Rhythmic arm cycling differentially modulates stretch and H-reflex amplitudes in soleus muscle

by

Andres Felipe Palomino
B.A. in Sport and Physical Activity, Sport National School, Colombia, 2004

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

MASTER OF SCIENCE (Kinesiology)

in the School of Exercise Science, Physical & Health Education

University of Victoria

Supervisory Committee

Rhythmic arm cycling differentially modulates stretch and H-reflex amplitudes in soleus muscle

by

Andres Felipe Palomino
B.A. in Sport and Physical Activity, Sport National School, 2004

Supervisory Committee

Dr. E. Paul Zehr (School of Exercise Science, Physical and Health Education, Uvic)
Supervisor

Dr. Sandra Hundza (School of Exercise Science, Physical and Health Education, Uvic)
Supervisor

Dr. Ryan Rhodes (School of Exercise Science, Physical and Health Education, Uvic)
Departmental Member

Dr. Brian Maraj (Faculty of Physical Education and Recreation, University of Alberta)
External Member

Abstract

Supervisory Committee

Dr. E. Paul Zehr (School of Exercise Science, Physical and Health Education, Uvic)
Supervisor

Dr. Sandra Hundza (School of Exercise Science, Physical and Health Education, Uvic)
Supervisor

Dr. Ryan Rhodes (School of Exercise Science, Physical and Health Education, Uvic)
Departmental Member

Dr. Brian Maraj (Faculty of Physical Education and Recreation, University of Alberta)
External Member

ABSTRACT

During rhythmic arm cycling soleus H-reflex amplitudes are reduced by modulation of group Ia presynaptic inhibition **(**Frigon et al, 2004). This reflex suppression is graded with the frequency of arm cycling (Loadman & Zehr 2007; Hundza & Zehr 2009) and 0.8 Hz is the minimum frequency to significantly reduce the soleus H-reflex (Hundza & Zehr 2009). Despite the data on modulation of the soleus H-reflex amplitude induced by rhythmic arm cycling, comparatively little is known about the modulation of stretch reflexes due to remote limb movement. Therefore, the present study was intended to explore the effect of arm cycling on stretch and H-reflex amplitudes in the soleus muscle. In so doing, additional information on the mechanism of action during rhythmic arm cycling would be revealed. Although both reflexes share the same afferent pathway, we hypothesized that stretch reflex amplitudes would be less suppressed by arm cycling because they are less inhibited by presynaptic inhibition (Morita et al, 1998). Failure to reject this hypothesis would add additional strength to the argument that Ia presynaptic inhibition is the mechanism modulating soleus H-reflex amplitude during rhythmic arm cycling. Participants were seated in a customized chair with feet strapped to footplates. Three motor tasks were performed: static control trials and arm cycling at 1 and 2 Hz. Soleus H-reflexes were evoked using single 1 ms pulses of electrical stimulation delivered to the tibial nerve at the popliteal fossa. A constant M-wave and ~6% MVC activation of soleus was maintained across conditions. Stretch reflexes were evoked using a vibratory shaker (ET-126; Labworks Inc). The shaker was placed over the triceps surae tendon and controlled by a custom written LabView program (single sinusoidal pulse at 100Hz). Results demonstrated that rhythmic arm cycling that was effective for conditioning soleus H-reflexes did not show a suppressive effect on the amplitude of the soleus stretch reflex. We suggest this indicates that stretch reflexes are less sensitive to conditioning by rhythmic arm movement, as compared to H-reflexes, due to the relative insensitivity of Ia presynaptic inhibition.

Table of Contents

List of Figures

Acknowledgments

First of all, I must thank all the members of the Rehabilitation Neuroscience Laboratory; Holly, Rinaldo, Tsuyoshi, Allen, Katy, Pamela, Geoff, Taryn, Trevor and Erika.

In particular, I want to thank Dr. Rinaldo Mezzarane, Dr. Nakajima Tsuyoshi and Holly Murray for their technical assistance throughout this project and for sharing their scientific knowledge.

Also, I owe a special thanks to Erika for providing valuable ideas for the set up of the shaker on the platform.

I would like to acknowledge my fellow graduate students in the School of Exercise Science, Physical Health & Education for their friendship and support. Also, I owe special thanks to Rebecca and Bev for their assistance regarding information, courses registration, etc.

I gratefully appreciate the time and valuable input of my committee members, Dr. Ryan Rhodes and Dr. Brian Maraj.

I would like to thank my supervisors Dr. E. Paul Zehr, and Dr. Sandra Hundza for giving me the opportunity to be one of their graduate students. I really appreciated their guidance, feedback, support and for sharing their expertise. Also, I must say thank you for giving me the opportunity to participate in National and International conferences. For me, it has been a privilege not only to be part of the Rehabilitation Neuroscience Laboratory, but also to learn from both of you.

This research was supported by grants to E.P.Z. from the Heart and Stroke Foundation of Canada (BC & Yukon) and the Natural Sciences and Engineering Research Council of Canada (NSERC).

Dedication

I would like to dedicate this thesis to my parents, family and my partner Kelly. Their support has made the completion of this thesis possible. Thank you!

CHAPTER 1: Introduction and review of literature

The study of human locomotion has been a focus in motor control research. One specific topic has been to study the neural control of rhythmic activities. In quadrupeds, it has been established that rhythmic motor output is regulated by spinal central pattern generators (CPGs). The output of locomotor CPGs is finely tuned by afferent feedback arising from movement and interactions with the environment. In muscle afferent pathways, presynaptic inhibition is an important mechanism that allows vertebrates to suppress selective information flow in some terminal branches (Rudomin & Schmidt 1999). This regulation of sensory feedback allows the central nervous system to respond with a motor output according to the specific need of the environment (Rudomin & Schmidt 1999). In contrast to reduced animal preparations, where intracellular measurement can be made, methodologies to study neural pathways and presynaptic inhibition in humans are more indirect. Modulation of spinal reflexes during the execution of rhythmic motor activities has been used to infer the activity of CPG circuits (Burke 1999; Burke et al. 2001; Zehr and Duysens 2004). Also, amplitude modulation of stretch and H-reflexes are commonly used to evaluate presynaptic inhibition of the Ia afferent monosynaptic synapse with alpha motoneurons (Stein 1995). It was revealed that rhythmic arm cycling at 1Hz significantly suppresses the soleus H-reflex amplitude compared to the amplitude during static control via presynaptic inhibition of Ia afferent transmission **(**Frigon et al. 2004; Loadman & Zehr 2007; Hundza & Zehr 2009). Even though the modulation of the soleus H-reflex in different tasks is well studied, there is a lack of information about the modulation of the stretch reflex induced by remote movement.

The purpose of this literature review is to outline the main features of central pattern generators and to compare and contrast the modulation of excitability in stretch and H-reflex pathways in different motor tasks. Finally, task and frequency dependent modulation of the H-reflex are described.

Central pattern generators (CPGs)

Central pattern generators (CPGs) are neuronal circuits within the spinal cord that can produce reciprocal, rhythmic motor pattern independent from supraspinal and peripheral inputs. CPGs are involved in motor tasks such as scratching, walking, running and swimming. The concept of CPGs was built based on the work Graham Brown (1911). In his experiments, cats with transacted spinal cords and cut dorsal roots, still produced rhythmic alternating contractions in ankle flexors and extensors of hindlimbs. One half of the center induces activity in flexors, the other in extensors muscles, coordinating the activation of these motoneuron pools. In quadrupeds it has been suggested that the neural control of locomotion is based on CPGs located in the lumbar and cervical spinal cord, with a CPG for each limb (Duysens & Van de Crommert 1998). These CPGs are responsible for generating the rhythm and shaping the pattern burst of motoneurons (Grillner et al. 1995; Grillner 1985). CPGs control each limb, and are interconnected via propriospinal pathways to ensure left-right and fore- and hindlimb coordination during quadrupedal movements.

Spinal interlimb neural pathways in cat, rat and human

As described above, quadrupedal locomotion has been ascribed to spinal CPGs controlling each limb. These CPG networks are connected between limbs in the cervical and lumbosacral regions (Miller et al. 1975; Cazalets & Bertrand 2000). Several studies have researched the neural mechanisms of interlimb coordination in animals. In addition, there have been studies in humans. For instance, Orsal et al. (1990) demonstrated that coupling between fore and hindlimb locomotor networks could be achieved in deafferented cats by interconnecting propriospinal pathways. Also, Akay et al. (2006) found that the fore and hind limbs of decerebrate cats walking on a transversely-split treadmill, are strongly coupled via asymmetrical organization of reciprocal cervico-lumbar pathways. These researchers found that reducing the speed of the front treadmill decreased the rate of stepping in both the fore and hind legs. Also, it was observed that 1:1 coordination of stepping was maintained over different speeds of the front treadmill. In contrast, slowing the rear treadmill by relatively small amounts, led to uncoupling of stepping in the fore and hind legs. The above mentioned scholars interpreted these results as the existence of asymmetric in the ascending and descending linkages between the neuronal system controlling stepping in the forelegs and hind legs.

It is well accepted that the coordination of all 4 limbs involving distant CPGs should require long interconnecting fibres (Falgairolle et al. 2006). In cats, this idea is supported by the presence of long caudorostral propriospinal tracts that have been identified in spinal columns and are presumed to participate in hindlimb-forelimb locomotor coupling (English et al. 1985). Also, Krutki et al. (1998) found propriospinal neurons from C6 projecting to sacral segments (S1/S2) with collaterals branching to lumbar segments (L4).

The existence of collaterals branching to segments of the lumbosacral enlargements raises the possibility that descending information can be relayed to several motor centers controlling different hindlimb muscles (Krutki et al. 1998). In monkeys, the organization of the long descending propriospinal tract is similar to the cat, although the distribution of neurons differs (Skinner et al. 1979). Some attempts have been made to compare monkeys to humans. However, Falgairolle et al. (2006) suggested that differences must exist since chimpanzees spend only 15% of the time on their hindlimbs. These results demonstrate a lack of true evolution to the bipedal mode of locomotion. Recently, it has been described that in the human spinal cord, caudal to the cervical enlargement, reticulospinal fibres are progressively replaced by propriospinal fibres but some still descend to the lowest sacral segments (Nathan et al. 1996). In summation, these studies provide evidence of neural connectivity between cervical and lumbosacral regions of the spinal cord in a variety of species.

