“Catch your dreams before they slip away”

Ruby Tuesday,
The Rolling Stones

University of Alberta

Sensorimotor integration in the human spinal cord

by

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Abstract

In this thesis sensorimotor integration in the human spinal cord was investigated in the intact (Chapters 2 and 3) and injured nervous systems (Chapter 4-stroke; Chapter 5-spinal cord injury (SCI)). In Chapter 2, I characterized a short-latency reflex pathway between sensory receptors of the lower leg and the erector spinae (ES) muscles of the lower back that may play a role in the maintenance of posture and balance. The ES reflexes were evoked bilaterally by taps applied to the Achilles' tendon and were modulated by task. Furthermore, these reflexes involved a larger contribution from cutaneous receptors in the lower limb, rather than muscle spindles. In Chapter 3, I investigated changes in reflex transmission along the H-reflex pathway throughout 10 s trains of neuromuscular electrical stimulation (NMES) using physiologically relevant frequencies (5-20 Hz) and during functionally relevant tasks (sitting and standing) and background contraction amplitudes (up to 20% MVC). The results of this study revealed strong post-activation depression of reflex amplitudes, followed by significant recovery during the stimulation, both of which were influenced by stimulation frequency and background contraction amplitude, but not task. During 10 Hz stimulation, reflex amplitudes showed complete recovery (i.e. back to their initial values), and at times, complete recovery occurred by the third reflex in the train. These results demonstrate that transmission along the H-reflex pathway is modulated continuously during periods of repetitive input. In Chapters 4 and 5, I studied the extent to which a novel stimulation protocol that incorporated wide pulse widths (1 ms) and high frequencies (up to 100 Hz) (wide-pulse NMES; WP-

NMES), could enhance electrically-evoked contractions through a “central contribution” in individuals with stroke or SCI. This central effect arises from the electrical activation of sensory axons, which in turn, reflexively recruit motoneurons in the spinal cord. After stroke, contractions evoked by WP-NMES were larger in the paretic arm than the non-paretic arm. After SCI, transmission along the H-reflex pathway was observed throughout trains of WP-NMES; direct evidence of a central contribution. These results suggest that maximizing the central contribution during WP-NMES may be useful for maintaining muscle quality after neurological injury.

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List of Abbreviations

AHP	afterhyperpolarization
ANOVA	analysis of variance
ASIA	American Spinal Injury Association
cES	contralateral erector spinae muscle
CNS	central nervous system
EC	eyes closed
EMG	electromyography
EO	eyes open
EPSP	excitatory postsynaptic potential
ES	erector spinae muscle
FMA	Fugl-Meyer assessment
GABA	gamma amino-butyric acid
H-reflex	Hoffmann reflex
iES	ipsilateral erector spinae muscle
$\mathbf{I_H}$	inwardly rectifying channel
IPSP	inhibitory postsynaptic potential
$\mathbf{M_{max}}$	maximum motor wave
MVC	maximum voluntary contraction
MVT	maximum voluntary torque
M-wave	motor wave
NMES	neuromuscular electrical stimulation
PAD	post-activation depression

PAD&R	post-activation depression and recovery
PIC	persistent inward current
PSI	presynaptic inhibition
PTP	post-tetanic potentiation
RI	reciprocal inhibition
rmANOVA	repeated measures analysis of variance
RT	radiating threshold
SCI	spinal cord injury
SD	standard deviation
SE	standard error
TS	triceps surae muscle
WP-NMES	wide-pulse neuromuscular electrical stimulation
χ^2	Chi –square analysis

Chapter 1: General Introduction

The most influential publication on sensorimotor integration within the nervous system remains, for many, the work published by Sir Charles Sherrington in 1906 titled, "The integrative action of the nervous system". Sherrington, a pioneer in the field of spinal circuitry and synaptic transmission, synthesized the anatomical and physiological findings of the day to present an organized and functional picture of the nervous system. Based on his belief in the Neuron Doctrine (Ramon y Cajal 1909, 1911), he introduced the idea of the spinal reflex: the simplest unit of integration in the nervous system. However, as Burke (2007) states in his review of Sherrington's work, Sherrington qualified the notion of a simple reflex as "probably a purely abstract conception, because all parts of the nervous system are connected together and no part of it is probably ever capable of reaction without affecting and being affected by various other parts, and it is a system certainly never absolutely at rest". Sherrington's ideas acted as the platform for the work of many other investigators who have gone on to provide more detailed descriptions of spinal circuits and a more in-depth understanding of the numerous mechanisms involved in synaptic transmission.

The work in my thesis is yet another extension of Sherrington's original ideas, in that the common theme is sensorimotor integration in the human spinal cord. Spinal circuitry and reflex transmission were investigated in the intact nervous system (Chapters 2 and 3) and the injured nervous system, including stroke (Chapter 4) and spinal cord injury (SCI; Chapter 5). In Chapter 2, I characterized a short-latency spinal reflex between the lower limb and lower back

musculature. In Chapter 3, I investigated changes in reflex transmission along the H-reflex pathway during neuromuscular electrical stimulation (NMES), related to postural state, stimulation frequency, and the level of background contraction. In Chapters 4 and 5, I applied my knowledge of sensorimotor integration and changing views of reflex transmission during NMES by testing the effects of a novel NMES protocol in individuals with stroke or SCI.

This general introduction consists of four main sections. The first two sections provide background information on the spinal circuitry and mechanisms of reflex transmission involved in responses to mechanical or electrical stimuli in the intact nervous system. The third section includes background information on the use of NMES for rehabilitation and describes the potential benefits of the novel NMES protocol, used in Chapters 4 and 5, for individuals with stroke or SCI. The last section focuses on changes in reflex transmission after stroke and SCI and how these changes affect responses to electrical stimulation.

1.1 Sensorimotor integration in the intact human nervous system: Spinal circuitry

Spinal reflexes are characterized by the rapid transmission of sensory information from skin, joint, or muscle receptors, to cells within the spinal cord which produce motor responses without involvement from the brain. When a mechanical or electrical stimulus is applied to the surface of the skin it is not possible to activate one afferent pathway in isolation, instead a mixed sensory volley is evoked (Burke et al. 1983). For this reason the spinal reflex pathways that are described in this section include Ia, Ib, Group II, and cutaneous afferents,

as well as reflex pathways that connect the upper and lower limbs ("interlimb reflexes").

1.1.1 Spinal projections of Ia afferents

Ia afferents originate from the primary endings of muscle spindles and transmit information related to changes in muscle length during human movement (Matthews 1972). Ia afferents have extensive monosynaptic projections onto motoneurons of homonymous and synergistic muscles (Eccles et al. 1957). They have also been shown to project to Ia inhibitory interneurons to facilitate reciprocal inhibition of the antagonist motor pool, to first-order primary afferent depolarization interneurons to evoke presynaptic inhibition on its own terminals, to Group Ib interneurons to promote inhibition of homonymous motoneurons, and to Group II propriospinal interneurons (Pierrot-Deseilligny and Burke 2005). Descending input does not modulate Ia afferent activity directly; rather it modulates the level of presynaptic inhibition on Ia afferent terminals through activation of primary afferent depolarization interneurons (Andén et al. 1966).

During human experimental protocols, Ia afferents are activated by a tap or vibration delivered to the muscle tendon, or electrical stimulation delivered to a peripheral nerve. The responses evoked by these stimuli are the tendon reflex, tonic vibration reflex, and Hoffmann reflex (H-reflex), respectively; the H-reflex was an outcome measure in Chapters 3 and 5 of this thesis. The H-reflex technique, developed by Hoffmann (1918, 1922), and named by Magladery & McDougal (1950), involves the application of surface electrical stimulation to a peripheral nerve trunk, proximal to the muscle belly (Misiaszek 2003). Ia afferents can be activated preferentially to motor axons when using low

stimulation currents and long pulse durations (0.5 -1 ms) (Panizza et al. 1989; Veale et al. 1973) due to the longer strength duration time constant and lower rheobase of sensory axons compared to motor axons (Panizza et al. 1992). The large synchronous volley that arrives at the motoneurons via the Ia afferents, recruits motoneurons synaptically according to the size principle (Bawa et al. 1984; Henneman et al. 1965) and produces an H-reflex. The early portions of the H-reflex have been shown to use a monosynaptic pathway; however it is possible for oligosynaptic pathways to be involved in the later portions, which include contributions from other afferents (Burke et al. 1984).

1.1.2 Spinal projections of Ib afferents

Ib afferents originate from Golgi tendon organs and relay information about active muscle loading (Jami 1992). Ib afferents, via a di-synaptic or tri-synaptic pathway that incorporates the Ib interneuron, can inhibit homonymous and synergistic motoneurons, and excite antagonistic motoneurons (Eccles et al. 1957). Ib afferents also project to first order primary afferent depolarization interneurons to facilitate presynaptic inhibition on Ib afferent terminals (Iles 1996; Mizuno et al. 1971), and to Group II propriospinal interneurons (Edgley and Jankowska 1987). A multitude of projections also converge onto the Ib interneuron which include: Ib afferents supplying synergistic and antagonistic muscles, excitatory input from Ia afferents and, with additional interneurons, excitatory input from cutaneous and joint afferents (Pierrot-Deseilligny and Burke 2005). Lastly, descending input onto the Ib interneuron is excitatory in nature from the corticospinal and rubrospinal systems, and inhibitory from the dorsal and noradrenergic reticulospinal systems (Jankowska 1992).

1.1.3 Spinal projections of Group II afferents

Group II afferents originate from the secondary endings of muscle spindles and transmit information related to changes in muscle length (Matthews 1972). Their electrical threshold is approximately two times greater than Ia afferents and they conduct slower than Ia afferents (Pierrot-Deseilligny and Burke 2005). Historically, Group II afferents were included in the "flexor reflex afferent" group due to the strong excitation of flexor muscles and inhibition of extensor muscles caused by their activation (Eccles and Lundberg 1959). Group II afferents project mainly to Group II interneurons, also known as propriospinal neurons. Group II interneurons receive excitatory input from Ia and Ib afferents (Edgley and Jankowska 1987), cutaneous and joint afferents (Jankowska 1992), and the corticospinal, reticulospinal, rubrospinal, and vestibulospinal systems (Davies and Edgley 1994). Descending noradrenergic pathways inhibit Group II interneurons (Jankowska and Riddell 1998). Presynaptic inhibition of Group II afferent terminals is also possible through activation of primary afferent depolarization interneurons (Jankowska and Riddell 1998). Lastly, Group II interneurons facilitate flexor motoneurons, and they can depress or facilitate extensor motoneurons (Eccles and Lundberg 1959; Jankowska 1992). Group II interneurons also have facilitatory projections to gamma motoneurons (Gladden et al. 1998).

1.1.4 Spinal projections of Cutaneous afferents

Cutaneomusclar reflexes involve the activation of mechanoreceptors in the skin, and their corresponding low threshold cutaneous afferents, by non-noxious stimuli. Cutaneomuscular reflexes often involve oligosynaptic pathways at the

level of the spinal cord, brainstem, and cortex and activation of these pathways has been shown to modulate spinal excitability based on functional state (Pierrot-Deseilligny and Burke 2005). Low threshold cutaneous afferents facilitate the activity of Ia interneurons (Rossi and Mazzocchio 1988), Group II interneurons (Jankowska 1992), and gamma motoneurons during certain functional tasks (Aniss et al. 1992). They also project onto Ib interneurons and can facilitate or inhibit Ib inhibition (Pierrot-Deseilligny et al. 1981; Pierrot-Deseilligny and Fournier 1986). Lastly, cutaneous afferents can depress the activity of first order primary afferent depolarization interneurons, thus decreasing the level of presynaptic inhibition on Ia afferent terminals (Rudomin et al. 1983).

1.1.5 Interlimb reflexes

Reflex pathways also exist within the human spinal cord that allow for rapid communication between the upper and lower limbs ("interlimb reflexes") during tasks such as walking and swimming, and are thought to assist with coordination of the limbs (Dietz et al. 2001; Wannier et al. 2001). Delwaide and Crenna (1984) studied interlimb reflexes in humans through the stimulation of cutaneous afferents in the median and sural nerves, while recording responses in the non-stimulated upper and lower limbs. They observed short-latency responses (~70 ms) in the soleus in response to median nerve stimulation and responses in the biceps brachii and triceps brachii muscles in response to sural nerve stimulation. Concurrent work done by Kearney and Chan (1979, 1981) showed interlimb reflexes in muscles of the arm evoked by cutaneous or muscle afferent stimulation in the lower limb. Currently, interlimb reflexes have been characterized in many muscles of the upper and lower limbs in response to

cutaneous stimulation (Zehr et al. 2001). Further evidence for interlimb reflex pathways in humans was provided by studies in which interlimb reflexes were reported in individuals with complete cervical spinal cord injury (Calancie 1991; Calancie et al. 1996). Interestingly, whether short-latency reflex connections exist between sensory receptors in the limbs and the muscles of the trunk has not been explored. The study described in Chapter 2 of this thesis was designed to test the hypothesis that spinal reflex pathways connect sensory receptors in the lower leg to the erector spinae muscles of the lower back. More specifically, I hypothesized that reflex pathways would connect spindles in the triceps surae muscles of the ankle to the ES muscles.

1.2 Sensorimotor integration in the intact human nervous system: Mechanisms involved in reflex transmission in the spinal cord

This section focuses on mechanisms that regulate reflex transmission in the spinal pathways described above. The most relevant mechanisms to this thesis, and those that are described specifically below, are: post-activation depression (PAD), presynaptic inhibition (PSI), reciprocal inhibition (RI), post-tetanic potentiation (PTP), recurrent inhibition, and persistent inward currents (PICs). Changes in these processes that occur after stroke or SCI are described in Section 1.4.

1.2.1 Post-activation depression (PAD)

It is well-established that the monosynaptic reflex can be facilitated or depressed based on stimulation frequency (Curtis and Eccles 1960; Lloyd and Wilson 1957; Lüscher et al. 1983). In humans, this type of depression is often elicited by delivering 2 pulses of electrical stimulation at various inter-stimulus

intervals. If the inter-stimulus interval is less than 10 s, the amplitude of the second reflex is typically depressed compared to the first (Rothwell et al. 1986), and this type of depression is called post-activation depression (PAD) (Crone and Nielsen 1989), homosynaptic depression (Beswick and Evanson 1957) or low frequency depression (Ishikawa et al. 1966). PAD is limited to the previously activated Ia afferent terminals, hence the use of the terms post-activation or homosynaptic depression. Currently, PAD is ascribed to a lower probability of neurotransmitter release at the 1a afferent-motoneuron synapse during repetitive activation of 1a afferents (Hirst et al. 1981; Hultborn et al. 1996; Kuno 1964).

PAD of reflex transmission may play a role in maintaining the sensitivity of the motoneuron during movement by reducing the amount of feedback converging onto the motoneurons (Hultborn and Nielsen 1998); however the evidence of PAD during functional tasks and different background contraction amplitudes is variable. For example, PAD has been reported to be unchanged (Goulart et al. 2000), reduced (Field-Fote et al. 2006) or absent (Stein et al. 2007) during standing compared to sitting, when similar background contraction amplitudes were held during both tasks. PAD has also been shown to be reduced or absent when seated participants held a voluntary contraction (Burke et al. 1989; McNulty et al. 2008; Rothwell et al. 1986; Stein et al. 2007; Trimble et al. 2000). A primary focus of Chapter 3 was to quantify the effect of task (sitting vs. standing), stimulation frequency (5-20 Hz), and contraction amplitude (relaxed to 20% MVC) on the immediate depression of soleus H-reflexes evoked by 10 s trains of stimulation. Interestingly, previous work has shown recovery of H-reflex

amplitudes during trains of tetanic stimulation (Klakowicz et al. 2006; Nozaki et al. 2003). In Chapter 3, I also characterized this recovery of reflex amplitudes during tetanic stimulation in relation to task, stimulation frequency, and background contraction.

1.2.2 Presynaptic inhibition (PSI)

PSI was first observed in the cat by Frank and Fuortes (1957) when they noticed depression of monosynaptic Ia excitatory postsynaptic potentials (EPSPs) in motoneurons that did not coincide with changes in the motoneuron excitability. Further investigations conducted by Eccles and colleagues (1962) showed that the inhibition was due to depolarization of the primary afferent terminals through an inhibitory action of primary afferent depolarization interneurons. Last-order primary afferent depolarization interneurons release the inhibitory neurotransmitter, gamma amino-butyric acid (GABA), which activates $GABA_A$ receptors on the 1a afferent terminal. This leads to an efflux of chloride ions and resultant depolarization of the afferent terminal. This early depolarization of the afferent terminal means there will be fewer voltage-sensitive sodium channels ready to activate when the next action potential arrives, thus decreasing the depolarization of the terminal. A smaller depolarizing current will reduce the influx of calcium ions and the subsequent release of neurotransmitter (Rudomin and Schmidt 1999).

PSI of Ia afferents changes in relation to functional state. PSI of Ia afferents in the soleus and quadriceps muscles has been shown to decrease at the beginning of a voluntary contraction (Meunier and Pierrot-Deseilligny 1989; Pierrot-Deseilligny 1997). It was suggested that full sensory feedback is

paramount at the beginning of a movement to help correct for small errors in the intended movement. As the contraction continues, PSI returns to normal or pre-movement levels. During walking, PSI of soleus Ia afferents shows a general increase, and this increase is further modulated throughout the step cycle (Capaday and Stein 1986; Faist et al. 1996). It is likely that PSI contributed to the results in this thesis because participants were required to perform specific tasks and maintain various background contraction amplitudes during various experiments.

1.2.3 Reciprocal inhibition (RI)

RI was first characterized by Sherrington (1897). Using a decerebrate cat preparation, he observed that contraction of one muscle was linked to the relaxation of its antagonist. This pathway was further described by Eccles and colleagues (1956) who used intracellular recordings to establish the presence of one inhibitory glycinergic interneuron in the RI pathway that projects onto motoneurons in the antagonist motor pool. This interneuron was coined the Ia interneuron. Ia interneurons have three distinguishing features: they receive monosynaptic input from Ia afferents, they project to motoneurons antagonistic to the muscle that they receive Ia input from, and they experience disynaptic recurrent inhibition from the motoneuron supplying the agonist muscle (Hultborn et al. 1971; Jankowska and Lindstrom 1972). Ia interneurons are also controlled by inputs from the corticospinal, rubrospinal, and vestibulospinal tracts (Hultborn et al. 1976; Illert and Tanaka 1978).

In humans, RI has been conclusively shown in muscles acting at the elbow (biceps brachii and triceps brachii; Katz et al. 1991) and at the ankle (tibialis

anterior and soleus; Crone et al. 1987). The output of the Ia interneuron changes during motor tasks due to descending input and the strength of the afferent input it is receiving. Descending input can affect the output of the Ia interneuron directly or by altering the level of PSI acting on the Ia afferent terminals (Kudina et al. 1993). The strength of the afferent feedback is increased by activation of gamma-motoneurons (Morin and Pierrot-Deseilligny 1977; Pierrot-Deseilligny and Burke 2005) and reduced due to mechanisms such as post-activation depression at the Ia afferent-Ia interneuron terminals during periods of repetitive firing (Lamy et al. 2005). For example, during a focused contraction of one muscle, RI that would normally be evoked by stretch of the antagonist muscle is reduced (Shindo et al. 1984). During muscle co-contraction, RI is markedly reduced in both muscles to allow for unopposed and necessary activation of both muscles (Nielsen and Kagamihara 1992). Lastly, during gait, RI is modulated in accordance with the phase of the step cycle (Petersen et al. 1999).

1.2.4 Recurrrent inhibition

Recurrent inhibition was first characterized by Renshaw (1941) based on experiments he conducted on animals with their dorsal roots sectioned. After delivering antidromic pulses to the motor axons of the homonymous muscle, he observed a short latency inhibition of the monosynaptic reflex in the homonymous motor pool. Eccles and colleagues (1954) determined this inhibition was initiated by the activation of recurrent collaterals on the motor axons; the recurrent collateral pathways activated inhibitory interneurons referred to as Renshaw cells. Renshaw cells receive input from several sources including: recurrent collaterals from a number of neighboring motoneurons (Eccles et al. 1954; Eccles et al.

1961), cutaneous and Group II muscle afferents (Ryall and Piercey 1971; Wilson et al. 1964), and descending input (Baldissera et al. 1981). The strongest projection from Renshaw cells is onto homonymous motoneurons, evident by large recurrent inhibitory postsynaptic potentials (IPSPs). Renshaw cells also project onto synergistic motorneurons and Ia inhibitory interneurons of antagonist motoneurons (Eccles et al. 1954).

Evidence from experimentation on humans suggests that recurrent inhibition leads to inhibition of the homonymous motor pool in muscles such as soleus (Bussel and Pierrot-Deseilligny 1977), quadriceps (Rossi and Mazzocchio 1991), and flexor carpi radialis and extensor carpi radialis (Katz et al. 1993). During strong contractions recurrent inhibition to the active motoneurons, along the homonymous pathway, is reduced (Hultborn and Pierrot-Deseilligny 1979). This reduced inhibition would allow for a high input-output gain for the motoneuron pool during strong contractions. In contrast, during weak contractions (Hultborn and Pierrot-Deseilligny 1979) and periods of co-contraction (Nielsen and Pierrot-Deseilligny 1996) recurrent inhibition increases, which would decrease the gain of the motoneuron pool. Additionally, increased levels of recurrent inhibition would decrease activity in Ia inhibitory pathways, allowing for increased activation of antagonistic muscles.

1.2.5 Post-tetanic potentiation (PTP)

PTP results in a prolonged increase in reflex amplitude following a period of repetitive muscle afferent stimulation (Lloyd 1949). In the cat, stimulation at ~ 300 Hz for 10-12 s was most effective for maximizing PTP (Lloyd 1949). The mechanisms which lead to PTP occur presynaptic to the motoneuron at the

afferent terminal (Curtis and Eccles 1960). The most widely accepted mechanism is an increase in neurotransmitter release after high frequency stimulation caused by a lower probability of failure to release neurotransmitter from the presynaptic terminal, coinciding with a higher probably of multi-quantal release (Hirst et al. 1981). PTP at the 1a afferent-motoneuron synapse has also been demonstrated in humans (Hagbarth 1962; Kitago et al. 2004; O'Leary et al. 1997; Van Boxtel 1986) and could increase the contribution from reflexively activated motoneurons to contractions evoked by the repetitive electrical stimulation used in Chapters 3, 4, and 5 of this thesis.

1.2.6 Persistent inward currents (PICs)

In addition to postsynaptic mechanisms, motoneuron excitability is also largely determined by the electrical properties intrinsic to the motoneurons (Heckman et al. 2009). One example of an intrinsic property is the PIC. PICs are mediated by voltage-sensitive persistent sodium and L-type calcium channels in the motoneuron (Hounsgaard et al. 1984; Li and Bennett 2003). PICs amplify synaptic input and, due to their slow inactivation rate, they help sustain motoneuron firing (Crone et al. 1988; Lee and Heckman 2000). The primary neuromodulatory systems responsible for PIC modulation are the serotonergic system, which originates in the raphe nucleus and releases serotonin, and the noradrenergic system, which originates in the locus coeruleus nucleus and releases norepinephrine (Holstege and Kuypers 1987). Increased monaminergic drive amplifies PICs, while decreased levels reduce PIC amplitudes (Heckman et al. 2005; Lee and Heckman 2000). Monaminergic drive is regulated by the state

of arousal (i.e. sleep vs. fight-or-flight response) and undergoes marked change after an injury to the central nervous system, such as stroke or SCI.

The importance of PICs for the discharge of human motoneurons has also been suggested, although only indirect measures are available (Collins et al. 2002; Gorassini et al. 1998, 2002; Kiehn and Eken 1997). After periods of tendon vibration (Gorassini et al. 1998) or NMES (Collins et al. 2001, 2002), human motor units exhibited self-sustained firing, which is a hallmark of PIC activation. An indirect method has also been developed to quantify PICs in humans (ΔF; Gorassini et al. 1998, 2002). Similar to the animal models, PIC amplitude in human motoneurons is also sensitive to monoaminergic drive. In cases where amphetamines were administered to participants, there was a large increase in PIC amplitude and a decrease in the time it took for the PIC to activate, compared to the participants who received a placebo treatment. A dose of caffeine, which is known to stimulate the release of serotonin and norepinephrine, also increased self-sustained firing in the tibialis anterior muscle in those that ingested it compared to a control group (Walton et al. 2002). In relation to this thesis, PICs may have been activated during the NMES used in Chapters 3, 4, and 5, and thus would have contributed to motoneuron recruitment during the electrically-evoked contractions.

1.3 Overview of Neuromuscular Electrical Stimulation

Currently in Canada, there are close to 300 000 people living with functional impairments caused by a stroke (Canadian Heart and Stroke Foundation) and 36 000 people affected by SCI (Canadian Paraplegic Association). Based on these statistics, the need for rehabilitative techniques that

maintain muscle health and function is evident. NMES is one such technique that is used to activate paralyzed muscle. An electric current, most often delivered via surface electrodes placed on the surface of the skin, depolarizes the motor axons that lie beneath the electrodes causing the muscle to contract. Typical NMES parameters include frequencies between 20-50 Hz and pulse widths of 200-300 μs (de Kroon et al. 2005). After stroke or SCI, NMES has been shown to assist with muscle strengthening, maintenance of bone mineral density, joint stability, activities of daily living, and exercise (Chae 2003; de Kroon et al. 2002, 2005; Dudley-Javoroski and Shields 2008; Glanz et al. 1996; Shields and Dudley-Javoroski 2006; Stein et al. 2006; Van Peppen et al. 2004).

Despite the advances made with NMES, limitations exist. During voluntary contractions or reflexive activation, motor units are recruited from smallest to largest according to the size principle (Henneman et al. 1965). During NMES, the motor unit recruitment order is subject to greater debate. A common assumption based on experiments involving direct stimulation of motor axons using implanted electrodes, is that the recruitment order is reversed (Gorman and Mortimer 1983). This view has been challenged by those that have shown the recruitment order during electrical stimulation is normal (Knaflitz et al. 1990; Thomas et al. 2002) or random, and likely depends primarily on the orientation of the axons in relation to the stimulating electrodes, and less on axon diameter (Feiereisen et al. 1997; Gregory and Bickel 2005; Jubeau et al. 2007). A non-physiological recruitment order would result in less activity of the small fatigue-resistant motor units compared to when the recruitment order is normal and small

fatigue-resistant units are activated first. Inactivity after injury leads to muscle atrophy and changes in motor unit characteristics (i.e. slow motor units take on characteristics of fast motor units) (Gordon and Pattullo 1993; Shields 2002). Weak and fatiguable muscle limits the use of NMES during activities of daily living or exercise where the stimulation is required to produce muscle contractions of sufficient amplitude over a relatively long period of time. Lastly, some individuals experience discomfort during the stimulation and therefore, do not readily use it. There is a need for NMES protocols that promote the orderly recruitment of motor units, leading to increased fatigue-resistance and reduced muscle atrophy, and that minimize discomfort. Several studies have investigated various stimulation protocols in an attempt to fulfil these requirements (Bickel et al. 2004; Kesar and Binder-Macleod 2006; Scott et al. 2007; Stein et al. 1992). The novel stimulation protocol used in Chapters 4 and 5 of this thesis is described below.

NMES protocols which utilize high frequencies (up to 100 Hz) and wide pulse widths (1 ms) (wide-pulse NMES; WP-NMES) enhance the amplitude of contractions through the addition of a "central contribution" in individuals with no neurological impairments (Collins 2007; Collins et al. 2001, 2002) and those with SCI (Nickolls et al. 2004). The high frequencies and wide pulse widths are thought to send a relatively large afferent volley to the spinal cord, increasing the reflexive recruitment of motoneurons and the likelihood of activating PICs in spinal motoneurons (Dean et al. 2007; Lagerquist et al. 2009). A potential advantage of using WP-NMES for rehabilitation, compared to traditional NMES,

may be the orderly recruitment of motor units adhered to during the central activation of motor units. The activation of the smallest motor units may also help reduce atrophy and generate muscle contractions that are more fatigue-resistant. Lastly, through the use of a wide pulse width, lower stimulus currents may be required to generate large muscle contractions which may reduce the discomfort of the stimulation. Chapters 4 and 5 of this thesis focus on the application of WP-NMES in individuals with stroke or SCI, respectively.

1.4 Sensorimotor integration in the injured human nervous system

In this section I describe changes in reflex transmission, that occur at the level of the spinal cord after stroke or SCI, which could influence the central contribution to contractions evoked by WP-NMES after stroke and SCI. The most relevant mechanisms to this thesis, and those that are described below, are: post-activation depression, presynaptic inhibition, reciprocal inhibition, recurrent inhibition, and persistent inward currents. Post-tetanic potentiation is not discussed below because little is known about changes in post-tetanic potentiation at the Ia-afferent motoneuron synapse after stroke or SCI.

1.4.1 Post-activation depression after stroke or SCI

After stroke and SCI, PAD in reflex pathways controlling the affected limbs was reduced compared to an unaffected or healthy control limb (Aymard et al. 2000; Lamy et al. 2009; Masakado et al. 2005; Schindler-Ivens and Shields 2000). Furthermore, PAD was only reduced in individuals with chronic SCI, not acute SCI (Schindler-Ivens et al. 2000). This finding supports the idea that the reduction in PAD after injury is caused by alterations at the 1a afferent-motoneuron synapse due to low levels of activity over time, and not solely the

immediate removal of descending drive (Hultborn and Nielsen 1998). Typical task-modulation of PAD is also impaired after injury. Individuals with SCI did not show a reduction of PAD during supported standing compared to sitting, as was observed in individuals with no neurological impairments (Field-Fote et al. 2006). PAD has been implicated as a mechanism of spasticity and a recent study found a correlation between PAD and spasticity after stroke (Lamy et al. 2009). In relation to Chapters 4 and 5 of this thesis, a reduction in PAD means that more of the afferent input generated during WP-NMES may reach the motoneurons of the affected limb and increase the central contribution to the electrically-evoked contractions.

1.4.2 Presynaptic inhibition after stroke or SCI

There is also a reduction of PSI on Ia afferent terminals in humans after stroke and SCI (Artieda et al. 1991; Aymard et al. 2000; Faist et al. 1994; Kagamihara and Masakado 2005; Lamy et al. 2009; Nakashima et al. 1989). Faist et al. (1994) attributed increased facilitation of the soleus H-reflex in response to a femoral nerve conditioning pulse in a group of individuals with paraplegia, compared to a group of individuals with no neurological impairments, to less PSI after SCI. Of relevance during WP-NMES, a reduction in PSI would result in a greater release of neurotransmitter in response to each action potential that arrives at the afferent terminals (Rudomin and Schmidt 1999). Similar to reduced PAD, less PSI could enhance the central contribution to contractions evoked by WP-NMES.

