Phase-Dependent Modulation of the Soleus H-Reflex Induced by Rhythmic Arm Cycling

by

Geoffrey Charles de Ruiter
B.Sc. in Biology, University of Northern British Columbia, Canada, 2006

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

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In the School of Exercise Science, Physical & Health Education

SUPERVISORY COMMITTEE

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ABSTRACT

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ABSTRACT

Rhythmic arm cycling is known to suppress the Hoffmann (H-) reflex in the soleus muscles of stationary legs; however, it is still unclear if this suppression is modulated by the phase of movement in the cycle path. In the present study we investigated phase-dependent modulation of the Sol H-reflex induced by rhythmic arm cycling. Modulation of the Soleus H-reflex was examined at 12 phases of the cycle path in 4 conditions; static arm positioning, as well as 3 arm cycling conditions, bilateral, ipsilateral and contralateral. H-reflexes were evoked and recorded at constant motor wave amplitudes across the conditions. Suppression of Sol H-reflex amplitude was dependent (main effect $p<0.0001$) upon the phase of movement during arm cycling, but not during static positioning. Results suggest that locomotor central pattern generators may contribute to the phasic reflex modulation observed in this study.

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DEDICATION

This thesis is dedicated to the effort and advancement of science and the future generations that will benefit from this item of information. Secondly this project dedicated to my closest and most loved family, including friends, you know who you are.

CHAPTER 1: Introduction and review of literature

The study of human locomotion is a highly defined field of motor control research as it incorporates many interconnecting topics. One specific research focus within the field of human locomotion has been activity-dependent reflex plasticity. Activity-dependent reflex plasticity can be defined as the change of synaptic connectivity due to prior synaptic activity (Johnson and Mitchell 2000). In a review of activity-dependent reflex plasticity, Zehr (2006) suggested that spinal reflex excitability is dependent on the type of motor task performed, i.e. is modulated with varying degree from sitting to standing to running. Further, reflex modulation dependent on the phase of movement has also been shown. In response to rhythmic activity, Crenna and Frigo (1984) initially demonstrated modulation of nociceptive reflexes based on the phases of the rhythmic movement cycle. Later, Capaday and Stein (1986) were first to demonstrate that the soleus Hoffmann (H-) reflex exhibited phase-dependency during the human gait cycle. This was repeated soon after by Crenna and Frigo (1987) and Capaday and Stein (1987), and then in arm muscles by Zehr et al. (2003). On the other hand, the influence of remote rhythmic limb activity on reflex amplitudes, also known as interlimb reflexes, remains unclear especially in regards to phase-dependency.

The purpose of this review is to outline the literature examining phase-dependent modulation of the H-reflex, specifically of the soleus muscle, in response to rhythmic activity. Within this review of literature the governing role of central pattern generators in regulating neural interlimb coupling and reflex modulation and plasticity of the spinal cord circuitry will be examined. Finally, the hypothesis that there will be phase-dependent modulation of soleus H-reflex in response to rhythmic arm cycling will be discussed.

Central pattern generators (CPGs)

In all forms of animal locomotion, rhythmic activity forms the basis of locomotion. Fish, dolphins, alligators, horses, birds, humans and apes use cyclical or repeating limb or body movements in order to propel themselves. The question remains, however, as to how exactly the nervous system directs rhythmic limb activity. Central pattern generators (CPGs) are known in many animals to be the primary locus shown to direct rhythmic activity. Although not initially interpreted as evidence for CPGs, Sherrington (1906) described rhythmic movement in spinalized dogs when an external mechanical or electrical irritant was applied behind the ear. This stimulus specifically produced rhythmic ear scratching of the hind legs when the same leg was moved into close proximity of the ear. Brown (1911) then showed that spinalized and deafferented cats were able to produce reciprocal flexion and extension activity in hind limb muscles. Since then, the existence of CPGs in animals has been examined and well characterized (reviewed in Grillner and Wallén 1985; Duysens 1998; Dietz 2003; Zehr et al. 2004), but yet questions still remain.

The simplest model of a CPG circuit was proposed by Brown (1911) and his half-centre model (Figure 1). The half-centre model consists of six neurons, which produce alternating patterns of flexion and extension around a joint. In this model, the cycle is initiated via tonic descending neural input commencing flexor and extensor interneuron activity (shown as F and E, respectfully). These flexor and extensor interneurons excite the pools of motoneurons and secondarily inhibitory interneurons (shown in solid black), which create a cycle of reciprocal inhibition of the flexor and extensor interneurons. In sum, this creates the reciprocal activation of flexor and extensor muscles to produce rhythmic movement.

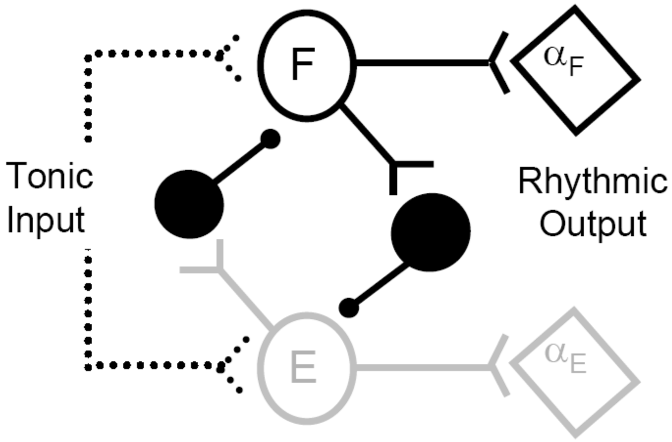

Figure 1. Schematic diagram of the half-center model proposed by Brown (1911) showing flexor (F) and extensor (E) interneurons, inhibitory interneurons (solid black), and the flexor and extensor α-motor neurons. Adapted from Zehr (2006).

The half-centre model best describes rhythmic activity at individual joints. For entire limbs, it has been proposed that many individual half-centre modules work in concert to produce more complex movement patterns including intralimb and interlimb interactions with segments spanning the spinal cord (Grillner 2006). Research has also added to and refined the concept of the CPG and has shown that many additional neurons are involved in its operation (Grillner 1981). Ultimately, the current proposed model has become more intricate than that of Brown's original model (reviewed in Barbeau et al. 1999). This includes the expansion of a multi-layered CPG, which accounts for rhythm generation and pattern formation (Lafreniere-Roula and McCrea 2005).

Due to the sometimes invasive nature of exploring CPGs in animals, such as tissue excising and lesioning, direct evidence for demonstrating the existence for CPGs in humans has been more difficult to acquire and harder to describe (Barbeau et al. 1999).