Coordination of arms and legs

During human walking, coordination exists in muscle activation between the legs and the arms (Dietz 2002; Wannier et al. 2001). This interlimb activation has been interpreted as existence of spinal interconnections between the upper and lower spinal CPGs that are engaged in the locomotor function (Wannier et al. 2001). One of the previous observations about human walking is that the upper limbs swing in alternation, with each arm swinging forward and back in phase with the contralateral limb (Carlet 1872). Elftman (1939) studied human walking and demonstrated that arm swing has consequential effect on the rotation of the body. Also, it was suggested that arm movement is not simply an aspect of forward movement but an integral part of the locomotion coordination. Fernandez-Ballesteros et al. (1965) also found that arm swinging was not a passive phenomenon, suggesting that arm movement is part of a centrally determined pattern of locomotion. Early theoretical work suggested that arm movement during walking is controlled by spinal CPG circuits (Jackson et al. 1978; Jackson 1983; Jackson et al. 1983). Distinct to animals where invasive methodologies such as direct cellular measurement can be made, in human studies indirect methodologies are employed to reveal the activity of spinal CPGs. One approach is to evoke spinal reflexes during rhythmic activities such as walking and arm cycling and observe the resulting pattern of modulation. Using this approach, it has been shown that there is a measurable impact of arm motion on lumbar spinal cord excitability that contributes to overall locomotor control (Zehr et al. 2009). This evidence has been obtained by studies of cutaneous (Balter & Zehr 2007) and muscle afferent (Zehr et al. 2007; Mezzarane et al. 2011) pathways.

The stretch reflex

Liddell and Sherrington (1924) first discovered the neural pathway mediating the stretch reflex in the decerebrate cat. They described a pathway with a single synapse in the spinal cord separating the Ia afferent fiber from homonymous alpha motoneurons, although oligosynaptic contributions to the response have been found as well (Burke et al. 1984). The stretch reflex is evoked by tapping a distal tendon. This stimuli stretches the muscle thereby stretching the muscle spindle, which leads to mechanotransduction and activation of the afferent endings. The action potentials travel along the afferent axons of the sensory neuron to synapse and excite motor neurons in the spinal cord. This process results in the motoneurons producing muscle contractions (Voerman et al. 2005). The stretch reflex is evoked in several muscles, however the most commonly used in clinical evaluation and motor control studies are the soleus and quadriceps (Voerman et al. 2005). The stretch reflex studies have been used to gain knowledge about spinal reflex pathways and properties of skeletal muscle spindles, and to develop quantitative tools for the assessment of spasticity (Bruin et al. 2006).

Mechanical stimulation, such as a tendon tap, activates the Ia afferents, and other receptors such as skin receptors and Golgi tendon organs (Burke et al. 1983). It has been reported that this type of stimulus produces dispersed action potentials in part because a given Ia afferent may fire several times at short intervals (5 ms) and multiple different afferents may be activated (Burke et al. 1983). As a result, the action potentials that travel along both the Ia and other afferents will arrive at the spinal cord unsynchronized (Burke et al. 1983). Hence, the excitatory postsynaptic potentials (EPSP) are produced by spatial (Enriquez-Denton et al. 2002) and temporal summation (Morita et al. 1998).

In the literature, it has been suggested that variation in strength, duration, direction and place of impact of the tendon tap may contribute to variations in the excitation of muscle spindles and consequently of the reflex responses (Stam & Tan 1987). Therefore, several methodological considerations are needed to ensure a consistent activation of the stretch reflex.

First, the stretch reflex response may vary according to the number of Ia afferents that are stimulated. Therefore, it is important to maintain similar synaptic input to alpha motoneurons. One method to achieve this is by maintaining the same displacement of the tendon tap. The displacement of the tendon tap is a reliable measure of the afferent response (Burke et al. 1981). Second, results for both soleus and flexor carpi radialis muscles show that impact velocity is an important variable in eliciting tendon reflexes (Archambeault et al. 2006). Third, the stretch reflex amplitude has been found to be dependent on excursion depth "depth of penetration" (Archambeault et al. 2006). Fourth, the place of mechanical contact along the tendon needs to be localized for each subject to ensure a reflex contraction of the studied muscle (Fornari & Kohn 2008).

The Hoffmann Reflex

The Hoffmann (H) reflex is considered to be the electrical analogue of the stretch reflex that bypasses the effects of gamma motoneurons and the muscle spindle discharge (Brooke et al. 1997; Schieppati 1987). The H-reflex is evoked through electrical stimulation of the mixed peripheral nerve, which results in a direct response from activation of the motor axons (M-wave), and a later reflex response, the H-reflex. The H-reflex is considered to be dominated by monosynaptic inputs (Misiaszek 2003). However in cats (Fetz et al. 1979; Jankowska et al. 1981a; Jankowska et al. 1981b) and in humans (Burke et al. 1983; 1984; Fukushima et al. 1982) the existence of oligosynaptic pathways from group Ia afferents onto homonymous muscles have been identified. In addition, it has been reported that during H-reflex studies, not only the Ia afferents are activated, but also Ib afferents serving golgi tendon organs, some large diameter cutaneous afferents and possibly some group II muscle spindle afferents (Misiaszek 2003). Also, electrical stimulation to evoke the H-reflex produces a synchronized discharge from a large number of Ia afferents. Each Ia afferent discharges once and is then slightly dispersed (Enriquez-

Denton et al. 2002). These researchers proposed that the EPSP evoked by electrical stimulation is caused mainly by spatial summation.

Many precautions are needed to correctly apply the H-reflex methodology. First, the H-reflex amplitude varies according to the number of Ia afferents being stimulated (Zehr 2002). Therefore, it is important to maintain similar activation of the afferent population in order to provide consistent synaptic input to alpha motoneurons. One method by which to achieve this is by maintaining constant M-wave amplitude from trial to trial. The M-wave is used to monitor and to control stimulus consistency (Abbruzzese et al. 1985; Brooke et al. 1997; Zehr 2002). Second, the H-reflex amplitude is influenced by the number of active motoneurons in the muscle being tested (Burke et al. 1989; Stein & Kearney 1995). It has been shown in the triceps surae that H-reflex amplitude increases linearly with background EMG up to 50% of maximum voluntary isometric contraction where after it plateaus or declines (Loscher et al. 1996). Therefore, it is important that participants maintain similar levels of tonic contraction while the H-reflex is evoked (Zehr 2002; Misiaszek 2003). Also, maintaining a tonic contraction during H-reflex testing serves to decrease the variability of the H-reflex amplitude (Burke et al. 1989) and gives a relative value to the state of activity of the motorneuronal pool (Schieppati 1987; Burke et al. 1989). Third, post activation depression may occur with repetitious stimulation as the amount of neurotransmitter at the Ia-alpha motoneuron synapse is depleted (Hultborn et al. 1996). This is controlled by having a time delay between stimulation of at least 3 seconds (Rossi-Durand et al. 1999). Overall, with careful experimental procedures, the H-reflex is a reliable tool for measuring the spinal reflex modulation.

Differences in the composition of the EPSP produced by mechanical and electrical stimulation

The afferent volleys produced by the electrical and mechanical stimulation differ in their relative contribution from other afferents in addition to the Ia afferent. Both electrical and mechanical stimulation may activate group Ib and group II afferents to a different extent. The electrical stimulation may additionally activate efferent alpha motor neuron axons, which through collaterals may lead to activation of Renshaw cells (Morita et al. 1998). In addition, Burke et al. (1983) suggested that the afferent volley of the stretch and H-reflexes contains activity arising from mechanoreceptors not only from the triceps surae, but also from mechanoreceptors in the skin, and from other muscles. The main difference in terms of afferent input is that the EPSP evoked by mechanical stimuli can be more contaminated by non-monosynaptic effects than the EPSP evoked by electrical stimulation (Burke et al. 1983; 1984).

Influences of gamma activation on stretch reflex amplitude

Stretch and H-reflex share the same afferent pathway (Pierrot-Deseilligny & Mazevet 2000). However, the stretch reflex amplitudes are altered by changes in gamma motoneuron activity affecting muscle spindle sensitivity (Zehr 2002). Previously, it had been established that voluntary contraction of a muscle activates the fusimotor neurones innervating that target muscle (Burke et al. 1979; Vallbo et al. 1979; Burke 1989). In fact, Vallbo (1974) examined the firing frequency of muscle spindles afferents in relation to isometric voluntary contraction of the finger flexor muscles. They found that spindle firing frequency increased with the contraction intensity. The dominant type of fusimotor outflow was the static type. But some indications of increased dynamic fusimotor outflow were also observed. In addition, Gregory et al. (1998) demonstrated that muscle length and different level of contraction produces changes in stretch reflex amplitudes. The striking results of this study suggest that a 5% MVC of soleus muscle activates the fusimotor system and 10% MVC produces a facilitation of the soleus stretch reflex. Gregory et al. (1990) also completed another study evaluating the stretch reflex amplitude. Muscle conditioning consisted of a maximum voluntary contraction with the foot dorsiflexed or plantarflexed by 30 degrees from the test position, after which the subject was asked to relax while the foot was held still for several seconds before being returned to the test position and then a tendon tap was given. After a contraction of the lengthened muscle the tendon jerk was smaller than after a contraction of the shortened muscle. Using this conditioning, Gregory and collaborators (1990) evoked the soleus H-reflex and opposite outcomes were observed. The researchers also conducted a parallel study in cats and found that the stretch reflex behaved the same as in humans. These observations led to the proposal that after a muscle contraction of a shortened muscle, there is an increase of muscle spindle sensitivity resulting from fusimotor activation. Therefore, gamma motor neuron activation potentiates the stretch reflex.

Despite some data suggesting that gamma activation potentiates the stretch reflex, contrary evidence also exists. Morgan et al. (1984) found that conditioning stimulation of gamma dynamic fibers in cats did not dramatically increase the response in Ia afferents when a tendon tap was provided. Also, Burke et al. (1981) demonstrated that in humans, remote movement or abrupt contraction of 60-70% maximum grip strength did not alter the afferent response of the soleus muscle.