1.4.3 Reciprocal inhibition after stroke or SCI

A number of studies have observed a reduction in RI after stroke and SCI (Boorman et al. 1991; Nakashima et al. 1989; Okuma et al. 2002; Okuma and Lee 1996). However, for individuals with SCI this reduction was often dependent on functional recovery (Okuma et al. 2002; Okuma and Lee 1996). When there was poor recovery of walking ability, a reduction of RI was found, possibly related to a decrease in the cortical facilitation of Ia inhibitory interneurons or an increase in the strength of mutual inhibitory connections between 1a inhibitory interneurons. When the recovery of walking ability was high, RI was found to increase after SCI. It was suggested that intensive training of the dorsiflexors during the rehabilitation process may have resulted in an upregulation of RI to the plantarflexors in an activity-dependent manner (Shindo et al. 1984; Tanaka 1974). The task-related modulation of RI is also altered after injury. RI typically increases during standing and at the beginning of voluntary contractions in individuals with no neurological impairments. In contrast, RI was not significantly different between sitting and standing (Perez and Field-Fote 2003) or between rest and the beginning of a voluntary contraction (Kagamihara and Masakado 2005) in individuals with SCI.

1.4.4 Recurrent inhibition after stroke or SCI

Homonymous recurrent inhibition has been tested in individuals with SCI and stroke, with inconclusive findings for both groups. One reason for this may be that many studies included patients with a variety of neurological disorders. In each study discussed below, the majority of the participants experienced either a stroke or a SCI. A study with a large cohort of individuals who had experienced a

stroke found recurrent inhibition to be either unchanged at rest or increased (Katz and Pierrot-Deseilligny 1982). After SCI, recurrent inhibition was increased at rest (Shefner et al. 1992). For both stroke and SCI, recurrent inhibition was modulated inappropriately during voluntary contraction (Katz and Pierrot-Deseilligny 1982). These changes in recurrent inhibition may be linked to the removal of tonic inhibitory input of Renshaw cells from the corticospinal tract (Katz and Pierrot-Deseilligny 1999). The NMES delivered in this thesis would have generated antidromic volleys in motor axons that could have triggered the recurrent inhibition pathway. Additionally, if recurrent inhibition was increased in any of the participants included in Chapters 4 and 5, the central contribution during WP-NMES could have been reduced.

1.4.5 Persistent inward currents after stroke or SCI

In chronic SCI animal models, monoaminergic drive to spinal neurons is severely diminished soon after the spinal cord transection and leads to a reduction of motoneuron excitability (Harvey et al. 2006; Hounsgaard et al. 1988). Over time, PICs develop on their own and restore the excitability of motoneurons despite the very low levels of serotonin and norepinephrine present within the spinal cord (Li and Bennett 2003; Murray et al. 2010). These changes in motoneuron excitability parallel the development of spasticity (Bennett et al. 2004). After human SCI, there is also evidence that PICs contribute to motor unit activity (Gorassini et al. 2004; Hornby et al. 2003; Nickolls et al. 2004; Zijdewind and Thomas 2003). Gorassini et al. (2004) used an indirect measure of PIC amplitude (ΔF, see Gorassini et al. 1998) in individuals with SCI and estimated that approximately 40% of the excitation to the motoneurons during a spasm is

provided through PIC activation. Furthermore, self-sustained firing of motor units has also been shown following WP-NMES (Nickolls et al. 2004) or voluntary contractions of paretic muscle (Zijdewind and Thomas 2003).

The role of PICs in motoneuronal excitability is less clear after stroke. After a stroke the disruption of corticospinal input may lead to an increased reliance on bulbospinal projections which provide monoaminergic input to the spinal cord (Cabaj et al. 2006; Dewald et al. 1995, 2001; Dewald and Beer 2001; Ono and Fukuda 1995; Sukal et al. 2007). If monoaminergic drive is enhanced, motoneuronal excitability should increase due to enhanced PICs (Heckman et al. 2005), thus increasing the reflex gain of motoneurons in the paretic limb. Consistent with this idea, McPherson et al. (2008) found that tonic vibration reflexes in the biceps brachii muscle were larger in the paretic arm compared to the non-paretic arm of individuals with chronic hemiparetic stroke. In contrast, another study found no difference in an indirect measure of PIC amplitude (ΔF measure) between the paretic muscle and the non-paretic or control muscle, during voluntary ramp contractions of the biceps brachii muscle (Mottram et al. 2009). Mottram et al. (2009) suggested that the increased number of spontaneously active motor units often recorded in the paretic limb may have been due to a low-level tonic depolarizing synaptic drive either of cortical or segmental origin, rather than enhanced PICs. Thus, there is presently no definitive evidence to support the idea that there are changes in motoneuron properties after stroke. If PICs are enhanced, meaning larger in amplitude and/or

more readily activated, after stroke or SCI, this may be another factor driving the central contribution to contractions evoked by WP-NMES in Chapters 4 and 5.

1.5 Summary and Thesis Objectives

The common theme throughout the chapters in this thesis is sensorimotor integration within the human spinal cord. The over-arching objective for this thesis was to investigate how sensory input is transformed into motor output in response to several different stimuli. This introduction has provided an overview of the spinal circuitry and mechanisms of reflex transmission involved in responses to mechanical perturbations or electrical stimulation in the intact and injured human nervous systems. An introduction to WP-NMES and its potential benefits for rehabilitation was also included. The specific objectives of each thesis chapter are outlined below.

Chapter 2: The objective of Chapter 2 was to determine if there are spinal reflex pathways between sensory receptors in the lower leg and the erector spinae muscles of the lower back. I hypothesized that reflex pathways connect spindles in the triceps surae muscles of the ankle to the erector spinae muscles. The task modulation and afferent origin of the reflexes in the erector spinae were also investigated.

Chapter 3: The objective of Chapter 3 was to investigate the effects of task (sitting and standing), stimulation frequency (5, 10, or 20 Hz), and background contraction amplitude (relaxed-20% MVC) on the depression and recovery of soleus H-reflexes during 10 s trains of stimulation. I predicted that reflexes would be significantly depressed immediately after the first reflex, but that reflex amplitude would recover over the 10 s stimulus train. I hypothesized that both the

depression and recovery would be influenced by stimulation frequency and background contraction level, but not task.

Chapter 4: The objective of Chapter 4 was to investigate whether WP-NMES augments muscle contractions after stroke. I hypothesized that WP-NMES would generate larger contractions in the paretic arm, compared to the non-paretic arm, due to changes in reflex transmission and motoneuron excitability that occur after stroke.

Chapter 5: The objective of Chapter 5 was to determine the extent to which muscle contractions are driven through reflex pathways during WP-NMES after SCI. I expected that the reflexive contribution to electrically-evoked contractions may be large after SCI due to changes in reflex transmission and motoneuron excitability. I hypothesized that transmission along the H-reflex pathway would be initially depressed, but would recover during the stimulation trains.

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Chapter 2: Reflex pathways connect receptors in the human lower leg to the erector spinae muscles of the lower back[1]

2.1 Introduction

The neural control of human movement is mediated in part by reflex pathways through the spinal cord. Some of these pathways transmit signals from sensory receptors in one limb to the musculature of the other three limbs. These pathways form the substrate for interlimb reflexes that are thought to contribute to the coordination of all four limbs during tasks such as walking (Dietz et al. 2001; see Dietz 2002; see Zehr and Duysens 2004). Interlimb reflexes are often studied by activating receptors in one limb and recording responses in the remote, non-stimulated limbs (Dietz et al. 2001; Haridas and Zehr 2003; Kearney and Chan 1979, 1981; Mienck et al. 1981; Zehr et al. 2001). Such reflexes have been observed in muscles of the upper limb from ankle joint displacement (Kearney and Chan 1981) and in all four limbs from electrical stimulation of cutaneous nerves at the wrist and ankle (Zehr et al. 2001). Whether reflex connections also exist between sensory receptors in the limbs and the muscles of the trunk has not been explored.

Activity of the trunk musculature is important for maintaining upright posture and ensuring stability during standing and walking (Floyd and Silver 1955; Waters and Morris 1972). The lower erector spinae muscles (ES), in particular, provide stability of the lumbar spine and together with the transverse abdominus and internal obliques correct for changes in the centre of mass

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(O'Sullivan et al. 2002) through a combination of spinal and cortical mechanisms. Spinal mechanisms include the stretch reflexes that can be demonstrated experimentally by taps applied over the ES muscles (Dimitrijevic et al. 1980; Zedka et al. 1999) or during more natural tasks, as a result of small movements of the trunk during arm movements (Zedka and Prochazka 1997). In contrast to these spinal mechanisms, after a more global postural perturbation ES activity has been characterized as part of an automatic postural response (Cordo and Nashner 1982) mediated in part through transcortical pathways (Diener et al. 1988). These responses are modulated by an interaction between central commands that depend on prior experience, and afferent feedback from the periphery (Deliagina et al. 2008; Horak et al. 1989; Jacobs and Horak 2007; Misiaszek 2006). Feedback from spindles in muscles around the ankle joint is thought to be especially important for postural control (Creath et al. 2005; Dietz et al. 1989; Fitzpatrick et al. 1992; Kavounoudias et al. 2001; Nashner et al. 1976). Trunk muscle activity in particular is thought to be heavily influenced by signals from spindles in muscles acting on the ankle joint (Kearney and Chan 1981), however, the neural pathways that mediate this control have not been well-defined.

The present experiments were designed to test the hypothesis that there are spinal reflex pathways between sensory receptors in the lower leg and the ES muscles of the lower back. In particular, we thought that reflex pathways would connect spindles in the triceps surae muscles (TS) of the ankle and the ES muscles. To test this, brief taps were applied to the Achilles' tendon ("tendon taps") to activate TS muscle spindles and electromyographic (EMG) activity was

recorded bilaterally from ES. After establishing the presence of the ES reflexes, we (1) investigated these reflexes during different tasks and conditions (i.e. sitting vs. standing, eyes open vs. eyes closed) to explore whether challenging the postural demands influenced their expression and (2) explored the afferent origin.

2.2 Methods

Sixteen subjects participated in this study (8 males and 8 females; 18-46 years) after providing informed, written consent. The study was conducted in 2 parts with 10 subjects participating in the first part and 8 in the second part. The experimental protocol was conducted in accordance with the standards set by the Declaration of Helsinki and was approved by the Health Research Ethics Board at the University of Alberta. All subjects reported no back pain or history of neurological disorders. Each experimental session lasted between 1.5-3 h.

2.2.1 Electromyography

EMG was recorded from the right soleus and bilaterally from the lower ES using disposable bipolar surface EMG electrodes (2.54 cm^2, A10043-P, Vermed Medical, Bellows Falls, VT). The soleus electrodes were placed below the gastrocnemius on the midline of the muscle. The ES electrodes were placed 2 cm lateral to the L4-L5 spinous processes according to the recommendations of Cholewicki et al. (1997). Reference electrodes (10.16 cm x 4.45 cm, Electrosurgical Patient Plate: Split, 3M Health Care, St. Paul, MN) were placed on both anterior superior iliac spines. Given that the stimulation was always delivered to the right foot, the right ES was defined as ipsilateral (iES) and the left ES as contralateral (cES). For the experiments in Part 1, EMG signals were

amplified 1000-5000 times and band-pass filtered between 10-1000 Hz (AMT-8, Bortec Biomedical, Calgary, AB). For the experiments in Part 2, EMG signals were amplified 1000-5000 times and band-pass filtered between 50-3000 Hz (Neurolog System; Digitimer, Welwyn Garden City, UK). Data were sampled at 2000 Hz using custom written software (LabView, National Instruments, Austin, TX) and stored on a computer for later analysis.

2.2.2 Maximal voluntary contractions

At the start of each session, subjects performed maximal voluntary contractions (MVCs) of soleus and ES while receiving verbal encouragement from the experimenters. For the soleus MVC, subjects were seated with their right knee and ankle at approximately 110º and 90º, respectively. The right foot was strapped to a stationary footplate and subjects performed maximal isometric plantarflexion contractions. For the ES MVC, a strap was looped around the subject's mid-back and attached to a metal frame in front of the subject. While seated, with a hip angle of approximately 90º, the subject extended their trunk (i.e. arched the lower back) against the strap to maximally engage ES. One to two practice trials were performed for each muscle to permit subjects to become familiar with how to maximally activate each muscle.

2.2.3 Part 1 Protocol: Erector spinae reflexes evoked by Achilles' tendon taps

These experiments were designed to quantify reflexes in ES evoked by taps applied to the Achilles' tendon. Data were collected while subjects were (1) standing with eyes open, (2) sitting with eyes open, (3) standing with eyes closed, and (4) sitting with eyes closed. The sitting and standing trials were included to determine whether reflex amplitude was modulated according to task. The eyes

closed condition was included to provide a greater challenge to postural stability and to increase the reliance on feedback from peripheral receptors for postural control (Fitzpatrick et al. 1994; Nagano et al. 2006). We predicted that ES responses would be largest while standing with eyes closed due to the increased postural demands of the task. During standing, the subjects were instructed to stand with their feet shoulder width apart, place equal weight on each foot, look straight ahead, and rest their hands at their sides. Prior to data collection the EMG activity in ES while standing was measured. Each subject matched this level of activity during seated trials by contracting the ES in the manner described for the MVC. During the eyes open trials, subjects used visual feedback of the low pass filtered EMG signal (0.3 Hz) displayed on a computer screen to maintain the desired level of activity. During the eyes closed trials, subjects received verbal cues from the experimenters when necessary to adjust their ES activity level to match the target level. The tendon taps were delivered manually by the experimenter, perpendicular to the right Achilles' tendon with a Taylor reflex hammer. The hammer was equipped with a force sensitive resistor and this signal was used to monitor the amplitude of the force applied during each tap, as well as to trigger data collection. The taps were delivered to evoke a consistent, robust stretch reflex in soleus as determined prior to data collection. Each trial consisted of 40 taps, separated by 3-5 s, delivered with a peak force of ~2 N. A set of control trials was also collected, while subjects were standing with eyes open, in which taps (n = 40) were delivered to the right calcaneus, below the lateral malleolus ("heel taps") in a similar manner as the tendon taps. The purpose of

these trials was to minimize the activation of muscle spindles in the triceps surae, while attempting to activate a similar proportion of cutaneous receptors as during the tendon tap. We predicted that the heel taps would evoke little or no reflex in soleus or ES. The 5 different trials were presented in a random order across subjects.

2.2.4 Part 2 Protocol: Erector spinae reflexes evoked by cutaneous nerve stimulation

These experiments were conducted to address the unexpected finding that ES reflexes evoked by tendon taps and heel taps were not significantly different. Thus, in 8 subjects we investigated whether ES reflexes could be evoked by electrical stimulation of cutaneous afferents from the foot. The sural nerve was chosen because it is a purely cutaneous nerve that innervates the dorsal lateral region of the foot, including the area where the heel taps were applied. We compared the amplitude and latency of ES reflexes evoked by sural nerve stimulation, tendon taps, and heel taps. All data were collected while subjects were standing with eyes open. The taps were applied to the tendon and heel as described for Part 1 (above). The electrical stimulation (4-pulses, 1 ms pulse width, 300 Hz) was delivered using a Digitimer DS7A constant current stimulator (Neurolog System; Welwyn Garden City, UK) through disposable bipolar electrodes (2.54 cm^2; A10043-P, Vermed Medical, Bellows Falls, VT) placed over the sural nerve. One electrode was placed posterior and inferior to the lateral malleolus and the other inferior to the lateral malleolus. Placement was adjusted for each subject to find the site at which there was a clear radiating paresthesia into the area of skin innervated by the sural nerve at the lowest stimulation

intensity (radiating threshold, RT). During data collection the stimulation was delivered at 2-3 times RT and the inter-stimulus interval was varied randomly between 3-5 s. This stimulation protocol is typical of that used to evoke cutaneous reflexes in previous studies (i.e. Zehr et al. 2001). In the present study the stimuli were non-noxious and did not evoke contractions of the local musculature that could be attributed to the activation of motor axons. Subjects completed 3 trials in which they received either; (1) tendon taps, (2) heel taps, or (3) sural nerve stimulation. In each trial they received 80 stimuli with an inter-stimulus interval of 3-5 s. The presentation order of the 3 different trials was randomized across subjects.

2.2.5 Data analysis

Data analyses were performed using custom-written Matlab software (The Mathworks, Natick, MA). All EMG data were rectified and low pass filtered (40 Hz, dual-pass Butterworth). The EMG recorded during the MVC for each muscle was averaged over a 500 ms window centered around the peak EMG. To quantify reflexes evoked by the different stimuli (tendon tap, heel tap, electrical stimulation) for each subject, iES, cES and soleus EMG were averaged over an interval from 100 ms before to 250 ms after the onset of the stimulus train. In 2 subjects who participated in Part 1, the ES EMG in some trials was contaminated by the signal associated with a heartbeat. The heartbeat EMG signals were easily identified as large distinct waveforms appearing simultaneously in both ES channels at random times in relation to the stimulation. When this occurred the sweep was removed from the analysis, thus average data for these subjects were calculated from 25-30 sweeps. For Part 2, 80 sweeps were collected in each trial

and the first 40 sweeps that were not contaminated by signals associated with the heartbeat were used for analysis, thus all average data for Part 2 were calculated from 40 sweeps. For all muscles, the background muscle activity was calculated as the average EMG over the final 100 ms prior to stimulus delivery. Responses were analyzed over fixed epochs, relative to stimulus onset, that were determined based on visual inspection of the data (see Fig. 2.1). For soleus, reflexes were analyzed over a window 25-75 ms after stimulus onset, consistent with a short-latency stretch reflex. For reflexes in ES in Part 1, data were sorted into early (15-35 ms), middle (35-75 ms), and late (75-125 ms) epochs. For the tendon tap and heel tap responses in Part 2, the latency of the late epoch was shifted slightly earlier (70-125ms) to more accurately capture the peak responses in this group of subjects. To quantify responses to stimulation of the sural nerve in Part 2, the epochs were slightly later (early: 25-45 ms; middle: 45-85 ms; late: 85-125 ms). For each response epoch, the peak latency, amplitude, and sign were calculated. Peak latency was calculated as the time from stimulus onset to the point of maximal excitation or inhibition. Amplitude was calculated by subtracting the average EMG over a 10 ms period, centered around the point of maximal excitation or inhibition, from the mean pre-stimulus EMG, and was expressed as a % MVC. The sign of the response was determined by whether the peak response was larger (positive) or smaller (negative) than the mean pre-stimulus EMG.

2.2.6 Statistical Analysis

For all subjects in Part 1, Chi-square analyses (χ^2) were used to determine whether there were significantly more excitatory responses compared to inhibitory responses, or vice versa, for each epoch. A response was considered significant

when the EMG in a given epoch remained outside of a ±2 SD band, centered around the mean pre-stimulus EMG activity, for a minimum of 5 ms. This 5 ms criterion has been used in previous studies for the analysis of cutaneous reflexes (Zehr et al. 2001; Haridas and Zehr 2003).

The subtracted and signed EMG values were used for statistical analyses of reflex responses across the group. Planned comparisons were used to identify significant differences between ES reflex amplitudes between the different conditions in Part 1. A three-way repeated measures analysis of variance (rmANOVA; Task: sitting and standing; Eyes: open and closed; Muscle: iES and cES) was performed for each epoch to provide the experimental error value for the planned comparison analyses. To compare the ES reflexes evoked by tendon taps with those by heel taps in each epoch, the error value for planned comparisons was extracted from a 2-way rmANOVA (Stimulation: tendon taps and heel taps; Muscle iES and cES). A two-way rmANOVA was used to compare soleus reflexes between conditions (Task: sitting and standing; Eyes: open and closed). For the experiments described in Part 2, separate two-way rmANOVAs (Muscle: iES and cES; Stimulation: tendon taps, heel taps and sural nerve stimulation) were used to compare ES response amplitudes and latencies evoked by tendon taps, heel taps and sural nerve stimulation for each epoch. Responses in soleus were compared using a one-way rmANOVA (tendon taps, heel taps and sural nerve stimulation). In Parts 1 and 2, background contraction levels for both soleus and ES were compared between conditions using rmANOVAs. Tukey's HSD tests were used for post-hoc comparisons of the ANOVA results when

significant main effects or interactions were identified. For all statistical analyses the significance level was $p \leq 0.05$. Descriptive statistics are reported as the mean ± 1 SD.

2.3 Results

2.3.1 Part 1: Tendon Taps

Reflexes recorded from ES and soleus in one subject while sitting with eyes open and holding a bilateral background contraction in ES are shown in Figure 2.1. For this subject, responses exceeded the 2 SD band centered around the pre-stimulus EMG (horizontal lines) and thus were considered significant in all three epochs for iES and cES. Figure 2.2 shows responses recorded from cES in another subject and highlights the qualitative similarity of reflexes recorded during sitting (panel A) and standing (panel B) with eyes open (thick lines) and closed (thin lines). For the sake of clarity, the 2 SD bands are not shown in this figure.

The taps applied to the Achilles' tendon elicited a stretch reflex in soleus in all subjects (Fig. 2.1c, 2.3d). These reflexes were always excitatory with an average amplitude of 160 ± 83% MVC and a peak latency of 48 ± 3 ms while standing with the eyes open. There were no significant differences in the amplitude (Fig. 2.3d) or latency of the reflexes recorded in soleus between conditions. There was also no significant difference between the background EMG activity in soleus when the subjects were standing during the tendon tap trials with the eyes open (6 ± 5% MVC) and the eyes closed (6 ± 4% MVC).

Taps applied to the Achilles' tendon evoked significant reflexes in iES and cES in all subjects. Significant reflexes consisted predominantly of early excitation, followed by a middle latency inhibition, and a subsequent period of excitation. Table 2.1 summarizes the frequency, sign, and latency of significant ES responses evoked by tendon taps for the group of 10 subjects. In iES, significant responses were evoked in 80, 93, and 88% of all trials for the early, middle, and late epochs, respectively. Significant responses were recorded from cES in 70, 83, and 95% of all trials for the early, middle, and late epochs, respectively. For iES, responses were excitatory (30/40 trials) in the early epoch significantly more often than they were inhibitory (2/40) [χ^2 (1, N=32) = 24.53, $p \leq 0.001$]. There were also significantly more excitatory responses in the early epoch for cES [22/40 excitatory vs. 6/40 inhibitory; χ^2 (1, N=28) = 9.18, $p \leq 0.01$]. During the middle epoch, iES responses were more often inhibitory (25/40 trials) than excitatory (12/40 trials) [χ^2 (1, N=37) = 4.59, $p \leq 0.05$]. The middle latency responses recorded from cES were also more often inhibitory (25/40 trials) than excitatory (8/40 trials) [χ^2 (1, N = 33) = 8.79, $p \leq 0.01$]. For the late epoch, excitatory responses predominated for both iES [26/40 excitatory vs. 7/40 inhibitory; χ^2 (1, N=35) = 12.63; $p \leq 0.001$] and cES [35/40 excitatory versus 3/40 inhibitory; χ^2 (1, N=38) = 26.97, $p \leq 0.001$].

For the group, there was no significant effect of task (standing vs. sitting) on the magnitude of responses in the early, middle, or late epochs for iES (Fig. 2.3). The cES muscle also showed no significant effect of task for responses in the early and middle epochs. In contrast, cES responses in the late epoch during

standing were significantly larger than during sitting (Fig. 2.3c). Responses in ES were not significantly different between eyes open and eyes closed for any condition. There were no significant differences in background ES EMG activity between conditions and the average background ES EMG activity measured for the group across conditions was 21% MVC (range: 17-24%).

Responses evoked in iES and cES were significantly different in amplitude, and these differences depended on task. In the early epoch, responses in iES were larger than those recorded in cES while sitting with eyes open and eyes closed (Fig. 2.1; Fig. 2.3a). During standing there were no significant differences between the early responses in iES and cES (eyes open or closed). As shown in Fig. 2.3b, responses in the middle epoch were not significantly different between iES or cES regardless of task. For the late epoch, the ES responses showed task-dependent changes that were opposite to those found for the early epoch. While standing (eyes open and eyes closed), the late responses in cES were larger than those in iES, but during sitting there were no significant differences in the late responses between iES and cES (eyes open or eyes closed) (Fig. 2.3c).

2.3.2 Tendon taps versus heel taps

In an additional set of trials, taps were applied to the lateral calcaneus while subjects were standing with eyes open. Figure 2.4 shows data for the group of 10 subjects averaged over the interval from 50 ms before to 200 ms after the taps (left panels) and quantified for each reflex epoch (right panels). The heel taps evoked reflexes in soleus that were 75% smaller than those evoked by the tendon taps ($p \leq 0.05$). In contrast, there were no significant differences in ES reflexes

evoked by tendon taps or heel taps in any epoch. Background soleus and ES EMG activity were not significantly different between the tendon tap and heel tap trials.

2.3.3 Part 2: Erector spinae reflexes evoked by cutaneous nerve stimulation

To determine whether the responses recorded from ES could be evoked by the activation of cutaneous receptors, a second series of experiments was conducted in which the amplitude and latency of ES reflexes evoked by tendon taps, heel taps, and sural nerve stimulation were compared in 8 subjects while standing with eyes open. The group traces in Figure 2.5 show responses in ES elicited by all three stimuli. There were no significant differences in the amplitudes of responses in any epoch between the different stimulus types. Similarly, there were no significant differences in the amplitudes of responses in either the early or late epochs between iES and cES. In the middle epoch, responses to tendon taps and heel taps were smaller in iES than cES. Responses to sural nerve stimulation were not significantly different between iES and cES in the middle epoch. The latency of the responses in each epoch were not different between iES and cES. However, response latencies in all three epochs for the sural nerve stimulation were significantly longer than responses to the tendon taps and the heel taps, which were not significantly different from each other. The mean latencies to the peak responses in the early epoch (averaged across iES and cES) were 24 ± 7, 26 ± 8 and 33 ± 7 ms for tendon tap, heel tap, and sural nerve stimulation, respectively. In the middle epoch, latencies were 54 ± 8, 51 ± 8, and 66 ± 11 ms for tendon tap, heel tap, and sural nerve stimulation, respectively. Response latencies in the late epoch were 90 ± 12, 87 ± 13, and 106 ± 11 ms for tendon tap, heel tap, and sural nerve stimulation, respectively. Responses in soleus

evoked by tendon taps were significantly larger than those elicited by heel taps and sural nerve stimulation, which were not significantly different from each other (Fig. 2.5e, f). Soleus reflexes evoked by the heel taps were on average 94% smaller than those evoked by tendon taps. The sural nerve stimulation generated a small net inhibition in soleus at this reflex epoch. There were no differences in the amount of soleus or ES background activity between the 3 stimulation conditions.

2.4 Discussion

These experiments are the first to characterize short-latency reflexes in muscles of the lower back initiated by the activation of sensory receptors in the lower leg. Taps applied to the right Achilles' tendon evoked reflexes bilaterally in ES, in addition to the well-known stretch reflex in ipsilateral soleus. Contrary to our prediction, taps applied over the lateral calcaneus ("heel taps") and electrical stimulation of the sural nerve (a cutaneous nerve of the foot) evoked reflexes in ES that were not significantly different in amplitude from those evoked by the tendon taps. We propose the reflexes in ES arise at least in part from the activation of cutaneous receptors in the foot and may contribute to the neural strategies used for the maintenance of posture and balance.

Taps applied to the Achilles tendon evoked reflexes bilaterally in the ES muscles in all subjects. In most cases, these reflexes consisted of a period of excitation in the early epoch, inhibition in the middle epoch, and excitation in the late epoch. This response pattern is qualitatively similar to the pattern observed for "interlimb" reflexes evoked in many muscles of the arms and legs by activation of sensory receptors in a stimulated limb, remote from the recording

site (Kearney and Chan 1979; Zehr et al. 2001). The early excitation in ES, which was maximal at a latency of 20-28 ms, and the middle epoch inhibition, maximal at a latency of 57-61 ms, are both consistent with transmission along pathways restricted to the spinal cord. The longer latency excitation peaked at 91-104 ms and may involve transcortical pathways (Nielsen et al. 1997). The early responses may represent a movement artifact caused by vibration in the body after each tap, rather than activation of neural pathways. We do not think this is likely because the early responses were not different between seated and standing trials; during the seated trials the wave of vibration after each tap would have dissipated into the chair on which the participant was sitting to a much greater extent and the early response would have been reduced or absent in this case if it was caused by vibration. Furthermore, based on examination of the raw EMG data, the onset latencies of the early responses were physiologically possible, in relation to the conduction velocities of muscle and cutaneous afferents. In Figure 2.4a and c, for example, the average onset of the early reponse for the group was not physiologically possible, but this occurred due to the off-line filtering of the EMG data. For this reason the peak latencies of the reflexes have been reported, instead of the onset latencies.

We hypothesized that reflex pathways connect sensory receptors in the lower leg with the ES muscles of the lower back. Specifically, we thought that reflexes would arise from the activation of spindles in the TS muscles that plantarflex the ankle. This idea was based on the well-established hypothesis that signals from muscle receptors around the ankle joint provide important

information for posture and balance and play a key role in triggering responses for balance correction (Creath et al. 2005; Diener et al. 1988; Dietz et al. 1989; Fitzpatrick et al. 1992; Kavounoudias et al. 2001; Nashner 1976; Schweigart and Mergner 2008). However, the tendon taps we utilized to activate TS muscle spindles will have also activated muscle spindles and cutaneous receptors over a large area of the leg and foot, as well as some TS Golgi tendon organs (Burke et al. 1983). Thus, to explore the afferent origin of the ES reflexes, "heel taps" were delivered to the right calcaneus directly below the lateral malleolus and electrical stimulation was applied to the sural nerve. We predicted the heel taps would activate a similar proportion of cutaneous receptors as the tendon taps, but fewer TS spindles (and no Golgi tendon organs), and responses in ES and soleus would be reduced accordingly. The sural nerve stimulation was chosen to provide a cutaneous input from the same region of the foot as the heel taps that, unlike the tendon and heel taps, was not contaminated by the activation of afferents from muscle spindle receptors or Golgi tendon organs. Despite a 75-94% reduction in the amplitude of the soleus stretch reflex elicited by heel taps, the ES reflexes were not significantly different in amplitude or latency than those evoked by tendon taps. The amplitude of ES responses in each epoch evoked by the tendon taps and heel taps were not significantly different from those evoked by the cutaneous volley generated by the sural nerve stimulation. However, the latencies of the responses in each epoch evoked by sural nerve stimulation were significantly longer than those evoked by tendon taps and heel taps. These results raise several possibilities regarding the origin of the ES responses: (1) The ES

reflexes may be part of a generalized startle response initiated by all three stimulus types. We do not believe this is the case because the responses occurred at a much shorter latency than would be expected from a traditionally defined startle response (Brown et al. 1991). Additionally, startle responses typically habituate within the first 2-6 stimuli (Brown et al. 1991) and thus could not account for the robust responses seen in individual subjects' data which represent the mean responses to 40 stimuli. (2) Both tendon and heel taps may have generated a wave of vibration through the musculoskeletal system activating muscle spindles in ES and generating a local stretch reflex in ES. However, the ES responses evoked by sural nerve stimulation, when there would be no vibration, argue against this possibility. Similarly, the ES responses to the heel taps and the sural nerve stimulation suggest the ES responses are not initiated by the movement associated with the soleus stretch reflex. (3) The responses evoked by sural nerve stimulation confirm that reflex pathways connect cutaneous receptors in the foot and ES motoneurons. Responses evoked by the tendon and heel taps were not significantly different in amplitude from those evoked by sural nerve stimulation, but had shorter peak latencies. The differences between the responses evoked by the taps and the sural nerve stimulation likely reflect differences in the afferent volley. The taps will have activated a different proportion of cutaneous receptors in a different temporal pattern than the electrical stimulation, and will also have activated muscle receptors (Burke et al. 1983). Responses in ES evoked by the tendon and heel taps were not significantly different, despite the heel taps evoking a substantially smaller soleus stretch

reflex, consistent with the activation of fewer TS muscle spindles than the tendon taps. Thus, the responses in ES do not appear to be dominated by the robust input from TS muscle spindles evoked by the tendon tap as we had initially predicted but, rather they may be predominantly cutaneous in origin. This is consistent with the emerging idea that feedback from force sensitive receptors in the skin of the foot may play a more important role in the control of human stance than feedback from ankle proprioceptors (Cnyrim et al. 2009).