Instead, most investigations have focused on indirect and inferential evidence. In a case study report by Calancie et al. (1994), a subject, after 17 years with an incomplete spinal cord injury (SCI) temporarily gained improved ability to produce stepping movements while lying supine with hips extended. EMG and kinetic analysis showed that leg movements were similar to that of normal walking. This has been repeated to some extent in other incomplete SCI individuals who, when supported over a treadmill and provided tactile sensory cues to the legs and feet, could produce hip and knee flexion and extension patterns at approximately 1 Hz (Maegele et al. 2002). Infants can also demonstrate walking-like patterns when supported and standing on a treadmill (Yang et al. 2004). This is striking because it occurs despite the lack of a fully developed coritcospinal tract, and thus the ability to cortically or volitionally control limb movements. In adults with complete SCI, electrical stimulation of the dorsal spinal cord (L2-L3) has been shown to initiate rhythmic stepping and reciprocally timed EMG bursts similar to those observed during walking (Gerasimenko et al. 2002; Dimitrijevic et al. 1998). Altogether, these observations support the existence of CPGs in humans and their likelihood of being located subcortically to direct rhythmic limb movement.

Supraspinal and somatosensory input to CPGs

There are generally two systems that are known to regulate and provide integral information to sculpt CPG output, the supraspinal and sensory afferent systems. Both these inputs are known to initiate and/or alter the frequency and amplitude of locomotion directed by CPGs (Barbeau et al. 1999; Duysens and Van de Crommert 1998; MacKay-Lyons 2002; Zehr 2005). From lampreys to primates the main location of initiation for CPG activity is from the mesencephalic locomotor region of the midbrain (brainstem) (reviewed in Whelan 1996). This has been tested through stimulation of the mesencephalic locomotor region by electrical stimulation or exogenous applications of neurotransmitters (Shefchyk and Jordan. 1985; Atsuta et al. 1990; Atsuta et al. 1991; reviewed in Gerasimenko et al. 2008).

Neural control of rhythmic locomotion involves many types of sensory feedback including nociception, and other cutaneous sensations, such as vibration or pressure. These feedback mechanisms specifically aid in generating and modifying CPGs and locomotion (Wolf and Pearson 1988). To demonstrate that CPGs may be initiated through the somatosensory system, many studies have been able to retrain spinalized cats and dogs to walk (reviewed in Wolpaw and Tennissen 2001). Additionally, recent research on partial or incomplete SCI has also demonstrated that through locomotor training a 4-year-old boy (Behrman et al. 2008) and rat pups (Brown et al. 2005) were able to regain partial walking ability.

It has been shown in cats and dogs that isolation of CPGs from afferent feedback via deafferentation continues to allow for locomotion to be generated through direct or supraspinal CPG stimulation, however and most interestingly, with reduced efficacy (Barbeau et al. 1999). Reduced efficacy of CPG activity from deafferentation was also shown in locust; when deafferented and placed in a wind tunnel, the locust was unable to maintain flight (Wolf and Pearson 1988; Wolf and Pearson 1989). Ultimately, supraspinal and somatosensory feedback are not exclusively required for CPG initiation and/or activity, although they do help to sculpt the output of CPGs during rhythmic tasks.

The stretch reflex

A highly studied topic in the field of somatosensory feedback has been the stretch reflex and its electrical analog the H-reflex, shown in Figure 2. Stretch reflexes are brief contraction of muscle fibres in response to mechanical stimuli induced by a rapid stretch of the homonymous muscle stretch receptors. The reflex is initiated by stretch sensitive receptors, known as muscle spindles, which when excited to threshold send action potentials along Ia afferent neurons to the spinal cord via the dorsal root leading to depolarization of α-motor neurons (indicated as α in Figure 2), which ultimately activates the muscle, producing a contraction.

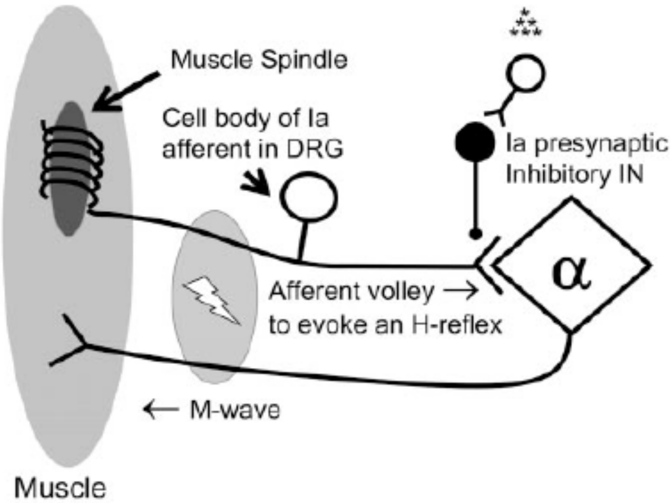

Figure 2. Schematic diagram of an H-reflex (electrical stimulation indicated via gray ellipse and super-imposed lightning bolt). Adapted from Zehr (2006).

Spinal reflexes have been widely used to investigate activity in the human nervous system (Wolpaw et al. 2006; Stein et al. 1993). For these types of studies, the H-reflex is often used because it offers control and predictability during the testing of spinal reflex excitability. The H-reflex is produced by electrical stimulation of a mixed peripheral nerve (shown within the gray ellipse and super-imposed lightning bolt of

Figure 2), which encompasses both afferent and efferent muscle neurons. Modulation of the H-reflex amplitude is now commonly believed to be from modulation of presynaptic inhibition (PSI) acting on the Ia primary afferent terminal (seen in Figure 2) (reviewed in Stein 1995; Brooke et al. 1997). The H-reflex is generally considered to be a monosynaptic connection; although, due to a relatively long post synaptic depolarization on the motorneuron, influences from other afferent fibers, such as from type II afferents or inhibition from activation of golgi tendon organs, may affect the latter segment of the H-reflex waveform indicating an oligosynaptic reflex (reviewed in Zehr 2002; Misiaszek 2003).

Neural interlimb coupling of CPGs and reflexes

Quadrupedal locomotion inherently demonstrates the need for coordination between all four limbs. This coordination is referred to as interlimb coordination, and anatomical and physiological evidence for its presence has been well established with propriospinal neurons connecting cervical enlargements to L2 and S2 (Skinner et al. 1980; Krutki et al. 1998) and from the sacral regions to C6, the cerebellum, and reticular formation (Grottel et al. 1998). Neurological evidence for interlimb coordination has been shown in the form of interlimb reflexes (Miller et al. 1973), which has been repeated with varying experimental protocols (Kearney and Chan 1981; Dietz et al. 1994). Interlimb reflex responses are evoked by electrically stimulating either the arms or legs, and recorded in a heteronymous limb segment. Interlimb reflexes have also been shown to affect the excitability of motor neurons in remote arms and legs (Miller et al. 1973; Schomburg and Behrends, 1978).