Modulation of the stretch and H-reflex

The modulation of both the stretch and H-reflexes has been studied widely. The result of the studies suggests that these two reflexes are modulated differently in cats than in humans. Akazawa et al. (1982) found in the mesencephalic walking cat that the modulation of the stretch and H-reflex were similar. Also, (Akazawa et al. 1982; see also Aldridge & Stein 1982) demonstrated that during spontaneous states of tonic contraction, the amplitude of the H-reflex paralleled that of the stretch reflex. In addition, (Enriquez-Denton et al. 2002) showed that excitatory postsynaptic potential (EPSP) in cats evoked by a single electrical stimulation of the tibial nerve or by fast muscle stretch were significantly depressed by conditioning stimulation of the posterior biceps and semitendinosus nerve (PBSt) stimulation. In addition, the previously mentioned researches observed a depression of the first of a series of EPSPs elicited by a train of electrical stimuli (27 stimuli; 250 Hz) to the gastrocnemius–soleus nerve as well as the initial part of the complex EPSP evoked by a slow stretch (0.2 mm, 1.6 mm/s) to the muscle. By contrast, in both the electrical and the stretch-evoked EPSPs, the last part of the potentials was much less depressed by the PBSt stimulation.

In contrast to cats, human studies suggest that these two reflexes are modulated differently. For example, the soleus H-reflex was strongly suppressed by biceps femoris tendon tap conditioning, while the stretch reflex was weakly depressed (Morita et al. 1998). In this study, the aforementioned researchers adjusted both the stretch and H-reflex to have the same amplitude in the control situation (2-3% of Mmax). Also, the tendon tap was advanced in relation to the tibial nerve stimulation by 5 ms, to take the longer latency of the stretch reflex in relation to the H-reflex into account. One of the main observations of this study is that even though both reflexes had very similar amplitudes, they were differently affected by presynaptic inhibition of the Ia afferent. In another study, the spinal reflex excitability of the soleus muscle was examined as human posture changed from a supine to a passive upright position. In fact, Shimba et al. (2010) demonstrated that the H-reflex amplitude in passive standing was smaller than in the supine posture condition. In contrast, the stretch reflex was significantly larger in the passive standing than in the supine posture condition. Other studies compared the standing position in relation to the stance phase of walking. Capaday and Stein (1986) found that the H-reflex during the standing position was greater than during the stance phase of walking. This suggests that high reflex amplitude on standing is required to maintain a stable posture. Meanwhile, the stretch reflex amplitude during the stance phase of walking and standing at matched soleus BEMG are similar (Sinkjaer et al. 1996). Also, the modulation of the stretch and H-reflex throughout the gait cycle has been studied. The greater amplitude of the stretch and H-reflex was obtained during the stance phase (Capaday & Stein, 1987; Crenna & Frigo 1987; Sinkjaer et al. 1996). However, differences were observed. Sinkjaer et al. (1996) found that during the late swing phase, the stretch reflex had an amplitude of ~50% of the maximal amplitude in the stance phase during walking. In contrast, the H-reflex amplitude is very small or totally absent during the swing phase (Capaday & Stein 1986; Crenna & Frigo 1987).

The stretch and H-reflexes are modulated by presynaptic inhibition of the Ia afferent. Morita et al. (1998) concluded that the main difference between the stretch and H-reflex is that the stretch reflex is less sensitive to presynaptic inhibition of the Ia afferent. Temporal dispersion and composition of the excitatory postsynaptic potential has been established as possible factors responsible for the differences in modulation between the two reflexes.

Task and frequency dependent modulation of the H-reflex

The Soleus H-reflex has been shown to be task and frequency dependent. Evidence of task-dependence comes from studies where the soleus H-reflex amplitude is modulated by a change in motor task. For instance, (Capaday & Stein 1986) showed that the H-reflex amplitude is more suppressed during running than when walking. In addition, task dependence has been shown in other motor activities such as rhythmic arm cycling. For example, it was demonstrated that rhythmic arm cycling at 1 Hz significantly suppresses the soleus H-reflex amplitude compared to the amplitude during static control **(**Frigon et al. 2004; Loadman & Zehr 2007; Hundza & Zehr 2009). This modulation can also be differentially expressed as facilitation in the tibialis anterior as compared with suppression in Soleus (Dragert & Zehr 2009). Frigon et al. (2004) demonstrated that arm cycling interacts with somatosensory conditioning. They used a conditioning stimuli to decrease (sural nerve) and to increase [(common peroneal nerve)(CP)] presynaptic inhibition of the soleus H-reflex pathway. In this experiment, Frigon and collaborators found that reflexes in soleus muscle, when sural and CP nerve stimulation were delivered alone, were not different between cycling and static trials. As a result, the significant suppression observed in H-reflex amplitude was not due to changes in motoneuron excitability suggesting that this modulation occurred at a premotoneuronal level by presynaptic inhibition of the Ia afferent. Moreover, it has been found that soleus H-reflex is graded with the frequency of arm cycling (Loadman & Zehr 2007). In fact, Hundza &

Zehr (2009) found that soleus H-reflex amplitude significantly differed from control at an average threshold of 0.8 Hz and that there is a linear relationship between frequency of arm cycling and suppression of the Soleus H-reflex. This suggests that presynaptic inhibition may be increased when a higher frequency of arm cycling is performed.

Despite the data on modulation of the soleus H-reflex amplitude induced by rhythmic arm cycling, comparatively little is known about the modulation of stretch reflexes due to remote movement. Therefore, the present study was intended to directly compare the effect of arm cycling on stretch and H-reflex amplitudes in the soleus muscle.

References

Abbruzzese G, Berardelli A, Rothwell JC, Day BL, Marsden CD (1985) Cerebral potentials and electromyographic responses evoked by stretch of wrist muscles in man. Exp Brain Res 58:544-551

Akay T, McVea DA, Tachibana A, Pearson KG (2006) Coordination of fore and hind leg stepping in cats on a transversely-split treadmill. Exp Brain Res 175:211-222. DOI 10.1007/s00221-006-0542-3

Akazawa K, Aldridge JW, Steeves JD, Stein RB (1982) Modulation of stretch reflexes during locomotion in the mesencephalic cat. J Physiol 329:553-567

Aldridge JW, Stein RB (1982) Nonlinear properties of stretch reflex studied in the decerebrate cat. J Neurophysiol 47:179-192

Archambeault M, de Bruin H, McComas A, Fu W (2006) Tendon reflexes elicited using a computer controlled linear motor tendon hammer. Conf Proc IEEE Eng Med Biol Soc 1:5068-5071. DOI 10.1109/IEMBS.2006.259923

Ballesteros ML, Buchthal F, Rosenfalck P (1965) The Pattern of Muscular Activity during the Arm Swing of Natural Walking. Acta Physiol Scand 63:296-310

Balter JE, Zehr EP (2007) Neural coupling between the arms and legs during rhythmic locomotor-like cycling movement. J Neurophysiol 97:1809-1818. DOI 10.1152/jn.01038.2006

Brooke JD, Cheng J, Collins DF, McIlroy WE, Misiaszek JE, Staines WR (1997) Sensori-sensory afferent conditioning with leg movement: gain control in spinal reflex and ascending paths. Prog Neurobiol 51:393-421

Brown TG (1911) The Intrinsic Factors in the Act of Progression in the Mammal. Proceedings of the Royal Society of London.Series B, Containing Papers of a Biological Character 84:pp. 308-319

Burke D, Adams RW, Skuse NF (1989) The effects of voluntary contraction on the H reflex of human limb muscles. Brain 112 (Pt 2):417-433

Burke D, Gandevia SC, McKeon B (1984) Monosynaptic and oligosynaptic contributions to human ankle jerk and H-reflex. J Neurophysiol 52:435-448

Burke D, Gandevia SC, McKeon B (1983) The afferent volleys responsible for spinal proprioceptive reflexes in man. J Physiol 339:535-552

Burke D, Hagbarth KE, Skuse NF (1979) Voluntary activation of spindle endings in human muscles temporarily paralysed by nerve pressure. J Physiol 287:329-336

Burke D, McKeon B, Skuse NF (1981) Dependence of the Achilles tendon reflex on the excitability of spinal reflex pathways. Ann Neurol 10:551-556. DOI 10.1002/ana.410100610

Burke RE (1999) The use of state-dependent modulation of spinal reflexes as a tool to investigate the organization of spinal interneurons. Exp Brain Res 128:263-277

Burke RE, Degtyarenko AM, Simon ES (2001) Patterns of locomotor drive to motoneurons and last-order interneurons: clues to the structure of the CPG. J Neurophysiol 86:447-462

Capaday C, Stein RB (1987) Difference in the amplitude of the human soleus H reflex during walking and running. J Physiol 392:513-522

Capaday C, Stein RB (1986) Amplitude modulation of the soleus H-reflex in the human during walking and standing. J Neurosci 6:1308-1313

Cazalets JR, Bertrand S (2000) Coupling between lumbar and sacral motor networks in the neonatal rat spinal cord. Eur J Neurosci 12:2993-3002

Crenna P, Frigo C (1987) Excitability of the soleus H-reflex arc during walking and stepping in man. Exp Brain Res 66:49-60

DeBruin H, Fu W, Galea V, McComas A (2006) Speculations surrounding a spinal reflex. J Neurol Sci 242:75-82. DOI 10.1016/j.jns.2005.11.018

Dietz V (2002) Do human bipeds use quadrupedal coordination? Trends Neurosci 25:462-467

Dragert K, Zehr EP (2009) Rhythmic arm cycling modulates Hoffmann reflex excitability differentially in the ankle flexor and extensor muscles. Neurosci Lett 450:235-238. DOI 10.1016/j.neulet.2008.11.034

Duysens J, Van de Crommert HW (1998) Neural control of locomotion; The central pattern generator from cats to humans. Gait Posture 7:131-141

Elftman H (1939) The Function of the Arms in Walking. Human Biology 11:529

English AW, Tigges J, Lennard PR (1985) Anatomical organization of long ascending propriospinal neurons in the cat spinal cord. J Comp Neurol 240:349-358. DOI 10.1002/cne.902400403

Enriquez-Denton M, Morita H, Christensen LO, Petersen N, Sinkjaer T, Nielsen JB (2002) Interaction between peripheral afferent activity and presynaptic inhibition of ia afferents in the cat. J Neurophysiol 88:1664-1674