Roles for feedback from cutaneous receptors of the foot in balance control have been proposed based on microneurographic recordings from the human tibial nerve (Kennedy and Inglis 2002) or sural nerve (Trulsson 2001) during stimulation of the foot sole. Cutaneous mechanoreceptors in the sole of the foot assist in detecting ground contact (Magnusson et al. 1990) and changes in the distribution of pressure (Kavounoudias et al. 1998). Postural sway increases when cutaneous input from the foot is reduced or eliminated through cooling of the foot (Magnusson et al. 1990) or through an ischemic block induced by inflating a cuff around the leg (Asai et al. 1992). Similarly, anesthesia of the sole of the foot resulted in significantly different EMG responses to perturbations applied during standing when compared to trials in which the foot was not anesthetised. Mechanoreceptors located along the lateral border of the foot, the region innervated by the sural nerve and the sites where the tendon and heel taps were applied, have been shown to be important in maintaining upright stance and postural control (Meyer et al. 2004; Trulsson 2001; Vedel and Roll 1982).

Increasing postural instability by having subjects stand with their eyes closed, which has been shown to increase body sway (Fitzpatrick et al. 1994; Nagano et al. 2006), did not alter the expression of the ES reflexes in the present study. It may be that the eyes closed condition did not present enough of a challenge to the postural system to result in a change in reflex expression. This is similar to other studies in which postural perturbations delivered during eyes open and closed conditions did not produce any differences in transmission through stretch reflex and transcortical pathways utilized for balance corrective responses (Carpenter et al. 1999; Keshner et al. 1987).

The ES muscles stabilize the spine and assist in postural corrections during sitting (Forssberg and Hirschfeld 1994; Preuss et al. 2005; Zedka et al. 1998) and standing (Cresswell et al. 1994). Presently, sensory input from one leg evoked ES reflexes bilaterally during sitting and standing. Thus, ongoing discharge of sensory receptors in the legs may contribute through these pathways to the continuous background synaptic drive to ES motoneurons. These heteronymous ES reflexes may also contribute to the generation of postural corrections, such as the bilateral ES activation that distributes forces on the pelvis and helps maintain a consistent distribution of the centre of mass (Dofferhof and Vink 1985; White and McNair 2002) during rotational perturbations (Carpenter et al. 1999) and walking (Dofferhof and Vink 1985). Although it is presently not known how these reflexes are expressed during walking, the amplitude of responses in ES were affected by changes in task, particularly the late responses in cES, as there was a significantly larger late cES response during standing compared to sitting.

Comparisons between iES and cES responses in both the early and late epochs revealed differences in amplitude that were also task-dependent. Such task-dependent differences in response amplitude may reflect a re-weighting of sensory inputs to meet the demands of the task (Cnyrim et al. 2009; Mahboobin et al. 2009; Misiaszek, 2006; Schweigart and Mergner 2008). A bilateral, but asymmetrical activation in ES has been demonstrated in other studies during rotational perturbations (Carpenter *et al*., 1999) and walking (Dofferhof and Vink 1985). Waters and Morris (1972) showed that the cES was significantly more active than the iES at heel strike which would counteract the rotational forces of the pelvis. The ES reflexes presently observed were driven by at least some of the same receptors that would be activated at heel strike.

2.4.1 Summary

These experiments characterized reflexes in the ES muscles of the lower back evoked by the activation of sensory receptors in the lower leg. These heteronymous reflexes were expressed bilaterally and changed in amplitude between sitting and standing. A comparison of reflexes evoked by tendon taps, heel taps, and sural nerve stimulation showed that reflex pathways connect cutaneous receptors of the foot and ES motoneurons and suggest that reflex connections from TS muscle spindles may be relatively weak. These reflex pathways between the legs and lower back musculature may play a role in the neural control of posture and balance.

Table 2.1 Frequency and latency of significant ES responses evoked by tendon taps across conditions for all subjects.

Conditions	iES			cES		
	Early epoch	Middle epoch	Late epoch	Early epoch	Middle epoch	Late epoch
Standing Eyes open N = 10	7 7+ / 0–	9 2+ / 7–	9 6+ / 3–	6 6+ / 0–	10 1+/ 9–	10 10+/ 0–
	24 ± 8ms	57 ± 4ms	99 ± 4ms	20 ± 8ms	59 ± 7ms	93 ± 10ms
Standing Eyes closed N = 10	9 9+ / 0–	10 5+ / 5–	9 7+ / 2–	7 6+ / 1–	8 1+ / 7–	10 10+ / 0–
	22 ± 5ms	57 ± 8ms	104±14ms	23 ± 6ms	57 ± 8ms	100±14ms
Seated Eyes open N = 10	7 7+ / 0–	9 2+ / 7–	8 7+ / 1–	8 5+ / 3–	8 3+ / 5–	9 7+ / 2–
	24 ± 4ms	60±12ms	96 ± 11ms	28 ± 6ms	59±10ms	99 ± 11ms
Seated Eyes closed N = 10	9 7+ / 2–	9 3+ / 6–	9 8+ / 1–	7 5+ / 2–	7 3+ / 4–	9 8+ / 1–
	26 ± 5ms	59±12ms	91 ± 8ms	28 ± 8ms	61±10ms	97 ± 12ms

Response frequency, prevalence of excitatory and inhibitory responses, and average response latencies for the different conditions in Part 1. For example, to describe responses in the early epoch for iES while standing with eyes open (first row), the response frequency was 7 (i.e. 7 out of 10 subjects displayed a significant ES response in that epoch). Out of these 7 significant responses, 7 were excitatory (7+) and 0 were inhibitory (0–). The average response latency was 24 ms and the standard deviation was ± 8 ms.

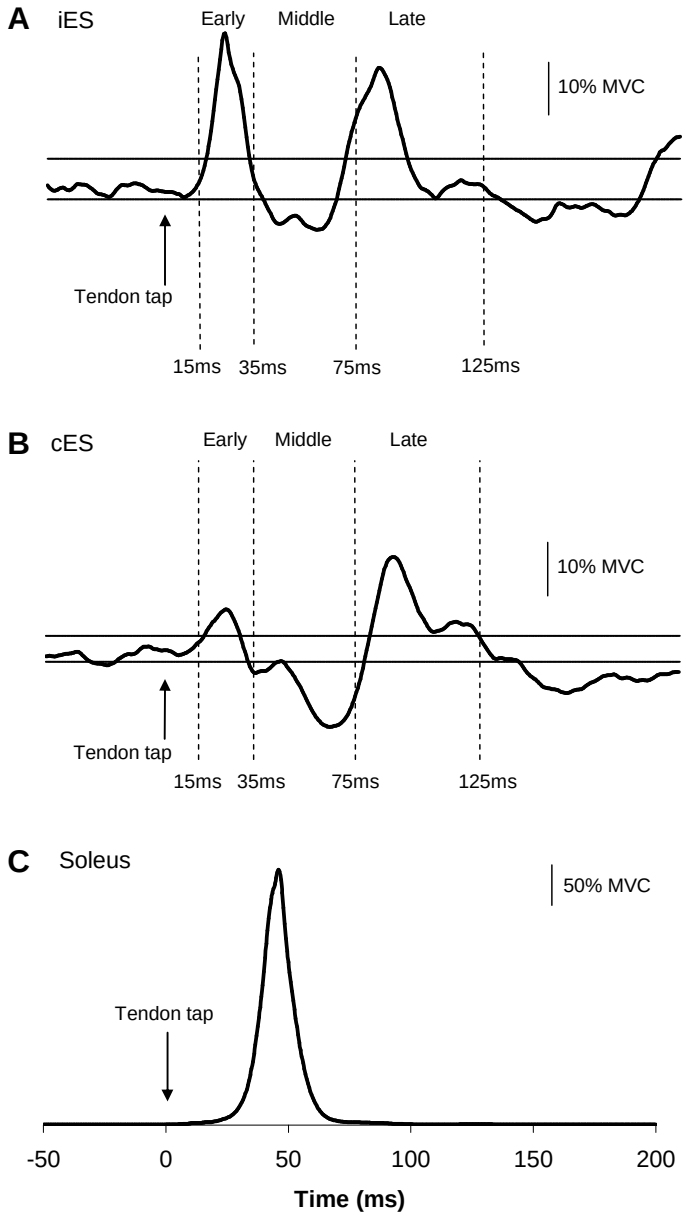

Figure 2.1 Reflexes evoked in iES, cES, and soleus by taps applied to the right Achilles' tendon in a single subject while sitting with eyes open. Each trace represents the average of 40 reflexes. Horizontal lines depict ± 2 SD of the background EMG recorded during the 100 ms prior to stimulus delivery.

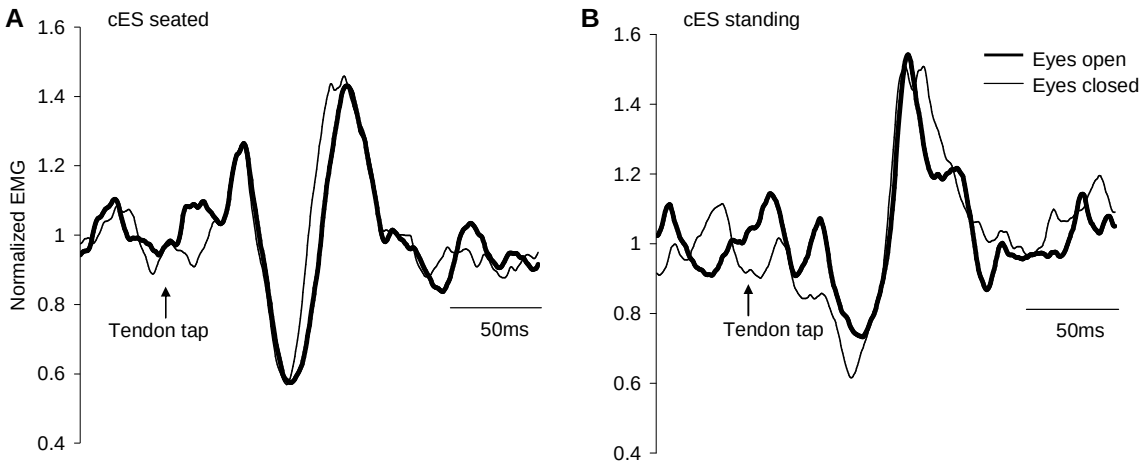

Figure 2.2 Data recorded from cES for a single subject. Reflexes were evoked by Achilles' tendon taps while the subject was seated (A) and standing (B) with the eyes open or eyes closed.

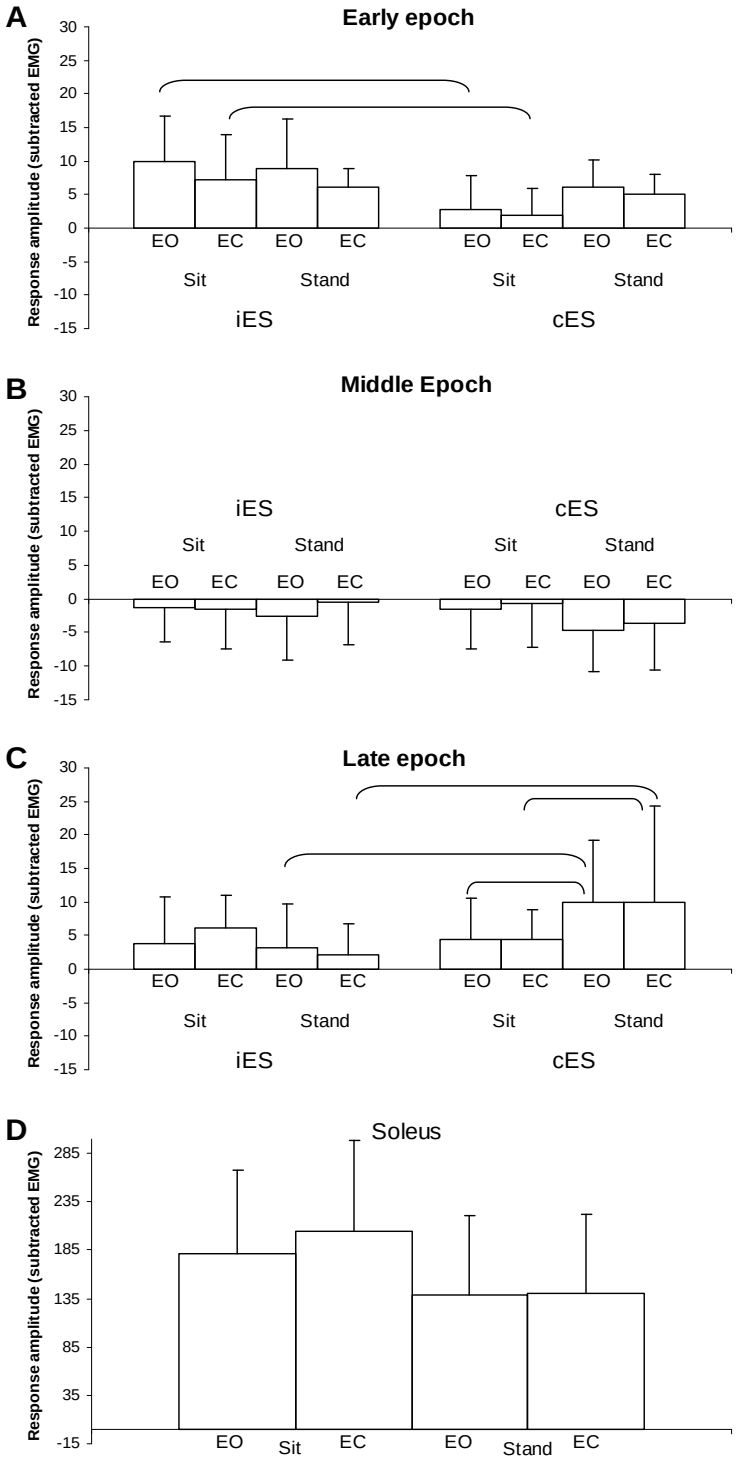

Figure 2.3 Amplitude of responses recorded from ES (early epoch panel A, middle epoch panel B, late epoch panel C) and soleus (D) averaged across the group for all conditions. Values are means ± 1 SD. Brackets identify responses that are significantly different from each other ($p \leq 0.05$). EO-eyes open; EC-eyes closed.

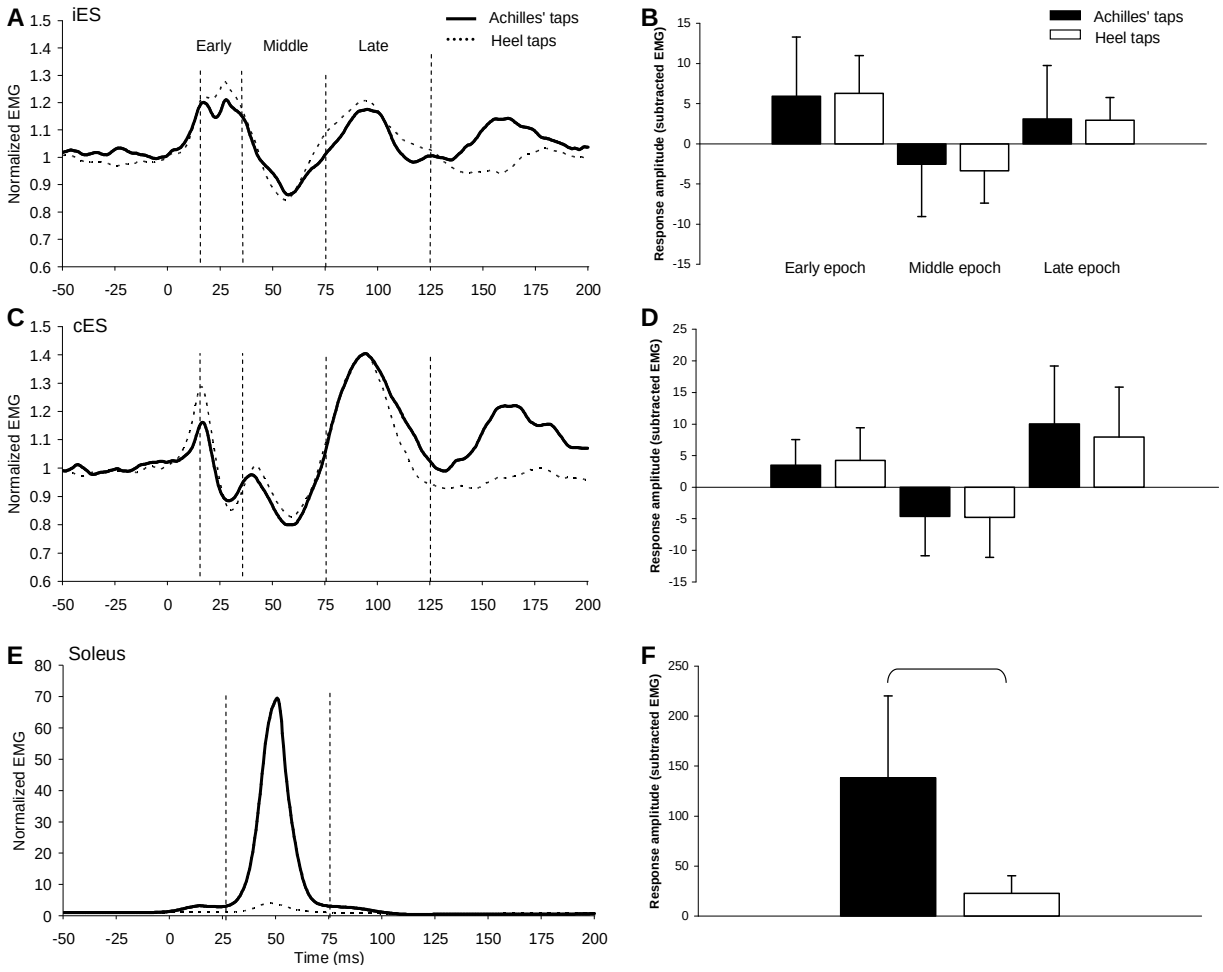

Figure 2.4 Group data showing reflexes recorded from iES (A and B), and cES (C and D), and soleus (E and F) evoked by Achilles' tendon taps or heel taps, applied to the lateral calcaneus, while subjects were standing with eyes open. Values are means ± 1 SD. Brackets identify responses that are significantly different from each other ($p \leq 0.05$).

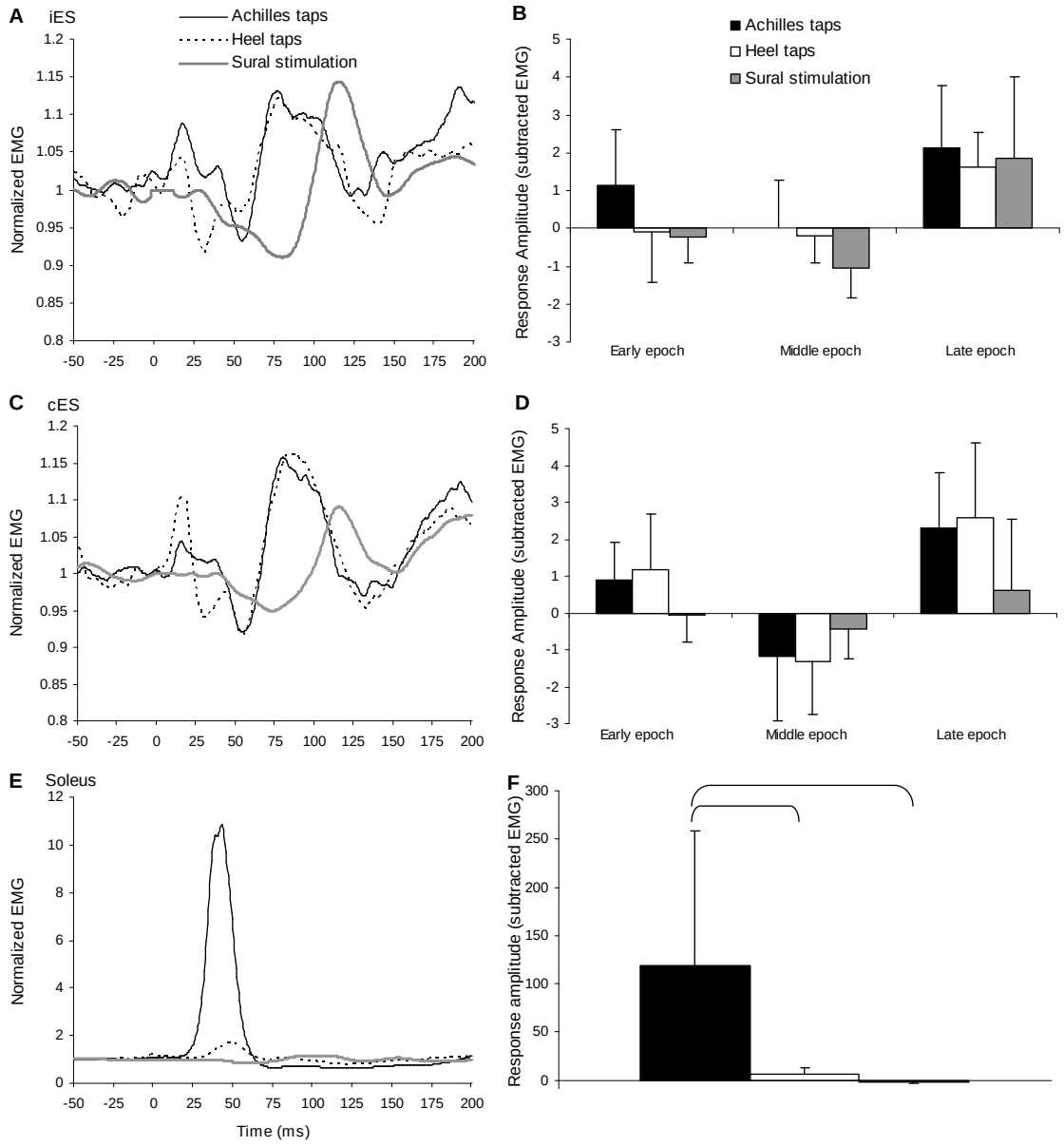

Figure 2.5 Group data showing the reflexes evoked by Achilles' tendon taps, heel taps, and sural nerve stimulation. Mean data are shown for responses recorded from iES (A and B), cES (C and D), and soleus (E and F). Brackets identify responses that are significantly different from each other ($p \leq 0.05$).

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Chapter 3: Post-activation depression and recovery of reflex transmission during human neuromuscular electrical stimulation[1]

3.1 Introduction

The integration of afferent feedback and motor output is a fundamental component of the neural control of human movement. It is clear that such sensorimotor integration is not hard-wired, but rather it depends on many factors including the task being performed (Capaday and Stein 1986; Hayashi et al. 1992; Krauss and Misiaszek 2007), the frequency of the afferent volley (Ishikawa et al. 1966; Magladery 1955), and the magnitude of the on-going voluntary contraction (Burke et al. 1989; Stein et al. 2007; Trimble et al. 2000). One of the strongest and most studied afferent projections to motoneurons is that of large diameter (Ia) afferents from muscle spindles. During natural movements, motoneurons receive trains of impulses from Ia afferents over a range of frequencies (Vallbo 1973) and the efficacy of synaptic transmission along this pathway depends strongly on impulse frequency (Curtis and Eccles 1960; Lüscher et al. 1983). In humans, reflex amplitude is progressively depressed when the frequency of the afferent volley increases above 0.1 Hz (Burke et al. 1989; Goulart et al. 2000; Ishikawa et al. 1966; Stein et al. 2007). This attenuation in reflex transmission, referred to as low frequency (Ishikawa et al. 1966), homosynaptic (Beswick and Evanson 1957), or post-activation depression (PAD; Crone and Nielsen 1989), is thought to be due to a presynaptic mechanism involving a decreased probability of neurotransmitter release from previously active Ia-afferent terminals (Hirst et al.

[1] The contributing authors to the work presented in this chapter were: Clair JM, Anderson-Reid JM, Graham C, and Collins DF.

1981; Hultborn et al. 1996; Kuno 1964). PAD is thought to play a role in attenuating synaptic transmission to maintain the sensitivity of motoneurons during movement (Hultborn and Nielsen 1998).

The most common way to study PAD in humans has been to compare the amplitude of two reflexes evoked at various inter-stimulus intervals (Burke et al. 1989; Crone and Nielsen 1989; Oya and Cresswell 2008; Ruegg et al. 1990; van Boxtel 1986). Relative to the first response, the second reflex is typically depressed by ~80% at frequencies of 4-10 Hz (Burke et al. 1989; Stein et al. 2007), and can be reduced further or even absent at frequencies above 10 Hz (Goulart et al. 2000). An alternative approach has been to deliver a brief train of impulses (up to 30) and to compare the amplitude of the first reflex to the mean of the subsequent reflexes (Ishikawa et al. 1966; Kohn et al. 1997; Rothwell et al. 1986). While both of these approaches have provided important information about frequency-dependant depression of reflex transmission, they do not shed light on how motoneurons respond over time during trains of electrical stimulation which more closely represent synaptic drive during natural movement. It is generally assumed that synaptic transmission at Ia afferent-motoneuron synapses remains depressed after the initial reflex. However, Klakowicz et al. (2006) showed that soleus H-reflex amplitude recovers partially from the initial depression by the end of a 7 s (20 Hz) stimulation train. Such reflex recovery would not be identified in traditional studies of PAD and has important implications for understanding how transmission along reflex pathways contributes to the control of human movement. In the current study we

investigated the "post-activation depression and recovery (PAD&R)" of transmission along the H-reflex pathway throughout 10 s trains of stimulation during different tasks, stimulation frequencies, and background contraction amplitudes.

Despite the strength and ubiquitous nature of PAD, its functional relevance for the neural control of human movement remains controversial. One reason for this controversy is that PAD was reduced or absent when seated participants held a voluntary contraction (Burke et al. 1989; Floeter and Kohn 1997; Hultborn et al. 1996; McNulty et al. 2008; Oya and Cresswell 2008; Rothwell et al. 1986; Ruegg et al. 1990; Stein et al. 2007; Trimble et al. 2000). It has been suggested that the contraction increases the number and discharge rate of active muscle spindles, which in turn, invokes or enhances PAD at previously inactive synapses and results in a tonic depression of synaptic transmission. Thus, when using experimental approaches to assess PAD, the first reflex is evoked at a time when synaptic transmission is already depressed and the ability to demonstrate any further depression decreases as contraction amplitude increases (Hultborn and Nielsen 1998; Stein et al. 2007). It has also been suggested that PAD is reduced (Field-Fote et al. 2006; Goulart et al. 2000) or absent (Stein et al. 2007) during standing compared to sitting, but the mechanism responsible for this task-dependence is less clear.

The present study was designed to quantify the effects of task (sitting vs. standing), stimulation frequency (5, 10, or 20 Hz), and background contraction amplitude (relaxed-20% MVC) on the PAD&R of soleus H-reflexes during 10 s

trains of stimulation. In general, we predicted that reflexes would be significantly depressed immediately after the first reflex, but that reflex amplitude would recover over the 10 s stimulus train. Our specific hypotheses related to task, frequency, and contraction level were: (1) PAD&R will not be influenced by task; there will be no difference between PAD&R of reflex amplitudes between sitting and standing. (2) PAD&R depends on stimulus frequency; there will be more depression and less recovery of reflex amplitudes as stimulation frequency increases. (3) PAD&R depends on the level of background contraction; there will be less depression and more complete recovery as contraction level increases. In addition to quantifying H-reflex amplitudes, we also quantified M-wave amplitudes as a measure of stimulus efficacy. The results of these experiments provide insight into the nature of transmission along the H-reflex pathway when motoneurons receive trains of impulses at physiologically relevant frequencies during functionally relevant tasks and contractions levels.

3.2 Methods

Eleven participants with no known neurological impairments (8 men and 3 women; 20-46 yrs) took part in this study after providing informed and written consent. The study was conducted in two parts with 8 participants involved in each part. The experimental protocols were conducted in accordance with the standards set by the Declaration of Helsinki and were approved by the Health Research Ethics Board at the University of Alberta. Each experimental session lasted approximately 3 h.

3.2.1 Electromyography

Surface electromyography (EMG) was recorded from the right soleus muscle using disposable bipolar surface EMG electrodes (2.54 cm^2, A10043-P, Vermed Medical, Bellows Falls, VT). The EMG signals were band-pass filtered from 30-3000 Hz and amplified 1000 times (Neurolog System; Digitimer, Welwyn Garden City, UK). A reference electrode was placed on the tibial plateau of the right leg (10.16 cm x 2 cm, Electrosurgical Patient Plate: Split, 3M Health Care, St. Paul, MN).

3.2.2 Maximum Voluntary Contractions

At the beginning of each experimental session, participants were instructed to plantarflex their right foot by pushing down in a gas pedal motion against a metal footplate using only their ankle muscles, until they reached their maximum and to hold this contraction for 1-2 s. Participants practiced this action and then performed 2-3 maximum voluntary contractions (MVCs) until 2 of their attempts were within 10% of each other. During all MVC trials the experimenters provided verbal encouragement to the participants to perform maximally.

