for maintenance of cortical representations in animals (Jacobs and Donoghue 1991) and pharmacological agents that up-regulate GABAergic inhibition in human subjects prevent the induction of cortical reorganization as seen during transient deafferentation (Ziemann et al. 2001; Werhahn et al. 2002).

1.5.3 Motor recovery after central lesions

The degree of functional recovery after neural injury is highly variable as some patients achieve full recovery whereas others see little to no improvements at all. Many factors influence the degree of recovery including the site and extent of the lesion, age and individual variations in the CNS. In agreement with the notion that the motor system is plastic and can be modified by experience, there is increasing evidence that plasticity of corticospinal connections may account for important functional improvements following SCI (Thomas et al. 2005; Everaert et al. submitted) Although plasticity in subcortical structures may also contribute to functional improvements, reorganization of cortical structures during functional recovery of human walking has been most promising. Although motor activity alone is not a prerequisite for functional reorganization, motor skill acquisition and motor learning are crucial elements which drive plasticity in the cortex (Nudo 2003; Perez et al. 2004; Jensen et al. 2005). Functional recovery has been linked with the re-emergence of the affected body part in the somatosensory and motor cortices.

Work from our lab has shown that 3-5 months of intensive treadmill training increases corticospinal connections in the SCI subjects who showed locomotor gains. TMS recruitment curves showed that the maximum MEP and intermediate-sized MEPs (i.e. MEP_{max} and ½MEP_{max}) in ankle flexors and knee extensors were significantly increased with the subjects who showed improvements in walking function. The percentage increase in the maximum MEP was positively correlated to the degree of locomotor recovery suggesting that the corticospinal tract contributed to part of the locomotor recovery. In a recent study, we have also shown that functional improvements of ambulatory capacity occur in parallel with increases in the size of the EMG activity (but decreases in duration) in the tibialis anterior and hamstring muscles (Gorassini et al. 2008; see Appendix C).

Constraint-induced movement therapy in stroke patients produces long-term improvements in hand function. A 12-day-period of constraint-induced movement therapy, consisting of intensive training while restraining the opposite unaffected arm, doubles the number of active TMS sites projecting to the paretic hand muscle (Liepert et al. 2000). These findings suggest a positive shift in the recruitment of adjacent cortical areas during functional recovery potentially due to decreases in cortical GABAergic activity or enhancements in synaptic strength. With long-term motor training, such as playing the piano, continued practice enhances performance by altering motor cortex organization through the creation of new synaptic connections (Rosenkranz et al. 2007).

1.5.4 Neural Stimulation

1.5.4.1 Peripheral nerve and muscle stimulation

Injury to the motor cortex during stroke often results in swallowing dysfunction. Peripheral stimulation of the pharynx for 10 minutes induces long-lasting excitatory effects (> 90 minutes) that similarly enhance functional recovery of swallowing (Fraser et al. 2002). Imaging data have revealed an increase in motor cortex excitability indicating that sensory stimulation contributes to the enhanced cortical drive to the pharyngeal muscle. In the lower limb, repetitive stimulation of the common peroneal nerve results in lasting facilitation of the MEP in the TA muscle (Khaslavskaia et al. 2002; Knash et al. 2003). In addition, recent work from our group has shown that electrical common peroneal nerve stimulation delivered over several months (via a neuroprosthesis for footdrop called the WalkAide) improves walking function and increases CST function in SCI and multiple sclerosis subjects (Stein et al. in press; Everaert et al. in press). These results support the notion that peripheral nerve stimulation combined with voluntary drive promotes functional recovery of walking.

1.5.4.2 Transcranial DC stimulation

Transcranial direct current stimulation (tDCS) applied directly over the motor cortex induces persistent changes in the human motor system in the upper (Nitsche and Paulus 2000; Liebetanz et al. 2002) and lower limb (Jeffery et al. 2007; see Appendix A). Increases in cortical excitability to anodal tDCS is abolished using either dextromethorphan, an NMDA receptor antagonist and carbamazepine, a drug that stabilizes the membrane potential in a voltage-dependent manner (Liebetanz et al. 2002). Cathodal tDCS, an intervention which depressesses MEPs in the upper limb is unaffected by carbamazepine, but is strongly influenced by dextromethorphan further supporting the importance of NMDA receptors in the tDC aftereffects. It is conceivable that the mechanism that increases short-term excitation following anodal tDCS may be associated with depolarizing the cell membrane in such a way that neurons in the motor cortex are more readily recruited during a given motor task. In the clinical population, 20 minutes of anodal tDCS improves motor function in the paretic hand of stroke patients as measured by the Jebsen-Taylor hand function test (Hummel et al. 2005).

1.5.4.3 Repetitive TMS

TMS induces a cascade of excitation and inhibition in the motor system. In addition to these transient changes, motor circuits may be altered using repetitive TMS (rTMS). Cortical excitability is generally suppressed following a period of low-frequency stimulation (1 Hz), but may be facilitated at 0.1 Hz, when paired with an intervention that potentiates the MEP (Ziemann et al. 1998c). Stimulating the motor cortex using high frequency stimulation at 5-20 Hz (Pasqual-Leone et al. 1994; Maeda et al. 2000) or short bursts delivered at the I-wave periodicity can facilitate the resting MEP (Thickbroom 2006; Hamada et al. 2008). Of all the methods, the technique of intermittent theta burst stimulations introduced by Huang et al. (2005) is among the most promising for increasing cortical excitably using rTMS. Low-intensity bursts of TMS at 50 Hz stimulated every 200 milliseconds produces long-lasting increases in the MEP and can alter motor performance in a reaction task. It is predicted that using theta burst stimulation to increase cortical excitability before intensive training may serve to jump-start cortical circuits thereby enhancing the amount of cortical reorganization that can occur with motor training.

1.5.4.4 Paired associative stimulation (PAS)

Models for LTP in animal preparations have revealed that long-term increases in synaptic strength are induced when the postsynaptic terminal is conditioned with a weak input before the arrival of a stronger input to the cell, whereas LTD is elicited when a stronger input arrives before the weak input (for review see Dan and Poo 2006). In the human motor cortex, LTP- and LTD-like plasticity have been induced using low-frequency nerve stimulation paired with TMS to the target muscle (Stefan et al. 2000, Wolters et al. 2003). This paradigm for LTP and LTD has been coined paired associative stimulation (PAS) and has been found to induce plasticity in the motor cortex likely acting through a change in synaptic strength between cortical neurons. For instance, the ability to induce LTP or LTD in the abductor pollicis brevis muscle depends on the latency between the peripheral electrical stimulation and the TMS pulse (see below). Conditioning the motor cortex using 90 pairs of stimulation at a rate of 0.1 Hz is capable of modulating the excitability of the motor system, with changes appearing within 30 minutes after PAS and persisting for an additional 60-90 minutes. Both dextromethorphan, an NMDA-receptor antagonist, and nimodipine, a blocker of L-type voltage gated Ca^{2+} channels, abolish the PAS-induced effect suggesting that the MEPs are modulated by LTP/LTD-like mechanisms (Stefan et al. 2002; Wolters et al. 2003). On the basis that descending corticospinal volleys and MEPs are modulated in parallel, PAS-induced increases and decreases in the MEP likely occur through cortical mechanisms (Di Lazzaro et al. 2009a, 2009b)

During PAS in humans, modifying the timing between TMS (strong input) and peripheral nerve stimulation (weak input) produces asymmetric changes in the excitability of the corticospinal tract (CST) (Wolters et al. 2003; Mrachacz-Kersting et al. 2007) similar to that described for hippocampal neurons (Dan and Poo 2006). For example, MEPs in muscles of the hand are increased when a TMS pulse is applied 25 to 30 ms after peripheral nerve stimulation such that TMS-induced firing

of CST neurons *coincides* with the arrival of afferent inputs at the motor cortex, i.e., under conditions of high calcium influx. In contrast, MEP responses are depressed at an interstimulus interval of 10 ms when the afferent inputs are timed to arrive immediately *after* the TMS inputs so that theoretically, the weak afferent input is activated during the afterhyperpolarization of the postsynaptic neuron, i.e., under conditions of low calcium influx (Wolters et al. 2003; Ziemann et al. 2004). Outside of this approximately ~20 ms interstimulus window, pairing TMS and peripheral nerve stimulation has no effect on CST excitability to upper extremities. PAS modifies TA MEPs when administered during walking (Stinear and Hornby 2005), however its effect in lower leg muscles had not been fully examined during a non-locomotor task.

The potential to increase or decrease the strength of CST connections makes PAS a possible therapeutic tool. PAS can facilitate MEPs after stroke, however, repeated exposure in 9 stroke patients (4 weeks of daily PAS) have revealed marginal functional and neurophysiological improvements to the TA muscle (Uy et al. 2003). As PAS and skilled-motor training are thought to share similar corticospinal networks (Ziemann et al. 2004), it is possible that PAS requires motor training to consolidate changes in cortical excitability. Presently, PAS has not been studied in individuals with an incomplete SCI.

1.6 THESIS OUTLINE

Studies on the motor cortex in the adult human brain have demonstrated that cortical reorganization takes place following injury to the nervous system. A change in the amount of afferent input reaching the sensorimotor cortex drives plasticity in the human CNS and induces cortical reorganization as evidenced by the altered MEP. Unmasking of latent synapses through modulation of GABAergic activity are likely associated with short-latency mechanisms, whereas long-term changes likely involve LTP/LTD-like mechanisms and synaptogenesis. The finding that MEPs are increased in SCI subjects showing locomotor gains suggests that spared corticospinal connections may contribute to strengthening ambulatory capacity after injury. Continuing to identify which neurological changes have clinical relevance remains an important challenge for advancing human motor rehabilitation.

1.6.1 Chapter 2

The overall goal of this thesis is to examine associative plasticity and the role of afferent regulation of corticospinal excitability in healthy individuals and after an incomplete SCI. This thesis begins by characterizing the effect of PAS in the ankle flexors of healthy subjects. Chapter 2 presents evidence that pairing electrical stimulation of the common peroneal nerve with TMS (in the form of PAS) can facilitate the MEP if the afferent input is timed to arrive at the motor cortex several tens of milliseconds *after* the cortical stimulus. We propose that in the leg motor cortex, facilitation of MEP responses from PAS occurs over a large range of interstimulus intervals due to the paired activation of sensory inputs with sustained, subthreshold activity of cortical neurons that follow a TMS pulse.

1.6.2 Chapter 3

To further elucidate the interaction of sensory afferents at the motor cortex, the following study investigates the activation of cortical circuits by peripheral sensory inputs in the human leg motor cortex. Chapter 3 provides evidence supporting the potentiation of cortical circuits following lower leg afferent stimulation. In particular, we show that increases in the MEP are due to both disinhibition and excitation of intracortical circuits.

1.6.3 Chapter 4

In Chapter 4, the afferent-induced MEP facilitation that occurs in healthy individuals is considerably diminished/absent after SCI, suggesting that supraspinal circuits contribute to the MEP facilitation. There was some evidence that TA MEPs in the SCI subjects could be potentiated by common peroneal nerve stimulation, but the effect was delayed by approximately ~10 ms as compared to non-injured controls. Given that spared connections exist after an incomplete SCI, we show that an intervention of PAS can induced short-term increases in corticospinal excitability in the injured subjects.

1.6.4 Chapter 5

As the three previous studies have investigated the effect of peripheral nerve stimulation on CST connections in the stimulated leg, Chapter 5 provides evidence that motor pathways in the opposite leg are also affected by afferent stimulation. In agreement with the notion that reflexes are not confined to a single limb, but complementary reflexes can be elicited in various muscles in the contralateral leg, we show that short trains of contralateral nerve stimulation (25 Hz train delivered to posterior tibial nerve) depresses the size of the cutaneomuscular reflex elicited in the opposite soleus muscle. Such a reduction in the response occurs at a latency of approximately ~65 ms, in-line with the inhibition of spinal circuitry. H-reflexes in the soleus muscle were unchanged while MEPs were reduced suggesting that the inhibition occurred at a premotoneuronal level. In this chapter, we characterize the crossed inhibitory pathway to the human soleus muscle using cutaneomuscular and corticospinal inputs. We show that contralateral afferent inputs inhibit the spinal circuitry to the soleus muscle and can be measured using peripheral and central inputs having diffuse premotoneuronal projections.

1.6.5 Chapter 6

Chapter 6 examines cortical inhibition in SCI subjects. We provide evidence that intracortical inhibition evaluated using paired-pulse TMS is reduced after SCI. As a spinal cord lesion increases the motor threshold assessed using surface EMG, the actual SICI-producing intensities were also increased after SCI. In addition, we show that the “U” shaped curve (describing the relationship between the conditioning intensity and the amount of inhibition) is preserved after SCI and is centered on the

same “relative intensity” as the uninjured controls (i.e. 80% of the active motor threshold).

1.6.6 Chapter 7

In the last experimental chapter, we examine the recruitment of cortical inhibition of healthy subjects using very-low intensity subthreshold TMS. Chapter 7 shows that two subthreshold TMS pulses at a 7 ms interval enhance the size and reliability of the EMG suppression in the pre-contracted TA muscle without increasing the preceding EMG. We propose that low intensity paired-pulse TMS might be beneficial for investigating the contribution of cortical cells actively involved in motor control, in particular during tasks that rely less heavily on voluntary drive.

1.6.7 Chapter 8

Chapter 8 summarizes our results and describes their contribution towards the study of human motor control. In this chapter we also propose new research questions based on our findings. Parts of this thesis have been previously published: Chapter 2 in Roy et al. (2007), Chapter 3 in Roy and Gorassini (2008) and Chapter 7 in Roy (2009). In addition, the three Appendices have been published in Jeffery et al. (2007), Poon et al. (2008) and Gorassini et al. (2009).

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CHAPTER 2: Role of sustained excitability of the leg motor cortex after transcranial magnetic stimulation in associative plasticity

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2.1 INTRODUCTION

Increases in the excitability of the corticospinal tract (CST) can be induced in conscious human subjects by repeatedly pairing low-frequency stimulation of a peripheral nerve with activation of the primary motor cortex by transcranial magnetic stimulation (TMS) in an intervention termed 'paired associative stimulation' (PAS; Stefan et al. 2000). PAS-induced facilitation occurs when inputs from peripheral nerve and TMS arrive at the motor cortex nearly simultaneously, with the hypothesis being that both inputs activate a common neuron within the motor cortex (Stefan et al. 2002). The ensuing increase in motor evoked potentials (MEPs) from CST activation is thought to be mediated by increases in synaptic strength between coincidentally activated neurons via mechanisms related to associative long-term potentiation (LTP).

Several studies in both *in vitro* and *in vivo* systems have shown that modifications in the synaptic strength between coincidently activated neurons depend on the temporal ordering of pre- and postsynaptic activation (reviewed in Dan and Poo 2006). For example, in cultured hippocampal neurons, LTP is induced if postsynaptic firing generated by a strong input occurs immediately *after* the activation of an excitatory postsynaptic potential (EPSP) generated by a weak input (Bi and Poo 1998). In this situation, the EPSP generated by the weak input occurs during the action potential of the postsynaptic neuron which strongly depolarizes the postsynaptic membrane and facilitates the influx of calcium through *N*-methyl-D-aspartic acid (NMDA) receptors involved in LTP induction (reviewed in Linden 1999). In contrast, long-term depression (LTD) occurs if postsynaptic firing just *precedes* presynaptic activation where the EPSP generated by the weak input occurs during the afterhyperpolarization of the postsynaptic neuron, which may dampen NMDA receptor-mediated calcium influx to induce LTD (see also Dan and Poo 2004).

During PAS in humans, modifying the timing between TMS (strong input) and peripheral nerve stimulation (weak input) produces similar asymmetric changes in the excitability of the CST as described above for hippocampal neurons (Wolters et al. 2003; Ziemann et al. 2004; Mrachacz-Kersting et al. 2007). For example, MEP responses in hand muscles are increased when a TMS pulse that produces firing of CST neurons occurs at the same time as excitation of the motor cortex from median nerve stimulation (Stefan et al. 2000; Stefan et al. 2002). The weak input from afferent stimulation is proposed to be strengthened because it occurs during the

period of strong depolarization of the postsynaptic neuron activated by TMS. Likewise, MEPs in the tibialis anterior (TA) muscle are increased when a TMS pulse is applied 45 to 55 ms after common peroneal nerve (CPN) stimulation such that TMS-induced firing of CST neurons coincides with the afferent excitation at the motor cortex (Mrachacz-Kersting et al. 2007). In contrast, MEP responses are depressed when the stimulation order is reversed whereby TMS inputs are timed to arrive at the motor cortex immediately *before* afferent inputs so that theoretically, the weak afferent input is activated during the afterhyperpolarization of the postsynaptic neuron (Wolters et al. 2003; Ziemann et al. 2004; Mrachacz-Kersting et al. 2007). Outside of this approximately 20 ms interstimulus window, pairing TMS and peripheral nerve stimulation has no effect on CST excitability to upper extremities (Wolters et al. 2003, see also Dan and Poo 2006 for similar results *in vitro*).

Similar to PAS in humans, Jackson et al. (2006) showed that the connectivity between two populations of neurons in the primate motor cortex could be modified by synchronizing cortical activity at two distant sites in a manner consistent with Hebbian synaptic potentiation. By using action potentials recorded from one site in the motor cortex to trigger electrical stimuli at distant site, repeatedly pairing the two events, over one day or more, produced a shift in the stimulus-evoked motor output of the recording site to resemble the motor output produced at the site of stimulation. In some cases, persistent changes in connectivity could be induced for more than one week when electrical stimuli were delivered up to 100 ms after neural firing. These findings are of interest as they challenge the notion that there exits a narrow temporal relationship governing synaptic plasticity between synchronized populations of neurons in the motor cortex of freely behaving animals. Likewise, preliminary reports in the leg have suggested that PAS in humans can increase MEP responses in the TA muscle when afferent inputs arrive at the motor cortex up to 30 ms *after* cortical stimulation (Mrachacz-Kersting et al. 2007; see also Perez et al. 2003). To further investigate these effects, we tested whether PAS-induced facilitation of the leg motor cortex can occur if afferent inputs arrive at the level of the motor cortex at long intervals *after* a pulse of TMS. Similar to PAS paradigms timed to provide synchronous excitation in cortex, we find that CST excitability to leg muscles was strongly enhanced when afferent inputs reached the motor cortex many milliseconds after cortical stimulation. Surprisingly, such facilitation occurred over a wide range of interstimulus intervals comparable to that observed *in vivo* (Jackson et al. 2006). In this paper, we examine if continued subthreshold activity in the leg motor cortex following a single pulse of suprathreshold TMS is sufficient to induce associative facilitation in the cortex when conditioned with afferent inputs from the lower leg.

2.2 METHODS

2.2.1 Subjects

Twenty healthy volunteers (12 male and 8 female) with an average of 26 ± 6 years participated in the study for a total of 74 separate experimental sessions. All subjects gave their written informed consent, and the protocol was approved by the Human Ethics Research Board at the University of Alberta.

2.2.2 Recording and stimulation

EMG activity was recorded from the tibialis anterior (TA) and soleus (SOL) muscles on the examined leg (16 right and 4 left) and the contralateral TA using pairs of surface Ag-AgCl electrodes (Kendall LTP, Chicopee, MA). Electrodes were placed over the muscle belly 1.5 cm apart and parallel to the long axis of the muscle. EMG signals were amplified 1000 times and band-pass filtered between 10 and 1000 Hz (Octopus, Bortec Technologies, Calgary, AB). Raw EMG signals were digitized at 5 kHz using Axoscope hardware and software (DigiData 1200 Series, Axon Instrument, Union City, CA) and stored on a personal computer for off-line analysis. EMG activity from the TA muscle was also rectified, low-pass filtered at 0.3 Hz and transmitted to an oscilloscope so subjects could monitor their EMG activity during the experiment.

Electrical pulses of 1 ms in duration were delivered to the CPN using a constant-current stimulator (DS7A, Digitimer Ltd., Hertfordshire, UK). Stimuli were delivered using a cathode (Kendall-LTP ES40076) placed over the CPN near the head of the fibula with a large anode (Axelgaard Manufacturing Co., Ltd.) placed on the medial side of the knee below the patella.

TMS was delivered using a Magstim 200 (The Magstim Co., Dyfed, UK) and a double-cone coil (P/N 9902-00: external wing diameter 110 mm) oriented in an antero-posterior direction. Paired-pulse TMS (described below) was performed using two Magstim 200 stimulators connected to a Bistim module (The Magstim Co.). The coil was placed over the leg area of the motor cortex with the handle orientated vertically and a few degrees from the mid-sagittal plane. The optimal stimulus site (usually located 1 cm lateral and 1 cm posterior to the vertex) was identified over the contralateral motor cortex using a suprathreshold stimulation intensity. The coil was suspended from an overhead gantry and stabilized on the head using two foam pads and a chin strap.

2.2.3 Protocol for PAS intervention

Subjects were seated comfortably in a chair with the examined leg bent at the knee and with the foot secured to a foot-plate. The intervention targeted the TA muscle at rest and delivered electrical stimuli to the CPN paired with TMS over the contralateral motor cortex. Stimulation of the CPN was delivered at 300% perceptual threshold (6 ± 2 mA) or 150% of motor threshold if the former was below motor threshold. TMS was delivered at an intensity that produced an MEP of approximately 0.3-0.6 mV in the relaxed TA (54 ± 7% maximum stimulator output: MSO). PAS consisted of 90 pairs of stimuli delivered at 0.1 Hz over 15 min (see also Kujirai et al. 2006).

2.2.4 Effect of timing TMS in relation to CPN stimulation

The influence of varying the timing between both stimuli applied during PAS on corticospinal excitability was investigated at six main interstimulus intervals (ISIs)

and one control ISI (see below). Eight subjects were tested at each of the main ISIs and a total of eighteen subjects participated in this part of the study. The order of interventions at each ISI was pseudorandom within and across subjects, and at least three days elapsed between consecutive sessions in a single subject. Since conduction velocities in the nervous system vary between subjects, all ISIs were adjusted according to the latency of the MEP in the TA muscle (30 ± 2 ms). The time interval between the CPN stimulus (weak inputs to cortex) and the TMS pulse (strong inputs to cortex) on average were equal to i) -40 ms (MEP latency minus 70 ms), ii) 0 ms (MEP latency minus 30 ms), iii) 20 ms (MEP latency minus 10 ms), iv) 35 ms (MEP latency plus 5 ms), v) 40 ms (MEP latency plus 10 ms), and vi) 60 ms (MEP latency plus 30 ms). In addition, five subjects were tested at the control ISI of -170 ms, where the interaction between TMS and peripheral nerve stimulation should be negligible (see Discussion).

2.2.5 Excitability of the CST

CST excitability following PAS was investigated at rest and during voluntary contraction (active trials). MEPs at rest were recorded in blocks of twenty stimuli at the intensity used during PAS. Active MEPs were recorded in blocks of ten stimuli while subjects maintained a small contraction in TA corresponding to 10% of their maximum voluntary contraction (MVC). The stimulus intensity was set to elicit MEP responses of approximately 1.0 mV (43 ± 5% MSO) in the contracted TA. Two sets of resting MEPs and one set of active MEPs were recorded at baseline, and single sets were recorded at 0, 10, 20, 30 and 60 min after PAS. Stimuli were delivered every 8 s. The size of the MEP was measured as the peak-to-peak amplitude of the non-rectified EMG response. MEPs were averaged and expressed as a percentage of baseline values before PAS at each of the five time points listed above. During active trials, the level of motor activity just preceding an MEP was evaluated from the mean value of the rectified EMG recorded in a 50-ms period just before the stimulus. Average levels of motor activity evaluated after PAS were within 1 SD of pre-PAS values, and up to 5% of the responses were discarded to match EMG levels.

2.2.6 Sustained excitability of the CST after suprathreshold TMS

In five subjects, we investigated how long corticospinal projections to the TA muscle remain excitable following a single pulse of suprathreshold TMS. To examine this, we evaluated the size of the MEP response when conditioned by a prior suprathreshold TMS. The intensities of the conditioning and test stimuli were set to produce a resting MEP of approximately 0.3-0.6 mV (similar to PAS) when given alone. ISIs of 20, 30, 50, 70, 80, 90 and 100 ms were tested. Five paired stimuli were applied at each ISI in a pseudorandom order interleaved with ten unconditioned test stimuli. Stimuli were delivered every 8 s. CST excitability was evaluated from the peak-to-peak amplitude of the test MEP in the paired conditions and expressed as a percentage of the unconditioned test (control) MEP.

2.2.7 Subthreshold PAS

To test whether PAS-induced facilitation of MEP responses occurred because of pairing of CPN inputs with cortical activity that was subthreshold to CST activation, we administered a modified intervention of subthreshold-PAS in eight subjects using a protocol similar to that used in the hippocampus to produce heterosynaptic LTP *in vitro* (Huang et al. 2004). Subthreshold-PAS consisted of three CPN stimuli paired with a single pulse of TMS over the contralateral motor cortex at 80% of the active motor threshold (AMT; 23 ± 4% MSO). AMT was defined as the lowest stimulus intensity that evoked an MEP with an amplitude > 50-100 µV in at least 3 of 6 consecutive stimuli during voluntary contraction. The three CPN stimuli (100 Hz; at 300% sensory threshold) were applied over a period of 15 to 35 ms before the TMS pulse. Subthreshold-PAS was administered at rest and consisted of 60 paired stimuli given at a rate of 0.2 Hz (lasting 5 min).

2.2.8 Intracortical and spinal mechanisms

In eight subjects, we investigated possible intracortical and spinal mechanisms responsible for PAS-induced facilitation using an ISI of 20 ms, where consistent facilitation of MEP responses occurred. Paired-pulse TMS was delivered using a conditioning stimulus of 95% AMT (33 ± 7% MSO). Test stimuli were given at an intensity that produced unconditioned MEPs of approximately 0.3-0.6 mV (57 ± 8% MSO). Short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) were evaluated using ISIs of 3 and 9 ms, respectively (Stokic et al. 1997). At each ISI, ten conditioned and ten unconditioned stimuli were delivered in a pseudorandom order. Stimuli were given every 8 s. ICF, SICI and MEPs were evaluated in the relaxed TA at baseline and 15 and 30 min after PAS. Since the size of the unconditioned MEP influences the excitability of intracortical circuits (Stefan et al. 2002), the intensity of the test stimulus was adjusted after the intervention (55 ± 8% MSO) to match the size of the unconditioned MEP recorded before PAS. SICI and ICF were evaluated from the peak-to-peak amplitude of the conditioned MEP and expressed as a percentage of the unconditioned MEP.

Excitability of the H-reflex, the electrical analogue of the stretch reflex pathway, was investigated in the TA muscle. The stimulus intensity was adjusted to produce an H-reflex at around 50% of the maximal H-reflex amplitude during tonic dorsiflexion at 10% MVC. Thirty H-reflexes were evaluated before and 25 min after PAS. Stimulations were given every 2 s. The average size of the peak-to-peak H-reflex was measured with matched M-wave and background EMG.

2.2.9 Statistical analysis

Two-factor repeated measures ANOVAs (analysis of variance), treating the time of measurement and the motor state (rest or active) as within-subject factors, were used to compare the size of the MEP response after PAS at the different ISIs and for the single ISI used during subthreshold-PAS. When the interaction effect (time x state) was significant, the motor states were analyzed separately using one-way repeated

measured ANOVAs (within-subject factor: time). A two-factor repeated measures ANOVA (within-subject factors: time, leg) was used to compare MEP responses in the stimulated and non-stimulated TA muscle. Post hoc *t*-tests (two-tailed) were used to evaluate the MEP response size at the different time-points compared to baseline. Post hoc testing was performed on the pooled data (rest and active) at ISIs showing a significant time effect with no interaction or state effects. The size of the MEP response following pairs of suprathreshold TMS and the effect of a 20 ms ISI on MEP, SICI, ICF and H-reflex responses were all analysed using *t*-tests (two-tailed). The significance level was set at $p < 0.05$. All data are given as means ± SD.

2.3 RESULTS

2.3.1 Changes in corticospinal excitability

Corticospinal excitability projecting to TA was altered following PAS as a function of the ISI employed during the intervention. Figure 2-1A shows average traces of the resting MEP from one representative subject at the six main ISIs tested. In agreement with data published in the lower leg (Mrachacz-Kersting et al. 2007), MEP facilitation was not produced when PAS was delivered at an ISI of 60 ms, when the afferent volley from nerve stimulation arrived at the contralateral motor cortex ahead of the TMS pulse by approximately 10 ms (bottom trace in Fig. 2-1A). However, MEP facilitation was produced when afferent inputs arrived at the motor cortex *after* TMS-induced activation, at ISIs ranging from -40 to 40 ms (traces from top to bottom). At these ISIs, the afferent inputs are estimated to arrive at the motor cortex 90 to 10 ms after the TMS pulse, respectively. Such findings were consistent across subjects as shown for the group data in Figure 2-1B, which plots the average size of the MEP after PAS relative to baseline values at the various ISIs tested. Similar to the 60 ms ISI, MEP facilitation did not occur at an ISI of -170ms where afferent inputs are estimated to arrive at the motor cortex approximately 220 ms after the TMS pulse.

2.3.2 Time course of MEP changes after PAS

When MEP responses were facilitated at ISIs of -40 to 35 ms (solid symbols in Fig. 2-2A), MEPs began to increase immediately after the intervention (at time 0) and persisted for at least 60 min (last time point tested). MEP responses measured at rest typically increased during the first 20 min and remained elevated at the 60-min time point, ranging from 41 to 67% of pre-PAS values. Faster decreases occurred following PAS at an ISI of 40 ms, where MEP increases were less consistent (open circles in Fig. 2-2*A*). At PAS intervals where no visible MEP facilitation occurred (ISIs of -170 and 60 ms; open squares and triangles in Figs 2-2*A*), MEP responses remained close to pre-PAS values (at 100%) except for a transient decrease measured 0 min at the -170 ms ISI ($n = 5$).

When examining group data across the various ISIs (Fig. 2-2*B-H*), separate two-factor repeated measures ANOVAs (in both resting and active states) showed that peak-to-peak MEP amplitudes were significantly increased at ISIs of -40 ms

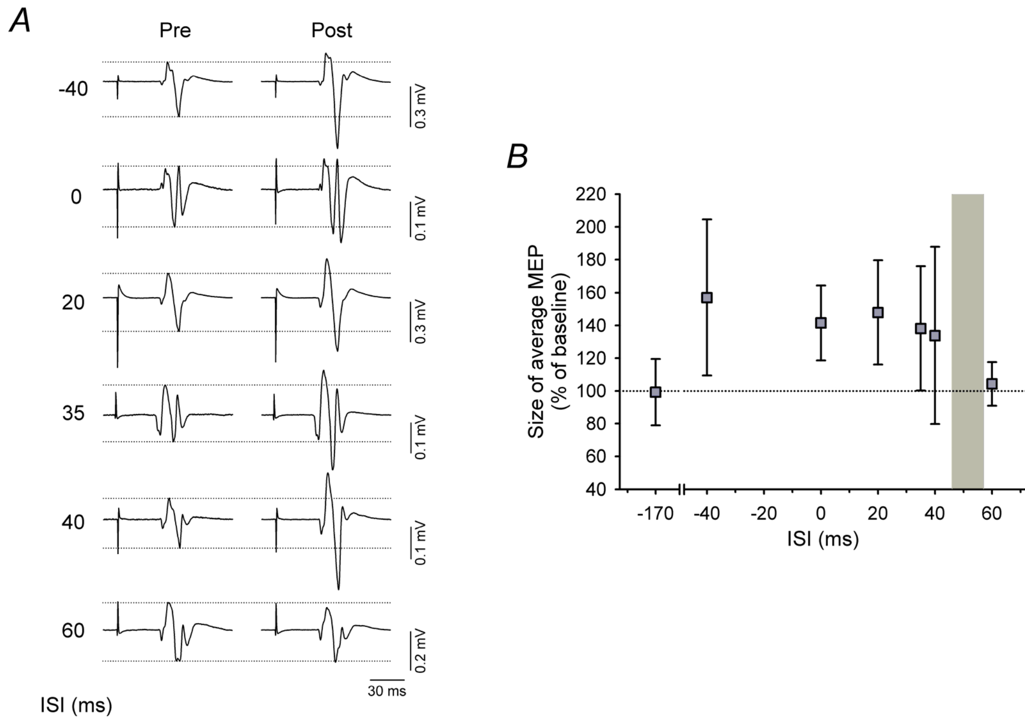

Figure 2-1. Changes in MEP response after PAS

A, Traces show the average MEP response collected in the relaxed TA muscle in one representative subject before (Pre) and after (Post) PAS at ISIs of -40 to 60 ms (top to bottom in ascending order). *B*, Graph shows the group resting MEP collected at the seven ISIs. Group means were calculated from the average MEP in each subject recorded after PAS (0 to 60 min post). The ordinate in (*B*) shows the size of the MEP as a percentage of the baseline value and the abscissa shows the ISI between the CPN stimulation and the TMS pulse. Shaded area in (*B*) indicates the estimated coincident arrival of both inputs in the contralateral motor cortex (Mrachacz-Kersting et al. 2007). Data are from eight subjects at the main ISIs (-40 to 60 ms) and five subjects at an ISI of -170 ms. Statistical analysis comparing the different ISIs was not performed as data at each ISI was obtained from groups of overlapping but different subjects.

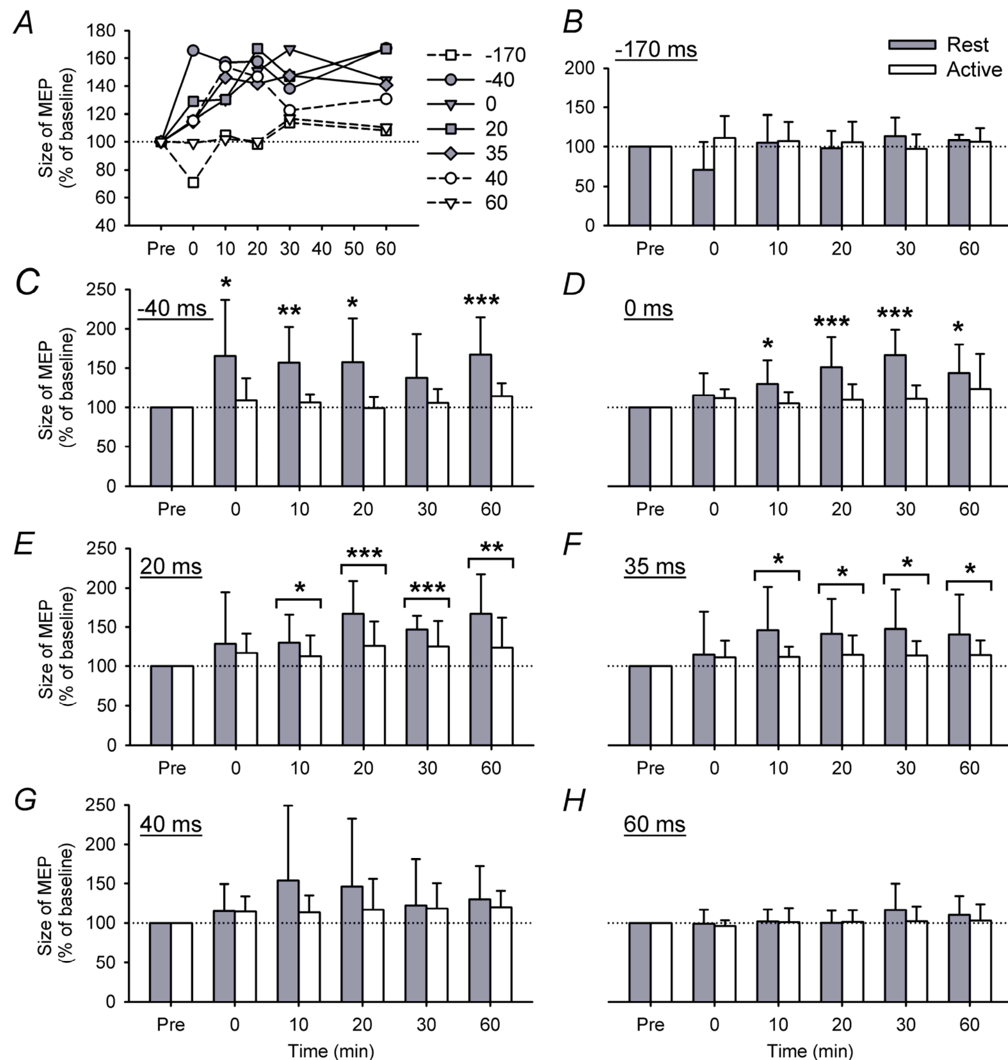

Figure 2-2. MEP facilitation as a function of the intervention ISI

A, The time course of MEP responses collected at rest. ISIs showing significant MEP facilitation in (*A*) are shown in gray. The ordinate shows the size of the MEP as a percentage of baseline values and the abscissa shows the time at which measurements were taken (0, 10, 20, 30 and 60 min after PAS). *B to H*, Bar graphs show the peak-to-peak amplitude of the MEP response collected in the relaxed (rest: gray bars) and contracted (active: white bars) TA muscle following PAS at ISIs of -170 to 60 ms. There was a significant interaction effect (time x state) at ISIs of -40 ms ($F(5,35) = 2.67$, $p < 0.05$) and 0 ms ($F(5,35) = 4.77$, $p < 0.005$) and post hoc analysis showed significant facilitation at rest. There was a significant time effect at ISIs of 20 ms ($F(5,35) = 5.70$, $p < 0.001$) and 35 ms ($F(5,35) = 2.50$, $p < 0.05$) with no interaction or state effects. At these ISIs, post hoc testing at each time-point was performed on the pooled data. Asterisks indicate time-points showing significant changes in comparison to baseline (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$).

(F(5,35) = 5.59, $p < 0.001$), 0 ms (F(5,35) = 4.92, $p < 0.005$), 20 ms (F(5,35) = 5.70, $p < 0.001$) and 35 ms (F(5,35) = 2.50, $p < 0.05$). These results indicate that corticospinal excitability was increased by PAS when the afferent volley reached the contralateral motor cortex an estimated 90, 50, 30 and 15 ms after the TMS pulse, respectively. The interaction effect (time x state) was significant at ISIs of -40 ms (F(5,35) = 2.67, $p < 0.05$) and 0 ms (F(5,35) = 4.77, $p < 0.005$), and MEP responses showed significant increases following PAS at ISIs -40 ms (F(5,35) = 4.30, $p < 0.005$) and 0 ms (F(5,35) = 4.42, $p < 0.005$) in the relaxed muscle, but not during tonic dorsiflexion (one-way ANOVAs: all $p > 0.07$). When MEPs were facilitated at ISIs of -40 to 35 ms, the majority of subjects demonstrated a 20% or greater increase in the resting MEP (data for each ISI is shown in Table 2-1). Changes in MEP responses were more variable at a 40 ms ISI, where the afferent volley is estimated to reach the contralateral cortex 10 ms *after* the TMS pulse, with only 3/8 subjects showing a 20% increase. The size of the MEP in the relaxed and contracted muscle was unchanged at ISIs of 60 and -170 ms, where the interaction between the afferent volley and cortical stimulation was likely minimal (see Discussion). Baseline MEPs were 0.5 ± 0.3 mV at rest and 1.1 ± 0.6 mV during voluntary contraction and were similar between the seven ISIs.

2.3.3 MEP responses in non-stimulated TA

TMS could evoke MEP responses in the non-stimulated TA muscle due to the adjacent position of the right and left leg motor cortices (data not shown). Appreciable MEP responses (> 0.1 mV) in the non-stimulated TA could be recorded at rest in fifteen experiments at facilitatory intervals of -40 to 35 ms. There was no significant interaction (time x leg) or differences in the MEP response between the simulated and non-stimulated leg. However, MEP responses in the non-stimulated TA were more variable compared to the stimulated muscle and were increased by +23 ± 59% versus +51 ± 40%, respectively.

2.3.4 Sustained excitability of the CST after suprathreshold TMS

The strength of corticospinal projections to lower leg muscles was increased after PAS protocols in which sensory inputs to the motor cortex arrived after inputs evoked from TMS (at ISIs from -40 to 35 ms). Thus, we examined if, after a single pulse of suprathreshold TMS, the motor cortex supplying the TA muscle remains excitable for an appreciable amount of time so that coincident excitation from sensory inputs arriving many milliseconds after TMS can occur. We evaluated the size of the MEP response when the motor cortex was conditioned by a prior suprathreshold TMS. As shown in Figure 2-3A for a single subject, the MEP response in the relaxed muscle could be facilitated by up to 80 ms following a conditioning TMS pulse. Interestingly, the maximum resting MEP response that could be evoked in this subject was 1.2 mV, while the conditioned MEP was increased to 2.6 mV, demonstrating a large degree of facilitation. MEP responses were consistently and significantly facilitated in all subjects when the conditioning stimulus was applied 20 to 50 ms before the test stimulus (all $p < 0.05$) showing that CST excitability is

ISI (ms)	Average change in MEP (%)	Range (%)	Subjects showing ≥ 20% increase
-170	-1 ± 20	-22 to 25	1/5
-40	57 ± 48	7 to 159	7/8
0	42 ± 23	-2 to 68	7/8
20	48 ± 32	14 to 107	6/8
35	38 ± 38	-18 to 75	6/8
40	34 ± 54	-18 to 134	3/8
60	6 ± 14	-13 to 27	2/8

Table 2-1. Effect of PAS at different ISIs on the size of the MEP response

The mean MEP was evaluated in each subject from the responses collected in the relaxed TA muscle 0 to 60 min after PAS.

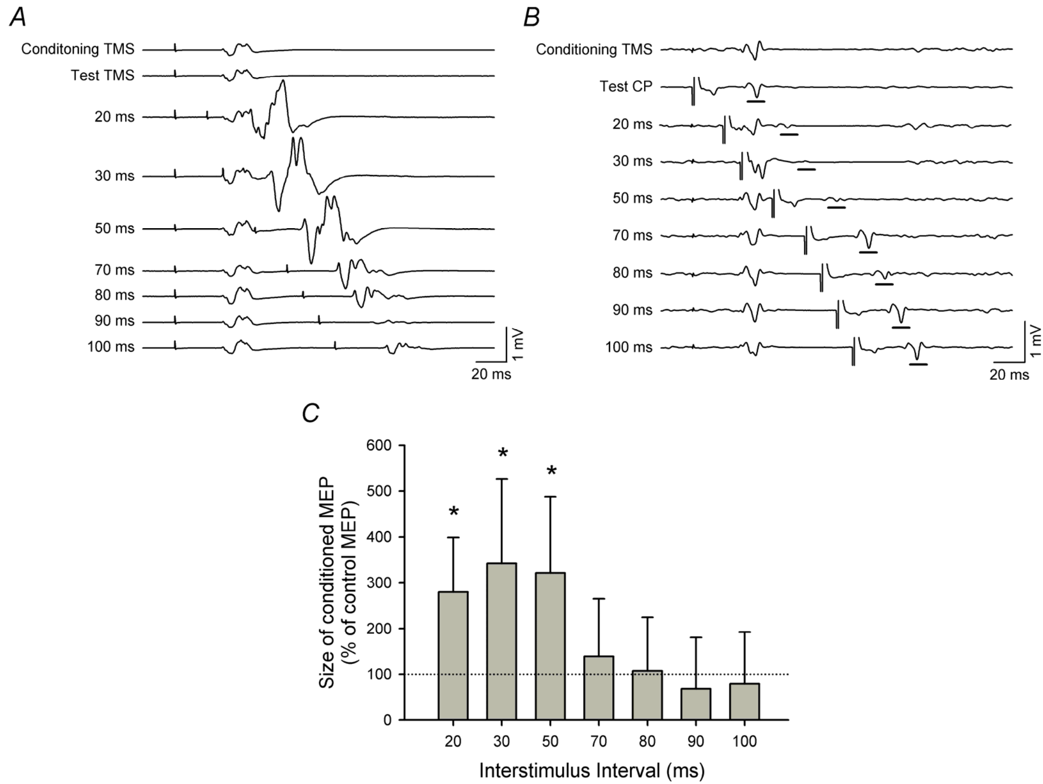

Figure 2-3. Effect of suprathreshold TMS on CST excitability

Raw sweeps in one representative subject show the conditioning effect of activating the CST using a single pulse of suprathreshold TMS on the TMS-induced MEP (*A*) and the H-reflex (*B*) in the TA muscle. MEPs were strongly facilitated (> 200%) at ISIs of 20 to 50 ms in (*A*), whilst the H-reflex was strongly depressed in (*B*). The expected time-period of the H-reflex response is marked below each sweep (horizontal line). *C*, Bar graph shows the size of the test MEP response when conditioned by suprathreshold TMS. The ordinate shows the size of the conditioned MEP response, as a percentage of the unconditioned control MEP response. The abscissa shows the ISI between conditioning and test stimuli. Data are from five subjects. Asterisks indicate significant differences compared to the control MEP (*$p < 0.05$).

strongly enhanced for a considerable period of time after TMS-induced activation (Fig. 2-3*C*). To examine whether this MEP facilitation occurred as a result of increased excitability of segmental interneurons and/or motoneurons to the TA muscle, we also evaluated the size of the TA H-reflex when conditioned by suprathreshold TMS in the same representative subject. Since the H-reflex in the TA muscle is more readily evoked during background activity than at rest, the size of the conditioning MEP was matched during voluntary dorsiflexion. In contrast to MEP responses, the H-reflex was strongly depressed when the conditioning TMS pulse was applied 20 to 50 ms before the CPN stimulus (Fig. 2-3*B*) suggesting that the paired-TMS facilitation was likely supraspinal in origin.

2.3.5 Subthreshold PAS

As shown in Figure 2-1, MEP facilitation occurred in PAS protocols where CPN inputs reached the motor cortex well after the discharge of corticospinal neurons from TMS. Because descending corticospinal volleys from the leg area of the motor cortex are recorded only up to 10 to 15 ms following suprathreshold TMS (Di Lazzaro et al. 2001), there should be little or no firing of corticospinal neurons at ISIs where afferent inputs reach the motor cortex 15 to 90 ms after a TMS pulse, i.e., at ISIs of 35 to -40 ms, respectively. Thus, it seems possible that facilitation of MEP responses at these intervals occurred when pairing CPN inputs with cortical activity that was subthreshold to corticospinal activation. To examine if MEP facilitation in the TA muscle is possible with subthreshold TMS, we used a technique where subthreshold TMS was paired with a 20-ms train of CPN stimuli. This paradigm is similar to that used by Huang et al. (2004) to produce LTP in hippocampal slice preparations. We used 60 sets of paired stimuli delivered at faster rate (0.2 Hz) to shorten the time of the intervention. In one subject, off-line analysis revealed that MEPs were too variable to establish a consistent baseline and were omitted from the analysis. In the remaining seven subjects, MEP responses gradually increased following subthreshold-PAS (individual subjects displayed in Fig. 2-4A). The interaction effect (time x state) was significant ($F(5,30) = 8.93$, $p < 0.001$; Fig 2-4B) and post hoc tests showed significant MEP facilitation both at rest ($F(5,30) = 13.18$, $p < 0.001$) and during tonic dorsiflexion ($F(5,30) = 2.63$, $p < 0.05$). At rest, MEPs plateaued 30 to 60 min after PAS and showed an average increase of 85 ± 42% at 60 min. In the contracted muscle, MEP responses increased immediately after subthreshold-PAS and were facilitated by 21 ± 16% over the 60-min period that followed the intervention. At baseline, MEPs recorded at rest and during voluntary contraction were 0.4 ± 0.2 mV and 1.1 ± 0.5 mV, respectively, and were similar to that used in the suprathreshold PAS protocols.

2.3.6 Strength of intracortical and spinal circuits

In a separate series of experiments, we evaluated the strength of intracortical and spinal circuits following PAS using an ISI of 20 ms (where the largest and most consistent facilitation occurred) to determine the loci of increased CST excitability (e.g. cortex or spinal cord). Despite significant increases in MEP responses 15 and 30 min after PAS (41 and 37% respectively, Fig 2-5A; $n = 8$), there were small but

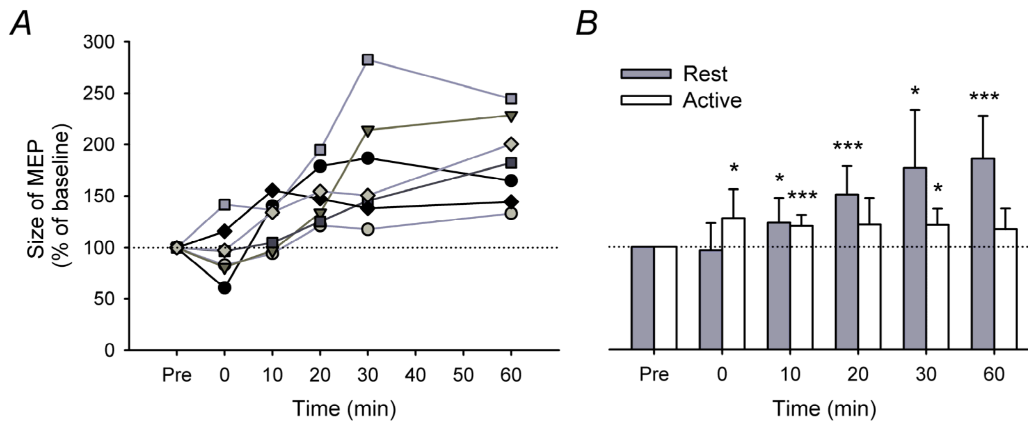

Figure 2-4. MEP facilitation after subthreshold-PAS

A, Individual subject data showing MEP increases in the relaxed TA muscle following subthreshold-PAS. *B*, Bar graph showing group MEP data collected at rest (gray bars) and during voluntary contraction (active: white bars). The ordinate shows the size of the MEP response expressed as a percentage of the baseline MEP. The abscissa shows the time at which measurements were taken (0, 10, 20, 30 and 60 min after subthreshold-PAS). Data are from seven subjects. The interaction effect (time x state) was significant ($F(5,30) = 8.93$, $p < 0.001$) and post hoc testing showed significant MEP facilitation at rest ($F(5,30) = 13.18$, $p < 0.001$) and during background contraction ($F(5,30) = 2.63$, $p < 0.05$). Asterisks indicate significant differences compared to baseline values (*$p < 0.05$, ***$p < 0.005$).

