Rhythmic arm cycling training improves walking and interlimb integrity in chronic stroke

by

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Abstract

Training locomotor pattern generating networks (CPGs) with body weight supported treadmill training or through arm and leg cycling improves walking in chronic stroke. These outcomes are presumed to result from enhanced interlimb connectivity and CPG function. The extent to which rhythmic arm training activates interlimb CPG networks for locomotion remains unclear and was assessed by studying chronic stroke participants before and after 5-weeks of arm cycling training. Strength was assessed bilaterally via maximal voluntary isometric contractions in the legs and hands. Muscle activation during arm cycling and transfer to treadmill walking were assessed in the more affected (MA) and less affected (LA) sides via surface electromyography. Changes to interlimb coupling during rhythmic movement were evaluated using modulation of cutaneous reflexes elicited by electrical stimulation of the superficial radial nerve at the wrist. Bilateral soleus stretch reflexes were elicited at rest and during 1Hz arm cycling. Clinical function tests assessed walking, balance and motor function. Results show significant changes in function and neurophysiological integrity. Training increased bilateral grip strength, force during MA plantarflexion and muscle activation. 'Normalization' of cutaneous reflex modulation was found during arm cycling. There was enhanced activity in the dorsiflexor muscles on the MA side during swing phase of walking. Enhanced interlimb coupling was shown by increased modulation of MA soleus stretch reflexes amplitudes during arm cycling after training. Clinical evaluations showed enhanced walking ability and balance. These results are consistent with training-induced changes in CPG function and interlimb connectivity and underscore the need for arm training in the functional rehabilitation of walking after neurotrauma.

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Dedication

This thesis is dedicated to my parents, Michelle and Stephen, who have always supported me and who instilled in me the belief that there is nothing that cannot be accomplished with hard work and perseverance.

Chapter 1: Introduction and Literature Review

Introduction

Stroke and heart disease result in around 340 000 Canadians admitted to hospital every year (CIHI, 2013) and these remain the leading causes of death and hospitalization. Many laboratories are working tirelessly towards prevention techniques and the development of treatments that can be given immediately after injury to limit the spread of damage. A concurrent problem that requires equal attention is how to rehabilitate function for those in whom damage has already occurred. As of 2009, there were about 1.6 million Canadians living with the effects of stroke (PHAC). After a short stay in the hospital, stroke survivors and their families are often left to navigate the world of rehabilitation alone, which is a major source of frustration and difficulty (Cardiac Care Network 2014). Those with loss of walking ability and who are motivated to try to regain lost function may find their way to body weight support treadmill training programs, which have indeed been shown to provide benefits after neurological injury (Dietz et al., 1998; Moseley et al., 2003; Wirz et al., 2005; Duncan et al., 2011). The limitation of these conventional walking therapies is two-fold. First, they are often very expensive as it is common to require the support of several physiotherapists, many hours per treatment, and many treatment sessions. Second, facilities with the necessary equipment are relatively inaccessible as there are very few of them across Canada. As such, there is a need for the development of therapies that will provide a similar benefit to walking, that are cost effective and easily accessible in most communities.

A proposed way to improve walking after stroke without actually training walking is to train a different rhythmic movement that accesses the same underling neural

mechanisms required for locomotion. In normal human locomotion, continuous movement is achieved through a combination of descending supraspinal input, regulatory neuronal oscillators within the spinal cord, and afferent sensory feedback (Nielson 2003, Zehr & Duysens, 2004). These oscillators exist for each limb and are likely connected by long and short-range propriospinal interneurons that allow for communication between the arms and the legs during locomotion (Zehr et al., 2016). After stroke, the initiating command sent from higher motor centers is often disrupted due to damage from the injury. The spinal networks, however, have been shown to remain at least partially intact (Barzi & Zehr, 2008; Mezzarane et al., 2014). Importantly, studies in cats have shown that these networks, in the absence of descending control, can be activated to produce patterns of rhythmic muscle activation similar to what is seen in walking (Brown, 1911). Walking, arm and leg cycling and swimming have been shown to share common characteristics raising the possibility that any of these types of rhythmic movement that require coordinated movement between the limbs could potentially activate a shared neural core (Zehr, 2005). A previous study in this lab has shown that combined arm and leg cycling operates under similar conditions to other rhythmic movements, and that training in this task can produce beneficial effects in walking in people with chronic stroke (at least six months post infarct)(Klarner et al., 2014, Klarner et al., 2016).

These studies all involved training the arms and legs together to improve walking, something not commonly applied in traditional therapies post-stroke. Instead treadmill walking is performed while participants grip parallel bars and partially support their weight. This is different from what occurs in normal locomotion, where the arms are free to swing on their own. In fact, it has been shown that this type of walking, in comparison

with training in which a participant wears a body weight support harness that leaves the arms free, is significantly less effective at activating the muscles of the legs (Visintin & Barbeau, 1994). The arms, once thought to have a passive role in walking, have been shown to be active contributors to the maintenance of smooth, rhythmic gait (Fernandez-Ballesteros et al. 1965; Kuhtz-Buschbeck and Jing 2012; Zehr et al., 2016). As such, a question that was posed following the completion of the combined arm and leg cycling training was whether or not similar results might be achieved by training only the arms at a cycling task. This thesis will review the evidence that interlimb networks, long established in other species, are present in humans, highlight the influence of the arms on the lower limbs, and propose an answer to the question posed above.