3.2.3 Nerve Stimulation

Electrical stimulation was delivered to the tibial nerve in the right popliteal fossa through disposable bipolar surface EMG electrodes (2.54 cm^2, A10043-P, Vermed Medical, Bellows Falls, VT) using a constant-current stimulator (DS7A; Digitimer, Welwyn Garden City, UK). Each stimulus train was delivered for 10 s (1 ms pulse width) at 5, 10, or 20 Hz. Each trial consisted of three identical stimulation trains separated by 30 s. A 2 minute rest period was incorporated between each trial to minimize muscular fatigue. Stimulation intensity was set at

the beginning of each trial to evoke a motor wave (M-wave) of ~5% of the maximum M-wave (M_{max}) in response to 3 single pulses delivered approximately 5 s apart. Data for soleus M-wave/H-reflex recruitment curves were collected in each experiment (n = 50 stimuli; 1 ms pulse width; 5-7 s inter-stimulus interval) while the participant was seated with their soleus relaxed. Stimulation delivery and data collection were controlled by custom written software programs (LabView, National Instruments, Austin, TX). All data were sampled at 5000 Hz and stored on a computer for later analysis.

3.2.4 Part 1 Protocol: Effects of task and stimulation frequency

Part 1 of this study was designed to assess the effects of task (sitting and standing) and stimulation frequency (5, 10, and 20 Hz) on the PAD&R of soleus H-reflexes. For the standing trials, participants stood with their feet hip width apart, hands at their sides, and looked straight ahead. For the seated trials, subjects sat on the chair of a Biodex dynamometer (System 3, Biodex Medical Systems Inc. Shirley, NY, USA) with their knee and ankle at 110 and 90º, respectively, and also looked straight ahead. While sitting the participants maintained a background contraction in soleus to match the EMG measured during standing. Visual feedback of the low pass filtered (3 Hz) soleus EMG signal was displayed on a computer screen to help the participants hold the desired level of activity. The stimulation trials included all combinations of task (sitting and standing) and frequency (5, 10, and 20 Hz). Thus, there were six stimulation trials and these were delivered in a random order across participants.

3.2.5 Part 2 Protocol: Effect of background contraction levels in soleus during sitting

Part 2 of this study was designed to assess the effect of different levels of background contraction on the PAD&R of soleus H-reflexes. Each participant was seated (see above) and received trains of 10 Hz stimulation while they were relaxed or holding a 1, 5, 10, or 20% MVC soleus contraction. Visual feedback of the soleus EMG (see above) was provided to help the participants maintain the desired contraction.

3.2.6 Data Analysis

Data analysis was performed post-hoc using custom written Matlab software (The Mathworks, Natick, MA). The average of a 500 ms window centered around the peak filtered (low pass, 40 Hz) and rectified EMG in the largest MVC trial was used to calculate the soleus MVC. Background contraction levels were quantified by measuring the filtered (low pass, 40 Hz) and rectified EMG over a 1 s period, centred around 1 s prior to the stimulation trains in each trial, and normalizing these values to the soleus MVC. The largest M-wave amplitude measured from the recruitment curve trial was considered to be M_{max}. The peak-to-peak amplitude of each M-wave and H-reflex evoked during each stimulus train was measured and then normalized to M_{max}.

To generate group mean M-wave and H-reflex amplitudes, the first (M_1 or H_1) and second (M_2 and H_2) responses for a given condition were averaged separately. For each participant and stimulation pattern the amplitude of the first (M_1 or H_1) and second (M_2 or H_2) responses were averaged over the three stimulation trains in each trial. Additionally, after the first response in each

stimulation train, all responses were averaged over 0.5 s intervals to generate 20 data bins as shown in Figure 3.1. A diagram of this averaging procedure for H-reflex data is shown in Figure 3.1. One measure was used to quantify the initial reflex depression (PAD) and 3 measures were used to quantify the reflex recovery. To quantify PAD, the amplitude of the second response was compared to that of the first response. The time course of recovery was assessed using 2 measures. To characterize the "fast" recovery of reflex amplitude that occurred within the first 0.5 s of the stimulus train, the amplitude of the second response was compared to the mean of Bin 1. To characterize the "slow" recovery that often occurred over the duration of the 10 s stimulation, Bin 1 was compared to Bin 20. To determine whether the recovery of reflex amplitude was "complete" (i.e. returned to the amplitude of the first response), Bin 20 was compared to the mean amplitude of the first response. In Part 2 of this study, the fast recovery was also investigated with a greater temporal resolution by comparing the amplitudes of the responses evoked by the first 6 stimulation pulses in each stimulus train.

3.2.7 Statistical Analysis

A one-way repeated measures analysis of variance test (rmANOVA) was used to compare the levels of background contraction between sitting and standing for the experiments described in Part 1 of this study. A one-way rmANOVA was also used to compare the different levels of background contraction in Part 2.

Separate rmANOVAs were performed for M-wave and H-reflex data[2]. To assess PAD&R in Part 1, a three-way rmANOVA was used to test for significant effects of task (sitting and standing), stimulation frequency (5, 10, 20 Hz), and time (first response, second response, Bin 1, Bin 20) on response amplitude. For Part 2, a two-way rmANOVA was used to assess the influence of background contraction (relaxed, 1, 5, 10, 20% MVC) and time (first response, second response, Bin 1, and Bin 20) on response amplitude. To test for changes in H-reflex amplitude over the first six responses in Part 2, separate two-way rmANOVAs were used with background contraction (relaxed, 1, 5, 10, 20% MVC) and time (responses 1–6) as factors. Tukey's HSD tests were performed when appropriate on significant interactions or main effects identified by the rmANOVA analyses. The alpha level for all statistical analyses was set at 0.05. Descriptive statistics are reported in the text as the mean ± 1 SD.

3.3 Results

We investigated the effects of task, stimulation frequency and background contraction on the depression and recovery of soleus H-reflexes throughout 10 s trains of electrical stimulation. M-waves were also quantified as a measure of stimulus efficacy. Data from a single participant are shown in Figure 3.2 for one 10 s train of 10 Hz stimulation delivered when the participant was seated and

[2] The M-wave and H-reflex data were analyzed separately based on the results from simplified repeated measures analysis of covariance tests (ANCOVAs). I used the difference between the first and second M-wave as the covariate in each condition, in a repeated measures ANCOVA to assess changes between the first and second H-reflexes (i.e Sitting 5 Hz, 10 Hz, 20 Hz; Standing 5 Hz, 10 Hz, 20 Hz; Background contraction – rest, 1, 5, 10, 20 % MVC). Changes in the M-wave amplitude between the first and second responses did not significantly account for changes in the H-reflex amplitude between the first and second responses across conditions.

holding a background soleus contraction of ~15% MVC. In the top panel, the amplitudes of M-waves and H-reflexes evoked by each stimulus pulse are shown by the open circles and filled diamonds, respectively. Soleus EMG recorded at the beginning and end of the stimulation train is shown in the bottom panel. This participant showed depression of both the M-wave and the H-reflex from the first to the second stimulus pulses. Although M-waves remained depressed for the duration of the stimulation, H-reflex amplitude recovered. As early as the third stimulus pulse (200 ms after the first pulse), reflex amplitude had recovered from 19 to 84% of H_1. Throughout the stimulation reflex amplitude varied, but there was a trend for a slow recovery of reflex amplitude over the 10 s, ending with the last H-reflex being larger than the first H-reflex (108% of H_1).

3.3.1 Part 1: Effects of task and stimulation frequency

The group data for all combinations of task and stimulation frequency across the full 10 s stimulation period are shown in Figure 3.3. In general, there was more H-reflex depression with 20 Hz stimulation, more H-reflex recovery with 10 Hz stimulation, and no apparent differences in PAD&R between sitting and standing. Soleus background contraction levels were not different between sitting (12 ± 4% MVC) and standing trials (11 ± 4% MVC) [$F_{(1,7)} = 0.84$, $p > 0.1$] (data not shown). For M-wave amplitude, there was a main effect of frequency [$F_{(2,14)} = 8.54$, $p < 0.001$] and no main effects of task or time (data not shown). The main effect of frequency, with the data collapsed across task and time, revealed a general depression of M-wave amplitude during 10 [$p < 0.001$] and 20 Hz [$p < 0.05$] stimulation, compared to 5 Hz stimulation.

The analysis of the depression of H-reflex amplitude identified a significant interaction between frequency (5, 10, and 20 Hz) and time (H_1, H_2, Bin 1, Bin 20) [$F_{(6,42)} = 18.93$, $p < 0.001$], and no significant effect of task. The results of the post-hoc analysis performed on this interaction are shown in Figure 3.4. Response depression occurred during 10 and 20 Hz stimulation only. There was a significant difference between H_1 and H_2 at 10 [$p < 0.001$] and 20 Hz [$p < 0.001$], but not at 5 Hz. H_2 at 20 Hz was also significantly smaller than H_2 at 10 Hz [$p < 0.001$], indicative of greater depression during 20 Hz stimulation.

The post-hoc analysis of the frequency by time interaction also showed that H-reflex amplitude recovered from the initial depression during stimulation at 10 and 20 Hz (Fig. 3.4). The 5 Hz stimulation did not evoke significant depression and reflex amplitude did not change significantly throughout the stimulation. During 10 Hz stimulation, fast reflex recovery occurred because Bin 1 was significantly larger than H_2 [$p < 0.05$], and slow recovery was also evident because Bin 20 was significantly larger than Bin 1 [$p < 0.05$]. Furthermore, Bin 20 and H_1 were not significantly different, thus, complete recovery of reflex amplitude occurred by the end of the 10 Hz stimulation. During 20 Hz stimulation, fast recovery occurred because Bin 1 was significantly larger than H_2 [$p < 0.05$], but slow recovery was not evident because Bin 20 was not significantly different than Bin 1. During the 20 Hz stimulation, reflex amplitude did not recover completely as Bin 20 was significantly smaller than H_1 [$p < 0.001$].

3.3.2 Part 2: Effect of background contraction

Figure 3.5 shows the mean amplitudes of M-waves and H-reflexes recorded during 10 Hz stimulation while participants were seated and holding different contraction levels in soleus. Qualitatively, M-waves showed initial depression for all contraction levels and no recovery, while H-reflexes showed more depression of reflex amplitude at lower levels of background contraction and similar recovery across most contraction levels. The five contraction levels, averaged across the group, were 0.4 ± 0.3, 1.8 ± 0.3, 5.3 ± 1.1, 9.8 ± 0.6, and 17.7 ± 1.7% MVC. There was a significant main effect of contraction level [$F_{(4,28)}$= 459.9; $p < 0.01$] and post-hoc tests revealed that all contraction levels were significantly different from each other (data not shown). For M-waves, there were significant main effects of contraction level [$F_{(4,28)} = 4.1$, $p < 0.05$] and time [$F_{(3,21)}= 27.5$, $p < 0.01$] (data not shown). The main effect of contraction, collapsed across time, showed that M-wave amplitudes during the relaxed, 1%, and 5% MVC conditions were significantly smaller than during the 20% MVC condition [$p < 0.05$ for all comparisons]. Post-hoc analysis of the main effect of time showed that M_1 was significantly larger than the M-waves at the other three time points (M_2, Bin 1, and Bin 20; $p < 0.001$ for all comparisons) when the data were collapsed across all contraction levels. M-waves did not recover from this initial depression as M_2, Bin 1, and Bin 20 were not significantly different from each other.

For H-reflexes, there was a significant interaction between contraction level and time [$F_{(12,84)}= 12.3$, $p < 0.01$] (Fig. 3.6). H-reflex depression occurred at all contraction levels, except 20% MVC. H_2 was significantly smaller than H_1 in

the relaxed state [$p < 0.01$], and at 1% MVC [$p < 0.01$], 5% MVC [$p < 0.01$], and 10% MVC [$p < 0.01$]. During the 20% MVC contraction, reflex amplitude did not change significantly throughout the stimulation and thus, these data are not discussed further. In regards to recovery, although H-reflexes were initially depressed during the 10% MVC condition there was no recovery of H-reflex amplitude. The second H-reflex was not different from Bin 1 (fast recovery) and Bin 20 was not different from Bin 1 (slow recovery). The three lower contraction levels did show significant recovery. In the relaxed condition there was no significant fast recovery (H_2 was not different from Bin 1), but there was slow recovery as Bin 20 was significantly larger than Bin 1 [$p < 0.001$]. For the 1 and 5% MVC contractions, fast, but not slow, recovery occurred. Bin 1 was significantly larger than H_2 ($p < 0.01$), however Bin 20 and Bin 1 were not significantly different. Reflexes did not recover completely (i.e. back to H_1 amplitude) for any contraction amplitude.

As mentioned in the description of the single participant data in Figure 3.2, reflex amplitudes varied throughout the stimulation and a marked recovery of H-reflex amplitude was observed in some participants by the third response. While the variability in reflex amplitude often appeared to be random, in some subjects a "pattern" emerged in which reflex amplitudes occasionally alternated between large and small (see Fig. 3.7) or between large, medium, small (data not shown). Figure 3.7 provides an example of data from a participant in whom the third reflex was 19% larger than the first reflex and a striking alternation of reflex amplitude, between ~30% M_{max} and 5% M_{max}, emerged while they were seated

and holding a contraction of ~5% MVC in soleus. While a detailed analysis of these apparent patterns in reflex expression was beyond the scope of the present study, we did quantify the fast recovery of reflex amplitude with a higher temporal resolution than permitted by the comparison of H_2 to Bin 1. We compared the amplitudes of the first 6 H-reflexes across the group and these results are shown in Figure 3.8. In this figure, significant differences between H_1 and all other responses are identified by the brackets but, for clarity, all other significant differences are not shown on the figure and are described below. The analyses of the first 6 H-reflexes identified a significant interaction between contraction level and time [$F_{(20,140)}= 9.5$, $p < 0.01$]. During the relaxed condition, H_1 was significantly larger than all other responses, and none of the other responses differed from each other. Thus, reflexes were depressed and did not recover within the first 6 responses and no alternation of reflex amplitude emerged. At 1% MVC, H_1 was significantly different from all other responses, and H_2 was significantly different from H_3 and H_5, but not H_4 or H_6. This illustrates the emergence of alternating reflex amplitudes. At the 5% MVC level, complete recovery occurred by the third pulse and a strong alternating pattern developed. Complete recovery was shown by the lack of difference between H_1 and H_3 amplitudes. A strong pattern was highlighted by significant differences between all of the even numbered reflexes and odd numbered reflexes, not including the first response. Similarly, during the 10% MVC contraction, complete recovery of reflex amplitude occurred because H_1 was not different from H_3 and H_5, and an alternation of reflex amplitude was evident because H_2 was

different from H_3 and H_5. Lastly, there were no significant differences between responses for the 20% MVC condition.

3.4 Discussion

The results of the present study revealed that the depression of transmission along the H-reflex pathway, commonly known as PAD, was followed by significant recovery of reflex amplitude during 10 s trains of electrical stimulation. Changes in task (sitting or standing) had no effect on the depression or recovery of soleus H-reflexes. On the contrary, stimulation frequency and the level of background contraction significantly influenced PAD&R. Although many studies have investigated PAD, this is the first study specifically designed to characterize the recovery of reflex amplitude during repetitive, reflexive activation of motoneurons. It is likely that there is a complex interplay between the mechanisms responsible for the depression and recovery of reflex transmission (Lloyd 1949, 1958), which depends both on the frequency of the afferent volley and the magnitude of the ongoing voluntary contraction. A better understanding of this modulation of reflex transmission during repetitive input will provide new insight on how reflexes contribute to the control of voluntary movement.

In the current study, in addition to measuring the amplitude of the H-reflex evoked by each stimulus pulse, we also measured each corresponding M-wave. M-wave amplitude is commonly used as a measure of stimulation efficacy in H-reflex studies (Misiaszek 2003). Despite this, few studies have measured M-

waves when assessing PAD, and those that did measure M-waves reported no change in amplitude when H-reflexes were depressed (Floeter and Kohn 1997; Ishikawa et al. 1966; Jeon et al. 2007; McNulty et al. 2008; Trimble et al. 2000). In our study, although M-waves did not change during stimulation at 5 Hz, they were depressed during 10 and 20 Hz stimulation for all levels of background contraction. Plausible reasons that previous studies of PAD in humans have not reported a similar depression of M-waves are that in some cases the amplitude of the second M-wave was not reported (Trimble et al. 2000), stimulation frequencies above 5 Hz were not tested (Floeter and Kohn 1997; McNulty et al. 2008), or trials were excluded if the M-wave amplitude changed more than 2% between pulses (Jeon et al. 2007). A depression of M-wave amplitude during 20 Hz stimulation was recently reported when electrical stimulation was delivered using wide (500 and 1000 μs), but not narrow (50 and 200 μs), pulse widths. This finding suggests that the M-wave depression stems from mechanisms involving the ability to activate motor axons beneath the stimulating electrodes, rather than reduced transmission across the neuromuscular junction (Lagerquist and Collins, in press), and that movement of the electrodes between pulses, as a result of the muscle contraction, was not the main factor causing the M-wave depression. In the current study, a decreased ability to activate motor axons during repetitive stimulation, represented by the depression of the M-wave, raises the possibility that there may have also been a reduction in the ability to recruit sensory axons, which could have contributed to the H-reflex depression. However, since motor and sensory axons have different properties (Burke et al. 2001), it is difficult to

translate changes in motor axon activation during repetitive stimulation to respective changes in sensory axon activation. Additionally, in some trials, depression of the M-wave was not observed, while marked depression of the H-reflex was present. Furthermore, for each condition, we assessed whether the immediate depression of the H-reflex that was often observed was associated with changes from the first to the second M-wave. To do this we conducted an analysis of covariance using the change from the first to the second M-wave in each condition as the covariate. Changes in the M-wave amplitude between the first and second responses did not significantly account for changes in the H-reflex amplitude between the first and second responses across conditions.

3.4.1 Effect of task on the depression and recovery of soleus H-reflexes

In support of our first hypothesis, PAD&R of reflex amplitudes were not influenced by task. We presently found no task-dependent differences in reflex depression or recovery between sitting and standing when background contraction and M-wave amplitudes were matched. Previous data available on the task-dependence of PAD have been variable. Stein and colleagues (2007) found no depression of reflex amplitude when participants stood and held a soleus contraction of 15-20% MVC, but depression was evident when participants were seated and held similar levels of background contraction. Goulart et al. (2000), did observe PAD during standing and found no differences in PAD between sitting and standing when participants held similar background contractions between tasks. However, neither of these studies (Goulart et al. 2000; Stein et al. 2007) tested whether contraction levels were significantly different between tasks and M-waves were not measured during the PAD protocols. In another study,

PAD was not different when participants were sitting or lying prone, over a range of background contraction levels (Trimble et al. 2000). Lastly, in a study in which M-waves amplitudes were controlled, PAD was not different when participants were lying prone with the soleus relaxed or standing while the tested leg was non-weight bearing (Jeon et al. 2007). It may be that differences in PAD previously attributed to task (Stein et al. 2007) may not be related to task per se*;* instead, if background contractions were larger during standing, the reduced PAD may have been more related to the well-known and strong effect of contraction on PAD (Burke et al. 1989; McNulty et al. 2008; Trimble et al. 2000) (see "Effect of background contraction" section below).

3.4.2 Effect of frequency on the depression and recovery of soleus H-reflexes

Our hypothesis about the relationship between PAD&R and stimulation frequency was supported by the finding that there was more depression and less recovery of soleus H-reflexes as stimulation frequency increased. While participants held a contraction of ~10% MVC, we found no reflex depression during stimulation at 5 Hz, significant depression during 10 Hz stimulation, and further depression during 20 Hz stimulation. These results are consistent with the well known frequency-dependence of PAD (Burke et al. 1989; Crone and Nielsen 1989; Ishikawa et al. 1966; Rothwell et al., 1986; Van Boxtel 1986), although our study is one, of only a few, to quantify PAD in humans at frequencies at or above 10 Hz (Goulart et al. 2000; Ishikawa et al. 1966; Jeon et al. 2007; Stein et al. 2007). During natural movements muscle spindle afferents commonly discharge over the range of frequencies presently tested (5-20 Hz). The present findings of depression, followed by significant recovery, of reflex transmission during 10 and

20 Hz stimulation suggest that on-going modulation along the Ia afferent pathway plays a role in the control of natural movements. While other studies have found significant depression at frequencies less than 5 Hz, in most of these cases the participants were relaxed (Burke et al. 1989; Ishikawa et al. 1966; Rothwell et al. 1986; Van Boxtel 1986). The lack of PAD during 5 Hz stimulation in our study may again reflect the strong influence of contraction on the ability to measure PAD.

The recovery of reflex amplitude also scaled with stimulation frequency. We quantified reflex recovery over two time courses: a fast recovery that occurred within the first 0.5 s after the initial depression and a slow recovery that occurred between the first 0.5 s and the last 0.5 s of the stimulation. Reflex amplitudes did not change throughout 5 Hz stimulation. In contrast, during 10 Hz stimulation PAD was followed by significant fast and slow recovery, and by the end of the stimulation reflex amplitudes were not significantly different from that of the first reflex, indicative of complete recovery. During 20 Hz stimulation only fast recovery of reflex amplitude occurred, and by the end of the stimulation reflexes remained significantly depressed compared to the first reflex.

This relationship between PAD&R and stimulation frequency may be explained by several factors including the ability to repetitively activate axons beneath the stimulating electrodes, mechanisms that control the presynaptic release of neurotransmitter, and mechanisms that regulate motoneuron excitability. Axonal excitability fluctuates when axons transmit trains of action potentials and these changes may contribute to the PAD&R we observed. At

different intervals after an action potential, human sensory and motor axons express a relative refractory period (~3-4 ms), a supernormal period (~ 4-20 ms), and a subnormal period (~20-150 ms) (Burke et al. 2001; Kiernan et al. 1996). The subnormal period, in which axonal excitability is decreased, may have influenced the PAD&R we observed because the inter-stimulus intervals for 10 and 20 Hz were 100 and 50 ms, respectively. At both of these stimulation frequencies, the current reaching the axons after each stimulus pulse, except the first, would have arrived at a time of decreased axonal excitability. Furthermore, during trains of stimulation the effects of the subnormal period are summative, eventually leading to a plateau of axonal hyperpolarization (Bergmans 1970; Bergmans and Bostock 1994). Such axonal hyperpolarization may have decreased the ability to activate axons repetitively, and thus the strength of the synaptic drive, more so during 20 Hz than 10 Hz stimulation. This may have contributed to greater depression and less recovery during 20 Hz stimulation.

The efficacy of synaptic transmission along the Ia afferent pathway also strongly depends on impulse frequency (Curtis and Eccles 1960; Lüscher et al. 1983). Such frequency-dependence is related to several mechanisms that control the presynaptic release of neurotransmitter. Activated Ia afferents can evoke presynaptic inhibition on their own terminals (Eccles et al. 1962), however this is not typically believed to contribute to the reflex depression in studies of PAD because the time course of presynaptic inhibition (up to 400 ms) was often shorter than the inter-stimulus intervals (Hultborn et al. 1996). In the present study, the inter-stimulus intervals were 200, 100, and 50 ms for 5, 10, and 20 Hz,

respectively. Therefore, presynaptic inhibition could have been involved in the reflex depression observed in the current study. The presynaptic mechanism most often associated with PAD is a decreased probability of neurotransmitter release from previously active Ia afferent terminals (Hirst et al. 1981; Hultborn et al. 1996; Kuno 1964). In terms of facilitation of neurotransmitter release, post-tetanic potentiation results in a prolonged increase in reflex amplitude following a period of repetitive afferent stimulation (Lloyd 1949). This is thought to be caused by a lower probability of failure to release neurotransmitter from the presynaptic terminal, coinciding with a higher probably of multi-quantal release (Hirst et al. 1981). In humans, post-tetanic potentiation is typically evoked by delivering stimulation at frequencies greater than 100 Hz for seconds to minutes (Hagbarth 1962; Kitago et al. 2004; O'Leary et al. 1997; Van Boxtel 1986); although it has been shown during 3 s stimulation trains delivered at lower frequencies (10, 30 Hz) (Hughes et al. 1957). We believe that post-tetanic potentiation may be involved in the reflex recovery currently observed, however because the reflex recovery during 20 Hz stimulation was less than during 10 Hz stimulation, the processes driving the depression may have been stronger than those leading to the recovery. This highlights the relevance of the interplay between depression and facilitation along the H-reflex pathway.

At the level of the motoneuron three additional mechanisms may influence the PAD&R that we observed. Firstly, the duration of the afterhyperpolarization (AHP) may contribute. The AHP can last up to ~100 ms for soleus motoneurons (Matthews 1996) and increases when motoneurons discharge repetitively

(Gustafsson 1974; Ito and Oshima 1962; Wienecke et al. 2009). Thus, during the 10, and particularly the 20 Hz stimulation, the ability of the afferent volley to depolarize the motor pool was likely reduced by the AHP. Secondly, recurrent inhibition, induced by antidromic volleys in motor axons generated by the stimulation or through the reflexive activation of motoneurons, could have reduced the excitability of the motoneurons to repetitive input (Bussel & Pierrot-Deseilligny 1977; Eccles et al. 1954). The influence of antidromic recurrent inhibition on the current results was likely small due to the low stimulation intensity used. Finally, a gradual increase in the excitability of the motor pool during the stimulation may have contributed to the reflex recovery through the activation of persistent inward currents. Persistent inward currents enhance motoneuron excitability by amplifying synaptic input and helping to sustain motoneuron firing (Crone et al. 1988; Lee and Heckman 2000). Persistent inward currents are thought to be involved in the discharge of human motoneurons (Collins et al. 2002; Gorassini et al. 1998, 2002; Kiehn and Eken 1997) and have been indirectly shown to contribute to muscle activation during periods of tendon vibration (Gorassini et al. 1998) or neuromuscular electrical stimulation (Collins et al. 2001, 2002). PICs have also been shown to 'warm-up' or be more readily activated during repeated voluntary contractions or repetitive stimulation (Gorassini et al. 1998). In the present study, the activation of persistent inward currents during 10 and 20 Hz stimulation could be contributing to the recovery of soleus H-reflex amplitudes.

3.4.3 Effect of background contraction on the depression and recovery of soleus H-reflexes

The hypothesis that H-reflex depression would scale inversely with increases in contraction level was supported by the current results. As the background contraction increased from rest, less depression of soleus H-reflexes was observed, and during the 20% MVC contraction there was no depression. Our findings correspond with previous studies that found less depression with increasing levels of background contraction (McNulty et al. 2008; Stein et al. 2007), including contractions as large as 50% MVC in soleus (Trimble et al. 2000). As others have suggested, the reduction of PAD during a voluntary contraction may be due to tonic depression of the Ia afferent terminals caused by muscle spindle activation during the contraction (Hultborn and Nielsen 1998; Stein et al. 2007). In the present study, we suggest that we measured PAD during contractions of 1-10% MVC because not all Ia afferent terminals were tonically depressed across this range of background contraction levels, and the terminals that were not depressed during the contraction were still susceptible to exhibit PAD. The relationship between motor unit size and PAD may also help explain the reduction in PAD as contraction amplitude increases. Most studies have shown that small motor units exhibit more PAD than large motor units (Crone et al. 1990; Floeter et al. 1997; Lloyd and Wilson 1957; Van Boxtel 1986; cf. McNulty et al. 2008) and as contraction amplitude increased, more of the small motor units were recruited for the contraction. This means at higher contraction amplitudes, there would be fewer small motor units capable of expressing PAD in response to the test pulses, because the Ia afferent terminals that synapse onto

these small motor units were depressed by the background contraction. Conversely, the Ia afferent terminals of the larger units may not have been depressed, but these terminals do not normally exhibit a great degree of PAD.

Our hypothesis that the recovery of H-reflex amplitude would be more complete as contraction level increased was not supported by comparisons of reflex amplitude throughout the 10 s stimulation trains. During the relaxed condition there was significant slow recovery, during the 1 and 5% contractions there was significant fast recovery, and no recovery of reflex amplitude occurred during the 10% MVC contraction. The absolute recovery of reflex amplitude (i.e. the increase in % Mmax) was similar during the relaxed, 1%, and 5% MVC contraction levels. Based on these results, reflex recovery may be influenced more by stimulation frequency, rather than background contraction.

Interestingly, the analysis of the first 6 reflexes in each stimulus train established that complete recovery of reflex amplitude was possible by the third reflex. This finding was surprising because the mechanism most often attributed to PAD is a decreased probability of neurotransmitter release (Hirst et al. 1981; Hultborn et al. 1996; Kuno 1964). During 10 Hz stimulation, if neurotransmitter depletion were involved, for recovery to occur by the third pulse, a time course of ~ 300 ms for vesicle reuse would be necessary. Vesicle recycling has been shown to occur on a time course of 300 ms to 1 s for the Calyx of Held, a large glutaminergic synapse commonly used to study synaptic efficacy in animal models (Kavalali et al. 2007), however vesicle recycling at this synapse may not be representative of the Ia afferent-motoneuron synapse. It is evident that further

work is required to verify the mechanisms behind the reflex depression and recovery observed in the current study. Additionally, immediate recovery of reflex amplitude was only observed in some participants. Other participants showed sustained depression, after the first reflex, over the full 10 s stimulation train. Lastly, it is possible that the alternation of reflex amplitude could be an artifact of our stimulating procedures in which large synchronous afferent volleys are generated at a fixed rate, as compared to dispersed, asynchronous volleys which would be more representative of natural firing patterns.

3.4.4 Summary

We studied PAD&R of reflex transmission by delivering trains of stimulation at physiologically relevant frequencies during functionally relevant tasks and contraction levels. Transmission along the H-reflex pathway was strongly influenced by stimulation frequency and background contraction amplitude. On the contrary, there were no task-dependent differences in PAD&R of reflex amplitudes between sitting and standing. After the initial PAD, reflex amplitude recovered completely by the end of the 10 Hz stimulation, which emphasizes that transmission along the H-reflex pathway does not remain depressed after the first pulse during repetitive stimulation, as implied in previous studies of PAD. Additionally, in some cases there was a complete recovery of reflex amplitude by the third pulse within a stimulation train; a finding that is not consistent with classical ideas regarding the mechanism of PAD. Our results suggest that there is an on-going interplay between depression and facilitation of transmission along the H-reflex pathway during trains of repetitive input which has not been considered in previous studies of PAD in humans. Here we have

shown that this balance between depression and facilitation depends strongly on the frequency of the afferent input and the magnitude of the background contraction, but is relatively insensitive to changes in task.