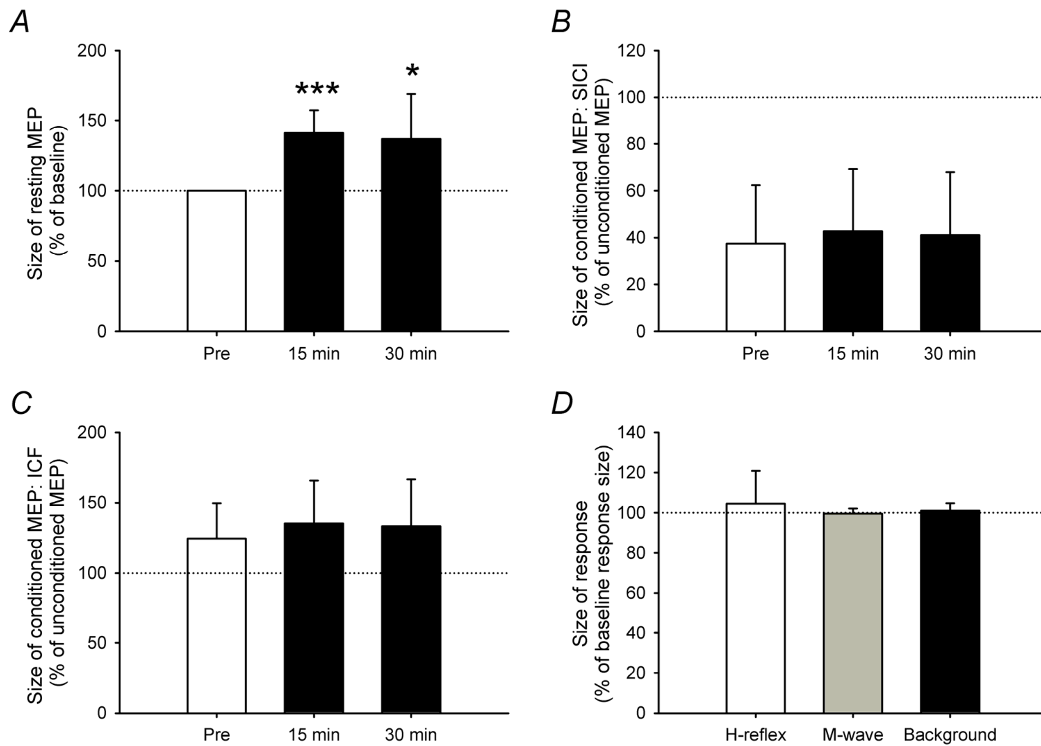

Figure 2-5. Effect of PAS on MEP, SICI, ICF and H-reflex

Bar graphs show the effect of PAS at an ISI of 20 ms on the size of the MEP response (*A*), SICI (*B*), ICF(*C*) and H-reflex (*D*) recorded in the TA muscle. The ordinate in (*A*) shows the size of the MEP response as a percentage of the MEP before the intervention. The ordinate in (*B-C*) shows the size of the conditioned MEP as a percentage of the unconditioned response. The abscissa in (*A-C*) shows the time at which measurements were taken. The ordinate in (*D*) shows the size of the response recorded 25 min after PAS as a percentage of the response size before the intervention, and the abscissa shows the amplitude of the H-reflex (clear column), M-wave (gray column) and the level of background EMG (black column). Data are from eight subjects. Asterisks indicate significant differences compared to baseline values (*$p < 0.05$, ***$p < 0.005$).

insignificant decreases in short-interval intracortical inhibition (SICI; Fig. 2-5B; paired *t*-test, all $p > 0.08$) and small but insignificant increases in intracortical facilitation (ICF; Fig 2-5C; paired *t*-test, all $p > 0.2$) after PAS. The size of the unconditioned MEP (0.4 ± 0.2 mV) used to evaluate SICI and ICF was unchanged throughout the experiments (paired *t*-test, all $p > 0.9$). Similar to SICI and ICF, the size of the H-reflex was unchanged 25 min after PAS (*t*-test, $p = 0.4$; open bar in Fig. 2-5D).

2.4 DISCUSSION

A conditioning intervention of low-frequency PAS delivered at rest can significantly increase the size of the MEP in lower leg muscles. Similar to the hand, MEP facilitation in the leg evolved rapidly over the first 10 min and persisted for many minutes after the intervention. Surprisingly, unlike PAS in the hand, facilitation in the leg was produced over ISIs when sensory inputs arrived at the motor cortex over an estimated range of 15 to 90 ms *after* TMS-induced firing of CST neurons. We argue that following a TMS pulse, the continued activity of the leg motor cortex that is subthreshold to CST neuron activation is sufficient to induce associative facilitation in the motor cortex when paired with afferent excitation from peripheral nerve stimulation. In fact, it was possible to induce strong MEP facilitation when sensory afferent inputs were directly paired with subthreshold TMS.

2.4.1 Effect of interstimulus interval on MEP facilitation

Similar to previous reports in the hand and leg, PAS in this study did not affect MEPs if the peripheral volley arrived at level of the motor cortex before the cortical stimulus at an ISI of 60 ms (Wolters et al. 2003; Mrachacz-Kersting et al. 2007). It is likely that excitation of the motor cortex from a CPN stimulus did not produce sustained periods of cortical activity. Thus, when using a 60 ms ISI, there was likely no coincident activation of the motor cortex from CPN and TMS inputs and, hence, no PAS-induced changes in cortical excitability.

Mrachacz-Kersting et al. (2007) have demonstrated that PAS in the leg strengthens corticospinal connections to the TA muscle when inputs from CPN stimulation are timed to coincide with inputs from cortical activation. Although the arrival time of the afferent input in the human motor cortex is not directly established, the first negative potential (N40) over the sensory cortex following peroneal nerve stimulation has an average latency of 42 to 47 ms (Shaw and Synek 1985; Mrachacz-Kersting et al. 2007). Since some central processing is also required to transmit the afferent input to the motor area (Nielsen et al. 1997; Petersen et al. 1998; Wolters et al. 2005), a 4-10 ms has been adopted to estimate the arrival time of the CPN volley in the contralateral motor cortex (Mrachacz-Kersting et al. 2007). This indicates that a TMS pulse should be delivered 46-57 ms after CPN stimulation to condition the motor cortex with coincident peripheral and central inputs. Likewise, ISIs from 45 to 55 ms have been shown to facilitate CST projections to the TA muscle following 30 min of low-frequency (0.2 Hz) PAS. Although an ISI of 55 ms does not produce consistent increases in corticospinal excitability in all subjects, adjusting the ISI in each

individual using somatosensory evoked potentials and adding a central processing delay of 6 ms has been shown to produce consistent MEP facilitation (Mrachacz-Kersting et al. 2007).

In this study, we demonstrated that MEP responses could also be facilitated at ISIs where the afferent excitation in the cortex was estimated to arrive over a range of 15 to 90 ms *after* the TMS pulse, assuming an average delay of 50 ms based on Mrachacz-Kersting et al. (2007). We propose that afferent inputs arriving tens of milliseconds after a TMS pulse still encountered subthreshold activity in the motor cortex so that coincident excitation of common neurons was possible. Evidence for prolonged activity of the cortex was supported by the paired-TMS responses. Following a single pulse of suprathreshold TMS, the strength of CST projections to lower leg muscles was strongly enhanced by over 200% for at least 50 ms after the cortical stimulus (and perhaps longer in pre-corticospinal neurons). Such sustained excitability is likely cortical in origin given that descending corticospinal volleys are increased (Nakamura et al. 1997; Di Lazzaro et al. 2002) and spinal excitability is depressed (Fuhr et al. 1991; Ziemann et al. 1993, see also Fig. 2-4B) for many tens of milliseconds following a single pulse of suprathreshold TMS. This increase in cortical excitability may occur as a result of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptor activation given the time course of AMPA and NMDA mediated EPSPs are around 100 ms (Clark et al. 1994; Edmonds et al. 1995; Kohn et al. 2002). By 200 ms after TMS, corticospinal activity likely returns to baseline values and cortical activation by CPN and TMS inputs becomes relatively independent once again. This is supported by a lack of change in MEP responses when CPN and TMS inputs to the motor cortex are separated by approximately 220 ms (at an ISI of -170 ms).

In the hand motor cortex, if inputs from peripheral nerve activation arrive at the motor cortex an estimated 5 to 15 ms *after* TMS, MEP responses are reduced (Wolters et al. 2003; Ziemann et al. 2004). The suppression of MEP amplitudes has been attributed to spike-dependent LTD mechanisms where the activation of weak (sensory) inputs occurs during the afterhyperpolarization of a common cortical neuron activated by a strong (TMS) input. Such time-dependent reduction in MEP responses has been reported in the leg if suprathreshold TMS is given 40 ms after CPN stimulation, again when inputs from peripheral nerve stimulation are estimated to arrive 10 ms after TMS (n = 5 subjects; Mrachacz-Kersting et al. 2007). In contrast to these results, our group data failed to show suppression of the MEP response at an ISI of 40 ms. As 3/8 subjects showed strong facilitation which washed out the smaller inhibition observed in 3/8 subjects, it is possible that we did not see an overall suppression of MEP responses because we did not test PAS at the correct ISI for each subject. Adjusting the ISI using somatosensory evoked potentials may be useful for targeting this inhibitory interval. Here, we cannot rule out that a narrow and distinct time-window may exist for each subject to induce PAS inhibition in the leg motor cortex at rest, which is in contrast to more consistent PAS-induced inhibition that can be produced during walking (Stinear and Hornby 2005; Prior and Stinear 2006). In addition, facilitation of MEPs using a 40 ms ISI in some subjects may have occurred due to coincident activation of the somatosensory cortex by TMS and afferent inputs (Wolters et al. 2005) given the close proximity of the leg motor and sensory cortices.

2.4.2 Facilitation of MEP responses from subthreshold PAS delivered at rest

The idea that continued activity in the motor cortex after suprathreshold TMS is sufficient to enhance cortical excitability when paired with sensory inputs is supported by the finding that MEP responses were increased when sensory inputs were paired with subthreshold TMS. This facilitation may have also occurred as result of using the 20-ms train of CPN stimulation or a faster rate of stimulation (0.2 Hz). However, recent evidence in humans suggests that cortical inputs subthreshold to CST neuron firing are important for PAS-induced increases in cortical excitability. Subthreshold-PAS in the hand, applied at a rapid-rate (5 Hz: Quartarone et al. 2006) or at low frequency (0.1 Hz) using antero-posterior stimulation combined with background contraction (Kujirai et al. 2006) has been shown to facilitate MEP responses. Moreover, it has been argued that later indirect (I) waves play an important role in associative plasticity given that antero-posterior magnetic stimulation tends to activate cortical circuits that preferentially elicit I3 waves (Sakai et al. 1997). Here, sustained excitability of I3-related cortical circuitry activated by antero-posterior stimulation over the leg motor cortex may have been involved in facilitating cortical excitability when paired with afferent volleys from the periphery.

2.4.3 Site of origin of PAS-induced changes in MEP responses

In the hand, PAS-induced MEP facilitation is likely cortical in origin, given that MEPs are facilitated when evoked with TMS and not from TES (Stefan et al. 2000; Ridding and Uy 2003). The fact that we observed MEP facilitation with subthreshold-PAS using TMS at 80% of active motor threshold also points to a cortical origin of facilitation, given that there are no descending volleys evoked at rest with TMS below active motor threshold (Di Lazzaro et al. 1998) and likely no paired activation of corticospinal and peripheral inputs to the spinal cord. Furthermore, the excitability of the motoneuron pool, as measured using the F-wave and/or the H-reflex in the TA muscle (Mrachacz-Kersting et al. 2007) and the abductor pollicis brevis muscle (Stefan et al 2000; Wolters et al. 2003) are unchanged following PAS despite concomitant changes to the size of the MEP response (although see Meunier et al. 2007). In line with these results, we did not observe large changes in the H-reflex response (5% increase) despite a 40% facilitation of resting MEPs in the relaxed muscle and a 21% increase in the active MEP observed in a separate experiment (see Fig. 2-2). Although we did not demonstrate that short-interval intracortical inhibition and intracortical facilitation were altered following PAS (similar to the hand), this does not necessarily mean that excitability changes did not occur at a cortical level. It is likely that cortical circuits recruited with short-interval intracortical inhibition, and potentially intracortical facilitation (Di Lazzaro et al. 2006) did not reflect changes in cortical excitability induced by PAS to the leg motor cortex.

2.4.4 Differences between the hand and leg areas of the motor cortex

In the primary motor hand area, there exists a precise and narrow (≈ 20 ms) temporal relationship between the arrival of afferent and cortical inputs resulting in PAS-

induced facilitation and inhibition of MEP responses (Wolters et al. 2003). This finding is in contrast to the related structures of the leg, which showed increases in MEPs over a wide range (≈ 80 ms) of PAS time intervals. However, it is unlikely that the broad temporal relationship between pairs of inputs are unique to the related structures of the lower leg as artificially pairing neural activity in the wrist area of the primate motor cortex at comparable intervals (≈ 100 ms) can increase connectivity between two distant sites (Jackson et al. 2006). Here we propose a few explanations why the leg, in comparison to the hand, exhibited PAS-induced facilitation over a large window of time intervals. First, the time course of MEP facilitation in the present study gradually increased following PAS and MEPs were more pronounced after 10-20 min. It is possible that MEP facilitation in the hand at ISIs where the afferent input arrived at the motor cortex tens of milliseconds after the TMS pulse was not observed by Wolters et al. (2003) because MEP responses were only collected immediately after PAS. Secondly, afferent inputs from the lower leg may be stronger in modifying cortical excitability as electrical stimulation of leg afferents modifies CST excitability of the leg motor cortex more rapidly compared to the related structures of the hand. For instance, at least 1.5 hours of repetitive electrical stimulation of the ulnar nerve is necessary to produce consistent increases to the size of the MEP in the abductor digiti minimi and first dorsal interosseous muscles (Ridding et al. 2000), whereas a similar stimulation of the CPN over a shorter 30-min period produces persistent increases (up to 60 min) to the size of the MEP in the TA muscle (Khaslavskaia et al. 2002; Knash et al. 2003). Thirdly, because TMS preferentially activates horizontally oriented interneurons in the outer layers of the cortex, which trans-synaptically activate corticospinal neurons (Day et al. 1987, 1989), the mechanism of cortical activation may provide an additional explanation for these differences. In the hand, PAS-induced changes in CST excitability are linked to the direction of TMS currents because subthreshold antero-posterior stimuli have been shown to induce MEP facilitation in the first dorsal interosseous muscle, whereas similar posterior-anterior currents have no significant effect (Kujirai et al. 2006). Geometry of a double-cone versus a figure-of-eight coil alters inputs to CST neurons and likewise alters descending corticospinal volleys that comprise the MEP (Terao et al. 2000). A double-cone coil may preferentially promote PAS-induced facilitation at ISIs where the afferent excitation reaches the motor cortex *after* the stronger TMS pulse. In line with these findings it may be worthwhile examining the effects of coil size and geometry on PAS-induced changes in cortical excitability. Likewise, it would be useful to investigate the temporal relationship between the arrival of afferent and cortical inputs resulting in PAS-induced facilitation and inhibition of MEP responses in hand muscles by measuring MEP responses at later time-points after PAS.

2.4.5 Clinical implications

The potential to increase the strength of corticospinal connections to leg muscles by PAS makes this technique a possible therapeutic tool. Previous studies have shown that the connectivity of CST neurons is crucial for functional motor recovery after subcortical insult (Thomas and Gorassini 2005; Ward et al. 2006). Future studies using peripheral and/or CNS stimulation prior to rehabilitative training, as shown

using transcranial direct current stimulation (Hummel et al. 2005) and repetitive TMS (Kim et al. 2006), shows potential for improving functional recovery of leg muscles after injury to the CNS when facilitation of cortical circuitry is required.

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CHAPTER 3: Peripheral sensory activation of cortical circuits in the leg motor cortex of man

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3.1 INTRODUCTION

Integration of sensory afferent feedback into the generation of motor commands from the primary motor cortex is important given the severe disruption of gait and fine motor skills in patients with large-fibre sensory neuropathies (Rothwell et al. 1982; Sanes et al. 1985; Lajoie et al. 1996). Although the process by which sensory inputs shape the activity of cortical networks is unknown, studies in monkeys have shown that both proprioceptive and cutaneous sensory stimulation can strongly excite or inhibit ongoing neuronal activity in the primary motor cortex during precise voluntary movement (Lemon 1981; Boudreau and Smith 2001). In humans, altering sensory input to the motor cortex via repetitive peripheral nerve stimulation (Ridding et al. 2000; Knash et al. 2003) or transient limb deafferentation (Brasil-Neto et al. 1992) can temporarily alter motor cortex excitability as assessed by transcranial magnetic stimulation (TMS). Likewise, permanent nerve injury or limb amputation is thought to promote cortical re-organization (Chen et al. 2002), potentially via changes in the excitability of intracortical inhibitory interneurons that link functional motor areas (Jacobs and Donoghue 1991; Schneider et al. 2002).

Recently, several human studies using paired-pulse transcranial magnetic stimulation (TMS) techniques have examined how sensory afferent inputs from the hand influence both excitatory and inhibitory cortical networks of the primary motor cortex. For instance, when afferent inputs from electrical stimulation first arrive at the motor cortex, motor evoked potentials (MEPs) from TMS are depressed in what is called ‘short-latency afferent inhibition’ or SAI (Tokimura et al. 2000). Since late descending corticospinal volleys are also depressed, SAI is thought to occur through supraspinal mechanisms (Tokimura et al. 2000) that involve both cholinergic and GABAergic systems distinct from networks producing short-interval intracortical inhibition (SICI) (Di Lazzaro et al. 2000, 2007). Other forms of afferent-induced inhibition of the motor cortex include long-latency afferent inhibition (LAI) that occurs when afferent inputs are stimulated 200 ms before a TMS pulse (Chen et al. 1999; Sailer et al. 2002) and surround inhibition from vibration applied to adjacent hand muscles as reflected in reduced MEPs and increases in SICI (Rosenkranz and Rothwell, 2003).

In addition to the inhibitory effects of afferent feedback at the cortex, afferent input can strengthen ongoing EMG activity through a transcortical loop (for review see Christensen et al. 2000). Muscle stretch and/or cutaneous stimulation in the upper and lower limb can access corticospinal circuits to facilitate the MEP (Nielsen et al. 1997;

Petersen et al. 1998; Rosenkranz and Rothwell 2003; Kessler et al. 2005). Such facilitation is most pronounced in muscles supplied by the nerve, though similar changes can be observed in neighbouring muscles (Deletis et al. 1992). In the leg, afferent feedback contributes to muscle activity (Sinkjaer et al. 2000) and the long-latency reflex is thought to be involved in lifting the foot over obstacles and stabilising the supporting limb in the stance phase of gait (Christensen et al. 2000). In terms of cortical networks, MEP facilitation from hand muscle afferents is associated with a reduction in SICI, but paradoxically in an increase in long-interval intracortical inhibition (LICI) (Rosenkranz and Rothwell 2003; Rothwell and Rosenkranz 2005). Increases in intracortical facilitation (ICF), a complex motor circuit whose origin is currently a matter of debate (Di Lazzaro et al. 2006), occur in wrist motor areas following vibration of homonymous afferents (Rosenkranz et al. 2003), whereas increases in ICF measured from muscles of the wrist are only observed when antagonist muscle afferents are electrically stimulated (Aimonetti and Nielsen 2001). Currently, the role of sensory stimulation on intracortical networks in the leg area of the primary motor cortex has not been systematically studied.

In this study, we explored the time-course of MEP facilitation and inhibition following electrical stimulation of the tibial (at ankle) and posterior tibial (at knee) nerves with the former having strong heteronymous inputs to ankle dorsi- and plantar-flexors (Pierrot-Deseilligny and Burke 2005). Conditioning TMS with a prior nerve stimulus produced bi-directional changes in the MEP with depression at short intervals (similar to SAI in the hand) and potentiation at longer-intervals, the latter occurring at latencies a few milliseconds after the arrival of afferent inputs at the somatosensory cortex. Responses elicited with direct corticospinal tract stimulation were depressed by the peripheral nerve stimulus at both short and long interstimulus intervals, suggesting that the MEP inhibition from peripheral nerve stimulation was mainly spinal in origin whereas MEP facilitation was mediated by cortical circuits. Because the effects of leg afferent stimulation appeared to be mainly excitatory on the motor cortex, we examined if MEP facilitation was associated with decreases in the activation of cortical networks producing SICI and LICI and increases in the excitability of ICF networks. We also examined which excitatory cortical networks were activated by leg afferents, i.e., those involved in the production of earlier or later corticospinal volleys, by using MEP conditioning techniques at the periodicity of indirect (I) waves (Ziemann et al. 1998). Parts of this study have been presented in abstract form (Roy and Gorassini 2008).

3.2 METHODS

3.2.1 Subjects

Nineteen healthy subjects (9 females) aged 21 to 53 (29 ± 8 years, means ± SD) participated in this study. All subjects gave written informed consent and the protocol was approved by the Human Ethics Research Board at the University of Alberta. Subjects were comfortably seated with the examined leg (17 right and 2 left) slightly bent at the knee and with the foot secured to a footplate. Responses were recorded in the left leg of two subjects because MEPs were more easily elicited at rest compared to the right leg.

3.2.2 EMG recordings

Surface electromyography (EMG) was recorded from the tibialis anterior (TA), soleus (SOL) and abductor hallucis (AH) muscles using pairs of surface Ag-AgCl electrodes (Kendall, Chicopee, MA). EMG signals were amplified 1000 times and filtered with a band-pass of 10 to 1000 Hz (Octopus, Bortec Technologies, Calgary, Canada). EMG signals were digitized at 5 kHz using Axoscope hardware and software (Digidata 1200 Series, Axon Instruments, Union City, CA) and stored on a personal computer for off-line analysis. During voluntary contraction, EMG from the target muscle was rectified, low-pass filtered using a 100 ms time-constant (NL703, Digitimer, Hertfordshire, UK) and displayed on an oscilloscope.

3.2.3 Transcranial magnetic stimulation (TMS)

TMS was performed using two Magstim 200 stimulators connected to a Bistim module (Magstim, Dyfed, UK) and a custom-built figure-of-eight coil (P/N 15857: bat wing with 90 mm external wing diameter). This figure-of-eight coil provides more focal stimulation to the leg motor cortex. The coil was placed over the leg area of the motor cortex. The optimal stimulus site was identified using a stimulation intensity that was slightly above threshold in the resting muscle. The coil was secured in place throughout the experiment and oriented to deliver anterior-posterior directed current in the brain. Single and paired pulses of TMS were delivered as described below.

3.2.4 Peripheral nerve stimulation

Peripheral nerves were stimulated using a constant-current stimulator (DS7A, Digitimer). The tibial nerve (TN), innervating the foot, was activated at the ankle with the cathode placed below the medial malleolus and a large anode on the lateral aspect of the ankle (0.2-ms pulse) as done by Yang and Stein (1990). The TN intensity was 1.5 × motor threshold (MT) in the AH muscle or 2 × MT if the M-wave was < 0.1 mV at the 1.5 MT intensity. Stimulation did not elicit cutaneous sensation on the lateral side of the foot via the sural nerve. The posterior tibial nerve (PTN) was stimulated using bipolar surface electrodes in the popliteal fossa (Poon et al. 2008) to minimize current spread to other nerves. The PTN stimulus intensity was just above MT (1-ms pulse). The H-reflex in the TA muscle was elicited by stimulating the common peroneal nerve near the head of the fibula with the anode placed on the medial side of the knee just below the patella (1-ms pulse). This arrangement provided more diffuse stimulation (compared to bipolar stimulation) and was used to test general spinal cord excitability to the TA muscle.

3.2.5 Corticospinal tract stimulation

The corticospinal tract was stimulated non-invasively at the cervicomedullary junction in 5 subjects. In 3 of the subjects, it was possible to elicit cervicomedullary

evoked potentials (CMEPs) with TMS to the brainstem, which is less disturbing than direct electrical stimulation. In these subjects, magnetic stimuli were delivered using a Magstim 200 stimulator and a double cone coil placed over the inion of the skull with the current flowing in a downward direction (Taylor and Gandevia, 2004). In the other 2 subjects, electrical stimulation of the corticospinal tract was done using a 100 µs electrical pulse (320-400 V, D185 stimulator, Digitimer) delivered through a pair of silver cup electrodes (10 mm diameter) fixed over the mastoids with the cathode on the left.

3.2.6 Somatosensory evoked potentials

Cortical potentials evoked by TN stimulation at the ankle were recorded with a pair of silver cup electrodes (10 mm diameter) placed on the scalp, with the anode over the vertex and the cathode 5 cm more anterior, a placement similar to that used in Nielsen et al. (1997). The signals were amplified 50,000 times and were filtered between 2 to 1000 Hz. Usually 200-300 sweeps were averaged. The arrival time of the evoked potential was measured as the time of the first negative peak, referred to as the P40 (Hauck et al. 2006).

3.2.7 Study design

3.2.7.1 Experiment 1: Time-course of afferent conditioning of TA MEPs

In 8 subjects, heteronymous afferent inputs from the TN that innervates the medial and plantar surface of the foot were used to condition MEP responses evoked in the TA muscle. Electrical stimuli to the homonymous common peroneal nerve were not used because they strongly depressed the TA MEP, likely due to strong spinal inhibitory effects. The TMS intensity was set to approximately half the maximum MEP response in the resting TA muscle (0.2-0.6 mV). Fourteen conditioning-test intervals between the nerve stimulus and the TMS pulse were used from 25 to 100 ms (see Fig. 3-1). Six conditioning TN stimuli were delivered at each conditioning-test interval intermixed with 12 single test stimuli. For all trials, the time between consecutive stimuli was 5 s.

TMS recruitment curves were collected for two conditions: without and with TN stimulation. The TN stimulus was delivered at a fixed conditioning-test interval that produced the largest MEP facilitation in each subject (at an ISI between 37.5-55 ms). Because absolute MEP amplitudes for a given intensity varied between subjects, the stimulation intensities used to produce the recruitment curves were normalized with respect to the resting TA motor threshold (RMT). RMT was determined before the recruitment curve was obtained and was defined as the lowest stimulus intensity that evoked an MEP with an amplitude > 50 µV in at least 3 of 5 consecutive stimuli (Perez et al. 2004). TMS intensities were then increased from 80% to 150% of the RMT in steps of 10%, with 5 MEPs collected at each intensity.

3.2.7.2 Experiment 2: Site of afferent-induced MEP inhibition and facilitation

To investigate the site (i.e. cortex or spine) of afferent-induced MEP inhibition at short ISIs (near 35 ms) and MEP facilitation at longer ISIs (near 50 ms), TN stimulation was also used to condition H-reflexes and CMEPs. Since TA H-reflexes and CMEPs in the TA muscle are more readily evoked in the pre-contracted muscle, all responses were collected while subjects maintained a tonic dorsiflexion corresponding to 10% of their maximum voluntary contraction (MVC). During tonic contraction, the time-course effect of TN stimulation on MEPs and H-reflexes was measured in 10 subjects. The H-reflex was adjusted to approximately half the maximum H-reflex response (0.2-0.6 mV) to show both inhibition and facilitation following the nerve stimulus. To compare similar-sized responses, the TMS stimulus intensity was adjusted to produce small, but reliable, MEPs of 0.5-0.75 mV in the contracted muscle. Twelve conditioning-test intervals from 25 to 100 ms were tested for both MEPs and H-reflexes (see Fig. 3-3). For each experiment, 6 conditioning TN stimuli were delivered at each conditioning-test interval intermixed with 12 single test stimuli. SEPs were also recorded in 9 subjects following TN stimulation.

TN conditioning of evoked responses from direct activation of the corticospinal tract were also examined in 5 of the 10 subjects (the majority on separate experimental sessions) to determine if the MEP inhibition and/or facilitation were cortical in origin. CMEPs were elicited by stimulating the corticospinal tract at the brainstem (see Corticospinal tract stimulation above). For brainstem stimulation, the duration of the conditioning-test interval was increased by 3-5 ms to account for the transmission delay from the motor cortex to the cervicomedullary junction and to match the arrival-time of the MEP and CMEP volleys at the motoneuron pool (Taylor et al. 2002). Two ISIs were tested, one that produced MEP inhibition (near 35 ms) and one that produced MEP facilitation (near 50 ms). MEPs and CMEPs were investigated separately in a block of trials consisting of two randomly intermixed conditions (with test stimulus alone or conditioned by the PTN stimulus), each with ten responses. Responses were collected during voluntary contraction.

3.2.7.3 Experiment 3: Intracortical mechanisms of afferent-induced MEP facilitation

Because it was determined that the major effect of leg afferent stimulation was to increase cortical excitability, we examined which cortical networks were associated with afferent-induced increases in MEP responses. The effect of afferent input on SICI, ICF, paired-pulse facilitation (PPF), LICI and I-wave facilitation was investigated using the appropriate paired-pulse TMS protocols (see below). These experiments were each performed at rest under two different conditions: with and without TN stimulation. TN stimulation was adjusted in each subject to a conditioning-test interval that produced MEP facilitation in the resting TA muscle (ISI near 50 ms), as described above. The afferent input was synchronised to arrive at the cortex at the same time as the test TMS stimulus as done by Aimonetti and Nielsen (2001). Because activity of intracortical circuits and corticospinal recruitment depend on the size of the test stimulus (Ziemann et al. 1998; Chen 2004), the TN

condition was also repeated (if necessary) with the test stimulus intensity adjusted (TS-ADJ) to match the MEP amplitude produced at baseline without TN stimulation.

The effect of TN stimulation on SICI and ICF was investigated in 5 subjects using a subthreshold conditioning stimulus preceding a suprathreshold test stimulus by 3 ms and 10 ms, respectively (Kujirai et al. 1993). The conditioning stimulus intensity was adjusted to 90-95% of the active motor threshold (AMT). AMT was defined as the lowest stimulus intensity that elicited a distinguishable MEP > 50-100 μV in at least 3 of 5 consecutive stimuli in the tonically contracted TA muscle. For both experimental conditions, 10 trials were recorded at each paired-pulse interval intermixed with 10 test stimuli.

The effect of afferent stimulation on paired-pulse TMS facilitation (PPF: ISIs from 10 to 50 ms) and LICI (ISIs from 60 to 150 ms) was investigated using pairs of suprathreshold TMS in 8 subjects on a different experimental session (see Fig. 3-4C). The two pulses were given at the same intensity. For both experimental conditions, 4 paired-pulse stimuli were delivered at each interval intermixed with 8 single-pulse test stimuli. LICI at 100 ms was further tested using a weaker conditioning stimulus such that the conditioned MEP was inhibited by approximately 40-60%. Ten paired-pulse stimuli and 10 test stimuli were delivered for each condition: without and with afferent input.

I-wave facilitation (also known as short-interval intracortical facilitation: SICF) was investigated in 6 subjects (5 recorded in the same session as SICI and ICF) using a paired-pulse protocol described by Ziemann et al. (1998) with a suprathreshold test stimulus preceding a subthreshold conditioning stimulus. Five interstimulus intervals were tested: 1.5, 2.9, and 4.9 ms to capture the peaks of the I-wave periodicity and 2.1 and 3.7 ms to capture the intervening troughs (Chen and Garg 2000). The first TMS pulse served as the test stimulus. The intensity of the second pulse was set to 95% of RMT. For both experimental conditions, a block of trials consisted of 8 paired stimuli for each interval intermixed with 16 test stimuli.

3.2.7.4 Experiment 4: Effect of posterior tibial nerve (PTN) stimulation on MEPs in SOL

The effect of afferent input on cortical excitability to SOL was investigated in 8 subjects. The afferent input was provided by stimulating the homonymous PTN in the popliteal fossa. The conditioning-test paradigm was similar to that described in Exp. 1 with ISIs from 20 to 100 ms (see above). Five subjects showing MEP decreases at an ISI of 30 ms were further tested at a variety of intervals between of 24 and 40 ms (see Fig. 3-6). The TMS intensity was adjusted to produce SOL MEPs of 0.1 mV, which was approximately half the size of the maximum resting MEP response. Since MEPs in SOL are much smaller than the antagonist TA, we ensured that the SOL MEPs had a different latency and waveform compared to the responses recorded in TA. Recruitment curves of increasing TMS intensities were collected for both conditions: without and with PTN stimulation. The PTN stimulus was delivered at a fixed conditioning-test interval that produced MEP facilitation in each subject (at an

ISI between 40-55 ms). TMS intensities were then increased from 88% to 124% of the RMT (in steps of 4%) and at 130% RMT to measure the maximum MEP responses, with 5 MEPs collected at each intensity.

3.2.8 Data analysis and statistics

The size of the MEP, H-reflex and CMEP was measured as the peak-to-peak amplitude of the non-rectified response. The level of background activity was measured from the rectified EMG in a 50-ms period before the stimulus and assessed to ensure there was a comparable level of EMG activity across conditions. Statistical analysis was performed on either the actual responses or after the data was expressed as a percentage of the test response. Both peak inhibition and facilitation were evaluated in each subject around the ISIs the produced significant changes in the MEP.

The time-course of the effect of TN stimulation on resting MEP responses in Exp. 1 was adjusted in each subject to accommodate for differences in the time required for the afferent input to reach the motor cortex (see Fig. 3-1B, C). The afferent-conditioned MEP profile (over all the ISIs) was first interpolated in steps of 0.5 ms using a linear interpolation technique. A given MEP profile was then shifted to the right or left by an amount that was equal to the difference between a subject's MEP latency in the AH muscle and the latency of the group average, which was 40.4 ± 1.3 ms. For example, the conditioned MEP profile from a subject with an MEP latency of 42.4 ms was shifted to the left by 2 ms. After shifting the profiles (by 2.6 ± 0.7 ms on average), the values on the curves were averaged at the original ISI values of 20, 30, 32.5 ms etc., all the way up to 100 ms. In the example subject, the interpolated values at 22, 32, 34.5 ms and so on were used when calculating the group average. MEP profiles during voluntary contraction (Exp. 2) were not adjusted because the facilitatory window was broader. Similarly, MEP profiles in the SOL muscle in response to PTN stimulation were not adjusted because the variability in SOL MEP latencies across subjects, and likely the variability in the time afferent inputs reached the motor cortex, were much less compared to TN stimulation.

For single pulse MEP and H-reflex data collected with TN stimulation over the range of ISIs used, Friedman analysis of variance (ANOVA) with 'ISI' as within-subject factor was used since the data were non-normally distributed across the intervals. For this data, post-hoc comparisons were done using Wilcoxon tests. The recruitment curve data and the paired-pulse data (SICI, ICF, PPF, LICI, and I-wave facilitation) were analyzed using two-way repeated measures ANOVAs on either the actual or normalized MEP amplitudes, with post-hoc comparisons done using paired t-tests (two-tailed). Differences in the effect of TN stimulation on brainstem and cortical responses were analyzed using a two-way repeated measures ANOVA with 'site of stimulation' (cortex and brainstem) and 'type of ISI' as within-subject factors. MEP data in SOL collected with PTN stimulation was analyzed using a one-way repeated measures ANOVA with 'ISI' as within-subject factor. The significance level was set at $P < 0.05$ and data are given as means ± SEM.

3.3 RESULTS

3.3.1 Experiment 1: Time-course of afferent conditioning on TA MEPs

Electrical stimulation of the TN at the ankle modified the size of the peak-to-peak TA MEP. The intensity of the test stimulus (70 ± 3% of the maximum stimulator output: MSO) was adjusted to elicit MEP responses on the steep portion of the recruitment curve, which was 0.30 ± 0.04 mV on average in the resting muscle. Fig. 3-1A shows the effect of TN stimulation on the test MEP in a single subject. Stimulating the TN 35 ms before applying a pulse of TMS to the motor cortex depressed the test MEP, whereas a conditioning stimulation at ISIs of 45 and 60 ms facilitated the test MEP. Surrounding these conditioning-test intervals, the MEP showed little change at ISIs of 30 and 80 ms. This trend was similar for all subjects in Fig. 3-1B, showing individual subject data across all ISIs.

In the averaged group data (Fig. 3-1C; see data analysis in Methods), a Friedman ANOVA showed a significant effect of the ISI ($P < 0.001$). TA MEPs were significantly depressed at intervals from 32.5 to 37.5 ms (by -55 ± 6% at peak inhibition) and facilitated at 45 and 50 ms (by +123 ± 42% at peak facilitation) (Wilcoxon test; $P < 0.05$). All subjects showed a > 20% reduction and a > 20% increase in the MEP at one of the inhibitory and facilitatory ISIs, respectively. MEPs in AH and SOL muscles were also modulated in a parallel manner, showing strong significant inhibition and non-significant facilitation (data not shown). To characterise how TN afferents excite the corticospinal tract, we evaluated its effects on the TMS recruitment curve (Fig. 3-1D). Afferent stimulation at an ISI near 50 ms significantly increased MEP responses at TMS intensities of RMT and 110% RMT ($P < 0.05$). Over these intensities, the MEP was increased by 0.10 ± 0.02 mV on average.

3.3.2 Experiment 2: Site of afferent-induced MEP inhibition and facilitation

To determine whether the MEP inhibition and facilitation shown in Fig. 3-1 occurred at the level of the cortex and/or the spinal cord, we examined the effect of TN stimuli on the excitability of subcortical networks using both H-reflexes and CMEPs. Because H-reflexes and CMEPs are more readily evoked in the pre-contracted TA muscle, the effect of afferent stimulation on TA MEPs was repeated during a small voluntary contraction of 10% MVC. The modulation in the MEP was similar in the contracted muscle (Fig. 3-2A; see below). When examining the excitability of spinal interneurons and motoneurons to the TA muscle using the H-reflex, TN conditioning resulted in non-significant changes to the size of the TA H-reflex (Fig. 3-2B; Friedman ANOVA; $P = 0.64$). At most ISIs, the TN conditioning input had a depressive effect on the H-reflex, though 5 out of 10 subjects revealed a small increase at conditioning-test intervals 5-15 ms longer than the ISIs that first produced MEP facilitation. The small reflex facilitation is shown as arrows for 3 example subjects in Fig. 3-2C-E. The size of the average test H-reflex was 0.44 ± 0.05 mV and was smaller than the average test MEP of 0.62 ± 0.05 mV ($P < 0.05$).

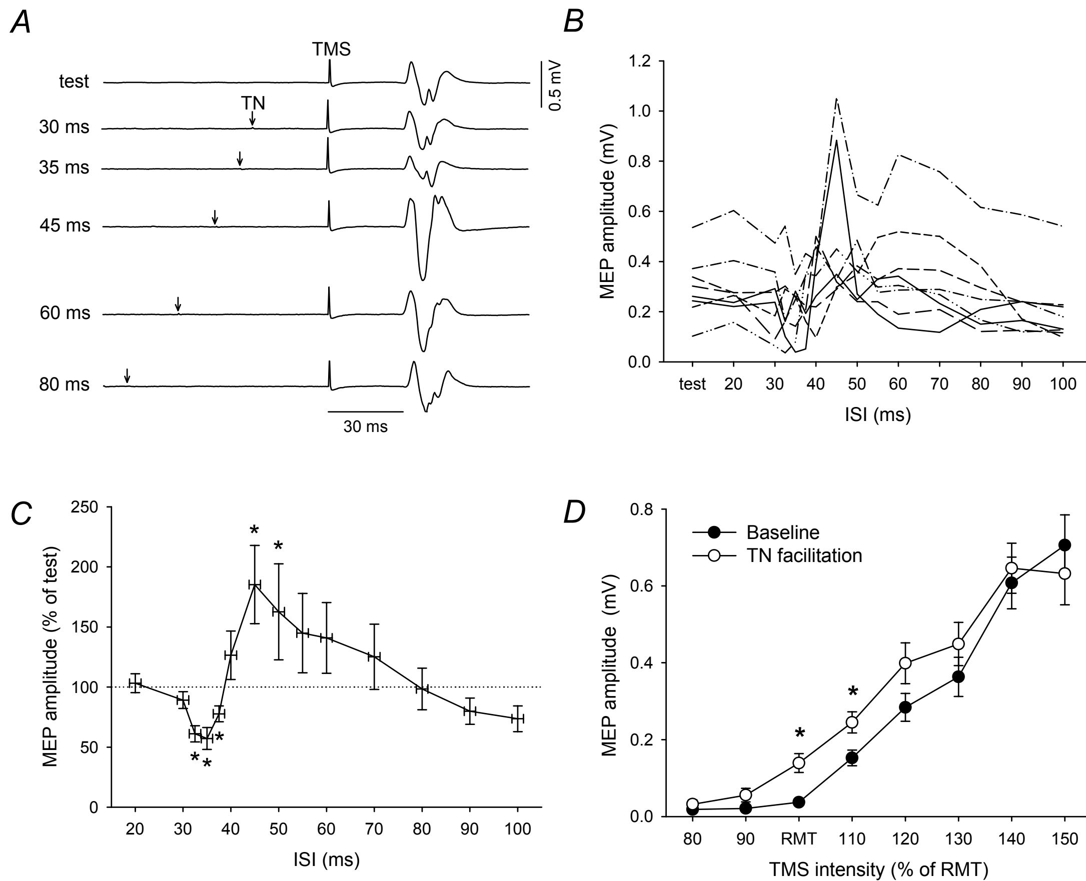

Figure 3-1. Effect of TN stimulation on MEP responses in TA

A, Raw sweeps showing average TA MEPs in one subject when preceded by a TN stimulus. The ISI influenced the size of the MEP by producing inhibition at 35 ms, facilitation at 45 and 60 ms and had no sizeable effect at 30 and 80 ms. Individual subject data at ISIs from 20-100 ms are shown in (*B*). *C*, To account for each subject's conduction delay, the curves were shifted in time using the latency of the MEP in the AH muscle (see Methods). *D*, Effect of TN stimulation on the TMS recruitment curve. The average RMT was 60 ± 3% MSO and the TN stimulus was delivered at a fixed interval that produced MEP facilitation in each subject. Data are from 8 subjects. MEPs at 150% RMT in (*D*) could only be elicited in 6 subjects. Asterisks indicate significant differences compared to baseline without TN stimulation (*P < 0.05).

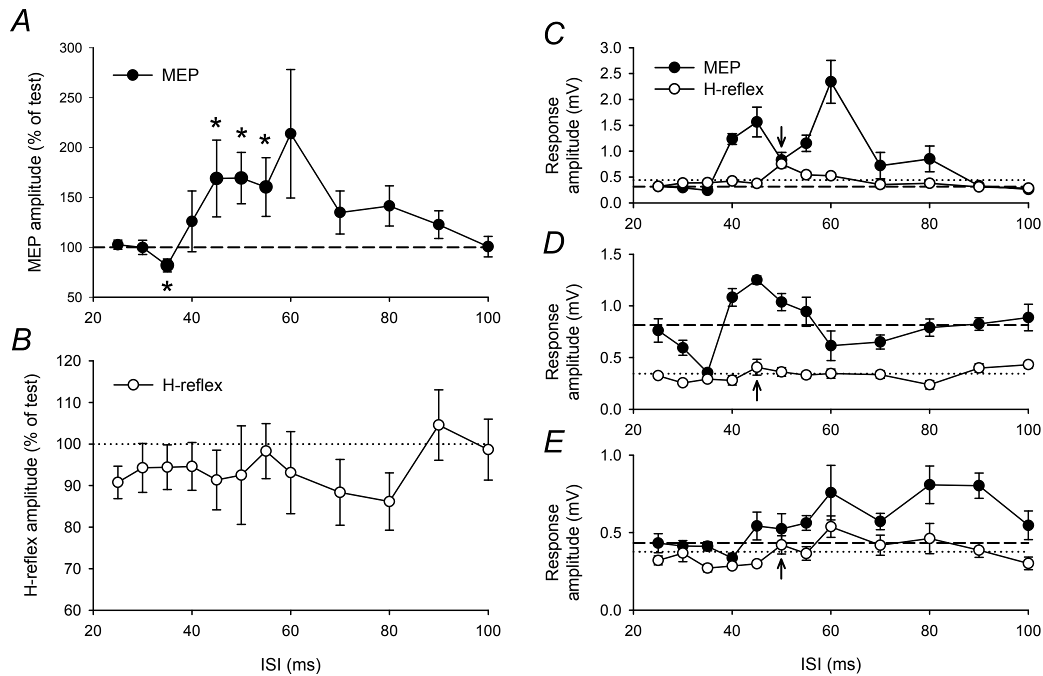

Figure 3-2. Effect of TN stimulation on MEPs and H-reflexes

Size of the average peak-to-peak MEP (A) and H-reflex (B) in TA when conditioned by a TN stimulus during background contraction. Data in (A,B) are from 10 subjects. C-E, Individual subject data showing MEP (●) and H-reflex responses (○) and the size of the unconditioned test MEP (black dashed line) and test H-reflex (black dotted line). The arrows show the increase in the H-reflex that occurred after the initial increase in the MEP response. Asterisks indicate significant differences from the test MEP (*$P < 0.05$).

As stated above, the effect of TN stimulation on MEPs was similar in the contracted muscle (Fig. 3-2A), but with the afferent input producing facilitation over a broader window of ISIs during contraction compared to rest. TA MEPs were significantly affected by the conditioning stimulus (Friedman ANOVA; $P < 0.005$) and were significantly facilitated at ISIs of 45 to 55 ms (by +145 ± 60% at peak facilitation) (Wilcoxon test; $P < 0.05$). This corresponded to intervals that were a few to several milliseconds longer than the average SEP latency (41.5 ± 0.9 ms, N = 9), indicating that MEP facilitation occurred after the arrival of the afferent input at the somatosensory cortex. Prior to the onset of the facilitation, MEPs were depressed at the 35 ms ISI (by -31 ± 5% at peak inhibition) ($P < 0.05$). Likewise, the size of the MEP in the homonymous AH was modified over a large range of intervals (Friedman ANOVA; $P < 0.005$), reflecting significant inhibition at 35 ms and a broad facilitatory window from 45 to 90 ms (data not shown). During dorsiflexion, MEPs in the antagonist SOL muscle were also significantly modulated (Friedman ANOVA; $P < 0.01$) and showed significant facilitation at ISIs of 45 and 55 ms (data not shown). The congruent results described in the three muscles were not likely due to cross-talk since the MEP waveforms and latencies differed in each muscle.

TN conditioning of responses evoked from stimulating the corticospinal tract at the cervicomedullary junction (i.e. CMEPs) was also measured and compared to TN conditioning of MEPs elicited over the motor cortex (Fig. 3-3). As shown for the single subject in Figure 3-3A, MEP (top traces) and CMEP (bottom traces) responses were equally suppressed at a short ISI (near 30 ms, see legend for details), suggesting that suppression of MEP responses by TN stimulation at this ISI occurred at a subcortical site. In contrast, TN stimulation near 55 ms prior to stimulating the brainstem failed to facilitate the CMEP, even though the cortical MEP was facilitated by +115%, suggesting that MEP facilitation at this longer interval occurred at a cortical site. In the group data (Fig. 3-3B), a two-way repeated measures ANOVA showed a significant interaction effect between 'stimulation site' (cortex and brainstem) × 'type of ISI' (inhibitory and facilitatory ISIs) ($F(1,4) = 17.65$, $P < 0.05$). At the facilitatory ISI, the conditioned MEP was significantly larger than the conditioned CMEP ($P < 0.05$), the latter in fact being suppressed compared to the unconditioned CMEP. At the inhibitory ISI, there was no significant difference in the size of the conditioned MEP compared to the conditioned CMEP ($P = 0.7$), and both responses were significantly reduced compared to 100% ($P < 0.05$). Average test MEP and CMEP amplitudes were 0.66 ± 0.07 mV and 0.58 ± 0.15 mV, respectively, and were not significantly different.