Interlimb coupling has not only been shown in reflex studies involving limbs, but also by coupling between cervical and lumbar locomotor CPGs. In experiments performed by Juvin et al. (2005), cervical and lumbar CPGs were found to be interconnected in the isolated spinal cords of newborn rats. Specifically, propriospinal neurons connecting to lumbar CPGs were shown to direct the phasic activity of cervical CPGs. In addition, when the two sections were decoupled via chemical nerve block, rhythmic activity of the cervical CPG continued to produce regular locomotor-like activity, but with decreased oscillatory frequency.

Although evidence of interlimb coupling is widely shown in many non-human animal species, the evidence of interlimb coordination and coupling in humans is more limited and indirect; however evidence does exist. Elftman (1939) originally described the connection of arms to legs as a biomechanical coupling to decrease vertical axis rotation of the body during gait. Elftman (1939) also discussed that this pattern was not simply passive, i.e. pendular, but active as indicated by arm muscle forces producing torque. This active arm movement was later supported by Fernandez-Ballesteros et al. (1965), who demonstrated rhythmic EMG burst patterns in the shoulders during walking. Observations of walking, crawling on all fours, and variations of swimming have also shown that regardless of the type of locomotor activity, arm and leg coordination remains coupled in upper versus lower limb frequencies (Wannier et al. 2001). This suggests that the arm and leg segments are linked via two coupled oscillators (CPGs), which are similar as observed in quadrupedal locomotion (Wannier et al. 2001).

Anatomical research of the human spinal cord has shown long propriospinal neurons connecting cervical and lumbar enlargements (Nathan et al. 1996). Additionally, Zehr et al. (2001) showed evidence for coupling between interlimb neural centres, by electrically evoking reflexes and measuring interlimb responses. Cutaneous nerves were electrically stimulated at either the ankle or the wrist in separate trials in order to observe a reflex response in heteronymous limbs. It was found that reflex responses occurred bilaterally and between the arms and legs. Some reflex latencies were also too short for the response to be transcortical, suggesting propriospinally mediated interlimb reflexes. Special populations, such as individuals with SCI, have also been extremely valuable for demonstrating interlimb coupling. SCI individuals have been shown to demonstrate the ability to produce interlimb reflexes with absent or reduced influence of supraspinal intervention (Calancie 1991; Calancie et al. 1996).

Modulation of H-reflex amplitudes in different tasks has been used as another indication for evidence for interlimb coupling. In an experiment with neurologically intact participants, Zehr et al. (2007) found that rhythmic cycling of the legs modulated the flexor carpi radialis H-reflex of the stationary arm. Research performed by Frigon et al. (2004) found that cycling of the arms suppressed the soleus H-reflex, again indicating interlimb reflex coupling. This interlimb reflex coupling is not only in the cycling paradigm, but many rhythmic movements can suppress/modulate interlimb H-reflexes. One example is from Baldissera et al. (1998), where rhythmic flexion-extension movements of the foot induced cyclic modulation of the ipsilateral flexor carpi radialis H-reflex. Overall remote limb movement suppressed reflex amplitudes; however the amount of suppression was dependent on the position of the foot. Varied or graded suppression of interlimb reflexes has also been shown to be relative to the frequency of movement. Specifically, increased frequency of rhythmic cycling of the arms has been shown to produce a graded suppression of H-reflexes of the soleus (Loadman and Zehr 2007; Hundza and Zehr 2009).

Phase-dependent modulation of the H-reflex

Neural control can be probed by the study of intralimb and interlimb reflex responses and both intra- and interlimb modulations of H-reflexes have been shown to be task- and phase-dependent (for reviews see Brooke et al. 1997; Zehr 2002; Zehr et al. 2004). Task dependency is the modulation of a reflex specific to the type of activity being performed, for example, across walking, running or cycling. More specifically, task-dependency is the change of reflex sign or amplitude between different motor tasks (Zehr et al. 2004). Phase-dependent modulation is the significant change in reflex amplitude at various positions along a movement cycle. Phase-dependence is shown in Figure 3 where Soleus H-reflex amplitudes, normalized to M_{max} (maximum recorded value of the motor wave) are almost entirely diminished at 60-80% of the cycle phase (McIlroy et al. 1992). Phase-dependent responses have been shown in both electrically-evoked cutaneous reflexes (Crenna and Frigo 1984; Belanger and Patla 1984) and mechanical reflex responses (Crenna and Frigo 1987). A review by Brooke et al. (1997) stated quite succinctly the principal goal of phase-dependency “may be to maximize the trade-off between avoiding the source of the strong stimulus and preserving both balance and the rhythm of locomotion.”

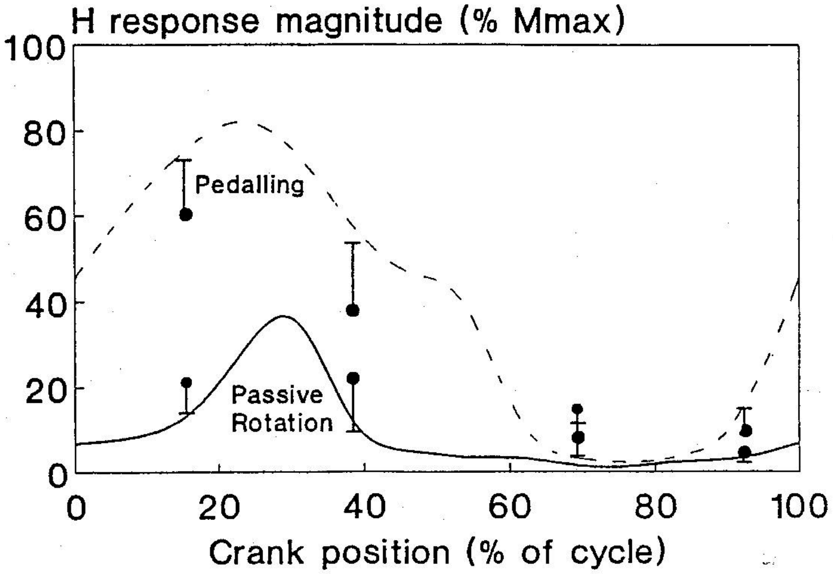

Figure 3. Soleus H-reflex level (normalized as a percentage of M_{max}) during active and passive cycling showing phase-dependent modulatory suppression at 60-80% of the cycling position (McIlroy et al. 1992).