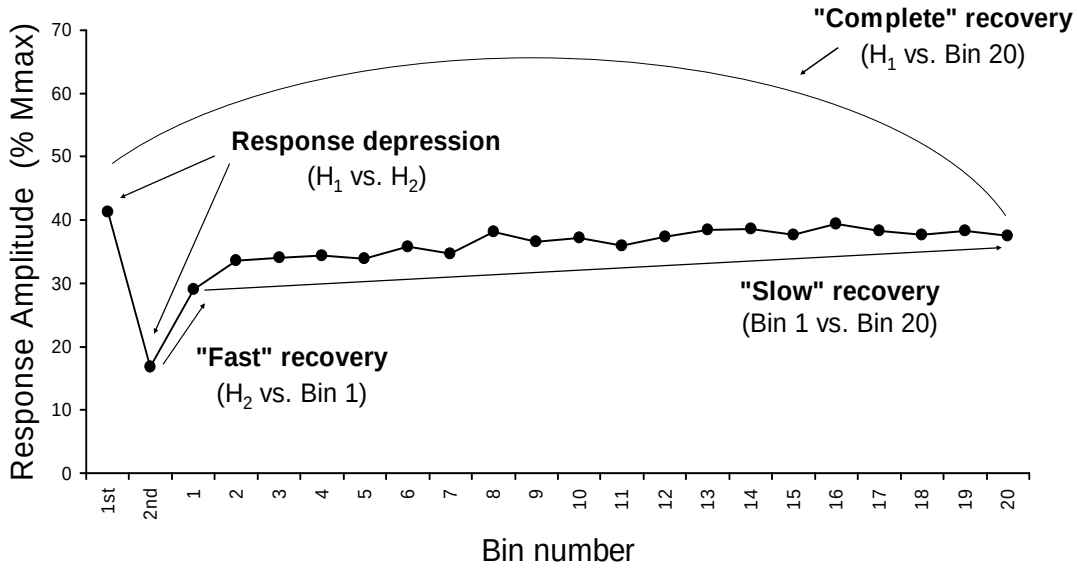

Figure 3.1 A diagram illustrating the method used to quantify reflex depression and recovery during 10 s trains of stimulation. The initial depression was assessed by comparing the first (H_1) and second (H_2) reflexes. "Fast" recovery was assessed by comparing the second reflex and Bin 1 (responses averaged over the first 0.5 s). "Slow" recovery was assessed by comparing Bin 20 (responses averaged over the last 0.5 s) to Bin 1. "Complete" recovery was assessed by comparing Bin 20 to the first reflex.

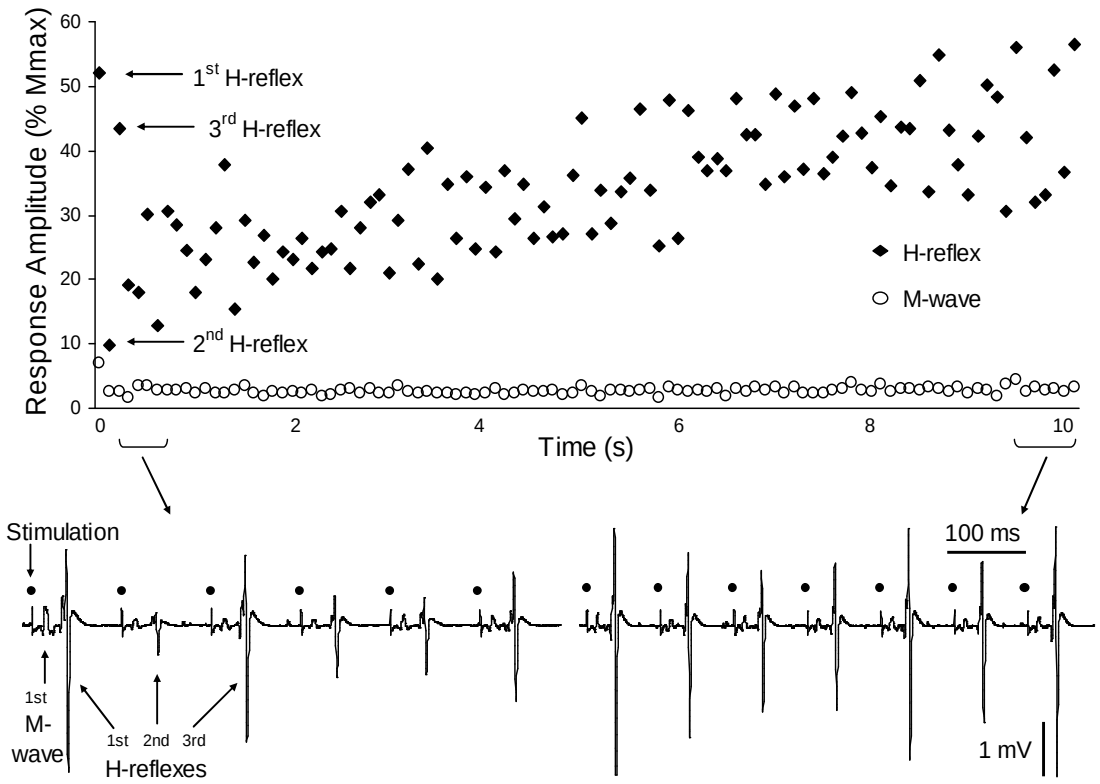

Figure 3.2 Data from a single participant during one 10 s train of 10 Hz stimulation, delivered when the participant was seated and holding a background soleus contraction of ~15% MVC. In the upper panel, M-waves and H-reflexes evoked by each stimulus pulse are shown by the open circles and filled diamonds, respectively. In the lower panel, raw soleus EMG from the beginning and end of the stimulation train are shown.

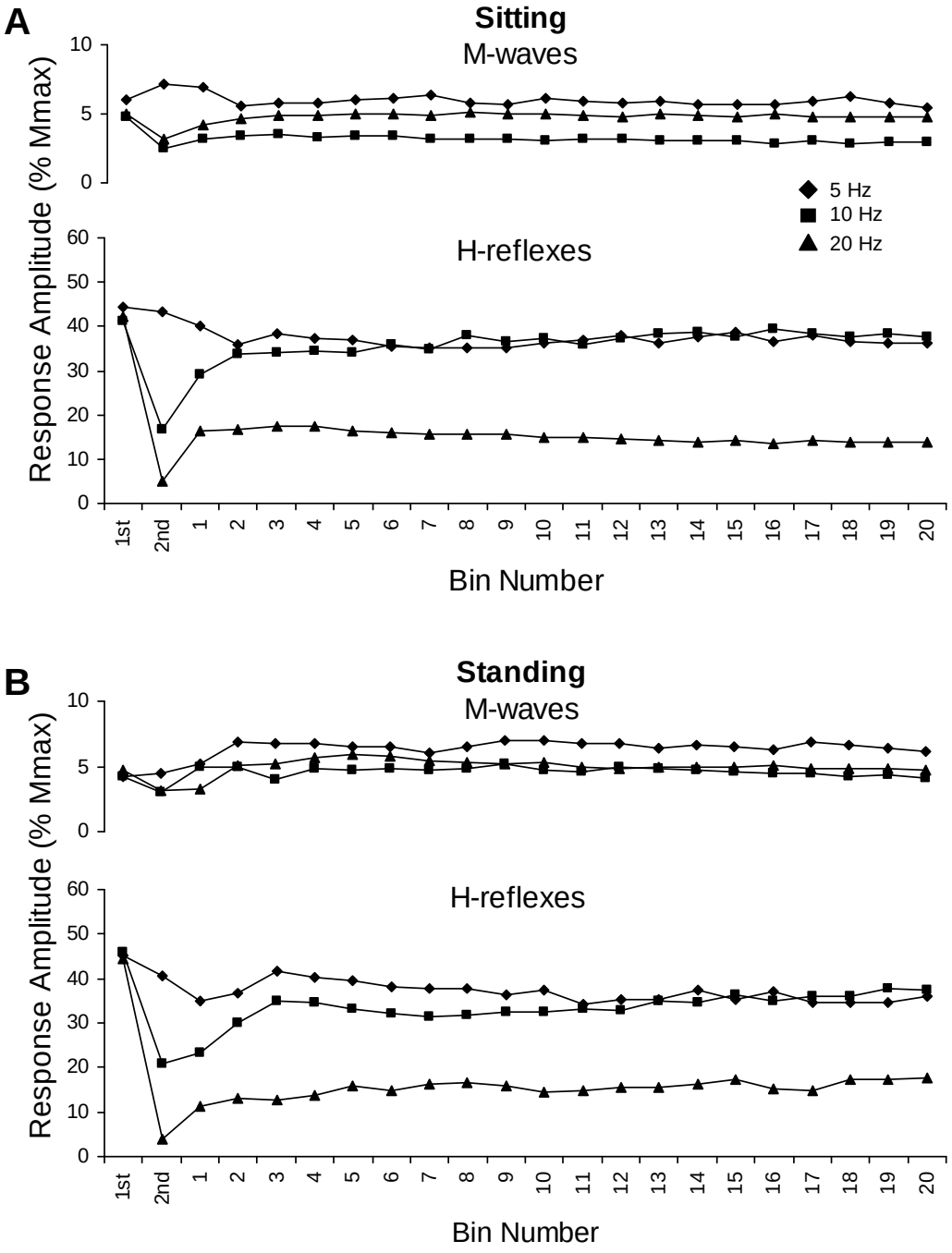

Figure 3.3 Group average M-wave and H-reflex amplitudes during 10 s trains of stimulation delivered at 5, 10, and 20 Hz during sitting (A) and standing (B). The mean of the first and second responses are shown, followed by the mean of responses averaged over 0.5 s bins. Error bars have been omitted for clarity.

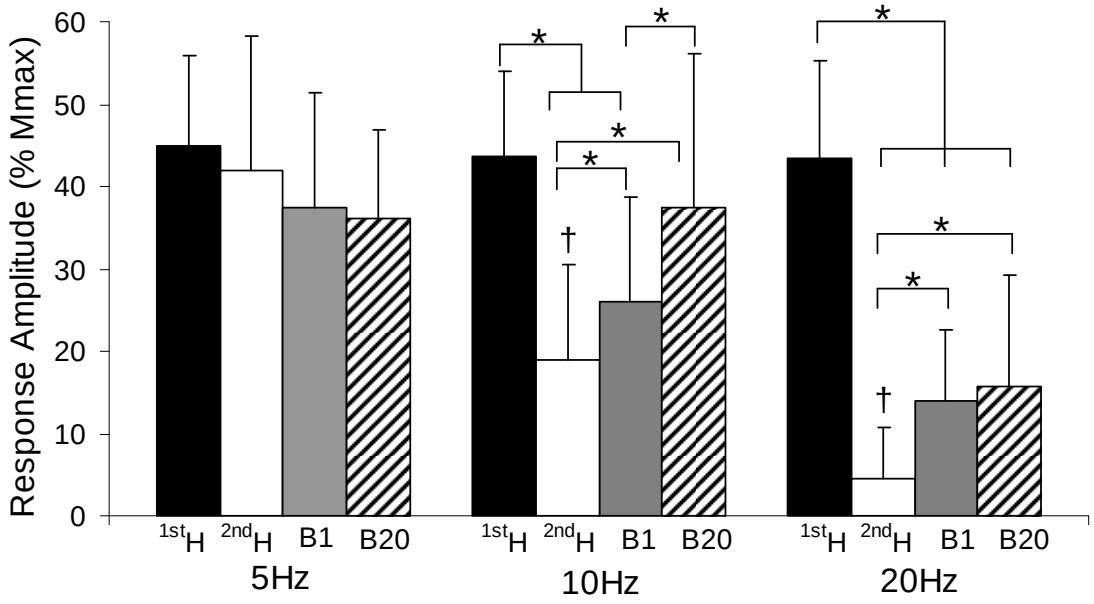

Figure 3.4 Group data illustrating the significant interaction of stimulation frequency and time on H-reflex amplitudes. Columns marked by asterisks or crosses were significantly different from each other. 1 SD is shown.

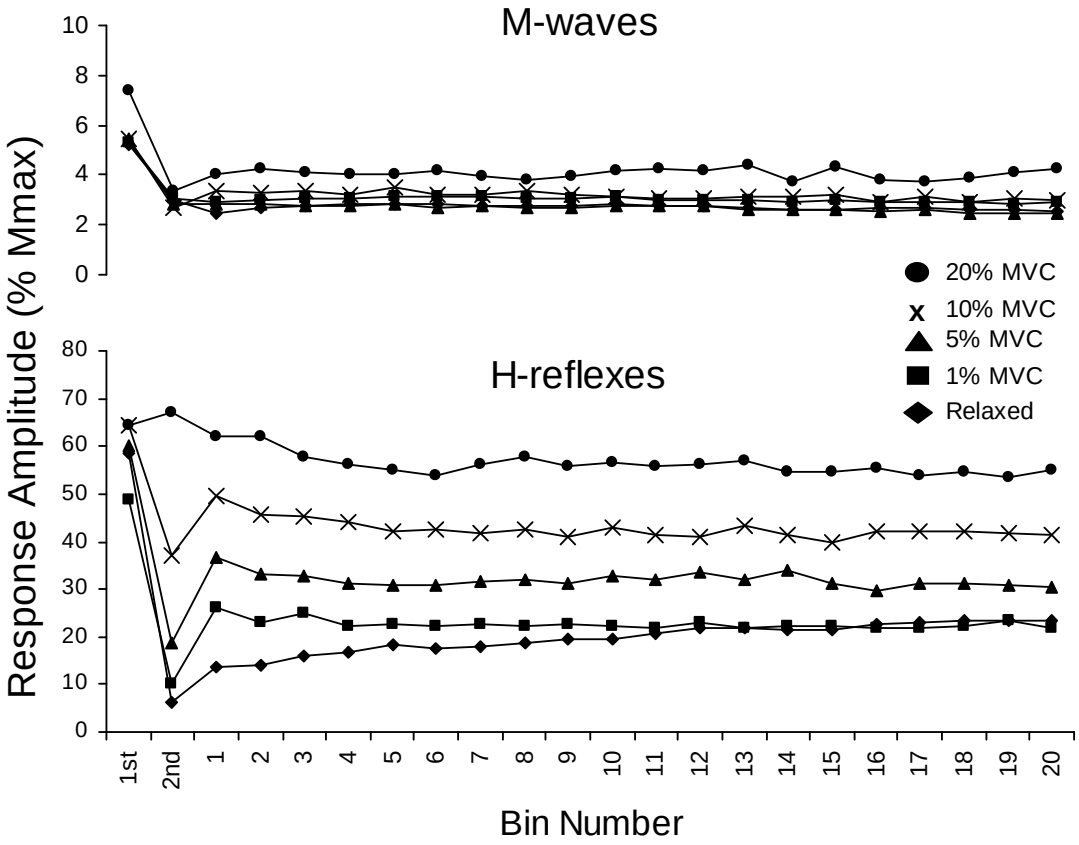

Figure 3.5 Group average M-wave and H-reflex amplitudes during 10 s trains of 10 Hz stimulation during different levels of soleus background contraction (relaxed, 1, 5, 10, and 20% MVC). The mean of the first and second responses are shown, followed by the mean of responses averaged over 0.5 s bins. Error bars have been omitted for clarity.

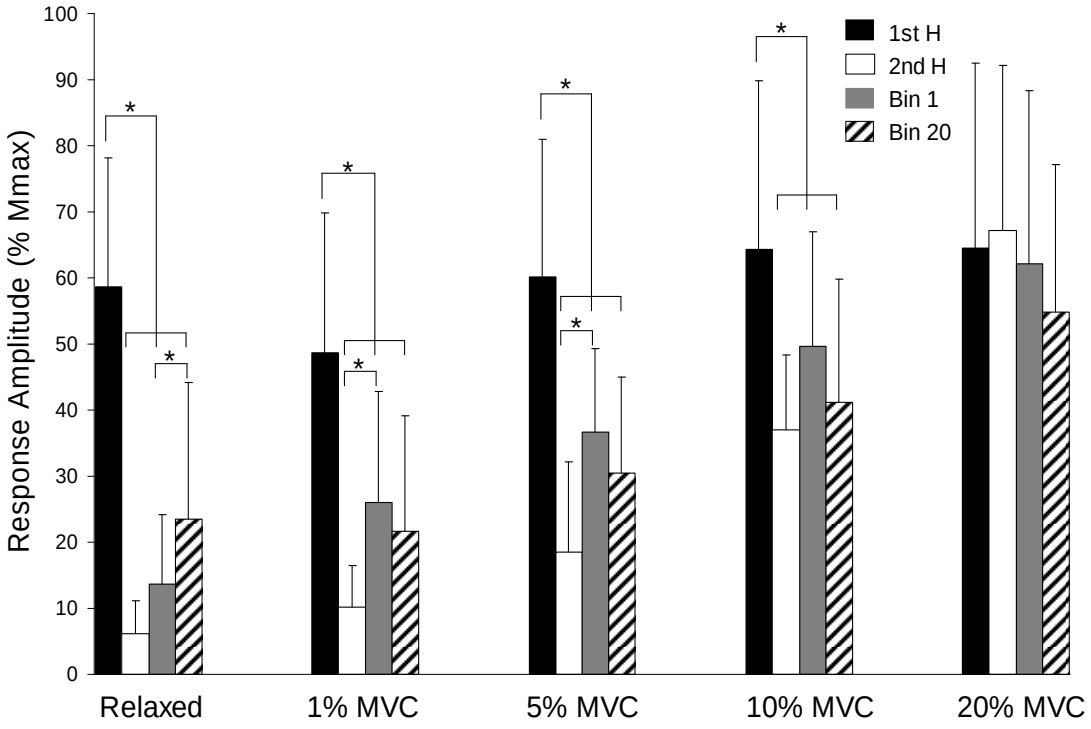

Figure 3.6 Group data illustrating the significant interaction of background contraction and time on H-reflex amplitude. Columns marked by asterisks were significantly different from one another. 1 SD is shown.

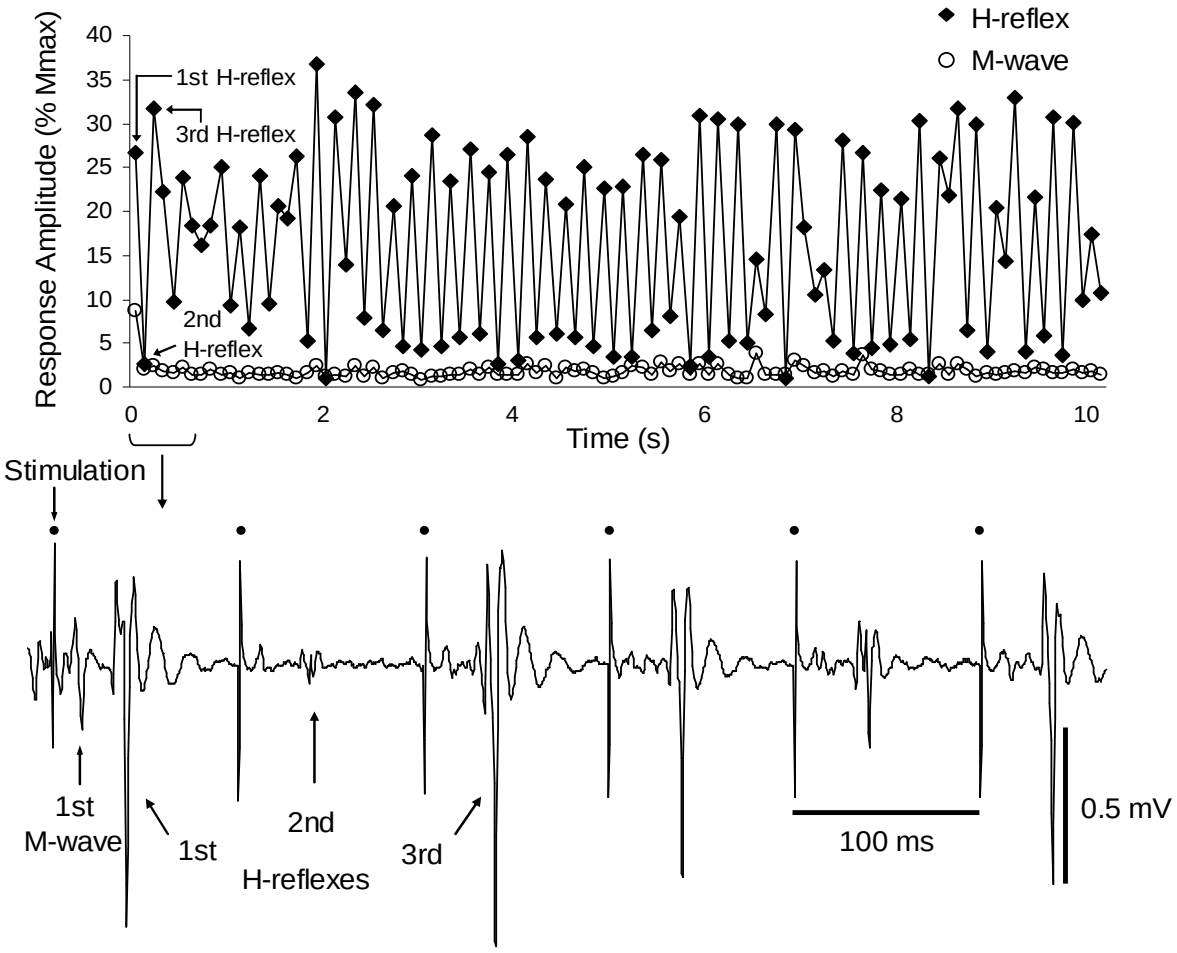

Figure 3.7 Data from a single participant during a 10 s train of 10 Hz stimulation delivered when the participant was seated and holding a background soleus contraction of ~5% MVC. The data in this figure illustrates the strong alternation of reflex amplitudes that was observed in some participants. In the upper panel, M-waves and H-reflexes evoked by each stimulus pulse are shown by the open circles and filled diamonds, respectively. In the lower panel, raw soleus EMG from the beginning of the stimulation train is shown.

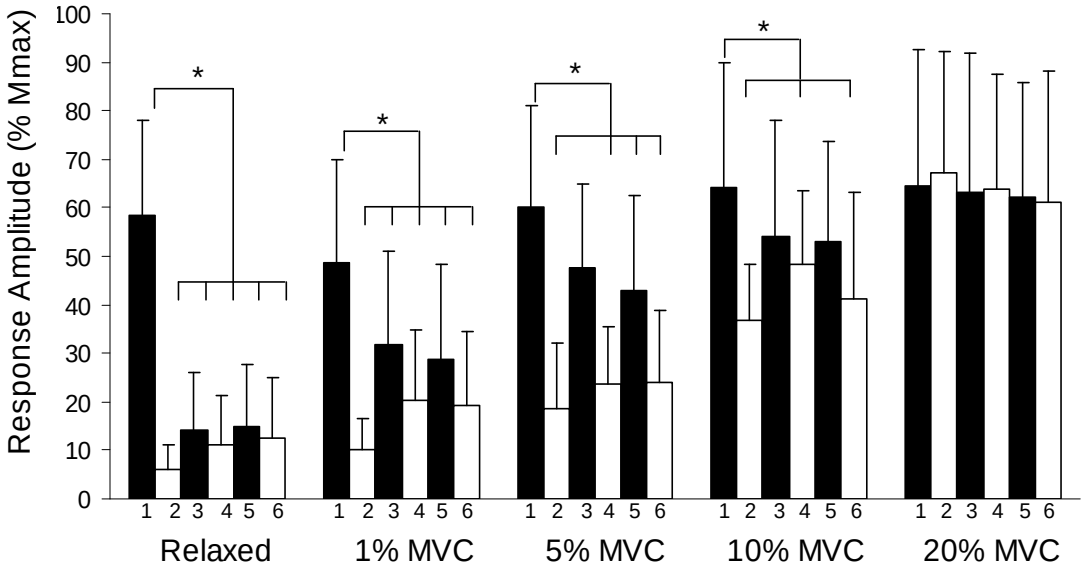

Figure 3.8 Group data illustrating the significant interaction of background contraction and time (i.e. first 6 responses) on H-reflex amplitude. Significant differences between the first reflex (H_1) and all other responses are marked by asterisks, but, for clarity, all other significant differences are not shown on the figure and are described in the text only. 1 SD is shown.

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Chapter 4: The effects of wide-pulse neuromuscular electrical stimulation on elbow flexion torque in individuals with chronic hemiparetic stroke[1]

4.1 Introduction

Individuals who have experienced a stroke often have difficulty generating sufficient and appropriate joint torques required to produce functional movements in the paretic upper limb. This diminished function may be related to muscle weakness or paralysis (Adams et al. 1990; Andrews and Bohannon 2000; Bohannon and Smith 1987; Colebatch and Gandevia 1989; Fellows et al. 1994a, 1994b; Gowland et al. 1992) and abnormal muscle co-activation patterns that lead to abnormal joint torque couplings. In the paretic upper limb these joint torque couplings, such as the coupling of shoulder abduction and elbow flexion or shoulder adduction and elbow extension, result in a debilitating loss of independent joint control, especially in moderately to severely impaired stroke survivors (Beer et al. 1999; Dewald et al. 2001; Dewald and Beer 2001; Roby-Brami et al. 2003). Neuromuscular electrical stimulation (NMES) has been applied to muscles affected by stroke to assist with muscle strengthening and activities of daily living (Chae 2003; de Kroon et al. 2002, 2005; Glanz et al. 1996; Popovic et al. 2009; Stein et al. 2006; Van Peppen et al. 2004). Traditionally, parameters used to stimulate muscles affected by stroke include pulse widths of 200-300 μs and frequencies of 20-50 Hz (de Kroon et al. 2005). During NMES the size of the evoked contraction is often limited by the

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individual's discomfort, as discomfort increases with increasing stimulation currents. Relatively high currents are sometimes required to generate muscle contractions sufficient to produce functional movements however, especially considering the need to overcome co-contraction and abnormal joint torque couplings (Keller et al. 2005). Fatigability of NMES induced contractions also limits contraction amplitude due to the non-physiological order in which motor axons are activated during NMES (Feiereisen et al. 1997; Gregory and Bickel 2005; Jubeau et al. 2007). Thus, there is a need for the continued development of NMES techniques that generate large muscle contractions, while minimizing discomfort and muscular fatigue.

NMES that incorporates higher frequencies (up to 100 Hz) and wider pulse widths (1 ms) (wide pulse NMES; WP-NMES), than those traditionally used for electrical stimulation, can enhance NMES-evoked contractions in individuals with no neurological impairments (Collins et al. 2001, 2002; Collins 2007) and in those with a spinal cord injury (Clair et al. 2006; Hornby et al. 2007; Nickolls et al. 2004) through an increase in the "central contribution". This central effect is thought to develop due to the recruitment of spinal motoneurons by the electrically-evoked afferent volley travelling along reflex pathways through the spinal cord (see Collins 2007). Mechanistically, the high frequencies and wide pulse widths are thought to send a relatively larger afferent volley to the spinal cord than traditional NMES, augmenting contraction amplitude by increasing H-reflex amplitudes (Klakowicz et al. 2006) and potentially increasing the activation of persistent inward currents (PICs) in spinal neurons (Collins et al.

2001, 2002; Collins 2007). A potential advantage of using WP-NMES for rehabilitation, compared to more traditional NMES, is that lower stimulus currents may be sufficient to generate functional muscle contractions. Furthermore, synaptic activation of motoneurons follows the size principle (Henneman et al. 1965) thereby recruiting fatigue-resistant motor units first, which may help generate contractions that are more fatigue-resistant (Lagerquist et al. 2009). The extent to which the central nervous system (CNS) contributes to contractions evoked by WP-NMES in individuals who have experienced a stroke has not been tested.

Several changes occur in the CNS after stroke that may enhance the central contribution to contractions evoked by WP-NMES in the paretic limb. These changes include presynaptic changes in inhibitory mechanisms that regulate the transmission of afferent input to motoneurons and putative postsynaptic changes in the excitability of the motoneurons themselves. Presynaptic changes include reduced post-activation depression (Aymard et al. 2000; Lamy et al. 2009; Masakado et al. 2005), presynaptic inhibition (Artieda et al. 1991; Aymard et al. 2000; Kagamihara and Masakado 2005; Lamy et al. 2009; Nakashima et al. 1989) and reciprocal inhibition (Nakashima et al. 1989; Okuma and Lee 1996). Together these changes would enhance synaptic efficacy, effectively allowing a larger afferent volley to reach the motoneuron. There may also be postsynaptic changes in motoneuron excitability due to changes in the intrinsic properties of motoneurons (McPherson et al. 2008) that arise from a disruption in the control of descending monoaminergic pathways (Benecke et al. 1991; Dewald et al. 1995;

Fries et al. 1993; Kline et al. 2007; Mazevet et al. 2003; Turton et al. 1996; Turton and Lemon 1999). This increased excitability would amplify the motoneuron's response to afferent input. Taken together, these presynaptic changes in reflex transmission and postsynaptic changes in motoneurons could increase reflex excitability, thus a given afferent volley would lead to a larger motor output.

The current study was therefore designed to investigate whether WP-NMES augments muscle contractions after stroke. These experiments were prompted in part by the finding that tonic vibration reflexes were larger in the paretic arm compared to the non-paretic arm in individuals with chronic stroke (McPherson et al. 2008). The tonic vibration reflex and WP-NMES both generate contractions through the reflexive recruitment of motoneurons, thus we expected that WP-NMES would also generate larger contractions in the paretic arm compared to the non-paretic arm. Specifically, we hypothesized that stimulation incorporating a 1 ms pulse width would generate larger contractions in the paretic arm versus the non-paretic arm, but contractions generated using 0.1 ms pulses would not be different between arms. In these trials, stimulus current intensity was adjusted across pulse widths, in each arm, to produce similar torque during a brief 250 ms stimulation train at 100 Hz. We also hypothesized that stimulation delivered using 1 ms pulses at 100 Hz would evoke contractions with a larger central contribution compared to stimulation using 1 ms pulses at 20 Hz. The central contribution was quantified by comparing the torque at the beginning of a stimulation train (1.5-2.0 s into the 12 s train) where most of the torque is generated through peripheral mechanisms (i.e. direct motor axon depolarization),

to the torque at the end of the stimulation train (11.5-12.0 s) where the reflexive recruitment of motoneurons augments contraction amplitude. This method of quantifying the central contribution has been utilized for similar stimulation protocols in previous studies (Baldwin et al. 2006; Dean et al. 2007; Klakowicz et al. 2006). For the 100 Hz stimulation using 1 ms pulses we predicted the torque would be larger at the end of the stimulation than the beginning, in both arms, and that this difference would be largest in the paretic arm. We predicted there would be no difference in the torque from the beginning to end of the 20 Hz stimulation using 1 ms pulses in either arm. The results of the current study provide further evidence of an increase in reflex excitability after stroke and have implications for the use of NMES in the neurorehabilitation of stroke.