3.3.3 Experiment 3: Intracortical mechanisms of afferent-induced MEP facilitation

Because MEP facilitation at the longer ISIs is likely cortical in origin (re: Exp. 2), we evaluated the influence of the afferent inputs on the excitability of intracortical circuits. First, the amount of SICI and ICF was examined when the TN stimulus was timed to arrive at the motor cortex at the same time as the test pulse. When the size of the test stimulus was matched to the unconditioned test MEP amplitude (TS-ADJ, see legend for details), TN stimulation reduced SICI and increased ICF (Fig. 3-4A). Normalizing the data by the test MEP size (Fig. 3-4B), there was a significant

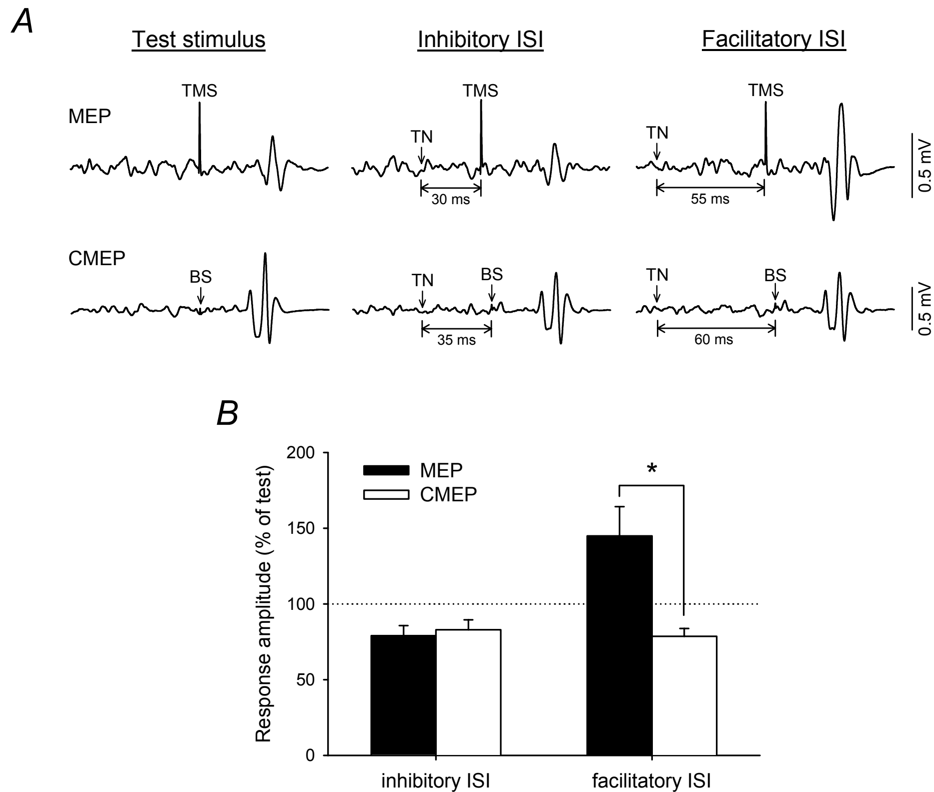

Figure 3-3. Effect of TN stimulation on MEPs and CMEPs

A, Average TA MEPs (top traces) and CMEPs (bottom traces) in a single subject when conditioned by a preceding TN stimulus. MEPs are depressed at the inhibitory 30 ms ISI (middle top) and potentiated at the facilitatory 55 ms ISI (right top), whereas the CMEPs elicited by an appropriately timed brainstem stimulus (BS) were depressed (bottom traces). B, Group data from 5 subjects showing the effect of TN stimulation on MEPs (black bars) and CMEPs (white bars) in the contracted TA muscle. The inhibitory and facilitatory ISIs were near 35 and 50 ms, respectively and were different for each subject. MEPs and CMEPs were both significantly reduced at the inhibitory ISI. Asterisks indicate significant differences in the size of the MEP compared to the CMEP (*$P < 0.05$).

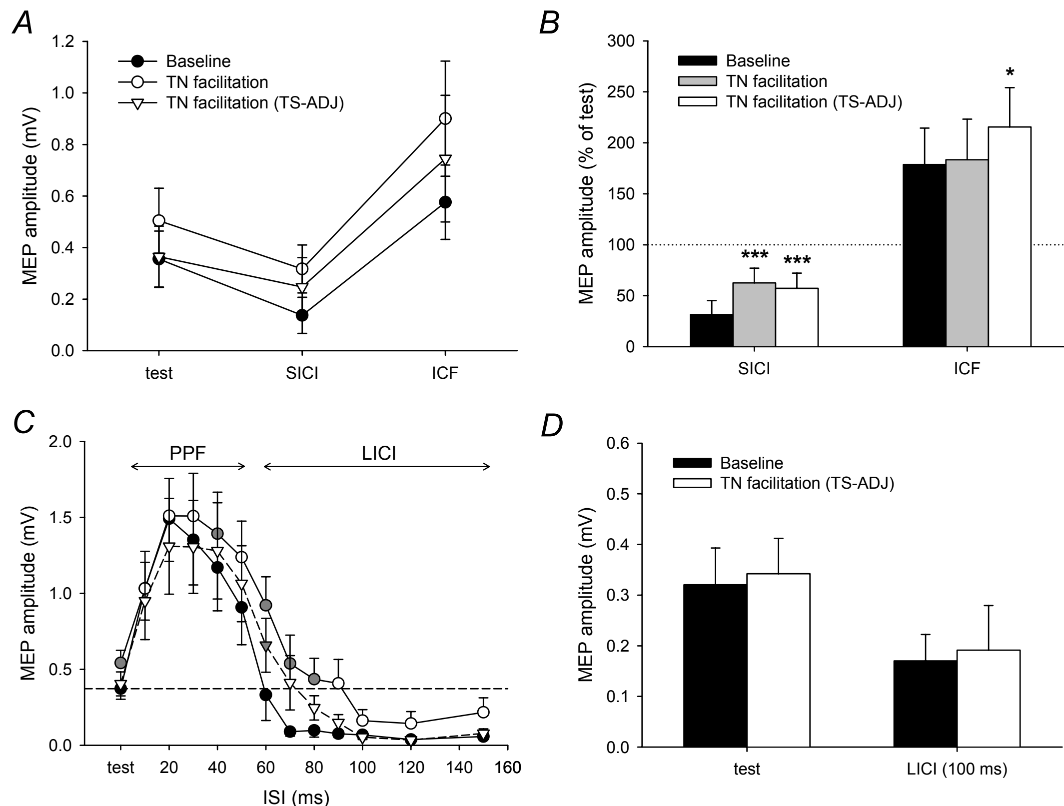

Figure 3-4. Effect of TN stimulation on intracortical circuits

Influence of TN stimulation at facilitatory ISI on SICI and ICF (*A, B*) and the time-course of PPF and LICI (*C, D*) in the resting TA muscle. SICI and ICF were evaluated at ISIs of 3 and 10 ms, respectively and show the actual MEP amplitudes in (A) and expressed as a percentage of the test MEP in (B). MEPs in TA were collected at baseline without TN stimulation (●; black bars), with TN facilitation (○; grey bars) and with TN facilitation when the test stimulus was adjusted (TS-ADJ) to match the baseline test MEP (▽; white bars). Only the intensity of the test stimulus was adjusted in the matched condition (compare the test MEP of ● and ▽ in *A*, *C*). The TN input was given at a fixed interval of 40-55 ms before the test TMS pulse. The broken line in (*C*) represents the size of the average test MEP. *D*, LICI at an ISI of 100 ms was evaluated using a weaker conditioning stimulus which produced 40-60% inhibition. Data are from 5 subjects in (A,B) and 8 subjects in (C, D). Asterisks or grey symbols indicate significant differences compared to baseline without TN stimulation (*$P < 0.05$, ***$P < 0.005$).

interaction effect between 'condition' (without and with TN stimulation) and 'ISI' (two-way ANOVA: $F(1,4) = 26.81$, $P = 0.01$). The afferent input reduced SICI from 69 to 43% ($P < 0.05$) and increased ICF from 179 to 215% ($P < 0.05$).

The effect of TN stimulation on PPF and LICI was also examined in the resting TA muscle. In unconditioned trials (Fig. 3-4C, filled circles), paired-pulses of suprathreshold TMS produced a sequence of strong MEP facilitation (> 200%) at PPF intervals of 10-40 ms followed by a suppression of test MEP responses at LICI intervals of 60-150 ms, as shown previously (Wassermann et al. 1996; Nakamura et al. 1997). When the second cortical stimulus (test stimulus) was conditioned by an appropriately timed TN stimulation, there was enhancement of the MEP response at select intervals from 40 to 80 ms (Fig. 3-4C, grey circles). When the size of the test MEP was matched in the conditioned trials (TS-ADJ, see legend), a two-way repeated measures ANOVA showed a significant interaction effect 'condition' × 'ISI' ($F(12,84) = 2.96$, $P < 0.005$) showing that neuronal populations mediating PPF or LICI were affected by the afferent input (Fig. 3-4C, triangles). Post-hoc paired t-tests showed significant MEP facilitation at the 60 ms ISI (grey triangles) and half of the subjects still showed a > 20% increase at 70 and 80 ms. To test LICI at conditioning intensities that did not fully depress the MEP, the conditioning stimulus at 100 ms was decreased so that the MEP was depressed by only 40 to 60%. Using equal-sized test MEPs (Fig. 3-4D), there was no effect on LICI following the afferent stimulus ($P = 0.6$).

To estimate the effect of afferent excitation on the recruitment of cortical inputs producing the descending corticospinal volleys, or I-waves from TMS, we examined the effect of TN stimulation on MEP facilitation at the I-wave periodicity. TN stimulation facilitated MEP responses at ISIs of 1.5, 2.7 and 4.9 ms, likely corresponding to the peak I-wave activity (Fig. 3-5A). Facilitation persisted when the size of the test MEP was matched to levels without afferent stimulation (TS-ADJ, see legend). When the data was normalized to the test MEP (Fig. 3-5B, white bars), a two-way repeated measures ANOVA showed a significant 'condition' effect ($F(1,5) = 15.06$, $P < 0.05$), revealing that the TN stimulus modified the amount of MEP facilitation at the I-wave periodicity. Compared to the pre-TN condition (baseline), facilitation using the adjusted test MEP size was significantly enhanced at ISIs of 1.5, 2.7, 4.9 ms, (all $P < 0.05$) with 5 out of 6 subjects showing an average > 20% increase. Intervals between the three presumed I-wave peaks (i.e. 2.1 and 3.7 ms) were non-significantly enhanced (all $P > 0.09$).

3.3.4 Experiment 4: Effect of PTN stimulation on MEPs in SOL

The profile of inhibition and facilitation of SOL MEPs following stimulation of the PTN at the knee was similar to that measured in the TA muscle in response to TN stimulation at the ankle. Fig. 3-6A shows the effect of the PTN stimulus on SOL MEPs in all the subjects. In the averaged group data (Fig. 3-6*Ba*), there was significant effect of the peripheral input on the SOL MEPs (two-way ANOVA: $F(11,77) = 5.61$, $p < 0.001$) and MEPs were significantly increased at ISIs of 45 to 60 ms ($P < 0.05$). In Figure 3-6*Bb*, a larger number of conditioning-test intervals

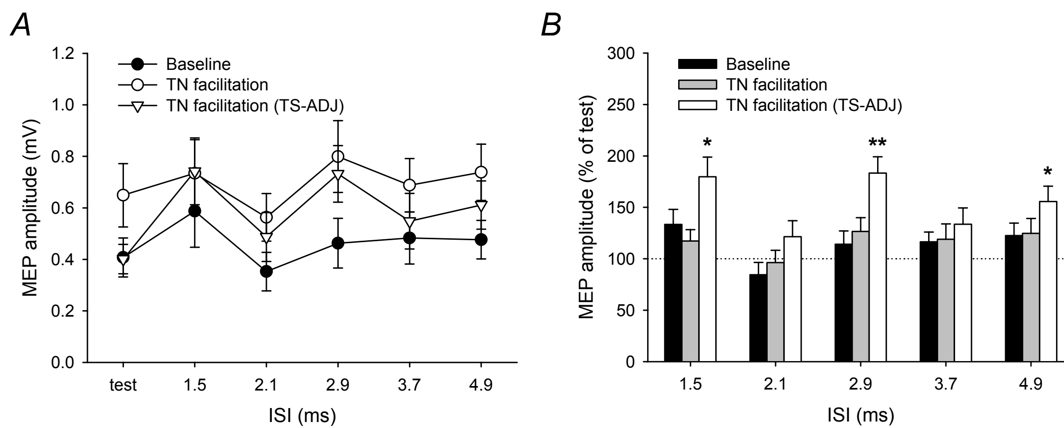

Figure 3-5. Effect of TN stimulation on I-wave facilitation

Effect of TN inputs on MEP facilitation at the I-wave periodicity in the TA muscle. Experimental details are described in the caption of Fig. 3-4. Data are from 6 subjects. Asterisks indicate significant differences compared to baseline without TN stimulation (*P < 0.05, **P < 0.01).

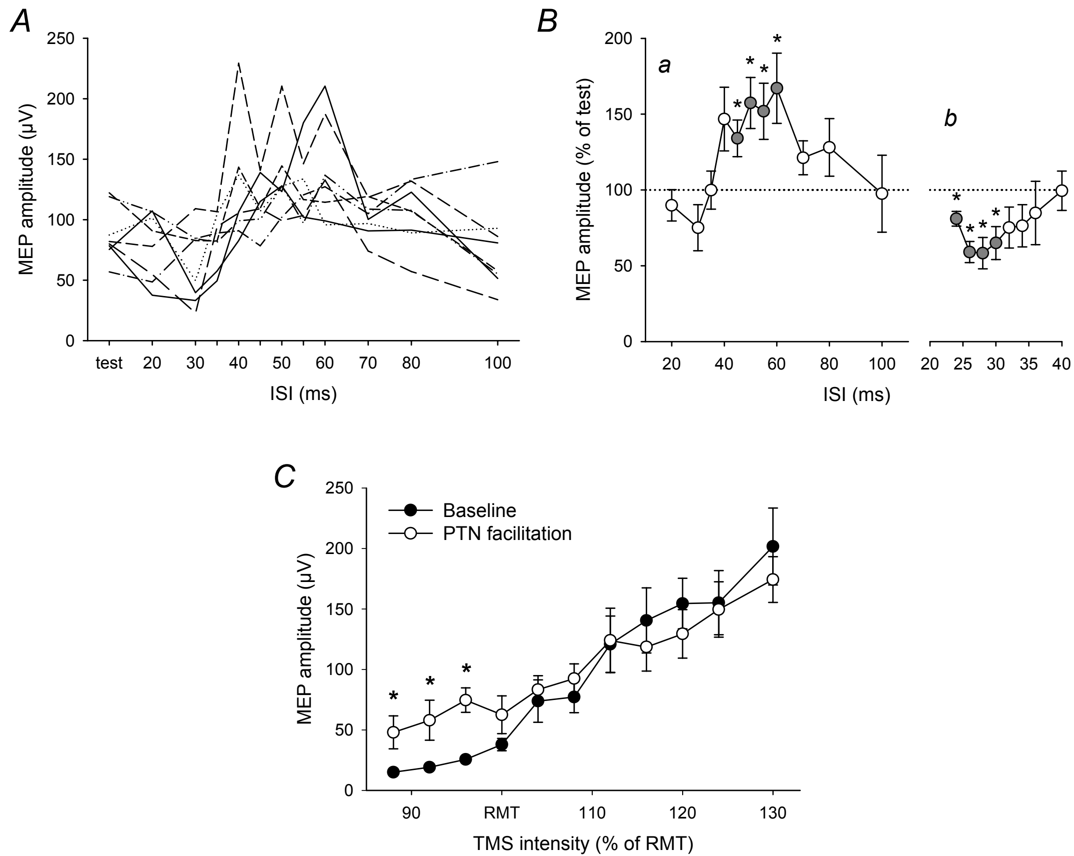

Figure 3-6. Effect of PTN stimulation on MEP responses in SOL

A, Individual subject data showing the effect of PTN stimulation on SOL MEPs. The non-adjusted group average is shown in (*Ba*). Five subjects showing decreases at an ISI of 30 ms were further tested at a variety of ISIs between 24 to 40 ms (*Bb*). *C*, Graph shows the TMS recruitment curve in SOL recorded at baseline without TN stimulation (●) and with TN stimulation (○). The TN stimulus was given at a fixed conditioning-test interval that produced MEP facilitation in each subject, based on (*A*). The TMS intensity is expressed as a percentage of the RMT in SOL, which was 67 ± 3% MSO on average. Data in (*A,C*) are from 8 subjects. Asterisks indicate significant differences compared to the test MEP (*B*) and between the two conditions (*C*) (*P < 0.05).

between 24 and 40 ms were examined and there was a significant effect of the 'ISI' over these inhibitory intervals (one-way ANOVA: $F(9,36) = 3.65$, $P < 0.005$) with post hoc t-tests showing significant MEP depression at intervals of 24 to 30 ms ($P < 0.05$). The facilitatory PTN input also altered the recruitment curve in the SOL muscle (Fig. 3-6C; two-way ANOVA: $F(10,70) = 2.66$, $P < 0.01$). Conditioning the SOL MEP at low TMS intensities, ranging from 88% to 96% RMT, significantly increased MEP amplitudes by 41 ± 13 μV on average ($P < 0.05$). The facilitatory effect observed for MEPs ranging from 50-100 μV was however weaker than what was shown in Figure 3-6*Ba*, potentially due to the inherent variability of the resting MEP. In contrast, at high TMS intensities (≥ 116% RMT), MEPs in the SOL muscle tended to be slightly, but not significantly, depressed by the peripheral stimulus.

3.4 DISCUSSION

In agreement with previous studies, MEP responses in lower limb muscles were both suppressed and increased by a preceding sensory nerve stimulus depending on the conditioning-test interval that was used (Deletis et al. 1992; Kasai et al. 1992; Nielsen et al. 1997). Similar time-dependent changes were observed in muscles with heteronymous (TN→TA, SOL) and homonymous (TN→AH; PTN→SOL) nerve activation. A novel finding was that the facilitation of corticospinal tract connections was only enhanced when activated at the cortex and not at the pyramidal decussation, supporting the evidence that MEP facilitation was cortical in origin. We further show that the MEP facilitation was associated with afferent-induced depression of intracortical inhibitory circuits (SICI) and facilitation of putative intracortical excitatory circuits (ICF). The present data further suggest that sensory afferents from the leg have access to excitatory cortical networks that synapse close to pyramidal tract neurons as evidenced from increases in paired pulse facilitation at early, as well as, middle and late cycles of I-wave periodicity. Finally, responses elicited from stimulation to the motor cortex and brainstem by conditioning afferent inputs at short ISIs (near 35 ms) were depressed to a similar degree suggesting that afferent-induced depression of MEPs (i.e. SAI in leg) mainly occurs via a spinal mechanism. We discuss below that afferent inputs from the leg have a mainly excitatory and diffuse action on the leg area of the primary motor cortex.

3.4.1 Site of sensory afferent induced facilitation of the MEP

The evidence from direct corticospinal tract stimulation suggests that afferent-induced MEP facilitation at ISIs between 45 to 55 ms occurs through cortical mechanisms since responses were only facilitated when elicited over the motor cortex and not at the pyramidal decussation in the brainstem, which is thought to recruit similar descending corticospinal axons (Taylor et al. 2002). In fact, TN stimulation at an ISI near 50 ms markedly depressed the CMEP (Fig. 3-3B), and thus, the magnitude of TN facilitation of descending volleys evoked from TMS over the motor cortex was probably underestimated because of the simultaneously occurring spinal inhibition. Similar decreases in evoked responses from transcranial electric stimulation have been reported when the sural nerve is stimulated 50 ms earlier (Nielsen et al. 1997). Further evidence supporting a lack of MEP facilitation by spinal

networks at ISIs near 50 ms was provided by the lack of TA H-reflex facilitation by TN stimulation (Fig. 3-2B), assuming that there was some degree of overlap between H-reflex and corticospinal tract inputs onto TA motoneurons (Morita et al. 1999; Petersen et al. 2002). It is interesting that in several subjects there was an abrupt, but small, recovery in the size of the H-reflex at conditioning intervals that were 5-15 ms longer than the intervals that first produced the MEP facilitation. This increase likely involves contributions from the transcortical reflex loop (Nielsen et al. 1997) and requires several extra milliseconds for the afferent input to depolarize and activate pyramidal tract neurons. Finally, the latency of the MEP facilitation is also consistent with a cortical mechanism because the shortest conditioning-test interval that facilitated each subject's MEP was on average 4.1 ± 1.4 ms longer than the latency of the respective somatosensory evoked potential from TN stimulation (41.5 ms). This suggests the presence of an afferent pathway that passes through the somatosensory cortex to the motor cortex and corroborates with a previously reported central processing delay of 4-10 ms (Nielsen et al. 1997; Petersen et al. 1998; Mrachacz-Kersting et al. 2007).

3.4.2 Site of sensory afferent induced inhibition of the MEP

With both TN and PTN stimulation, there was period of MEP inhibition that preceded the facilitatory window analogous to SAI in the hand (Tokimura et al. 2000). However, unlike SAI in the hand, the inhibition of MEPs in leg muscles was likely not mediated by afferent inhibition of cortical networks given that TN stimulation equally depressed brainstem and cortically evoked responses in the TA muscle. SAI in the hand at an ISI of 20 ms is in line with a fast thalamo-cortical pathway (Tokimura et al. 2000; Kessler et al. 2005). If cortical in origin, inhibition of leg MEPs with TN stimulation occurring at ISIs near 35 ms would also have to go through a fast thalamo-cortical route because the latency for afferent inputs to the sensory cortex was 40 ms or more (see above). A spinal post-synaptic mechanism is more likely given recent findings where TMS and PTN inputs inhibit the SOL H-reflex to the same degree when delivered at an ISI of 30-40 ms (Poon et al. 2008). Because the amount of inhibition was the same for the two different conditioning pre-synaptic inputs, the common depression is likely occurring at a post-synaptic site. For instance, in conditioning of MEP responses, the motoneurons that are first activated by the peripheral stimulus are likely in a state of refractoriness when the descending corticospinal inputs arrive 30-40 ms later. Although the brainstem stimulation and H-reflex data provide some indication of a spinal mechanism, it is nonetheless possible that the short-latency inhibition might not be as prominent in the contracted muscle given that suppression of the TA MEP was less pronounced in the active muscle (-31 ± 5%) compared to rest (-55 ± 6%). Thus, it would be worthwhile to record from epidural electrodes over the spinal cord to examine whether any of the descending corticospinal volleys are in fact depressed by the leg afferents at these short ISIs (Tokimura et al. 2000).

3.4.3 *Effect of afferent input on cortical circuits*

The activation of cortical networks by afferent inputs from the leg was also demonstrated by the modification of SICI and ICF by TN stimulation. At an ISI that produced MEP facilitation in the TA muscle, SICI was decreased and ICF was increased, suggesting that increases in MEP responses by peripheral nerve stimulation were mediated by a decrease in the activation of inhibitory cortical networks and an increase in the activation of excitatory cortical networks. However, there has been some question as to whether ICF at 10 ms (used in this study) is in fact mediated by cortical networks given that descending volleys evoked from ICF at 10 to 15 ms are not altered despite increases in the size of the MEP (Di Lazzaro et al. 2006). Because the brainstem and H-reflex data show that the site of afferent-induced MEP facilitation in the leg is mainly cortical in origin, the fact that ICF was increased by afferent inputs suggest that the neurons mediating ICF at 10 ms are indeed cortical in origin.

When probing the excitability of neuronal populations mediating PPF and LICI, MEPs of large amplitude (≈ 1.5 mV) were produced at ISIs of 10 to 40 ms followed by inhibition starting at 60 ms. When comparing MEPs at intervals exhibiting maximal PPF, the afferent input produce little enhancement in the MEP response (except at 40 ms). This is potentially due to a ceiling effect as the recruitment curve data indicates that TA MEPs > 0.5 mV on average were not enhanced by the sensory input. At longer ISIs, the afferent excitation increased the size of the MEPs normally susceptible to long-interval inhibition. The observed MEP facilitation at the 60 ms ISI, evaluated using a matched test MEP, could have arisen either by a direct disinhibition of LICI networks or by a prolongation of the PPF window by the afferent input. To further examine this question, we tested LICI at a longer 100 ms ISI when the MEP was only partially depressed by the conditioning TMS pulse. In such a case, LICI was unchanged by the peripheral stimulus. Hence, it seems likely that continued activation of PPF neurons triggered from the first TMS pulse could have been potentiated by afferent inputs arriving more than 80 ms later to facilitate the second MEP. This result may also elucidate the underlying mechanism behind our previous findings where repetitively pairing cortical and peripheral nerve inputs in an intervention of paired-associative stimulation (PAS) facilitated MEPs in lower leg muscles when afferent inputs arrived at the motor cortex up to 90 ms after the TMS pulse (Roy et al. 2007).

3.4.4 *Effect of afferent input on I-wave facilitation*

In the upper and lower limb, the effect of afferent excitation on I-wave facilitation has not been reported previously. In the present study, afferent inputs potentiated the amount of MEP facilitation at early (1.5 ms), middle (2.9 ms) and late (4.9 ms) intervals of I-wave periodicity. On the basis that MEP facilitation reflects the periodicity of indirect waves (i.e. I1, I2, I3 waves) initiated by the first TMS pulse (Di Lazzaro et al. 1999; Hanajima et al. 2002), the facilitation of the MEP at an ISI of 1.5 ms suggests that afferent inputs synapse onto interneurons that can potentiate the earlier descending volleys. Excitatory inputs from hand afferents likely also activate interneurons that enhance the early I-wave cycle but this remains to be tested. So far

only a reduction of I-wave facilitation at 1.5 ms has been reported following stimulation of the digit, which further depresses the MEP (Zittel et al. 2007). Similar to hand afferents, leg afferents also access more distant cortical interneurons as evidenced by MEP facilitation at 2.9 and 4.9 ms intervals and by the modification of SICI, which preferentially reduces the size of later I-wave inputs onto corticospinal neurons (Nakamura et al. 1997; Di Lazzaro et al. 1998; Hanajima et al. 1998).

3.4.5 Physiological considerations

We have shown that cortical excitability is enhanced using afferent stimulation from the lower leg in a manner consistent with the transcortical loop of the long-latency reflex. Interestingly, the afferent facilitation of the leg motor cortex was predominantly non-specific and diffuse, occurring from stimulation of mixed nerves supplying both homotopic (TN→AH; PTN→SOL) and distant heterotopic (TN→TA and SOL) muscles. This is in contrast to the upper limb where the facilitation of the motor cortex by peripheral nerve stimulation, even of mixed nerves, is more muscle specific (Classen et al. 2000; Aimonetti and Nielsen 2001; Tamburin et al. 2001). Further studies are required to determine if more specific activation of leg muscle afferents by vibration produces more focal, or even inhibitory, activation of cortical networks as has been shown in the wrist and hand (Rosenkranz et al. 2003; Rosenkranz and Rothwell 2003). Moreover, afferent activation of the leg motor cortex is predominantly excitatory, potentially mediated by both disinhibition and potentiation of excitatory intracortical circuits. In trauma such as spinal cord injury, much of this facilitation is likely abolished due to damage of ascending connections (Hayes et al. 1992). As afferent sensory input from the leg is important for lower limb motor function such as standing and walking (Thoumie and Do 1996), motor dysfunction after spinal cord injury may result not only from disruption of descending efferents but also from a lack of afferent-induced facilitation of cortical motor networks. Thus, regulating the amount of sensorimotor integration may be important in enhancing motor rehabilitation following injury to the central nervous system. Based on the present results, SICI and ICF may be involved in shaping these changes when muscle afferent input is provided from the lower leg.

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CHAPTER 4: Afferent regulation of motor cortex excitability to the ankle flexor is reduced with incomplete spinal cord injury

4.1 INTRODUCTION

Cortical representations are plastic and are continuously modified by experience (Buonomano and Merzenich 1998). In general, sensorimotor areas are allocated based on the proportional *use* or *disuse* of a pathway in line with the mechanism of use-dependent plasticity studied in the human motor cortex (Butefisch et al. 2000). A steady stream of sensory input maintains the integrity of cortical networks; while in contrast, alterations in afferent activation promote sensorimotor reorganization (Brasil-Neto et al. 1992; Ziemann et al. 1998; for review see Chen et al. 2002). After an incomplete spinal cord injury (SCI), damage to the ascending and/or descending pathways induces widespread modifications to the sensorimotor system (Levy et al. 1990; Topka et al. 1991; Curt et al. 2002; although see Brouwer and Hopkins-Rosseel 1997). Imaging experiments have shown that the activity in the primary motor cortex is reduced shortly after SCI and progressively reappears over the course of motor recovery (Jurkiewicz et al. 2007). With functional recovery of walking, SCI subjects show strengthening of corticospinal projections as seen by an increase in the motor evoked potential (MEP) elicited using transcranial magnetic stimulation (TMS) (Thomas and Gorassini 2005; Wirth et al. 2008; Everaert et al. in press).

In agreement with other studies, we have previously shown than MEPs in leg muscles can be facilitated in uninjured individuals by prior stimulation of afferent pathways from homonymous and/or neighbouring muscles (Deletis et al. 1992; Nielsen et al. 1997; Petersen et al. 1998; Roy and Gorassini 2008). Moreover, these findings have been recently confirmed in the upper extremity following median nerve stimulation; an interaction that is thought to involve muscle spindle afferents (Devanne et al. 2009). In both upper and lower limbs, sensory-induced MEP facilitation is partly mediated by decreases in the activation of inhibitory intracortical pathways (Rosenkranz & Rothwell, 2003) and facilitation of excitatory intracortical pathways (Aimonetti and Nielsen 2001; Rosenkranz et al. 2003; Roy and Gorassini 2008; Devanne et al. 2009). Following a complete and a nearly complete spinal cord lesion, afferent projections in the dorsal column are impaired. The disruption to the ascending pathways can abolish the sensory-induced MEP facilitation produced in ankle flexors (Hayes et al. 1992). Although it is likely that the potentiation of cortical circuits by peripheral nerve stimulation is graded according to the severity of the injury, the interaction of afferent inputs on corticospinal circuits has yet to be investigated in SCI subjects having some spared ascending pathways.

Repetitive electrical stimulation of the common peroneal nerve (CPN) in uninjured individuals produces facilitation of the MEP in ankle flexors, which can last for one hour or more (Khaslavskaia et al. 2002; Knash et al. 2003). In addition, a recent study

has shown that CPN stimulation delivered over the course of several months, via a neuroprosthesis for foot drop, improves walking function and increases corticospinal tract function in SCI and multiple sclerosis subjects (Stein et al. in press; Everaert et al. in press). By pairing sensory afferent excitation with a cortical stimulus, it has been possible to accelerate the rate of MEP potentiation in an intervention called paired associative stimulation (PAS; Stefan et al. 2000). In the lower leg, pairing afferent inputs from the CPN with a TMS pulse increases MEPs in ankle flexors when the afferent input is timed to arrive at the motor cortex *with* or *shortly after* the cortical stimulus (Mrachacz-Kersting et al. 2007; Roy et al. 2007). In the upper limb, this MEP facilitation involves the activation of the N-methyl-D-aspartate (NMDA) receptor (Stefan et al. 2002), reminiscent of long-term potentiation (LTP) in animal experiments (for review see Dan and Poo 2006), and may give insight into the LTP-like properties of the human motor cortex (Rosenkranz et al. 2007). After stroke, PAS can potentiate MEPs in wrist muscles (Castel-Lacanal et al. 2007, 2009) and there is preliminary evidence that repeated exposure to PAS (daily sessions for 4 weeks) increases corticospinal connections to leg muscles (Uy et al. 2003). Presently, PAS-induced changes have not been reported in individuals with an incomplete spinal cord lesion.

In this study, we investigated whether afferent inputs can modulate motor cortex excitability in SCI subjects by pairing peripheral nerve stimulation with TMS. By delivering repeated pairs of peripheral and central stimulation, we also examined whether an intervention of PAS can induced short-term plasticity in incomplete SCI subjects. Here, we provide evidence that 1) afferent input from the CPN can reach the motor cortex to potentiate the MEP and 2) PAS can transiently increase in the resting MEP response after SCI. Parts of the data from the uninjured controls have been presented elsewhere (Roy and Gorassini et al. 2008).

4.2 METHODS

4.2.1 Subjects

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 subjects. Most of the subjects with an incomplete SCI were recruited to participate in locomotor rehabilitation and the experiments were done at various stages during their training. Experiments involving short-term conditioning of the MEP by sensory inputs were done on a rest day between training sessions and all PAS experiments were done when the subjects were no longer training. Experiments done in the same the subject were conducted at least 5 days apart.

Our sample comprised of 22 subjects with an incomplete SCI (6 female) aged 20 to 69 (45.3 ± 13.2; mean ± SD; Table 4-1) and 13 uninjured control subjects (5 females) aged 20 to 68 (33.9 ± 13.8; mean ± SD). All participants who had sustained damage to the spinal cord were classified as either ASIA C or D (Table 4-1). Data from muscles on the right and left leg in a single SCI subject were considered to be independent because of the asymmetry in the lesion location.

Code/ Sex	Age	Years Post Injury	Cause of Injury	Injury Level	ASIA Score	Medication	Side(s) Tested
1M	56.9	34.2	Trauma	C5-6	D	oxybutynin, tamsulosin	left
2M	44.7	4.9	Trauma	C3-5	C	baclofen, gabapentin, oxybutynin	right
3M	27.3	1.7	Trauma	T6-10	C	baclofen	right, left
4M	47.1	23.0	Trauma	C5, C6	C	none	right, left
5M	45.0	2.4	Trauma	L2, L3	D	gabapentin, hydromorphine	right, left
6F	44.2	1.0	Surgical Bleed	T2-T4	C	baclofen,	right, left
7F	48.1	1.3	Trauma	C6	C	baclofen, gabapentin, tolterodine	right, left
8M	41.7	1.1	Trauma	C3-4	C	baclofen, gabapentin, oxybutynin, oxycodone	right, left
9M	20.9	1.1	Trauma	C7	C	baclofen, oxybutynin	right
10F	69.4	2.5	Surgery	T4, L5	D	oxybutynin, oxycodone	right, left
11M	63.3	20.0	Trauma	C4-5	D	none	right, left
12M	33.6	1.1	Trauma	C4-5	C	baclofen, pregabalin	right, left
13M	60.7	2.4	Trauma	C5	D	baclofen, pregabalin	right, left
14M	44.1	17.6	Trauma	T12	D	none	right, left
15M	25.0	1.0	Trauma	T4,11,12	C	gabapentin	right, left
16F	42.7	1.9	Trauma	C6,7	C	none	right
17M	56.8	1.2	Trauma	C6/7, T8	C	baclofen, dantrium, oxybutynin	right
18M	52.3	2.6	Trauma	T5/6	C	tamsulosin	right
19M	41.6	1.6	Trauma	C3, C4	D	baclofen, pregabalin	right
20F	58.5	4.9	Idiopathic transverse myelitis	T2-12	D	baclofen, gabapentin	right
21M	23.4	1.0	Trauma	T6-9	C	none	right, left
22F	50.1	1.8	Tumor removal	T6	D	baclofen, oxybutynin	right, left

Table 4-1. Details of subjects with incomplete SCI

The age of the subject and the number of years after the subject sustained a spinal cord injury measured at the time of the experiment are shown. *Medication*: baclofen and dantrium are antispastistic agents; gabapentine, hydromorphine, oxycodone and pregabalin help alleviate pain; oxybutynin and tolterodine are used in the treatment of overactive bladder symptoms; tamsulosin treats the symptoms of an enlarged prostate.

4.2.2 Recordings and stimulation

Surface electromyography (EMG) was collected from the tibialis anterior (TA), soleus and abductor hallucis muscles using pairs of Ag-AgCl electrodes (Kendall, Chicopee, MA). All measurements were taken from the TA muscle. The signals were amplified (500 to 2k gain) and filtered (10-1000 Hz band-pass) (Octopus, Bortec Technologies, Calgary, Canada) and were digitized at a rate of 5 kHz using Axoscope hardware and software (Digidata 1200 Series, Axon Instruments, Union City). Rectified and heavily smoothed EMG (100-ms time constant) from the TA muscle was displayed on an oscilloscope on a fast time sweep to help the subjects maintain a steady contraction. At the start of each experiment, the subjects generated 3 maximum isometric contractions (2-3 s in duration) using visual feedback to assess their maximum voluntary contraction (MVC).

TMS was applied to the contralateral motor cortex using a double cone coil or a custom made figure-of-eight coil (P/N 15857: 90 mm wing diameter). The coil was chosen based on the severity of the lesion and its ability to elicit MEPs below the injury. Single pulse TMS was delivered using a MagStim 200 stimulator (Magstim, Dyfed, UK). The optimal spot to the TA muscle was identified in each recording session using an intermediate TMS intensity and was marked on the scalp using a felt tipped pen (generally 1-2 cm lateral of vertex with the figure-of-eight and 1 cm more posterior with the double cone). The site of stimulation was similar between days. The coil was generally orientated to induce postero-anterior currents in the brain (the control subjects shown in Fig. 4-1A were tested using antero-posterior currents). Peripheral nerves were stimulated using two constant-current stimulators (DS7A, Digitimer). The common personeal nerve (CPN) was stimulated with the cathode just below the head of the fibula (1-ms pulse). The tibial nerve (TN) was stimulated at the ankle with the cathode below the medial malleole (0.2-ms pulse).

4.2.3 Recruitment curves

TMS recruitment curves were evaluated in 22 SCI subjects (35 legs) to characterise the integrity of the spared corticospinal tract. The recruitment curves were collected during a tonic dorsiflexion that was generally at ~15% of the subject's MVC. The applied stimulation intensity was generally increased from below motor threshold to 80% of the maximum stimulator output (MSO) using the double cone coil or to 100% MSO using the figure-of-eight coil (in increments of 5 to 10% MSO). Four stimuli were given at each intensity and were delivered every 5-6 s. MEP_{max} was obtained from the TMS intensity that produced the largest peak-to-peak response. The double cone at 80% MSO was frequently used to verify MEP_{max} elicited using the smaller figure-of-eight coil. MEP_{max} was evaluated in 5 uninjured controls (10 legs) for comparison.

4.2.4 Afferent conditioning of MEPs

We have previously shown that heteronymous nerve inputs from the TN at the ankle can facilitate TA MEPs in uninjured control subjects (Roy and Gorassini 2008; open symbols in Fig. 4-1A). Here, we examined such conditioning of MEP responses in 8 individuals after an incomplete SCI. The TMS intensity in the SCI subjects was adjusted to the steep portion of the recruitment curve and was near ½MEP_{max}. The unconditioned MEPs in the 10 uninjured controls were small (well below ½ MEP_{max}) but typically twice that of the SCI subjects. The TN was stimulated at 1.5-2 × motor threshold (MT) (see Roy and Gorassini 2008) and the interval between the nerve stimulus and the TMS pulse was varied from 30 to 80 ms in 10-ms increments. A minimum of 6 responses were collected at each interval intermixed with a minimum 12 single pulse test responses. TA H-reflexes were collected to investigate the effect of the afferent input on motoneuron pool excitability, in a similar manner to the MEP. To evaluate the effect of homonymous nerve inputs on the TA MEP, the CPN was stimulated at 1.2 x MT and its interaction with the MEP was also evaluated at interstimulus intervals (ISIs) ranging from 30 to 80 ms in the SCI subjects and 30 to 100 ms in the uninjured controls. Sixteen SCI subjects (6 of the subjects conditioned with the TN) and 4 uninjured controls were tested with CPN stimulation.

4.2.5 Paired associative stimulation

Since afferent stimulation paired with TMS can facilitate MEPs in the TA muscle in uninjured controls (Mrachacz-Kersting et al. 2007; Roy et al. 2007), we examined whether PAS can modify corticospinal excitability after SCI. Thirteen SCI subjects were recruited and two interventions were tested (12 of these subjects were also used in the CPN experiments). PAS was expected to increase MEPs while PAS-sham (described below) was expected to leave the MEPs unchanged. A similar proportion of subjects were first administered PAS instead of PAS-sham. Baseline MEPs were collected at rest using TMS intensities that produced a peak-to-peak amplitude of 0.1-0.2 mV. In subjects where appreciable resting MEPs > 50 µV could not be elicited, the corticospinal tract was evaluated using a double cone coil set to 80% MSO, a stimulation strength that was maximally tolerable for the subjects. In one subject who could only elicit a flicker of voluntary activation, *resting* MEPs were collected when the muscle was quiet, but when the subject was trying to flex the ankle. In the 12 subjects who could maintain a steady contraction, active MEPs were also measured at 15% MVC using a TMS intensity that was near ½MEP_{max}. A minimum of 10 responses were collected for each condition.

PAS consisted of 120 paired afferent and cortical stimuli. To enhance the strength of the afferent input reaching the cortex, and the likelihood that the input would be synchronized with the cortical stimulus, the CPN (at 1.2 x MT, 1-ms pulse width) was stimulated 3 times, either 40, 50 and 60 ms before the TMS pulse or 50, 60 and 70 ms before the TMS pulse. The different intervals were chosen to account for the extent of the spinal cord lesion and were selected based on the MEP_{max} value along with the MEP latency. Subjects with low-amplitude MEPs and/or longer MEP latencies (≥ 40 ms) were given the 50 to 70 ms ISI. The TMS intensity during PAS

was generally near ½MEP_{max} during a voluntary contraction. For SCI subjects with higher TMS thresholds, the TMS intensity was increased to a maximum 65% MSO using the double cone coil since repeated stimulation (120 pulses) at higher intensities would not be well tolerated by the volunteers. There were no systematic difference between the PAS intervals (40 to 60 ms or 50 to 70 ms) and the amount of facilitation produced by PAS. Since voluntary drive enhances the induction of PAS (Kujirai et al. 2006; Mrachacz-Kersting et al. 2007), the stimuli were triggered when the voluntarily activated EMG in the TA muscle crossed a pre-defined threshold (near 15% of MVC). The subjects were cued to dorsiflex their ankle using a computer program (every 5 s). Two blocks of 60 stimuli were delivered with a 4 minute break in-between. During PAS-sham, the afferent excitation (20-ms train) arrived at the cortex many tens of milliseconds *before* the TMS pulse; ISIs from 80 to 200 ms were randomly distributed. Post MEPs were measured 10 and 20 minutes after the intervention.

4.2.6 Data analysis

MEP and H-reflex responses were analyzed peak-to-peak. The level of background EMG was evaluated in the 100-ms window before the stimulus. To calculate MVC, the rectified EMG was first smoothed using a 500-ms sliding average. MVC was then quantified as the maximum activity produced by each of the bursts. Statistical comparisons were done using paired t-tests, with the exception of the resting MEP from the PAS experiment which was analysed using the Wilcoxon signed-rank test since the data was non-normally distributed. Relationships between the different measurements were evaluated using the Pearson product–moment correlation (r). Data are means ± SEM. Significance was set at $P < 0.05$.

4.3 RESULTS

4.3.1 Recruitment curves

To compare the amount of corticospinal tract integrity within the SCI population examined in this study, recruitment curves were obtained by plotting the mean peak-to-peak MEP against the corresponding TMS intensity. As shown for an example subject with a C3-4 lesion (subject 8M in Table 1), the size of the MEP response measured during a background contraction of ~15% MVC generally followed a sigmoid curve and plateaued at high stimulation intensities (i.e. MEP_{max}; Figs. 4-1A&B; see also Thomas and Gorassini 2005). Figure 4-1C shows that the mean MEP_{max} in the injured subjects (0.65 ± 0.08 mV) was considerably reduced as compared to the uninjured controls (2.6 ± 0.4 mV; $P < 0.0001$). However, there was some overlap in MEP_{max} values between both groups which may have been partly due to an overlap in the level of background EMG used (see Fig. 4-1D). Although not systematically tested in each subject, when plotting MEP_{max} as a function of the actual background EMG (in µV) for the whole group, there was a strong correlation between the MEP_{max} value and the level of background EMG in both injured subjects ($r = 0.73$; $P < 0.0001$) and uninjured controls ($r = 0.93$; $P < 0.0001$); indicating that

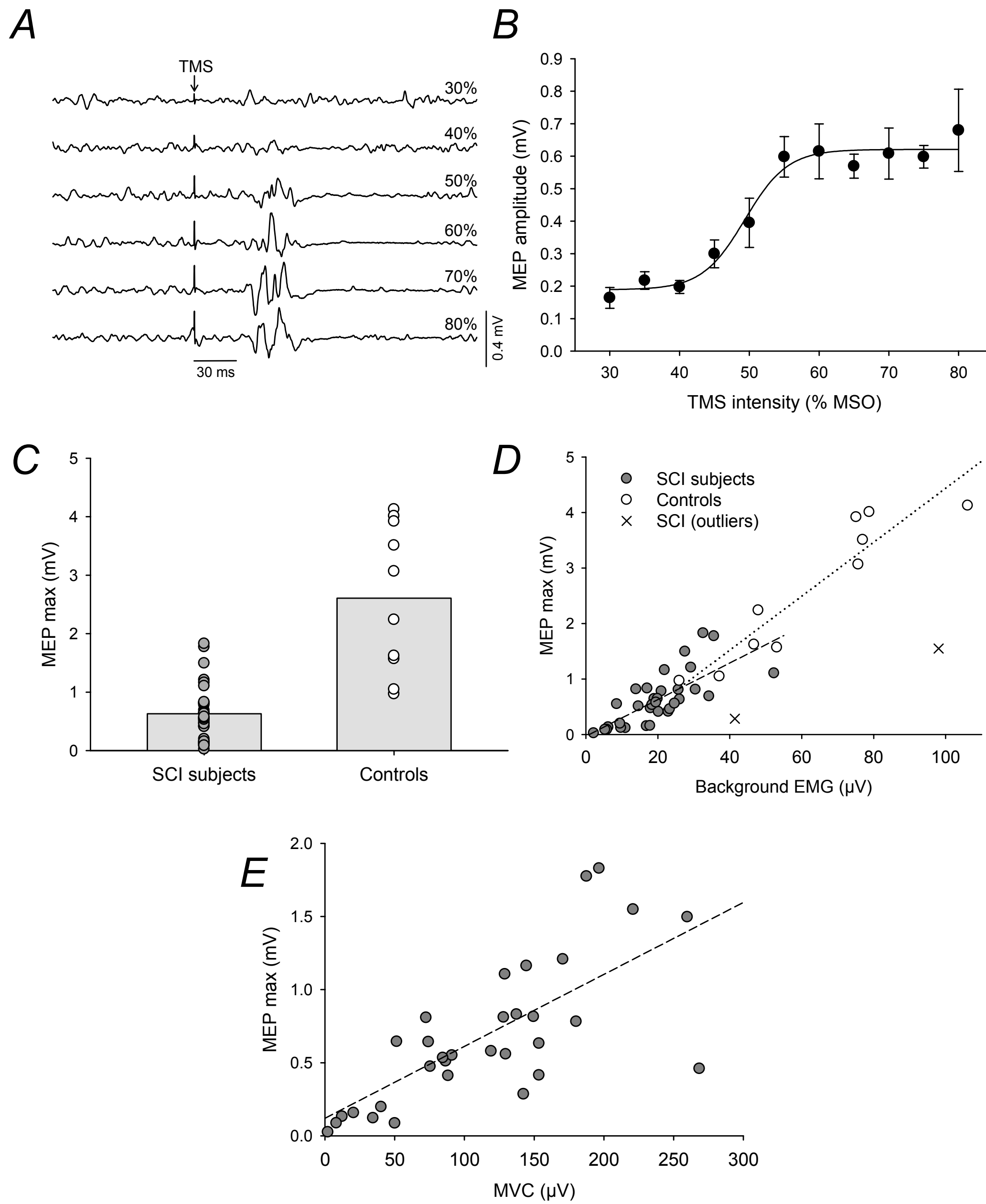

Figure 4-1. TMS recruitment curve and MEP_{max} in SCI subjects and uninjured controls

A, Raw sweeps showing the TMS recruitment curve in an individual with a C3-4 spinal cord lesion (subject 8M in Table 1). The sweeps are the average of 4 recordings and the average peak-to-peak MEPs are shown in (*B*). The sigmoid function is shown to highlight the overall shape of the recruitment curve (see Devanne et al. 1997). *C*, MEP_{max} measured in 22 SCI subjects (35 legs) and 5 uninjured controls (10 legs). The individual data points are shown using the symbols and the vertical bars represent the mean. *D*, Scatter plot and regression lines showing the relationship between MEP_{max} and the level of background EMG in both

the MEP_{max} value was partially linked to the absolute amount of background EMG activity produced by the tonic contraction at 15% of MVC (see Discussion). The MEP_{max} in the SCI subjects was also positively correlated to the maximum voluntary EMG that could be produced by the paretic muscle (Fig. 4-1E; $r = 0.72$; $P < 0.0001$), thus indicating that individuals with less corticospinal tract damage could also elicit the strongest voluntary contractions.

4.3.2 Heteronymous (TN) nerve stimulation

Stimulating the TN at the ankle had no noticeable effect on the TA MEPs recorded in the SCI group (8 subjects, filled symbols in Fig. 4-2A). This lack of effect from prior heteronymous nerve stimulation is in direct contrast to the uninjured controls (open symbols) which showed considerable MEP facilitation in the contracted muscle at a latency that is consistent with a transcortical loop (open symbols in Fig. 4-2A). For the uninjured subjects, the small MEPs were significantly increased when the peripheral nerve was stimulated 45 to 50 ms before the TMS pulse and was suppressed at the 35 ms ISI (as compared to the test MEP; all $P < 0.05$). The H-reflexes conditioned by a prior sensory stimulus were not significantly altered in either the SCI subjects or the uninjured controls (Fig. 4-2B).

4.3.3 Homonymous (CPN) nerve stimulation

In the injured individuals (16 subjects), there was some indication that homonymous CPN stimulation could modulate corticospinal output to the TA muscle. Since the size of the MEP amplitude is related to the voluntary muscle strength (see above), and hence to the severity of the spinal cord lesion (Curt et al. 1998), the SCI subjects were grouped according to their MEP_{max} value (either > 0.5 mV or < 0.5 mV). In both SCI groups, most MEPs were suppressed by the peripheral nerve stimulation (most points fell below the horizontal line), but by amounts that were generally weaker than the uninjured controls (Fig. 4-2D). During the period when the MEP was suppressed (ISIs from 30 to 70 ms), there was a tendency for the homonymous CPN inputs to provide excitatory drive to the TA MEP at a latency of 50 ms, a transmission latency that is consistent with the interaction of the sensory volley at the level of the cortex.

Continuation of Fig. 4-1 caption:
SCI subjects (grey symbols and dashed line) and uninjured controls (open symbols and dotted line). The two SCI subjects (× symbols) whose data was outside the 99% prediction interval were omitted from the linear regression (outlier on the left was injured from transverse myelitis while the outlier on the right was tested at ~40% of MVC). *E*, Shows the linear relationship between MEP_{max} and the size of the maximum voluntary contraction (MVC) in the SCI subjects (data not available from 3 limbs).