As discussed earlier, alterations in the level of Ia PSI is considered to be a main influence in H-reflex modulation during movement (Nielsen and Kagamihara 1993; Frigon et al. 2004; reviewed in Brooke et al. 1997; Stein 1995). In the cat model, Gossard (1996) performed an experiment showing that phase-dependent modulation of the soleus H-reflex was caused via fluctuating PSI. Gossard (1996) then stated that CPGs were involved in the modulation of Ia primary afferents through PSI (topic reviewed in Stein 1995). In humans, this concept has also been discussed by Zehr et al. (2004) stating that CPG influences provide a plausible mechanism for reflex phase-dependency through premotoneuronal gating via PSI.

Two motor tasks have been used to explore the process of CPG phase-dependent control of H-reflex amplitudes: passive limb movement and stationary limb positions. Phase-modulated H-reflexes during passive movement has been suggested to indicate that peripheral afferent feedback generated by the discharging of kinesthetic receptors during movement is an initiator of suppression (Brooke et al. 1997). However it has also been argued that passive limb movement may not fully exclude contributions from central motor commands including the supraspinal or spinal inputs which could initiate muscle activity and influence reflex modulation (Schneider et al. 2000). Recently, two studies have shown that afferent feedback is not a primary contributor of interlimb H-reflex suppression. Hundza and colleagues demonstrated that altered afferent input through applying vibration to arm muscles (Ia afferents) (Hundza et al. (2008) and increasing arm cycling load (Ib afferents) (Hundza and Zehr (2007), did not increase the suppression of the soleus H-reflex. Further Hundza and Zehr (2009) found a graded increase in suppression of the soleus H-reflex amplitude with increased frequencies of arm cycling. Such a linear relationship would be expected if CPG input contributed to the signal responsible for the reflex modulation.

Supraspinal control may also be a possible source of H-reflex modulation; however many studies have shown, in individuals with spinal cord injury, that H-reflex amplitudes are modulated in response to activity or movement (Knikou et al. 2007; Knikou and Conway 2001; Knikou et al. 2006). This again suggests that the source of reflex modulation is directed through mechanisms found in the spinal cord.

Intralimb phase-dependent suppression of the H-reflex initiated through rhythmic movement has been well documented in the arms and legs of humans (Capaday and Stein 1986; Crenna and Frigo 1987; McIlroy et al. 1992; Zehr et al. 2003; Xia et al. 2005). As described above, evidence for interlimb suppression of H-reflexes has been well established (McIlroy et al. 1992; Cheng et al. 1998; Hiraoka 2001; Zehr et al. 2003; Frigon et al. 2004; Zehr et al. 2007; Loadman and Zehr 2007; Javan and Zehr 2008);

however, studies specifically exploring the influence of remote rhythmic limb movement on interlimb H-reflex phase-dependency have been somewhat limited. Studies have examined the effect of leg cycling (Zehr et al. 2007) and arm cycling (Zehr et al. 2003) on phase-dependent modulation of FCR H-reflex amplitudes. Other work has examined phase-dependent inter-leg H-reflex modulation during passive movements (Cheng et al. 1998). This thesis will explore the influence of remote rhythmic arm movement on H-reflex modulation in leg muscles. Such interlimb H-reflex modulation has only been explicitly explored twice, and with very limited control measures. These studies, performed by Hiraoka (2001) and Hiraoka and Iwata (2006), examined the effects of phase of arm swing movement on the modulation of the soleus H-reflex amplitude while participants were seated. Results showed modulation of the H-reflex amplitude while the arm was at the end of the backward swing phase and beginning of the forward swing phase. This would be translated into or around the location of ipsilateral heel-strike during walking. A lack of methodological control, however, was seen in this experiment and thus the results must be interpreted with caution. Specifically, Hiraoka (2001) did not control or report motoneuron pool excitability of the Sol and other muscles, as well as maximum M-wave (M_{max}), control of M-wave amplitudes, and maximum H-reflex (M_{max}) amplitudes. In a follow-up experiment, Hiraoka and Iwata (2006) added controls for Sol muscle motoneuron pool excitability and M-wave amplitudes during H-reflex stimulation. Results from their second experiment did corroborate the positions of phase-dependent suppression found in their first experiment. Two other studies investigated the influence of rhythmic arm cycling on interlimb soleus H-reflex modulation; however, phase-dependency was not the primary objective of these studies and thus was only explored in a limited number of phases of the movement cycle. The first experiment was performed by Frigon et al. (2004) and showed greater suppression of soleus H-reflex amplitude during shoulder flexion as compared with shoulder extension during rhythmic arm cycling. This shoulder position in the most flexed position turns out to be opposite to the shoulder position found to be the most modulated by Hiraoka (2001) and Hiraoka and Iwata (2006). Lastly Loadman and Zehr (2007), as a part of a larger study examined soleus H-reflex amplitude at four positions spaced equidistantly across the movement cycle. Results of this segment of the study indicated no significant difference in modulation of the soleus H-reflex between phases of the arm cycle, although a trend towards phase-dependence was seen.

Recently the was detected an effect of rhythmic arm activity on the excitability of the human lumbosacral spinal cord during combined arm and leg cycling (Balter & Zehr 2007). This effect was only observed at specific discrete phases of movement using sampling across 12 phases of locomotion. This observation has re-ignited interest into definitively investigating phasic modulation of reflexes in stationary leg muscles during arm cycling. Thus the purpose of this thesis was to provide a more comprehensive examination into interlimb phase-dependent suppression of the Sol H-reflex.

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CHAPTER 2: Manuscript

Introduction

The H-reflex is commonly used as a neural probe to examine connections by measuring the conditioning influence of limb activity on excitability of the reflex pathway. In humans, interlimb linkages (from one limb to the other limbs) have been demonstrated by soleus H-reflex suppression during rhythmic arm movement (Frigon et al. 2004; Loadman and Zehr 2007; Hundza and Zehr 2009a). When evoked in a moving limb, modulation of the H-reflex amplitude has been consistently shown to be dependent on the phase of movement during human rhythmic movements (Capaday and Stein 1986; Crenna and Frigo 1987; McIlroy et al. 1992; Brooke et al. 1992; Zehr et al. 2003; Xia et al. 2005). In comparison the influence of remote limb movement on the phase-dependent nature of reflex modulation evoked in a stationary limb has remained less clear. Phase-dependent modulation of the soleus H-reflex was not seen with contralateral passive leg cycling (Cheng et al. 1998) nor in FCR H-reflex responses with contralateral active or passive arm cycling (Zehr et al. 2003), however phase-dependent modulation of the FCR H-reflex was seen with rhythmic foot movement (Baldissera et al. 1998) but not with rhythmic leg cycling (Zehr et al. 2007).