4.2 Methods

4.2.1 Participants

NMES was applied via surface electrodes over the right and left biceps brachii muscles of 14 individuals who had experienced a hemiparetic stroke resulting in upper limb paresis. Four of these subjects disliked the feeling of the electrical stimulation and withdrew from the study. Thus, only data from the remaining 10 participants were used for the data analyses (7 males and 3 females; age range: 53-83 yrs). Each participant provided informed, written consent. The experiments were conducted in accordance with the standards set by the Declaration of Helsinki for research involving human participants and were approved by the Institutional Review Board of Northwestern University.

Inclusion criteria were the following: (1) A cortical or sub-cortical stroke at least one year prior to the study (range: 26-255 months post-stroke; mean: 102 months). (2) An upper limb Fugl-Meyer Assessment (FMA) score for the paretic limb of less than 50 (out of 66). In this study scores ranged from 10-46, which indicates moderate to severe impairment (Fugl-Meyer et al. 1975). (3) An FMA score for the non-paretic limb of 66, which indicates no impairment. (4) Passive range of motion in both limbs of at least 90º for shoulder flexion, shoulder abduction, and elbow flexion. (5) No inflammatory conditions at the shoulder, elbow, wrist, or fingers as screened for by applying overpressure at the end of the range of motion. (6) No recent changes in medications used to manage hypertension. (7) Not taking medications to treat spasticity.

4.2.2 Experimental Protocol

The position of the participant and the equipment used in the current study closely follow that used by McPherson and colleagues (2008). Participants were seated in the chair of a Biodex dynamometer system (Biodex Medical Systems, Shirley, NY, USA) with seatbelts placed over the shoulders and across the waist to help maintain a consistent upright posture for the duration of the experiment. Both feet were supported by a foot rest. Participants wore a custom-made fibreglass cast over the forearm, wrist, and hand of the arm receiving stimulation in order to minimize movement during the experiment. The cast also allowed for tight coupling between the arm and the 6 degrees-of-freedom load cell used to measure joint torques (JR3, Model 45E15A, Woodland, CA, USA). The load cell was attached to the cast at the wrist and orthogonal forces and moments generated in the x, y, and z planes were recorded. The arm was positioned with 75º of

shoulder abduction, 40º horizontal shoulder flexion, and 90º of elbow flexion. The weight of the arm was supported throughout the experiment to help participants remain fully relaxed.

To monitor whether participants remained relaxed throughout the experiment, surface electromyography (EMG) was recorded via electrodes placed over the muscle belly with a 1 cm inter-electrode distance (Delsys, 16-channel Bagnoli EMG System, Boston, MA, USA). In the arm receiving stimulation, the muscles recorded from were: brachioradialis, biceps brachii, lateral head of the triceps brachii, long head of the triceps brachii, anterior deltoid, intermediate deltoid, posterior deltoid, and pectoralis major.

The paretic and non-paretic arms were tested on separate days. For a given participant, the sessions were conducted at the same time of day, between 1 and 5 days apart. The non-paretic arm was tested on the first day because stimulation intensities for both arms were matched to a percentage of the maximum voluntary elbow flexion torque in that arm. Participants started each experiment by performing maximal voluntary isometric elbow flexion contractions, using visual feedback of their maximal voluntary torque (MVT) provided on a computer monitor. Verbal encouragement to perform maximally was provided by the experimenters. The participants completed as many trials as necessary to record three maximal contractions within 10% of each other (typically 3-5). For trials during the main protocol, participants were instructed to relax to minimize muscle activity in the arm being tested and to stay as still as possible.

NMES was delivered through bipolar surface electrodes (oval 3.81 x 6.35 cm, Uni-patch Superior Silver Electrodes, Wabasha, MN, USA) over the biceps brachii muscle. The electrodes were positioned over the proximal and distal ends of the muscle belly to allow for the biceps brachii EMG electrodes to be placed between the stimulating electrodes. A Compex Motion stimulator (Keller et al. 2002) was used to control the parameters of the stimulation. Stimulation intensity was set based on the peak torque generated during a 250 ms stimulus train (25 pulses at 100 Hz; 1 or 0.1 ms pulse width). These short trains were chosen because they provided an indication of primarily peripheral motor axon activation in each arm (Baldwin et al. 2006; Collins et al. 2001, 2002). This procedure for matching stimulus intensity was used because we could not measure EMG during the stimulation due to the presence of large stimulation artifacts, and therefore could not use M-wave amplitude as an indication of stimulation intensity. Intensity was adjusted to generate peak torque during the short train in both arms that was approximately 6% of the non-paretic MVT. This stimulation intensity was chosen because it was the highest that was comfortable for participants in pilot experiments. In 7 out of 10 subjects this was achieved by using the same stimulation current for both arms. For the other 3 subjects to generate the same torque in both arms the current had to be increased by 2, 6, and 12 mA for the paretic arm.

Four stimulation patterns were delivered: 20-100-20 Hz (4 s each phase, 1 ms pulse width); 20-100-20 Hz (4 s each phase, 0.1 ms pulse width); 20 Hz for 12 s (1 ms pulse width); and 100 Hz for 12 s (1 ms pulse width). The 20-100-20 Hz

(1 ms) stimulation pattern was included based on previous work that showed a sustained increase in torque after the 100 Hz stimulation period due to the central recruitment of motoneurons (Collins et al. 2001, 2002). The same stimulation pattern was delivered using a narrower pulse width (20-100-20 Hz, 0.1ms) to assess the effect of pulse width on the evoked contraction. The torque evoked by each of these stimulation patterns was compared between arms. The 20 and 100 Hz constant frequency patterns were included to assess the effect of stimulation frequency on the evoked contraction. The central contribution has been quantified in other studies by measuring the difference in the torque between the beginning and end of the stimulation train (Baldwin et al. 2006; Dean et al. 2007; Klakowicz et al. 2006); an increase in torque throughout the stimulation train was evidence of an increasing central contribution. In the present study, this difference in torque was compared between the 20 and 100 Hz stimulation patterns within each arm and for each stimulation pattern between arms.

In a single trial, one stimulation pattern was delivered 5 times with 2 minutes of rest between each stimulation train. The order of delivery of the stimulation patterns was randomized across subjects. The experimenter visually inspected the torque and all EMG channels after each trial to determine if that trial was acceptable. A trial was rejected, and subsequently re-collected, if there was muscle activity prior to the stimulation. Three participants had difficulty turning off all muscle activity in their paretic arm prior to each trial. In these cases, as many trials as time permitted were re-collected and the trials with the least amount of activity were used for analyses. A trial was also rejected, and

subsequently re-collected, if the participant moved before the trial finished (i.e. coughed, moved their head) or fell asleep.

4.2.3 Data Collection and Analyses

Data were sampled at 1000 Hz. EMG data were amplified 1000 times, high pass filtered (cut-off frequency, 20 Hz) (16-channel Bagnoli EMG System), and then low pass filtered using an eighth-order low-pass Butterworth filter (cut off frequency, 500 Hz) (Model 9016; Frequency Devices, Havelhill, MA). Custom written Matlab software (The Mathworks Inc., Natick, MA, USA) was used to analyze the torque and biceps brachii EMG data. A Jacobian-based algorithm was used to convert the load cell forces and moments measured at the wrist into elbow flexion torque. The torque data were filtered using a digital 8^{th} order low pass Butterworth filter (cut-off frequency, 50 Hz). All torque data were then normalized to the MVT of the non-paretic arm. This normalization was performed for two reasons: (1) the MVT of the paretic arm can be unreliable and may not truly represent the force generating capacity of the muscle due to reduced central drive, the possibility of increased co-contraction between elbow flexors and extensors, and changes in muscle properties (Dietz and Sinkjaer 2007; Sinkjaer and Magnussen 1994); and (2) it was not possible to measure the electrically-stimulated maximum torque in the paretic arm due to pain tolerance of the participants. For statistical analyses, torque data were averaged over two 500 ms windows centred around: 1.5 s (Time 1) and 11.5 s (Time 2) into the stimulus train (see shaded regions Figure 4.2a).

Biceps brachii EMG data were full-wave rectified and then smoothed using a causal one-sided moving average filter (window duration, 250 ms). This was

followed by baseline correction which involved subtracting the average biceps brachii EMG over the first 250-500 ms in a trial, during which the subject was at rest and there was no stimulation, from all other EMG data within that trial. The EMG data were then normalized to the maximum EMG recorded during maximum voluntary contractions in the non-paretic arm. The EMG was quantified prior to the stimulation to verify if the muscle was fully relaxed. Although analysis of the EMG recorded during the stimulation was not possible due to interference from the stimulation artifacts, a comparison of EMG activity before and after stimulation was made. Data were averaged over two 500 ms windows centred around 1 s prior to (pre) and 1 s after (post) the stimulation. The EMG data from muscles other than the biceps brachii (i.e. brachioradialis, lateral head of the triceps brachii, long head of the triceps brachii, anterior deltoid, intermediate deltoid, posterior deltoid, and pectoralis major) were only used to determine whether participants were relaxed prior to the start of each trial. One participant's EMG data were excluded due to poor signal quality.

4.2.4 Statistical Analysis

A two-way repeated measures analysis of variance test (rmANOVA) was used to assess differences in torque evoked by the 250 ms train used to set stimulus intensity based on the following two factors: arm (2 levels: paretic and non-paretic) and pulse width (2 levels: 1 and 0.1 ms). A three-way rmANOVA was used to assess differences in torque during the 12 s stimulation trains based on the following three factors: stimulation pattern (4 levels: 20-100-20 Hz (1 ms pulses), 20-100-20 Hz (0.1 ms pulses), 20 Hz (1 ms pulses), 100 Hz (1 ms pulses)), arm (2 levels: paretic and non-paretic), and time (2 levels: Time 1 and

Time 2). A similar three-way rmANOVA was used to assess changes in biceps brachii EMG activity with the following three factors: stimulation pattern (4 levels: 20-100-20 Hz (1 ms pulses), 20-100-20 Hz (0.1 ms pulses), 20 Hz (1 ms pulses), 100 Hz (1 ms pulses)), arm (2 levels: paretic and non-paretic), and time (2 levels: pre-stim and post-stim). The Huynh-Feldt correction was applied if the data violated the assumption of sphericity for rmANOVA. Tests of simple effects, followed by simple comparisons, if necessary, were used post-hoc to assess significant 3-way interactions identified in the rmANOVA results. Tukey's HSD tests were performed on significant 2-way interactions or main effects when appropriate. Descriptive statistics are reported as the mean ± 1 SE. All statistical tests were conducted with an alpha level of 0.05.

4.3 Results

4.3.1 Stimulation intensity

For all trials stimulus intensity was adjusted such that a 250 ms stimulus train (25 pulses at 100 Hz) evoked torque of approximately 6% of the non-paretic MVT. Examples of the torque recorded during these short trains in the paretic and non-paretic arms of a single participant are shown in Figure 4.1b (inset). For the group of participants, the torque evoked by the 250 ms train was not different between the paretic and non-paretic arms for either pulse width [$F_{(1,9)} = 4.186$, $p = 0.071$]. For trials that used a 1 ms pulse width, the evoked contractions were 6.4 ± 0.5 and 5.9 ± 0.1% of the non-paretic MVT in the paretic and non-paretic arms, respectively. For trials that used a 0.1 ms pulse width, the evoked contractions

were 5.7 ± 0.4 and 6.1 ± 0.1% of the non-paretic MVT in the paretic and non-paretic arms, respectively.

4.3.2 Single participant torque data

Elbow flexion torque evoked for all stimulation patterns in the paretic and non-paretic arms of one participant is shown in the main panels of Figure 4.1. A clear effect of pulse width on torque generation in the paretic arm was evident for this participant. For all stimulation patterns that incorporated the wide (1 ms) pulse width there was more torque evoked in the paretic arm than the non-paretic arm (dark vs. light trace, respectively, Fig. 4.1a, c, d). In contrast, when the stimulation was delivered with a 0.1 ms pulse width, torque generated in the paretic and non-paretic arms was not different (Fig. 4.1b). For this participant, there was no influence of stimulation frequency on the amplitude of the central contribution because the 100 Hz constant frequency stimulation pattern (1 ms pulses) did not show a greater increase in the amplitude of the central contribution compared to the 20 Hz constant frequency stimulation pattern (1 ms pulses) in either arm.

4.3.3 Group torque data: Effect of pulse width

The torque evoked by each stimulation pattern, averaged over the 10 participants, is shown in Figure 4.2. The shaded areas in Figure 4.2a represent the time windows over which data were quantified for statistical analyses. There was a significant three-way interaction between stimulation pattern, arm, and time for the torque generated by NMES [$F_{(1,12)} = 4.43$, $p < 0.05$]. To assess the effect of pulse width on torque, we used post-hoc analyses of this interaction to compare the two-way interaction of arm by time for the two stimulation patterns that used

the same stimulation frequencies, but different pulse widths: 20-100-20 Hz (1 ms pulse width) and 20-100-20 Hz (0.1 ms pulse width). The arm by time interaction for the 20-100-20 Hz (1 ms pulse width) pattern was significant [$F_{(1,12)} = 5.79$, $p < 0.05$]. Simple comparisons analyses revealed that for this stimulation pattern more torque was evoked in the paretic arm than the non-paretic arm at Time 1 [$F_{(1,12)} = 33.9$, $p < 0.05$] and Time 2 [$F_{(1,12)} = 85.1$, $p < 0.05$]. These statistically significant differences can be seen by comparing the dark and light traces in Figure 4.2a and each pair of black and white columns in Figure 4.3A. For the 20-100-20 Hz stimulation pattern that used a 0.1 ms pulse width, significant interactions or main effects were not found which indicates that the torque was not different between arms at either Time 1 or Time 2 (Fig. 4.2b; each pair of black and white columns in Fig. 4.3b).

4.3.4 Effect of stimulation frequency

To assess the effect of stimulation frequency on torque, we used post-hoc analyses of the significant 3-way interaction mentioned above, to compare the two-way interaction of arm by time for the two stimulation patterns that used the same pulse width, but different stimulation frequencies: 20 Hz (1 ms pulse width) and 100 Hz (1 ms pulse width). For the 20 Hz stimulation pattern no significant interactions or main effects were found which indicates that the torque was not different between arms at Time 1 or Time 2 (Fig. 4.2c; Fig. 4.4a). For the 100 Hz stimulation pattern the main effects of arm [$F_{(1,12)} = 5.66$, $p < 0.05$] and time [$F_{(1,12)} = 14.36$, $p < 0.05$] were significant. The main effect of arm showed that the torque was larger in the paretic arm compared to the non-paretic arm throughout the stimulation train. The main effect of time highlighted the

significant decline in torque from the beginning (Time 1) to the end (Time 2) of the stimulation train in both arms (Fig. 4.2d; each pair of columns in Fig. 4.4b). This decrease was not significantly different between arms. These main effects are not shown in Figure 4 for clarity.

To investigate whether the 4 s period of 100 Hz stimulation during the 20-100-20 Hz (1 ms pulse width) pattern resulted in a prolonged increase in torque, post-hoc analyses of the significant 3-way interaction mentioned above was used to compare the two-way interaction of arm by time during the 20-100-20 Hz (1 ms pulse width) stimulation pattern. The arm by time interaction was significant [$F_{(1,12)} = 5.79$, $p < 0.05$] and simple comparisons analyses revealed that the torque increased significantly from Time 1 to Time 2 in the paretic arm only [$F_{(1,12)} = 10.04$, $p < 0.05$] (dark trace, Figure 4.2a; black columns in Fig. 4.3a). When a 0.1 ms pulse width was used, an increase in torque after the 4 s period of 100 Hz stimulation was not observed in either arm.

4.3.5 Group EMG data

Some participants had difficulty completely relaxing the muscles in their paretic arm, therefore the average group pre-stimulus biceps brachii EMG recorded from the paretic arm, across all trials, was 11 ± 3% of the EMG recorded from the non-paretic arm during the maximum voluntary contraction. For the non-paretic arm the average group pre-stimulus EMG was 2 ± 0.4% of the non-paretic maximal EMG. To assess whether the stimulation caused a sustained increase in EMG amplitude once the stimulation had been turned off, we measured EMG over two half second windows centred around: 1 s before (pre) and 1 s after (post) stimulation. There was a significant stimulation pattern by

time interaction [$F_{(3,24)} = 3.51$, $p = 0.031$] and no main effect of arm (data not shown). With the data from both arms grouped together, there was significantly more biceps brachii EMG 1 s after the 100 Hz stimulation pattern as compared to 1 s before the stimulation. There was also more activity 1 s after the 100 Hz stimulation as compared to 1 s after the 20 Hz stimulation. There were no differences in the EMG amplitudes before and after the 20 Hz constant frequency stimulation using a 1 ms pulse width or the 20-100-20 Hz stimulation using a 1 or 0.1 ms pulse width.

4.4 Discussion

In the current study a novel stimulation paradigm (WP-NMES) was delivered to the biceps brachii muscles of the paretic and non-paretic arms in individuals with chronic hemiparetic stroke. We predicted that after a stroke, contractions evoked by WP-NMES would be larger in the paretic arm compared to the non-paretic arm due to reduced inhibition of afferent input to motoneurons (see section on "Presynaptic changes" below) and increased motoneuron excitability (see section on "Postsynaptic changes" below). Consistent with our first hypothesis, more torque was generated in the paretic arm compared to the non-paretic arm when a 1 ms pulse width was used. There was no difference in torque between arms when a 0.1 ms pulse width was used. Our second hypothesis, which stated that higher stimulus frequencies would increase the central contribution more than lower frequencies, was not supported. As predicted, there was no difference in torque from the beginning to the end of the stimulation for each arm during constant frequency stimulation at 20 Hz using a 1

ms pulse width. However, for both arms during the 100 Hz constant frequency stimulation (1 ms pulses), there was a decline in torque from the beginning to the end of the stimulation train, contrary to our prediction. Interestingly, when only 4 s of 100 Hz stimulation was incorporated into the 12 s stimulation train (1 ms pulses), torque was larger in the paretic arm compared to the non-paretic arm. Based on these results, NMES that incorporates wide pulses and brief periods of high frequency stimulation may prove to be an effective way to enhance electrically-evoked contractions for rehabilitation for individuals who have had a stroke, while not generating appreciable levels of fatigue. Additional research characterizing the rate of torque reduction over longer periods of time, using wide versus narrow pulse durations, is currently underway.

4.4.1 WP-NMES enhances the central contribution to contractions after stroke

The present experiments were prompted by two previous findings: (1) In individuals with no neurological impairments, WP-NMES enhances electrically-evoked contractions, compared to more traditional NMES that uses narrower pulse widths and lower stimulus frequencies, due to an increased central or reflexive activation of motoneurons (Collins et al. 2002; Klakowicz et al. 2006); and (2) After a stroke, tonic vibration reflexes were larger in the paretic arm than the non-paretic arm (McPherson et al. 2008). We believe the larger contractions evoked by WP-NMES in the present study share a similar central mechanism with the enhanced tonic vibration reflexes and that the sensory volleys evoked during the stimulation (vibration or electrical) resulted in greater reflexive recruitment of motoneurons in the paretic arm than the non-paretic arm. In the present study, contractions were larger in the paretic arm when 1 ms pulses were used, but not

when 0.1 ms pulses were used. This effect of pulse width occurred despite adjusting the stimulation intensity so that a brief 100 Hz stimulus train delivered using both pulse widths evoked contractions of equal amplitude in both arms. We matched contraction amplitudes during the short trains to generate contractions in both arms that had a similar contribution from the immediate, peripheral contribution (i.e. direct motor axon depolarization). The reflexive contribution to contractions during the short trains should have been minimal because in individuals with no neurological impairments it develops slowly (over seconds) when stimulation is applied over the muscle (Baldwin et al. 2006), as in the present study. There is some evidence that after stroke motor axons change and there is a reduction in the efficacy of inwardly rectifying channels (I_H) in the paretic arm versus the non-paretic arm (Jankelowitz et al. 2007). A reduced I_H current on motor axons in the paretic arm would mean the axons show less accommodation to hyperpolarizing currents and would be more difficult to activate repetitively. Since the stimulation intensity was adjusted to recruit a similar proportion of motor axons with the different pulse widths in both arms, changes to motor axons after stroke would, if anything, reduce contraction amplitude during NMES. Thus, we believe that the enhanced contractions in the paretic arm in the present study were not due to a purely peripheral mechanism, but resulted from an increased recruitment of motoneurons centrally.

If the effect of pulse width in the paretic arm is not due to a difference in activating motor axons, it must be, at least initially, due to a differential ability of the two pulse widths to depolarize sensory axons. Longer pulse widths depolarise

sensory axons preferentially to motor axons due to greater axon diameters of the largest sensory nerves resulting in a longer strength duration time constant and lower rheobase (Dawson 1956; Krarup 2004; Lagerquist and Collins 2008; Panizza et al. 1992; Veale et al. 1973). Unlike motor axons, there is no evidence for differences in properties of sensory axons between the paretic and non-paretic arms (Jankelowitz et al. 2007). Accordingly, changing the pulse width should have a similar effect on the afferent volley for the paretic and non-paretic arms and cannot explain how increasing the pulse width enhanced contractions in the paretic arm only. Instead, if increasing the stimulation pulse width increased the afferent volley in both limbs equally, the afferent volley generated with these stimulus parameters in the non-paretic arm must not have been large enough to increase the reflexive recruitment of motoneurons and generate a measurable increase in contraction amplitude. For the paretic arm, pre- and postsynaptic changes in the spinal cord may have increased reflex excitability resulting in a significantly larger contraction during WP-NMES. Alternatively, as yet unidentified changes in sensory axons between the paretic and non-paretic limb may account for the effect of wider pulse widths in the paretic arm only.

Stimulation frequency also had an effect on the torque evoked in the paretic arm. Both the 20 and 100 Hz constant frequency stimulation patterns were delivered using 1 ms pulses, however, only during 100 Hz stimulation was more torque evoked in the paretic arm than the non-paretic arm. The afferent input during 100 Hz stimulation would be greater than during 20 Hz stimulation due to the increased number of volleys sent to the spinal cord for a given time period;

however, based on the lack of changes in sensory axons after stroke (see above), the afferent volleys would have likely been similar between arms during the 100 Hz stimulation. Therefore, for the paretic arm, changes in presynaptic and postsynaptic mechanisms in the spinal cord may be responsible for the enhanced torque evoked during the 100 Hz stimulation.

Contrary to our second hypothesis, in the paretic arm during the 100 Hz constant frequency stimulation pattern using a 1 ms pulse width, there was a significant decline in torque from the beginning (Time 1) to the end (Time 2) of the stimulation. We believe the current findings do not indicate a lack of a central contribution during 100 Hz constant frequency stimulation after stroke. Rather, we speculate the motoneurons in the paretic arm were activated very quickly during the stimulation trains due to the increased afferent mediated input they were receiving. These factors may have allowed for maximal central activation during the first few seconds of stimulation (i.e. within the Time 1 window) which would not permit further activation by Time 2. We believe that the decline in the torque represents peripheral muscle fatigue overriding the central contribution. For this reason, as is discussed later, brief periods of high frequency stimulation may be most effective for augmenting torque through an enhanced central contribution in the paretic arm.

4.4.2 Presynaptic changes may enhance contractions evoked by WP-NMES after stroke

WP-NMES will activate large diameter afferents from muscle and cutaneous receptors and both of these may contribute to the central recruitment of motoneurons during the stimulation. After a stroke, changes occur in the spinal

cord that are presynaptic to motoneurons and influence the regulation of afferent input to motoneurons. The net effect of these changes is that more afferent input will reach motoneurons of the paretic arm versus the non-paretic arm for a given input to the spinal cord. This increased afferent drive, combined with our WP-NMES protocol designed to maximize afferent activation, may contribute to the larger muscle contractions evoked in the paretic arm in the current study.

During NMES, post-activation depression (Crone and Nielsen 1989; Hultborn et al. 1996) manifests as an immediate decrease in reflex amplitude, after the first reflex (Klakowicz et al. 2006), and is purported to be due to a lower probability of neurotransmitter release at the Ia afferent and motoneuron synapse (Hirst et al. 1981; Kuno 1964; Lev-Tov and Pinco 1992). After a stroke, post-activation depression in reflex pathways controlling the paretic limb is reduced compared to the non-paretic limb (Aymard et al. 2000; Lamy et al. 2009; Masakado et al. 2005) and this would increase the efficacy of synaptic transmission from afferents to motoneurons during repetitive electrical stimulation. There is also a decrease in presynaptic inhibition on Ia afferent terminals in humans after stroke (Artieda et al. 1991; Aymard et al. 2000; Kagamihara and Masakado 2005; Lamy et al. 2009; Nakashima et al. 1989). Similar to reductions in post-activation depression, reduced presynaptic inhibition would also increase neurotransmitter release each time an action potential reaches the afferent terminal (Rudomin and Schmidt 1999) and thus enhance afferent drive to motoneurons during WP-NMES. Increases in post-tetanic potentiation at afferent terminals could also contribute to the enhanced contractions in the paretic

arm. However, little is known about post-tetanic potentiation after stroke, and thus, the role of post-tetanic potentiation in the current results is not clear. Lastly, there is reduced disynaptic Ia reciprocal inhibition in the paretic limb in humans after stroke (Nakashima et al. 1989; Okuma and Lee 1996).

4.4.3 Postsynaptic changes may enhance contractions evoked by WP-NMES after stroke

In addition to changes that occur presynaptic to the motoneuron after a stroke, there may be postsynaptic changes that affect motoneurons themselves. In recent years, even in non-pathological states, motoneuronal excitability has been shown to vary over a wide range, in part by regulating the strength of an intrinsic property known as the persistent inward current (PIC) (Hultborn et al. 2004). There is indirect evidence that PICs also contribute to the discharge of human motoneurons (Collins et al. 2002; Collins 2007; Gorassini et al. 1998) and that the extent to which they do so can be enhanced by increasing monoanimergic drive to the spinal cord through oral administration of amphetamines (Udina et al. 2010) or caffeine (Walton et al. 2003). After a stroke, the disruption of corticospinal input may lead to an increased influence on motoneuron excitability of bulbospinal projections that provide monoaminergic input to the spinal cord (Dewald et al. 1995; Dewald and Beer 2001; Ellis et al. 2007; Kline et al. 2007; Zaaimi et al. 2009). If monoaminergic drive is enhanced after stroke, motoneuronal excitability, and potentially the central contribution to contractions evoked by WP-NMES, may increase due to multiple mechanisms. These mechanisms could include increased subthreshold depolarization, reduction of the action potential threshold, reduction of the spike afterhyperpolarization, or

augmentation of PIC amplitude itself (Fedirchuk and Dai 2004; Heckman et al. 2005, 2008; Heckman 2003; Powers and Binder 2001). It seems unlikely that an increase in the amplitude of the PIC is responsible for the changes in reflex excitability noted here, as recent evidence provided by Mottram and colleagues (2009) showed no difference in an indirect measure of PIC amplitude between the paretic muscle and the non-paretic or control muscles during voluntary ramp contractions of the biceps brachii muscle. Mottram et al. (2009) did however suggest that increases in the number of spontaneously active motor units often seen in the paretic limb after stroke may be due to a low-level tonic depolarizing synaptic drive either of cortical or segmental origin. If monoaminergic in nature, such a tonic drive to motoneurons innervating the paretic muscles would be consistent with the findings of McPherson et al. (2008) who demonstrated that tonic vibration reflexes in the biceps muscle were larger in the paretic arm compared to the non-paretic arm. Thus, although there is presently no definitive evidence for changes in PIC amplitude between limbs after stroke, increased monoamine-mediated motoneuron excitation, potentially facilitating the ease with which PICs are elicited, may still be present. Regardless, both pre- and postsynaptic changes could contribute to the enhanced contractions in the paretic arm and the present study was not designed to distinguish between the two. Finally, recurrent inhibition has most often been shown to be normal or increased in individuals with hemiparetic stroke (Katz and Pierrot-Deseilligny 1982, 1999). If recurrent inhibition was increased on the paretic motoneurons, this would have made the motoneurons less responsive to the repetitive input generated during the

stimulation (reflexive or antidromic). Despite this, the responses were still enhanced in the paretic arm during WP-NMES.

4.4.4 Clinical Significance

This work represents the first time WP-NMES has been used to generate contractions for individuals who have experienced a stroke and is a first step towards the potential application of WP-NMES for rehabilitation in this population. By using WP-NMES it may be possible to generate larger muscle contractions in the paretic arm for a given stimulus current. The larger muscle contractions may help overcome the abnormal joint torque couplings after stroke that limit functional movements (Dewald et al. 1995; Dewald and Beer 2001). Additionally, producing these contractions with lower stimulation currents, due to the central activation of motoneurons during WP-MNES, may also reduce discomfort for the individual. Electrically-evoked muscle contractions that involve a large central contribution from the recruitment of motoneurons in the spinal cord may also be more fatigue-resistant due to the physiological motor unit recruitment order followed with synaptic activation, in which small fatigue-resistant motor units are activated first. This type of recruitment is preferable to that which occurs during contractions that primarily involve the direct depolarization of motor axons underneath the stimulating electrodes and employs a random motor unit recruitment order. In the present study, since torque declined during the 12 s train of 100 Hz constant frequency stimulation, but was augmented after a shorter (4 s) burst of 100 Hz stimulation, including brief periods of high frequency stimulation may be the most effective way to augment muscle contractions in the paretic limb while limiting peripheral fatigue.

A potential drawback of using WP-NMES after stroke may be that some muscles have stronger reflexive input and thus may generate contractions with a larger central contribution than others. For example, there are more Ia afferent projections to slow motor units than fast motor units (Eccles et al. 1957; Pierrot-Deseilligny and Burke 2005) and the size of the H-reflex varies for different muscles (Jusić et al., 1995; Palmieri 2002). Lastly, further studies are required to determine whether discomfort is reduced during WP-NMES based on the idea that lower stimulus currents may be employed during WP-NMES due to the use of a 1 ms pulse width and the addition of the central contribution to the contraction amplitude.

4.4.5 Future Directions

These experiments have shown that WP-NMES enhances contractions in the paretic arm after stroke, ostensibly due to an enhanced reflexive recruitment of motoneurons. A valuable extension of the current study would be a more thorough investigation of the stimulation parameters to determine the optimal combination of stimulation pulse width, frequency and intensity for maximizing the central contribution to electrically-evoked contractions after stroke. Gaining a better understanding of the afferent origin of the central contribution (i.e. muscle vs. cutaneous afferents) would also be of interest and may lead to improved methods for enhancing the evoked contractions. Studies in which reflex responses and motor units are recorded from the paretic limb during WP-NMES may help verify the central contribution to the evoked contractions and may provide insights into the pre- and postsynaptic changes that occur in the spinal

cord after stroke. Finally, experiments designed to test muscle fatigue and the recruitment characteristics of single motor units during WP-NMES in the paretic limb are needed to determine the extent to which WP-NMES reduces muscle fatigue after stroke.