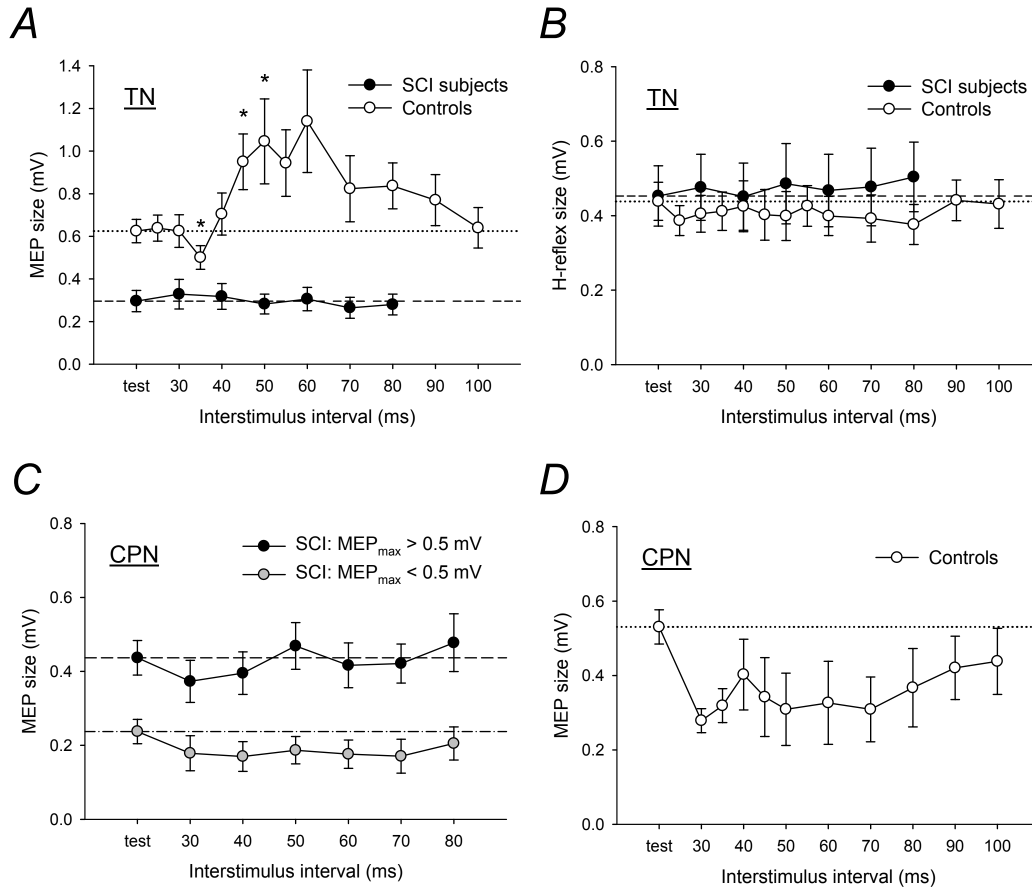

Figure 4-2. Effect of peripheral nerve stimulation on MEPs and H-reflexes

MEPs (*A*) and H-reflexes (*B*) conditioned by TN stimulation in SCI subjects (filled symbols) and 10 uninjured controls (open symbols). Data in (*A*) and (*B*) are from 8 and 7 SCI subjects, respectively. *C*, Displays the effect of CPN stimulation on MEPs in 11 SCI subjects having an MEP_{max} > 0.5 mV (filled symbols) and 5 subjects with an MEP_{max} < 0.5 mV (grey symbols). *D*, Shows the effect CPN stimulation on MEPs in 4 uninjured controls. The horizontal lines represent the test MEP in each group. Asterisks indicate significant differences as compared to the test MEP (*P < 0.05).

Indeed, the local increase in the MEP (i.e. excitation/disinhibition) was more pronounced in the SCI subjects having stronger corticospinal projections (dark symbols in Fig. 4-2C) and the average MEP response was 22.1% larger than the MEP response measured over the intervals of 30 to 40 ms (N = 11; P = 0.028). Evidence for excitatory CPN inputs onto the MEP was considerably weaker in the SCI subjects having fewer spared corticospinal connections (grey symbols in Fig. 4-2C). In the 5 SCI subjects tested with both peripheral nerves (i.e. TN and CPN) and having an MEP_{max} > 0.5 mV, only the MEP conditioned by CPN stimulation were facilitated/ disinhibited by the afferent input (data not shown). Although of the level of background EMG can strongly affect the MEP_{max} (see Fig. 4-1D), there was no distinguishable correlation between the background EMG activity and amount of sensory-induced MEP facilitation/disinhibition in the SCI subjects, potentially since the majority of the subjects were tested with a background contraction between 20 and 30 μV (data not shown).

Individuals with an intact spinal cord showed a local increase in the MEP (i.e. disinhibition) at the 40 ms ISI (Fig. 4-2D) indicating the spinal trauma may have delayed the excitatory interaction of the sensory input with the MEP by approximately ~10 ms. In the uninjured controls, the local increase in the MEP from CPN stimulation at the knee (at 40 ms) occurred earlier than with TN stimulation at the ankle (at 45 ms) likely due to the shorter distance between the stimulation site and the cortex (compare open symbols in Figs. 4-2A&D).

4.3.4 Paired associative stimulation

An intervention of PAS was tested in 13 SCI subjects to investigate whether afferent stimulation below the lesion paired with TMS could increase corticospinal excitability to the ankle flexor. To measure changes in corticospinal excitability, MEPs were evoked in the resting muscle using a double cone coil (in 9 subjects; 76.4 ±1.8% MSO) or a figure-of-eight coil (in 4 subjects; 93.8 ± 4.7% MSO). Figure 4-3A shows that an intervention of PAS rather than PAS-sham increased the group's mean resting MEP. The normalized resting MEPs were significantly facilitated 10 min after the intervention (Wilcoxon test: P = 0.039), but the facilitation tended towards baseline values at 20 min (Wilcoxon test: P = 0.087 in Fig. 4-3A and P = 0.196 in Fig. 4-3C). Resting MEPs measured 10 to 20 min after PAS were facilitated by >20% in 7 out 13 subjects (solid lines in Fig. 4-3C), while only 3 of the subjects showed similar increases following the sham treatment (solid lines in Fig. 4-3D). Although the resting MEPs could be increased by PAS, no effect was carried over to the contracted MEP. Neither PAS or PAS-SHAM had any effect on the MEPs evaluated during a voluntary contraction, which were 429 ± 78 and 461 ± 75 μV at baseline, respectively (Fig. 4-3B; t-tests: all P > 0.19).

As shown in Figure 4-3C, some subjects with initial resting MEPs that were small in amplitude (< 50 μV) displayed MEP potentiation with PAS, whereas others with higher resting MEPs (> 100 μV) did not, suggesting that the degree of spinal cord damage did not affect the facilitatory effect of the PAS. However, when the MEP_{max} value was plotted against the percentage increase in MEP (10 and 20 min average

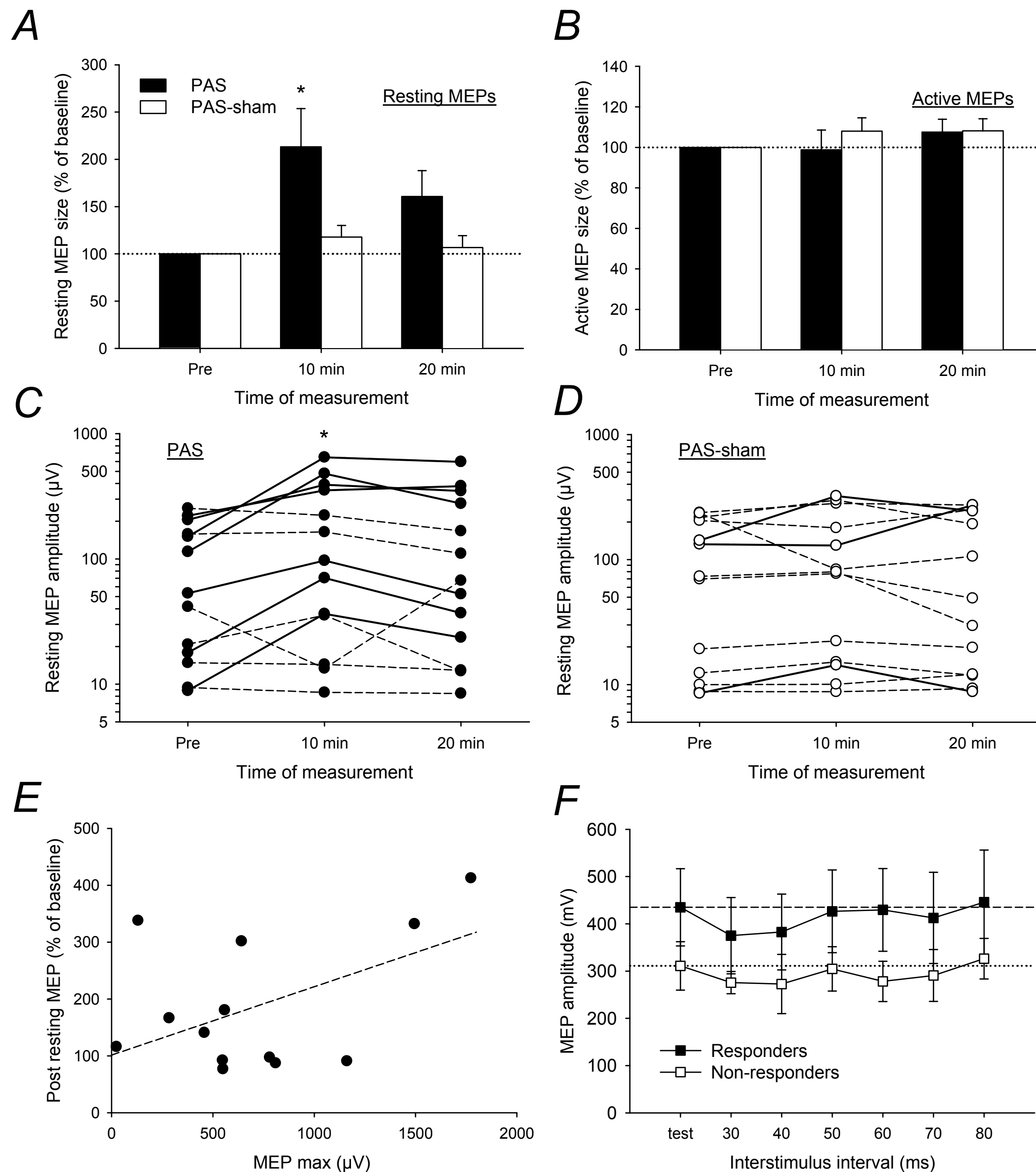

Figure 4-3. The effect of PAS on the MEP in SCI subjects

Graphs show the effect PAS (*black bars*) and PAS-sham (*white bars*) on the average MEP at rest (*A*) and during a voluntary contraction (*B*). Individual subject data showing the resting MEPs *before* and *after* PAS (*C*) and PAS-sham (*D*); with subjects showing > 20% MEP facilitation represented by solid lines. *E*, Scatter plot shows the PAS-induced changes to the MEP in relation to the MEP_{max} values. The dashed line shows the linear regression of the PAS data (r = 0.52; P = 0.068). *F*, short-term conditioning of the MEP by CPN stimulation (12 subjects tested). The profiles are grouped according to the subjects that showed a >20% PAS-induced facilitation (Responders: 7 subjects) and those that did not (Non-responders: 5 subjects). Asterisks indicate significant differences as compared to the baseline MEP (Wilcoxon test: *P < 0.05).

post PAS), there was a non-significant tendency for the strength of the corticospinal pathway to be related to the magnitude of the PAS effect (Fig. 4-3E; $r = 0.52$; $P = 0.068$). Moreover, it is also possible that part of the variability in the PAS induction might be explained by the strength of the spared ascending pathway. To test this, we examined the short-term conditioning of the MEP by CPN stimulation.as done in Fig. 4-2C. In general, the magnitude of the sensory-induced MEP facilitation/disinhibition was greatest in the subjects who showed >20% PAS-induced facilitation (Responders in Fig. 4-3F; 7 subjects) rather than the individuals who were only marginally affected by PAS (< 20%, Non-responders: 5 subjects). In fact, in the Responder group, the MEP was facilitated/disinhibited over a broader range of ISIs (~20 ms) compared to the Non-responders. Likewise, the MEP response measured at 50 and 60 ms (compared to the MEP at the 30 to 40 ms intervals) was twice as large as the Non-responders.

4.4 DISCUSSION

In the present study, we show that after incomplete SCI, the afferent-induced MEP facilitation produced by TN stimulation at the ankle is considerably diminished in the TA muscle as compared to the uninjured controls, suggesting that supraspinal circuits contribute to the extra excitation produced by sensory nerve stimulation. In a group of SCI subjects with larger test MEPs, we also provide evidence that afferent inputs from homonymous CPN stimulation can provide excitatory drive to the TA MEP, but the effect is delayed by approximately ~10 ms as compared to the uninjured controls. By pairing CPN and TMS inputs, we show that an intervention of PAS can transiently increase corticospinal connections of SCI subjects with functionally intact descending motor and ascending sensory pathways.

4.4.1 Recruitment curves and background EMG

MEP responses elicited using a stimulation intensity that was maximally tolerable (i.e. using a double cone stimulation at 80% MSO) were negligible or absent in the resting TA muscle of several SCI subjects (see resting MEPs in Figs. 4-3C&D), and contrasts the markedly larger resting MEPs of uninjured individuals (see Roy and Gorassini 2008). This indicates that the *resting* MEP response may be a sensitive measure of damage to the descending corticospinal system (Brouwer and Hopkins-Rosseel 1997; Davey et al. 1999; see also Calancie et al. 1999). In contrast, when MEP_{max} responses were measured during a low background contraction (< 60 μV), comparably sized responses were occasionally evoked in SCI subjects and uninjured controls. For example, SCI subjects with comparatively higher background contractions (with respect to the SCI group) had similar MEP_{max} amplitudes as uninjured subjects using comparatively low background contractions (with respect to the uninjured group). However, at high levels of background contraction (> 100 μV) SCI subjects will invariably have much lower MEP responses (see outlier subject in Fig. 4-1D and Davey et al. 1999; van Hedel et al. 2007). Nonetheless, these findings highlight that when comparing the maximum connectivity of the corticospinal tract (MEP_{max}) within the same group it is important to match low contraction levels in

terms of a set amount of absolute EMG (in μV). Using a set percentage of MVC may introduce variability because different subjects will have different absolute levels of MVC and hence, different absolute levels of background EMG (in μV). When comparing corticospinal function between SCI and uninjured subjects, it may be better to compare responses at higher levels of background EMG, such as 40% of MVC (van Hedel et al. (2007), to reveal differences between the two groups.

4.4.2 Afferent input

Afferent stimulation of the TN at the ankle can facilitate TA MEPs at a latency that is a few milliseconds longer that the somatosensory evoked potential latency (i.e. P40 occuring near 40 ms; Hauck et al. 2006) and the interaction is consistent with a transcortical loop (Roy and Gorassini 2008). There is additional evidence that part of this facilitation is mediated at a cortical level on the basis that cortical MEPs and not subcortical responses are potentiated by the afferent input from the leg (Nielsen et al. 1997; Petersen et al. 1998; Roy and Gorassini 2008). In the present study, the lack of MEP facilitation in the SCI subjects with damaged ascending sensory pathways suggests that supraspinal neurons rostral to the injury site are important for such MEP potentiation. The lack of facilitation may have occurred because of a loss of afferent input reaching the cortex, or alternatively, because the descending volleys potentiated by the sensory inputs were attenuated by the injury. Admittedly, the group of SCI subjects tested with TN stimulation had a small test MEP response. However, smaller MEPs closer to 0.3 mV can be strongly facilitated in the relaxed muscle of uninjured subjects (Roy and Gorassini 2008) suggesting that the smaller MEPs in the SCI subjects were not responsible for the diminished sensory-induced facilitation.

For homonymous CPN stimulation, MEP responses in the TA muscle of uninjured individuals were generally depressed, potentially due to a post-synaptic motoneuron effect caused by stimulating the homonymous nerve (Poon et al. 2008). Likewise, the facilitatory effect induced by CPN stimulation was less persistent than what was found for the TN, potentially due to the greater spinal inhibition caused by activating the homonymous nerve. Despite such underlying inhibition, there was evidence that the MEP in the uninjured controls received excitatory inputs from the CPN when it was activated 40 ms before the cortical stimulus. This latency is consistent with the arrival of the afferent input at the motor cortex, and the mechanism is general accordance with the results obtained when stretching the TA muscle (Petersen et al. 1998; van Doornik et al. 2004; Zuur et al. 2009). In the SCI subjects, the MEPs tended to be less inhibited by the CPN stimulus potentially due to the impairment of descending activation of spinal inhibitory mechanisms (reviewed in Pierrot-Desseilligny and Burke 2005; Knikou 2007; Norton et al. 2008).After spinal trauma, the CPN-induced MEP facilitation/disinhibition was delayed by approximately ~10 ms and was related to the severity of the injury. For example, individuals with stronger corticospinal connections (i.e. MEP_{max}) exhibited larger afferent-induced MEP facilitation/disinhibition as compared to those with weaker connections, which were more reminiscent of the subjects tested with TN stimulation. There was also a tendency for the MEP to receive stronger excitatory inputs from the homonymous CPN inputs as compared to the heteronymous TN in the 5 subjects having an

MEP_{max} > 0.5 mV and support the notion that afferent connections to the TA muscle may be more strongly regulated by homonymous rather than heteronymous inputs (see also Deletis et al. 1992). The relative strength of the interaction may however be obscured by the inhibitory effect produced by electrically stimulating the homonymous nerve (see Poon et al. 2008).

4.4.3 Paired associative stimulation

An intervention of PAS potentiated the MEPs recorded at rest and this increase persisted for 10 minutes after the intervention. As a large number of subjects were taking baclofen at the time of the experiment, it is possible that the drug may have hindered the induction of the MEP potentiation given that baclofen, a GABA-B receptor antagonist, decreases PAS-induced facilitation in uninjured subjects (McDonnell et al. 2007).Although the effect of PAS was variable and facilitated MEPs by more than 20% in only 7 out 13 subjects, MEPs were not significantly altered by the sham treatment, suggesting that appropriately timed afferent and cortical inputs were important for modifying the strength of the corticospinal connections. We cannot determine if the MEP facilitation from PAS occurred at cortical or subcortical levels. However, given that PAS increases the size of the descending corticospinal volleys (Di Lazzaro et al. 2009), but has no effect on the H-reflex recorded in the TA muscle (Mrachacz-Kersting et al. 2007; Roy et al. 2007), it is likely that part of the MEP facilitation involves cortical elements. However, changes to spinal excitability, as shown in wrist flexors following PAS, cannot be entirely excluded (Meunier et al. 2007). In contrast to the resting MEP response, none of the MEPs measured during a voluntary contraction were altered following PAS. Since MEPs in the contracted TA muscle can be facilitated following PAS in uninjured subjects but by smaller amounts than during rest (Roy et al. 2007), it is possible that the facilitatory effect in the SCI subjects was washed away by the voluntary contraction, as is the case in the upper limb (Stefan et al. 2000; Ridding and Taylor 2001).

The amount of facilitation produced by PAS tended to be related to the strength of the descending corticospinal pathway; though large MEPs were not a necessary requirement for the potentiation to occur (see Fig. 4-3C). In line with a subject's volitional muscle strength, it is likely that MEP_{max} can provide insight about the integrity of the corticospinal tract and may help predict whose motor system will respond to PAS. Moreover, another important determinant of a subject's response was the integrity of the spared ascending sensory connections to the motor cortex. In general, larger PAS facilitation tended to be more prevalent in the group of subjects where the afferent input could interact with and excite/disinhibit the cortical MEP. Thus, the amount of afferent-induced cortical facilitation in combination with the strength of the corticospinal system may help predict if a SCI subject will respond to PAS or not.

4.4.4 Summary and clinical implications

The present findings provide evidence that PAS can facilitate MEP responses in some SCI subjects having functionally spared descending and ascending inputs to the motor cortex. As has been shown for repetitive TMS and transcranial direct current stimulation in stroke, priming the nervous system with PAS prior to motor rehabilitation may facilitate the effects of motor training (see Hummel et al. 2008). However, the resting MEP in the SCI subjects was only increased for 10 min after the intervention, which would not leave much time to perform extensive motor training. Potentially using a larger number of PAS conditioning stimuli to overcome the impaired afferent excitation or using other forms of cortical facilitation such as repetitive TMS (Huang et al. 2005; Hamada et al. 2008) or transcranial direct current stimulation (Nitsche and Paulus 2000; Jeffery et al. 2007) may induce more robust periods of cortical facilitation in a larger percentage of SCI subjects.

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CHAPTER 5: Activation of inhibitory spinal circuitry to the soleus muscle by contralateral afferent stimulation

5.1 INTRODUCTION

Reflex pathways can provide strong regulation and coordination of limb movements (reviewed in Rossignol et al. 2006). Such reflexes are not confined to the ipsilateral limb but can also be elicited in various muscles of the contralateral limb (Burke et al. 1991; Zehr et al. 2001a; Pierrot-Desseilligny and Burke 2005). The most commonly known of these pathways is the crossed extensor reflex whereby afferent stimulation elicits powerful flexion of the stimulated limb and contralateral extension (Sherrington 1910). In addition to the circuits that *excite* contralateral motoneurons, animal experiments have revealed a crossed inhibitory pathway in both anesthetised and behaving animals (Curtis et al. 1958; Jankowska et al. 2005; Edgley and Aggelopolous 2006; Frigon and Rossignol 2008). For instance during feline locomotion, stimulation of cutaneous nerves produces short-latency (~13 ms) inhibitory responses in the contralateral extensor EMG (Frigon and Rossignol 2008). Such crossed inhibition is strongly modulated throughout the step cycle and may facilitate locomotor-generating circuitry to help in the timing of EMG activity during walking. For example, the bombardment of afferent inflow that is elicited when the foot first makes contact with the ground (Prochazka and Gorassini 1998) may help to inhibit extensors in the contralateral limb at the end of stance to help facilitate the onset of swing. The fact that this crossed inhibitory pathway is abolished by the removal of descending inputs (Aggelopolous et al. 1996) makes this pathway an important one to study as a part of the dis-coordination in stepping following spinal cord injury, which may be explained, in part, by a lack of crossed inhibition.

In human subjects, cutaneous and muscle afferents can similarly inhibit motor activity in the contralateral limb (Burke et al. 1991; Zehr et al. 2001a; Pierrot-Desseilligny and Burke 2005). For example, a large barrage of afferent excitation produced by tapping the Achilles tendon leads to inhibition in the contralateral soleus (SOL) muscle, as seen by a persistent (>150 ms) reduction in the amplitude of the H-reflex (Koceja and Kamen 1992). Likewise, stimulation of foot afferents (sural and superficial peroneal nerves at the ankle) can suppress ongoing EMG activity in flexor and extensor leg muscles (Burke et al. 1991; Zehr et al. 2001a). Finally, electrical stimulation of the contralateral posterior tibial and common peroneal nerves (cPTN and cCPN) also produces inhibition of the SOL H-reflex but the inhibition only occurs at a very long-latency (~500 ms; Slivko and Teteryatnik 2005) and is present after an earlier period of facilitation (Robinson et al. 1979; Koceja and Kamen 1992; see also Delwaide et al. 1981) . Thus, it is clear that peripheral nerve stimulation can inhibit the excitability of contralateral motoneurons, but the site or mechanism of this inhibition remains unclear.

The goal of the present study was to characterize the human crossed inhibitory pathway which was first identified by Curtis et al. (1958) in anaesthetized preparations and by Frigon and Rossignol (2008) in behaving animals. To provide enough afferent input to drive the inhibitory pathway, we activated the cPTN and cCPN using a train of electrical stimuli (25 Hz for 700 ms). To test if contralateral afferent inputs mediate the suppression of extensor EMG activity by activating spinal inhibitory pathways, we first examined the effect of stimulating the contralateral afferents on the cutaneomuscular reflex (CMR) evoked in the SOL muscle since the CMR activates inhibitory interneuronal circuits in the spinal cord (Gibbs et al. 1995; Bennett et al. 1996). Likewise, because crossed inhibitory pathways are facilitated by descending inputs (Aggelopolous et al. 1996), we also characterized the effects of contralateral afferent stimulation on spinal circuits activated by corticospinal inputs. In this study, we show that contralateral nerve stimulation in the leg facilitates inhibitory spinal circuitry to ankle extensor motoneurons as evidenced by a further suppression of the spinal inhibitory component of the cutaneomuscular reflex and corticospinal responses.

5.2 METHODS

5.2.1 Subjects

Twenty-one healthy volunteers (11 females) with an average age of 29.5 ± 6.8 years (means ± SD) participated in the study. Subjects gave informed consent and the protocol was approved by the Human Ethics Research Board at the University of Alberta. Subjects were comfortably seated with their legs slightly bent at the knee and both feet secured to a foot plate. For all experiments, the conditioning stimuli were delivered to the left leg and test responses were evoked in the right.

5.2.2 EMG Recordings

Surface EMG was collected from both SOL and tibialis anterior (TA) muscles and the right abductor hallucis muscle (for the Cutaneomuscular reflex) using pairs of Ag-AgCl electrodes (Kendall, Chicopee, USA). The signals were amplified 1000 times and filtered using a 10-1000 Hz band-pass (Octopus, Bortec Technologies, Calgary, Canada). EMG signals were digitized at 2.5 kHz using Axoscope hardware and software (Digidata 1200 Series, Axon Instruments, Union City, USA).

5.2.3 Cutaneomuscular reflex

To test if contralateral afferent inputs mediate their suppression of extensor EMG activity by activating spinal inhibitory pathways, we examined the effect of stimulating the contralateral posterior tibial nerve (cPTN) on the CMR evoked in the SOL muscle since the CMR activates inhibitory interneuronal circuits in the spinal cord (Gibbs et al. 1995; Bennett et al. 1996). In 8 subjects, the cPTN (left) was activated using a stimulus train (25 Hz for 700 ms) to produce a barrage of contralateral afferent inputs, similar to that done using muscle vibration (Rosenkranz

and Rothwell 2003; Rosenkranz et al. 2003). The cathode was placed in the popliteal fossa and the large anode was located just below the patella (1-ms pulses). The conditioning nerve stimulation (at both 0.8 and 1.0 × MT) was started 500 ms before evoking the test CMR in the right SOL. To evoke the CMR, the right tibial nerve at the ankle was stimulated through bipolar electrodes placed just below the medial malleolus (5 x 0.2-ms pulses at 300 Hz). The CMR intensity, which was near the noxious threshold, was adjusted to produce periods of early-latency inhibition and middle-latency excitation (see Fig. 5-1A). The CMR was evoked while the subjects maintained a tonic plantarflexion at 5-10% of their maximum voluntary contraction (MVC). The smoothed and rectified SOL EMG was displayed on an oscilloscope to help the subjects maintain a steady contraction. A minimum of 30 responses were collected for each condition (test alone or condition-test responses). Stimuli were randomly delivered every 3-5 s. All peripheral nerves were stimulated using a constant-current stimulator (DS7A, Digitimer).

5.2.4 MEP and H-reflex

We also examined the effects of contralateral afferent stimulation on inhibitory spinal circuits activated by corticospinal inputs (see Iles and Pisini 1992; Nielsen and Petersen 1995). In these experiments we also compared the effect of stimulating the contralateral common peroneal nerve (cCPN) that innervates the TA muscle to determine if contralateral afferents supplying flexor muscles and the dorsum of the foot also activate inhibitory pathways to the SOL muscle (see also Frigon and Rossignol 2008). The left cCPN was activated through bipolar electrodes placed just below the fibula head (1-ms pulses). Transcranial magnetic stimulation (TMS) was applied to the left motor cortex (supplying the right SOL muscle) using a custom made figure-of-eight coil (P/N 15857: 90 mm external wing diameter; Magstim, Dyfed, UK) and a MagStim 200. The optimal spot to activate the right SOL was identified using a low stimulation intensity and was generally 1-2 cm lateral to the vertex. The coil was secured in place and orientated to induce postero-anterior currents in the brain. The MEP response in the right SOL was adjusted to ½ MEP_{max} during a weak plantarflexion at 5-10% of MVC. To characterize the effect of the stimulation intensity, the cPTN and cCPN were stimulated at 6 intensities from 0.4 to 1.4 × MT in steps of 0.2 x MT. Twelve conditioned responses were collected at each intensity and intermixed with 18 test responses. The interval between consecutive stimuli was 5-6 s.

Because the inhibitory contralateral pathway is likely mediated through the activation of spinal inhibitory pathways, as a control we measured the comparative affect of contralateral afferent stimulation on the more direct SOL H-reflex which likely bypasses the inhibitory interneuronal circuits. H-reflexes in the test SOL muscle were set to be of comparable size to ½ MEP_{max}. Contralateral nerve stimulation at the different intensities (0.4 to 1.4 x MT) was performed as described for the MEP responses above. In a separate series of experiments, H-reflexes in 8 subjects (7 subjects that had not be been tested using the smaller H-reflex) were tested near ½ H_{max}, a size that is more sensitive to changes in motoneuron excitability (Crone et al. 1990). Twenty H-reflexes and 20 MEPs near ½ MEP_{max} were collected without (control) and with cPTN stimulation at 0.9 × MT.

5.2.5 *Plantarflexion EMG on crossed MEP inhibition*

To further verify that the inhibitory contralateral pathway suppresses SOL activity by facilitating inhibitory interneurons upstream from the motoneuron, the effect of voluntary drive on the crossed MEP inhibition was tested since voluntary drive can modify the contribution of direct (corticomotoneuronal) versus indirect (polysynaptic) corticospinal pathways to the SOL muscle (Morita et al. 2000). For instance, at rest and during weak plantarflexion, a large portion of the SOL MEP is likely evoked through the long-latency, polysynaptic pathway. As the strength of the contraction increases, the monosynaptic corticomotoneuronal pathway likely contributes a larger extent of the MEP (Nielsen and Petersen 1995; Morita et al. 2000). The TMS intensity was set to elicit small but reliable MEPs at rest (~50 μV) and was tested at various plantarflexion strengths ranging from rest to 50% of MVC. At each contraction strength, 20 MEPs were collected without (control) and with cPTN stimulation (at 0.9 × MT). Additional MEPs at rest were collected using a stronger TMS intensity near ½ MEP_{max} to again activate more direct, higher-threshold corticomotoneuronal pathways (Cowan et al. 1986).

5.2.6 *Muscle vibration on MEP*

To examine if activation of contralateral muscle afferents using mechanical stimulation also inhibits the SOL MEP, we examined the effect of contralateral Achilles tendon vibration on the SOL MEP. Muscle vibrations were produced using a voice coil driven by a signal generator (AFG 3022, Tektronix Inc., Beaverton, USA) and an audio amplifier. Weak and strong vibrations (80 Hz) were applied using a 5 mm diameter probe and started 500 ms before the test stimulus (700 ms duration). The TMS intensity was adjusted to produce small MEPs (~150 μV) in the weakly contracted SOL muscle (~5% MVC). Twenty responses were collected for each condition. Responses to electrical cPTN stimulation at 0.9 × MT, which also activates cutaneous afferents, were also collected for comparison.

5.2.7 *Cortical inhibition*

We examined if a part of the MEP suppression by contralateral afferent excitation was also mediated by the activation of cortical inhibitory circuits by examining the effects of cPTN stimulation on short-interval intracortical inhibition (SICI) and EMG suppression by subthreshold TMS. SICI was tested in 8 subjects using the Kujirai paradigm (Kujirai et al. 1993) with an interstimulus interval of 3 ms using two MagStim 200 (or 200^2) stimulators connected to a BiStim module (or Bistim^2). The test stimulus was adjusted to ½ MEP_{max} during a tonic contraction (at ~5-10% MVC). The conditioning pulse was adjusted to produce strong SOL MEP inhibition and was generally near 80% of the active motor threshold. The active motor threshold was defined as the intensity which evoked an MEP in the contracted muscle approximately ~50% of the time. SICI was evaluated without (control) and with cPTN stimulation (700 ms train at 0.9 × MT). If the contralateral stimulation

depressed the test MEP by >10%, the intensity of the test stimulus was increased to match the test (control) MEP. Twenty responses were collected for each condition. To examine the effect of contralateral afferent excitation on other inhibitory intracortical pathways, very low-intensity subthreshold TMS was used to inhibit the averaged rectified EMG (Davey et al. 1994; Roy 2009) in 9 subjects (one additional subject). The stimulation intensity was below the MEP threshold and was adjusted to suppress the ongoing-EMG. A minimum of 80 trials were collected without (control) and with cPTN stimulation (at 0.9 × MT). Stimuli were delivered every 3 s.

5.2.8 Ankle Flexor MEP

Because contralateral nerve inputs can also regulate spinal activity to ankle flexor muscles (Delwaide et al. 1981), we examined whether TA MEPs would also be modulated by a train of cPTN and cCPN stimulation at 0.8 and 1.2 × MT, two intensities that produced appreciable MEP suppression in the SOL muscle. The TMS intensity was set to elicit small but consistent MEPs in the weakly dorsiflexed muscle (~5% MVC). Twenty responses were collected for each condition.

5.2.9 Data analysis

The data was analyzed offline within Matlab (The MathWorks). CMR responses were rectified and averaged before being expressed as a percentage of the background EMG. The level of background EMG was measured in the 200-ms window that preceded the stimulus. The early-latency CMR inhibition was measured from 50-90 ms, as done by Jones and Yang (1994). The CMR data was binned in 10-ms windows to determine the onset of the crossed effect. The size of the MEP and the H-reflex was measured peak-to-peak. The MEP latency was estimated by visual inspection of the average unrectified sweeps and was usually where the EMG activity diverged by >1 SD from the mean. SICI was evaluated as a percentage of the test MEP. TMS-induced EMG suppression was measured from the ‘averaged rectified EMG’ as obtained using rectification and the Hilbert transform (see Roy 2009). The inhibitory window was defined as the points where the average rectified EMG was below the background EMG (with an onset near 40 ms; see Fig. 5-6C). Statistical comparisons were done using paired t-tests (two-tailed). Data are given as means ± SEM.

5.3 RESULTS

5.3.1 Cutaneomuscular reflex

The CMR response was elicited in the right SOL by stimulating the tibial nerve below the medial malleolus during a tonic contraction. Electrical stimulation near the noxious threshold (17.2 ± 1.8 mA) suppressed the background EMG activity for a few tens of milliseconds, typically starting at ~50 ms (grey solid line in Fig. 5-1A). The EMG activity following the suppression was facilitated at a latency of ~90 ms, as shown in the example subject in Figure 5-1A. When the CMR was evoked in combination with cPTN stimulation (25 Hz train), both the magnitude and duration of

the underlying inhibition were increased (solid and dashed black lines in Fig. 5-1A). In 8 subjects, cPTN stimulation delivered at 0.8 and 1.0 × MT further depressed the average background EMG occurring from 50 to 90 ms (40-ms window during the inhibitory component of the CMR) by 18.0 ± 4.8% and 20.9 ± 4.7%, respectively (all P < 0.05). To determine the onset of the enhanced inhibition by cPTN stimulation, the data was averaged using 10-ms bins (Fig. 5-1B). The additional EMG inhibition induced by cPTN stimulation was first observed at the 70-80 ms bin for the 0.8 × MT intensity (grey triangle) and the 60-70 ms bin for the 1.0 × MT intensity (grey circles in Fig. 5-1B; all P < 0.05).

5.3.2 MEP and H-reflex

Because crossed inhibitory circuits are facilitated by descending inputs in animals (Aggelopolous et al. 1996), we examined if descending activation of SOL motoneurons by TMS, which also activates preceding interneuronal circuits in humans (Iles and Pisini 1992; Nielsen and Petersen 1995), were also inhibited by contralateral nerve inputs. The TMS intensity (78.6 ± 5.3% of the maximum stimulator output: MSO) was adjusted to elicit MEPs on the steep portion of the recruitment curve, which was 3.5 ± 0.6% of M_{max} and around 0.3 mV in amplitude (grey lines in Fig. 5-2A, left column). As shown for the single subject data, cPTN stimulation (extensor afferents) more strongly reduced SOL MEPs compared to cCPN stimulation (flexor afferents, see black dashed lines in Fig. 5-2A). MEPs were significantly reduced by cPTN stimulation at intensities ranging from 0.4 to 1.4 × MT (black bars in Fig. 5-2B) with a 22.6 ± 4.8% suppression of the MEP on average (all P < 0.05). The effect was similar but weaker for cCPN stimulation (open bars) and MEPs were only significantly depressed at 0.8 × MT (P = 0.022).

H-reflexes that were of comparable size to the MEP responses (5.6 ± 1.4% of M_{max} producing MEPs of ~0.45 mV) were used to evaluate whether the train of contralateral afferent stimulation also affected more direct, monosynaptic reflex pathways. In contrast, neither the cPTN nor cCPN stimulation affected the size of the H-reflex response as shown for the single subject data in Figure 5-2A (right column) or for the group data in Figure 5-2C. Larger H-reflexes at ½ H_{max} (along the ascending limb of the recruitment curve), which are more sensitive to changes in spinal excitability (Crone et al. 1990), were re-evaluated in 8 subjects (7 different subjects from above). Similar to the previous data, the larger H-reflexes (22 ± 2% of M_{max} producing MEPs of ~2 mV;) were unchanged by the afferent input at 0.9 × MT (P = 0.5), while in these same subjects the MEPs were significantly depressed by 11.4 ± 3.0% (P = 0.010).

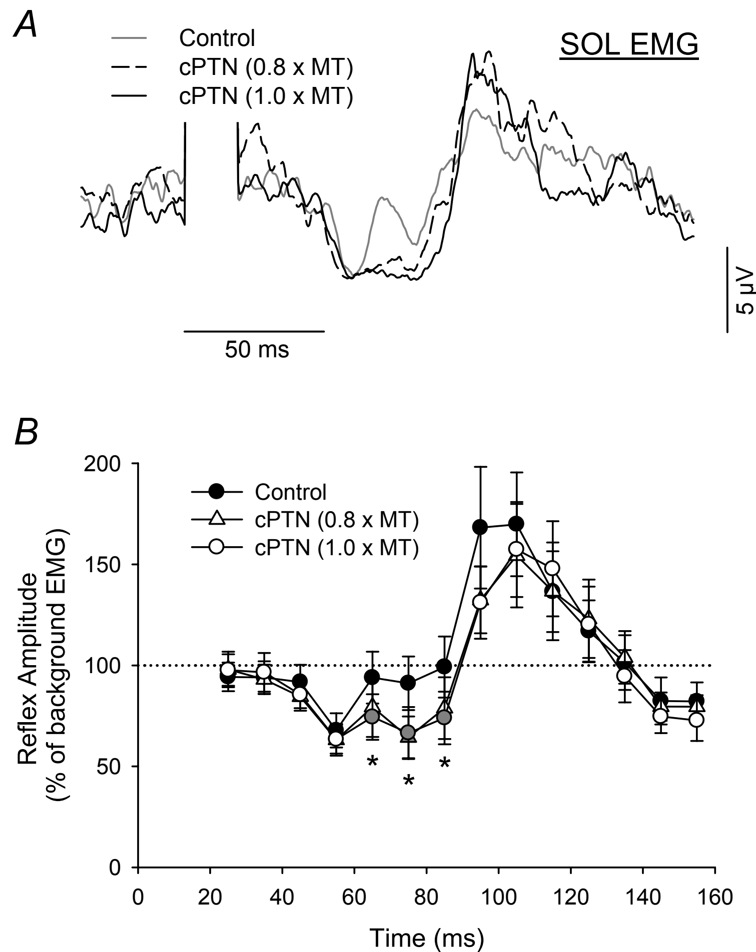

Figure 5-1. CMR in the SOL muscle combined with cPTN stimulation

A, Time course of the rectified CMR response (grey solid line) and when paired with cPTN stimulation at 0.8 × MT (black dashed line) and 1.0 × MT (black solid line). Each trace is the average of 30 sweeps. *B*, Graph shows the EMG measured in 10-ms bins following the test stimulus that occurred at 0 ms (data adjusted to the middle of the bin) for the 3 conditions: control (filled circles), cPTN stimulation at 0.8 × MT (open triangles) and 1.0 × MT (open circles). Data are from 8 subjects. Asterisks and symbols in grey represent significant differences as compared to the control response. In the 0.8 × MT condition, only the 75 ms data point (hidden) was significantly different (*$P < 0.05$).

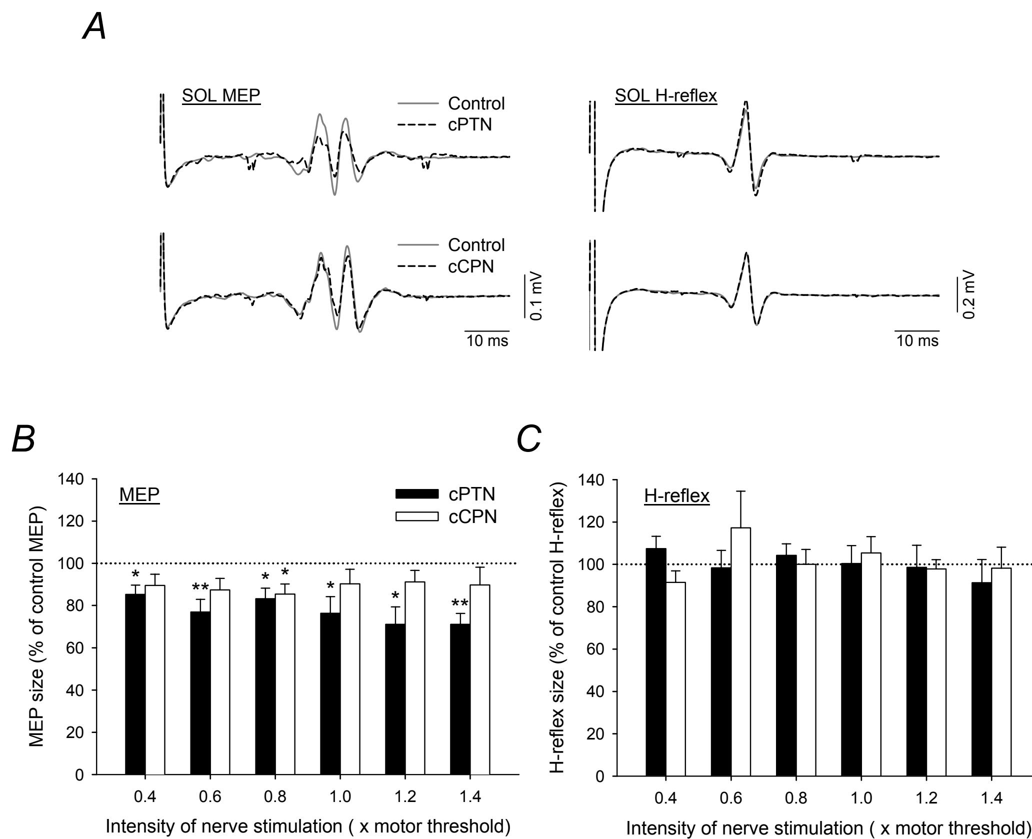

Figure 5-2. Effect of contralateral nerve stimulation on MEPs and H-reflexes in the SOL muscle during a weak plantarflexion

A, Single subject data showing that MEPs (left traces) and not H-reflexes (right traces) were inhibited with contralateral afferent stimulation (at 0.8 × MT). The MEP was more strongly reduced by cPTN stimulation. Bar graphs show changes in the MEP (*B*) and H-reflex (*C*) with cPTN (black bars) and cCPN stimulation (white bars) delivered at the different intensities. Data are from 7 subjects. Asterisks indicate significant differences as compared to the control response (* $P < 0.05$; ** $P < 0.01$).

5.3.3 Plantarflexion EMG on MEP

Given that the more direct monosynaptic pathway (*i.e.* H-reflex) was not affected by contralateral afferent stimulation, we examined the effect of increasing voluntary drive on the strength of the MEP inhibition because increasing descending drive can favour the contribution of monosynaptic, corticomotoneuronal *versus* polysynaptic corticospinal activation of the MEP (see Discussion). MEPs in 7 subjects were evaluated using a TMS intensity (74.7 ± 1.7% MSO) that elicited small MEPs when evoked at rest (~50 µV; < 1% of M_{max}, Fig. 5-3A) and with a latency of around 33 ms (Fig. 5-3C). Increasing background contractions from 2.5 to 50% MVC progressively increased the size and decreased the latency of the MEP response. For instance, during a strong plantarflexion at 50% of MVC, the average MEP was increased to near 15% of M_{max} (Fig. 5-3A) and its latency was reduced by 2 ms as compared to the resting response (Fig. 5-3C), signifying a greater contribution of the cortico-motoneuronal pathway in the MEP response (see Morita et al. 2000). During cPTN stimulation, the MEPs were only significantly reduced at rest or during weak plantarflexions at 2.5 and 7.5% of MVC (Fig. 5-3B, $P < 0.05$). The inhibitory effect was weak and non-significant when the contractions were considerably strengthened (at 25 and 50% of MVC; all $P > 0.09$). The latency of the MEP was also generally delayed by the afferent input at rest or during a weak plantarflexion. The cPTN input significantly prolonged the MEP latency by an average of 1.3 ms only during rest and at the 7.5% MVC level (open symbols in Fig. 5-3C, all $P < 0.05$).

The inhibitory effect was more variable and diminished when evaluated using a TMS intensity that elicited appreciably larger MEPs at rest (~100 µV; near ½ MEP_{max}; 86.9 ± 1.8% MSO) with 2 out of 7 subjects showing >10% facilitation (data not shown). At the stronger TMS intensity, the larger resting MEP was non-significantly suppressed by cPTN stimulation and was 88.0 ± 9.3% of the control MEP ($P = 0.24$), potentially due to the stronger involvement of the monosynaptic pathway (see Discussion).

5.3.4 Vibration on MEP

High frequency (80 Hz) vibration was applied to the contralateral Achilles tendon to determine if mechanical activation of contralateral muscle afferents (Ia and/or group II) contributed to the MEP inhibition. Weak, but not strong, tendon vibration slightly suppressed the SOL MEP in the 8 subjects ($P = 0.027$, Fig. 5-4). In comparison to the inhibition produced using electrical cPTN stimulation ($P = 0.0031$), the effect produced by the tendon vibration was half as strong.

5.3.5 Cortical inhibition

To determine if a part of the MEP inhibition was due to activation of inhibitory circuits at the level of the cortex (see Discussion), we evaluated intracortical inhibition using SICI and TMS-induced EMG suppression. SICI was evaluated in 8 subjects during a voluntary plantarflexion (at 5-10% of MVC). During cPTN

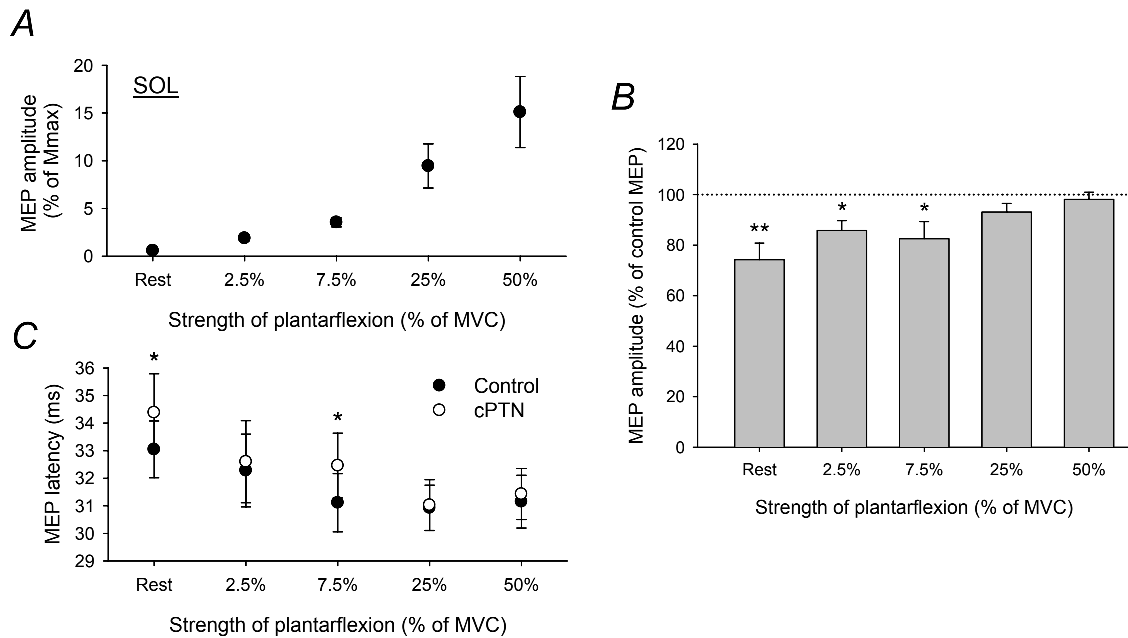

Figure 5-3. Effect of cPTN stimulation as a function of plantarflexion EMG

A, Graph shows the size of the SOL MEP collected at rest and during voluntary contraction from 2.5 to 50% of MVC. The MEP size is expressed as a percentage of the maximum M-wave. *B*, Bar graph shows changes in the MEP, expressed as a percentage of the control MEP, when conditioned with cPTN stimulation. *C*, Shows the latency of the control MEP (filled symbols) and the MEP conditioned with cPTN stimulation (open symbols). Data are from 7 subjects and asterisks indicate significant differences from the control MEP (*P< 0.05; **P<0.01).