Hiraoka (2001) studied the influence of rhythmic arm swing on soleus H-reflexes and found phase-dependent reflex modulation; however some key variables were not controlled or monitored. Most importantly, motoneuron pool excitability of the Sol muscle was not controlled. Consistency of maximum M-wave (M_{max}), across-experiment constant M-wave controls, maximum H-reflex (H_{max}) values, and background EMG values were also not reported. Frigon et al. (2004) performed a related experiment, with arm cycling, and found that reflex amplitude was significantly more suppressed with shoulder flexion (i.e. arm outstretched) compared to shoulder extension which was opposite to Hiraoka's findings (2001). Hiraoka and Iwata (2006) then performed an almost identical experiment to the 2001 study, adding controls for Sol muscle motoneuron pool excitability and constant M-wave amplitudes during H-reflex stimulation. Results from this experiment corroborated the phase-dependent suppression found in their first experiment. Recently, limited phase-dependent suppression of the Sol H-reflex was found when sampling 4 positions of the arm movement cycle (Loadman and Zehr 2007). A hallmark of rhythmic motor output is phase-dependent reflex control; accordingly, it has remained puzzling as to why our earlier studies did not reveal distinct phasic modulation.

Recently we detected an effect of rhythmic arm activity on the excitability of the human lumbosacral spinal cord during combined arm and leg cycling (Balter & Zehr 2007). This effect was only observed at certain phases of movement and by using sampling across 12 phases of locomotion. This observation has re-ignited interest into definitively investigating phasic modulation of reflexes in stationary leg muscles during arm cycling. The purpose of the current experiment was to provide a more comprehensive examination into the possible phasic nature of interlimb suppression of the Sol H-reflex. It was hypothesized that with higher resolution of 12 positions, phase modulation of the Sol H-reflex response could be demonstrated. Additionally, the application of a higher resolution (12 phase) approach to the question of phase dependency also afforded the opportunity to further explore the issue of task dependence based upon arm use. While Loadman and Zehr (2007) did not show strong task dependency amongst different arm cycling conditions, our current high resolution approach could reveal task dependent modulation between bilateral (BL), ipsilateral (IL) and contralateral (CL) arm cycling. Therefore Soleus H-reflex amplitude was examined during rhythmic BL, IL and CL arm cycling conditions to determine the influence of different loci during rhythmic arm movement.

Methods and Materials

Subjects

Eleven participants (mean age 26, range 21-38, 5 men and 6 women) with no known history of neurological or metabolic disorders were recruited for this study. All participants provided informed 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 completed a physical activity readiness questionnaire (PAR-Q, Canadian Society for Exercise Physiology, Revised 2002) to assess the ability to participate in the study.

Protocol

The experimental methodology and protocol was similar to that described in previous experiments involving arm cycling and H-reflex stimulation (Zehr et al. 2003; Frigon et al. 2004; Loadman and Zehr 2007). Participants were seated in a customized wheelchair that restricted unwanted movements of the lower limbs and trunk (Frigon et al. 2004; Loadman and Zehr 2007) and an over-the-shoulder seatbelt. The feet were strapped to footplates maintaining a neutral ankle angle of approximately 90 degrees. A custom-made arm cycling ergometer (described in Zehr et al. 2003) was positioned directly in front of the subjects. The crank arms of the cycling ergometer were constrained to move together at 180 degrees out of phase. Arm cycling was performed in a forward direction. Participants cycled at 60 rpm (1 Hz) and were occasionally guided by verbal feedback along with visual frequency feedback provided by an oscilloscope (Hameg 20MHz, HM205-3, Frankfurt/Main, Germany). Low-level (~10% of maximum voluntary contraction (MVC)) activation of the Sol muscle was maintained via visual feedback via a custom written LabVIEW 8.0 program (National Instruments, Austin, USA).

H-reflex recruitment curves were obtained at the beginning and end of each experiment. An M_{max} trial was also performed halfway through the experiment. With the ipsilateral (left) arm in the 3 o'clock position when viewed from the participants' right side, six M-wave static control trials were performed: one in the beginning of the experiment; one after all the experimental trials; and 4 spaced equally throughout. Participants performed 4 tasks: static positioning of the arms; bilateral (BL) arm cycling; ipsilateral (IL) arm cycling; and contralateral (CL) arm cycling. The twelve static (ST) trials were performed at each of the phases in random order and corresponded to each hour of a clock face. For movement trials, the three arm cycling conditions were also performed in random order. IL and CL cycling trials involved single arm cycling while the other arm rested in the participant's lap.

H-reflex stimulation

Soleus (Sol) H-reflexes were evoked using single electrical 1 ms square-wave pulses delivered to the tibial nerve in the popliteal fossa of the left leg. Stimulation was delivered using bipolar UNI-GEL surface electrodes (Thought Technologies Ltd., Montreal, Canada) with a Grass S88 stimulator (Astro-Med® Inc., Brossard, Canada), and Grass SIU5 isolation unit connected in series with a CCU1 constant current unit. Nerve stimulation was delivered pseudo-randomly between 2 and 4 seconds for all control, ST and movement trials. Stimulation current was measured by a mA-2000 Noncontact Milliammeter (Bell Technologies, Orlando, USA).

H-reflex and M-wave recruitment curves were constructed from 40 sweeps to determine M_{max} and H_{max} (mean of the largest values) and constant M-wave amplitudes, which corresponded to approximately 70% H_{max} on the ascending limb of the recruitment curve. M-wave amplitude was then continuously monitored and maintained across all ST, movement, and control trials to ensure consistent stimulation intensity. For both control and ST trials 8 sweeps were recorded. Cycling (movement) tasks were recorded via 125 sweeps. The data were then partitioned into 12 equidistant phases that represent the clock face, resulting in 8-15 sweeps per phase. In addition, M_{max} recordings (5 sweeps) were taken in the middle of the experiment.