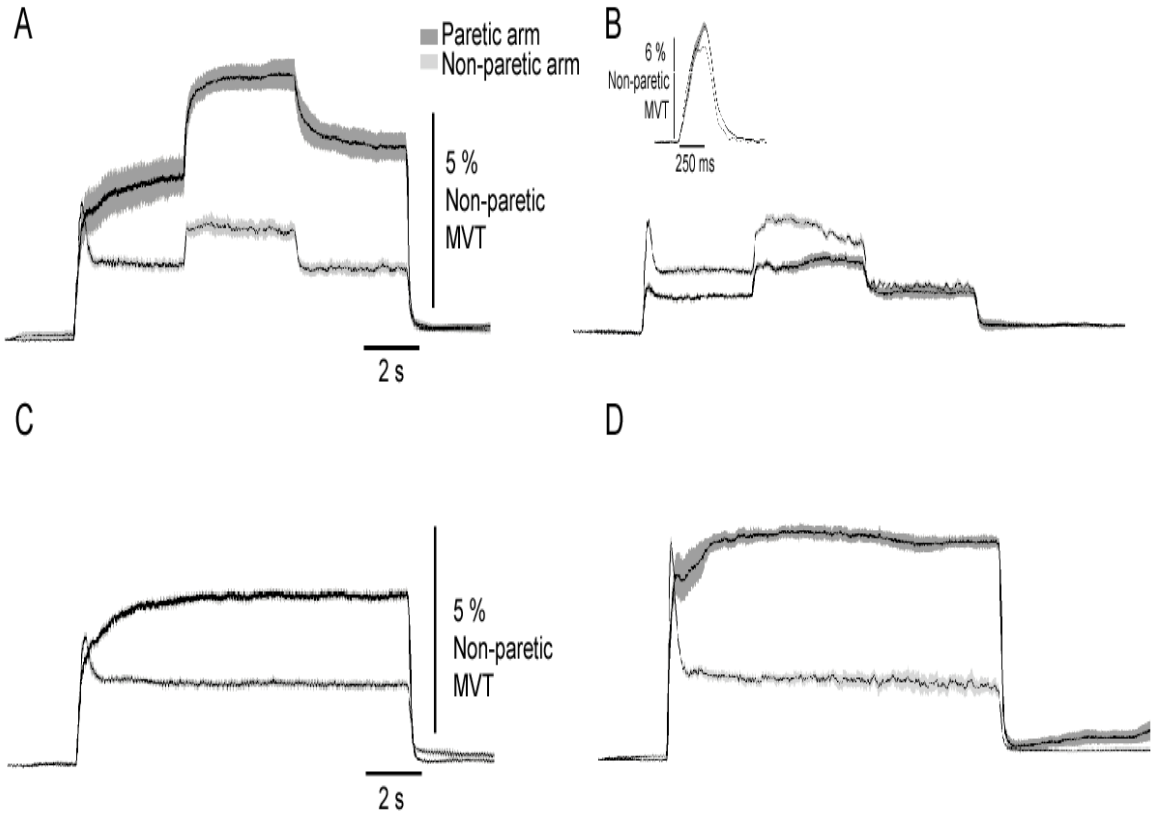

Figure 4.1 Torque generated by each stimulation pattern in a single participant. Torque in the paretic arm is shown by the dark trace and in the non-paretic arm by the light trace. Each panel shows the torque generated by a specific stimulation pattern: A) 20-100-20 Hz (1 ms pulse width); B) 20-100-20 Hz (0.1 ms pulse width); C) 20 Hz constant frequency (1 ms pulse width); and D) 100 Hz constant frequency (1 ms pulse width). The inset in panel B shows the torque evoked in each arm by the short stimulus train (25 pulses at 100 Hz; 1 ms pulse width) used to set stimulus intensity. Each trace is an average of 5 repetitions of each stimulation pattern. The shaded bands represent ± 2 SE. MVT = maximum voluntary torque.

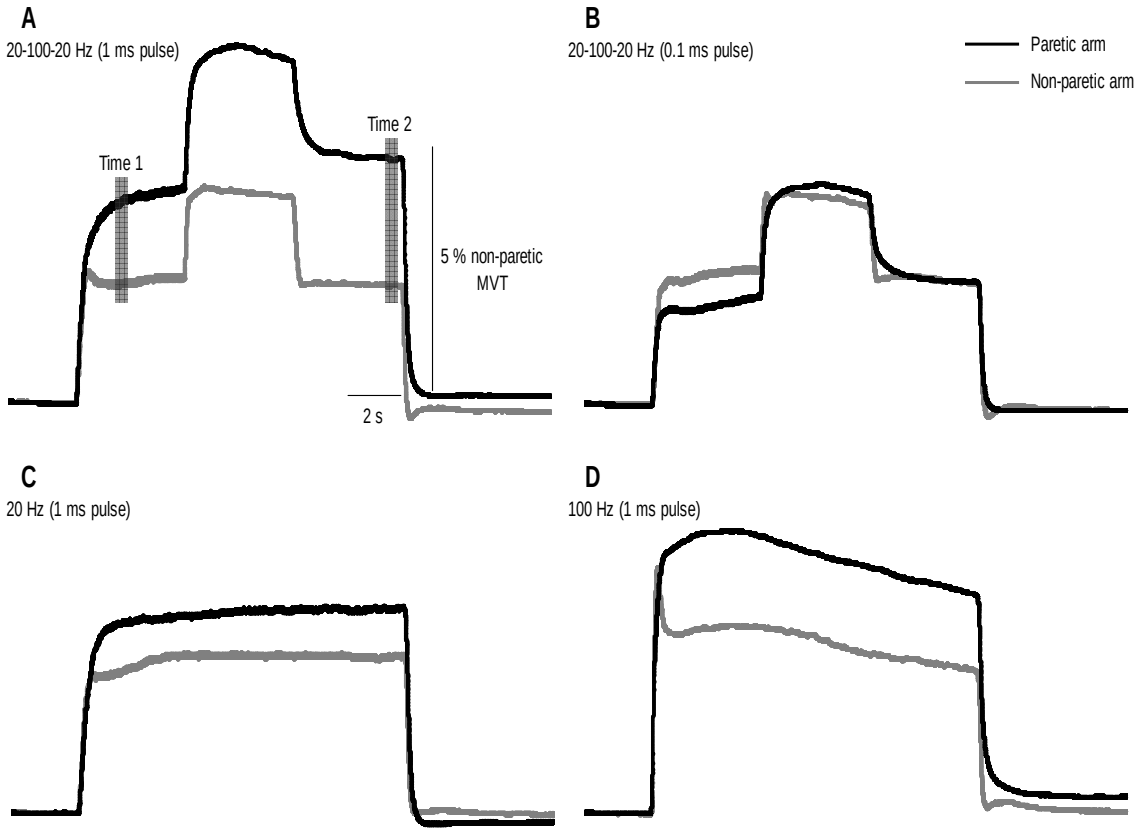

Figure 4.2 Torque generated throughout each stimulation pattern averaged across the group. Torque in the paretic arm is shown by the dark trace and in the non-paretic arm by the light trace. Each panel shows the torque generated by a specific stimulation pattern: A) 20-100-20 Hz (1 ms pulse width); B) 20-100-20 Hz (0.1 ms pulse width); C) 20 Hz constant frequency (1 ms pulse width); and D) 100 Hz constant frequency (1 ms pulse width). Error bars have been omitted for the sake of clarity. The shaded regions in Panel A represent the time periods (Time 1 and Time 2) over which data were quantified for statistical analyses (see Figures 4.3 and 4.4). MVT = maximum volutary torque.

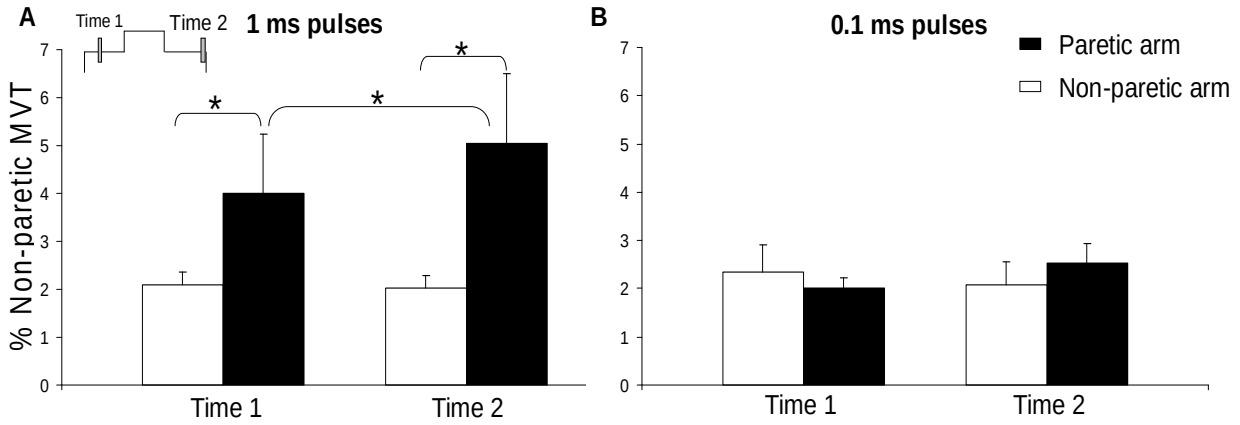

Figure 4.3 Group data showing the effect of pulse width (1 vs. 0.1 ms) on torque generation for the 20-100-20 Hz stimulation pattern. Torque evoked in the paretic arm is represented by the black columns; in the non-paretic arm by the white columns. Panel A shows torque evoked using 1 ms pulses at Time 1 (1.5 s into the stimulation train) and Time 2 (11.5 s into the stimulation train). Panel B shows the torque evoked at Time 1 and Time 2 by stimulation using 0.1 ms pulses. 1 SE is shown. Data columns connected by brackets are significantly different from each other ($p < 0.05$). MVT = maximum voluntary torque.

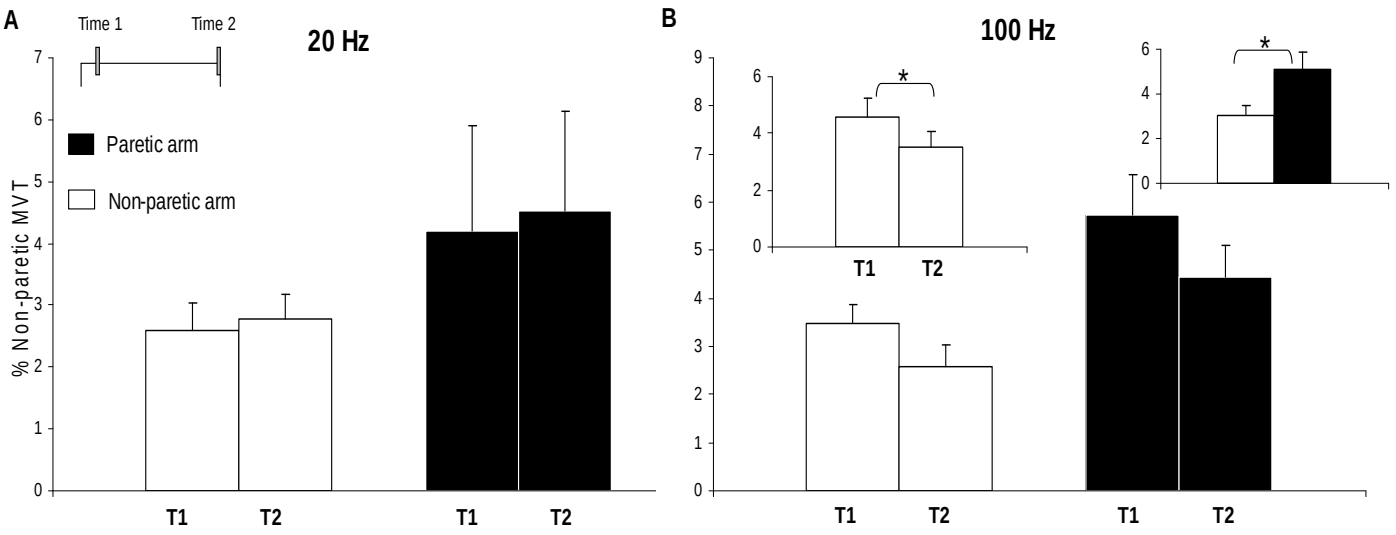

Figure 4.4 Group data showing the effect of stimulation frequency (20 vs 100 Hz) on torque generation for the group. Torque evoked in the paretic arm is represented by the black columns; in the non-paretic arm by the white columns. Panel A shows the torque evoked by 20 Hz stimulation (1 ms pulse width) for each arm at the beginning (Time 1; T1) and end (Time 2; T2) of the stimulation train. Panel B shows the torque evoked by 100 Hz stimulation (1 ms pulse width) for each arm at Time 1 and Time 2. The main effects of time and arm are shown in the separate insets. 1 SE is shown. For the 100 Hz stimulation pattern, there were significant main effects of arm and time which are not shown here for clarity (see text). MVT = maximum voluntary torque.

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Chapter 5: Reflexive contributions to contractions evoked by wide-pulse neuromuscular electrical stimulation in individuals with chronic spinal cord injury[1]

5.1 Introduction

Neuromuscular electrical stimulation (NMES) is often applied over paralyzed muscles of individuals with chronic spinal cord injury (SCI) to assist with activities of daily living and exercise (Sheffler and Chae 2007). NMES activates muscle via the direct depolarization of motor axons beneath the stimulating electrodes. In recent years it has become evident that there can also be a "central contribution" to electrically-evoked contractions (Collins 2007; Maffiuletti 2010; Vanderthommen and Duchateau 2007). NMES delivered using wide pulse widths (1 ms) and high frequencies (up to 100 Hz) (wide pulse NMES; WP-NMES) has been shown to enhance this central contribution to the evoked contractions (Collins et al. 2001, 2002). This central effect arises from the electrical activation of sensory axons, which in turn, reflexively recruit motoneurons in the spinal cord. During WP-NMES, a central contribution has been shown in individuals with no neurological impairments (Collins et al. 2001, 2002) and in those who have experienced a spinal cord injury (Nickolls et al. 2004) or stroke (Clair et al. submitted, Chapter 4). Contractions that have a large central contribution may be more fatigue-resistant than those that are generated primarily by the direct activation of motor axons (Lagerquist et al. 2009), because the reflexive recruitment of motoneurons follows the size principle in which

[1] The contributing authors to the work presented in this chapter were: Clair JM, Lagerquist O, and Collins DF.

fatigue-resistant motor units are recruited first (Henneman et al. 1965). Additionally, orderly motor unit recruitment during NMES may help reduce disuse atrophy by increasing the activity in these small motor units.

In individuals with no neurological impairments, much of the central contribution to electrically-evoked contractions comes from transmission along the H-reflex pathway (Klakowicz et al. 2006), at least when the stimulation is delivered over a nerve trunk (Baldwin et al. 2006). After an initial depression of the H-reflex, consistent with the well-known phenomenon of post-activation depression (Crone and Nielsen 1989), reflex amplitude recovered to approximately 50% of its initial value by the end of 7 s of stimulation at 20 Hz (Klakowicz et al. 2006). Recently we have characterized, in more detail, the depression and recovery of reflex amplitude during 10 s trains of stimulation and have shown that after the initial depression, reflexes recover and contribute to the evoked contraction during stimulation at 10 and 20 Hz (Clair et al. Chapter 3). This recovery of reflex amplitude was suggested to occur due to a combination of post-tetanic potentiation of neurotransmitter release at Ia afferent-motoneuron synapses and the activation of persistent inward currents (PICs) on the motoneurons themselves. The extent to which H-reflexes contribute to contractions evoked by WP-NMES after SCI has not been investigated.

H-reflexes may be larger during WP-NMES in individuals with SCI, compared to individuals with no neurological impairments, due to the changes that occur in the central nervous system (CNS) after SCI. For example, there is less post-activation depression of reflex amplitude in individuals who have had a

SCI (Field-Fote et al. 2006; Ishikawa et al. 1966; Schindler-Ivens and Shields 2000). This, combined with reduced presynaptic inhibition (Aymard et al. 2000; Faist et al. 1994; Kagamihara and Masakado 2005), suggests that there may be a greater release of neurotransmitter from afferent terminals for a given afferent input. After a SCI there may also be changes in motoneuron properties which increase motoneuron excitability. Acutely after SCI, motoneuron excitability is largely depressed due to the loss of brainstem mediated monoaminergic input (Harvey et al. 2006; Hounsgaard et al. 1988). Recent work has shown that over time large amplitude PICs develop that re-establish motoneuron excitability (Murray et al. 2010). Using an indirect measure of PIC amplitude in humans with SCI, it was estimated that during a spasm, approximately 40% of the excitation to motoneurons comes from PICs (Gorassini et al. 2004). Additionally, self-sustained firing of motor units, a hallmark of PIC activation, has been shown following electrical stimulation (Nickolls et al. 2004) or voluntary contractions of paretic muscle (Zijdewind and Thomas 2003). The combination of reduced presynaptic inhibitory mechanisms and enhanced motoneuron excitability, may lead to a greater central contribution to muscle contractions evoked by WP-NMES after SCI.

The purpose of the present study was to determine the extent to which muscle contractions are driven through reflex pathways during WP-NMES after SCI. Stimulation was delivered over the tibial nerve in the popliteal fossa using 1 ms pulse widths, to maximize the activation of sensory axons (Lagerquist and Collins 2008; Panizza et al. 1992; Veale et al. 1973), and two stimulation patterns:

20 Hz for 12 s ("constant frequency pattern") and 20-100-20 Hz for 4 s in each phase ("burst-like pattern"). The constant frequency pattern was used to assess reflex amplitudes during trains of electrical stimulation that were similar to traditional stimulation protocols. The burst-like pattern was used because previous work has shown a sustained increase in torque (Collins et al. 2001, 2002; Nickolls et al. 2004) and reflex amplitude (Klakowicz et al. 2006) after the 100 Hz stimulation due to the central recruitment of motoneurons. We hypothesized that transmission along the H-reflex pathway would be initially depressed, but would recover during both stimulation patterns. We also hypothesised that H-reflexes would be larger by the end of the burst-like pattern compared to the constant frequency pattern, due to an enhanced central contribution from the 100 Hz "burst". The results of these experiments show that H-reflexes can contribute when NMES is used to generate contractions for individuals who have had a SCI. Enhancing the synaptic recruitment of low threshold motor units using WP-NMES may help generate contractions that are more fatigue-resistant and reduce disuse atrophy.

5.2 Methods

5.2.1 Participants

NMES was applied to the right tibial nerve in the popliteal fossa to activate the triceps surae muscles of 12 individuals who had experienced a spinal cord injury (SCI). Three individuals were withdrawn from the study because H-reflexes were not evoked consistently, or at all, when single pulses of electrical stimulation were delivered over the range of stimulus intensities used to generate

M-wave/H-reflex recruitment curves (see below). Therefore, data from the remaining 9 participants (7 males and 2 females; age range: 18-52 yrs) were used for the data analyses (see Table 5.1: SCI Participant Characteristics). All injuries occurred as result of trauma at least 9 months prior to participating in this study and were located in the cervical region of the spinal cord. Four participants had complete injuries and five had incomplete injuries. Seven of the participants were taking Baclofen to treat spasticity. Participants were recruited from The Steadward Centre at the University of Alberta, a facility that provides physical activity and sport programs for individuals with disabilities, where they were taking part in functional electrical stimulation assisted exercise programs. Each participant provided informed, written consent. The experiments were conducted in accordance with the standards set by the Declaration of Helsinki for research involving human participants and were approved by the Health Research Ethics Board at the University of Alberta.

5.2.2 Experimental Protocol

Experimental procedures were performed on the right leg in 8 participants. In 1 participant procedures were performed on the left leg due to limited range of motion of their right knee and ankle. Surface electromyography (EMG) was recorded from the soleus muscle using disposable bipolar surface EMG electrodes (2.54 cm^2, A10043-P, Vermed Medical, Bellows Falls, VT). The EMG signals were band-pass filtered from 30-3000 Hz and amplified 1000-2000 times (Neurolog System; Digitimer, Welwyn Garden City, UK). A reference electrode was placed on the tibial plateau (10.16 cm x 2 cm, Electrosurgical Patient Plate: Split, 3M Health Care, St. Paul, MN).

Electrical stimulation was delivered to the tibial nerve in the popliteal fossa through disposable bipolar surface EMG electrodes (2.54 cm^2, A10043-P, Vermed Medical, Bellows Falls, VT) using a constant-current stimulator (DS7A; Digitimer, Welwyn Garden City, UK). Stimulation delivery and data collection were controlled by custom written software (LabView, National Instruments, Austin, TX). All data were sampled at 5000 Hz and stored on a computer for later analysis. For all trials, participants were seated on the chair of a Biodex dynamometer (System 3, Biodex Medical Systems Inc. Shirley, NY, USA) with their knee and ankle at 110 and 90º, respectively. Seatbelts were placed over their shoulders and across their waist to help maintain a consistent upright posture for the duration of the experiment. Participants were instructed to remain relaxed throughout the stimulation trials. Each experiment began by collecting data for soleus M-wave/H-reflex recruitment curves by delivering 50 single pulses (1 ms pulse width), each separated by 5-7 s, at intensities ranging from below M-wave and H-reflex threshold to 1.5 times the minimum current required to elicit the maximum M-wave (M_{max}). In all other trials, 12 s stimulus trains were delivered using a 1 ms pulse width and two stimulation patterns: 1) 20 Hz for 12 s ("constant frequency pattern") and 2) 20-100-20 Hz (4 s each phase; "burst-like pattern"). For 4 participants 15 Hz was used instead of 20 Hz because the latencies of their H-reflexes were such that during 20 Hz stimulation the H-reflexes overlapped with the stimulation artefacts and accurate quantification of reflex amplitude was not possible. Each trial consisted of three identical stimulation trains separated by 30 s. Stimulation intensity was set at the

beginning of each trial to evoke an M-wave of 10-15% of M_{max} in response to 3 single pulses delivered approximately 5 s apart.

5.2.3 Data Analysis

Custom written Matlab software (The Mathworks, Natick, MA) was used to perform data analysis post-hoc. Response amplitudes during the stimulation were quantified and compared using procedures we have described previously (Clair et al. Chapter 3). The peak-to-peak amplitudes of each M-wave and H-reflex were measured and then normalized to the largest M-wave amplitude (M_{max}) obtained from each participant's recruitment curve. Responses were not analyzed during the 100 Hz stimulation of the burst-like pattern due to contamination of the EMG by the stimulus artefacts. For each participant and stimulation pattern the amplitude of the first (M_1 or H_1) and second (M_2 or H_2) responses were averaged over the three stimulation trains in each trial. Additionally, after the first response in each stimulation train, all responses were averaged over 0.5 s intervals to generate 24 data bins as shown in Figure 5.1. To characterize the changes in reflex amplitude, one measure was used to quantify the initial reflex depression (post-activation depression) and 3 measures were used to quantify the reflex recovery. To quantify post-activation depression, the amplitude of the second response was compared to that of the first response. The time course of recovery was assessed using 2 measures. To determine whether responses recovered immediately after the initial depression ("fast" recovery), the amplitude of the second response was compared to the mean of the responses over the first 0.5 s of stimulation (Bin 1). Any "additional" recovery after Bin 1, was assessed by comparing the mean of the responses during the last 0.5 s of

stimulation to the mean of Bin 1. The recovery of reflex amplitude was "complete" if the mean of Bin 24 was not different from the mean amplitude of the first response. For the burst-like pattern, data collected during the 100 Hz burst (Bins 9-16) were not included in the analyses due to the contamination of the EMG by the stimulation artefacts, as described above.

5.2.4 Statistical Analysis

Separate repeated measures analysis of variance tests (rmANOVAs) were performed for M-wave and H-reflex data. A 2-way rmANOVA was used to test for significant effects of stimulation pattern ("constant frequency" and "burst-like") and time (first response, second response, Bin 1, Bin 24) on response amplitude. Tukey's HSD tests were performed when appropriate on significant interactions or main effects identified by the rmANOVA analyses. Post-hoc analyses of main effects were not conducted when there was a significant interaction. The alpha level for all statistical analyses was set at 0.05. Descriptive statistics are reported as the mean ± 1 SE.

5.3 Results

Data from a single participant are shown in Figure 5.2 for one 12 s train of stimulation during the constant frequency (panel A) and burst-like (panel B) stimulation patterns. The amplitudes of M-waves and H-reflexes are shown by the open circles and filled diamonds, respectively. During both stimulation patterns, there was depression of the H-reflex amplitude immediately after the first response. Recovery of the H-reflex amplitude, however, was dependent on stimulation pattern. During the constant frequency pattern there was partial

recovery of reflex amplitude by the end of the train, although, the H-reflex amplitude at the end of the train was still approximately 50% smaller than H_1 (Fig. 5.2a). During the burst-like pattern, recovery of reflex amplitude was also evident, however this recovery was "complete", because the reflex amplitudes after the brief period of 100 Hz stimulation were similar, at times even larger, than H_1 (Fig. 5.2b). In contrast to these robust changes in H-reflex amplitude, M-waves remained relatively stable throughout the stimulation.

For the group, mean M-wave and H-reflex amplitudes quantified over the 12 s trains are shown in Figure 5.3. Both M-waves and H-reflexes were depressed after the first response. H-reflexes recovered from this initial depression only after the 100 Hz stimulation. For M-wave amplitude, there was a significant main effect of time [$F_{(3,24)} = 6.26$, $p < 0.01$], no main effect of stimulation pattern, and no significant interaction. Post-hoc analysis of the main effect of time, with the data collapsed across both stimulation patterns (data not shown), revealed that the first M-wave (M_1: 11.9 ± 1.6% M_{max}) was significantly larger than the second M-wave (M_2: 6.9 ± 1.5% M_{max}) [$p < 0.01$], M-waves averaged over the first 0.5 s of stimulation (Bin 1: 7.3 ± 1.9% M_{max}) [$p < 0.05$], and at the end of the 12 s stimulation train (Bin 24: 6.6 ± 1.8% M_{max}) [$p < 0.01$]. M-waves were not significantly different between M_2, Bin 1, and Bin 24.

For the H-reflex data, there was a significant interaction between stimulation pattern and time [$F_{(3,24)} = 4.18$, $p < 0.05$]. The results of the post-hoc analyses performed on this interaction are shown in Figure 5.4. Significant reflex depression occurred during both stimulation patterns. The second H-reflex was

depressed by 57% and 51%, compared to the first reflex, during the constant frequency pattern [$p < 0.001$] and the burst-like pattern [$p < 0.001$], respectively. Reflexes did not recover during the first 0.5 s of the stimulation for both stimulation patterns; H_2 was not different from Bin 1. Reflex amplitudes remained depressed for the remainder of the constant frequency pattern (H_2, Bin 1 and Bin 24 were not significantly different). During the burst-like pattern, recovery of reflex amplitude was observed after the first 0.5 s of stimulation; Bin 24 was larger than Bin 1 [$p < 0.001$], indicating that the 4 s "burst" of 100 Hz stimulation had an effect on reflex recovery. Complete recovery was also achieved during the burst-like pattern because H_1 and Bin 24 were not significantly different. Lastly, H-reflexes were larger by the end of the burst-like pattern (55% M_{max}) compared to the end of the constant frequency pattern (37% M_{max}) [$p < 0.001$].

5.4 Discussion

In the present study we examined the reflexive contribution to muscle contractions evoked during WP-NMES in a group of individuals with chronic SCI. In support of our first hypothesis, transmission along the H-reflex pathway contributed to contractions evoked by both the constant frequency and burst-like patterns. In support of our second hypothesis, the central contribution was greater after the 100 Hz stimulation during the burst-like pattern compared to the same time period during the constant frequency stimulation pattern. The rationale for our prediction that H-reflexes would contribute to contractions evoked by WP-NMES after SCI was based on two lines of evidence. Firstly, a central

contribution during WP-NMES has been shown in individuals with no neurological impairments (Clair et al. Chapter 3; Klakowicz et al. 2006) and in individuals with SCI (Nickolls et al. 2004) or stroke (Clair et al. submitted, Chapter 4). Secondly, transmission along the H-reflex pathway (Calancie et al. 1993; Ishikawa et al. 1966; Schindler-Ivens and Shields 2000) and motoneuron excitability (Gorassini et al. 2004; Li and Bennett 2003; Murray et al. 2010) change after chronic SCI, such that the central contribution to contractions evoked by WP-NMES may be larger for individuals with SCI compared to those with no neurological impairments, although a direct comparison between groups was not part of the current study. Below we discuss the pre- and postsynaptic changes that influence reflex transmission after SCI and that could affect the central contribution to contractions evoked by WP-NMES.

5.4.1 Pre- and postsynaptic changes may enhance contractions evoked by WP-NMES after SCI

Post-activation depression (Crone and Nielsen 1989) was shown to be reduced after chronic SCI; during 2 s of 10 Hz stimulation, H-reflex amplitudes were depressed by 49%, after the first response, as compared to 93% in individuals with no neurological impairments (Schindler-Ivens and Shields 2000). In the current study we observed similar depression of reflex amplitudes after SCI (54%) when stimulation was delivered at 15-20 Hz. Additionally, in a previous study using a stimulation protocol similar to that used in the current study, we found a 90% reduction of reflex amplitudes in individuals with no neurological impairments (Clair et al. Chapter 3). Given that post-activation depression is reduced after SCI, sensory input generated during WP-NMES would have a larger

affect on motor pool excitability after SCI because there would be less inhibition of afferent transmission. Thus, a reduction in post-activation depression at the Ia afferent terminals could be contributing to the reflexive contribution observed in the current study. There is also a reduction in presynaptic inhibition after SCI (Faist et al. 1994; Kagamihara and Masakado 2005). Individuals with paraplegia showed greater facilitation of the soleus H-reflex amplitude in response to a femoral nerve conditioning pulse, compared to individuals with no neurological impairments (Faist et al. 1994). Similar to reductions in post-activation depression, less presynaptic inhibition at the Ia afferent terminals would result in increased neurotransmitter release for a given input to the afferent terminal (Rudomin and Schmidt 1999).