Falgairolle M, de Seze M, Juvin L, Morin D, Cazalets JR (2006) Coordinated network functioning in the spinal cord: an evolutionary perspective. J Physiol Paris 100:304-316. DOI 10.1016/j.jphysparis.2007.05.003

Fetz EE, Jankowska E, Johannisson T, Lipski J (1979) Autogenetic inhibition of motoneurones by impulses in group Ia muscle spindle afferents. J Physiol 293:173-195

Fornari MC, Kohn AF (2008) High frequency tendon reflexes in the human soleus muscle. Neurosci Lett 440:193-196. DOI 10.1016/j.neulet.2008.05.075

Frigon A, Collins DF, Zehr EP (2004) Effect of rhythmic arm movement on reflexes in the legs: modulation of soleus H-reflexes and somatosensory conditioning. J Neurophysiol 91:1516-1523. DOI 10.1152/jn.00695.2003

Fukushima Y, Yamashita N, Shimada Y (1982) Facilitation of H-reflex by homonymous Ia-afferent fibers in man. J Neurophysiol 48:1079-1088

Gossard JP, Cabelguen JM, Rossignol S (1990) Phase-dependent modulation of primary afferent depolarization in single cutaneous primary afferents evoked by peripheral stimulation during fictive locomotion in the cat. Brain Res 537:14-23

Gregory JE, Mark RF, Morgan DL, Patak A, Polus B, Proske U (1990) Effects of muscle history on the stretch reflex in cat and man. J Physiol 424:93-107

Gregory JE, Wise AK, Wood SA, Prochazka A, Proske U (1998) Muscle history, fusimotor activity and the human stretch reflex. J Physiol 513 (Pt 3):927-934

Grillner S (1985) Neurobiological bases of rhythmic motor acts in vertebrates. Science 228:143-149

Grillner S, Deliagina T, Ekeberg O, el Manira A, Hill RH, Lansner A, Orlovsky GN, Wallen P (1995) Neural networks that co-ordinate locomotion and body orientation in lamprey. Trends Neurosci 18:270-279

Hultborn H, Illert M, Nielsen J, Paul A, Ballegaard M, Wiese H (1996) On the mechanism of the post-activation depression of the H-reflex in human subjects. Exp Brain Res 108:450-462

Hundza SR, Zehr EP (2009) Suppression of soleus H-reflex amplitude is graded with frequency of rhythmic arm cycling. Exp Brain Res 193:297-306. DOI 10.1007/s00221-008-1625-0

Jackson KM (1983) Why the upper limbs move during human walking. J Theor Biol 105:311-315

Jackson KM, Joseph J, Wyard SJ (1983) The upper limbs during human walking. Part 2: Function. Electromyogr Clin Neurophysiol 23:435-446

Jackson KM, Joseph J, Wyard SJ (1978) A mathematical model of arm swing during human locomotion. J Biomech 11:277-289

Jankowska E, Johannisson T, Lipski J (1981a) Common interneurones in reflex pathways from group 1a and 1b afferents of ankle extensors in the cat. J Physiol 310:381-402

Jankowska E, McCrea D, Mackel R (1981b) Oligosynaptic excitation of motoneurones by impulses in group Ia muscle spindle afferents in the cat. J Physiol 316:411-425

Krutki P, Grottel K, Mrowczynski W (1998) Lumbar collaterals of neurons of the C6 segment projecting to sacral segments of the cat spinal cord. J Physiol Paris 92:37-42. DOI 10.1016/S0928-4257(98)80021-1

Liddell EGT, Sherrington C (1924) Reflexes in Response to Stretch (Myotatic Reflexes). Proceedings of the Royal Society of London Series B, Containing Papers of a Biological Character 96:212-242. DOI 10.1098/rspb.1924.0023

Loadman PM, Zehr EP (2007) Rhythmic arm cycling produces a non-specific signal that suppresses Soleus H-reflex amplitude in stationary legs. Exp Brain Res 179:199-208. DOI 10.1007/s00221-006-0782-2

Loscher WN, Cresswell AG, Thorstensson A (1996) Excitatory drive to the alpha-motoneuron pool during a fatiguing submaximal contraction in man. J Physiol 491 (Pt 1):271-280

Mezzarane RA, Klimstra M, Lewis A, Hundza SR, Zehr EP (2011) Interlimb coupling from the arms to legs is differentially specified for populations of motor units comprising the compound H-reflex during "reduced" human locomotion. Exp Brain Res 208:157-168. DOI 10.1007/s00221-010-2467-0

Miller S, Van Der Burg J, Van Der Meche F (1975) Coordination of movements of the kindlimbs and forelimbs in different forms of locomotion in normal and decerebrate cats. Brain Res 91:217-237

Misiaszek JE (2003) The H-reflex as a tool in neurophysiology: its limitations and uses in understanding nervous system function. Muscle Nerve 28:144-160. DOI 10.1002/mus.10372

Morgan DL, Prochazka A, Proske U (1984) Can fusimotor activity potentiate the responses of muscle spindles to a tendon tap? Neurosci Lett 50:209-215

Morita H, Petersen N, Christensen LO, Sinkjaer T, Nielsen J (1998) Sensitivity of H-reflexes and stretch reflexes to presynaptic inhibition in humans. J Neurophysiol 80:610-620

Nathan PW, Smith M, Deacon P (1996) Vestibulospinal, reticulospinal and descending propriospinal nerve fibres in man. Brain 119 (Pt 6):1809-1833

Orsal D, Cabelguen JM, Perret C (1990) Interlimb coordination during fictive locomotion in the thalamic cat. Exp Brain Res 82:536-546

Pierrot-Deseilligny E, Mazevet D (2000) The monosynaptic reflex: a tool to investigate motor control in humans. Interest and limits. Neurophysiol Clin 30:67-80

Rossi-Durand C, Jones KE, Adams S, Bawa P (1999) Comparison of the depression of H-reflexes following previous activation in upper and lower limb muscles in human subjects. Exp Brain Res 126:117-127

Rudomin P, Schmidt RF (1999) Presynaptic inhibition in the vertebrate spinal cord revisited. Exp Brain Res 129:1-37

Schieppati M (1987) The Hoffmann reflex: a means of assessing spinal reflex excitability and its descending control in man. Prog Neurobiol 28:345-376

Shimba S, Kawashima N, Ohta Y, Yamamoto S, Nakazawa K (2010) Enhanced stretch reflex excitability in the soleus muscle during passive standing posture in humans. J Electromyogr Kinesiol 20:406-412. DOI 10.1016/j.jelekin.2009.04.003

Sinkjaer T, Andersen JB, Larsen B (1996) Soleus stretch reflex modulation during gait in humans. J Neurophysiol 76:1112-1120

Skinner RD, Coulter JD, Adams RJ, Remmel RS (1979) Cells of origin of long descending propriospinal fibers connecting the spinal enlargements in cat and monkey determined by horseradish peroxidase and electrophysiological techniques. J Comp Neurol 188:443-454. DOI 10.1002/cne.901880307

Stam J, Tan KM (1987) Tendon reflex variability and method of stimulation. Electroencephalogr Clin Neurophysiol 67:463-467

Stam J, van Crevel H (1989) Measurement of tendon reflexes by surface electromyography in normal subjects. J Neurol 236:231-237

Stein RB (1995) Presynaptic inhibition in humans. Prog Neurobiol 47:533-544

Stein RB, Kearney RE (1995) Nonlinear behavior of muscle reflexes at the human ankle joint. J Neurophysiol 73:65-72

Vallbo AB (1974) Human muscle spindle discharge during isometric voluntary contractions. Amplitude relations between spindle frequency and torque. Acta Physiol Scand 90:319-336

Vallbo AB, Hagbarth KE, Torebjork HE, Wallin BG (1979) Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. Physiol Rev 59:919-957

Voerman GE, Gregoric M, Hermens HJ (2005) Neurophysiological methods for the assessment of spasticity: the Hoffmann reflex, the tendon reflex, and the stretch reflex. Disabil Rehabil 27:33-68

Wannier T, Bastiaanse C, Colombo G, Dietz V (2001) Arm to leg coordination in humans during walking, creeping and swimming activities. Exp Brain Res 141:375-379. DOI 10.1007/s002210100875

Zehr EP (2002) Considerations for use of the Hoffmann reflex in exercise studies. Eur J Appl Physiol 86:455-468. DOI 10.1007/s00421-002-0577-5

Zehr EP, Duysens J (2004) Regulation of arm and leg movement during human locomotion. Neuroscientist 10:347-361. DOI 10.1177/1073858404264680

Zehr EP, Hundza SR, Vasudevan EV (2009) The quadrupedal nature of human bipedal locomotion. Exerc Sport Sci Rev 37:102-108. DOI 10.1097/JES.0b013e31819c2ed6

Zehr EP, Klimstra M, Johnson EA, Carroll TJ (2007) Rhythmic leg cycling modulates forearm muscle H-reflex amplitude and corticospinal tract excitability. Neurosci Lett 419:10-14. DOI 10.1016/j.neulet.2007.03.045

CHAPTER 2: Manuscript

Introduction

During rhythmic motor tasks such as walking, swimming, and running, coordination is required between the legs and arms. In animals, this coordination has been ascribed to neural linkages that connect cervical and lumbosacral networks in the spinal cord (Gernandt and Megirian 1961; Gernandt and Shimamura 1961). Growing evidence in human experiments suggests that locomotion is controlled by interlimb neural coupling as observed in quadrupedal gait (Dietz 2002; Zehr and Duysens 2004; Zehr and Haridas 2003). When the legs and arms are moving simultaneously, the neural pathways that may connect the upper with the lower limbs can be “swamped” by the effects of limb movement. To reduce this interference, an effective experimental design involves exploring the effect of rhythmic arm movement on stationary legs. Previously, it was demonstrated that rhythmic arm cycling significantly suppresses the soleus Hoffman (H-) reflex amplitude in stationary legs compared to the amplitude during static control **(**Frigon et al. 2004; Loadman & Zehr 2007; Hundza & Zehr 2009). Moreover, it has been found that soleus H-reflex is graded with the frequency of arm cycling (Loadman & Zehr 2007; Hundza & Zehr 2009) and 0.8 Hz is the minimum frequency to significantly reduce the soleus H-reflex (Hundza & Zehr 2009).