EMG

EMG was recorded using UNI-GEL bipolar surface electrodes (Thought Technologies Ltd., Montreal, Canada). EMG activity was recorded from ipsilateral soleus (iSol), tibialis anterior (iTA), vastus lateralis (iVL), biceps femoris (iBF), and anterior deltoid (iAD), and contralateral soleus (cSol), tibialis anterior (cTA) and anterior deltoid (cAD) muscles. Maximum voluntary contractions (MVCs) were obtained at the end of the experiment in all recorded leg muscles for EMG normalization and analysis. Grounding electrodes were placed on the skin of the participant over nearby regions of bony protuberances, such as the lateral malleolus, patella, or clavicle. Pre-stimulus (20 ms) EMG from each muscle was used to determine background EMG (bEMG). Soleus EMG signals were amplified 500 times and band pass filtered 100-1000 Hz, while the other monitored muscles were amplified 5000 times, band pass filtered 100-300 Hz using a P511 Grass Instruments AC Amplifier (Astro-Med® Inc., Brossard, Canada) and full wave rectified.

Kinematics and cycling frequency

Task range of motion was recorded in all participants from both the ipsilateral and contralateral elbow joints using electronic single axis SG110 goniometers and a K100 amplifier (Biometrics Ltd, Gwent, United Kingdom). Cycling frequency was determined using an optical sensor encoding ergometer crank arm position in the movement cycle.

Data acquisition and analysis

Data were sampled at 5000 Hz with a 12-bit A/D converter controlled by a custom-written LabVIEW 8.0 program (National Instruments, Austin, USA). Peak-to-peak amplitudes of Sol M-waves and H-reflexes were determined using a custom-written Matlab® program (The MathWorks™ Inc, Natick, USA). Values in each phase from each participant were then averaged and normalized to M_{max} to allow for inter-subject comparisons (Frigon et al., 2004).

Statistics

STATISTICA (StatSoft®, Tulsa, USA) was used to perform a 4 x12 (task x phase) repeated measures analysis of variance (ANOVA) for Sol H-reflex, M-wave amplitudes, and bEMG. A 1 x 4 (task) repeated measures ANOVA for kinematics, and 1 x 3 repeated measures ANOVA for movement frequency stability. When significant main effects were found, Tukey's HSD *post hoc* test was applied. An α level of 0.05 was used for statistical significance.

Results

Phase-dependent modulation of the Sol H-reflex during arm cycling

The effect of phase of movement on the Sol H-reflex amplitude during BL arm cycling is shown for a single participant in Fig. 4. Each trace represents a reflex taken from a different phase (1-12 o'clock) of arm movement and shows varying levels of amplitude modulation across the cycle path. H-reflex amplitudes, expressed as percentages of M_{max}, were averaged across all participants and are displayed in Fig. 5 with all plots referenced to the IL ergometer handle position. Group data demonstrates that the strength of suppression of Sol H-reflex amplitude is dependent upon the movement phase during arm cycling (Main effect $p<0.0001$). A significant interaction was also found ($p<0.0001$). Using *post hoc* tests, phases within each task were compared to their respective 2 o'clock position, which was found to be the least suppressed position during IL, CL and BL conditions. *Post hoc* analysis of phase x task interaction showed no significant differences within ST, two in CL, seven in IL, and five in BL (indicated by asterisks in Figure 5). The phases of most suppression in each cycling condition were found at 7 o'clock in BL, 7 o'clock in IL, and 10 o'clock in CL

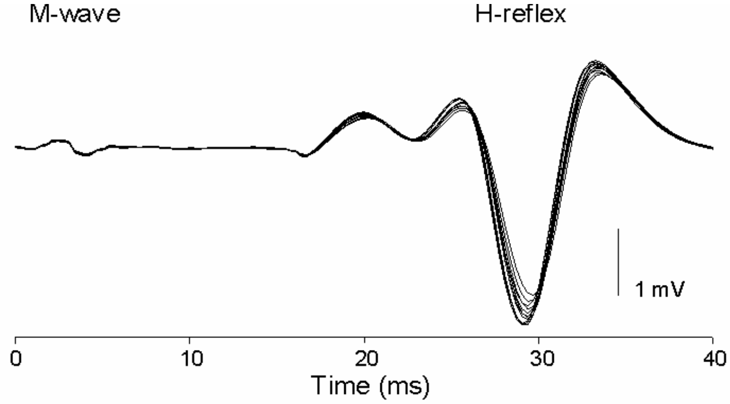

Figure 4. Sol H-reflex recordings from a single participant taken at each of the 12 phases of arm cycling. Each trace represents 8-15 averaged sweeps during the bilateral cycling trial. M-waves and H-reflexes are indicated.