Interlimb Coordination in Quadrupedal Locomotion

Interlimb coordination between the fore and hindlimbs in habiturally quadrupedal animals is a highly effective, well-tuned phenomenon that has evolved over millions of years. Anyone who has ever watched their pet dog or cat run can see that the limbs must move in coordination with one another; else Fido would constantly stumble and fall. What is perhaps less obvious is the perfectly timed neural mechanisms that operate within the nervous system to produce this coordination.

Interlimb coordination in quadrupeds has been a topic of neurophysiological study since the late 1900s. In 1911, T. Graham Brown released a paper in which he described how electrical stimulation of the spinal cord of decerebrate, deafferented cats resulted in the production of patterns of activity in muscles like those seen during normal locomotion. In this experiment, these patterns were produced without the influence of descending or peripheral input. Based on these findings, Brown concluded that a

mechanism within the spinal cord must be responsible for producing patterned locomotor activity. Brown proposed the "half-centre model" which attempted to describe the possible mechanistic underpinnings of this observed phenomena. According to the half-centre model, two groups of neurons or "half -centres" which by themselves have no ability to generate rhythm, exist within the spinal cord. When one of these groups is active (ex. an extensor half-centre), impulses are sent to excite extensor muscles and simultaneously inhibit neurons comprising the flexor half-centre. Brown proposed that a "fatigue mechanism" must exist which functions to slow the firing of extensor half centres, thereby releasing the flexor half-centre from inhibition and allowing it to dominate the next pattern of activity (Brown, 2011). In this way Brown had proposed, through deductive reasoning and indirect evidence, the existence of central pattern generators, or CPGs.

Many decades passed before Brown's half-centre model could be further expanded upon. In the 1960s, the development of intracellular recordings provided the first real means of putting the half-centre model to the test. For the first time, stimulation of cutaneous muscle afferents was shown to produce short bursts of rhythmic, alternating activity within flexor and extensor motoneurons (Jankowska et al. 1967). A CPG for each limb exists to produce rhythmic movement in that limb, and linkages exists between each of these CPGs to coordinate movement of the limbs (Shik & Orlovsky, 1976). Since the 1960s, genetic, molecular, pharmacological and imaging studies have provided further evidence for the existence and inner workings of CPGs. Since Brown's decerebrate cat experiment, the presence of CPGs has been established and studied in many invertebrates including lampreys, sea slugs, leeches and crayfish (Grillner 2006, Friesen & Kristan

2007, Hughes & Wiersma 1960). These species have been chosen for the relative simplicity of their nervous systems, which makes it more feasible for them to be studied at the cellular level.

'Fictive locomotion' refers to the ability of the isolated spinal cord, in the absence of descending command or afferent sensory input, to produce coordinated flexion and extension patterns in the limbs. Since Brown's original experiment, fictive locomotion has been used as one of several pieces of key evidence for the existence of central pattern generators. The neuronal basis of CPGs is thought to reside within the cervical and lumbar enlargements and function to coordinate movement bilaterally between the fore and hindlimbs (Yamaguchi 2004, Zehr et al., 2004a). When stimulated, these enlargements produce a motor pattern similar to that seen in locomotion, including alternating ipsilateral flexor/ extensor bursts with accompanying left/right alternations (Butt & Kiehn, 2003). Coordination between the fore and hindlimbs during movement is thought to be achieved by long propriospinal neurons within the spinal cord that run from the cervical to lumbar enlargements (Miller et al., 1973, Miller et al., 1975). However, it was recently discovered that fictive locomotion in neonatal rats can be interrupted by application of a sucrose blockade to thoracic segments of the spinal cord. Cervical and lumbar rhythms become independent, albeit stable and within a similar frequency range (Juvin et al., 2005). Because this blockade does not affect transmission of long propriospinal neurons that pass through the thoracic segment and cervicolumbar coordination is still disrupted, there must be additional mechanisms in place during interlimb coordination. A proposed possibility is the existence of short-projecting propriospinal interneurons (Juvin et al., 2012).

Interlimb coordination is often tested by applying an input to the nervous system (often electrical stimulation) at one location, and measuring its output in the form of facilitation or suppression of spinal reflexes at another location. In 1973, Miller et al. studied the effects of electrical stimulation of hindlimb nerves on monosynaptic reflexes in the pectoralis major and forelimb flexor muscles. They found that reflexes measured in these muscles were greatly facilitated by hindlimb stimulation (Miller et al., 1973). This facilitation was greater when the stimulated nerve was on the ipsilateral side of the body, as opposed to the contralateral side, indicating that although bilateral cervicolumbar coordination exists, it is perhaps not as strongly coupled as ipsilateral cervicolumbar coordination. Further studies have shown that interlimb connections such as these are also active during rhythmic tasks. Stimulation of cutaneous nerves in the forelimbs of decerebrate cats during walking produces phase modulated responses in the muscles of the hindlimb (Schomburg et al., 1978). These reflex responses in the hindlimb are modulated across the step cycle.