Despite the data on modulation of the soleus H-reflex amplitude induced by rhythmic arm cycling, comparatively little is known about the modulation of stretch reflexes due to remote limb movement. Therefore, the present study was intended to explore the effect of arm cycling on stretch and H-reflex amplitudes in the soleus muscle.

In so doing, additional information on the mechanism of action during rhythmic arm cycling would be revealed.

Stretch and H-reflex share the same afferent pathway (Pierrot-Deseilligny and Mazevet 2000). However, H-reflex amplitudes are independent of the gamma regulation of muscle spindle excitability (Pierrot-Deseilligny E., and D. Mazevet 2000; Zehr 2002), while stretch reflex amplitudes are altered by changes in gamma motoneuron activity affecting muscle spindle sensitivity (Zehr 2002). The few previous studies comparing both stretch and H-reflexes suggest that they are modulated differently. For example, the soleus H-reflex was strongly suppressed by biceps femoris tendon tap conditioning, while the stretch reflex was weakly suppressed (Morita et al. 1998). Also, Shimba et al. (2010) found that soleus H-reflex amplitude during passive standing was smaller than in the supine posture condition. However, the stretch reflex was significantly larger in the passive standing than in the supine posture condition. Sinkjaer and colleagues found no significant differences in stretch reflex amplitudes during the stance phase of normal walking and static standing postures (Sinkjaer et al. 1996). Conversely, Capaday and Stein (1986) found that the H-reflex during the static standing postures was greater than during the stance phase of walking. During walking, Sinkjaer et al. (1996) found that in the late swing phase the stretch reflex had an amplitude of ~50% of the maximal amplitude in the stance phase of during normal walking. Interestingly, the amplitude of the H-reflex, is small or totally absent during the swing phase and comparatively larger in stance phase (Capaday and Stein 1986; Crenna and Frigo 1987).

The central motor commands controlling the rhythmic arm cycling appear responsible for the modulation of the soleus H-reflex (Frigon et al. 2004; Zehr et al.

2004; Zehr and Duysens 2004; Loadman and Zehr 2007; Hundza & Zehr 2009) with the main mechanism being modulation of Ia presynaptic inhibition (PSI) (Frigon et al. 2004). The differential modulation of stretch and H-reflexes has been explained by the different sensitivity to presynaptic inhibition of the Ia afferent terminals (Morita et al. 1998) and the activation of gamma motoneurons. Given this, we hypothesized that stretch reflex amplitudes would be less suppressed by arm cycling because they are less inhibited by presynaptic inhibition (Morita et al. 1998). Failure to reject this hypothesis would add additional strength to the argument that Ia presynaptic inhibition is the mechanism modulating soleus H-reflex amplitude during rhythmic arm cycling.

Methods and Materials

Participants

Eleven participants (4 female and 7 male) aged 21-38 years and with no history of neurological or metabolic disorders, were recruited for this study. All participants gave written consent to a protocol approved by the Human Research Ethics Board at the University of Victoria and in accordance with the declaration of Helsinki. Participants also completed a Physical Activity Readiness Questionnaire (Canadian Society for Exercise Physiology, Revised 2002).

Protocol

The experimental methodology is similar to that described in previous experiments of arm cycling (Zehr et al. 2003; Frigon et al., 2004; Loadman & Zehr 2007; Hundza & Zehr 2009). Participants performed static control trials (i.e., arms were stationary) and bilateral arm cycling trials in a clockwise direction at 1 Hz and 2 Hz on a hydraulic arm ergometer (Zehr et al. 2003). Participants were seated in a custom chair to reduced unwanted movement of the trunk and legs while the hip and knee were positioned in flexion at approximately 90 and 120 degrees, respectively (Loadman & Zehr 2007). The feet were strapped to footplates independent of the chair and the right ankle was positioned at approximately 100 degrees. During each trial, participants maintained a consistent low-level tonic contraction [(average of 6% of maximum voluntary contraction (MVC)] of the right soleus muscle and obtained feedback using a rectified and filtered EMG signal provided through a visual oscilloscope, (LabView National Instruments)**.** During cycling trials, participants obtained visual feedback of the frequency of arm cycling through an analog oscilloscope (Hameg 20MHz, HM205-3, Frankfurt/MAIN, Germany). If required, verbal encouragement was provided to the participants to maintain the appropriate level of contraction and arm cycling frequency.

Static control trials

Static control trials were performed with the right arm at the 7 o’clock position, and the left arm at the 1 o’clock position, as described previously (Klimstra et al. 2009; de Ruiter et al. 2010). (See figure 1). These control trials were completed for both stretch and H-reflexes.

Arm cycling trials

Stretch and H-reflexes were evoked at the 7 o’clock position for the right arm, while participants performed arm cycling at the two test frequencies (1 Hz and 2 Hz). The reflex amplitudes obtained during arm cycling trials were compared to those from control trials. Presentation of all trials was randomized.

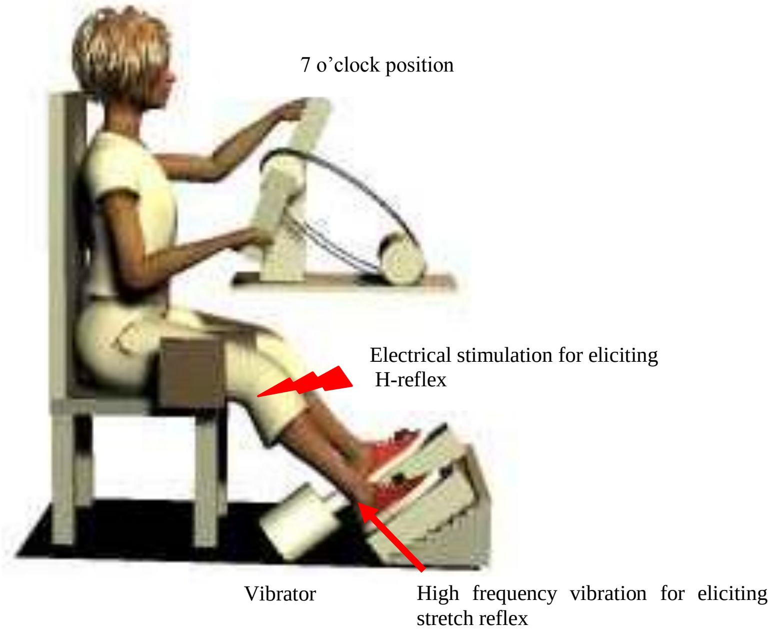

Figure 1: Schematic representation of the experimental design showing the right arm at the 7 o'clock position. Electrical stimulation was delivered at the tibial nerve to evoke the H-reflex. High frequency vibration was delivered at the achilles tendon to evoke the stretch reflex.

H-reflex

The tibial nerve of the right leg was stimulated with a single 1 ms square-wave pulse at the popliteal fossa using a bipolar surface electrode (Thought Technology Ltd.) and a Grass S88 stimulator (AstroMed Inc.) connected in series with a Grass SIU5 isolation unit, and a CCU1 constant current unit. Nerve stimulation was delivered pseudo-randomly with an inter-stimulus interval of 2 to 3 seconds during the static control trials. During arm cycling at 1 Hz, nerve stimulation was delivered every 2-3 cycles. At 2 Hz, nerve stimulation occurred every 4-6 cycles. Thus, a minimum of 2 seconds lapsed between nerve stimulations.

A constant motor response [(M-wave) (6.4% of M_{max} ±3.8 SD)], was maintained during all trials. This intensity evoked a H-reflex amplitude of approximately 83% of the H_{max} on the ascending limb of the recruitment curve. The M-wave was constantly monitored online with the use of an oscilloscope, adjusting the amplitude of the M-wave when needed to ensure consistent stimulation.

At the beginning and end of each experiment, H-reflex and M-wave recruitment curves (RC) were obtained by recording 30 sweeps of data, when subjects were in static control position. Maximal M-waves (M_{max}) and H-reflexes (H_{max}) were determined from the average of the three largest values. These values were used for data normalization and to verify that the H_{max} and M_{max} did not significantly change throughout the experiment.

Stretch reflex

Stretch reflexes were evoked using a high frequency vibration system consisting of a power amplifier and an electrodynamic shaker (ET-126; Labworks Inc), which was attached and fixed to a platform. The tip of the shaker was placed over the achilles tendon at the level between the medial and lateral malleoli. This experimental methodology is similar to that described in (Fornari & Kohn 2008 and Morita et al. 1998). Constant pressure was applied against the tendon to evoke a clear stretch reflex response throughout the experiment. The electrodynamic shaker was programmed to deliver a single sinusoidal pulse at a frequency of 100 Hz (custom written LabView software). An accelerometer was placed at the shaker’s tip to measure displacement, a proxy for the intensity of the high frequency vibration (Burke et al. 1981). High frequency vibration was delivered pseudo-randomly with an inter-stimulus interval of 2 to 3 seconds during both the static control and arm cycling trials.

A constant displacement of the vibration (2.8 mm ±0.016 SD) was maintained during all stretch reflex trials. The stimulation intensity evoked a stretch reflex amplitude of approximately 71% of the stretch reflex peak (SR_{peak}). The position of the tip of the shaker was marked on the participant's achillies tendon and this position was monitored throughout the experiment to ensure that each vibration was delivered to the same location. At the beginning and end of the experiment, stretch reflex RCs were obtained by recording 30 sweeps of data when subjects were in static control position. The SR_{peak} was determined by the average of the three largest values and this was used to verify that the SR_{peak} did not change throughout the course of the experiment.

Electromyography

Electromyographic (EMG) activity was recorded with surface electrodes (Thought Technologies Ltd.) using bipolar configuration from the following muscles on both sides of the body: soleus, (SOL), tibialis anterior (TA), vastus lateralis (VL), biceps femoris (BF) and anterior deltoid (AD). Ground electrodes were placed over the patella and clavicle. The skin underlying the surface electrodes was cleaned with alcohol to optimize signal clarity.

Data Acquisition and Analysis

Data were sampled at 5000 Hz with a 12-bit A/D converter controlled by a custom-written LabView program (National Instruments, Austin, Tx. USA). For all trials, 15 sweeps of data were recorded using a 100 ms sweep with 20 ms pre-stimulus to determine the level of muscle activation when the reflexes were evoked.