Post-tetanic potentiation at the Ia afferent-motoneuron synapse during repetitive stimulation (Lloyd 1949), resulting in an increased probability of neurotransmitter release (Hirst et al. 1981; Kuno 1964), may also partly account for the large H-reflex amplitudes during WP-NMES observed in the current study (Klakowicz et al. 2006). Currently, little is known about post-tetanic potentiation at the Ia afferent-motoneuron synapse after SCI. In the present study, we observed no recovery of reflex amplitude during constant frequency stimulation at 20 Hz. This result is not consistent with previous findings in individuals with no neurological impairments, in which recovery within the first 0.5 s of stimulation was reported during the same stimulation pattern (Clair et al. Chapter 3). This difference in the recovery of reflex amplitudes between these studies may be an indication of differences in post-tetanic potentiation after SCI.

In addition to changes in the regulation of afferent input, motoneuron properties change after SCI. For example, the activation of PICs is markedly diminished acutely after SCI (Harvey et al. 2006; Hounsgaard et al. 1988), but restored in the chronic state (Li and Bennett 2003). PICs after chronic SCI are often large in amplitude and difficult to turn off due to the lack of descending inhibitory inputs. Similarly after human SCI, enhanced PICs are thought to be involved in restoring motoneuronal excitability and to contribute to the generation of muscle spasms (Gorassini et al. 2004; Hornby et al. 2003; Nickolls et al. 2004; Zijdewind and Thomas 2003). Taken together, decreased inhibition of afferent transmission, along with enhanced motoneuron excitability, suggest the sensory input generated during WP-NMES after SCI may result in a larger than normally expected motor output. Future experiments are required to compare the central contribution to contractions evoked by WP-NMES in individuals with SCI and those with no neurological impairments.

Other factors that could have influenced the current results are a decrease in the duration of the afterhyperpolarization and changes in axonal excitability after SCI. Cats with chronic spinal transections showed a significant reduction in the duration of the afterhyperpolarization period (Cope et al. 1986; Czeh et al. 1978; Hochman and McCrea 1994). After human SCI if motoneurons exhibit similar changes in the duration of the afterhyperpolarization, during WP-NMES, motoneurons may be able to respond more robustly to repetitive input. Additionally, Lin et al. (2007) reported that motor axon excitability was decreased below the level of the lesion in individuals with chronic SCI. This may have

played a role in the depression of M-waves observed in the current study. If sensory axons exhibit comparable decreases in axonal excitability after SCI, this could also account for part of the depression of H-reflex amplitudes.

5.4.2 Implications for using WP-NMES for rehabilitation after SCI

To maximize transmission along the H-reflex pathway during WP-NMES, low stimulation intensities would reduce antidromic collision along the motor axons. Currently, we have not determined whether contractions generated during WP-NMES at lower stimulus intensities would be large enough to improve muscle quality after SCI or if they would be sufficient for functional movements; however, in individuals with no neurological impairments contractions of 40% MVC have been generated (Collins et al. 2002). The application of WP-NMES for generating functional movements may also be limited because precise methods to control the amplitude of the central contribution and to turn off contractions evoked by WP-NMES have not been determined.

There is also the possibility that muscle spasms could be enhanced due to the reflexive recruitment of motoneurons during WP-NMES after SCI. We do not think this is likely based on the current study and that by Nickolls et al. (2004) in which none of the participants reported a change in spasms during or after their involvement in the study. Alternatively, in the current study the stimulation may not have triggered spasms because a majority of the participants were taking Baclofen, a medication prescribed to reduce spasticity (Dario and Tomei 2004). Although we did not investigate the effect of Baclofen on the reflexive contribution to electrically-evoked contractions, long-term use of this medication has been shown to reduce motor unit tetanic forces (Thomas et al. 2010).

Additionally, because Baclofen acts primarily on the presynaptic afferent terminals it would likely reduce the central contribution during WP-NMES, in which case, the H-reflex contributions to electrically-evoked contractions may have been underestimated in the current study.

In the current study, a central contribution during 12 s trains of WP-NMES was demonstrated in individuals with chronic SCI. Transmission along the H-reflex pathway was affected by stimulation pattern since reflex amplitudes were 37% M_{max} by the end of the constant frequency pattern and 55% M_{max} by the end of the burst-like pattern. Slow recovery of reflex amplitude was only observed during the burst-like pattern and no recovery of reflex amplitude occurred during the constant frequency pattern. Hence, brief periods of 100 Hz stimulation were beneficial for enhancing the central contribution to contractions. In the future, delivering these brief bursts very early in a stimulation train may expedite the recovery of reflex amplitude and increase the reflexive recruitment of motoneurons throughout the stimulation, while also minimizing muscular fatigue. A potential benefit of WP-NMES is that the reflexive recruitment of motor units during WP-NMES occurs according to the size principle in which the small fatigue resistant motor units are recruited first. Maintaining the activity of small motor units may increase the fatigue-resistance of electrically-evoked contractions and may help reduce disuse atrophy after SCI. A central contribution to contractions during WP-NMES has been demonstrated in several different muscles in individuals with no neurological impairments (Baldwin et al. 2006; Blouin et al. 2009; Collins et al. 2002; Klakowicz et al. 2006; Maffiuletti 2010).

These results suggest that WP-NMES may be used to activate many different muscles after SCI, although this has not been tested. Further work is also required to determine the optimal combination of stimulus parameters (i.e. frequency, pattern, intensity, and pulse width) for evoking the greatest central contribution. Lastly, investigations of the fatigue properties and motor unit recruitment characteristics during WP-NMES are needed to verify the proposed benefits of WP-NMES over more traditional stimulation protocols.

Table 5.1 SCI Participant Characteristics

Code/ Sex	Age (yrs)	Time post-injury (yrs)	Injury Level	ASIA score*
1M	19	0.75	C_7	A
2M	18	0.75	C_{4-5}	B
1F	51	1.8	C_{5-6}	A
2F	28	5.75	C_{6-7}	B
3M	27	5.9	C_{5-6}	B
4M	34	9.7	C_{5-6}	B
5M	24	6.0	C_{7-8}	A
6M	52	5.1	C_{4-5}	A
7M	23	3.7	C_{4-5}	C

* American Spinal Injury Association Score

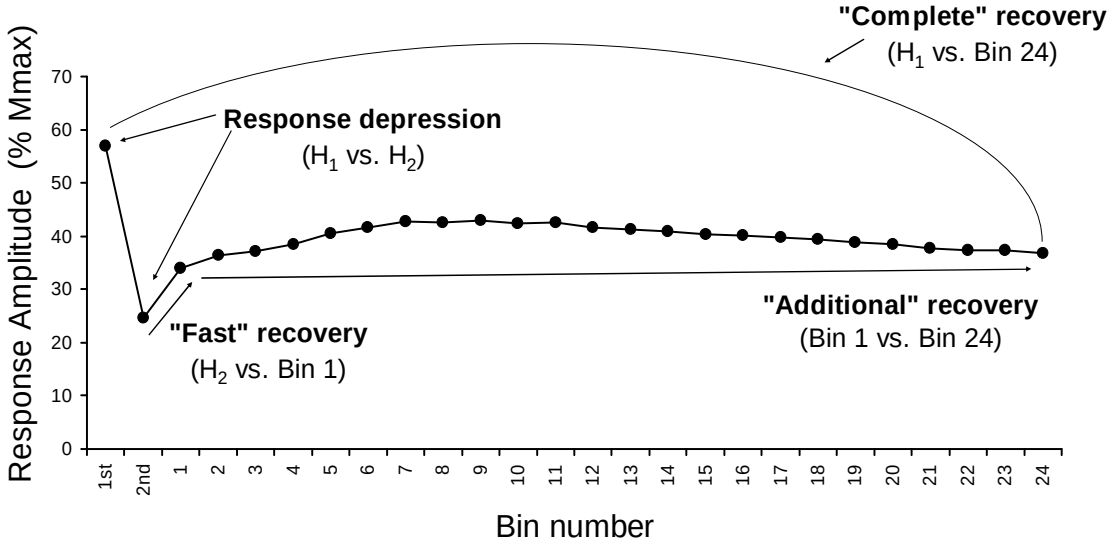

Figure 5.1 A diagram illustrating the method used to quantify reflex depression and recovery during 12 s trains of stimulation. The initial depression was assessed by comparing the first (H_1) and second (H_2) reflexes. "Fast" recovery was assessed by comparing the second reflex and Bin 1 (responses averaged over the first 0.5 s). "Additional" recovery, after the first 0.5 s of stimulation, was assessed by comparing Bin 24 (responses averaged over the last 0.5 s) to Bin 1. "Complete" recovery was assessed by comparing Bin 24 to the first reflex.

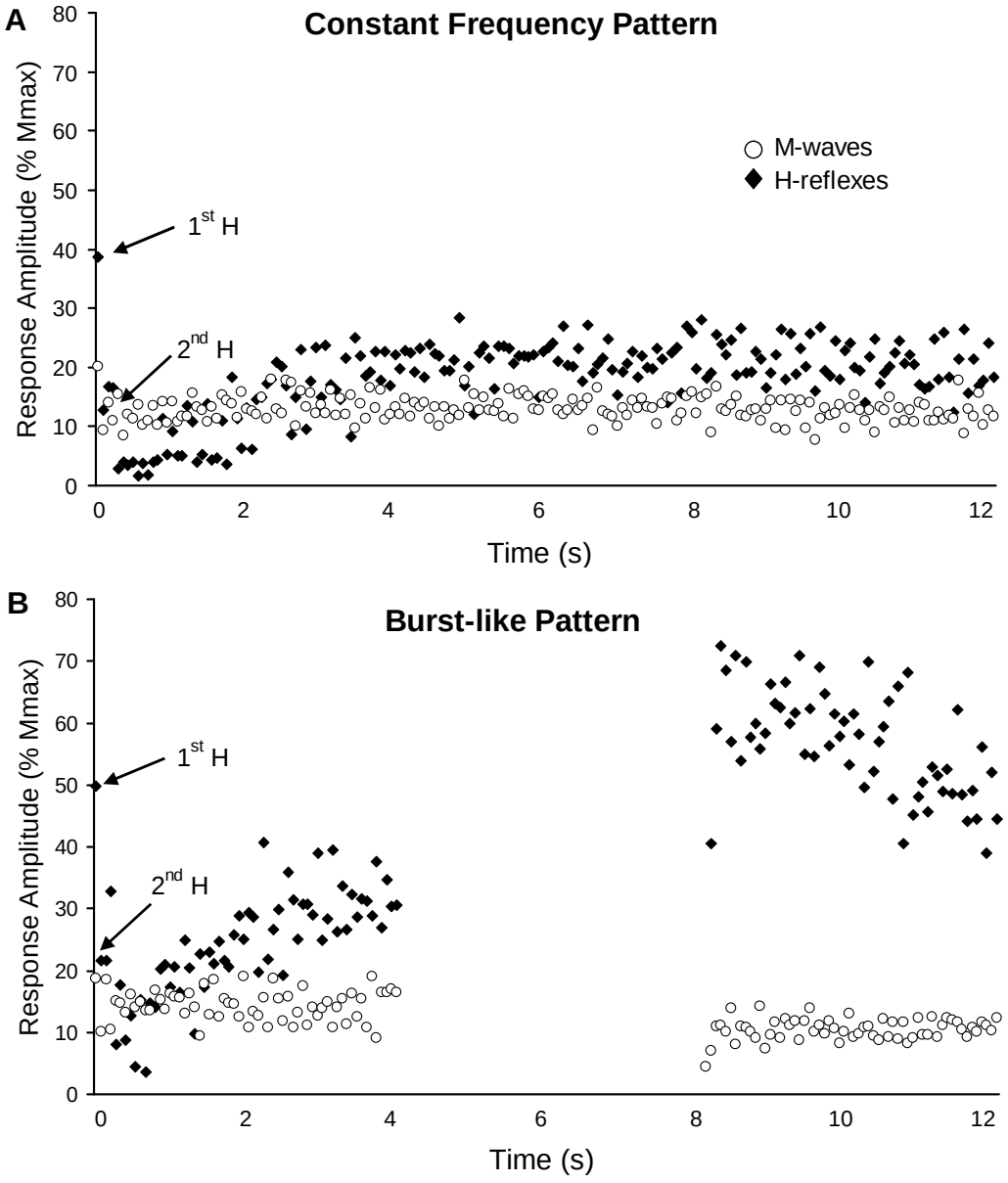

Figure 5.2 Soleus M-wave and H-reflex data from a single participant with incomplete SCI (C_5-C_6) during stimulation of the tibial nerve in the popliteal fossa using two different stimulation patterns. A) 20 Hz for 12 s using a 1 ms pulse width ("constant frequency pattern"). B) 15-100-15 Hz for 4 s each phase using a 1 ms pulse width ("burst-like pattern"). Stimulus intensity was set to evoke ~15% M_{max} with a single pulse. The M-wave and H-reflex evoked by each stimulus pulse are shown.

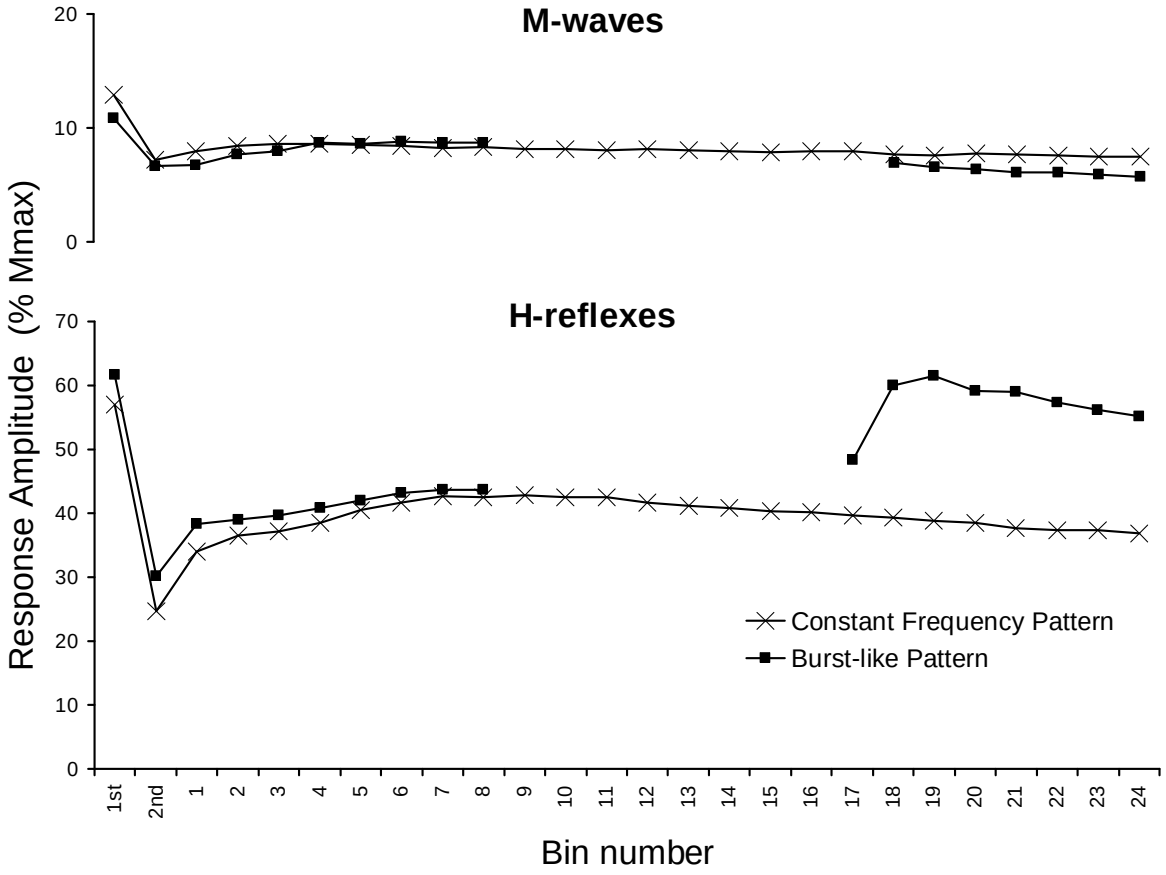

Figure 5.3 Group average M-wave (upper panel) and H-reflex amplitudes (lower panel) throughout the entire stimulation period for the constant frequency and burst-like patterns. The mean of the first and second responses are shown, followed by the mean of responses averaged over 0.5 s bins. Error bars have been omitted for clarity.

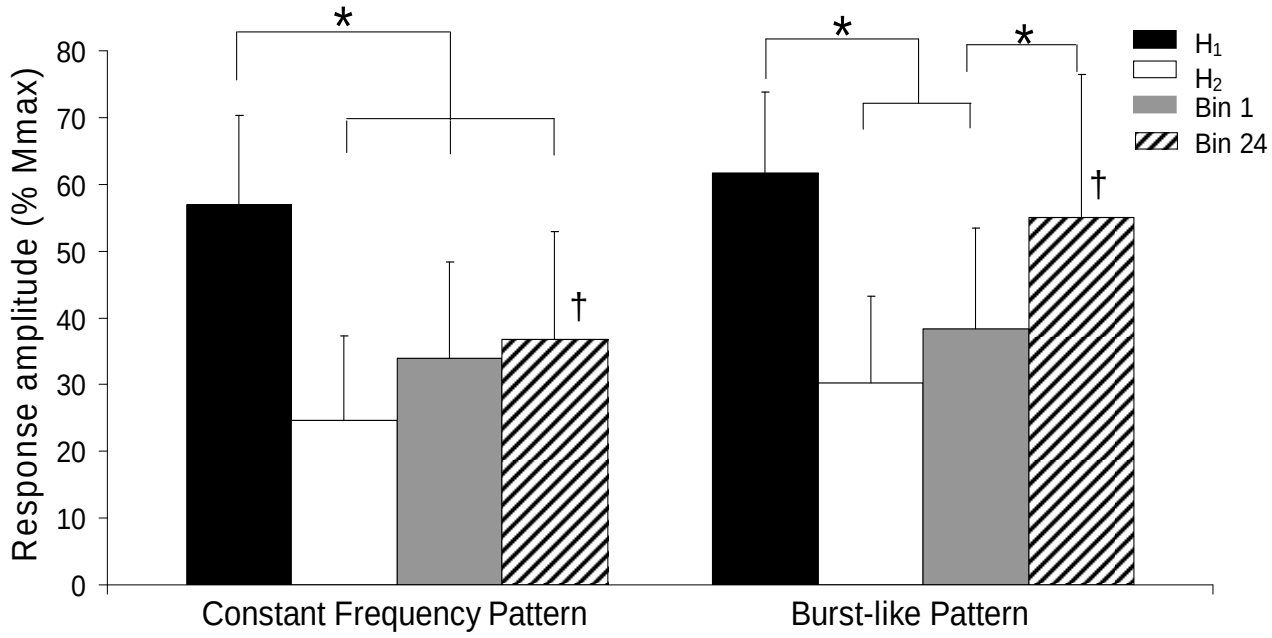

Figure 5.4 Group data showing the effect of stimulation pattern and time on H-reflex amplitudes. Four time points are shown for each stimulation pattern (H_1, H_2, Bin 1, and Bin 24). 1 SE is shown. Columns marked by asterisks or crosses were significantly different from each other.

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Chapter 6: General Discussion

The common theme that links the chapters of this thesis is sensorimotor integration in the human spinal cord. Sensorimotor integration in the intact nervous system was the focus of Chapters 2 and 3, where as the injured nervous system, due to stroke or spinal cord injury (SCI), was the focus of Chapters 4 and 5. Mechanical perturbations and surface electrical stimulation were used as non-invasive probes of the nervous system and allowed for further characterization of spinal circuitry and reflex transmission in both the intact and injured states. Summarized below are the major findings of each thesis chapter, followed by a discussion of the implications of these findings in terms of basic or clinical neurophysiology and possible future directions.

6.1 Spinal circuitry in the intact human spinal cord

The objective of Chapter 2 was to determine whether spinal reflex pathways connect sensory receptors in the lower leg to the erector spinae (ES) muscles of the lower back. I found that taps applied to the Achilles' tendon elicited short-latency reflexes bilaterally in the ES muscles. These reflexes were larger in the ES muscle contralateral to the stimulation site and were modulated based on body position, but not due to the presence or absence of visual input. Most surprisingly, taps delivered to the lateral calcaneus evoked reflexes in the ES muscles that were not different from those evoked by the Achilles' taps. Stimulation of the sural nerve, a purely cutaneous nerve, also evoked responses in the ES muscles that were similar to those evoked by the Achilles' tap. Based on these findings, I suggested that the reflexes elicited in the ES muscles involved a larger contribution from cutaneous receptors in the lower limb, rather than muscle

spindles. I proposed that these reflexes may play a role in postural stability and the maintenance of balance; however, this remains to be determined.

Future investigations on the nature of the short-latency ES reflexes could include a detailed assessment of these reflexes during functional tasks, such as walking. In regards to the afferent origin of the short-latency reflexes in ES, determining the relative contributions of cutaneous receptors versus muscle spindles would be of interest. In additional experiments not discussed in Chapter 2, techniques such as applying a topical anaesthetic to the skin of the foot and ankle or freezing the foot in ice water did not adequately remove contributions from cutaneous receptors. Thus, to assess the contribution from muscle spindles located in muscles of the lower leg, the best method to selectively eliminate contributions from the cutaneous receptors would be to deliver a local anaesthetic to the cutaneous nerves of the lower leg and foot.

6.2 Reflex transmission in the intact human spinal cord

The objective of Chapter 3 was to study transmission along the H-reflex pathway throughout 10 s trains of electrical stimulation delivered at physiologically relevant stimulation frequencies during functionally relevant tasks and background contraction amplitudes. Changes in transmission were assessed by quantifying the post-activation depression and recovery of reflex amplitudes (PAD&R). Stimulation frequency and background contraction amplitude influenced PAD&R, but there was no difference in the modulation of transmission along the H-reflex pathway between sitting and standing tasks. The PAD I observed was, in most cases, consistent with previous reports (Burke et al. 1989;

Goulart et al. 2000; Ishikawa et al. 1966; McNulty et al. 2008; Trimble et al. 2000). The finding of reflex recovery, however, opposed the common assumption that during periods of repetitive afferent input the depression expressed after the first pulse is maintained for the duration of the input. I not only observed significant recovery of reflex amplitude, but during 10 Hz stimulation reflex amplitudes recovered completely, and at times, complete recovery occurred by the third reflex. These findings highlight that transmission predominantly along the Ia afferent pathway is continually modified during repetitive input and that the balance achieved between depression and facilitation of transmission is dependent upon stimulation frequency and background contraction amplitude. These results also demonstrate that modulation of transmission along the Ia afferent pathway may play a role during voluntary movements when Ia afferents are firing at frequencies of between 5 and 20 Hz and background contraction amplitudes up to 20 % MVC. Furthermore, the very fast reflex recovery observed within the first several stimulation pulses is not consistent with the classical mechanism used to describe PAD; a decreased probability of neurotransmitter release from previously active Ia afferent terminals (Hirst et al. 1981; Hultborn et al. 1996; Kuno 1964). This time course for the recovery of reflex amplitude may not coincide with the time course of neurotransmitter replenishment at afferent terminals (Kavalali 2007).

Based on the characterization of the recovery of reflex amplitude in Chapter 3, further investigations of the mechanisms driving this recovery process are warranted, especially, in terms of the very fast recovery. The use of computer

programs which can model reflex transmission at the Ia afferent-motoneuron synapse may be shed light on the mechanisms involved in the reflex recovery, in addition to determining the physiological parameters which could lead to the strong alternations in reflex amplitudes that were observed. Another avenue to explore could be the relationship between motor unit size and the amplitude of reflex recovery. Since more PAD has been reported in small motor units as compared to large motor units (Crone et al. 1990; Floeter and Kohn 1997; Lloyd and Wilson 1957; Van Boxtel 1986), the question remains as to whether the amplitude of recovery is also dependent on motor unit size. PAD&R assessed using single motor unit recordings during trains of electrical stimulation could help answer this question. These single motor unit recordings would also provide information about whether the strong alternations of reflex amplitude are expressed at the level of single motor units. Lastly, it would be interesting to study how transmission along the H-reflex pathway changes during trains of electrical stimulation in individuals with neurological impairments. In Chapter 5 of this thesis PAD&R of reflex amplitude were investigated in individuals with chronic SCI, however a wide range of stimulation conditions was not included. It may be that continual modulation of transmission along the H-reflex pathway allows for the reflexive recruitment of motoneurons to play a role in voluntary movement and during muscle contractions evoked by electrical stimulation for rehabilitation.

6.3 The effect of wide-pulse neuromuscular electrical stimulation on torque production and reflex transmission after stroke and SCI

The objective of the Chapter 4 was to determine the effect of wide-pulse neuromuscular electrical stimulation (WP-NMES) on torque production in individuals with chronic hemiparetic stroke. When a 1 ms pulse width was used elbow flexion torque was enhanced in the paretic arm compared to the non-paretic arm. Additionally, brief periods of 100 Hz stimulation were beneficial for augmenting torque production, as compared to longer periods of 100 Hz stimulation in which a fatigue-related decline in the torque was evident. A combination of changes in presynaptic inhibitory mechanisms that control reflex transmission at the Ia-afferent motoneuron synapse and possible increases in motoneuron excitability may contribute to the enhanced contractions in the paretic arm. This was the first time that WP-NMES has been tested in individuals with chronic stroke and the enhanced torque production in the paretic limb suggests that WP-NMES may be beneficial for rehabilitation (see below).

The objective of Chapter 5 was to determine the extent to which reflexes can contribute to contractions evoked by WP-NMES after SCI. The results showed a significant reflexive contribution to electrically-evoked contractions after SCI. There was significant depression of reflex amplitude at the beginning of the stimulation trains, similar to the results obtained in individuals with no neurological impairments (Chapter 3). After chronic SCI, significant recovery of reflex amplitude only occurred after a brief burst of 100 Hz stimulation. This large reflex recovery may be due to changes that occur in mechanisms controlling reflex transmission at the Ia afferent terminals or increases in motoneuron

excitability after SCI. This was the first time that a reflexive contribution to contractions evoked by WP-NMES after SCI has been shown, and these results point towards the use of WP-NMES for rehabilitation after SCI.

There are two applications of WP-NMES for rehabilitation after stroke or SCI: the first is using WP-NMES to maintain or improve muscle quality and the second is using WP-NMES to produce functional movements. The results presented in this thesis show promise for the use of WP-NMES for maintaining muscle quality, however the eventual use of WP-NMES for generating functional movements is more uncertain. This is because precise methods for controlling the amplitude of the contractions evoked by WP-NMES or the timing of the central contribution have not been developed. It is for this reason that the discussion below will focus on the first application of WP-NMES.

WP-NMES may be effective for maintaining muscle quality because the central contribution is thought to be generated via the reflexive recruitment of motor units according to the size principle (Henneman et al. 1965). This means that the small fatigue-resistant units would be recruited first and would be more active than if a random recruitment order was followed, such as for the direct activation of motor axons during NMES (Feiereisen et al. 1997; Gregory and Bickel 2005; Jubeau et al. 2007). Enhancing the orderly recruitment of motor units during WP-NMES may lead to muscle contractions that are more fatigue-resistant and, if used on a long-term basis, could reduce disuse atrophy; however, further work is required to confirm these potential implications. A study in which single motor units are recorded during WP-NMES in individuals with stroke or

SCI, would verify the recruitment order of motor units during WP-NMES. I predict that there would be a greater number of small motor units, compared to large motor units, recruited during electrically-evoked contractions that have a large central contribution versus contractions that have a small central contribution. Another future direction could be to compare the fatigue-resistance of contractions evoked by WP-NMES compared to more traditional NMES protocols (e.g. 100 μs pulse width, 20 Hz) in paralyzed muscle. The evidence available to date, in individuals with no neurological impairments, shows that contractions are more fatigue-resistant during WP-NMES when a central contribution is present (Lagerquist et al. 2009). Further work is also required to determine the optimal combination of stimulation frequency, pattern, pulse width, intensity, and duration for maximizing the central contribution. Part of this body of work may involve determining whether stimulation delivered over the nerve trunk or muscle belly is more effective for enhancing the central contribution to contractions evoked by WP-NMES after stroke or SCI. Lastly, the effect of WP-NMES could be investigated in individuals with other neurological impairments that have resulted in muscle fatigue, weakness, or atrophy, such as multiple sclerosis or amyotrophic lateral sclerosis.

The topic of spasticity and WP-NMES also warrants further investigation. It was suggested in Chapters 4 and 5 that the activation of persistent inward currents (PICs) on motoneurons may be one of the mechanisms driving the central contribution to muscle contractions. Since PICs have been shown to be involved in the generation of muscle spasms (Gorassini et al. 2004), there is some concern

that spasms may be exacerbated during WP-NMES. This does not seem likely because none of the participants in the Nickolls et al. (2004) study or in this thesis reported a change in spasms during or after a session of WP-NMES. Also, other studies have reported a reduction in spasticity after a single session of NMES (Ping Ho and Kam Kwan 2010) or repeated sessions (Aydin et al. 2005; Krause et al. 2008). A systematic study in which the effect of WP-NMES on spasticity is investigated would confirm or reject the current anecdotal reports. It would also be interesting to look at the effect of anti-spastic medications on the amplitude and time course of the central contribution. There is a possibility that the central contribution reported in Chapter 5 was under-estimated due to the majority of participants taking Baclofen. On the contrary, these medications may have decreased the likelihood of evoking spasms during WP-NMES.

Lastly, if positive results were obtained from the potential studies described above (i.e. in a single experimental session it was shown that during WP-NMES the central contribution increased the orderly recruitment of motor units and fatigue-resistance, and spasticity was not worsened), I would continue on with a study that investigates the long- term effects of using WP-NMES on muscle health after stroke or SCI. This could take the form of a 12 week training study that compares WP-NMES to more traditional NMES protocols (e.g. 100 μs pulse width, 20 Hz) in individuals with chronic stroke or SCI. This study would conclusively determine the applicability of WP-NMES for rehabilitation by answering the question of whether long-term use of WP-NMES significantly improves the fatigue-resistance of the muscle and decreases disuse atrophy.

6.4 Summary

The work presented in this thesis contributes to the field of sensorimotor integration within the human nervous system in the intact and injured states. In regards to the impact of this work, it is my hope that it raises awareness about the potential involvement of transmission along reflex pathways during voluntary and electrically-evoked muscle contractions for researchers, clinicians, rehabilitation specialists, and fitness professionals. This thesis emphasizes that the modulation of transmission along reflex pathways is a continual process, in which the balance between depression and facilitation is constantly changing and is dependent upon many factors, such as task (Chapter 2), stimulation frequency (Chapters 3, 4, 5) and background contraction (Chapter 3). Lastly, maximizing transmission along reflex pathways during WP-NMES may improve the efficacy of electrically-evoked contractions for individuals with neurological impairments.

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