Other studies have investigated interlimb coordination by means of gait characteristics, rather than reflexes. For example, coordination patterns between the limbs reveal the functional outcomes of neuronal interlimb connections. This is evident in transverse split-belt treadmill studies in the cat, which have shown that as the forelimbs are made to increase in speed, they take more steps, initiating a 2:1 stepping relationship with the hindlimbs. In contrast, when the hindlimbs are made to move faster, stride length increases in order to maintain a 1:1 stepping ratio with the forelimbs (Thibaudier et al., 2013; Thibaudier & Frigon, 2014). This clearly indicates both a tight coupling between the fore and hindlimbs, as well as the fact that there are likely constraints

imposed on the cervical compared to lumbar locomotor centers. Input to the nervous system at the lumbar level likely has a larger impact on cervical centers than the reverse, but movement in the forelimbs still plays an important role in regulating lumbar spinal networks.

Interlimb Coordination in Human Bipedal Locomotion

Interlimb coordination in humans, while readily apparent during rhythmic activities such as walking, running and swimming, has proven more difficult to mechanistically define due to methodological constraints. The necessity of noninvasive techniques means that the majority of evidence for CPGs and interlimb coordination in bipedal walking is indirect. Often it is achieved through the use of surface electromyography to evaluate interlimb reflexes. Reflexes produced in response to inputs such as electrical stimulation differ between tasks (task dependent) but share the common characteristic of exhibiting distinct patterns that are dependent on the phase of movement (phase-dependent) (Wannier et al., 2001; Dietz et al., 2001; Zehr et al., 2001; Zehr & Haridas, 2003; Haridas & Zehr, 2003; Klarner et al., 2014). It is increasingly clear that although quadrupedal and bipedal locomotion differ in specific characteristics, they likely share many of the same underlying neural mechanisms. Since the arms appear to serve no mechanical, propulsive purpose in upright bipedal walking, evidence is mounting that the actions of the arms are intimately integrated into human walking as a whole. It appears as though arm swing during locomotion is not merely a vestigial product of the evolution of bipedal walking from quadrupeds, but rather that it plays an important role in the production and maintenance of gait.

Evidence for Interlimb Neural Pathways in Humans

Much of the evidence for the existence of CPGs and interlimb coordination in humans comes from reflex studies. One commonly used marker of interlimb neural pathways is cutaneous reflexes. Cutaneous reflexes play an important functional role in that they allow afferent, sensory information applied to the skin to directly modulate the activity of muscles all over the body. In order to measure a cutaneous reflex in the anterior deltoid (for example), one would stimulate the radial nerve at the wrist and record the response via surface electromyography (sEMG) electrodes placed over the muscle belly. Important to note is that any muscle in the body may be chosen and as such, cutaneous reflexes provide a glimpse of how afferent information is taken in from the skin at one location and used to adapt the motor program for any given muscle. For example, stimulation of the radial nerve at the wrist can evoke reflexes in the muscles of the legs and vice versa (Zehr et al., 2001a). These reflexes can be measured on the ipsilateral side of the body (same side as where the stimulation is provided), or the contralateral side. Cutaneous reflexes exhibit three important characteristics that give evidence for the existence of CPGs in humans; 1) cutaneous reflexes are task dependent, differing for example whether one is sitting still or cycling the arms, 2) cutaneous reflexes are phase dependent, i.e. they are subject to modulation across the different phases of a particular movement (Wannier et al., 2001; Dietz et al., 2001; Zehr et al., 2001; Zehr & Haridas, 2003; Haridas & Zehr, 2003; Klarner et al., 2014). A third way in which cutaneous reflexes provide evidence for CPGs is that the phase dependent modulation seen during a given task is similar between the upper limbs, and similar between the lower limbs (Zehr & Kido, 2001; Zehr et al., 2001a). This is similar to patterns of modulation seen in the forelimbs and hindlimbs of quadrupedal species.

When cutaneous reflexes are evoked via stimulation of the hand or foot during a functional task like walking or stepping, reflexes are produced in the muscles of the arms and legs that are phase as well as task modulated (Haridas & Zehr, 2003). When stimulation is applied to the wrist during combined arm and leg cycling, reflexes in the lower limbs (particularly in the tibialis anterior) are subject to modulation by phase of arm movement (Balter & Zehr, 2007). This is similar to the effects seen in the reflexes evoked in the lower limbs during recumbent stepping (Zehr et al., 2007a). Task and phase dependency are hallmarks of CPG function in other species and as such, their presence in cutaneous reflexes provide indirect evidence for the existence of CPGs in humans.

Another reflex pathway which is often used to provide insight into the workings of the human nervous system is the stretch reflex, along with its electrical analogue the Hoffman reflex. The stretch reflex is functionally postulated as a postural reflex, allowing for automatic contraction (shortening) of a muscle in response to increasing skeletal muscle length. Within a lab setting, in order to observe the stretch reflex through surface EMG electrodes placed over the belly of the soleus muscle, one would provide stimulation to the Achilles tendon via a tap. Information from the tendon is sent to the spinal cord where 1a afferent fibers synapse with alpha motoneuron efferents which function to contract or relax opposing muscle groups. Although this reflex pathway makes only one synapse within the spinal cord, it is highly susceptible to activity at said synapse, and in turn the reflex itself is susceptible to modulation by events transpiring at cervical levels. The electrical analogue of the stretch reflex, the Hoffmann reflex (H-reflex), is evoked when a mixed nerve (containing the 1a afferent fiber) is stimulated

midway along the nerve by electrical stimulation, bypassing the muscle spindle (Palmieri et al., 2004). As such, the H-reflex is said to be more a measure of the excitability of the reflex arc, as opposed to the sensitivity of the fusimotor system. Both reflexes are influenced by the number of active motoneurons (Burke et al., 1989; Stein & Kearney, 1995), as well as by the amplitude of stimulation (Zehr, 2002).