Right soleus EMG activity was preamplified, and band pass filtered at 100-1000 Hz and this channel was not rectified. EMG activity in the other muscles was preamplified with a gain of 5,000 rectified and bass pass filtered at 100-300 Hz (P511 Grass Instruments, AstroMed Inc.).

Peak to peak amplitudes of the stretch and H-reflexes were analyzed off line (custom written software, Matlab), and the average M_{max} of each participant was used to normalize their respective stretch, H-reflex and M-wave amplitudes. A maximum voluntary contraction (MVC) of the SOL was obtained at the end of the experiment and was used for normalization of pre-stimulus EMG.

Statistics

STATISTICA (StatSoft, Tulsa, OK., USA), was used to perform a 2 x 3 (reflexes x conditions) repeated measures analysis of variance (ANOVA) to identify significant main effects between stretch and H-reflexes amplitudes during static and arm cycling trials and pre-stimulus EMG amplitudes. In addition, a 1 way (ANOVA) was performed on M-waves and displacement of the vibrations as an addition control measurement. Tukey’s HSD post hoc test was used to identify significant statistical differences between the conditions. Student’s paired t-tests were conducted to determine the differences between the M_{max}, H_{max} and SR_{peak} values pre- and post arm cycling. Statistical significance was set at $p \leq 0.05$.

Results

Stretch and H-reflex amplitudes during arm cycling

The effect of arm cycling on the soleus stretch and H-reflex amplitude for a single subject is shown in Fig. 2. Reflexes are the average of 15 sweeps of data during static (black line), 1 Hz (grey line) and 2 Hz (dotted line) arm cycling. These reflexes are shown superimposed in the figures. The stimulus artefact, M-wave, stretch and H-reflex are indicated. It appears that the H-reflex amplitude was progressively suppressed while the frequency of arm cycling increased. The effect of arm cycling at both 1 and 2 Hz on the stretch reflex amplitude was minimal when compared to the static control.

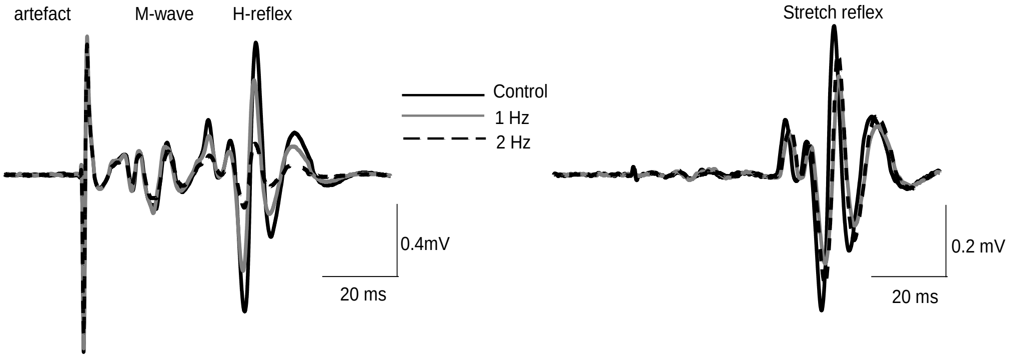

Figure 2: Soleus stretch and H-reflex amplitudes during static, 1 Hz and 2 Hz arm cycling in a single subject. Reflexes are represented for the average of 15 sweeps of data during static (black line) 1 Hz (grey line) and 2 Hz (dotted line). The stimulus artefact, M-wave, stretch and H-reflex responses are indicated.

The stretch and H-reflexes, M–wave amplitudes and displacement of the vibration averaged across all subjects are shown in Fig. 3. The group data have been normalized as a percentage of control (static) trial. The bars show 1Hz (black) and 2 Hz (grey) arm cycling for both reflexes. Asterisks indicate a statistically significant difference between arm cycling trials and static controls (identified by the black horizontal line at 100%). H-reflex amplitudes were graded with the frequency of arm cycling as seen in the single subject. H-reflex amplitude decreased to 67.8% at 1 Hz and 53.7% at 2 Hz (P = 0.0006, P = 0.0001) respectively. Arm cycling at either 1 Hz or 2 Hz did not induce significant suppressive effects on the soleus stretch reflex amplitude. The M-wave amplitudes and the displacement of the vibrations were stable across trials; there were no statistically significant differences seen comparing arm cycling and static control. In addition, the T-test showed that there were no statistical differences between pre-post M_{max}, H_{max} and SR_{peak}.

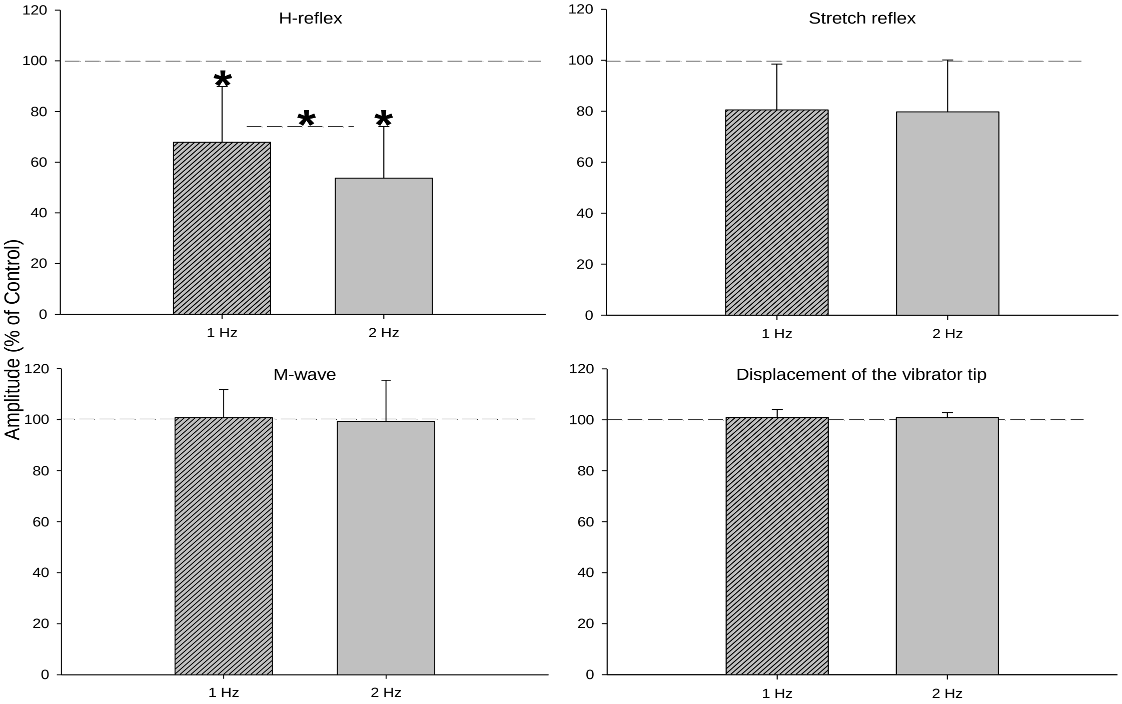

Figure 3: Soleus stretch and H-reflex peak to peak amplitudes at two frequencies of arm cycling. (a) Stretch and H-reflex amplitudes are expressed as a percentage of control (static). (b) In the second panel, M-wave (left) and displacement of the vibrator (right) are displayed in percentage of control (static). (c) Data are means and SD for all subjects (n = 11) recorded at 7 o'clock position (d) Asterisks (*) indicate statistically significant differences compared to static control (P<0.05).

Background EMG of leg muscles

The background EMG (bEMG) activity for all participants during static control and arm cycling trials for both stretch (grey bars) and H-reflexes (black bars) are shown in Fig. 4. The SOL bEMG was maintained at approximately 6% of the MVC throughout the experiment and there were no statistically significant differences between the static control, 1 Hz and 2Hz arm cycling trials. The TA, VL and BF muscle results were consistent at both static control and 1 Hz arm cycling. Post hoc analyses reveal a significant difference between static control and 2 Hz arm cycling, and between 1 Hz and 2 Hz arm cycling. Interestingly, the bEMG for all leg muscles behaved similarly for both stretch and H-reflex trials.