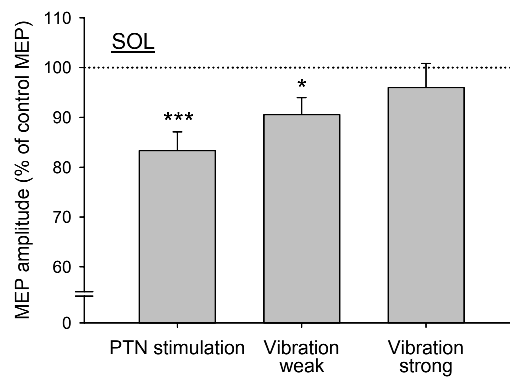

Figure 5-4. Comparing the effect of contralateral nerve stimulation *versus* vibration on SOL MEPs

Graph shows changes in the SOL MEP (1.5 ± 0.3% of M_{max}) with cPTN stimulation (at 0.9 × MT) and tendon vibration (at weak and strong intensities). Asterisks indicate differences compared to the control MEP (* P < 0.05; *** P < 0.005).

stimulation, the intensity of the test pulse was increased by 1-3% (as necessary) to match the size of the unconditioned MEP (see Fig. 5-5A). With matched test MEPs, SICI in the SOL muscle was unchanged with cPTN stimulation (P = 0.15; Fig. 5-5B). As shown for a single subject in Figure 5-5C, very low-intensity subthreshold TMS (at 65.8% of active motor threshold) suppressed the ongoing-EMG activity at a latency of approximately ~40 ms (solid grey line in Fig. 5C) with no effect on this suppression during cPTN (dark dashed line). In 9 subjects (including 8 of the subjects above), the TMS-induced EMG suppression (measured between the dashed vertical lines as in Fig. 5C) was unchanged by the contralateral nerve stimulation (P = 0.24; Fig. 5D).

5.3.6 Ankle flexor MEP

TA MEPs were evaluated to determine whether the contralateral stimulation had any affect on corticospinal circuits to the ankle flexor. In general, cPTN and cCPN stimulation weakly suppressed the TA MEP in the 8 subjects (Fig. 5-6), with TA MEPs significantly reduced only by cPTN stimulation at 1.2 × MT (P = 0.0083). In general, the inhibition in the TA muscle was considerably weaker than in the SOL muscle (see Fig. 5-2B).

5.4 DISCUSSION

The crossed inhibitory pathway was studied in the human leg by electrically stimulating contralateral nerves (25 Hz train). Stimulating the cPTN likely facilitated the activation of inhibitory circuits to the SOL motoneuron pool as seen by a reduction in the size of the MEP and by an enhancement of the early-latency inhibitory component of the CMR response. The two findings that contralateral afferent stimulation had no effect on H-reflexes and increasing voluntary drive to the SOL muscle diminished the strength of the MEP inhibition supports the notion that contralateral afferent inputs predominantly suppress SOL motoneuron activity along an inhibitory interneuronal pathway. We provide the first detailed evidence that mixed-nerve afferents can activate a crossed spinal inhibitory pathway to human SOL muscle and that the excitability of this pathway can be studied using corticospinal and CMR inputs.

5.4.1 Cutaneomuscular reflex

Afferent stimulation of the cPTN inhibited the CMR response, most notably during the early-latency inhibitory component of the CMR that occurs between 50 to 90 ms (see Fig. 5-1). The inhibitory period of the CMR response is often called the I1 component (Gibbs et al. 1995) and responses occurring at latencies earlier than 70-80 ms likely originate at the spinal cord (Pierrot-Desseilligny and Burke 2005). In the present study, cPTN stimulation enhanced the early inhibition at a latency of approximately ~65 ms and the first half of this inhibition is consistent with the facilitation of inhibitory spinal circuitry to the SOL motoneuron pool. The inhibitory

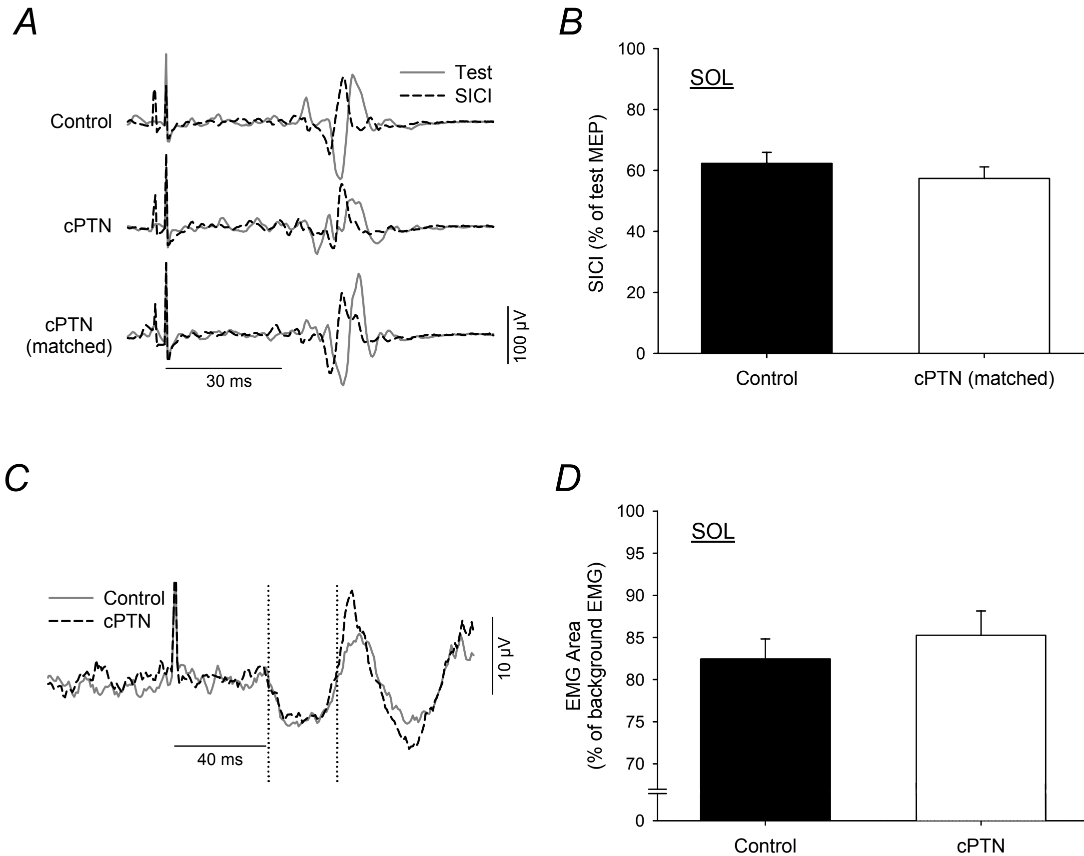

Figure 5-5. Stimulating the cPTN had no significant effect on cortical inhibition

A, Single subject data showing SICI tested during a background contraction (5-10% MVC). The test MEP was suppressed with cPTN stimulation (*middle trace*) and the test stimulus intensity was increased by 2% MSO (to 87% MSO) (*bottom trace*) to match the test MEP to the control condition (*top trace*). *B*, Group data from 8 subjects showing SICI when evaluated without (control) and with sensory stimulation (matched condition). The test MEP was 4.1 ± 0.5% of M_{max} and the conditioning pulse was 82.3 ± 0.4% of active motor threshold. *C*, Low-intensity subthreshold TMS depressed the background EMG in the single subject (between the vertical dotted lines). *D*, Group data from 9 subjects show the amount of TMS-induced inhibition without (control) and with cPTN stimulation. The subthreshold TMS intensity was 66.2 ± 0.9% of active motor threshold.

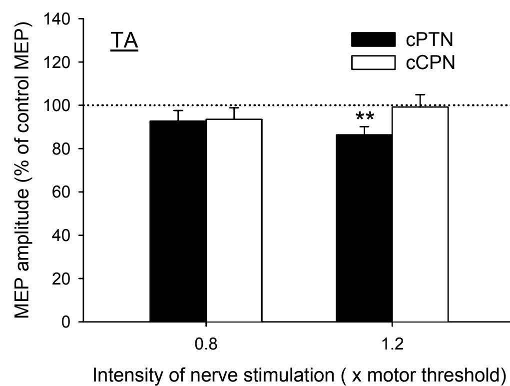

Figure 5-6. Effect of cPTN (*black bars*) and cCPN stimulation (*white bars*) on TA MEPs

Graph shows changes in the TA MEP (7.8 ± 1.2% of M_{max}) tested using a low TMS intensity (59.3 ± 3.0% MSO). Data are from 8 subjects. Asterisks indicate significant differences from the control MEP (** P < 0.01).

component of the CMR was not preceded by a prior excitatory response where effects from motoneuron synchronization could contribute to the observed EMG inhibition (Turker and Powers 2005). Given the inhibition was extended to 90 ms, it is also possible that the enhancement seen during the second half of the suppression involved supraspinal influences (although see Cortical Inhibition below).

5.4.2 Corticospinal inputs

We also examined the effect of contralateral afferent stimulation on spinal interneuronal circuits activated by the corticospinal system given the known facilitation of crossed inhibitory pathways by descending inputs (Aggelopolous et al. 1996). The fact that increasing plantarflexion strength reduced the amount of crossed inhibition of the MEP supports the notion that the MEP responses were produced by an appreciable activation of spinal interneurons. For instance, there is evidence that the lowest threshold for activating soleus motoneurons is via a long-latency, polysynaptic pathway rather than through a direct corticomotoneuronal pathway (Cowan et al. 1986; Nielsen and Petersen 1995; Morita et al. 2000). When the muscle is gradually contracted, Morita et al. (2000) have shown that the latency of the response gradually shortens by up to 4 ms in combination with an increase in the soleus MEP; as was the case for our data but by only 2 ms. At rest and during weak plantarflexion, a large portion of the soleus MEP is likely evoked through the long-latency, polysynaptic pathway. As the strength of the contraction increases, the monosynaptic corticomotoneuronal pathway likely contributes to a larger extent of the MEP (Nielsen and Petersen 1995; Morita et al. 2000). Here, the crossed inhibition was more consistently produced when the strength of the contraction was relatively weak. As the strength of the contraction or the TMS intensity was increased, thereby favouring the monosynaptic connection, much of the inhibition was washed away.

As compared to the SOL muscle where the dominant excitatory corticospinal projections are largely polysynaptic (Cowan et al. 1986), monosynaptic connections to ankle flexor motoneurons are considerably stronger (Bawa et al. 2002). As the effect of contralateral nerve stimulation was weaker in TA MEPs and the responses were only inhibited by cPTN stimulation at 1.2 × MT, it is conceivable that the lack of consistent MEP inhibition was due to a weaker engagement of the interneuronal circuitry to the TA motoneurons. Alternatively, as the strength of the crossed contralateral pathway varies between muscles (Edgley and Aggelopoulos 2006), the crossed inhibition may be weaker in the ankle flexor muscle as compared to the ankle extensors.

5.4.3 Afferents mediating crossed inhibition

During cPTN and cCPN stimulation, MEPs in the weakly contracted SOL muscle were suppressed. However, the MEP inhibition was stronger and more consistent when stimulating the cPTN, potentially due to the larger number of cutaneous afferents in the posterior tibial nerve innervating the sole of foot (see below). The inhibition of the MEP by cPTN stimulation was seen at intensities ranging from 0.4 to 1.4 × MT suggesting that the effect was first produced by low-threshold cutaneous

fibres and potentially by group I muscle afferents, which are generally activated using a single pulse at 0.5-0.6 × MT (Pierrot-Desseilligny and Burke 2005). When muscle spindles (groups I and II) were activated using low-amplitude vibrations (80 Hz train), the contralateral SOL MEPs were weakly suppressed, but by amounts that were nearly half of those produced using electrical stimulation. Interestingly, the inhibition was only observed when the vibrations were weak and was absent when the vibration intensity was increased; which was markedly different from the electrical stimuli which suppressed the MEP over all intensities. Together, these results are in agreement with the animal literature (Edgley and Aggelopoulos 2006; Frigon and Rossignol 2008) and suggest that low-threshold cutaneous inputs contribute more substantially to the crossed inhibitory pathway to the human SOL muscle.

5.4.4 Motoneuron and interneuron excitability

The inhibitory pathway onto motoneurons was evaluated using the H-reflex. Neither cPTN nor cCPN stimulation had any effect on the size of the H-reflex further suggesting that 1) the direct effect onto motoneurons produced by the stimulation was small and 2) the crossed inhibition was more strongly mediated via the activation of spinal interneuronal pathways. The lack of H-reflex inhibition contrasts findings by Koceja and Kamen (1992) who showed that the SOL H-reflex was suppressed by a mechanical tap to the contralateral Achilles tendon. Potentially, the large barrage of afferent excitation produced by the tap may have been stronger than the afferent input activated by the train of stimulation. Alternatively, it is possible that the activation of mixed nerve afferents may have produced a combination of inhibition and excitation, thereby obscuring the effect, given that cPTN and cCPN stimulation can produce H-reflex facilitation at similar latencies (~50 ms) to the tendon tap inhibition (Robinson et al. 1979; Koceja and Kamen 1992; Slivko and Teteryatnik 2005). At the motoneuron pool, it is likely that the small H-reflexes and the comparatively-sized MEPs innervated similar populations of motoneurons given that the SOL muscle is very homogeneous and its motoneurons do receive similar synaptic inputs from corticospinal and Ia neurons (Morita et al. 2000). Similarly, different mechanisms regulate H-reflexes and CMR responses evoked in the SOL muscle, and such pathways behave differently during a motor task (Zehr et al. 2001b; Zehr and Duysens 2004). There is evidence that presynaptic inhibition of Ia terminals can be reduced by cutaneous inputs to increase the size of the H-reflex (Pierrot-Desseilligny and Burke 2005) and potentially mask the crossed spinal inhibition; however such changes have only been reported in the ipsilateral limb (Iles 1996). Burke et al. (1994) has shown that descending drive during voluntary wrist extension is modulated by cutaneous stimulation. In agreement with the present results, the authors found that cutaneous superficial radial nerve stimulation depressed MEPs but had little effect on the H-reflex in wrist extensors. As descending commands can reach the motoneuron pool via a non-monosynaptic pathway, it is thought that part of the cutaneous-induced suppression to wrist muscles involves cervical propriospinal neurons.

5.4.5 *Cortical inhibition*

Stimulation of afferents in the left (conditioning) leg likely increased cortical excitability in the homonymous (right) motor cortex (Deletis et al. 1992; Nielsen et al. 1997; Petersen et al. 1998; Roy and Gorassini 2008). Since increasing cortical activity in one hemisphere (e.g., right motor cortex in our case) can actively inhibit the opposite hemisphere (e.g., left motor cortex supplying the right SOL; Perez and Cohen 2008, 2009), it is conceivable that part of the MEP inhibition from contralateral afferent stimulation might be happening at the level of the cortex. For instance, muscle vibration in the contralateral hand can depress MEPs in the opposite hand, in parallel with increases in SICI and interhemispheric inhibition (Swayne et al. 2006; although see Kossev et al. 2001). To test the possibility that suppression of the SOL MEP occurred at a cortical level, we examined cortical inhibition using SICI and subthreshold TMS. SICI in the plantarflexed muscle was unchanged by the contralateral nerve stimulation. SICI in the SOL muscle is nonetheless stronger at rest (Soto et al. 2006) and it is potentially less susceptible to changes in excitability during a contraction (as done here). However, since we also failed to observe increases in cortical inhibition using subthreshold TMS, which requires a voluntary contraction, the present data add to the body of evidence that the reduction in the MEP was primarily due to the inhibition of neurons that reside in the spinal cord. However, other cortical circuits, including those involved in long-interval intracortical inhibition and afferent inhibition still need to be examined to rule out the absence of a cortical mechanism.

5.4.6 *Conclusion and functional implications*

Trains of contralateral afferent stimulation activated the crossed inhibitory pathway in the human leg. The crossed inhibition was unlikely present in the motor cortex or at the motoneuron pool indicating that interneuronal pathways likely mediated the crossed inhibition. Inhibitory interneurons in the mid-lumbar spinal cord with crossed projections (Bannatyne et al. 2006) or activation of Ia inhibitory interneurons by excitatory commissural interneurons (Jankowska et al. 2005) are two possible candidates. Crossed inhibitory connections, in combination with crossed excitation, provide flexibility to the motor system. Its function, although speculative, may be to synchronize EMG activity in both limbs and/or slow down forward progression during locomotion by suppressing contralateral extensor activity (Frigon and Rossignol 2008). In the present study, we provide evidence that low-threshold afferents can inhibit the spinal circuitry to contralateral SOL motoneurons, and can be studied non-invasively in humans using corticospinal and cutaneomuscular inputs.

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CHAPTER 6: Short-interval intracortical inhibition to the ankle flexor after incomplete spinal cord injury

6.1 INTRODUCTION

Gamma-Aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the brain, and is crucial for maintenance of cortical representations in animals (Jacobs and Donoghue 1991; Capaday and Rasmusson 2003). In humans, regulation of GABAergic inhibition is vital for cortical plasticity given that pharmacological agents that up-regulate GABAergic inhibition prevent the induction of cortical reorganization (Ziemann et al. 2001; Werhahn 2002; for review see Chen et al. 2002). After incomplete spinal cord injury (SCI), changes to the ascending and descending pathways can induce cortical plasticity as seen by alterations in the motor cortical maps assessed using transcranial magnetic stimulation (TMS) (Levy et al. 1990; Topka et al. 1991; Brouwer and Hopkins-Rosseel 1997). Corticospinal connections to leg muscles affected by a spinal cord lesion are strengthened during functional recovery of walking (Thomas and Gorassini 2005; Wirth et al. 2008), and may in part be regulated by changes in GABAergic function (Thomas and Gorassini 2005; see also Belci et al. 2004).

There is some suggestion that cortical inhibition is reduced following SCI (Davey et al. 1998; Smith et al. 2000a). Such changes have been documented using subthreshold TMS and measuring the subsequent suppression of ongoing voluntary EMG activity. Low-intensity subthreshold TMS can temporarily inhibit the ongoing EMG (Davey et al. 1994), likely through the activation of inhibitory cortical neurons with oligo-, or possibly disynaptic, connections onto fast-conducting corticospinal tract neurons that drive the voluntary contractions (Butler et al. 2007). Within several weeks of SCI, cortical inhibition to muscles below the injury may already be reduced on the basis that the onset of EMG suppression by subthreshold TMS is considerably delayed. In fact, the onset of the EMG suppression in SCI subjects is ~25 ms longer than the MEP latency (~13 ms in healthy controls), suggesting that the early part of the cortical inhibition is diminished due to the injury (Davey et al. 1998; Smith et al. 2000a). However, a greater involvement of slowly-conducting corticospinal tract axons to voluntarily activated EMG may also explain the greater delay in EMG suppression after SCI.

In support of a reduction in intracortical inhibition, there have been two single-subject reports showing that short-interval intracortical inhibition (SICI), evaluated using paired-pulse TMS (Kujirai et al. 1993), is also suppressed after SCI (Shimizu et al. 2000; Saturno et al. 2008). Such paired-pulse data may provide a more direct measure of intracortical inhibition as it measures the magnitude of MEP inhibition from a prior subthreshold, conditioning TMS pulse. Although the results are in agreement with the EMG-suppression data of Davey's group, these latter findings

may be hampered by the fact that SICI was only tested at a single conditioning intensity. It has become increasingly important to study SICI over a range of conditioning intensities to rule out the contribution of short-interval intracortical facilitation (SICF) which can impinge on the SICI-mediated MEP inhibition (Ortu et al. 2008; Peurala et al. 2008; Roy 2009). Moreover, because the intensity of the conditioning stimulus is based on active motor threshold, damage to the spinal cord will raise the stimulus intensity required to produce an active motor response and invariably, increase the intensity of the conditioning stimulus compared to uninjured controls. Therefore, in the present study we systematically examined SICI in the lower limb of SCI subjects using conditioning intensities that were above and below the motor threshold of uninjured subjects. We provide evidence that, during a voluntary contraction, SICI in the ankle flexor is reduced after SCI as compared to uninjured controls. However, questions arose as to how much the MEP suppression by a prior subthreshold TMS is purely mediated by cortical mechanisms.

6.2 METHODS

6.2.1 Subjects

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 subjects. Most of the subjects with an incomplete SCI were recruited to participate in locomotor rehabilitation and the experiments were done at various stages during their training. All experiments were done on a rest day between training sessions. Our sample comprised of 12 subjects with an incomplete SCI (3 female) aged 20 to 69 (48.3 ± 12.8; mean ± SD; Table 1) and 5 healthy control subjects (2 females) aged 20 to 68 (37.2 ± 19.0; mean ± SD). Inclusion criteria for the study were that subjects must have sustained damage to the spinal cord and all participants were classified as either ASIA C or D (Table 1). Data from muscles on the right and left leg in a single subject were considered to be independent because of the asymmetry in the lesion location in the SCI subjects.

6.2.2 Recordings and stimulation

Surface electromyography (EMG) was collected from one or both tibialis anterior (TA) muscles using pairs of Ag-AgCl electrodes (Kendall,Chicopee, MA). The signals were amplified (500 or 1k gain) and filtered (10-1000 Hz band-pass) (Octopus, Bortec Technologies, Calgary, Canada) and were digitized at a rate of 5 kHz using Axoscope hardware and software (Digidata 1200 Series, Axon Instruments, Union City). Rectified and heavily smoothed EMG (100-ms time constant) from the TA muscle was displayed on an oscilloscope at a fast time sweep to help the subjects maintain a steady contraction.

TMS was applied to the contralateral motor cortex using a custom made figure-of-eight coil (P/N 15857: 90 mm external wing diameter) or a double cone coil. The coil was chosen based on the severity of the lesion and its ability to elicit maximum MEPs below the injury (see below). Single and paired-pulse TMS were delivered using two

Code/ Sex	Age	Years Post Injury	Cause of Injury	Injury Level	ASIA Score	Medication	Side(s) Tested
1M	56.9	34.2	Trauma	C5-6	D	oxybutynin, tamsulosin	left
2F	48.1	1.3	Trauma	C6	C	baclofen, gabapentin, tolterodine	left
3M	41.7	1.1	Trauma	C3-4	C	baclofen, gabapentin, oxybutynin, oxycodone	right, left
4F	69.4	2.5	Surgery	T4, L5	D	oxybutynin, oxycodone	right
5M	63.3	20.0	Trauma	C4-5	D	none	right, left
6M	33.6	1.1	Trauma	C4-5	C	baclofen, pregabalin	right, left
7M	60.7	2.4	Trauma	C5	D	baclofen, pregabalin	right, left
8M	44.1	17.6	Trauma	T12	D	none	right, left
9M	25.0	1.0	Trauma	T4,11,12	C	gabapentin	right
10F	42.7	1.9	Trauma	C6,7	C	none	right
11M	52.3	2.6	Trauma	T5/6	C	tamsulosin	right
12M	41.6	1.6	Trauma	C3, C4	D	baclofen, pregabalin	right

Table 6-1. Details of subject with incomplete SCI

The age of the subject and the number of years after the subject sustained a spinal cord injury measured at the time of the experiment are shown. *Medication*: baclofen is an antispastistic agent; gabapentine, oxycodone and pregabalin help alleviate pain; oxybutynin and tolterodine are used in the treatment of overactive bladder symptoms; tamsulosin treats the symptoms of an enlarged prostate.

MagStim 200 stimulators connected to a BiStim module (Magstim, Dyfed, UK). The optimal spot to the TA muscle was identified using an intermediate TMS intensity and was marked on the scalp using a felt tipped pen (generally 1-2 cm lateral of vertex with the figure-of-eight and 1 cm more posterior with the double cone). The coil was held in place by the experimenter and was orientated to induce postero-anterior currents in the brain.

6.2.3 Short interval intracortical inhibition

SICI was tested in the contracted TA muscle using the Kujirai protocol (1993) with an inter-pulse interval of 3 ms. Since resting MEPs are often diminished or absent after a spinal cord lesion, SICI was tested during a voluntary contraction corresponding to ~15% of the subjects maximum voluntary contraction (MVC). Twelve SCI subjects (17 legs) and 5 healthy controls (10 legs) were tested. The intensity of the test stimulus was near ½MEP_{max} and the conditioning stimulus was referenced to the active motor threshold (AMT), which was defined as the TMS intensity which produced clearly distinguishable MEPs in the contracted muscle approximately ~50% of the time. The maximum MEP response (MEP_{max}) was also assessed using the double cone coil set to 80% of the maximum stimulator output (MSO), an intensity that was maximally tolerable, or the figure-of-eight coil at 100% MSO.

SICI was generally tested using the figure-of-eight coil. Due to an elevated threshold in a few of the SCI subjects, 3 of the limbs (17.6 % of the experiments) were tested using the double cone coil. Similarly, 2 of the uninjured control limbs were tested using the double cone coil (20% of the experiments) to control for the type of coil. Since a spinal cord lesion increases the MEP threshold (Davey et al. 1998) and neuronal circuits that mediate SICF can impinge on the cortical inhibition (Ortu et al. 2008; Peurala et al. 2008; Roy 2009), which are tested at supra-threshold intensities, we evaluated SICI using various conditioning intensities that ranged from 60 to 110% of AMT. Intensities of 50 to 120% of AMT were used in the uninjured control subjects to bracket the intensities used in the SCI group. A minimum of 6 paired-pulse responses were collected at each intensity, which were intermixed with 18 single pulse responses.

6.2.4 Data analysis

MEPs were analyzed peak-to-peak. The level of background EMG was evaluated from the 100-ms window before the stimulus. Because the amount of SICI was considerably reduced after injury, appreciable amounts of SICI in the SCI subjects was considered to be >5% whereas this value was set to >15% in the uninjured controls. The chosen cutoffs were close to half of the inhibition produced at intensities of 70 to 90% of AMT in both groups of subjects. The number of conditions producing appreciable amounts of inhibition were expressed as a percentage of the total number of times the intensity was tested (see Figs. 6-1C and 6-2C). Statistical comparisons were done using paired t-tests. Relationships between

the different measurements were evaluated using the Pearson product–moment correlation. Data are means ± SEM. Significance was set at $P < 0.05$.

6.3 RESULTS

SICI was measured in 17 limbs afflicted by spinal cord trauma and compared to responses from 10 limbs of uninjured control subjects. Since a spinal cord lesion interferes with the threshold to elicit a motor response from TMS, SICI was evaluated over a range of conditioning intensities that was above and below the motor threshold of uninjured subjects (Fig. 6-1). Likewise, it was necessary to measure SICI in the SCI subjects during a small background contraction to obtain an appreciable test MEP. The intensity of the test MEP was near ½MEP_{max} in both SCI subjects (72.8 ± 3.1% MSO) and uninjured controls (62.3 ± 2.6% MSO). AMT in the SCI subjects was 57.4 ± 2.6% MSO and was significantly higher than the uninjured control group which was 49.2 ± 1.6% MSO ($P = 0.034$).

Figure 6-1A shows the effect of paired-pulse TMS in SCI subjects evaluated at the different conditioning intensities. In general, most of the SCI subjects showed a "U" shaped relationship between the conditioning intensity and the amount of inhibition, which was generally centered on 80% of AMT. In the group average, the test MEP was significantly reduced by 14.5 ± 3.7% at 80% AMT (marked by grey symbol in Fig. 6-1A; $P = 0.0013$). At 110% of AMT, the stronger conditioning stimulus facilitated the MEP in line with the recruitment of SICF ($P = 0.037$). In the uninjured subjects, the MEPs were significantly reduced over a larger range of conditioning intensities from 60 to 90% of AMT (Fig. 6-1B), and the MEPs were facilitated at 120% of AMT (all $P < 0.05$). Similar to the SCI subjects, inhibition in the uninjured controls was maximal at 80% of AMT and the test MEP was reduced by 35.6 ± 6.6%. Figures 6-1C show the distribution of TMS intensities that induced >5% inhibition in the SCI subjects and >15% inhibition in the uninjured controls. The TMS intensities that resulted in the largest amounts of inhibition were remarkably similar between the groups (in terms of AMT), with SICI being most prevalent at 80% of AMT. In particular, the test MEPs were reduced by >5% in 14 of the 17 limbs afflicted by the lesion when the conditioning stimulus was set to 80% of AMT.

In the SCI subjects, neither MEP_{max} (Fig. 6-1D), the test MEP size (Fig. 6-1E), or the level of background EMG (Fig. 6-1F) was correlated with the maximum SICI in each of the subjects (open symbols), indicating the neither the severity of the injury or the measurement parameters confounded the results. As well, none of these relationships were significantly correlated in the uninjured controls (grey symbols in Fig. 6-1D-F).

Figure 6-2A compares the average recruitment of SICI in both SCI and uninjured subjects plotted as function of AMT. Although the MEPs were modulated in a similar "U" shaped pattern, SICI in the SCI subjects was significantly lower at conditioning intensities between 60 to 90% of AMT as compared to the intact subjects (all $P < 0.05$). Indeed, the maximum SICI was considerably stronger in the uninjured controls (41.6 ± 4.9% inhibition) rather than the SCI subjects (20.2 ± 3.2% inhibition; $P = 0.00090$). Interestingly, when the SICI curves were plotted as a function of the mean stimulation intensity (expressed in MSO), the spinal cord lesion shifted the curve to

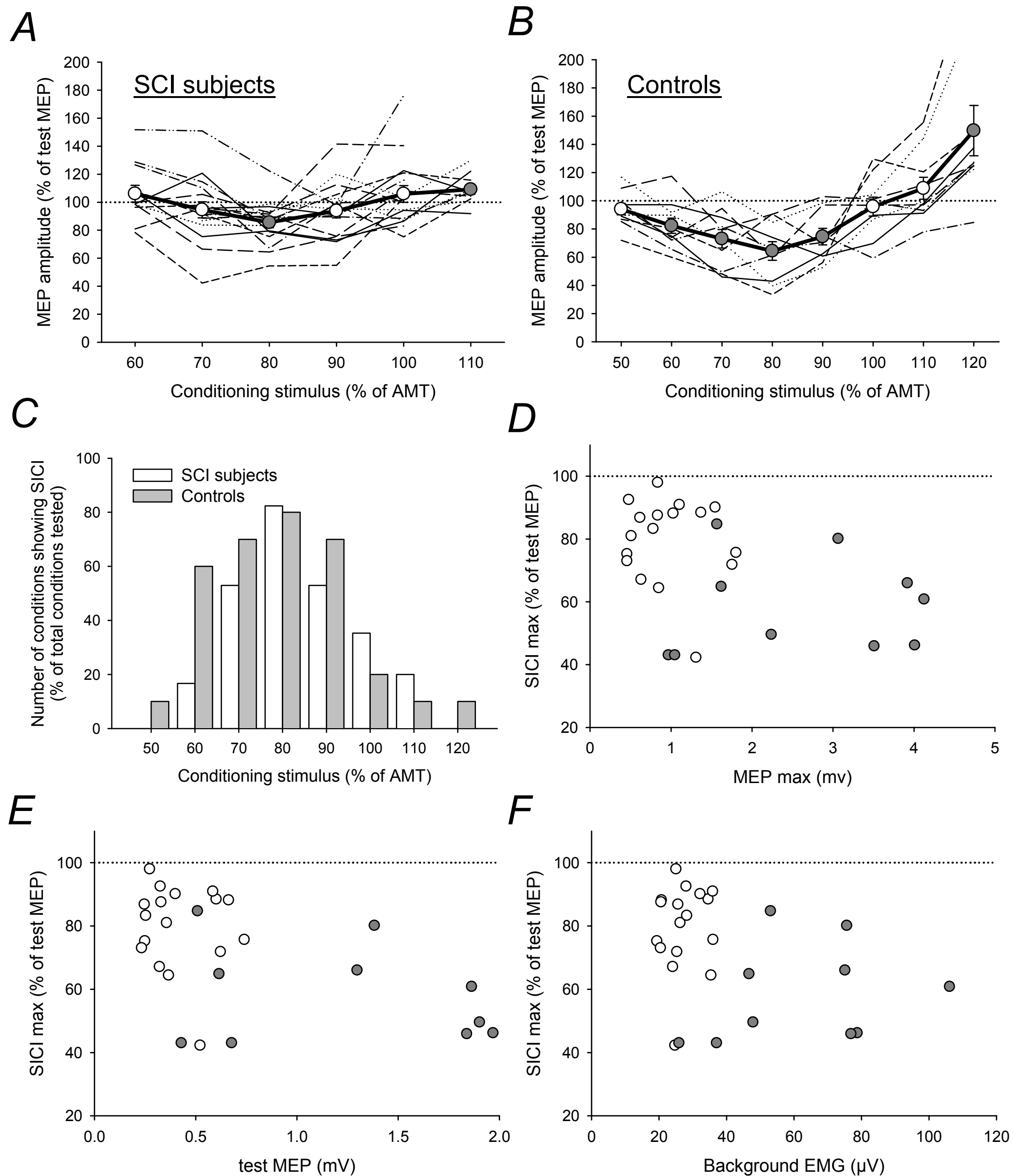

Figure 6-1. SICI in SCI subjects and uninjured controls evaluated over a range of conditioning intensities

Graphs display the individual paired-pulse inhibition in both SCI subjects (*A*) and uninjured controls (*B*), expressed as a percentage of the test MEP. Thick solid lines and open symbols represent the average. Symbols in grey represent significant differences from the test MEP ($P < 0.05$). The two data points not shown in (*B*) were 231 and 271% (at 120% of AMT). *C*, Bar graphs display the percentage of conditions showing appreciable inhibition in the SCI subjects (white bars; >5% SICI) and uninjured controls (grey bars; >15% SICI). The maximum SICI plotted as a function of MEP_{max} (*D*), the size of the test MEP (*E*) and the level of background EMG (*F*) in the injured (open symbols) and uninjured subjects (grey symbols).

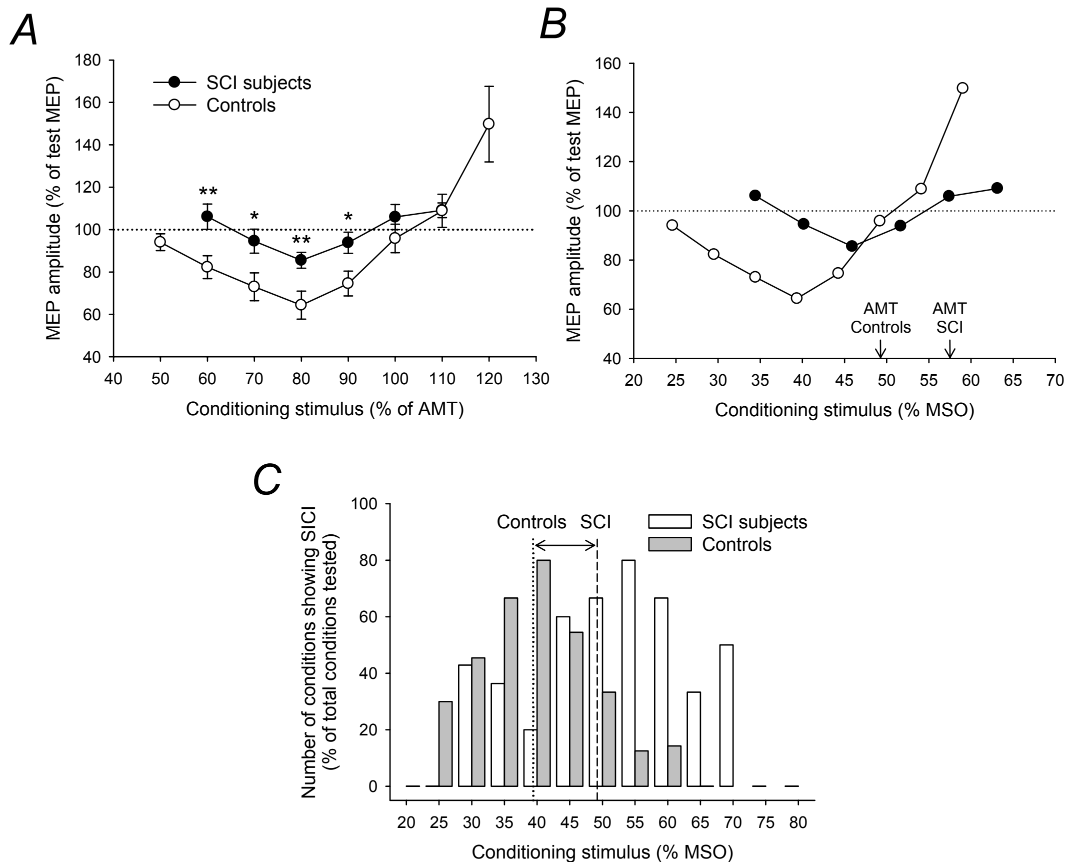

Figure 6-2. Comparing the magnitude and distribution of SICI in the SCI subjects and the uninjured controls

A, graph shows the average amount of SICI in the SCI subjects (closed symbols) and the uninjured controls (open symbols) plotted as a function of AMT. *B*, Displays the same curves shown in (*A*) but plotted in terms of MSO. *C*, Bar graph shows the distribution of intensities which produced appreciable inhibition in the SCI subjects (>5% SICI; white bars) and uninjured controls (>15% SICI; grey bars). The x-axis represents the centre of the bins and the data is expressed as a percentage of the total conditions tested. The vertical lines represent the mean intensity which produced SICI in the SCI subjects (dashed line) and the uninjured controls (dotted line). Asterisks indicate significant differences between the two groups (* P < 0.05; **P < 0.01).

the right suggesting that higher TMS intensities were required to recruit SICI. Likewise, when the incidence of appreciable SICI from Figure 1C was plotted in terms of actual MSO intensities (Fig. 6-2C), the mean MSO intensity producing appreciable SICI in the SCI subjects (49.2 ± 1.6% MSO) was significantly higher than the uninjured controls (39.4 ± 1.5% MSO; P = 0.000054).

6.4 DISCUSSION

6.4.1 SICI after SCI

In agreement with the previous case-study reports (Shimizu et al. 2000; Saturno et al. 2008), a spinal cord lesion significantly reduced the amount of paired-pulse inhibition. As compared to the uninjured controls, SICI was reduced at conditioning intensities ranging from 60 to 90% of AMT, and at its peak was reduced by half. Although AMT was increased by the injury, the "U" shaped recruitment of SICI (relating the conditioning intensity to the amount of inhibition) was very similar in both groups, suggesting that circuits mediating SICI maintained some residual activity after the injury. For both injured and uninjured subjects, the average inhibition was maximal at 80% of AMT, and amplitude of SICI was 14.5 % and 35.6%, respectively.

When the amount of SICI was plotted as a function of the actual TMS intensity, the "U" shaped curves were no longer aligned (see Fig. 6-2B). In fact, the MEP inhibition in the SCI subjects was produced at significantly higher TMS intensities. In addition, lower conditioning intensities, similar to those employed in the uninjured subjects (i.e. < 40% MSO), were less likely to produce inhibition in the SCI subjects. Such findings are in agreement with Davey et al. (1998) and Smith et al. (2000a) who showed that higher subthreshold TMS intensities are required to suppress the voluntary background EMG after an incomplete injury. Because afferent regulation of motor cortex excitability is impaired following injury (see Chapter 4), it is possible that the threshold for intracortical inhibitory circuits may have increased due to the loss of afferent inputs reaching the motor cortex. SICI is mediated by GABA-A receptor activation (Ziemann et al. 1996; Di Lazzaro et al. 2000) and GABAergic circuits participate in cortical reorganization following the removal of afferent inputs, such as during transient limb deafferentation (Ziemann et al. 1998; for review see Chen et al. 2002). Although we have recently shown in uninjured controls that sensory input from the leg reduces SICI (Roy and Gorassini 2008), after SCI it is possible that the incomplete lesion may down-regulate the activity of GABA-related circuits that project to muscles affected by the injury (see also Smith et al. 2000a, 2000b) to help maximize the recruitment of excitatory pathways.

Conditioning the motor cortex at 110% and/or 120% of AMT produced an increase in the MEP response that is consistent with the recruitment of SICF. Given that intracortical SICF (Di Lazzaro et al. 1999) was equivalent in both subject groups at 110% of AMT, such findings provide evidence that the decreased SICI in the SCI subjects was not due to an increase in SICF.

6.4.2 Matching conditions

When evaluating SICI in the resting muscle, there is evidence both for and against the importance of matching the size of the test MEP (Daskalakis et al. 2002; Roshan et al. 2003; Garry and Thompson 2009). Here, the MEPs were collected during a tonic contraction, and there was no relationship between the test MEP size and the maximum SICI value. Thus, the different sizes of test MEPs did not likely contribute to the SICI differences between the two groups. Since a spinal cord lesion impedes voluntary muscle strength, we made an effort to control for the amount of voluntary drive between the different subjects (15% of MVC); though as result, the actual background EMG was unmatched between the groups. However, since SICI is favoured under conditions of low EMG activity (Ortu et al. 2008), it is also possible that the inhibition measured in the uninjured control group may have been underestimated because of their stronger contractions. Matching for the level of background of EMG between the two groups would have likely shown an even larger reduction in SICI after SCI. Indeed, it would be beneficial to measure SICI while matching for the test MEP size and the level of background EMG to ensure that a reduction in SICI is the result of a decrease in cortical inhibition, and not because of technical limitations of the paired-pulse technique (work in progress).

6.4.3 Does the spinal cord contribute to SICI?

SICI was first reported by Kujirai et al. (1993) and there has been ample evidence from spinal reflexes and epidural recordings of descending corticospinal volleys to suggest that SICI is cortical in origin (Nakamura et al. 1997; Di Lazzaro et al. 1998). Similarly, descending volleys in primates are prominently reduced by a prior motor cortex stimulation (at a 3.3 ms ISI) in line with a supraspinal mechanism (Patton and Amassian 1954). In the present study, SICI was more prevalent in the SCI subjects using a stronger conditioning stimulus. Indeed, it is possible that the requirement for a stronger TMS pulse can be explained by an increase in the threshold for intracortical inhibitory circuits (as described above), or alternatively, it may indicate that inhibitory spinal circuits activated by descending pathways (from subthreshold TMS) can contribute to SICI. Data from several groups have shown that motor cortex stimulation activates spinal inhibitory interneurons projecting to lower limb motoneurons, particularly to leg/hindlimb extensors (Cowan et al. 1986; Preston et al. 1967), at a latency that is 1-2 ms longer than the monosynaptic excitation (Iles and Pisini et al. 1992; Nielsen and Petersen 1995) Such short-latency inhibition of the H-reflex in ankle muscles by TMS can be produced at intensities below motor threshold (Nielsen et al. 1993; personal observations). Likely, the H-reflex involves a similar but non-overlapping proportion of the motoneuron pool engaged by the paired-pulse MEP. The rightward shift in the "U" shaped SICI profile seen in the SCI subjects (see Fig. 6-2B) may provide indirect support of a spinal inhibitory mechanism which was engaged by descending pathways activated by the subthreshold stimulus (before the arrival of the test MEP). Thus, it is worthwhile investigating why the threshold for SICI is higher in individuals with a spinal cord lesion even though the "U" shaped relationship is well preserved (see also Smith et al. 2000a). This data may be relevant in light of the fact that intracortical inhibition (as shown here) and spinal inhibitory

mechanisms are both reduced after an incomplete SCI (reviewed in Pierrot-Desseilligny and Burke 2005; see also Norton et al. 2008).

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CHAPTER 7: Suppression of EMG activity by subthreshold paired-pulse transcranial magnetic stimulation to the leg motor cortex

A version of this chapter has been published.
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7.1 INTRODUCTION

Very low-intensity transcranial magnetic stimulation (TMS) that is subthreshold to a motor response can inhibit ongoing EMG activity in a voluntarily contracted muscle without producing any preceding excitation (Davey et al. 1994). The suppression of voluntary motor activity is thought to occur through the activation of intracortical GABAergic interneurons (Classen and Benecke 1995) and acts to reduce motor output to the target muscle. In surface EMG, the onset of the suppression occurs approximately 10 ms after the motor evoked potential (MEP) latency (Petersen et al. 2001), which raises the question of whether low-intensity TMS only affects slowly-conducting corticospinal pathways. However, Butler et al. (2007) have recently shown with motor unit recordings, which have a greater temporal resolution compared to surface EMG (Pierrot-Deseilligny and Burke 2005), that the latency of the inhibition is actually only 2-3 ms later. Thus, low-intensity subthreshold TMS likely inhibits the ongoing activity of fast-conducting corticospinal cells; the pathway that is considered to provide descending drive to motoneurons during voluntary movements (Butler et al. 2007, see comment in Rothwell 2007).

The use of subthreshold TMS to suppress voluntary activity offers a method to evaluate the contribution of the corticospinal pathway during a motor task (Nielsen 2002; Butler et al. 2007). This approach is particularly relevant since the motor system is *relatively* unperturbed by the cortical stimulus, but can still depress the EMG activity in lower leg muscles by 17-20% during a task such as walking (Petersen et al. 2001). However, since a large number of stimuli (~100) and relatively few TMS intensities can yield a distinguishable period of inhibition, it is beneficial to devise approaches to increase the size and robustness of the depressed EMG activity. In the present study, we investigated whether pairs of subthreshold TMS to the leg motor cortex could enhance the amount of EMG suppression in the tibialis anterior (TA) muscle. We hypothesised that the inhibition would be increased by adding of a second stimulus and thus characterized the effects of pairs of subthreshold TMS at interstimulus intervals (ISIs) ranging from 1 to 12 ms on the voluntary EMG activity in the TA muscle.

7.2 METHODS

7.2.1 Subjects

Ten healthy volunteers (6 of whom were female) aged 21-41 (27 ± 6; mean ± SD) were recruited in this study. All subjects gave their informed consent to participate in the study and the protocol was approved by the Human Ethics Research Board at the University of Alberta. The experiments conformed to the Declaration of Helsinki.

7.2.2 Experimental setup

Subjects were comfortably seated with the right leg slightly bent at the knee and the foot secured to a footplate. Surface electromyography (EMG) was recorded from the tibialis anterior (TA) muscle on the right leg using Ag-AgCl bipolar surface electrodes (BiPole, Bortec Technologies, Calgary, Canada). EMG signals were amplified 1000 times and filtered using a band-pass of 10 to 1000 Hz (Octopus, Bortec Technologies). EMG signals were digitized using Axoscope hardware and software at a rate of 2.5 kHz (Digidata 1200 Series, Axon Instruments, Union City, USA) and stored on a personal computer for off-line analysis. To maintain a constant level of contraction, the EMG was rectified, low-pass filtered using a 100 ms time-constant (NL703, Digitimer, Hertfordshire, UK) and displayed on an oscilloscope. Transcranial magnetic stimulation (TMS) was delivered using two Magstim 200 stimulators connected to a Bistim module and a double-cone coil (Magstim, Dyfed, UK). The optimal site of stimulation was identified over the left motor cortex using an intensity that was slightly above the threshold in the pre-contracted muscle. The coil was secured in place and orientated to induce antero-posterior currents in the brain.

7.2.3 Protocol

A single pulse of subthreshold TMS was delivered during tonic dorsiflexion, corresponding to 5-10% of the subject's maximum voluntary contraction. Active motor threshold (AMT) was defined as the intensity that produced a distinguishable MEP in 50% of the trials. The stimulus intensity was then adjusted to suppress the level of background EMG while ensuring that the activity preceding the suppression was not enhanced, so as to avoid any post-activation inhibition of motoneurons (Pierrot-Deseilligny and Burke 2005). Both magnetic stimulators were set to the same percentage of the maximum stimulator output (MSO). The effect of paired-pulses of very low-intensity TMS was evaluated at seven ISIs ranging from 1 to 12 ms (*i.e.* 1, 2, 3, 5, 7, 9 and 12 ms). Pulses were given in blocks of 25 stimuli (~ 1.5 min per block). Short breaks were provided between blocks to mitigate the effect of fatigue since central fatigue can develop during sustained submaximal contractions (see Taylor and Gandevia 2008). One hundred stimuli were delivered at each ISI administered in a pseudorandom order and intermixed with 100 single TMS pulses. The time between consecutive stimuli was 3-4 s.

7.2.4 Data Analysis

The EMG data was analysed off-line using Matlab (The MathWorks, Inc, Natick, MA, USA). To improve the clarity of the EMG suppression, two approaches were used to generate the 'average rectified EMG': rectification and the Hilbert transform. Suppression of EMG occurs as a modulation in the EMG envelope and a Hilbert transform is an effective envelope extraction technique (Myers et al. 2003). Rectification and the Hilbert transform were applied separately to the EMG recordings. All rectified EMG and transformed data were subsequently averaged. The onset and end of the suppression were estimated by visual inspection of the EMG recordings and were usually at points which crossed the level of background EMG. The mean level of background EMG was measured in the 300-ms window that preceded the stimulus. Since EMG facilitation produced with subthreshold TMS (> 70% of AMT) is less pronounced than what is seen at higher TMS intensities, the onset and end of the excitation were defined as the MEP latency and the onset of the suppression, respectively. This approach had a bias for EMG values that were slightly above the background level since the end of excitation (*i.e.* onset of suppression) was usually at points having a negative slope which crossed the level of background EMG. EMG suppression and facilitation were measured in terms of area and expressed as a percentage of the background EMG activity. EMG suppression and facilitation were analyzed separately using a one-way repeated measures analysis of variance (ANOVA) treating the stimulation protocol as within-subject factor and post-hoc paired *t*-tests (2-tailed). The relation between the stimulus intensity and the amount of EMG facilitation at ISIs of 1-3 ms was measured using the Pearson product-moment correlation (*r*).