As with cutaneous reflexes, the modulation of the stretch reflex by remote activity can be used to infer how interlimb coordination is achieved during human locomotion. The stretch reflex pathway can be acted upon at two different locations. First, modulators can act at the level of the synapse between the 1a afferent nerve and the alpha motoneuron. Alternatively, activation of interlimb pathways might act upon the fusimotor system to increase sensitivity of muscle spindles to stretch (Mezzarane et al., 2014). One mechanism by which the stretch reflex (or Hoffmann reflex) is modulated is through presynaptic inhibition (PSI) by the neurotransmitter gamma aminobutyric acid (GABA) (Capaday & Stein 1986; Crenna & Frigo 1987; Frigon et al., 2004). An increase in presynaptic inhibition at the synapse has the known effect of suppressing the H-reflex, whereas an inhibition of PSI leads to a potentiation of the H-reflex (Lundberg et al., 1987; Stein, 1995). There is evidence that PSI can be modulated via the actions of sensory afferents during active and passive limb movements (Stein, 1995; Brooke et al., 1997b.) In turn, an increase in PSI at the synapse activated by movement of the upper limbs can modulate the amplitude of the stretch (or Hoffmann) reflex produced in the lower limbs.

Even within static tasks, it would seem as though the legs are “listening in” to what the arms are doing through the mechanisms explored above. H-reflexes in leg

muscles have been shown to exhibit modulation in response to postural changes of the arms (Delwaide et al., 1977; Eke-Okoro, 1994). Additionally, during a task in which the arms are held in static swing positions, soleus H-reflexes are modulated differentially according to the position of the arms (Eke-Okoro, 1994).

Rhythmic movement of the upper or lower limbs also serves to drive modulation of lower limb H- reflexes. While there is evidence that during walking, arm swing modulates H-reflexes in the lower limbs (Hiraoka, 2001), some of the more compelling evidence of arm movement effecting reflex pathways in the lower limbs comes from studies of arm cycling. In 2004, Frigon et al. investigated the effect of rhythmic arm cycling on the H-reflex elicited in the stationary soleus muscle. The authors found that the H-reflex in the soleus muscle is significantly suppressed during arm cycling. As this was compared to soleus H-reflexes elicited during static arm positioning, this study provides evidence that rhythmic movement of the arms impacts lumbar spinal reflex excitability in a manner that is task dependent (Frigon et al., 2004). Soleus H-reflex modulation during arm cycling is achieved via an increase in segmental 1a PSI (Frigon et al., 2004). A follow up study from this lab examined whether parameters of arm cycling such as phase, amplitude and frequency of movement differentially modulate the soleus H-reflex. The authors found that the modulation in the H-reflex seen during arm cycling is not phase dependent when examined at equidistant points, rather there seems to be a general descending suppressive effect (Loadman & Zehr 2007).

A study by Javan & Zehr added an interesting piece of information to the puzzle that is the effect of arm movement on lower limb reflexes. As with previous studies, participants performed rhythmic arm cycling while their feet were secured in a stationary

position and H-reflexes were elicited bilaterally in the soleus muscles (Javan & Zehr 2007). This time, however, participants cycled continuously for a 30-minute period and the investigators continued to sample the H-reflex for a time after the cessation of arm cycling. They found that H-reflexes continued to be suppressed for up to twenty minutes following the cessation of movement (Javan & Zehr 2007). In a second part of the experiment, the addition of cutaneous stimulation to the superficial radial nerve at the wrist effectively cancelled the prolonged suppression (Javan & Zehr 2007). This study provides two important pieces of information. The first is that short-term plasticity can be induced in reflex pathways following a period of rhythmic, continuous arm movement. This could have important implications for rehabilitation. If similar effects can be induced in individuals experiencing spasticity, hyperreflexia could possibly be reduced through long-term training of the arms. Second, because superficial radial stimulation generally facilitates the soleus H-reflex by reducing PSI, we can predict that the prolonged persistent suppression of the H-reflex is likely due to a prolonged increase in the level of PSI (Javan & Zehr, 2007). As such, this study also provides a possible mechanism by which movement of the arms can induce short-term plasticity in the reflex pathways of the legs.

Within a stroke population, fewer proof of principle studies have been undertaken and "norms" can be more difficult to establish due to the wide variety of survivor presentations which are dependent on location and size of injury. There have, however been a few studies that have looked at whether or not arm cycling elicits a similar suppressive effect in lower limb spinal reflexes within chronic stroke (at least 6 months post stroke). A study by Barzi & Zehr had chronic stroke participants cycle at 1 and 1.5

Hz while H-reflexes were elicited in the soleus muscles bilaterally (Barzi & Zehr, 2008). The authors were able to show that a similar suppression of the H-reflex is induced during arm cycling, although they noted the suppression was less strong than that seen in a neurologically intact population. This study suggests that the mechanisms underlying the ability of the arms to modulate reflexes in the legs is at least partially preserved after stroke.