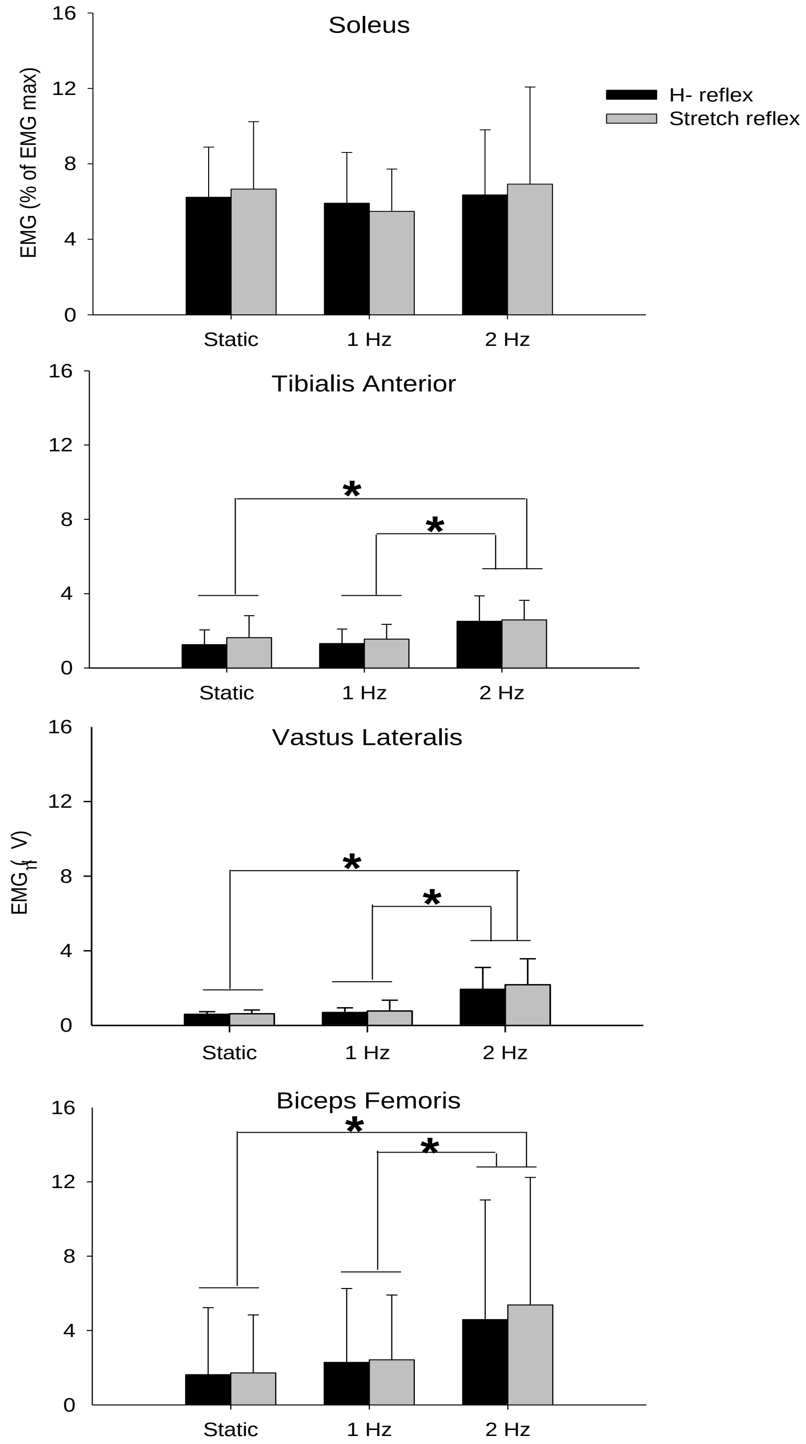

Figure 4: Background EMG for stretch and H-reflex. (a) The background EMG activity for stretch (grey bars) and H-reflex (black bars) are indicated. (b) Data are means and SD for all subjects (n = 11) (c) Asterisks (*) indicates statistically significant differences across conditions (P<0.05).

Discussion

In the present study a major novel result was obtained. Rhythmic arm cycling that was effective for conditioning soleus H-reflexes did not show a suppressive effect on the amplitude of the soleus stretch reflex. We suggest this indicates that stretch reflexes are less sensitive to conditioning by rhythmic arm movement, as compared to H-reflexes, due to the relative insensitivity of Ia presynaptic inhibition.

Methodological considerations

In this study, several methodological considerations were used to ensure control during the experiments and to integrate the changes in stretch and H-reflex amplitude due to arm cycling. First, the M-waves were maintained at the same level during all trials, this was indicated by a lack of significant differences, suggesting constancy of test reflex stimulus to the Ia afferents across the H-reflex trials (Brooke et al. 1997; Zehr 2002). Also, Burke et al. (1981) suggested that a constant displacement of the vibrator is a reliable measure of the afferent response for the stretch reflex. This observation was confirmed through control experiments (N=2). A trial, consisting of ten soleus stretch reflexes evoked in seated subjects, was conducted every five minutes for one hour. In total, one hundred and twenty stretch reflexes were evoked. The amplitude of the first ten stretch reflexes were not statistically different from the last ten reflexes. Second, the bEMG of the soleus muscle was consistent (6% MVC) throughout the experiment. This was done to ensure similar soleus motoneuronal activation, and to decrease the variability, latency and to optimize the stretch and H-reflex response from trial to trial. (Burke et al. 1989; Funase & Miles 1999).

Finally, since heteronymous muscle activity influences the soleus H-reflex amplitude (Hultborn et al. 1987a; Crone & Nielsen 1994; Morita et al. 1998; Pierrot-Deseilligny & Mazevet 2000), bEMG activity was recorded to monitor possible effects on the soleus stretch and H-reflex amplitude. The TA, VL and BF muscles showed no statistically significant differences from static control relative to 1 Hz arm cycling. Therefore, the bEMG activity of the above muscles cannot be implicated as a source of the significant suppression seen on H-reflex nor the relatively minimal level of suppression seen on the stretch reflex amplitudes between static control and 1 Hz arm cycling. However, at 2Hz the TA, VL and BF muscles during both reflexes testing sessions were significantly different relative to static control (See Fig. 4). This modulation of bEMG activity seen at 2 Hz arm cycling appeared to cause a passive recruitment of motor unit activity in lower limb muscles. This occurred when subjects' upper limbs increased the frequency of movement on recumbent stepping machines (Kao & Ferris 2005) and in arm cycling studies (Loadman & Zehr 2007; Hundza & Zehr 2009). The increase in bEMG in the TA could lead to an inhibitory response on the soleus H-reflex amplitude due to reciprocal inhibition. However, during constant soleus contraction, this effect is reduced (Tanaka 1974; Petersen et al. 1998; Pierrot-Deseilligny & Burke 2005). Moreover, any suppression caused via reciprocal inhibition could be countered by heteronymous Ia facilitation arising from the VL as described by Bergmans et al. 1978; Hultborn et al. 1987b; Meunier et al. 1993. In addition, in previous experiments of arm cycling, the changes in the bEMG in TA, VL and BF muscles has been shown to be unrelated to changes in soleus H-reflex amplitude (Hundza & Zehr

2009), and are therefore unlikely to have been responsible for the suppression seen on H-reflex at 2 Hz arm cycling presented in this study.

Why are stretch reflexes less sensitive to conditioning by rhythmic arm movement compared to H-reflexes?

In the present study, the stretch reflexes amplitudes were not significantly modulated by arm cycling in contrast to the H-reflexes. Previously, it had been proposed that during rhythmic arm cycling, soleus H-reflex amplitudes were reduced due to descending influences increasing presynaptic inhibition of Ia afferent transmission (Frigon et al. 2004). In addition, it has been suggested that central pattern generators (CPG) involved in generating rhythmic arm movement might be responsible for the descending modulation affecting reflex pathways in the lumbar spinal cord (Frigon et al. 2004; Loadman & Zehr 2007; Hundza & Zehr 2009). However, even though stretch and H-reflexes share the same monosynaptic afferent pathway (Pierrot-Deseilligny and Mazevet 2000) we found that in contrast to H-reflex, the stretch reflex is not affected by arm cycling that conditions H-reflexes. In another study, where both reflexes were compared, Shimba et al. (2010) found that the H-reflex amplitude in passive standing was smaller than in the supine postures position; meanwhile, the stretch reflex was significantly larger in the supine postures than in the passive standing condition. The different modulation observed between the stretch and H-reflex may be explained by the different sensitivity to PSI of the Ia afferent terminals and the activation of gamma motoneurons.

The previous statement can be supported by former findings that suggest that the stretch reflex is less sensitive to Ia presynaptic inhibition than the electrically evoked reflex. This could be due to the different temporal dispersion and composition of the afferent input produced by the two stimuli (Morita et al. 1998). That is, vibration over the achilles tendon may produce disperse action potentials. The same Ia afferent may fire several times at short intervals (5 ms), thus the action potentials that travel along the Ia afferents will arrive at the spinal cord unsynchronized (Burke et al. 1983). Therefore, the excitatory postsynaptic potentials (EPSP) are produced by spatial (Enriquez-Denton et al. 2002) and temporal summation (Morita et al. 1998). In contrast, the H-reflex is known to evoke a synchronized activation of Ia afferents. Each afferent discharge only once (Enriquez-Denton et al. 2002) and they are slightly dispersed, producing EPSPs largely by spatial summation (Morita et al. 1998). Morita et al. (1998) also found that in humans Ia presynaptic inhibition produced by applying a tendon tap to the biceps femoris evoked a significant depression of the soleus H-reflex, but weakly suppression of the stretch reflex. Interesting, Enriquez-Denton et al. (2002) evaluated the effect of preceding activity in afferent fibers on the magnitude of presynaptic inhibition in cats. They found 9.6% reduction of presynaptic inhibition when a train of five pulses was applied to homonymous nerve preceded by single pulse test stimulation on the MG motoneuron. As a result, it is possible that presynaptic inhibition of the Ia afferent may more effectively reduce the EPSP produced by synchronized action potentials evoked by the H-reflex than the disperse burst of action potentials produced by the stretch reflex (Morita et al. 1998).

Another possible explanation for the reduced amount of suppression seen in the stretch reflex amplitude during arm cycling may be due to differences in the composition of the EPSP produced by the high frequency vibrator and the electrical stimulation. Stretch reflex may have a higher proportion of Group II and other non-monosynaptic influences in the EPSP than the H-reflex (Burke et al. 1983; 1984). Morita et al. (1998) suggest that both stretch and H-reflex activates afferents other than Group Ia, such as Group Ib, group II, and cutaneous afferents. Also, (Burke et al. 1983) proposed that both reflexes could activate, not only mechanoreceptors from the triceps surae, but also skin mechanoreceptor. As evidenced by (Cavallari et al. 1987), group Ia and II may have non-monosynaptic projection to homonymous motoneurons, and because group II afferents are not affected by presynaptic inhibition of the Ia afferent (Baldissera et al. 1981), the stretch reflex would therefore be less affected by PSI coming from arm cycling.

Finally, the different modulation seen during stretch and H-reflex might be due to the gamma regulation. Previously, Gregory et al. (1998) demonstrated that muscle length and different level of contractions produce different reflex sizes. Those scholars suggested that 5% MVC activates the fusimotor system. Also, they found that during 10% MVC, this activation is great enough to produce a facilitation of the soleus stretch reflex in humans. In the present study, the potential of activation of the fusimotor system exists due to tonic contraction of the soleus muscle (6% MVC). Also, the increased frequency of arm cycling might enhance the activation of the soleus gamma motor neuron. This increase of gamma motor neuron may lead to an excitability of the muscle spindles, producing greater amplitude of the stretch reflex. This bigger reflex size may be counteracted by increased presynaptic inhibition coming from arm movement. This may explain why in contrast to the H-reflex, the stretch reflex amplitude during 2 Hz was similar to 1 Hz arm cycling. Finally, to obtain a more conclusive observation about the modulation of the soleus stretch reflex due to remote movement, a bigger sample size would be needed in future experiments.

In summary, stretch and H-reflexes may be affected differently by presynaptic inhibition of the Ia afferent due to the differences of temporal dispersion, composition of the afferent volley and gamma motor neuron activation.