We evaluated the signal-to-noise ratio (SNR) of the quantified inhibition for the single-pulse and the 7 ms paired-pulse conditions. Various numbers of sweeps (*i.e.* 10, 20, …, 100) were used to yield the rectified average EMG. The SNR refers to the squared ratio of the signal (*i.e.* amplitude of the depressed EMG below the background level) and the background noise (*i.e.* variability in the EMG signal above and below the background level measured before the stimulus) and was evaluated in decibels using the following equation

$$SNR(dB) = 20\log_{10}\left(\frac{RMS_{signal}}{RMS_{noise}}\right).$$

Both signal and noise were evaluated over an equivalent duration of time and calculated using the root mean square (RMS). For consistency, the inhibition was measured over the period that was chosen from the 100 sweeps. Differences in the SNR between the two conditions (single and 7 ms paired-pulse) were assessed using a two-way repeated measures ANOVA and post hoc paired *t*-tests. The significance level was set at $P < 0.05$ and data are given as means ± SEM.

7.3 RESULTS

A single pulse of very low-intensity TMS to the leg motor cortex suppressed the average rectified EMG without producing any preceding excitation (see Methods). The TMS intensity used for the single pulse was 73 ± 1% of AMT (AMT = 35 ± 1% MSO) and depressed the level of ongoing background EMG by -14.9 ± 1.6%. The effect was weak in 2 out of the 10 subjects showing < 10% inhibition. The average latency of the suppression was 38.1 ± 0.7 ms, and for each subject, was on average 9.0 ± 0.7 ms longer than the MEP latency. The duration of the suppression was ~20 ms.

Figure 7-1A shows the effect of delivering paired-pulses of subthreshold TMS in two different subjects at the optimal intensity determined for the single pulse (see above). Compared to the single pulse response, the amount of EMG suppression (shaded areas in Fig. 7-1A) was enhanced using paired-pulses for subject 1 (at ISIs of 2 to 12 ms) and subject 2 (at ISIs of 3, 7, 9 and 12 ms). Over these ISIs, the average inhibition was increased by +23%. At the shorter intervals (1-3 ms), paired-pulses also produced EMG facilitation during the period that immediately preceded the suppression in both subjects. This EMG facilitation was most prominent for subject 2 at ISIs of 2 and 3 ms.

Given that EMG excitation might interfere with the period of inhibition (see below), the depression was not quantified at intervals of 1-3 ms which were prone to facilitation. Paired-pulse TMS at the remaining ISIs tended to reduce the EMG activity above the amount produced using a single pulse (filled symbols in Fig. 7-1B). A one-way repeated measures ANOVA showed a significant effect of the stimulation protocol on the size of the suppression ($F(4,36) = 5.48$, $P = 0.001$). The inhibition was significantly greater using the 7 ms interval ($P = 0.0008$) and the EMG activity was suppressed by an additional +42% compared to the single-pulse. In addition, the 7 ms protocol significantly extended the period of inhibition elicited using the single pulse by 3.9 ± 1.5 ms ($P = 0.027$), but had no effect on the onset of the suppression.

Prior to the onset of the inhibition, the short intervals of 1 to 3 ms also produced a brief increase in EMG activity with a latency of ~30 ms (open symbols in Fig. 7-1B). Over all conditions, there was a significant effect of the stimulation protocol on the amount of EMG excitation (one-way ANOVA: $F(7,63) = 4.07$, $P = 0.001$) and the level of EMG was significantly higher for the 2 ms condition than the single pulse ($P = 0.002$). By visual inspection of the recordings, 9 out of 10 subjects showed some indication of descending excitation at one of the three ISIs, albeit only trace amounts in 3 of the subjects. Interestingly, the average EMG excitation measured over ISIs of 1-3 ms was positively correlated with the stimulus intensity when expressed as a percentage of AMT ($r = 0.65$; $P = 0.042$).

Since techniques that increase the size of the suppression might be beneficial for studying the contribution of corticospinal inputs onto motoneurons, we examined the SNR of the suppression. For the single and 7 ms paired-pulse protocols, there was a significant interaction effect between the protocol and the number of trials used to compute the average rectified EMG (two-way ANOVA: $F(9,81) = 11.91$, $P = 0.013$; Fig. 7- 2). For both protocols, the SNR increased in relation to the number of trials,

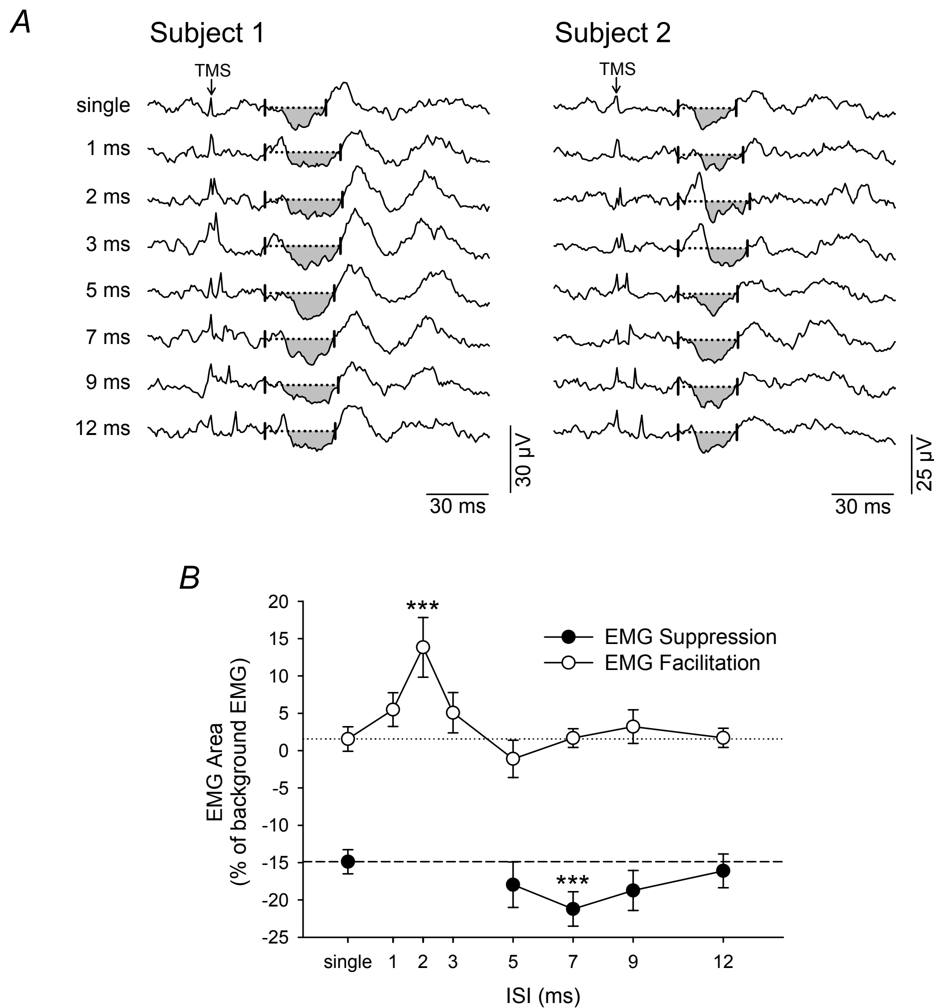

Figure 7-1. EMG suppression using paired-pulse TMS

A, Raw sweeps from two subjects showing the effect of low-intensity single (*top trace*) and paired-pulse TMS on the average rectified EMG activity in the TA muscle. The single pulse depressed the ongoing background EMG (shaded area) in the two subjects by 19% (on average). The paired-pulse intervals are labelled and each trace is the average of 100 sweeps. Vertical solid lines delineate the period between the MEP latency and the end the suppression. The horizontal dotted lines indicate the level of background EMG. Horizontal time-scale bars (bottom right) also represent the baseline (0 μV) for the lowest trace. *B*, Group data showing the amount of EMG suppression (●) and short-latency EMG facilitation that preceded the inhibition (○) as a percentage of background EMG activity. EMG suppression at ISIs of 1-3 ms was not quantified as many of the responses were prone to preceding excitation. Horizontal lines indicate the average EMG suppression (broken line) and EMG facilitation (dotted line) produced by the single pulse. The mean level of background EMG was 24.8 ± 1.9 μV and did not differ between the conditions. Asterisks indicate significant differences compared to single-pulse values (***$P < 0.005$).

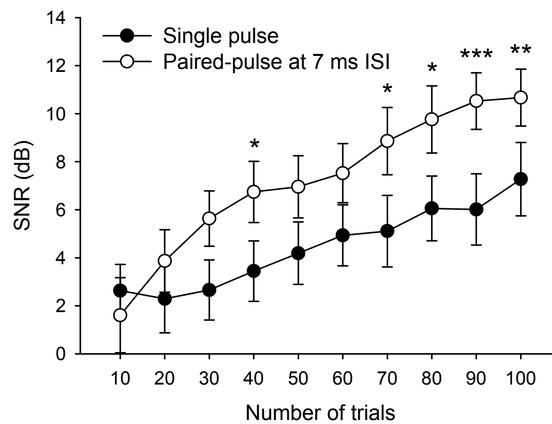

Figure 7-2. SNR of the TMS-induced EMG suppression

Graph shows the SNR of the quantified inhibition as a function of the number of sweeps used to compute the rectified average EMG. Paired-pulse TMS at a 7 ms ISI (○) showed higher SNR than the single pulse data (●). Asterisks indicate significant differences between the two conditions (*P<0.05, **P<0.01, ***P<0.005).

and paired-pulse TMS at the 7 ms ISI yielded a significantly higher SNR for 40, 70, 80, 90 and 100 trials versus the single pulse (all $P < 0.05$).

7.4 DISCUSSION

A single pulse of very low-intensity TMS to the leg motor cortex suppressed the average rectified EMG activity starting ~9 ms after the MEP latency and corroborates previous reports (Davey et al. 1994; Petersen et al. 2001). The presented results demonstrate that delivering a second pulse of subthreshold TMS at an ISI of 7 ms can enhance the amount of EMG suppression without producing any preceding excitation. The optimal paired-pulse interval for EMG suppression was a few milliseconds longer than anticipated, likely due to the presence of short-interval intracortical facilitation (SICF) impinging on the cortical suppression.

Many studies have shown that cortical inhibitory neurons have a lower threshold to TMS than excitatory neurons (Davey et al. 1994; Ziemann et al. 1996; Petersen et al. 2001; Butler et al. 2007; Ortu et al. 2008). Here, the EMG suppression was elicited at a mean intensity of 0.73 × AMT and was below the threshold for single-pulse excitation. The EMG activity in the TA muscle was decreased by -14.9% for single-pulse TMS and was comparable to that shown during walking (Petersen et al. 2001). In agreement with our hypothesis, adding a second TMS pulse further reduced the average EMG. The EMG suppression at the 7 ms interval was -21.2% and was +42% larger than the single-pulse response. With subthreshold single-pulse stimulation, inhibition of TA EMG is thought to occur at the level of the cortex given that TMS and not transcranial electrical stimulation can produce such suppression (Petersen et al. 2001). As low-intensity paired-pulse TMS at an interval of 7 ms failed to produce any early facilitation, it is likely that the extra suppression occurred because of inhibitory neurons that reside in the cortex. Potentially, the second TMS pulse enhanced the EMG suppression due to the recruitment of inhibitory cells not activated by the first stimulus and/or because a fraction of the inhibitory neurons were re-activated by the second TMS pulse. The net result was likely a reduction in the size and duration of the descending volleys driving the motoneurons during the voluntary contraction. Similarly, paired-pulse TMS at the 7 ms ISI increased the SNR above single-pulse values indicating more robust EMG suppression. In fact, nearly twice as many single-pulse sweeps were needed to yield a SNR equivalent to the double-pulse data (see Fig. 2).

When paired-pulses of low-intensity TMS were delivered at ISIs of 1-3 ms, we observed a brief excitation in the EMG response occurring with a latency of ~30 ms. Increases in the EMG were significant at the 2 ms ISI and nearly all subjects exhibited some degree of facilitation at one of the three intervals. Over intervals of 1-3 ms, the size of the excitation was positively correlated with the stimulus intensity. Similar MEP facilitation occurs in the form of SICF with paired-pulse TMS (at ISIs < 5 ms) generally produced using higher stimulation intensities (Ziemann et al. 1998; Ilic et al. 2002; Roy and Gorassini 2008). The threshold for SICF is higher than for intracortical inhibition (Awiszus et al. 1999; Ortu et al. 2008), though trace amounts of EMG facilitation have also been reported in the contracted muscle of the little

finger at an intensity of 0.70 × AMT (ISI of 1.5 ms; Ilic et al. 2002). SICF likely explains the present EMG excitation as it was produced over short intervals at a similar intensity (0.73 × AMT) and reflects non-synaptic facilitation of the initial axon segment of excitatory interneurons that were depolarized by the first stimulus and were therefore hyperexcitable at the time of second pulse (Hanajima et al. 2002). In the present study, when subthreshold pulses were separated by 5 ms or more, the EMG activity preceding the suppression was no longer enhanced. Indeed, when two pulses were delivered 7 ms apart, the cortical pathway mediating the extra inhibition was unlikely affected by SICF. At the 12 ms interval, the amount of EMG suppression tended towards single-pulse values and was unable to overcome the late EMG increase observed following the suppression (see Fig. 1A and Davey et al. 1994), thus suggesting that 7 ms is the optimal interval for suppressing EMG activity using very low-intensity paired-pulse TMS.

In the present study, we show that pairs of subthreshold TMS at a 7 ms interval enhances the size and reliability of the EMG suppression in the pre-contracted TA muscle without increasing the preceding EMG. On the basis of these results, paired-pulse TMS might be beneficial for investigating the contribution of cortical cells actively involved in motor control, in particular during tasks that rely less heavily on voluntary drive. We speculate that short trains of repetitive TMS with an inter-pulse interval of 7 ms might further inhibit the cortical path producing EMG during a voluntary contraction and more readily probe the contribution of corticospinal inputs to a given motor task.

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CHAPTER 8: General discussion

8.1 THESIS SUMMARY

This thesis investigated associative plasticity and the role of afferent regulation of corticospinal tract (CST) output to lower limb muscles in both healthy individuals and after an incomplete spinal cord injury (SCI). In addition, we examined intracortical and spinal inhibition in our investigation of the human motor pathways controlling leg function. This chapter begins by giving an overview of our results and then discusses their contribution to the study of human motor movement. The main findings have been subdivided into 4 sections and are as follows.

8.1.1 Afferent regulation of corticospinal excitability (Chapters 3&4)

- Afferent stimulation of the tibial nerve (TN) at the ankle increases motor evoked potentials (MEPs) in the tibialis anterior (TA) muscle by exciting cortical networks, potentially by decreasing short interval intracortical inhibition (SICI) and increasing both intracortical facilitation and short-interval intracortical facilitation
- The afferent-induced facilitation of the TA MEP occurs at rest and during a tonic contraction
- TN stimulation facilitates the MEP elicited using transcranial magnetic stimulation (TMS) but not brainstem responses
- Afferent-induced MEP facilitation with common peroneal nerve (CPN) or TN stimulation is diminished/absent in SCI subjects
- The amount of MEP potentiation in the SCI subjects following CPN stimulation is graded according to the severity of the lesion and is delayed by approximately ~10 ms as compared to healthy individuals

8.1.2 Paired associative stimulation in leg muscles (Chapters 2&4)

- PAS in the lower leg increases TA MEPs over a range of conditioning intervals and TA MEPs are increased when the conditioning afferent inputs arrive at the motor cortex many tens of milliseconds *after* the cortical stimulus
- PAS-induced MEP facilitation occurs without changes to SICI, intracortical facilitation and the H-reflex
- PAS administered in SCI subjects induces short-term increases in the resting MEP but has no effect on the MEP evaluated during a voluntary contraction

8.1.3 Inhibition to the soleus muscle by contralateral afferents (Chapter 5)

- Electrical stimulation to the contralateral posterior tibial nerve (PTN; 25 Hz for 700 ms) activates inhibitory spinal circuits to the human soleus (SOL) muscle.
- The contralateral afferent input enhanced the inhibitory component of the cutaneomuscular reflex at a latency that is consistent with the facilitation of inhibitory spinal interneurons
- SOL MEPs were depressed by contralateral afferent stimulation likely through the interneuronal pathway to the SOL motoneurons.

8.1.4 Intracortical inhibition (Chapters 6&7)

- SICI is reduced after an incomplete SCI
- The "U" shaped relationship between the intensity of the conditioning stimulus and the amount of inhibition is preserved in SCI subjects when expressed as a function of the active motor threshold
- The motor cortex of SCI subjects needs to be conditioned with a stronger TMS pulse to inhibit the test MEP
- Cortical inhibition in healthy individuals evaluated using very low-intensity subthreshold TMS can be enhanced using two pulses delivered 7 ms apart

8.2 AFFERENT REGULATION OF CORTICOSPINAL EXCITABILITY

8.2.1 Discussion

The increased demand for stability during human standing and walking may explain the importance of afferent feedback in controlling muscle activity in the lower limb. For instance, the long-latency reflexes may be involved in lifting the foot over obstacles and stabilizing the supporting limb in the stance phase of gait (Christensen et al. 2000). In addition, integrating sensory feedback into the generation of motor commands from the primary motor cortex is important given the severe disruption of gait and fine motor skills in patients with large-fibre sensory neuropathies (Rothwell et al. 1982; Sanes et al. 1985; Lajoie et al. 1996). In cats and monkeys, sensory inputs modulate the discharge of corticospinal cells projecting to hindlimb muscles (Fetz et al. 1980; Palmer et al. 1985). Although functional activation of sensory connections to cortical networks cannot be measured directly in intact humans, there is considerable evidence to support the existence of such pathways.

The contribution of transcortical reflexes in the control of the TA muscle has been systematically studied by the group of Jens Nielsen in Copenhagen, Denmark and many of the findings are discussed in a review article by Christensen et al. (2000). Subcortical mechanisms including polysynaptic spinal and spino-bulbo-spinal reflexes may also contribute to the EMG and may provide an important adaptation following loss of supraspinal control. Regulating EMG activity from subcortical structures can occur at latencies that are consistent with the transcortical loop, and for this reason, the contribution of cortical inputs can be difficult to tease out. In Chapters 3&4, we provide several lines of evidence that electrical stimulation of

peripheral nerves in the leg can activate the TA muscle through a transcortical loop. Firstly, MEPs in the resting or contracted TA muscle are strongly facilitated when the tibial nerve is stimulated 45 to 55 ms before the TMS pulse (40 ms for the CPN), a latency that is consistent with the arrival of the afferent input at the motor cortex. In fact, the TA MEPs are facilitated at a latency that is >4 ms longer than the arrival of the afferent input at the somatosensory cortex. Secondly, cortical MEPs and not subcortical responses are potentiated following peripheral nerve stimulation and are in agreement with reports using sural nerve stimulation (Nielsen et al. 1997) and the long-latency TA stretch reflex (Petersen et al. 1998). Thirdly, afferent inputs can alter the cortical circuitry that mediates SICI, intracortical facilitation (see also Aimonetti and Nielsen 2001; Rosenkranz et al. 2003) and short-interval intracortical facilitation in a manner that is consistent with the up-regulation of cortical excitability. Lastly, we show that the MEP potentiation that occurs following TN or CPN stimulation in healthy individuals is greatly diminished in SCI subjects with disrupted ascending sensory pathways. In particular, the MEP potentiation produced by CPN stimulation was graded according to the severity of the injury was delayed by approximately ~10 ms (compared to the non-injured controls), thus supporting the contribution of supraspinal structures in the sensory-induced MEP facilitation.

When afferent inputs from electrical stimulation first arrive at the motor cortex, MEPs are depressed in what is called short-latency afferent inhibition (SAI; Tokimura et al. 2000). In the upper limb, this occurs at a latency of approximately ~20 ms and occurs via cholinergic neurons (Di Lazzaro et al. 2000). In the lower limb, we observed inhibition at approximately ~27 and ~35 ms when the TN was activated at the knee and ankle, respectively. Although we only examined the site of the inhibition in one set of experiments, our results were unable to support a cortical mechanism given that both brainstem and TMS responses were inhibited to the same degree (by approximately 10%; see Chapter 3). Further experiments still need to be done to systematically examine the site of such short-latency inhibition to the TA muscle. Although it is unclear whether the inhibitory phenomenon seen in the leg is directly related to SAI in the upper limb, it may be worthwhile to examine the pharmacological origin of the afferent-induced inhibition produced in leg muscles.

8.2.2 Future directions

In animals, skilled training strengthens horizontal intracortical connections in the regions of the motor cortex that are associated with the motor training (Rioult-Pedotti et al. 1998). Similarly, human motor training increases the size of the MEP reaching the TA muscle, both in healthy individuals (Perez et al. 2004; Jensen et al. 2005) and after an incomplete SCI (Thomas and Gorassini 2005; Wirth et al. 2008; Everaert et al. submitted). As afferent inputs modify the strength of corticospinal connections and drives cortical plasticity (see Chapter 1), we are presently investigating whether the connectivity of afferents onto sensorimotor networks are modified by locomotor training. As skilled versus strength training of lower limb muscles results in different neurophysiological changes (Jensen et al. 2005), we are presently examining whether two training regimes (involving 2 months of overground or precision walking) produces specific changes to the connectivity of the sensorimotor pathway after SCI.

In addition, there is preliminary evidence from Jen Nielsen's group in Denmark that afferent stimulation of the PTN at the knee can interfere with skilled motor training involving ballistic ankle contractions (unpublished observations). Since it remains unclear whether cortical and/or spinal circuits are responsible for this motor interference, examining motor learning in SCI subjects may be useful for investigating the mechanism of the motor interference given that a spinal cord lesion impedes part of the sensory input from reaching the sensorimotor cortex.

8.3 PAIRED ASSOCIATIVE STIMULATION IN LEG MUSCLES

8.3.1 Discussion

Unlike PAS in the hand, we found that TA MEPs were facilitated at ISIs between 40 to -40 ms, intervals where the sensory afferent inputs were *estimated* to arrive at the motor cortex 0 to 80 ms *after* the TMS-induced firing of cortical neurons (based on Chapter 3 and is 10 ms earlier than what was estimated in Chapter 2). Likewise, it was possible to induce strong MEP facilitation when the afferent inputs were directly paired with subthreshold TMS suggesting that the effect can be induced at a cortical site. Following a single TMS pulse, the corticospinal system remains active for many tens of milliseconds after a suprathreshold stimulus. In Chapter 3, we have also shown that CST excitability is further enhanced and prolonged in duration (by some tens of milliseconds) when combined with afferent inputs from the lower leg. Based on these findings, it is possible that the continued activation of corticospinal neurons triggered by the TMS pulse were potentiated by the afferent inputs arriving up to 80 ms later, thereby producing the PAS-induced MEP facilitation.

Although not mentioned in Chapter 2, it is possible that the observed MEP facilitation following PAS may be related to a similar phenomenon reported by Ziemann et al. (1998), whereby low frequency TMS (0.1 Hz), which on its own has no clear effect on the MEP, can become excitatory when combined with a manipulation that increases the MEP. Ziemann et al. (1998) showed that low-frequency TMS when paired with transient limb deafferentation further enhances the facilitation of the biceps MEP. In our experiments, low-frequency TMS (0.1 Hz), which on its own was likely too weak to potentiate the TA MEP, may have become facilitatory when combined with the afferent excitation from the lower limb so long as the two inputs could interact. However, given that the leg motor cortex was facilitated following most interventions, more control experiments would have been helpful to evaluate the saliency of each input (i.e. TMS, peripheral nerve stimulation or voluntary drive) for modifying cortical excitability. This would have been useful to rule out the effect of short-trains of peripheral nerve stimuli when employed with subthreshold TMS (i.e. subthreshold-PAS experiment). However, given the "relatively" small number of peripheral nerve stimuli used in this experiment, it seems unlikely that the CPN stimuli alone were responsible for the persistent MEP potentiation.

Stinear and Hornby (2005) were the first to report changes in the leg MEPs following PAS. The authors showed that TA MEPs during walking could be facilitated or depressed depending on the interstimulus interval between the CPN stimulus and the

TMS pulse. However, further studies in the same lab have shown that TA MEPs are predominantly inhibited when PAS is administered during walking (Prior and Stinear 2006). Such findings make sense in light of the fact that afferent connections are strongly inhibited during walking (Pierrot-Desseilligny and Burke 2005). At rest or during a voluntary contraction, the data from our study and Mrachacz-Kersting et al. (2007) suggest that that low-frequency PAS in lower leg muscles is more often excitatory rather than inhibitory. Although it is still unclear why the intervals that produce excitation/inhibition in the leg differ from the upper limb, properly-timed afferent and cortical inputs at the motor cortex can facilitate corticospinal connections to hand and leg muscles. Descending volleys produced by TMS are more difficult to elicit from the leg motor cortex as compared to the hand due to the orientation and depth of the pyramidal tract neurons in the leg motor cortex. In addition, the recruitment order of descending volleys differs considerably between leg and hand motor areas (Nielsen et al. 1995; Di Lazzaro et al. 1998; Terao et al. 2000; see also Di Lazzaro et al. 2008). While TMS over the hand region has been well characterized, much less is known about the specific activation of motor cortex neurons destined to muscles in the lower extremity. In the experiments described in Chapter 2, PAS was administered using a double cone coil which has side loops that can affect neighbouring neurons (Epstein 2008). A flat figure-of-eight coil is more common for the hand motor cortex and has side fields that can be safely ignored. Indeed, differences in the shape of the coil and the strength of the TMS pulse may have been partly responsible for producing the dominant facilitatory effect observed in the leg.

In agreement with the hand literature (Stefan et al. 2002; Russmann et al. 2009), we were unable to detect changes in SICI following an intervention of PAS. However, since short-interval intracortical facilitation can contaminate SICI (see Ortu et al. 2008; Peurala et al. 2008), testing SICI using a weaker conditioning intensity (well below 95% of AMT) would be beneficial to rule out alterations in GABA-A receptor inhibition. Recently, it has been shown that PAS-induced facilitation is partly mediated by decreases in long-interval intracortical inhibition and alterations in long-latency afferent inhibition (Russmann et al. 2009). Presently, the role of these two inhibitory pathways on associative plasticity in the leg motor cortex has yet to be examined.

In Chapter 4, we showed that TA MEPs were facilitated by an intervention of PAS in 7 out of 13 SCI subjects. Although the effect was short-lasting and the MEPs tended towards baseline values at 20 min, these results suggest that sensorimotor connections can be facilitated by spared sensory connections after SCI. In the future, it may be worthwhile to increase the number of pairings to add to the robustness of the facilitation. The potential to modify the strength of the CST connections by pairing afferent and cortical inputs suggests that PAS may have therapeutic potential and may serve as a priming tool for neural plasticity. Administering PAS in the upper limb of stroke patients can facilitate MEPs for 30-60 minutes (after the end of the intervention) and may be helpful for assessing the excitability of the corticospinal system after injury (Castel-Lacanal et al. 2007, 2009).

8.3.2 Future directions

Both walking function (Stein et al. 2006) and corticospinal excitability to the TA muscle are increased with CPN stimulation (Khaslavskaia et al. 2002; Knash et al. 2003). In addition, Everaert et al. (in press) has recently shown that several months of CPN stimulation increases MEPs in SCI (and multiple sclerosis) subjects showing locomotor improvements. In addition, cortical stimulation using repetitive TMS and transcranial direct current stimulation in stroke patients can transiently increase hand and arm function (Kim et al. 2006; Hummel et al. 2005). Together, these findings suggest that non-invasive brain stimulation may contribute to restoring motor function after injury. In line with these techniques, PAS may be a useful priming tool for increasing corticospinal connections to leg muscle after a spinal cord lesion and may serve as a potential adjunct to rehabilitative training. Presently, the use of PAS in combination with motor training has yet to be investigated after SCI.

8.4 INHIBITION OF THE SOLEUS MUSCLE BY CONTRALATERAL AFFERENTS

8.4.1 Discussion

Stimulation of cutaneous afferents in both anesthetised and walking animals activates a crossed inhibitory, spinal pathway to extensor muscles in the hindlimb (Curtis et al. 1958; Frigon and Rossignol 2008). In chapter 5, we showed that a long train of electrical stimulation to the contralateral PTN (25 Hz for 700 ms) activates inhibitory spinal circuits to the human soleus muscle. We present evidence that contralateral leg afferents facilitate inhibitory interneuronal circuitry to the soleus motoneurons, and the excitability of this pathway can be studied using spinal circuits activated by cutaneomuscular and corticospinal inputs. Potentially, the crossed inhibitory pathway may sculpt descending excitation and locomotor output during human walking. In walking cats, such crossed inhibition is strongly modulated throughout the step cycle and may help synchronize EMG bursts during walking (Frigon and Rossignol 2008). In human subjects, soleus MEPs are reduced during the stance phase of gait (Capaday et al. 1999), and since presynaptic inhibition is absent on the terminals of corticospinal neurons (Nielsen and Petersen 1994; Jackson et al. 2006), it is possible that spinal inhibitory interneurons may regulate the descending excitation during walking.

8.4.2 Interhemispheric inhibition of the leg motor cortex

Although not stated previously, the experiments described in Chapter 5 were initially directed by the notion that increasing cortical activity in one hemisphere (potentially using peripheral nerve stimulation) has the corresponding effect of actively inhibiting the opposite hemisphere (Perez and Cohen 2008, 2009). In addition, removal of sensory input via transient deafferentation has the reverse effect and increases MEPs in contralateral limb muscles through a reduction in interhemispheric inhibition (Werhahn et al. 2002). Although it is not currently possible to investigate the presence of interhemispheric inhibition in the leg motor cortex using paired TMS

(due to the close proximity of the leg motor cortices), it may be useful to study the response using the cross extensor reflex (as the conditioning stimulus). For instance, sural nerve stimulation facilitates the target sensorimotor cortex (Nielsen et al. 1997), and at strong intensities elicits ipsilateral flexion and contralateral extension. In complete SCI subjects, sural nerve stimulation produces facilitation of the H-reflex in the opposite limb indicating that spinal excitatory pathways are activated to the contralateral soleus muscle (Roby-Brami and Bussel 1990). If the cortical MEP is suppressed by the contralateral stimulation (despite the strong spinal facilitation) then such a result would support the presence of a supraspinally-mediated inhibition. Moreover, recent experiments have shown that TA MEPs can be suppressed by a strong dorsiflexion in the opposite leg (Perez et al. 2008). These changes do not affect the H-reflex and occur in parallel with a reduction in SICI suggesting that cortical structures contribute to the MEP inhibition. Provided that the various spinal pathways are relatively unchanged by the contraction, such models may be useful for assessing the strength of the interhemispheric linkage between the leg motor cortices.

8.5 INTRACORTICAL INHIBITION

8.5.1 Discussion

Intracortical inhibition is the most frequently studied of the neuronal networks associated with human motor control (see Rothwell et al. 2009). Intracortical inhibition is impaired in motor disorders (Berardelli et al. 2008) and likely plays an integral part in cortical plasticity (Ziemann et al. 2001; Teo et al. 2009). In Chapter 6, we showed for the first time that the "U" shaped relationship between the intensity of the conditioning stimulus and the amount of inhibition persists after SCI. Although the inhibition was considerably reduced in the SCI subjects, the "U" shaped profile was centered on the same "relative intensity" in both healthy and injured subjects (i.e. 80% of the active motor threshold). This result was a little surprising in light of the fact that the motor cortex of SCI subjects needed to be conditioned with a stronger TMS pulse to inhibit the test MEP. As described in Chapter 6, there is strong evidence that SICI is a cortical phenomenon, but on the basis of the change in the inhibitory threshold after SCI, it is possible that SICI may also have a spinal component (see below).

Another way of probing cortical inhibitory circuits is to use the method of EMG suppression introduced by Davey et al. (1994) whereby the background EMG activity is briefly inhibited following a pulse of subthreshold TMS. In an earlier study (unpublished experiments), we used low-intensity subthreshold TMS to inhibit the background EMG at the onset of a muscle relaxation given that SICI was shown to be increased at this time (Buccolieri et al. 2004). Co-activation of muscles is exaggerated during voluntary movement in SCI subjects (Alexeeva et al. 1997), and understanding this mechanism may be useful in reducing the amount of co-activation. Although we were unable to detect differences in the amount of EMG suppression occurring at the onset the relaxation, I thought that it might be useful to introduce a technique that would increase the size and duration of the EMG suppression. In the short study described in Chapter 7, I investigated whether adding a second TMS pulse could be useful for further inhibiting the background EMG in the TA muscle. I

show that pairs of subthreshold TMS delivered 7 ms apart produced the maximum EMG suppression and increased the size of the inhibition by +42% as compared to the single pulse. The reduction in the EMG at the 7 ms paired-pulse interval occurred without any short-latency excitation suggesting that the two stimuli increased the activation of cortical inhibitory neurons in isolation.

8.5.2 *Future directions*

Although the methods of EMG suppression and SICI share many similarities, including the threshold of inhibition, presently a systematic comparison of both techniques has yet to be performed. There is some preliminary evidence from Zuur et al. (2008) suggesting that EMG suppression and SICI are regulated by different mechanisms during standing, walking and muscle relaxation (done differently from our experiment). Chapter 6 presents the first systematic evaluation of SICI in SCI subjects, but many questions still remain to be answered. Firstly, we still need to understand why the actual threshold for inhibition is considerably higher after SCI as compared to non-injured individuals. Whether this is due to a reduction in activity of the intracortical circuits or a contribution of spinal inhibition in SICI still remains to be explored. Examining the effect of subthreshold TMS pulses on the H-reflex may be useful for probing such putative mechanisms. Although the nature of the interaction (i.e. excitation or inhibition) produced using subthreshold TMS can vary based on the state of the motor system, there is good evidence that weak TMS or transcranial electric stimulation can inhibit motoneurons via a short-latency disynaptic pathway (Cowan et al. 1986; Iles and Pisini 1992, Nielsen et al. 1993). To our knowledge, it is presently unclear whether SICI and the inhibition of motoneurons activated by CST inputs are related. Secondly, given that SICI is reduced after SCI, it would be useful to investigate whether SICI is related to the severity of the injury. We presently have data from the left and right legs of 5 SCI subjects and there is a tendency for the amount of inhibition to be greater in the stronger as compared to the weaker limb ($P = 0.19$). Thirdly, given that SICI is important in motor learning, it may be worthwhile investigating whether intracortical inhibition in the leg is also modified with locomotor recovery. Altering the activation of intracortical inhibitory circuits may promote cortical reorganization following permanent nerve injury. Similarly, it is plausible that rehabilitative strategies that target GABAergic inhibition may assist in promoting cortical plasticity and sensorimotor recovery after central nerve injury.

8.6 *BIBLIOGRAPHY FOR CHAPTER 8*

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APPENDIX A: Effects of transcranial direct current stimulation on the excitability of the leg motor cortex

A version of this chapter has been published.
Jeffery DT, Norton JA, Roy FD, and Gorassini MA., *Exp Brain Res* 182: 281–287, 2007

Involved in data collection, statistical analysis and editing the manuscript, my total contribution was ~15%.

A.1 INTRODUCTION

Recovery of motor function after stroke or partial spinal cord injury is correlated with the strengthening of spared corticobulbar and corticospinal connections (Fraser et al. 2002; Thomas and Gorassini 2005). Using procedures to temporarily increase or decrease the excitability of these connections, as an adjunct to conventional methods of rehabilitation therapy, may serve to shorten recovery time and improve the absolute recovery level in patients with stroke or partial spinal cord injury (Hummel and Cohen 2006; Talelli and Rothwell 2006). However, the degree of cortical facilitation or suppression, the optimal target site, and the best strategy for modifying the excitability of cortical motor networks to optimize motor recovery needs further study.

Several non-invasive strategies aimed at modifying corticospinal excitability have emerged in recent years, including: repetitive transcranial magnetic stimulation (TMS) (Pascual Leone et al. 1994), paired associative stimulation (PAS) (Stefan et al. 2000) and transcranial direct current stimulation (Nitsche and Paulus 2000). Transcranial direct current stimulation (tDCS) is one of the simplest emerging technologies for modifying cortical excitability. Consisting of a controlled current source and two electrodes, a tDCS unit is portable and easy to use. The stimulation elicits minimal discomfort and only a mild tingling sensation that usually disappears after a few seconds (Nitsche et al. 2003a). Facilitation of cortical excitability lasting up to 90 min, as evidenced by increases in motor evoked potentials (MEPs) elicited by TMS, can be achieved with only 13 min of stimulation (Nitsche and Paulus 2001).

By varying the position and polarity of the electrodes, tDCS has been adapted to produce a wide variety of effects (Nitsche et al. 2007). Stimulation with the anode over the hand motor cortex contralateral to the target muscles and the cathode over the ipsilateral orbit (anodal tDCS) increases the excitability of the corticospinal projections to the hand. In contrast, with the cathode over the motor cortex and the anode over the orbit (cathodal tDCS), corticospinal excitability is decreased (Nitsche et al. 2005). In the hand, anodal tDCS has been shown to improve motor skills after stroke when combined with motor rehabilitation (Hummel et al. 2005), decrease absolute reaction time in a serial reaction time test (Nitsche et al. 2003b) and induce

sustainable increases in cortical excitability (Nitsche and Paulus 2000, 2001). However, there have been no published studies to date concerning the effects of tDCS on the leg area of the motor cortex. Because the leg area of the motor cortex has a deeper location and a more vertical orientation relative to the scalp than the hand motor cortex, we investigated if tDCS can modify corticospinal excitability of the leg motor cortex in a manner similar to that seen for the hand area. In this study we show that anodal stimulation (2 mA for 10 min) can increase the excitability of the leg corticospinal system but that cathodal and sham stimulation have minimal, and no effect, respectively, on the excitability of the corticospinal tract as assessed using single-pulse TMS.

A.2 METHODS

Ethical approval for this study was obtained from the Human Ethics Research Committee at the University of Alberta and all procedures were carried out according to the Declaration of Helsinki (http://www.wma.net/e/policy/b3.htm, 2006). All subjects gave informed consent before participating in the study and were screened for possible contra-indications such as history of epilepsy and neurological damage.

A.2.1 Subjects

Eight subjects (5 male) aged 25.3 ± 6 years on average volunteered for this study. All subjects took part in three tDCS trials consisting of anodal polarization, cathodal polarization and sham stimulation as described below. Subjects, but not researchers, were blind to the nature of the trial and the sequence of trials was randomized between subjects. The minimum period between sessions for any one subject was 3 days. In seven of the eight subjects stimulation was applied to the left motor cortex supplying the right leg. In the remaining subject, tDCS was applied to the right motor cortex because TMS of the left motor cortex produced a large MEP response in both the left and right TA muscle. Because responses in this subject followed the same trend as the other anodal, cathodal, and sham trials, data from this subject was included in the overall group average. All subjects reported right leg dominance.

A.2.2 Electromyography (EMG) recording

Electromyography signals were recorded via disposable recording electrodes (Kendall Soft-E H59P/H69P) placed 1.5–2.0 cm apart on the right and left tibialis anterior (TA) and vastus lateralis (VL) muscles. Signals were amplified (×1,000) and bandpass filtered (10–1,000 Hz) (Octopus, Bortec Technologies, Calgary, AB, Canada) and collected on a PC. The signals were digitized at 5 kHz using AxoScope hardware and software (DigiData 1200 Series, Axon Instruments, Union City, CA, USA).

A.2.3 Non-invasive cortical stimulation

tDCS was delivered using a constant-current stimulator (CX-6650, Rolf Schneider Electronic, Germany) via two saline soaked sponge electrodes (35 cm^2) placed on the scalp. Prior to positioning the electrodes, the skin was rigorously prepared by a combination of cleaning and light abrasion to reduce the skin impedance. Target skin impedance was ≤200 kΩ for a DC signal. Skin preparation was repeated until the target impedance was met. The stimulation intensity was set to 2 mA and was applied over a 10-min period. The current was ramped up to 2 mA over a 10 s period and a similar but descending current ramp was used at the end of the trial to minimize any sensation for the subject. At the beginning of sham trials, a 10 s ramp up to 2 mA current was applied. This was followed by 10 s of stimulation at 2 mA, after which the investigator dialed the current to zero, out of the field of view of the subject, over another approximately 10 s. At the end of the sham trials, the subjects received stimulation from a low intensity (100 μA) current for another 10 s to mimic the sensation of the ramp-down current in the anodal and cathodal tDCS trials. Subjects reported experiencing similar sensations under the electrodes at the beginning and the end of anodal, cathodal and sham trials, and were unable to differentiate between polarities. The anode or cathode electrode was centered over the leg area of the primary motor cortex contralateral to the TA muscle of interest for anodal or cathodal stimulation respectively. The centre position of this electrode (hotspot) was determined as the point where the largest MEP in the TA muscle could be elicited by TMS (see below). The second electrode was placed above the contralateral orbit of the eye, ipsilateral to the TA muscle of interest as per Nitsche and Paulus (2000, 2001).

TMS was applied using a MAGSTIM 200 (The MAGSTIM Company Limited) or a MES-10 (CADWELL) and a double cone coil (external wing diameter 110 mm for MAGSTIM 200 and 90 mm for CADWELL) oriented in the anterior–posterior direction. Motor evoked potentials (MEPs) were recorded before the intervention and at 0, 10, 30 and 60 min post-intervention. MEPs were recorded first at rest and then while the subject maintained a voluntary contraction in the TA muscle corresponding to 10% of their maximum voluntary contraction (MVC). Rectified and low-pass filtered (0.3 Hz) EMG was displayed on an oscilloscope and used by subjects to consistently match background contractions. Mean MEP amplitudes were calculated from the average of 20 peak-to-peak MEP responses recorded at rest and 10 MEP responses recorded during voluntary contractions.

The coil placement that best elicited a response in the TA (hotspot) was determined over the contralateral motor cortex using a stimulation intensity that produced a MEP of approximately 0.1 mV peak-to-peak. The hotspot was, on average, approximately 1 cm posterior and 1 cm lateral to the vertex. The same hotspot was used throughout each trial but varied between subjects and trials. TMS intensities used at rest were on average 59 ± 11% of maximum stimulator output (%MSO), which produced average baseline MEP amplitudes of 0.38 ± 0.2 mV. During background contractions, stimulation intensities used were on average 50 ± 10% MSO, which produced average MEP amplitudes of 0.91 ± 0.4 mV. These MEP amplitudes were chosen to target the mid-point of the slope region of the sigmoidal recruitment curve (Devanne et al. 1997).

A.2.4 Data analysis

Axoscope files were imported into MATLAB (The Mathworks, Inc, Natick, MA, USA). Custom MATLAB code was written to calculate the peak-to-peak amplitude of the MEPs. The level of background EMG was evaluated as the average root mean squared value of the EMG activity in a 100 ms period before the TMS pulse. The resulting data were then exported into an Excel spreadsheet, where each individual sweep was evaluated to ensure appropriate levels of background EMG activity during contraction trials. Likewise during rest trials, MEP waveforms were examined in Axoscope to ensure that there was no activity in adjacent or contralateral muscles. No more than two sweeps were removed from any one set of 10 or 20 trials and outliers that met no elimination criteria were included as normal variability. The averages resulting from this analysis were normalized with respect to baseline levels recorded before the intervention.

A.2.5 Statistical analysis

A three-way repeated measures analysis of variance (ANOVA) was used to test the effect of tDCS on corticospinal excitability treating the polarity of the electrodes (anodal/cathodal/sham), the state of the motor system (rest/background) and the time of measurement as within-subject factors. Post hoc paired t tests (two-tailed) were used to evaluate changes in the size of the MEP response at the different time points compared to baseline. A one-way ANOVA was used to test the consistency of baseline MEP amplitudes using polarity (anodal/cathodal/sham) as the within-subject factor. Significance was set at $P < 0.05$. Statistical analysis was performed using SPSS software (SPSS Inc., IL, USA).

A.3 RESULTS

Anodal stimulation, with the anode over the leg area of the motor cortex, resulted in a persistent increase in the size of the MEP recorded in the TA muscle. The top graph in Fig. A-1 shows in a representative subject that the MEPs evoked 30 min after anodal tDCS were larger than the MEPs evoked before the stimulation. In contrast, cathodal stimulation did not produce noticeable changes in MEP responses as shown for the same subject on a different recording day (Fig. A-1, middle graph: 30 min post-tDCS). Likewise, sham stimulation, where current was applied only over a short period at the beginning and then again at the end of stimulation, had no effect on the MEPs recorded in the relaxed TA muscle as shown once again for the same subject on a different recording day (Fig. A-1, bottom graph). In all cases, subjects were unable to determine either the polarity of the stimulation (anodal or cathodal) or whether sham stimulation was being applied. Anodal and cathodal tDCS at 2 mA for 10 min was well tolerated by all subjects.

Figure A-2 presents average data from all subjects across all three conditions. A three-way repeated measures ANOVA using within-subject factors of polarity (anodal/ cathodal/sham), state (rest/background) and time, showed a significant interaction effect (polarity × time) [$F(8,56) = 3.274$, $P < 0.005$] indicating that MEP

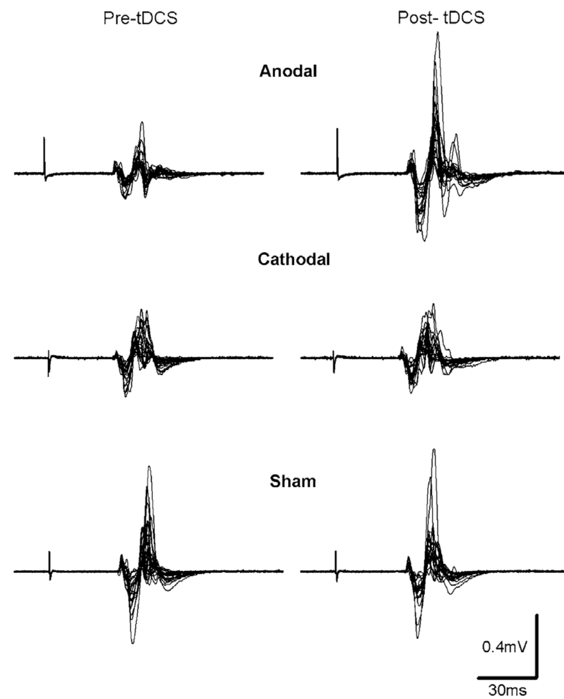

Figure A-1. Traces showing changes in the MEP after tDCS

Illustrative traces of TA MEPs (overlay of 20 trials) evoked at rest from a single subject before and 30 min after anodal and cathodal tDCS of the motor cortex (*top* and *middle graphs*, respectively). *Bottom graph* representative data from the same subject before and 30 min after sham tDCS. Sustained increases in MEP responses were produced from anodal tDCS only.

responses were modulated after tDCS according to the polarity of the stimulation. The state and time interaction effects (state x time) were non-significant [$F(4,28) = 0.282$, $P = 0.887$], indicating that tDCS produced similar changes in the MEP response recorded in both resting and contracted muscles.

Following anodal tDCS, post hoc analysis showed significant increases in the average MEP response of both resting (Fig. A-2a) and active states (Fig. A-2b) from baseline at 10, 30 and 60 min after the intervention (all $P < 0.05$). MEP facilitation at the 60-min time point following anodal tDCS was increased by 59 and 35% above baseline for resting and active MEPs respectively ($P < 0.05$). Resting MEPs following cathodal tDCS remained unchanged except for a small decrease (17%) at the 60-min time point ($P < 0.05$). MEPs recorded during a background contraction after cathodal stimulation showed no significant change across all time points. Likewise MEP responses recorded at rest or with a background contraction were unchanged following sham stimulation. When MEPs were averaged across all time points for each condition, only MEPs following anodal tDCS were significantly greater than baseline (Post: Fig. A-2a, b, $P < 0.05$).

Separate one way ANOVAs showed no significant differences in the size of the MEP responses recorded before anodal, cathodal or sham tDCS at rest [$F(2,21) = 0.27$, $P = 0.8$] or with background contraction [$F(2,21) = 0.37$, $P = 0.7$] (values given in legend of Fig. A-2). Moreover, for MEPs recorded during a background contraction, the level of background EMG activity varied on average by less than 3% of pre-tDCS values for all time points following anodal, cathodal and sham stimulation experiments, revealing that background excitability was well controlled throughout all experiments.

A.4 DISCUSSION

This study shows that, similar to the hand corticospinal tract, 10 min of anodal tDCS can be used to increase excitability of the leg corticospinal tract for at least 60 min, even when MEP responses are recorded during a background contraction. In contrast to the hand, marked decreases in the strength of corticospinal projections to the leg did not occur when using equivalent intensities of cathodal stimulation. We discuss why the leg area of the motor cortex may be less prone to inhibition with tDCS compared to the hand area of the motor cortex.

A.4.1 Facilitation of the leg motor cortex from anodal tDCS

To induce facilitation of the hand motor cortex with anodal tDCS, the majority of previous studies have used 1 mA current instead of the 2 mA current used in the present study (reviewed in Priori 2003). Our pilot experiments using 1 mA of current during anodal tDCS were not effective in increasing MEP responses in leg muscles, likely because not enough current penetrated deep enough to affect the leg area. However, after anodal tDCS at 2 mA, leg MEP responses recorded at rest were facilitated to a similar extent (by ~40%) to that recorded in the hand. Likewise, leg MEP responses remained elevated for at least 1 h when tDCS was applied for 10 min.