In one of the very few studies to evaluate the effects of arm cycling on soleus stretch reflexes, Mezzarane et al. had chronic stroke participants cycle at 1Hz while stretch reflexes were elicited bilaterally. Interestingly, in contrast to what is seen with H-reflexes, the authors found that soleus stretch reflexes are modulated in a bidirectional manner during arm cycling. About half of the participants had increases in stretch reflex amplitude during cycling, and the others experienced a suppression (Mezzarne et al., 2014). There was no effect of more affected (MA) vs less affected (LA) side on the direction of modulation (MA side is the side of the body contralateral to the hemisphere in which the stroke occurred, where one typically sees larger deficits). The results of this study suggest that while the lower limbs H-reflexes appear to be modulated mainly by presynaptic inhibition, stretch reflexes likely receive additional modulation via the fusimotor system (Mezzarne et al., 2014).

Taking together the studies investigating the effects of rhythmic upper limb movement on cutaneous and stretch reflexes in the lower limbs, it is clear that interlimb pathways are at least partially responsible for interlimb coordination, and that these pathways modulate reflex arcs via mechanisms such as presynaptic inhibition. Additionally, these pathways appear to be at least partially preserved in stroke, and seem

to be subject to similar modulatory mechanisms (Barzi & Zehr, 2008; Mezzarane et al., 2014). The bilateral coupling between the arms and between the legs that is maintained by these interlimb pathways will be the topic of discussion in the next section.

Bilateral Coordination of the Arms and the Legs

Evidence of locus of control similar to that in quadrupeds for bipedal walking is perhaps most obvious in the legs as opposed to the arms. The literature shows that a very tight bilateral coupling of the legs is necessary for proper smooth, rhythmic gait as humans are dependent on the coordination of the legs in order to move (Zehr et al., 2016). Even during split-belt treadmill walking, where one belt is set to a faster pace, the legs maintain alternating coordination (Dietz & Duysens 1994; Prokop et al. 1995; Erni & Dietz, 2001). On a split belt treadmill with different stepping rates, the leg on the slower belt will spend more time in stance phase, whereas the leg on the faster belt will spend more time in swing, maintaining a 1:1 stepping relationship (Thelen et al., 1987; Dietz et al., 1994b; Prokop et al., 1995; Yang et al., 2005a). This phenomenon is similar to what has been observed in hindlimb stepping in cats and as such, functional coupling of the legs in humans likely shares a common neural core with lumbar coupling in quadrupeds (Thibaudier et al., 2013; Thibaudier & Frigon, 2014). In addition, movement in one leg has the ability to modulate reflexes and effect muscle activation and force production in the contralateral limb. Movement of one leg, be it passive or active, has a suppressive effect on soleus stretch reflexes in the contralateral limb (Brooke et al. 1992; Collins et al. 1993; Cheng et al. 1998; Misiaszek et al. 1998). Taken together, these studies indicate that the legs are tightly coupled bilaterally during locomotion, and activity in one limb has the power to modulate reflexes in the contralateral limb.

There has been a recent accumulation of evidence in support of the idea that coordination between the arms during walking or cycling is very similar to the mechanisms of control that coordinate movement between the legs during a similar task (Zehr & Duysens, 2004, Zehr et al., 2016). Cutaneous reflexes elicited in the arms during walking and cycling show patterns of phase dependency that are independent of background EMG, i.e. not dictated by the muscle activity (Zehr & Kido, 2001; Zehr & Haridas, 2003). While these patterns exist, the arms do not seem to be as tightly coupled as the legs. In contrast to phenomena observed in the legs, H-reflexes elicited in a stationary arm do not seem to be affected by passive movement in the contralateral limb, although they are modulated with active movement (Zehr et al., 2003). This suggests that although the arms are likely coordinated bilaterally through similar neural mechanisms that coordinate the legs, there is far weaker coupling between the arms. This weaker coupling makes sense given the tight bilateral coordination required of the legs to produce walking, as well as the ability of the arms to produce independent skilled movements.

The Importance of the Arms in Bipedal Human Locomotion

It is conceivable that bipedal walking would share common characteristics of quadrupedal walking, as early primates evolved to require the use of the upper limbs for skilled reaching and grasping tasks. Indeed, it has been argued that during walking, human and quadrupedal locomotion are controlled similarly, and are simply uncoupled when skilled upper limb movements are required (Dietz, 2002).

Two lines of thought have emerged around the role that the arms play in human locomotion. The first claims that arm swing is merely an evolutionary by-product of

forearm swing left over from quadrupedal walking. This theory suggests that the role of the arms is to prevent the jerky, uncoordinated gait that would exist without their control (Jackson, 1983). Alternatively, an argument has been made for arm swing having both active and passive components. The passive component may have arisen in order to counteract lower limb torque, whereas the active component may be controlled by cervical locomotor centers and serve to contribute to gait maintenance (Zehr et al., 2016). In this way, the interlimb coupling present in the quadruped between the forelimbs and hindlimbs would exist in some form as the common core of interlimb coordination in humans. It may be that the use of the arms for climbing in early primates evolved to offset torque generated by the lower limbs during bipedal walking (Zehr et al., 2016). Early work by Elftman in 1939 investigated the torque produced by the arms during walking. He found that contrary to popular belief, arm swing was not passive, but rather that it involved active muscle contractions. Studies have since shown that whole-body angular movement around a vertical axis is induced by the lower limbs during locomotion, and that this rotation is offset by upper body movements (Hinrichs 1987, Hinrichs et al., 1987). The ability of the arms to actively offset rotational perturbations is thought to require neural coordination (Zehr & Duysens, 2004). Elftman's work was further corroborated in 1985, when researchers showed that even when arm movements are constricted during walking, the muscles continue to display a rhythmic pattern of activation (Fernandez-Ballesteros et al. 1965; Kuhtz-Buschbeck and Jing 2012).