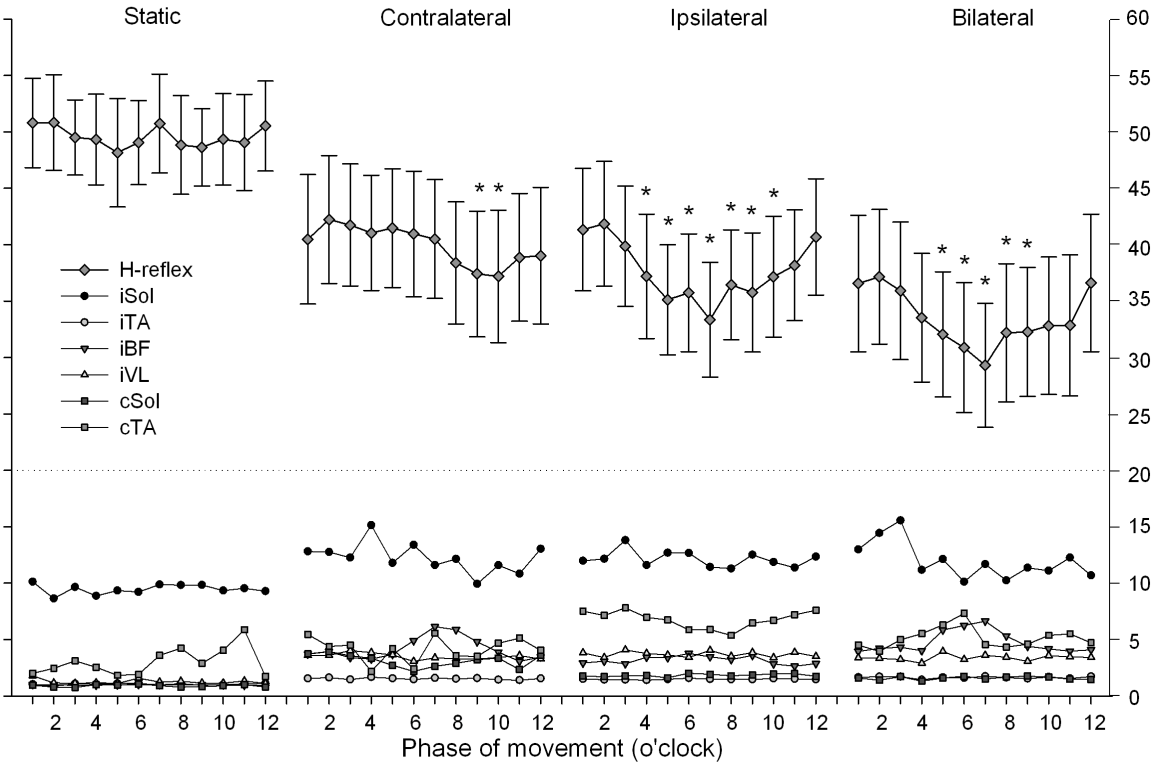

Figure 5. Phase-dependent suppression of the soleus (Sol) H-reflex within contralateral, ipsilateral and bilateral arm cycling conditions, with static results displayed for reference. Position 12 o'clock indicates the left (ipsilateral) ergometer handle top dead center when viewed from participant's right. The actual contralateral arm position of most suppression during cycling is then translated to the 3 and 4 o'clock phase. Standard errors of the mean are displayed. Significant differences are in reference to the 2 o'clock (the least suppressed position) and are indicated by *asterisks* ($p<0.05$). Additionally displayed are corresponding background EMG (bEMG) activity of ipsilateral (i) soleus (Sol), tibialis anterior TA), vastus lateralis (VL), biceps femoris (BF), anterior deltoid (AD) and contralateral (c) Sol, TA and AD.

Task-dependent modulation of the Sol H-reflex during arm cycling

Across all phases H-reflex amplitudes were reduced to ~80% in CL, ~75% in IL, and ~65% in BL all relative to ST. Overall, suppression of cycling tasks were significantly different from ST, but not significantly different from each other (main effect for task; CL cycling, $p=0.003$; IL cycling, $p=0.0004$; BL cycling, $p=0.0002$).

When examining the 7 o'clock phase (most suppressed phase in the BL task), there was significant suppression of Sol H-reflex amplitude in all arm cycling task compared to ST (main effect for task $p<0.0001$; *post hoc* CL cycling, IL cycling, and BL cycling $p<0.0001$) (As shown in Fig. 6). There was no significant difference in reflex amplitude when comparing BL to IL at the 7 o'clock phase ($p=0.15$), but significant differences were seen when comparing CL to IL and BL ($p<0.0001$). A significant task x phase interaction was seen with *post hoc* analysis showing BL differed from IL at 4 phases (1, 2, 6, and 11), BL differs from CL in 9 phases (2-9, and 11), and IL differs from CL for 3 phases (5-7).

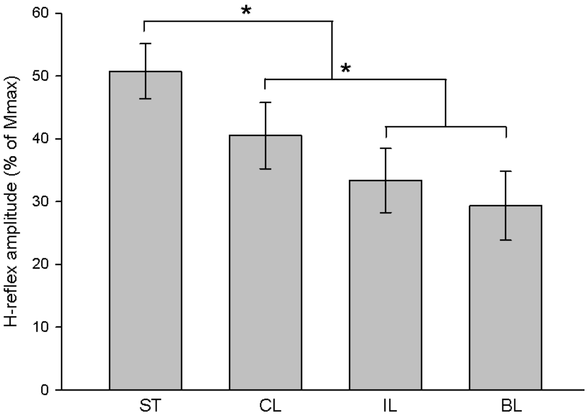

Figure 6. Task dependent suppression of Sol H-reflex amplitude. Static positioning (ST) is significantly different from Contralateral (CL), ipsilateral (IL) and bilateral (BL) arm cycling. CL cycling is significantly different from both IL and CL, and IL and BL cycling are not significantly different from each other. All values are normalized to maximum M-wave values (M_{max}). Statistical significance at $p<0.05$ indicated by *asterisks*. Given with reference to the phase of largest suppression (7 o'clock) during BL**.**

H-reflex experimental controls

M_{max} values recorded at the beginning, middle and end of the experiment were not statistically different ($p=0.6$). Normalized H_{max} values ($p=0.2$) and H/M ratio (H_{max}:M_{max}) ($p=0.7$) recorded pre and post experiment did not change. No significant differences were seen throughout the experiment within the 6 M-wave control trials ($p=0.1$) and constant M-wave control values across all experimental trials (*p*=0.8). On average, H-reflexes were found to be ~83% of H_{max} on the ascending limb of the H-reflex-current recruitment curve.

Kinematics and cycling frequency controls

Angular excursions of the ipsilateral (left) elbow were consistent when comparing ST positioning, BL and IL cycling (*p*=0.48). The contralateral (right) elbow also did not show significant difference when comparing ST positioning, BL, and CL cycling (*p*=0.2). Arm cycling cadence was consistent across trials. Repeated measures ANOVA was performed on BL, IL, and CL cycling frequencies and showed no significant difference between (*p*=0.8).

EMG

Over the course of the experiment, ipsilateral (i) Sol muscle background EMG (bEMG) was maintained at approximately 10% MVC, while other leg muscles had activity of less than 5% of MVC during the experiment. Main effects for task were found within the iSol (*p*=0.02), iTA (*p*=0.01), iBF (*p*=0.03), and iVL (*p*=0.01) muscles. When significant differences were found, they were different to static control, i.e. between cycling conditions there were no significant differences in level of bEMG. A main effect for phase was found for iBF (*p*<0.0001). There were no observed changes in CL leg muscle bEMGs. Background EMG values averaged across subjects are displayed in Figure 5.

Discussion

In the present study we investigated phase-dependent modulation of the Sol H-reflex induced by rhythmic arm movement. Effects of static arm positioning, as well as conditioning evoked during 3 cycling conditions CL, IL, and BL were examined. The major finding was that phase-dependent modulation of Sol H-reflex amplitude occurred during all rhythmic arm cycling tasks but not during static postures.

Methodological considerations

Control M-wave amplitudes remained constant throughout the experiment suggesting no significant change in corresponding H-reflex stimulation. M_{max} values also remained stable over the course of the experiment and were used for normalization to reduce inter-subject variability (Zehr 2002). As M_{max} has also been shown to change at different muscle lengths and limb positions (Gerilovsky et al. 1989), the lower limbs were immobilized via restraints at the hips and ankles.