Functional Implications

The findings in the present study are consistent with the idea put forward by Ferris et al. (2006) that rhythmic arm movements have the potential to enhance the coordination of the lower limbs muscle activation after neurotrauma. Our results confirm that arm cycling suppresses the soleus H-reflex when legs are stationary and that this suppression is related to the frequency of arm cycling as seen previously by (Hundza and Zehr 2009). Therefore, it might be important to consider the frequency of arm cycling not only to increase the intensity of the therapeutic intervention, but also to increase the interlimb neural coupling. Even though the soleus H-reflex was significantly suppressed, the stretch reflex was not modulated by arm cycling. These further suggest that these two reflexes respond differently to remote movement due to the different sensitivity to presynaptic inhibition of the Ia afferent and the regulation of gamma motoneurons. Nevertheless, it has been demonstrated that prolonged arm cycling (~30 min), induces persistent suppression effect of the soleus H-reflex amplitude up to 20 minutes after the end of arm movement (Javan and Zehr 2008). In addition, arm cycling may influence lumbar networks mediating H-reflex excitability after stroke (Barzi and Zehr 2008). Recently, experiments were conducted using subjects that had suffered strokes. It was revealed that arm cycling suppresses the soleus stretch reflex in 50% of this population (Unpublished data, Mezzarane et al. 2011). Further studies are required to examine if arm cycling has the potential to decrease spasticity.

Acknowledgments This work was supported by grants to E.P.Z. from the Heart and Stroke Foundation of Canada (BC & Yukon) and the Natural Sciences and Engineering Research Council of Canada (NSERC).

References

Baldissera F, Hultborn H, Illert M (1981) Integration in spinal neuronal system. Handbook of Physiology. The Nervous System. Motor Control. Washington, DC: Am. Physiol. Soc 509-595

Barzi Y, Zehr EP (2008) Rhythmic arm cycling suppresses hyperactive soleus H-reflex amplitude after stroke. Clin Neurophysiol 119:1443-1452. DOI 10.1016/j.clinph.2008.02.016

Bergmans J, Delwaide PJ, Gadea-Ciria M (1978) Short-latency effects of low-threshold muscular afferent fibers on different motoneuronal pools of the lower limb in man. Exp Neurol 60:380-385

Borst JG, Sakmann B (1998) Facilitation of presynaptic calcium currents in the rat brainstem. J Physiol 513 (Pt 1):149-155

Brooke JD, Cheng J, Collins DF, McIlroy WE, Misiaszek JE, Staines WR (1997) Sensori-sensory afferent conditioning with leg movement: gain control in spinal reflex and ascending paths. Prog Neurobiol 51:393-421

Burke D, Adams RW, Skuse NF (1989) The effects of voluntary contraction on the H reflex of human limb muscles. Brain 112 (Pt 2):417-433

Burke D, Gandevia SC, McKeon B (1984) Monosynaptic and oligosynaptic contributions to human ankle jerk and H-reflex. J Neurophysiol 52:435-448

Burke D, Gandevia SC, McKeon B (1983) The afferent volleys responsible for spinal proprioceptive reflexes in man. J Physiol 339:535-552

Burke D, McKeon B, Skuse NF (1981) Dependence of the Achilles tendon reflex on the excitability of spinal reflex pathways. Ann Neurol 10:551-556. DOI 10.1002/ana.410100610

Capaday C, Stein RB (1986) Amplitude modulation of the soleus H-reflex in the human during walking and standing. J Neurosci 6:1308-1313

Cavallari P, Edgley SA, Jankowska E (1987) Post-synaptic actions of midlumbar interneurones on motoneurones of hind-limb muscles in the cat. J Physiol 389:675-689

Crone C, Nielsen J (1994) Central control of disynaptic reciprocal inhibition in humans. Acta Physiol Scand 152:351-363

Curtis DR, Eccles JC (1960) Synaptic action during and after repetitive stimulation. J Physiol 150:374-398

Cuttle MF, Tsujimoto T, Forsythe ID, Takahashi T (1998) Facilitation of the presynaptic calcium current at an auditory synapse in rat brainstem. J Physiol 512 (Pt 3):723-729

de Ruiter GC, Hundza SR, Zehr EP (2010) Phase-dependent modulation of soleus H-reflex amplitude induced by rhythmic arm cycling. Neurosci Lett 475:7-11. DOI 10.1016/j.neulet.2010.03.025

Dietz V (2003) Spinal cord pattern generators for locomotion. Clin Neurophysiol 114:1379-1389

Dietz V (2002) Do human bipeds use quadrupedal coordination? Trends Neurosci 25:462-467

Enriquez-Denton M, Morita H, Christensen LO, Petersen N, Sinkjaer T, Nielsen JB (2002) Interaction between peripheral afferent activity and presynaptic inhibition of ia afferents in the cat. J Neurophysiol 88:1664-1674

Ferris DP, Huang HJ, Kao PC (2006) Moving the arms to activate the legs. Exerc Sport Sci Rev 34:113-120

Fornari MC, Kohn AF (2008) High frequency tendon reflexes in the human soleus muscle. Neurosci Lett 440:193-196. DOI 10.1016/j.neulet.2008.05.075

Frigon A, Collins DF, Zehr EP (2004) Effect of rhythmic arm movement on reflexes in the legs: modulation of soleus H-reflexes and somatosensory conditioning. J Neurophysiol 91:1516-1523. DOI 10.1152/jn.00695.2003

Funase K, Miles TS (1999) Observations on the variability of the H reflex in human soleus. Muscle Nerve 22:341-346

Gernandt BE, Megiriand D (1961) Ascending propriospinal mechanisms. J Neurophysiol 24:364-376

Gernandt BE, Shimamura M (1961) Mechanisms of interlimb reflexes in cat. J Neurophysiol 24:665-676

Gregory JE, Wise AK, Wood SA, Prochazka A, Proske U (1998) Muscle history, fusimotor activity and the human stretch reflex. J Physiol 513 (Pt 3):927-934

Hultborn H, Meunier S, Morin C, Pierrot-Deseilligny E (1987) Assessing changes in presynaptic inhibition of I a fibres: a study in man and the cat. J Physiol 389:729-756

Hultborn H, Meunier S, Pierrot-Deseilligny E, Shindo M (1987) Changes in presynaptic inhibition of Ia fibres at the onset of voluntary contraction in man. J Physiol 389:757-772

Hundza SR, Zehr EP (2009) Suppression of soleus H-reflex amplitude is graded with frequency of rhythmic arm cycling. Exp Brain Res 193:297-306. DOI 10.1007/s00221-008-1625-0

Javan B, Zehr EP (2008) Short-term plasticity of spinal reflex excitability induced by rhythmic arm movement. J Neurophysiol 99:2000-2005. DOI 10.1152/jn.01315.2007

Kao PC, Ferris DP (2005) The effect of movement frequency on interlimb coupling during recumbent stepping. Motor Control 9:144-163

Klimstra MD, Thomas E, Stoloff RH, Ferris DP, Zehr EP (2009) Neuromechanical considerations for incorporating rhythmic arm movement in the rehabilitation of walking. Chaos 19:026102. DOI 10.1063/1.3147404

Loadman PM, Zehr EP (2007) Rhythmic arm cycling produces a non-specific signal that suppresses Soleus H-reflex amplitude in stationary legs. Exp Brain Res 179:199-208. DOI 10.1007/s00221-006-0782-2

Mendell LM (1984) Modifiability of spinal synapses. Physiol Rev 64:260-324

Meunier S, Pierrot-Deseilligny E, Simonetta M (1993) Pattern of monosynaptic heteronymous Ia connections in the human lower limb. Exp Brain Res 96:534-544

Morita H, Petersen N, Christensen LO, Sinkjaer T, Nielsen J (1998) Sensitivity of H-reflexes and stretch reflexes to presynaptic inhibition in humans. J Neurophysiol 80:610-620

Peshori KR, Collins WF, Mendell LM (1998) EPSP amplitude modulation at the rat Ia-alpha motoneuron synapse: effects of GABAB receptor agonists and antagonists. J Neurophysiol 79:181-189

Petersen N, Morita H, Nielsen J (1998) Evaluation of reciprocal inhibition of the soleus H-reflex during tonic plantar flexion in man. J Neurosci Methods 84:1-8

Pierrot-Deseilligny E, Mazevet D (2000) The monosynaptic reflex: a tool to investigate motor control in humans. Interest and limits. Neurophysiol Clin 30:67-80

Rudomin P, Schmidt RF (1999) Presynaptic inhibition in the vertebrate spinal cord revisited. Exp Brain Res 129:1-37

Schneider C, Lavoie BA, Capaday C (2000) On the origin of the soleus H-reflex modulation pattern during human walking and its task-dependent differences. J Neurophysiol 83:2881-2890

Shimba S, Kawashima N, Ohta Y, Yamamoto S, Nakazawa K (2010) Enhanced stretch reflex excitability in the soleus muscle during passive standing posture in humans. J Electromyogr Kinesiol 20:406-412. DOI 10.1016/j.jelekin.2009.04.003

Sinkjaer T, Andersen JB, Larsen B (1996) Soleus stretch reflex modulation during gait in humans. J Neurophysiol 76:1112-1120

Stein RB (1995) Presynaptic inhibition in humans. Prog Neurobiol 47:533-544

Stuart GJ, Redman SJ (1991) Mechanisms of presynaptic inhibition studied using paired-pulse facilitation. Neurosci Lett 126:179-183

Tanaka R (1974) Reciprocal Ia inhibition during voluntary movements in man. Exp Brain Res 21:529-540

Zehr EP, Collins DF, Frigon A, Hoogenboom N (2003) Neural control of rhythmic human arm movement: phase dependence and task modulation of hoffmann reflexes in forearm muscles. J Neurophysiol 89:12-21. DOI 10.1152/jn.00416.2002

Zehr EP, Duysens J (2004) Regulation of arm and leg movement during human locomotion. Neuroscientist 10:347-361. DOI 10.1177/1073858404264680

Zehr EP, Haridas C (2003) Modulation of cutaneous reflexes in arm muscles during walking: further evidence of similar control mechanisms for rhythmic human arm and leg movements. Exp Brain Res 149:260-266. DOI 10.1007/s00221-003-1377-9

Zehr PE (2002) Considerations for use of the Hoffmann reflex in exercise studies. Eur J Appl Physiol 86:455-468. DOI 10.1007/s00421-002-0577-5