The ability of the arms to drive activation in the legs was investigated in a neurologically intact population trained in recumbent stepping (Huang & Ferris, 2003). A recumbent stepper allows the arms and legs to be coupled bilaterally, and as such

conditions can be tested where the arms drive the legs and vice versa. This study involved three movement conditions with simultaneous EMG recordings from the muscles of the lower limbs. In condition one participants moved the arms and legs actively at an easy pace; in condition two, participants actively moved the arms at an easy, medium or hard pace (self-driven condition); and the third condition involved both arms and legs moving passively through the stepping motions as movement was driven externally by an investigator (Huang & Ferris, 2003). The authors found that EMG amplitudes in the muscles of the lower limbs were always higher in the self-driven conditions than in the external ones. Additionally, they found that as resistance and upper limb activity increased, so did EMG amplitude in the passive lower limbs (Huang & Ferris, 2003). These results suggest that rhythmic activation of the upper limbs can drive activation of muscles of the lower limbs. This has implications for clinical populations, who might be able to train their arms to increase muscle activation in the legs.

Another study investigating the effects of arm movement on muscle activation in the legs utilized an interesting design in which participants laid on their sides with their feet suspended in an exoskeleton, while their hands "walked" on an overhead treadmill (Sylos-Labini et al., 2014). The authors found that hand walking elicited activity in the proximal leg muscles that was similar in timing to patterns seen during normal locomotion in about 58% of people. Additionally, the authors were able to rule out that these activations were entirely a by-product of torso rotation using externally imposed trunk movements and biomechanical modelling (Sylos-Labini et al., 2014). Interestingly, even when leg movements were blocked by the investigator, and for a short time after

arm walking ceased, patterns of EMG activity in the leg muscles persisted (Sylos-Labini et al., 2014). These results speak once again to the idea that rhythmic activation of the arms has a role in driving locomotor-like activity in the lower limbs.

Interlimb Training in a Clinical Setting

While there have been studies that have looked at the contributions of the arms to walking in a clinical population within a single session (Visintin & Barbeau, 1994), fewer studies have examined whether interlimb connections can be trained over a period time to bolster locomotion. A recent study looked at whether or not long-term training of interlimb pathways could produce a measureable transfer to walking in a chronic stroke population (at least six months post infarct) (Klarner et al., 2014, Klarner et al., 2016). Participants trained for 30 minutes at a time, three days a week for five weeks on a combined arm and leg cycling ergometer (Sci-fit Pro 2). Exercise was of moderate intensity, below the level required to improve cardiovascular fitness in a stroke population (Pang et al., 2006, Gordon et al., 2004), making it more likely that any changes seen post intervention where not simply a by-product of increased cardiovascular fitness. Following five weeks of training, there were improvements in strength in all four limbs, as well as an increase in muscle activation in some of the muscles of the lower limbs. Clinical status as evaluated via walking and balance tests improved, as people were able to walk further and faster following the training (Klarner et al., 2016). Additionally, there were global changes to treadmill walking, including an increase in joint range of motion, and changes to stride frequency and duration. Within the changes to stride duration on the less affected side, there was a decrease in time spent in stance and an increase in swing duration, a phenomenon more reflective of normal locomotion

(Klarner et al., 2016). Cutaneous interlimb reflexes elicited during walking were also evaluated as markers of change in neurological integrity. Results from average cutaneous reflexes show a "normalization" of facilitative and suppressive phases of the lower limb muscles that are functionally correlated with transitions from swing to stance and vice versa (Klarner et al., 2016).

Taken together, the results from this study indicate that it is indeed possible to train interlimb networks at a rhythmic task that will provide a transfer of effects to walking within a clinical population. It remains to be determined whether, in order to achieve these improvements, all four limbs must be trained together, or whether training only the upper or lower limbs is sufficient to activate these networks

Conclusions

Human locomotion is achieved via a combination of descending supraspinal command, afferent sensory feedback, and CPGs within the spinal cord that regulate continuous movement (Nielson 2003, Zehr & Duysens, 2004). In addition, these CPGs coordinate movement of the limbs via interlimb networks. These networks have been shown in animal models, and indirect reflex studies suggest their activity in humans during rhythmic tasks such as walking and cycling as well (Zehr et al., 2001a; (Duysens et al., 1992; Brown & Kulkulka, 1993; Tax et al., 1995). These studies have provided evidence that not only does movement in the lower limbs affect the upper limbs, the reverse is also true. The arms are capable of modulating reflexes as well as muscle activation within the legs (Frigon et al., 2004; Ferris et al., 2006; Loadman & Zehr, 2006; Javan & Zehr, 2007). More and more the arms are being shown to play an active role in the maintenance of bipedal gait. Recent work has encouraged the use of the arms in

combination with the legs in rehabilitative practices to improve walking within a chronic stroke population (Klarner et al., 2014; Klarner et al., 2016). However, little work has been done to investigate what contributions training the arms alone can have in a clinical setting.