Levels of motoneuron pool excitation have been shown to be highly influential on the amplitude of the H-reflex (Capaday and Stein 1987a,b). To ensure similar levels of motoneuron pool excitability, Sol muscle activation was maintained at approximately 10% MVC across phases of movement. Throughout the experiment all participants were seated in a custom wheelchair that limited lower leg movements and corresponding muscle activity. This was done to limit influences of local muscle activity (Iles and Roberts 1987; Crone and Nielsen 1994; reviewed in Pierrot-Deseilligny and Mazevet 2000). Though there were no observed changes in CL bEMG, significant differences were found in IL leg muscles showing bEMG to be higher in iSol, iTA, iBF, iVL muscles in cycling conditions versus that of static control. Given there was no significant difference in the level of bEMG between cycling conditions, fluctuations in bEMG did not confound the results. It has commonly been demonstrated that increases in motoneuron pool excitability of the Sol leads to increased H-reflex amplitudes, however our results, and as similar to other experiments has showed general suppressive effect with arm cycling (Frigon et al. 2004; Loadman and Zehr 2007; Hundza and Zehr 2009a). This also indicates the observed increases in Sol bEMG during cycling conditions to be of minor importance based on the overall reflex suppression seen.

In summary, we are confident that the effects seen in this experiment are due to the specific tasks of rhythmic arm cycling and are not due to confounding methodological factors.

Phase-dependant modulation of Sol H-reflexes induced by rhythmic arm movement

In the current experiment, the phase of most suppression seen during IL rhythmic arm movement was found just prior to and at the end of shoulder extension (approximating the 7 o'clock position used here), which closely matches that of Hiraoka (2001) and Hiraoka and Iwata (2006). Our results for CL arm cycling showed the most suppression of the reflex at the 3 and 4 o'clock positions (i.e. 9 and 10 o'clock position with reference to IL ergometer hand crank position), which aligns with the results found in Hiraoka and Iwata (2006). That is, the greatest suppression occurs when the CL limb is transitioning from shoulder flexion towards extension.

In our previous work we detected only minor fluctuations in the strength of suppression using two (Figon et al. 2004) or four phases (Loadman and Zehr 2007) of movement. Here, however, using 12 phases of movement, reflex modulation during arm cycling was more clearly shown to have distinct phase-dependent minima and maxima. Our earlier studies did not sample reflexes at the clear trough (7 o'clock) and peak (2 o'clock) in amplitude discovered here in BL cycling. With both the IL and BL cycling conditions showing the largest suppression at the 7 o'clock position, this could indicate that the main source of BL cycling reflex modulation originates from activity related to the IL limb.

Work in our laboratory has indicated that although the actual movements/kinematics are different between different rhythmic arm activities, such as arm cycling and walking, the underlying central neural control mechanisms are conserved and are thus very similar (Zehr et al. 2009; Klimstra et al. 2009). This could account for why different paradigms, such as arm swing employed by Hiraoka (2001) and Hiraoka and Iwata (2006) and arm cycling here, showed corresponding patterns of H-reflex phase-dependence.

If the phases of movement showing the largest effects were translated into foot positions during gait, then during IL and CL arm movement the phase of largest Sol H-reflex suppression would occur at about heel-strike. Because phase-dependency has been proposed as a limb protective measure to avoid further effects of perturbation or injury and maintain stability (Brooke et al. 1997), during heel-strike this suppression would limit possible stretch reflex activity of, in this case, the Sol muscle. Finally, when compared to studies involving intralimb suppression of the Sol H-reflex during gait, the largest effects are found at approximately this position (Capaday and Stein 1987a; Crenna and Frigo 1987; Garrett et al. 1999; Kawashima et al. 2006).

Task-dependent modulation of Sol H-reflex

As in our previous experiment (Loadman and Zehr 2007), we showed that, overall, the modulation of Sol H-reflex amplitude was significantly different between ST and BL, IL, and CL cycling, but cycling tasks were not different from each other. In the current study, when further examining the task x phase interaction, we compared the 7 o'clock phase (the most suppressed phase in BL cycling) across all tasks. It was shown that the cycling tasks were significantly different from ST positioning. In addition, BL and IL were not significantly different from each other, while both were different from CL. This seems to point to a common source of phase/task suppression between the IL and BL tasks when incorporating the effect of phase-dependency. Also, BL was most dissimilar from CL (9 phases significantly different), while IL showed fewer differences from both BL and CL tasks (with four and three phases different, respectively). These observations could suggest that both IL and CL tasks may collectively produce the modulation seen in BL cycling, i.e. the effect from IL and CL limbs may contribute additively to the greater reflex suppression seen in the BL condition. However, this was not tested explicitly in this experiment.

Source of the Sol H-reflex phase-dependency

Locomotor central pattern generators (CPGs) are involved in the modulation of the excitability of Ia primary afferents through presynaptic inhibition (PSI) (Gossard 1996). In humans, CPG activity related to rhythmic arm movement has been suggested as a plausible contributing mechanism for task- and phase-dependent reflex modulation (Zehr et al. 2004). Additionally, the lack of any suppressive effect seen with static positioning further suggests that mechanisms strictly related to rhythmic movement are the primary locus of the interlimb effects described here. This notion is corroborated in other experiments where static positioning of limbs did not produce phase modulation of the H-reflex, while rhythmic movement did (Capaday and Stein 1986; Zehr et al. 2003). This is further supported by evidence that the source of the signal mediating the general suppression of the Sol H-reflex is not related to afferent feedback (Hundza et al. submitted), but instead appears to be influenced by the central rhythm generation commands regulating frequency of arm movement (Hundza and Zehr, submitted). Additional evidence shows afferent feedback (particularly that arising from muscle spindles) do play a modulating role in H-reflex excitability in animals (Misiaszek et al. 1995a). Indirect evidence in humans using passive movement also corroborates this point (McIlroy et al 1992, Misiaszek et al. 1995b, Brooke et al. 1996).

Future research

With the results of this experiment, there are many new experimental avenues that may be explored. As we have previously shown that forward versus backwards arm cycling are directed under common neural control mechanisms (Zehr and Hundza 2005), it would be of interest to examine the effect of the three arm cycling conditions while performing in reverse. This would allow for further examination of the specific locus of control directing the phasic modulation seen in this experiment. Similarly, extensive work performed by Brooke and colleagues (reviewed in Brooke et al. 1997) has revolved around passive or “involuntary” rhythmic limb movement and its influence on the H-reflex. Therefore it may shed more light on the neural control mechanisms employed by the body when combined with the results of backwards arm cycling. Specifically if phasic modulation of the Sol H-reflex remained during involuntary movement, then CPGs would be a less likely mechanism for phase-dependant modulation. Finally, the study of BL cycling with both hands in the same phase (i.e. not 180° out of phase as performed in this experiment) may help to demonstrate if any possible interactions exist between the arms and if they are additive. If this is so, the determined BL Sol H-reflex suppression profile seen in this experiment may be altered.

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