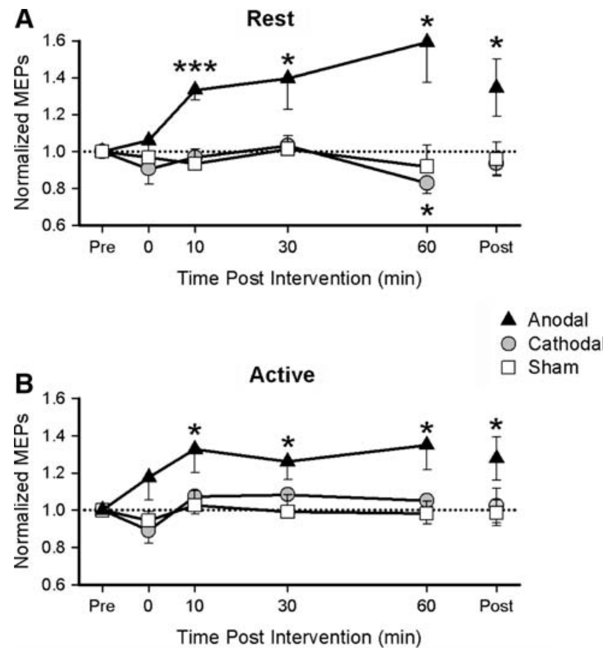

Figure A-2. Time-course of MEPs after tDCS

Group results illustrating changes in the relative amplitudes of the MEPs evoked by TMS following 10 min of anodal (*triangles*), cathodal (*circles*) and sham (*squares*) tDCS compared to pre-stimulation. Data is the average of MEP responses recorded from eight subjects at rest (*closed circles*) and during a background contraction (*open triangles*). The average resting MEP amplitude before tDCS application for all three conditions were similar (anodal: 0.33 ± 0.2 mV, cathodal: 0.39 ± 0.2 mV, sham, 0.42 ± 0.3 mV) as were the pre-tDCS MEPs recorded during a background contraction (anodal: 0.89 ± 0.3 mV, cathodal: 0.83 ± 0.4 mV, sham: 1.01 ± 0.5 mV). Also shown to the right of the time course measurements is the average of all of the normalized post-tDCS measurements. Asterisks indicate significant increases in the MEP response compared to baseline (*$P < 0.05$, ***$P < 0.005$).

Given the similar magnitude and duration of MEP facilitation, it is likely that anodal tDCS of the hand motor cortex at 1 mA and anodal tDCS of the leg motor cortex at 2 mA produces similar effects that may include activity-dependent increases in excitatory glutamatergic cortical pathways and suppression of intracortical inhibitory pathways (Liebetanz et al. 2002; Nitsche et al. 2005).

A.4.2 Lack of consistent inhibition of leg motor cortex from cathodal tDCS

A surprising finding from this study was that, although anodal tDCS facilitated leg MEP responses, cathodal tDCS at the same intensity only produced a small inhibitory effect at the 60 min time point after stimulation. The hyperpolarizing current field applied across primary and secondary leg motor areas likely failed to reduce intracortical facilitation and augment intracortical inhibition, two factors that are associated with the suppression of MEPs in the hand motor cortex in response to cathodal tDCS (Nitsche et al. 2005).

The difficulty in reducing cortical excitability by cathodal tDCS in the leg motor cortex may be due to the fact that there are fewer inhibitory circuits available to suppress compared to the hand motor area. For instance, the hand area of the motor cortex may have more prominent surround inhibition to aid in the execution of precise and individuated finger movements compared to the more gross motor movements required in the leg and foot (Matsumura et al. 1991; Hallett 2003; Tokimura et al. 2000). Alternatively, the organization of inhibitory (and excitatory) circuits in the leg motor cortex may be equivalent to that in the hand area but due to the differences in the orientation and position of the leg motor cortex, cathodal stimulation from surface electrodes may be less effective in facilitating inhibitory, or suppressing facilitatory, intracortical circuits in the leg area. For instance, the strength of the hyperpolarizing current field near the leg area of the motor cortex during cathodal stimulation is likely less than the strength of the depolarizing current field during anodal stimulation (Miranda et al. 2006) and this difference may be less pronounced in the more superficial hand sensorimotor cortex. Thus, in the leg area of the sensorimotor cortex, stronger cathodal tDCS may be required to suppress MEP responses compared to the strength of anodal tDCS required to facilitate MEP responses. Further experiments where electrode position and current intensity are modified are needed to resolve these issues.

A.4.3 Safety concerns

Before using 2 mA of tDCS in our experiments, we carefully considered the safety implications. The dangers of electrical stimulation with varying currents have been carefully examined over the past 25 years and include direct measures of neural damage in animal models (Yuen et al. 1981; Agnew and McCreery 1987; McCreery et al. 1990). In contrast, there have been relatively few direct studies concerning the safety of DC cortical stimulation (Priori 2003). The safety limits calculated for varying currents by McCreery's group involved high frequency stimulation with a charge-balanced biphasic waveform. These findings have been generalized to DC stimulation by simply extending the length of one "phase" of the stimulation to that

of the entire intervention. The problem with this generalization is that the total charge of tDCS, which has been considered probably the most important parameter (Nitsche et al. 2003c), is not equivalent to the total charge produced by a high frequency, charge-balanced biphasic stimulation. There is some possibility that exposing the cortex to an extended depolarizing or hyperpolarizing field may have a different effect on the integrity of cortical tissue than the rapid switching polarities of a charge-balanced biphasic waveform.

Despite the potential differences between high frequency and DC stimulation, we feel it is safe to apply 2 mA of tDCS for 10 min based on previously published data. For example, a recent study using 2 mA tDCS for 20 min per day over 5 consecutive days to treat central pain reported no adverse motor or cognitive effects (Fregni et al. 2006). Likewise, 9-13 min of anodal or cathodal tDCS did not produce measurable changes in: (1) brain edema or alterations of the blood brain barrier that were detectable by MRI (Nitsche et al. 2004), (2) neuronal damage as detected by neurone specific enolase (Nitsche et al. 2003d) and (3) processing, psychomotor speed and emotional state (Iyer et al. 2005). Although tDCS has been documented to have no ill effects thus far, a more extensive and direct anatomical evaluation of its safety limits should be performed in animals so that clinicians can have specific guidelines for calculating appropriate dosage.

A.4.4 Summary

The ability to increase corticospinal excitability makes 2 mA anodal tDCS a potential tool for facilitating motor rehabilitation in the leg; however, 2 mA of cathodal tDCS may have minimal effects when suppression of cortical excitability is required. Further studies will be needed to determine its efficacy and appropriateness in various rehabilitation strategies.

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APPENDIX B: Interaction of paired cortical and peripheral nerve stimulation on human motor neurons

A version of this chapter has been published.
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Involved in data collection, data analysis and writing parts of the manuscript, my total contribution was ~20%.

B.1 INTRODUCTION

Motor neurons serve as the final common pathway for producing movement and the output of motor neurons is conveniently monitored using surface electromyograms (EMG). As a result many human studies have been directed at particular inputs to motor neurons, such as the largely monosynaptic connections from muscle spindle afferents that produce the H-reflex (Pierrot-Deseilligny and Burke 2005), and the descending connections from motor cortex that produce a motor evoked potential (MEP) (Di Lazzaro et al. 2004). Interaction between these inputs at spinal and cortical levels has been studied (Deuschl et al. 1991; Deletis et al. 1992; Petersen et al. 2002), although a systematic comparison of corticospinal and afferent inputs to lower leg muscles is less well studied and may give some insight into presynaptic effects that which will be different for each input (Jackson et al. 2006) and post-synaptic effects that will be common. Pairing stimuli to these two pathways at different intervals can clarify the time course of facilitation and depression in each pathway. The purpose of this paper is to compare and contrast the facilitation and depression of two major inputs to spinal motor neurons and determine whether they arise from presynaptic or postsynaptic mechanisms. This topic is important in relation to studies that have attempted to condition the H-reflex up or down (Chen et al. 1999) and those that have used paired associative stimulation of peripheral and cortical sites to strengthen descending connections in control subjects or those with motor disabilities (Stefan et al. 2000; Uy et al. 2003; Mrachacz-Kersting et al. 2007; Roy et al. 2007). We have also shown recently (Stein et al. 2006; Stein et al. 2007) that daily use of a stimulator to correct the condition of foot drop leads to enhanced maximum voluntary contraction and MEPs. Foot drop is a common condition that occurs in many central nervous system disorders, such as stroke, incomplete spinal cord injury and multiple sclerosis. Thus, finding the optimal combination of peripheral and central stimulation to strengthen corticospinal pathways would have direct clinical application.

The H-reflex has been studied for eighty years since the initial work of Hoffmann (Hoffmann 1918). Pairs of stimuli have also been applied and the response to the second stimulus is well known to be depressed for inter-stimulus intervals of up to 10 s. This is far too long to be due to refractoriness of the motor neurons and has been

attributed to a phenomenon known as homosynaptic depression (Curtis and Eccles 1960) or post-activation depression (Hultborn et al. 1996). These authors suggest that the release of transmitter by the second stimulus is depressed in those and only those muscle afferents that have been activated by the first stimulus. The extent of post-activation depression differs for different muscles and is absent in some (Rossi-Durand et al. 1999; Pierrot-Deseilligny and Burke 2005) but has mainly been studied in resting muscles. Even in those muscles such as soleus that show a marked depression after stimulating the tibial nerve, post-activation depression can be reduced to less than 1 s or eliminated by asking a subject to contract a muscle voluntarily, particularly in a functional situation such as standing (Stein and Thompson 2006). Using a steady, weak voluntary contraction also has the advantage that the state of the motor neurons is better defined than at rest. Resting motor neurons may be just below threshold or strongly enough inhibited that they are almost unable to fire. Therefore, we have used a steady level of background contraction except when specifically comparing the resting and tonic states.

Measurement of MEPs has become common in the past twenty years, since the introduction of transcranial magnetic stimulation (TMS) (Day et al. 1989; Petersen et al. 2003; Di Lazzaro et al. 2004; Reis et al. 2008). TMS provides a non-invasive, relatively non-painful way to measure descending connections from the motor cortex. TMS excites populations of inhibitory and excitatory neurons in the motor cortex (for review see Chen 2004) and changes in these cortical circuits can be assessed using paired pulses of TMS (e.g. Kujirai et al. 1993; Stokic et al. 1997; Chen et al. 1998). Suprathreshold, paired-pulse TMS results in strong facilitation of the second response when pairs are delivered up to 50 ms apart (Nakamura et al. 1997; Roy et al. 2007). This increase in the MEP is in stark contrast to the H-reflex depression occurring with pairs of peripheral nerve stimuli and warrants further investigation. Since any facilitation observed with pairs of TMS pulses could arise from cortical or subcortical processes, we tried to distinguish between these possibilities by stimulating the pyramidal tract at the level of the brain stem.

The heterosynaptic process, by which TMS creates a descending corticospinal volley, may be closer to natural muscle recruitment than direct pyramidal tract activation (Di Lazzaro et al. 2004). Electrical stimulation of pyramidal tract neurons at the level of the cortex or the brainstem are alternative methods for eliciting descending volleys in the corticospinal tract (Di Lazzaro et al. 2001; Taylor and Gandevia 2004), but are much more painful than motor cortex stimulation with TMS (Day et al. 1989; Di Lazzaro et al. 2004). The TMS level we used was high enough to produce about half the maximum excitation that could be elicited, so that facilitation (or depression) could be measured as an increase (or decrease) in the MEP. We also used a stimulus to a peripheral mixed nerve that produced a similar sized H-reflex to compare the two inputs accurately at the same level of voluntary activity (15-20% of the maximum voluntary contraction).

Prolonged peripheral nerve stimulation in the lower leg can increase the MEP in control and disabled subjects (Khaslavskaia et al. 2002; Knash et al. 2003; Stein et al. 2006). This effect is particularly effective in the presence of voluntary drive (Khaslavskaia and Sinkjaer 2005) which further enhances the amount of MEP facilitation. Repetitive pairing of peripheral and central stimuli at selected intervals in

the relaxed or contracted muscle, known as paired associative stimulation, can also increase the MEP in the lower leg (Stefan et al. 2002; Mrachacz-Kersting et al. 2007; Roy et al. 2007) or the hand (Stefan et al. 2002; Kujirai et al. 2006) for up to an hour. However, in this study we restricted the number of stimuli at each interval to limit the induction of plastic changes in the MEP. This paper compares the responses evoked in the soleus muscle by paired-pulse TMS and paired-pulse TN stimulation, as well as to paired TMS and TN stimulation at a variety of intervals. Depending on the conditioning stimulus, different synaptic pathways (i.e., corticospinal or reflex) were either facilitated or depressed.

B.2 METHODS

B.2.1 Subjects

Ten adult subjects (6 female, 4 male; ages 18-67 years) gave informed consent to participate in this non-invasive study in accordance with the Declaration of Helsinki. They were screened for neurological disorders or known physical conditions that may have been aggravated by external stimulation (such as injured knees). The NIH guidelines for application of TMS (Wassermann 1998) were followed to eliminate subjects with a tendency to epilepsy, or who had metal objects in their head. The experiment lasted approximately two hours per subject.

B.2.2 Set-Up and EMG recording

Subjects were seated with one leg placed in a stationary metal brace that held the knee and ankle at about 100° to maintain a consistent posture for all experiments (Knash et al. 2003). The tibialis anterior (TA) and soleus muscles were initially cleaned with alcohol before applying silver/silver chloride recording electrodes (3.3x2.2 cm; Kendall, Chicopee, MA). The TA electrodes were placed about 1/3 of the distance between the ankle and the knee over the belly of the muscle. The soleus electrodes were placed in the midline of the back of the leg just below the lower border of the calf muscles. The recording electrodes and a ground electrode were connected to an isolated pre-amplifier/amplifier (Octopus, Bortec Technologies, Calgary, Alberta) and amplified 1500 times. EMGs from the two muscles were filtered, digitized (DigiData 1200 Series, Axon Instruments, Union City, CA) and displayed on a personal computer running Axoscope 8 with a 5 kHz sampling rate. A rectified and low-pass filtered (3Hz) signal of the soleus EMG was displayed on an oscilloscope to monitor the level of background EMG during the experiment. Subjects were asked to extend (straighten) their ankle as hard as they could three times separated by a few seconds rest in between contractions. The subject's maximum voluntary contraction (MVC) was recorded as the highest value of the rectified and filtered EMG that could be maintained for more than 1 s. Subjects were then asked to maintain a tonic contraction (15-20% of MVC), which was displayed on the oscilloscope during each period of recording. As mentioned, the contraction ensures a constant activation of the soleus muscle, and decreases the variability of the MEPs (Darling et al. 2006).

B.2.3 Tibial nerve (TN) stimulation

Silver/silver chloride disposable electrodes (2.2 cm square; Jason, Huntington Beach CA) were placed at the best location for stimulating the tibial nerve behind the knee (cathode) and about 2 cm higher on the leg (anode). The stimulus intensity (SD9 Stimulator, Grass Intstruments, West Warwick RI) was set to a value that produced a peak-to-peak H-reflex that approximately matched the MEP. At the low-intensity stimulation used to match the size of the MEP (see the next section) an H-reflex can be recorded with minimal contamination from the M-wave that results from directly activating motor neurons. The threshold for motor neurons is somewhat higher than that for the primary muscle spindle afferents that produce the H-reflex. Soleus muscle was used for most of the measurements, since relatively large and comparable H-reflexes and MEPs can be obtained in normal subjects.

B.2.4 Transcranial Magnetic Stimulation (TMS)

To generate the TMS pulses, two Magstim 200 Mono-pulse units were attached to a Magstim BiStim (Magstim Company Ltd., Great Britain) and connected to a Magstim double cone coil. The coil was oriented to induce posterior-anterior currents in the brain. To find the best location to stimulate the motor cortex, the vertex was first identified on the subject's head. A position 1 cm posterior and 1 cm lateral on the side opposite to the secured leg was marked as the initial starting point for TMS activation. The TMS was moved in increments of 1 cm to find the coordinates that would evoke the largest MEP. If this position differed from the initial one, a new marker was placed and subsequently used for TMS. The TMS intensity that resulted in approximately 50% of the maximum MEP that could be evoked in the active muscle was then determined and this intensity was used throughout the experiment.

B.2.5 Paired Stimulation Protocol

All stimuli were applied at random intervals between 3 and 5 s, so subjects could not anticipate the timing of stimulation. The TMS and TN stimuli were controlled by separate analog delays, allowing for a wide range of intervals between stimuli. Ten subjects underwent the same protocols for three conditions: TN stimulation preceding TN simulation, TMS preceding TMS, and TN stimulation preceding TMS. Five subjects did an additional test: TMS preceding TN stimulation. To establish a baseline response, four unpaired electrical stimuli were applied to the tibial nerve (paired TN) and/or the motor cortex (paired TMS). This was followed by sets of four pairings at various intervals from 0 up to 70 ms and then another set of unpaired stimulations. If the initial and final control responses to unpaired stimuli varied substantially, the trial was repeated with the intervals taken in reverse order (from 70 to 0 ms). Control experiments were done in eight subjects testing conditions with TN preceding TMS and TMS preceding TMS in soleus muscle at rest. For these experiments the TN stimulus intensity was just above the motor threshold and the TMS intensity was set to 50% the maximum resting MEP. The TMS coil (custom-built figure-of-eight) induced currents in the anterior-posterior direction.

B.2.6 Direct corticospinal Tract Stimulation

Corticospinal tract axons were activated at the level of the brain stem using electrical and magnetic stimulation. Not all subjects showed responses at a tolerable level of stimulation. The responses were much clearer in the TA muscle than the soleus so we targeted the TA muscle in these experiments. The time course of facilitation is similar in the two muscles (Roy et al., unpublished observations). Responses were observed in two subjects to magnetic stimuli delivered over the inion using a double cone coil with the current flowing in a downward direction (Taylor and Gandevia, 2004). Responses to electrical stimulation, which is also thought to directly activate the corticospinal tract (see the Introduction), were observed in one subject using a 100 μs electrical pulse (350-400 V, D185 stimulator, Digitimer, Hertfordshire, UK) delivered through a pair of silver cup electrodes (10 mm diameter) fixed over the mastoids with the cathode on the left. Paired-pulse brain stem stimulations were done at inter-stimulus intervals of 5, 10, 15 and 20 ms, intermingled with single-pulse test stimuli. We will refer to the results from the three subjects as “direct corticospinal tract stimulation” and they will be compared to paired-pulse TMS over the motor cortex at the same inter-stimulus intervals, as described above.

B.2.7 Data Analysis

Sweeps recorded from the EMG electrodes were visualized in Axoscope, then processed in a custom Matlab program to analyze background contraction levels and peak-to-peak H-reflexes with TN stimulation or peak-to-peak MEPs with TMS (Fig. B-1). Peak-to-peak values could be measured for each sweep within the analysis window indicated by the vertical dashed lines and then averaged. During each experiment, the experimenters adjusted the parameters of the program to match the times where each subject’s H-reflex and MEP began and ended. The H-reflex usually began a few milliseconds before the MEP so the H-reflex determined the onset of the analysis period. The MEP usually persisted for a few milliseconds after the H-reflex so the MEP determined the end of the analysis period. In addition, the responses were rectified and averaged. The mean rectified background activity prior to the stimulus was also averaged and subtracted from the mean rectified H-reflex and MEP.

For short inter-stimulus intervals (0, 5, 10, 15 ms), the responses to the conditioning stimuli overlap those to the test stimuli. Simply subtracting unrectified responses is inadequate, since the MEPs and H-reflexes can have quite different waveforms (Fig. B-1). The H-reflex consists of a fairly synchronous wave, whereas the MEP consists of a series of direct and indirect waves. Positive or negative phases of one response could add to or cancel phases of the other response or of the ongoing EMG. A better, but not perfect method was to rectify and average the responses. As shown in Fig. B-2, the mean rectified response to a single stimulus (A) was shifted in time by the conditioning/test interval (B) and subtracted from the composite response to the paired stimuli (C). This allowed the response to the test stimulus to be estimated in isolation from that of the conditioning stimulus. The difference was then averaged over the time interval shown by the vertical dashed lines. A linear subtraction is only a first approximation since rectification is a non-linear operation. Also, the two

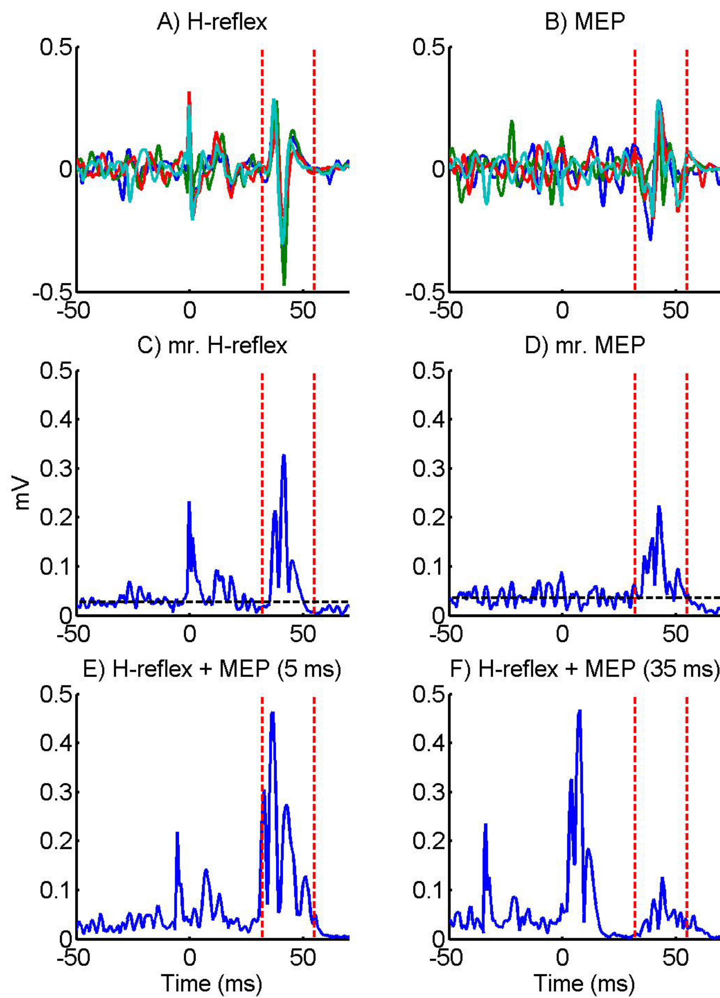

Figure B-1. H-reflexes and MEPs for one subject

Four superimposed traces for TN stimuli to elicit H-reflexes (*A*) and for TMS to elicit MEPs (*B*). Vertical dashed lines represent the interval chosen to measure peak-to-peak values on each trace and to measure the mean rectified (mr) H-reflex (*C*) and MEP (*D*). The horizontal dashed lines show the mean rectified background activity prior to the stimuli (at 0 ms), which was subtracted from the responses. A TN stimulus 5 ms prior to TMS elicited a larger response (*E*) whereas a TN stimulus 35 ms before the TMS elicited a smaller response (*F*). The TMS occurred at 0 ms in (*E*) and (*F*).

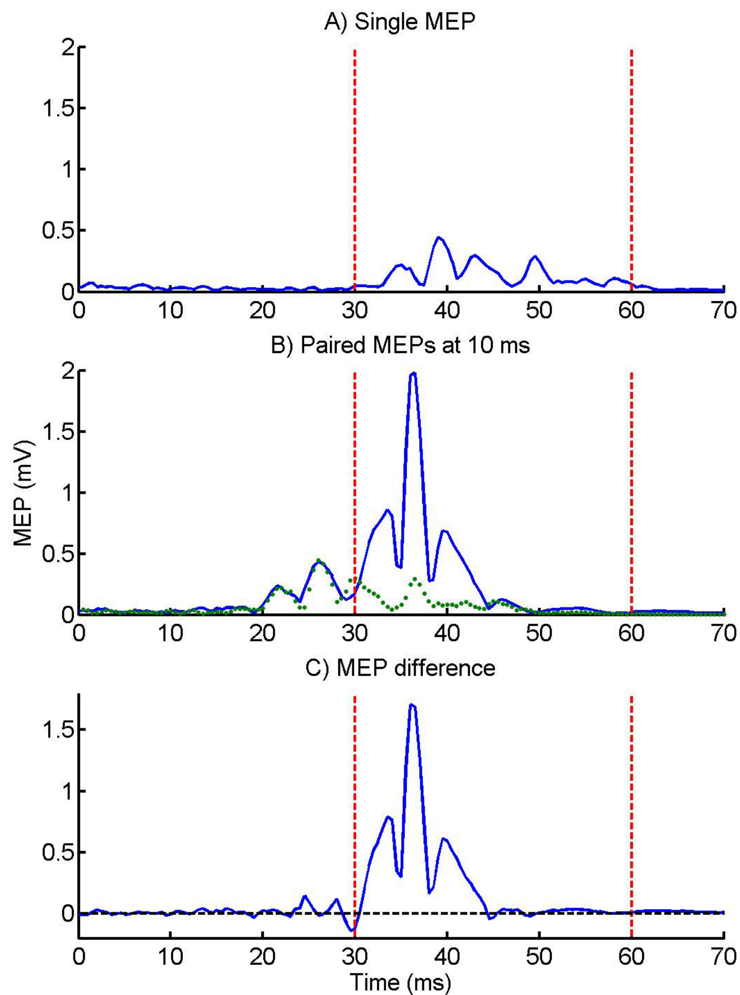

Figure B-2. Method for determining the contribution of the test stimulus for a short inter-stimulus interval

Part *A*) shows the mean rectified MEP to TMS, while part *B*) shows the mean rectified response to two TMS pulses at a 10 ms interval (solid line). The same trace as in *A*) has been superimposed after shifting it in time by 10 ms (dotted line). *C*) the two traces in *B*) have been subtracted to give the contribution of the second (test) TMS pulse, which is about 3 times as large as the response to an unpaired stimulus; compare the amplitudes in *A*) and *C*).

responses can cancel each other to some extent, if a negative wave in one is shifted in time enough to line up with a positive wave in the second response.

All responses are presented in terms of inter-stimulus intervals which are known precisely. Since the MEP responses can last for 30 ms (Fig. B-2) there is no single "arrival time" of the volley at the spinal cord. All statistical and graphical analysis was done through Microsoft Excel and SPSS software (SPSS Inc., IL, USA). Responses were normalized with respect to the response to the unpaired test stimulus (either from the peak-to-peak unrectified signal or the mean rectified signal, as appropriate) to account for differences in excitability of individual subjects. The effect of the inter-stimulus interval between the conditioning and test stimulus was assessed using a one way-repeated measures analysis of variance (ANOVA) treating the inter-stimulus interval as a within-subject factor. Paired t-tests (two-tailed) were used to test for significant changes at the different inter-stimulus intervals compared to the unpaired response and for comparing differences between the brain stem and TMS responses. Data are given as means ± standard errors (SE) and significance was set at $p < 0.05$.

B.3 RESULTS

Figure B-1 shows examples of one subject's responses to test stimuli applied at time 0. Four individual trials are superimposed for the H-reflex (A) and the MEP (B). The H-reflex is slightly larger but shorter lasting so the two responses are reasonably well matched. This was true for the population as a whole; the unpaired peak-to-peak H-reflex and MEPs were 0.85mV ± 0.11mV (mean ± SE) and 0.64mV ± 0.08mV, respectively. These levels also allow for both inhibition and facilitation to be observed (Zehr and Stein 1999). Mean rectified values are shown in (C) and (D) and the increased (E) and decreased (F) responses produced by TN stimuli 5 and 35 ms before a TMS stimulus. Figure B-2 illustrates how responses at short inter-stimulus intervals were modified to account for overlap between conditioning and test stimuli (details in Methods).

When delivering pairs of TN stimuli and TMS pulses, both the size of the conditioned H-reflex ($F(12,108) = 10.3$, $p < 0.001$) and MEP ($F(10, 90) = 13.9$, $p < 0.001$) depended on the interval between the conditioning and test stimuli. Figure B-3 compares the size of the response produced by the second stimulus at different intervals for both paired TN and TMS. The responses are normalized to the response to a single stimulus (i.e., TN or TMS). For paired TN stimulation, the second response is profoundly depressed except for the 5 ms interval and the responses are all statistically different from 1.0 ($p < 0.05$). In contrast, the MEPs in response to TMS pairs are generally facilitated and the facilitation is statistically significant for intervals between 10 and 25 ms ($p < 0.05$).

What is the cause of this striking difference between the two inputs to the soleus motor neurons? Does the prolonged facilitation with TMS arise at the level of the motor cortex or the spinal cord? One way to study these questions is to stimulate the corticospinal tract directly (see Methods). The responses in the three subjects studied

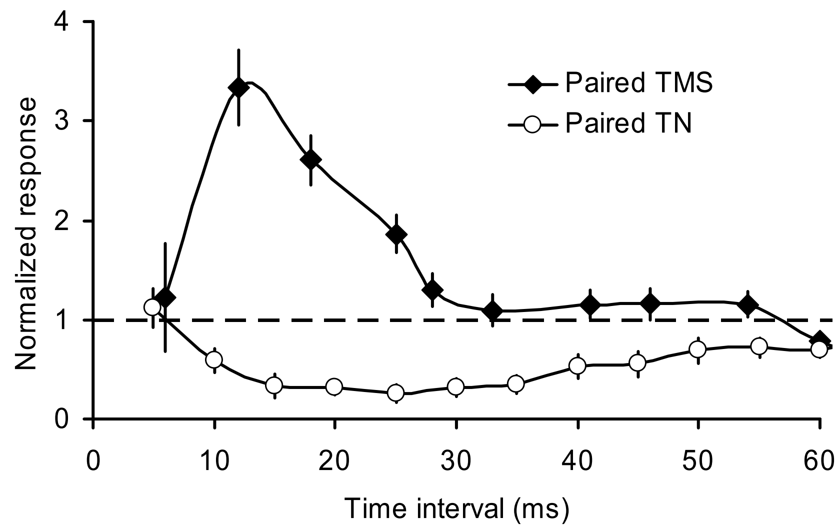

Figure B-3. Mean and standard errors of normalized responses in soleus muscle to the second of a pair of TMS or TN stimuli

For short intervals (5-15 ms) where the initial responses summed with the second response, a program was used to subtract the effects of the overlapping response (Fig. 2). Everything above the horizontal dashed line represents facilitation (response greater than 1.0) and everything below the line represents depression.

were clearer in TA than the soleus muscle, so the TA responses are presented. Also, since the direct corticospinal stimulation was more uncomfortable than TMS over the motor cortex, we limited our measurements to intervals up to 20 ms. In Figure B-4 paired stimuli directly activating the corticospinal tract showed suppression in the size of the response at inter-stimulus intervals of 10-20 ms that contrasted with the facilitation produced with pairs of TMS over the motor cortex. The responses to paired TMS in the TA muscle had a similar time course to those in soleus while the direct activation of the corticospinal tract at intervals > 5 ms are reminiscent of the peripheral nerve stimulation in Figure B-3. At interval of 10-20 ms, the effect of the type of stimulation was significant ($p < 0.05$), suggesting that the long-lasting facilitation observed with paired motor cortex stimulation is cortical in origin.

Figure B-5 shows the results when TN stimulation preceded TMS by various intervals. The average peak-to-peak H-reflex response, normalized to TMS values, across subjects was 1.47 ± 0.20. The linear sum of the MEPs and the H-reflex alone would be 2.47 (1.00 for the MEP normalized to itself plus 1.47 for the H-reflex normalized to MEP). However, a higher average normalized MEP of 3.15 ± 0.66 ($p < 0.05$) is observed despite subtracting the value of H-reflex (see Fig. B-2). This facilitation is quickly lost at an inter-stimulus interval of 10 ms, with a depression of the MEP in the 25-35 ms range ($p < 0.05$). There appears to be a late facilitation at intervals of 50–60 ms, but the values are not significantly greater than 1.0.

These experiments were also repeated with eight subjects at longer intervals when the soleus muscle was at rest. Figure B-6 compares MEPs from paired TMS to those from single TMS conditioned by TN stimulation in the resting muscle. The paired pulse TMS shows even a larger and longer lasting facilitation than during contraction (compare Fig. B-6 and Fig. B-3). The facilitation of the MEP was statistically significant at intervals from 10-40 ms ($p < 0.05$). However, the resting MEPs were an order of magnitude smaller (peak-to-peak values near 0.1, compared to 1.0 mV), so the facilitation occurred from a smaller initial value. When conditioned by a TN stimulus, the facilitation of the MEP was also more prominent at rest (Fig. B-6) than during voluntary contraction (Fig. B-5) and the values in Figure B-6 were significantly different from 1.0 at intervals of 50-60 ms ($p < 0.05$).

The presence or absence of facilitation at intervals of 10-25 ms seems to be determined by the conditioning stimulus (Fig. B-7). Three of the four conditions have been presented earlier, but the data are superimposed and data have been added for TMS preceding TN. Trials with TMS as the initial stimulus show a short latency facilitation which lasts for at least 25 ms. In contrast, trials with TN as a conditioning stimulus show a facilitation of less than 10 ms followed by a depression that persists until about 40 ms. We will now discuss the mechanisms underlying these results.

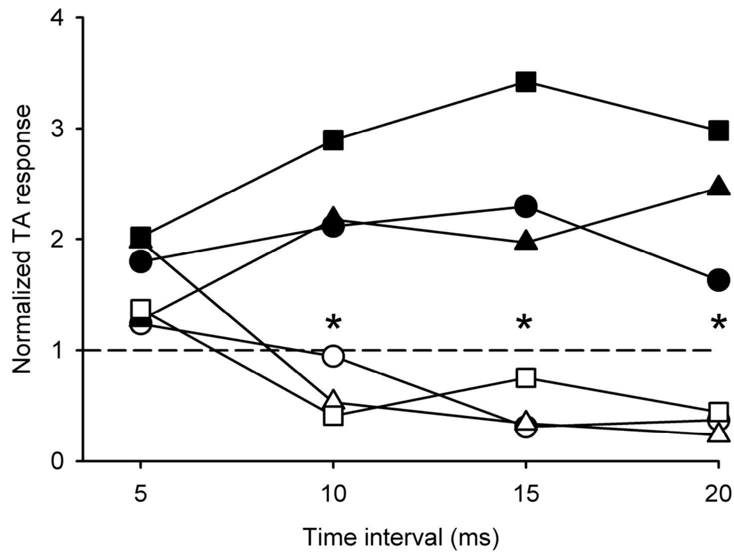

Figure B-4. Comparing paired corticospinal tract stimulation to paired TMS

Individual subject data (different symbols) showing TA EMG responses following paired-pulse TMS over the motor cortex (filled symbols) and pairs of stimuli (open symbols) directly activating the corticospinal tract at the level of the brain stem with TMS (two subjects) or by electrical stimulation over the cortex (one subject). The peak-to-peak responses to a single cortical TMS pulse and to direct activation of the corticospinal tract were 0.82 + 0.36 mV (mean + SD) and 0.61 + 0.28 mV, respectively. The data are normalized to the responses to single stimuli and no correction was made at short intervals for overlapping responses. Values between 1.0 and 2.0, for example at 5 ms, may just represent summation of responses, rather than true facilitation. Asterisks indicate significant differences in the response between the two stimulation sites ($p < 0.05$).

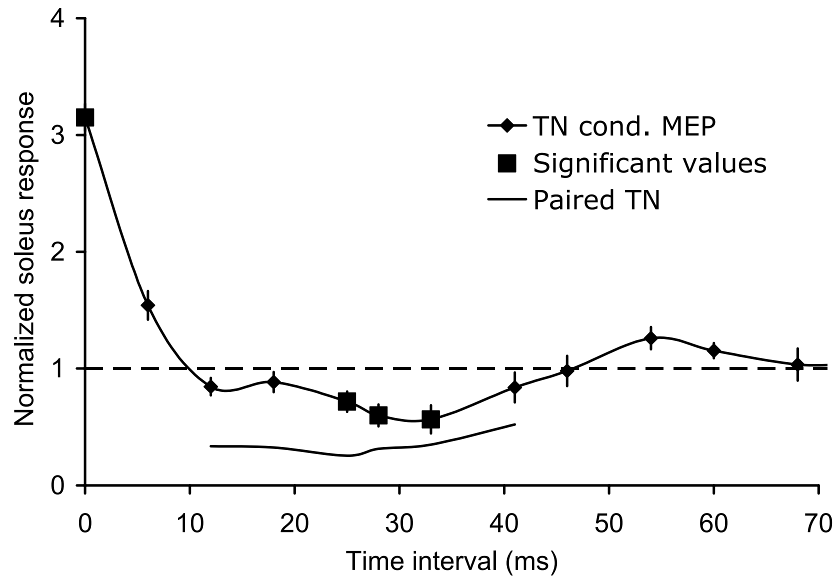

Figure B-5. Mean and standard errors of the normalized soleus MEPs, when conditioned by TN stimulation at various time intervals prior to TMS

There was a statistically significant facilitation at time 0, even after subtracting the effects of the overlapping H-reflex, and a significant depression at time intervals between 25 and 35 ms (shown as larger squares). Superimposed on the graph are the paired TN responses from Fig. B-3 for the range of intervals over which they were significantly more depressed than for the MEP with a prior TN stimulus.

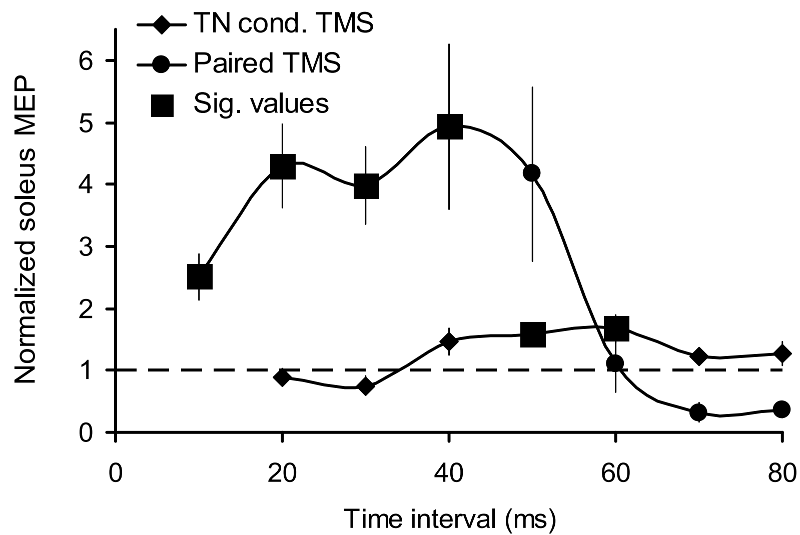

Figure B-6. TMS responses in the resting soleus muscle

Normalized MEPs in soleus muscle at rest when conditioned by TN (♦) or TMS (●). MEPs were much smaller at rest than during contraction (typically 0.1 mV, rather than 1 mV peak-to-peak), but were facilitated more when conditioned by a preceding TMS pulse. Data are from eight subjects. The facilitation was significant (■; $P < 0.05$) for the paired TMS at intervals from 10-40 ms and for TN conditioning at 50-60 ms.

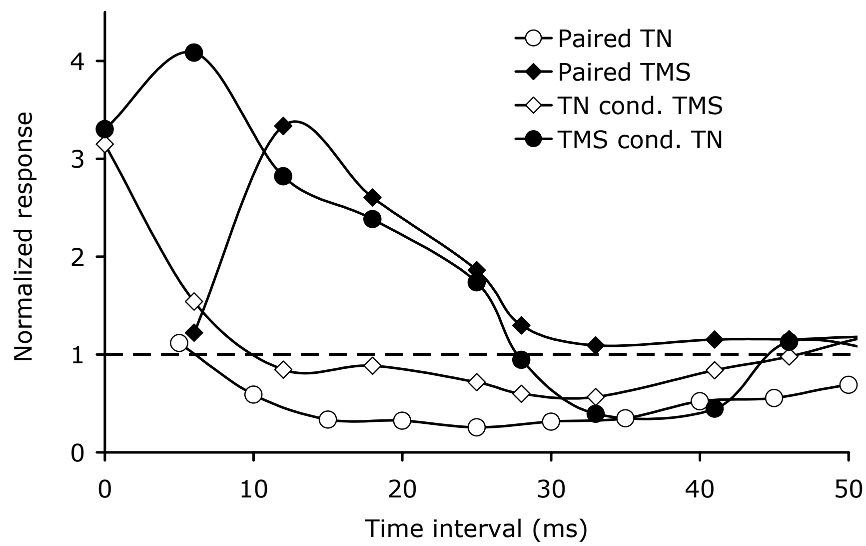

Figure B-7. Comparison of four different conditions

The responses that are conditioned by TN (open symbols) show a depression at intervals between 10 and 30 ms, whereas those conditioned by TMS (closed symbols) show facilitation. Further discussion in the text.

B.4 DISCUSSION

In this paper we studied the interaction of two inputs onto motor neurons that were matched to produce similar sized responses in the human soleus muscle. The two inputs were: stimulation, predominantly of muscle spindle afferents in the tibial nerve (TN) to produce an H-reflex, and TMS over the motor cortex to produce an MEP. Combining a TN stimulus and TMS produced markedly different responses than either alone. For example, a striking result (Fig. B-3) was that one TN stimulus produced a maintained depression of the H-reflex response to a second TN stimulus for at least 60 ms, whereas one TMS pulse produced a maintained facilitation of the second MEP for 25 ms. The TN stimulus could even depress a subsequent MEP elicited by TMS for up to 40 ms, while TMS was able to facilitate a subsequent H-reflex for 25 ms (Fig. B-7).

The depression produced by a TN stimulus can most easily be explained by a refractoriness of the soleus motor neurons produced by their prolonged afterhyperpolarization (McPhedran et al. 1965; Matthews 1996). If the neurons are hyperpolarized, a stronger second stimulus will be needed to activate them again. An alternative explanation is the phenomenon of post-activation or homosynaptic depression (Hultborn et al. 1996). However, we have argued elsewhere (Stein and Thompson 2006) that this depression is largely abolished once subjects maintain a voluntary contraction. Also, as implied by the name, homosynaptic depression applies to a depression of only the synapse that has been stimulated. However, Fig. B-7 clearly shows that stimulation of the TN depresses the response to TMS for up to 40 ms. This depression is consistent with a post-synaptic effect, such as an afterhyperpolarization, rather than a presynaptic phenomenon on the synapses that have been stimulated. Other post-synaptic effects such as Renshaw inhibition may also contribute to this depression (Windhorst 1996) or there may be inhibitory interneurons that are activated by both TMS and TN stimulation; these possibilities have not been tested in the present study.

How is TMS able to facilitate a subsequent response, despite the afterhyperpolarization of the motor neuron? In principle, the facilitation could be at a spinal or supraspinal level. Similar MEP facilitation, referred to as intracortical facilitation, can also be observed using paired-pulse TMS with a subthreshold conditioning stimulus (Kujirai et al. 1993); however the origin of this facilitation is still unclear (Di Lazzaro et al. 2006). Here, paired suprathreshold stimuli directly to the corticospinal tract at the level of the brainstem did not produce similar increases in the motor response (Fig. B-4), suggesting that the potentiation has a strong cortical component Instead, TA responses to paired stimuli to the corticospinal tract were depressed and were qualitatively similar to those observed in the soleus muscle following paired TN stimuli. Together, these findings are in agreement with motor unit data suggesting that pyramidal tract and monosynaptic Ia connections act on motor neurons in a directly comparable way (Petersen et al. 2002). Afferent stimulation may recruit different, but overlapping sets of motor units (Morita et al. 1999), but it is unlikely that afferent stimulation acts on one set of motor neurons that show depression and cortical stimulation acts on another set that shows facilitation.

Regarding the mechanism of this MEP facilitation, TMS over the motor cortex predominantly activates pyramidal tract neurons trans-synaptically through local interneurons (Di Lazzaro et al. 2004). TMS elicits a series of descending volleys in the pyramidal tract (Di Lazzaro et al. 2001) and differs from the more synchronous afferent volley elicited with TN stimulation. Since pairs of suprathreshold TMS at inter-stimulus intervals of up to 50 ms result in parallel increases in the size of the descending corticospinal volleys and the MEP, a cortical mechanism probably underlies this facilitation. We postulate that: 1) the continuing depolarization of intracortical neurons can overcome the afterhyperpolarization in pyramidal neurons that have fired, 2) that the depolarization discharges additional pyramidal tract neurons that were in the subliminal fringe of the first stimulus and 3) that these two factors are sufficient to facilitate a second soleus MEP for at least 25 ms following the first TMS pulse.

When TN stimulation precedes TMS, a tri-modal pattern is observed, consisting of early facilitation, depression and later facilitation (Fig. B-5). The large early facilitation (near 0 ms) can be easily explained, since the latencies of the pathways from the TN to soleus motor neurons and from the leg area of the motor cortex are similar. In fact, the largest facilitation (about 4-fold) was seen when the TMS preceded TN stimulation by about 5 ms (Fig. B-7), which is consistent with the slight latency difference in the two pathways. Thus, with near simultaneous stimulation the two inputs can sum at the spinal level and produce a response several times as large as TMS alone or TN alone (Deuschl et al. 1991; Nielsen et al. 1993; Petersen et al. 1998), even after subtracting the effect of the overlapping conditioning response (Fig. B-2). The subsequent depression, when TN is the conditioning stimulus, has been discussed above. The small, later facilitation when TN precedes TMS by 50-60 ms was not significant during voluntary contraction (Fig. B-5), but was significant when subjects were at rest (Fig. B-6). The timing is such that the afferent volley from TN stimulation could reach the sensory cortex, cross over to the motor cortex and facilitate the MEP produced by TMS (Deletis et al. 1992; Mrachacz-Kersting et al. 2007). An alternative explanation is that the TN stimulation may produce an afterdepolarization of the motor neuron following the period of afterhyperpolarization (Kernell 1965). TMS would then produce a facilitated MEP when superimposed on the afterdepolarization. An argument against this latter explanation is that the latency of the facilitation is similar (50-60 ms) at rest and during voluntary contraction. The timing of the afterdepolarization should change with different activity levels, as the motor neuron is depolarized more or less quickly. In contrast, the pathways to the motor cortex via the somatosensory cortex will be relatively invariant since they mainly depend on the conduction velocity of the neurons involved. The time-course of this facilitation agrees with the arrival of the sensory input reaching the cortex and corroborates findings from paired associative stimulation targeting the leg motor cortex (Mrachacz-Kersting et al. 2007).

In conclusion, our data and those of other studies are consistent with the ideas that: 1) the profound depression in the first 50-60 ms following TN stimulation is largely due to post-synaptic mechanisms such as the large, long afterhyperpolarization found in soleus motor neurons and 2) the facilitation following cortical stimulation is largely due to presynaptic mechanisms occurring in the sensori-motor cortex.

B.5 BIBLIOGRAPHY FOR APPENDIX B

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APPENDIX C: Changes in locomotor muscle activity after treadmill training in subjects with incomplete spinal cord injury

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C.1 INTRODUCTION

Body-weight-supported treadmill training (BWSTT) is an effective treatment method for retraining walking in individuals with motor-incomplete spinal cord injury (iSCI). Intensive training leads to greater ability to walk on the treadmill with less assisted body-weight support, faster walking speeds and better endurance (for review see Harkema 2001; Field-Fote 2000). Further, improvements on the treadmill eventually transfer to increased gains in over-ground walking. Kinematically, training leads to better symmetry of steps (Field-Fote et al. 2005), less stride-to-stride variability (Grasso et al. 2004), more normal excursion in joints of the lower extremity (Barbeau and Blunt 1991; Barbeau et al. 1993) and a toe trajectory that is more similar to the uninjured (Barbeau et al. 1993; Grasso et al. 2004). Clinically, subjects require less assistance with walking, both in terms of personal assistance and walking aids (Wernig et al. 1995; Behrman and Harkema 2000), and they show improved sitting and standing balance (Behrman and Harkema 2000; Dobkin et al. 2006). Improvements in walking are seen whether the training is in the acute (Dobkin et al. 2006; Grasso et al. 2004; Nymark et al. 1998) or chronic phase after injury (Wernig et al. 1995). Moreover, those who regain the ability to walk in the household or community maintain this improvement over several years, and some continued to improve after training (Wernig et al. 1998; Wirz et al. 2001; Hicks et al. 2005).

What are the neural mechanisms that bring about these walking improvements? Is the motor pattern reverting to an uninjured pattern, or is the nervous system finding new solutions? Answers to these questions remain incomplete; however studying the motor pattern through surface EMG has provided some answers. For example, qualitative reports on single subjects suggest better timing of muscle activation (Barbeau and Blunt 1991) and some reduction in co-contraction between antagonists (Dietz et al. 1994, 1998). Quantitative information exists for the gastrocnemius muscles, which show greater activation in the stance phase after training (Dietz et al. 1994, 1995). However, this change could be explained by the increasing ability of subjects to support more of their own body weight from pre- to post-training (Dietz et al. 1994, 1995, 1998) since extensor EMG is responsive to the amount of weight-bearing, even in individuals with complete spinal cord injury (Harkema et al. 1997).

In addition, these subjects were trained acutely after the injury, so spontaneous recovery could also have contributed to the increased EMG activity (Calancie et al 2000). More recently, Grasso and colleagues (2004) reported that subjects show marked improvement in the end-point trajectory of the toe during walking, but the muscle patterns that lead to this improvement are quite different from those seen in uninjured subjects walking under matched conditions (Ivanenko et al. 2003). Their interesting findings suggested that new motor patterns were learned during BWSTT. Perhaps different subjects find their own motor solutions that may not be similar to the uninjured or to other injured individuals. In the Grasso study, only composite EMG scores were reported (i.e., average change in amplitude ratios), so the training-induced changes in EMG activation in individual muscles and between subjects remain unclear.