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Chapter 2: Manuscript

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Introduction

Walking occurs via an initiating descending motor command from supraspinal centers that activates spinal networks and is modulated by afferent sensory feedback (Nielsen, 2003; Zehr & Duysens, 2004). Following stroke, descending motor commands and supraspinal regulation are interrupted and dysfunctional (Zehr, 2011). However, preserved networks in the spinal cord remain relatively intact and accessible (Klarner *et al.*, 2014). These spinal networks are presumed to regulate rhythmic limb activities, such as cycling or swimming (Zehr, 2005). At the heart of these spinal networks are presumed central pattern generating networks (CPGs) that assist in producing rhythmic coordinated movements of all 4 limbs (Dietz, 2002).

In humans, the evidence for CPG networks and interlimb connections between the arms and legs is indirect due to methodological constraints (see for review Klarner & Zehr (this issue), (Zehr & Duysens, 2004; Zehr, 2005, 2016)). Modulation of reflexes sampled during human locomotion reflect many of the hallmark characteristics of modulation induced by CPG regulation such as task and phase dependency (Dietz *et al.*, 2001; Wannier *et al.*, 2001; Zehr *et al.*, 2001; Haridas & Zehr, 2003; Zehr & Haridas, 2003). As such, an input that produces a reliable change in reflex modulation can be used to infer mechanisms within the spinal cord. Previous studies have shown that rhythmic arm movement modulates reflexes in the lower limbs that can be suppressive (Frigon *et al.*, 2004; Hundza & Zehr, 2009) but also facilitative (Dragert & Zehr, 2009). It has been suggested that rhythmic arm movement produces a strong, persistent descending input that modulates the level of presynaptic inhibition altering transmission between Group Ia

afferents in muscles and alpha motor neurons supplying the muscles of the lower limbs (Frigon *et al.*, 2004).

In recent years, the role of the arms in human locomotion has become a topic of renewed interest. Bipedal locomotion, although different from quadrupedal locomotion, nonetheless shares many of the same underlying characteristics. It has been argued that during locomotion, bipedal arm and leg coordination is similar to quadrupedal coordination, and that this coordination is simply uncoupled during a skilled upper limb task (Dietz, 2002). The arms, while once thought to be functionally passive during walking, have since been shown to be active contributors to the maintenance of smooth, rhythmic gait by offsetting the rotational torque produced by the lower limbs (Elftman, 1939). Arm swing has been shown to facilitate activation of muscles of the lower limbs, indicating that they are actively contributing to locomotion (Ferris *et al.*, 2006). The arms are thought to influence the legs via the same interlimb networks present in other animals (Zehr, 2016).

The neurological lesion occurring as a result of a stroke leads to permanent disability, including hemiparesis, foot drop, gait asymmetries and difficulty with activities of daily living (Zehr, 2011). Unfortunately, although rehabilitation continues to be useful even decades after stroke (Sun *et al.*, 2015), little therapy is typically provided beyond 6 months post-lesion. In chronic stroke, years of disuse can compound the already debilitating effects of the initial injury with walking being one of the most frequently impacted abilities. Post-stroke quality of life decreases directly with the inability to ambulate (Ada *et al.*, 2009).

Recently, rhythmic arm and leg cycling training in chronic stroke was shown to induce changes to muscle activation and reflex modulation in all four limbs which was seen alongside improved overall quality of walking (Klarner *et al.*, 2014; Klarner *et al.*, 2016b, a). These findings build on previous work that has shown in chronic stroke populations, movement of the arms can induce short-term changes in reflex excitability of the lower limbs (Barzi & Zehr, 2008; Mezzarane *et al.*, 2014). Clearly established interlimb networks, present in both reduced animal and neurologically intact populations, remain at least partially accessible after stroke (Zehr & Loadman, 2012). Additionally, rhythmic movement other than walking can activate these spinal networks (Zehr *et al.*, 2012; Klarner *et al.*, 2014; Klarner *et al.*, 2016a, b). This has important implications for those affected by chronic stroke whose walking function is below what is required to take part in treadmill training.

A remaining question is the role that rhythmically training the arms alone may play in the recovery of walking function. It is important to establish whether all four limbs need to be active during rhythmic training, or whether an individual who is unable to participate in active lower limb cycling can receive the benefits of interlimb connectivity using only arm cycling. Here we tested the working hypothesis that arm cycling training would transfer to improvement of interlimb neurological integrity and walking function.

Methods

Participants

Nineteen participants (72.5 ± 9.37 years; range: 57 to 87 years) with chronic stroke (104.65 ± 57.86 months post-stroke; range: 7 to 214 months post-stroke) were

recruited for this study, based on a similar recruitment number in a previous training study (Klarner *et al.*, 2016a, b). Participants were recruited through presentations at community stroke support groups, posters in medical offices and hospitals, and direct referral from community clinicians familiar with work in the laboratory. Exclusion criteria included the use of medication affecting muscle tone (botox, baclofen, etc.), pacemakers, epilepsy, and insulin-dependent diabetes. Participants ranged in physical ability level from low to high functioning (see Table 1 for clinical assessment scores). One participant completed all clinical tests except for the Chedoke McMaster Stroke Assessment due to injury of the primary caregiver unrelated to the intervention. Another participant was unable to complete the clinical post-test because of a back injury that occurred just prior and thus all associated clinical results were excluded from the data. Otherwise, there was high levels of adherence to the training protocol as evidenced by no participants dropping out during the training intervention.