The purpose of this study was to quantify the changes in EMG activity from several major muscle groups in the lower extremity before and after training in a group of chronically (>0.8 yrs) injured subjects, under walking conditions that were identical (i.e., matched for both walking speed and weight-support). We sought to determine the changes in EMG amplitude, the timing of muscle activity and the amount of co-contraction between antagonists. We also compared the frequency content of the EMG activity both before and after training; a measure that has not been compared in the past, to determine if training has any effect on the degree of clonic activity during walking. Subjects were separated into two groups: those who gained functional improvements in over-ground walking ability (responders) and those who did not (non-responders). To assess functional over-ground walking ability, we used the Walking Index for Spinal Cord Injury II (WISCI II) (Ditunno and Ditunno 2001) which was developed and validated for the SCI population. The WISCI II contains a larger number of discrete categories compared to other walking scales such as the modified Wernig scale (Hicks et al. 2005; Wernig et al. 1998) or the Hauser scale (Hauser et al. 1983). We also defined improvements in over-ground walking function as any increase in over-ground walking speed of more than 0.06 m/s, as this has recently been shown to be functionally relevant (Musselman 2007). Subjects who eventually showed clinical improvements in over-ground walking showed EMG patterns that were vastly different from uninjured controls and also displayed changes in amplitude and timing in distinct muscles that were not observed in subjects that did not respond to training.

C.2 METHODS

C.2.1 Subjects

All experiments were carried out with the approval of the Human Research Ethics Board at the University of Alberta and with the written, informed consent of the subjects. Approval from a family physician was also obtained before each subject participated in the training. Our sample comprised of 19 subjects with incomplete spinal cord injury (iSCI) and 6 neurologically intact, control individuals (Table C-1). Inclusion criteria for treadmill training were that subjects must have sustained damage to the spinal cord and have the ability to move at least one of the leg joints

Code/Sex	Age	Injury Level	ASIA Score	Years Post Injury	Initial MMS (60 max)	Weeks Training	BWS, lbs (%BWS)		Belt Speed, m/s		WISCI II Pre–Post (diff)	Overground Speed, m/s Pre–Post (diff)
							Initial	End	Initial	End		
1M	41	C3–5	C	0.8	35	10	50 (35%)	20 (14%)	0.22	0.36	8–13 (5)	0.05–0.28 (0.23)
2M	25	T7/8	C	1.8	35	19	90 (32%)	30 (11%)	0.45	0.36	12–16 (4)	0.34–0.53 (0.19)
3M	58	L1	C	3.0	19	10	150 (70%)	60 (28%)	0.36	0.54	1–3 (2)	N/T
4M	71	T5–9	D	0.8	46	14	150 (73%)	60 (29%)	0.54	0.54	4–8 (4)	N/T
5M	78	T4/5	D	2.0	46	10	20 (10%)	20 (10%)	0.36	0.72	13–19 (6)	0.65–0.78 (0.13)
6M	48	C4–5	D	10.0	32	12	120 (50%)	60 (25%)	0.36	0.36	12–16 (4)	0.57–0.60 (0.03)
7M	34	C1–3	D	0.8	44	26	20 (14%)	0 (0%)	0.22	0.59	6–19 (13)	0.33–1.20 (0.87)
8F	74	T4–5	D	7.4	45	12	50 (32%)	60 (38%)	0.22	0.27	13–13 (0)	0.10–0.16 (0.06)
9F	24	T10	C	1.0	38	17	60 (44%)	30 (22%)	0.22	0.45	6–9 (3)	0.15–0.32 (0.17)
10M*	50	C5–6	D	28.2	37	18	60 (50%)	30 (25%)	0.45	0.54	9–9 (0)	0.8–1.1 (0.3)
11M*	42	T11–12	C	1.2	18	20	110 (65%)	80 (47%)	0.63	0.63	0–4 (4)	N/T
12M	30	C6 and T10	C	3.8	11	12	130 (70%)	100 (54%)	0.36	0.54	0–0	0.0–0.0 (0.0)
13M	23	T4–5	C	0.9	2	11	80 (44%)	90 (50%)	0.36	0.72	5–5	N/T
14M	47	C5/6	D	20.0	23	13	90 (55%)	50 (30%)	0.22	0.22	9–9	0.11–0.10 (−0.01)
15M	66	C5/6	C	5.0	15	12	110 (55%)	110 (55%)	0.22	0.27	0–0	0.0–0.0 (0.0)
16M	56	C3 and C6	C	1.5	20	28	150 (75%)	150 (75%)	0.22	0.36	0–0	0.0–0.0 (0.0)
17F	44	T2–4	C	1.0	31	11	70 (50%)	50 (36%)	0.22	0.31	9–9	0.14–0.18 (0.04)
18M	21	C7	C	1.0	15	10	110 (70%)	90 (57%)	0.22	0.45	9–9	N/T
19M	42	C3–4	C	1.0	38	11	120 (53%)	300 (44%)	0.13	0.31	0–0	0.0–0.0 (0.0)
20F	32	N/A	E	N/A	60	N/A	40%	N/A	0.7	N/A	20	N/A
21F	41	N/A	E	N/A	60	N/A	40%	N/A	0.7	N/A	20	N/A
22F	55	N/A	E	N/A	60	N/A	40%	N/A	0.7	N/A	20	N/A
23M	28	N/A	E	N/A	60	N/A	40%	N/A	0.7	N/A	20	N/A
24M	33	N/A	E	N/A	60	N/A	40%	N/A	0.7	N/A	20	N/A
25M	32	N/A	E	N/A	60	N/A	40%	N/A	0.7	N/A	20	N/A

Table C-1. Demographic, injury, training and experimental details for all iSCI and control subjects.

Responders are in white columns, non-responders in light grey (12M-19M), controls in dark grey (20F – 25M). All iSCI subjects sustained trauma to the spinal cord except for subjects 8F and 17F where damage occurred due to transverse myelitis and a surgical bleed, respectively. Subject 9F also sustained a head trauma. Subject 7M performed over-ground training 2-3 times per week in the last 4 months of training. ASIA scores (Ditunno et al. 1994) include global sensory and motor scores from all body segments below the lesion. Years Post Injury is the time between the injury and the onset of training is indicated. MMS = the initial manual muscle strength. BWS = body weight support. The initial BWS and belt speed were used at each EMG testing session. The end BWS and belt speed were the values used during the final training sessions. N/A = not applicable, N/T = not tested. * subjects included in clonic analysis only.

(ASIA C and D; Ditunno et al. 1994). Exclusion criteria were: 1) orthopaedic problems that could affect walking ability; 2) bone density that was 30% or less of age-matched, non-injured subjects; 3) impaired mental capacity or severe depression; and 4) other medical contraindications to treadmill training (such as cardiovascular problems, pulmonary disorders, history of deep vein thromboses, etc.). In one subject (3M), who had an L1 lesion, data were excluded for the left TA and SOL due to lower motor neuron damage; however, the activity patterns in remaining muscles were similar to other subjects before training with no evidence of denervation activity (i.e., fibrillation potentials) in the raw EMG traces, so 3M was included in the analysis.

C.2.2 Training

Training consisted of one hour per day of partial BWSTT, as described elsewhere (Thomas and Gorassini 2005). The target frequency was 5 days per week; on average, subjects trained 3.3±1.3 (SD) days per week for an average of 14 ± 6 weeks. Training continued until subjects reached a plateau in walking function, which was defined as a lack of change in over-ground walking ability or speed (see Clinical Assessment below). In subjects in whom no improvements were seen, training lasted for a minimum of 10 weeks. For subject 7M, 2 to 3 treadmill sessions a week were replaced with over-ground training for the last 4 weeks.

Briefly, each training session consisted of body-weight support (BWS) combined with manual assistance of leg movements while the subject walked on a motorized treadmill. Depending on the need, one or two people were positioned at the lower limbs to provide stepping assistance by lifting the foot through swing, flexing the knee at the start of swing and/or stabilizing the knee during stance. A bungee cord tied to the harness and the support frame was sometimes used to help stabilize the subject's trunk; in other cases, a trainer manually stabilized the trunk. Features of stepping, such as base of support, weight shift between the two legs, step length, postural alignment, hip extension at the end of stance and foot contact during stance (heel to toe), were monitored by the therapist to ensure subjects stepped as efficiently and safely as possible. Subjects were encouraged to swing one or both of their arms when possible. If arm swing resulted in instability of the body, subjects were allowed to grasp horizontal bars positioned at chest level on each side to aid in balance control, but not for weight-bearing. Subjects walked at a slow pace, between 0.2 to 0.6 m/s (0.5 to 1.4 mph), enabling them to concentrate on voluntarily activating their muscles during walking. Rests were taken when needed, but subjects were encouraged to walk/rest at ~10 minute intervals. The amount of body weight support and/or stepping assistance were gradually decreased and the treadmill speed increased when the physical therapist noticed improvements in cardiovascular tolerance, better ability to volitionally control the limbs, smooth weight transition between limbs and better upright trunk alignment. The responders decreased the amount of BWS by 39.1 ± 32.7 lbs on average compared to the non-responders who only decreased the amount of BWS by 15.0 ± 16.9 lbs ($p = 0.07$, see Table C-1 for individual values). The average increase in walking speed at the end of training compared to the start of training was similar between the two groups (responders 0.12 ± 0.15 m/s vs. non-responders 0.15 ± 0.11 m/s, $p = 0.61$, Table C-1). The average amount of time that a

responder walked for during a training session also increased from 25.4±11.2 to 36.2±11.4 minutes with the non-responders increasing from 14.6±11.3 to 29.8±9.2 minutes (average differences not significant, p = 0.4). The amount of assistance given by the physical therapists was not systematically documented in all subjects.

C.2.3 Clinical assessments

Clinical assessments of walking function were performed by experienced physiotherapists. Functional over-ground walking ability was assessed by the WISCI II, a 21-point ordinal scale that incorporates the use of aids required to achieve the walking function (Ditunno and Ditunno 2001). As in our previous study (Norton and Gorassini 2006), we separated subjects who did not show an improvement in WISCI II score (non-responders) from those who did (responders). In addition, subjects that increased their walking speed by 0.06 m/s or more were also considered to be responders as this increment is considered functionally relevant (Musselman 2007). Manual muscle strength (MMS) was assessed by experienced physiotherapists who used the qualitative MRC scale (Medical Research Council 1976) to assess the strength of the major muscle groups in the leg (hip, knee and ankle flexors and extensors, 5 point maximum per muscle group). The maximum score obtainable with this method was 60; (6 muscles per leg x 2 legs x 5 points per muscle = 60). Subjects were also classified using the ASIA classification scale (Ditunno et al. 1994). All participants in this study were classified as either ASIA C or D (Table C-1).

C.2.4 EMG assessment

Irrespective of the length of the training program, comparisons of EMG activity while walking on the treadmill were made between the start and end of the treadmill training period using the initial (pre-train) BWS and treadmill belt speed values for each subject (Table C-1). EMG activity was also measured in 6 uninjured control individuals who walked on the treadmill at the average walking speed (0.3 m/s) and BWS (40%) of the iSCI subjects. In the control subjects, test/retest reliability of the EMG measures was established by using two instances of walking on a treadmill separated by at least a week in which subjects did not alter their exercise routine, to avoid any novel training effects. EMG measurements were also made while controls walked at a more natural speed (0.9 m/s) for comparison. In both iSCI and controls, EMG activity from at least 30 steps was recorded bilaterally from the tibialis anterior (TA), soleus (SOL), hamstrings (HAM) and quadriceps (QUAD) muscles using isolated EMG amplifiers (Octopus, Bortec Technologies, Calgary, AB). Signals were recorded using bipolar surface electrodes (Kendall H59P, Tyco, MA) and were amplified (500 to 1k) and filtered (band-pass 10-1000 Hz) before being stored on a PC using a Digidata1200 AD card and Axoscope software (Axon Instruments, Molecular Devices, Sunnyvale, CA). All data were sampled at a rate of 5 kHz. Changes in knee angle during stepping were recorded with an electrogoniometer (Biometrics Limited, Ladysmith, VA). Data from muscles on the right and left leg in a single subject were considered to be independent because of the asymmetry in lesion location in all subjects.

C.2.5 Peak and total EMG activity

Peak and total EMG activity were analyzed in 17 subjects (1M to 9F, 12M-19M) using custom-written software within the Matlab programming environment (The MathWorks, Natick, MA). The EMG signals were first rectified and then smoothed using a 150-ms sliding average (Fig. C-1A). This data was then re-sampled at 50 Hz. The knee angle was used to estimate the start and end of each step. To obtain the amount of peak EMG activity reached during each step, the maximum and minimum values in the rectified and smoothed EMG were automatically determined. The minimum values were used to characterize the level of background EMG (noise) in each step and were subsequently subtracted from the maximum EMG value to obtain a measure of peak EMG activation in each step. The peak EMG value for each step was then averaged across all steps for a given experiment. The total EMG area was calculated by summing all the points within a step after subtracting the mean level of background EMG (or noise). Likewise, the total EMG area for each step was averaged across all steps.

C.2.6 EMG burst duration and area

To quantify the percentage of time that a muscle was active during a step cycle (EMG burst duration), the data were first normalized to the step-length (with each step having 100 points, Fig. C-1B). The onset and end of the EMG burst were estimated by visual inspection of the normalized recordings (see dashed vertical lines in Fig. C-1B) and were expressed as a percentage of the total step cycle (out of 100%). The EMG burst area was calculated by summing all points within the delineated burst after subtracting the background EMG, then averaging across all steps. In some muscles (n = 3), it was not possible to measure the onset or end of the EMG burst due to contact artefact in the EMG signal.

C.2.7 Co-contraction

Co-contraction occurred when antagonistic muscles (TA and SOL or HAM and QUAD) were both active at the same time in the step cycle (see Fig. C-1B). Again, the duration of co-contraction between two antagonist pairs of muscles was expressed as a percentage of the total step cycle (100%), and values for each step were averaged for a given experiment.

C.2.8 Variability of muscle on and off times

Because the on or off time of an EMG burst can cross the boundary of a step cycle that is delineated by the knee angle data, we used circular statistics to assess the variability in on and off times for each muscle burst. Using this approach, the on or off time of a particular EMG burst was plotted on the edge of a unit circle at an angle indicating the point in the step cycle that the muscle becomes active or inactive (see Fig. C-6A). This process was repeated both before and after training for each muscle on and off time. To measure the degree of spread of the points (i.e., the angular

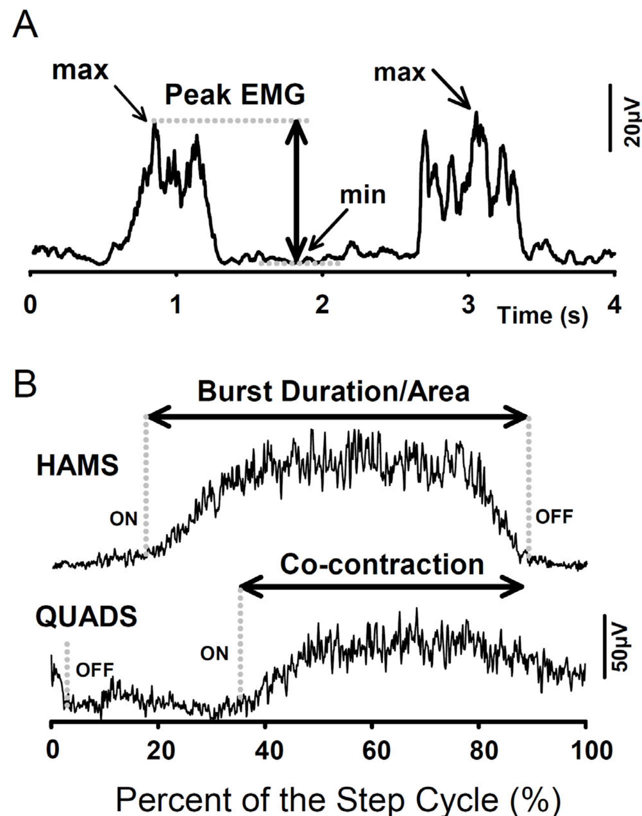

Figure C-1. Quantifying EMG burst duration and area

A, For each step cycle (as determined from the knee angle) the minimum value of the rectified and smoothed EMG was subtracted from the maximum value (dashed horizontal lines) to calculate the peak EMG activity for that step. The peak EMG values were then averaged for a given stepping trial. *B*, To calculate burst duration, the step cycle was first normalized to 100% and the time of onset of the burst was subtracted from the time at the end of the burst (vertical dashed lines) for each step. The burst duration values were then averaged for a given stepping trial. To calculate burst area, the points within the burst were summed. The amount of co-contraction was measured as the percentage of time in the step cycle a pair of antagonist muscles (TA/SOL and HAM/QUAD) were active at the same time.

dispersion), the length of the mean vector (*r)* was calculated which is represented as the distance from the origin of the circle to the centre of mass of the points (Zar 1999). In the example shown in Figure C-6A, a selection of onset times for a muscle is plotted on a circular measure of the gait cycle along with the associated *r* value. This normalized value (1 indicating no spread and 0 indicating no clustering) was used to determine if stepping became more regular after training.

C.2.9 Clonus

To determine if treadmill training had any effect on the amount of clonic EMG activity before and after training, custom-written scripts within the Matlab environment were used to rectify the data and apply a fast Fourier transform. The power in the 7.2 to 8.8 Hz band was then calculated per unit time (Hidler et al. 2002) for the 4 muscles of each leg during a walking trial (data with contact artefact was excluded). For each subject, we averaged the power spectrum of all 8 muscles together as there was not a statistical tendency for any particular muscle group to be any more clonic (contain appreciable power in the ~7 to 9 Hz range) than other muscle groups. Data from two additional subjects (10M and 11M, both responders) were included in the clonic EMG analysis (Table C-1). Power in the clonic frequency band was also measured from EMG activity generated during treadmill walking in the 6 uninjured control individuals in the same manner as for the iSCI subjects.

C.2.10 Statistics

Statistical analysis was performed using SigmaPlot 8.0 for Windows (SPSS Inc., Chicago, IL) and Matlab. For normally distributed data, statistical analysis was performed using paired Student's t-tests to compare parametric data within groups. Wilcoxon tests were used for non-parametric and non-normally distributed data and the unpaired Student's t-test was used to compare parametric data between responders and non-responders, responders and controls or non-responders and controls. When a Wilcoxon test was used, it is stated in the text; otherwise, Student's t-tests were used. In all cases a significance level of 95% is considered to be statistically significant and all data are given as means ± SD.

C.3 RESULTS

C.3.1 Changes in walking function

The responder group (subjects 1-9, Table C-1) was comprised of four ASIA C and five ASIA D subjects whereas the non-responder group (subjects 12-19) had seven ASIA C and only one ASIA D subject. Likewise, the responders had initial MMS scores for the leg (38±9) that were two times as great as the non-responders (19±11, $p < 0.01$, Wilcoxon test, see Table C-1 for individual values). The average time from the injury date to the start of training was lower in the responders compared to the non-responders (3.1±3 vs. 4.3±7 years, respectively) but the difference was not

significant ($p > 0.6$) and the number of weeks that subjects were trained (14.4±5 responders, 13.5±6 non-responders, $p > 0.7$) was also similar. The speed of the treadmill belt at which the testing was carried out was higher in the responders (0.33±0.12 m/s) compared to the non-responders (0.24±0.08) but the difference did not reach significance ($p = 0.11$). However, the non-responders did use a greater amount of percent BWS during testing compared to the non-responders (40±22% responders vs. 59±11% non-responders, $p = 0.04$). The average improvement in the WISCI II score for the responders was 4.6 points (from 8.3±4 to 12.9±5, $p < 0.01$, Wilcoxon test) with an average increase in over-ground walking speed of 0.24 m/s (from 0.31±0.3 to 0.55±0.4 m/s, $p < 0.05$, Wilcoxon test). Non-responders had no increases in WISCI II scores and an average increase in walking speed of less than 0.06 m/s, given that many did not walk over-ground before or after training (from 0.04±0.07 to 0.05±0.08 m/s, $p=0.58$, Wilcoxon test).

C.3.2 EMG activation in responders, non-responders and controls

Responders showed greater EMG activity during BWS treadmill walking *prior* to training compared to the non-responders. Typical results are presented in Figure C-2 (grey lines, pre training), which demonstrates that responders (left column) started out with greater EMG activity compared to non-responders (middle column), especially in the TA and HAM muscles for these two subjects. The amount of TA and SOL EMG activation in the responder is comparable to the amount generated in the control subject walking at a similar speed (0.3 m/s) and BWS (40%, right column), but the amount of HAM and QUAD activity is greater in the iSCI subjects, especially in the responder. In addition, the amount of EMG activity generated during treadmill walking increased substantially in the responder after training (Fig. C-2 black traces), especially in the TA and HAM muscles, in contrast to the non-responder whose EMG profiles (except for HAM) did not change appreciably in response to training.

C.3.3 Peak EMG Activation

As a group, the amount of peak EMG activation in the responders increased significantly for the TA and HAM muscles (Fig. C-3A; $p < 0.05$; Table C-2) but not for the SOL and QUAD muscles in response to training. In fact, the average peak EMG activation in TA and HAM after training was greater than the average values measured in the controls walking at 0.3 m/s as represented by the solid horizontal lines in Fig. C-3 (TA $p < 0.05$; HAM $p < 0.01$). The amount of SOL peak EMG activation did not change after training and was slightly higher than in controls ($p = 0.07$), in contrast to the QUAD values which were much higher than in controls both before ($p= 0.0001$) and after ($p = 0.02$) training. In the non-responders (Fig. C-3B), the initial peak EMG activation for all muscles was about half of that seen in the responders, with SOL and HAM values closer to the controls. Following training, there were small to negligible increases in peak EMG activation in the non-responders (Table C-2); however, none was statistically significant. Similar trends

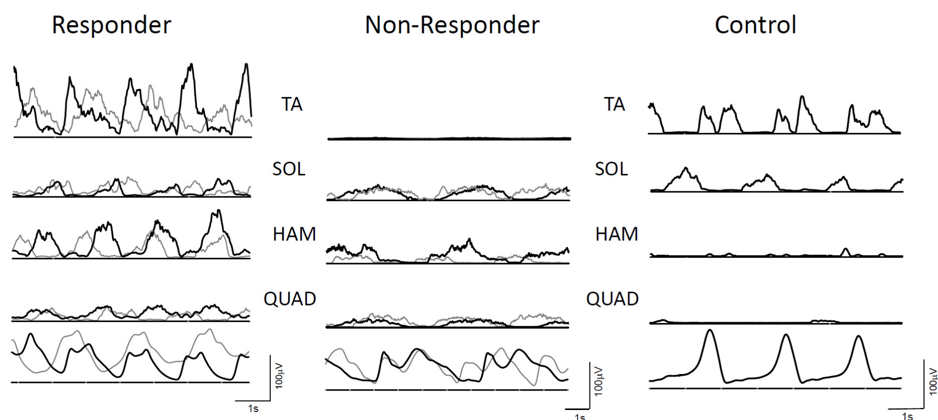

Figure C-2. EMG activity before and after treadmill training

EMG activity from the TA, SOL, HAM and QUAD muscles recorded during treadmill walking from a responder (left column) and non-responder (middle column) both before (grey lines) and after (black lines) treadmill training. Knee angle is shown in the bottom trace, and the onset of the swing is shown as an upward deflection in the knee angle. Example EMG recording in a control individual is shown in the right column. All EMG signals are displayed on the same y-axis scale.

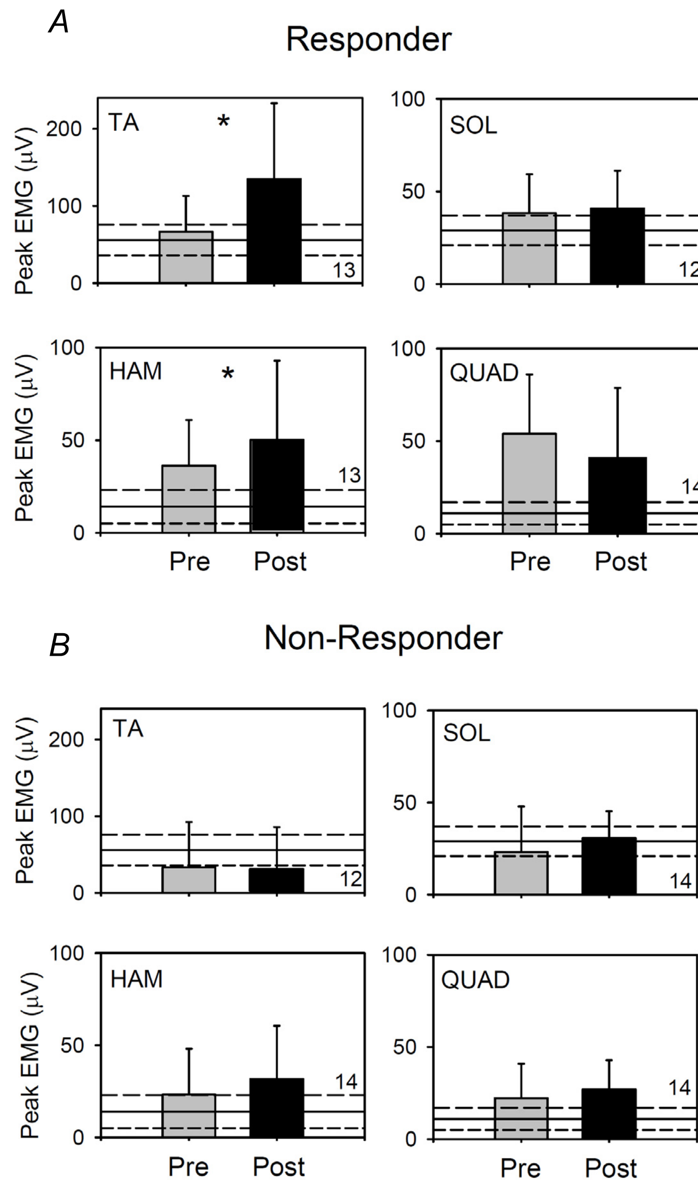

Figure C-3. Summary of the changes in peak EMG activation.

A, Responders: changes in peak EMG activity before (grey bars) and after (black bars) training. The numbers in the lower right of each graph represent the number of muscles included in each average. Solid and dashed horizontal lines represent mean and ± standard deviation values, respectively, for the control group. *B*, Non-responders. Same format as in *A*. (* $p < 0.05$).

Subjects	Peak EMG, μV		Total Area, μV		Burst Area, μV		Burst Duration, % Step Cycle	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Responders								
TA	67 ± 46	**135 ± 98**	1,900 ± 1,116	**3,877 ± 3,245**	1,284 ± 1,080	**2,424 ± 2,298**	54 ± 22	49 ± 18
SOL	38 ± 21	39 ± 22	1,731 ± 937	1,760 ± 945	1,088 ± 694	1,404 ± 796	70 ± 10	67 ± 19
HAM	36 ± 25	**50 ± 43**	1,419 ± 941	**1,996 ± 1,417**	788 ± 494	**1,275 ± 923**†	83 ± 10	**72 ± 17**#
QUAD	54 ± 32	40 ± 40	2,234 ± 1,364	1,963 ± 1,595	1,919 ± 1,299	1,692 ± 2,010	83 ± 11	**73 ± 13**
Nonresponders								
TA	33 ± 59	31 ± 55	1,282 ± 2,594	898 ± 1,442	695 ± 1,514	562 ± 731	41 ± 20	42 ± 22
SOL	23 ± 25	31 ± 15	1,175 ± 1,026	1,556 ± 944	810 ± 864	951 ± 555	71 ± 12	72 ± 9
HAM	23 ± 25	32 ± 29	1,049 ± 1,279	1,595 ± 1,593	665 ± 797	923 ± 985	63 ± 25	63 ± 21
QUAD	22 ± 19	27 ± 16	1,341 ± 1,274	1,283 ± 759	822 ± 683	900 ± 540	78 ± 16	69 ± 17
Control (0.3 m/s)								
TA	56 ± 20	N/A	1,963 ± 981	N/A	1,631 ± 775	N/A	62 ± 11	N/A
SOL	29 ± 8	N/A	959 ± 217	N/A	649 ± 175	N/A	46 ± 8	N/A
HAM	14 ± 9	N/A	534 ± 601	N/A	384 ± 423	N/A	47 ± 22	N/A
QUAD	11 ± 6	N/A	394 ± 253	N/A	326 ± 207	N/A	55 ± 24	N/A
Control (0.9 m/s)								
TA	79 ± 26	N/A	1,823 ± 644	N/A	2,225 ± 1,031	N/A	61 ± 4	N/A
SOL	58 ± 13	N/A	1,164 ± 245	N/A	1,252 ± 488	N/A	46 ± 11	N/A
HAM	18 ± 12	N/A	364 ± 394	N/A	366 ± 415	N/A	46 ± 17	N/A
QUAD	24 ± 21	N/A	462 ± 480	N/A	474 ± 653	N/A	44 ± 21	N/A

Table C-2. Data summarizing EMG analysis for responders, non-responders (before and after training) and control subjects (walking at 0.3 and 0.9 m/s for comparison).

Calculation of total area and burst area were done differently (see Methods) and are not directly comparable. Numbers marked in bold represent a statistically significant change post training compared to pre-training ($p < 0.05$, paired t-test). Differences in EMG values between iSCI subjects and controls are stated in the text. N/A = not applicable. # $p = 0.06$, † $p = 0.10$

occurred for total and burst EMG activation in a step cycle for both responders and non-responders (see Total Area and Burst Area, Table C-2). EMG values for controls walking at a more natural speed (0.9 m/s) are also shown in Table C-2 for comparison.

C.3.4 EMG burst duration

In addition to changes in the *amplitude* of EMG activity with treadmill training, there were also changes in the *timing* of EMG activity measured in the responders. For example, the percentage of time that a muscle was active in the step cycle (burst duration, see Methods) was reduced in some muscles following training, even though the treadmill speed was kept constant. There was a significant decrease in burst duration for the QUAD muscle after training and a strong trend to decrease in the HAM muscle (Figure C-4A, Table C-2). After training, the duration of HAM and QUAD burst activity decreased to reach amounts that were closer to values in the controls, whereas the TA and SOL burst durations remained below and above control values, respectively. In contrast, the burst duration times did not change with training in the non-responders, though there was a non-significant decrease in the burst duration of the QUAD muscle after training ($p = 0.14$; Fig. C-4B, Table C-2). Note that the large variability in the burst duration for the HAM and QUAD muscles in the control subjects was due to the fact that the burst duration could vary by as much as 50% of the step cycle between subjects. This variability in timing was likely of little functional consequence because the amplitude of HAM and QUAD activation is very low in the non-injured (Figure C-3). This is also in agreement with the low levels and high variability of muscle moments about the knee reported by others for walking in non-injured subjects (Winter 1991).

C.3.5 Co-contraction

We also examined the amount of time that there was coincident activity in muscles traditionally defined as antagonists (TA/SOL and HAMS/QUADS), a measure of co-contraction (see Methods). In responders, there was a greater amount of co-contraction in TA/SOL and HAM/QUAD compared to controls ($p < 0.05$, Fig. C-5A). In controls, there was a large degree of variability in HAM/QUAD co-contraction because, at the 0.3 m/s walking speed, HAM activity occurred either in the stance or swing phase with QUAD activity occurring mainly in the stance phase. In iSCI subjects, HAM activity mainly occurred in the stance phase alongside the QUAD activity. After training, as a group there were no net changes in co-contraction in the responders given that half of the muscle pairs increased and half of the muscle pairs decreased in co-contraction. Of note, in 6 of the 13 HAM/QUAD muscle pairs examined, a decrease in co-contraction of more than 15% of the total step cycle occurred after training. An example of a *decrease* in HAM/QUAD co-contraction for a responder subject is shown in Figure C-5C whereas an example of a HAM/QUAD *increase* in co-contraction is shown for the responder subject in Figure C-2. The amount of co-contraction for both TA/SOL and HAM/QUAD in the non-responders (Fig. C-5B) was lower compared to responders and more similar to control values given the shorter burst durations in the TA and HAM muscles.

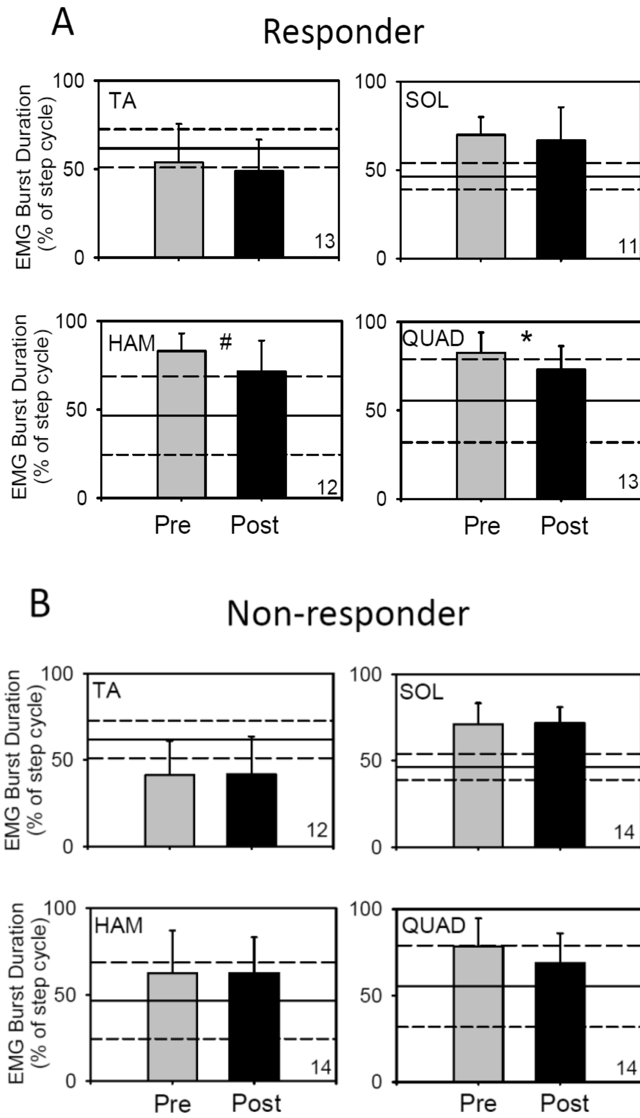

Figure C-4. Changes in EMG burst duration with training

Changes in EMG burst duration with training expressed as a percentage of the total step cycle that is normalized to 100%. Same format as in Figure 3. In some muscles (1 SOL, 1 HAM and 1 QUAD of responders), burst duration values could not be measured because of contact artefact affecting the EMG signal. Solid and dashed horizontal lines represent mean and ± standard deviation values, respectively, for the control group.* $p < 0.05$, # $p = 0.06$

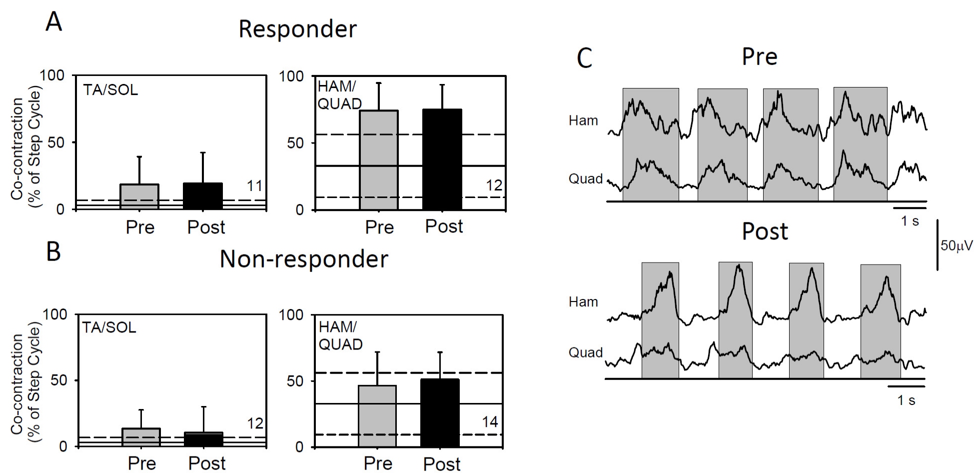

Figure C-5. Changes in co-contraction with training.

A, Responder group. Amount of co-contraction expressed as a percentage of normalized step cycle both before (grey bars) and after (black bars) treadmill training for the TA/SOL (left graph) and HAM/QUAD (right graph) muscle pairs. Same format as Figures C-3 and C-4. *B*, Corresponding data for the Non-responder group. *C*, Example data from Responder subject (1M) demonstrating decrease in co-contraction (marked by grey boxes) for the HAM/QUAD muscle pairs after training, which is mainly due to a decrease in burst duration of the HAM muscle.

In only 1 of the 12 HAM/QUAD muscle pairs studied in the non-responders was there a decrease in co-contraction of greater than 15% of the step cycle after training.

C.3.6 Regularity of EMG activation (on and off times of EMG burst)

To determine if training affected the regularity of stepping, the on and off times of an EMG burst for each subject were plotted on a circular representation of the step cycle (Fig. C-6A) and the angular dispersion (r), a measure of variability, was calculated (see Methods). There was less variability in the on and off times of muscle activity in the responders before training compared to the non-responders as reflected in higher r-values obtained by combining data from all muscles [0.92±0.07 vs. 0.89±0.1, $p < 0.05$, Figure C-6B & C-C (grey bars)]. After training several muscles in both responders and non-responders showed a statistically significant decrease in variability in either the on and off times, indicated by an increase in the r-value (black bars in Figure C-6B&C, $p < 0.05$ in all cases). After training, the amount of variability in the on and off times was similar between the two groups (0.96±0.04 responders vs. 0.94±0.07 non-responders, values combined for all muscles, $p = 0.17$).

C.3.7 Changes in clonic activity

In the EMG activity of all subjects, including the controls, power in the clonic frequency band (~7 to 9 Hz) was detected. An example of visible clonus in the EMG signal from the HAM muscle generated during the stance phase of walking is shown for a responder in Figure C-7A (before training). There were no differences between responders and non-responders in the amount of power in the clonic band before training (4.2±1.1 and 4.0±0.9 dB/s respectively, $p > 0.6$, Fig. C-7C grey bars). After training, the amount of power in the 7 to 9 Hz band decreased as shown for the responder subject in Figure C-7B. Similar trends occurred for both groups where the amount of clonic EMG activity decreased to 3.7±0.9 dB/s in the responders and to 3.5±0.9 dB/s in the non-responders (Fig. C-7C, black bars, $p > 0.05$). The iSCI subjects (combined data from responders and non-responders) showed significantly more power in the ~7 to 9 Hz band both before (4.1±1.0 dB/s) and after (3.7±0.9 dB/s) training than the controls (2.5±0.2 dB/s, all $p < 0.005$).

C.3.8 Reproducibility of EMG measures

Because EMG measurements were made on data acquired on different recording days, it was necessary to ensure that factors such as small differences in positioning and/or impedance of recording electrodes did not appreciably change the measured EMG values. Reproducibility of the measured peak EMG activity was examined by recording muscle activity during treadmill walking in 3 controls (at two intervals separated by at least one week). Muscle activity patterns in the controls should not change since no training was undertaken in the intervening period, allowing us to assess the test/retest reliability of our measures and to ensure that any changes in EMG activity were indeed a result of training. Peak EMG activation for any muscle

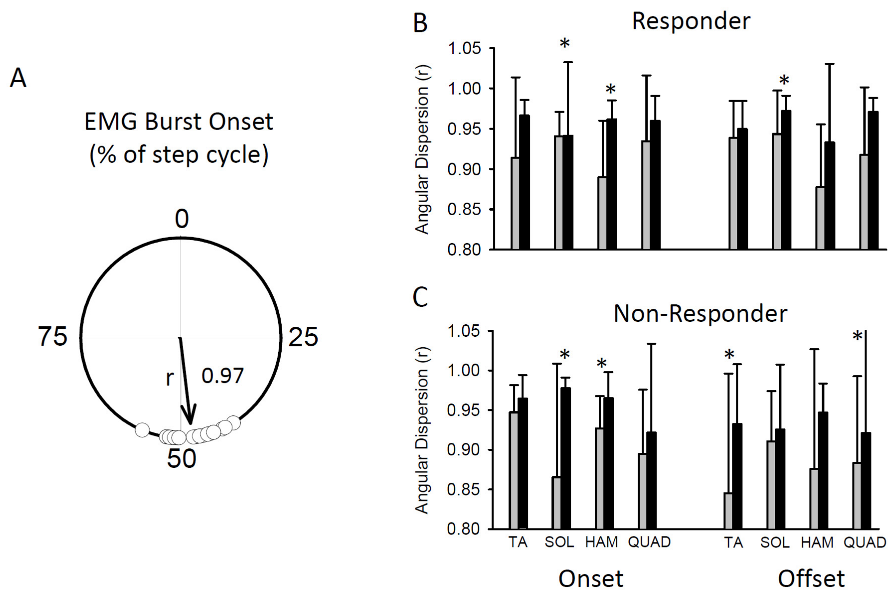

Figure C-6. Angular dispersion (*r*) was used to estimate the variability in onset and offset times for each muscle group.

A, Each dot on the circle represents the onset time of a muscle expressed as a percentage of the normalized step cycle. The vector points to the centre of gravity of the dots, the length being an indicator of the spread of the dots. *B*, Responders: there was a decrease in the variability of both the onset (left bars) and offset (right bars) times for all muscle groups as indicated by increased r values (pre: grey bars; post: black bars). Increases were significant in 3 cases (paired t-test). *C*, Non-Responders: Increases in r-values were significant in 4 cases. * $P<0.05$

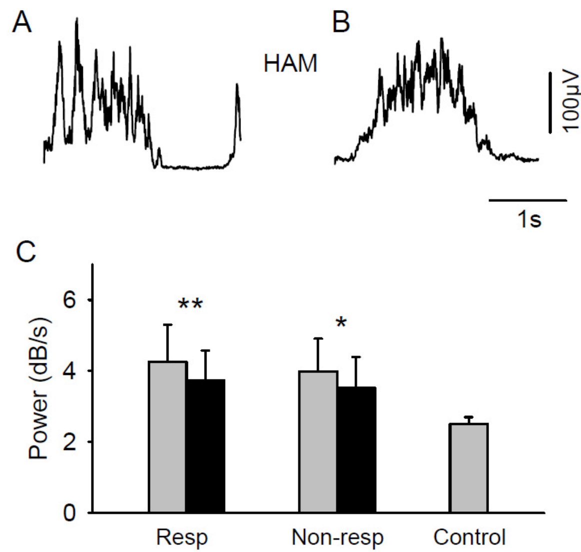

Figure C-7. Changes in clonic EMG activation from training.

Example HAM EMG during a single step before (*A*) and after (*B*) training, respectively. Although some clonic activity in the 7 to 9 Hz range remains after training it is reduced in amplitude. *C*, The amount of clonic activity decreased in both the responder and non-responders. Although clonic activity was reduced after training, it remained higher than in controls. * $p < 0.05$, ** $p < 0.005$

did not vary by more than 10% (all $p > 0.1$) and this is in accord with published guidelines on EMG test/retest reliability (Hallet et al. 1994).

C.4 DISCUSSION

Although responders and non-responders both developed a more regular and less clonic EMG pattern on the treadmill, only the responders showed increases in EMG activation and shortening of the EMG burst duration in select muscles. Interestingly, the responders also had vastly different EMG profiles in proximal muscles compared to uninjured controls, both before and after training. Therefore, the ability to modify the activation of muscles during walking after injury may predict if a patient can produce further compensations in response to training. Mechanisms responsible for the muscle activation patterns observed during walking after iSCI and the changes induced by training are discussed below.

C.4.1 Training altered EMG activity in certain muscles

Increases in muscle activation have been reported for iSCI subjects undergoing training at an earlier stage of recovery (Grasso et al. 2004: 1-6 months post-injury), but the changes were not reported for individual muscle groups, and subjects who responded positively to the training were averaged with those who did not respond. Moreover, spontaneous recovery could also have contributed to the changes during the relatively acute phase post-injury. The present study controls for spontaneous recovery by examining subjects 8 months or more post-injury who had already plateaued in their walking function. In the responders only, the TA and HAM muscles showed increases in peak, total and burst EMG activity. However, both responders and non-responders showed patterns of stepping that were equally less variable at the end of the training period compared to their initial values; hence, a decrease in variability of walking alone does not account for the changes in EMG activation observed in the responder group. The increase in the regularity of stepping may arise from sources intrinsic to the subject, such as improved modulation of muscle activity associated with the subject getting more accustomed to the treadmill, or from extrinsic factors, such as improved timing of assistance from the therapists. To avoid the increased regularity of stepping affecting our calculation of EMG amplitudes and timing, we examined each step individually rather than calculating these values from averaged EMG profiles. In addition, we were careful to ensure that the recording conditions before and after training were identical, particularly with regard to treadmill speed and body-weight unloading, which can affect both the amplitude and timing of EMG activation during treadmill walking (Ivanenko et al. 2003, 2006; Harkema et al. 1997). Changes in EMG activation as a result of changing walking speed are also shown for the control subjects walking at 0.3 and 0.9 m/s (Table C-2).

In the Grasso et al. 2004 study, a correlational analysis of muscle activity before and after treadmill training was presented. In that study, although the kinematics of the movement became more similar to the uninjured, especially the foot trajectory, the muscle activity did not show the same trend, and in some subjects, the activity pattern

actually became less like controls. In our recordings, it was surprising to note that the responders, who on average had twice the volitional muscle strength as the non-responders (see MMS scores in Table C-1), also displayed EMG activation patterns during walking that were vastly different from uninjured controls. In particular, the proximal HAM and QUAD activity which mainly occurred during stance was 3 to 4 times larger than in controls and likely reflects the greater need to control for postural instabilities resulting from reduced muscle strength and/or control of important muscles for walking. Unlike controls, the responders likely cannot take advantage of the limbs' biomechanics to help stabilize the body during stance and propel the limbs forwards in swing, but must use additional EMG activation. For example, the knee is often more flexed during stance in individuals with iSCI than in the uninjured, necessitating more extensor activity to prevent collapse (Winter 1980). Once the responders became more stable in their walking after training, some actually showed a decrease in QUAD muscle activity to reach values closer to the controls, although this was not a consistent trend across all subjects. In contrast, the non-responders were too weak to provide the added muscle activity needed to compensate for the inefficient biomechanics and ironically, had similar amounts of activation in proximal muscles compared to the uninjured controls. As discussed below, a lack of compensation from training in the non-responders may arise from an insufficient amount of residual descending pathways as evidenced by the more pronounced muscle weakness in these subjects compared to the responders.

C.4.2 Neuronal mechanisms producing changes in EMG activation

A prolonged period of treadmill training with partial body-weight support leads to an increase in over-ground walking ability that is accompanied by changes in amplitude and timing of select muscles. Observations from animals with anatomically complete spinal cord lesions indicate that the spinal cord contains neural circuits capable of generating a stepping pattern (Lovely et al. 1986). Treadmill training in these adult animals, which presumably occurs by training these neural circuits or central pattern generators (Barbeau and Rossignol 1987), leads to improved treadmill walking, but does not translate to over-ground, self-propulsive walking. Based on the animal findings (Barrière et al. 2008), a possible mechanism to explain our current observations is that we are training spinal circuits or reflexes to generate a better stepping pattern. The observed reduction in clonic activity suggests changes in spinal reflex pathways or circuitry may contribute to the improvements in walking function (Dimitrijevic et al. 1980; Barbeau et al. 1999). However, a decrease in the amount of clonic activity was not correlated with an improvement in function as a similar number of responders and non-responders demonstrated decreases in clonic activity.

An alternative and additional mechanism to produce improved walking may be an increase in the activation of descending pathways from the motor cortex and/or brainstem. This may also explain why changes only occurred in the TA and HAM, and not SOL muscles. Estimates of the cortical projections to the leg indicate that the SOL muscle receives fewer cortical inputs than the TA and the more proximal muscles (Brouwer and Ashby 1990; Bawa et al 2002). The SOL is predominantly a postural muscle whose activity is heavily modulated by spinal load-dependent reflexes (Duysens et al. 2000; Harkema et al. 1997) and the similar amounts of SOL

EMG activation between the stronger controls and weaker non-responders may reflect the spinally driven element of the SOL activity. Our earlier studies have demonstrated a relationship between functional recovery and descending corticospinal drive. The increase in the size of the maximal motor evoked potential in the TA and QUAD muscles from training was positively correlated with functional improvement in walking (Thomas and Gorassini 2005). The degree of common cortical drive during walking, as measured by coherence of the EMG signal in the 20-40 Hz bandwidth, was also found to be higher for the thigh muscles (HAM & QUAD) of iSCI subjects who responded to treadmill training (Norton and Gorassini 2006). Together with the current data, it suggests that muscles with greater descending drive from the motor cortex are also the ones that change the most with training.

In addition to training-induced increases in the activation of *excitatory* spinal circuits by spared corticospinal and brainstem inputs, increases in the activation of *inhibitory* spinal circuits by descending inputs may also have occurred in response to training. For example, decreases in the burst duration (and co-contraction) of proximal muscles may have been produced by a stronger descending activation of inhibitory interneurons controlling the duration of muscle timing during walking (Shefchyk and Jordan 1985).

C.4.3 Summary and clinical implications

Before training there was a difference in the amplitude of muscle activity during walking between the responders and the non-responders. This initial level of muscle activity produced during walking may be a useful predictor of subjects who will respond to the therapy, especially in the ASIA C class which is very broad and within which there are currently no predictors for who will benefit from treadmill training therapy. This is an issue we will address in a forthcoming publication.

This study has shown distinct changes in the way the damaged nervous system activates the muscles of the leg during walking that parallel functional recovery of walking skills after treadmill training. The altered pattern of EMG activity from training in iSCI subjects likely reflects changes at both spinal and supraspinal levels of the neural axis, although evidence for the former still needs to be established in the human. Finally, this study also highlights that functional gains in over-ground walking ability can be made several years after a spinal cord injury and raises the concern that protocols using both regeneration-based interventions (e.g., olfactory ensheathing cell graphs) and motor training in subjects with chronic injury must take into account the affects of training in isolation.

C.5 BIBLIOGRAPHY FOR APPENDIX C

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