Table 1

Summary of participant demographics and results from tests assessing clinical status

Participant	Sex	Age	MA side	Post-Lesion Time (Months)	Modified Ashworth (MA side) Ankle/Knee/Wrist/Elbow Pre	Modified Ashworth (MA side) Ankle/Knee/Wrist/Elbow Post	FAC (/6) Pre	FAC (/6) Post	Chedoke-McMaster Arm/Hand/Shoulder/Leg/Foot Pre	Chedoke-McMaster Arm/Hand/Shoulder/Leg/Foot Post	Monofilament (MA Side) Hand/Foot Pre	Monofilament (MA Side) Hand/Foot Post	Berg Balance Score (/56) Pre	Berg Balance Score (/56) Post
1	M	60	*R*	190	2/1/1/1	*2/1/1/1*	6	6	*3/5/6/6/3*	3/5/6/6/3	F3.61/J4.31	F3.61/J4.31	49	47
2	M	69	*L*	114	2/1/1+/2	*1+/1/1+/2*	5	5	*3/2/6/5/3*	3/2/6/5/3	T6.65/T6.65	T6.65/T6.65	44	46
3	M	71	*L*	58	2/1+/2/1+	*2/1+/2/1+*	4	4	*2/2/2/3/2*	2/2/2/3/2	T6.65/T6.65	T6.65/T6.65	25	24
4	F	81	*R*	43	1/1/0/1	*1/1/0/1*	5	5	*6/6/6/6/4*	6/6/6/6/4	F3.61/J4.31	F3.61/F3.61	42	44
5	F	61	*L*	78	1/1/0/0	*1/1/0/0*	6	6	*7/6/7/7/7*	7/6/7/7/7	J4.31/K4.56	J4.31/J4.31	53	56
6	M	82	*R*	89	1/0/0/0	*1/0/0/0*	2	2	*7/6/6/6/6*	7/6/6/7/6	J4.31/T6.65	J4.31/K4.56	55	55
7	F	57	*L*	96	1/0/0/1	*1/0/0/1*	6	6	*6/6/7/7/7*	6/6/7/7/7	J4.31/J4.31	F3.61/J4.31	56	56
8	M	81	*R*	150	0/0/0/0	*1/0/0/0*	6	6	*6/5/6/6/4*	6/6/6/6/4	J4.32/T6.65	K4.56/T6.65	48	50
9	M	65	*L*	86	4+/3+/3/2	*3/1+/2/1+*	4	5	*2/2/4/3/2*	2/2/4/3/2	F3.61/T6.65	F3.61/K4.56	21	36
10	M	72	*L*	68	1/0/0/1	*1/1/0/1*	6	6	*7/6/6/5/4*	7/7/6/5/5	J4.31/K4.56	J4.31/T6.65	55	55
11	M	87	*L*	70	2/1/0/0	*2/1/0/0*	3	5	*3/5/6/4/3*	3/5/6/5/4	J4.31/J4.31	J4.31/J4.31	46	49
12	M	72	*L*	155	2/0/0/0	*0/0/1/0*	4	3	***TIME CONSTRAINTS***				20	20
13	F	83	*R*	7	0/0/0/0	*0/0/0/0*	2	2	*3/5/6/2/2*	4/6/6/3/2	J4.31/K4.56	J4.31/T6.65	8	15
14	M	86	*L*	156	2/0/1+/0	*0/0/2/1*	4	4	*5/4/6/5/3*	4/5/7/5/4	J4.31/J4.31	J4.31/J4.31	48	50
15	F	67	*R*	164	2/2/1+/1+	*3/1/1+/2*	4	4	*3/2/5/4/3*	3/2/6/4/3	F3.61/T6.65	T6.65/T6.65	25	27
16	F	69	*R*	214	0/0/0/0	*1/0/0/0*	5	5	*7/7/7/6/6*	7/7/7/6/7	J4.31/J4.31	J4.31/J4.31	54	55
17	F	68	*R*	41	0/0/0/0	*1/0/0/0*	5	5	*5/7/4/6/7*	7/7/5/6/7	J4.31/J4.31	J4.31/J4.31	47	56
18	F	77	*L*	108	**SUSTAINED BACK INJURY NON-RELATED TO TRAINING INTERVENTION PRIOR TO POST-TEST**									
19	M	82	*L*	90	1/0/0/1+	*1/0/0/2*	5	5	*5/5/7/5/5*	5/6/7/5/5	J4.31/J4.31	K4.56/J4.31	52	49

Note: including a test for muscle tone (Modified Ashworth), functional ambulation category (FAC), physical impairment (Chedoke-McMaster scale), touch discrimination (Monofilament test) and balance (Berg Balance Scale) for stroke participants before and after arm cycling training. Abbreviations: MA, more affected; M, male; F, female; L; FAC, Functional Ambulation Category.

Before beginning arm cycling training, participants were screened with the Physical Activity Readiness Questionnaire (Canadian Society for Exercise Physiology, 2012) and if a response of 'yes' was given for any of the questions, physician's permission was obtained for that participant. The protocol was approved by the Human Research Ethics Committee at the University of Victoria and conducted in accordance with the declaration of Helsinki, with all participants providing informed, written consent.

Training Protocol

The protocol and experimental design utilized in this study were similar to a previously described experiment in which participants trained using combined arm and leg cycling (Klarner *et al.*, 2016a, b). Participants performed asymmetrical arm cycling training on a Sci-Fit Pro 2 ergometer with the foot pedals removed and seat height adjusted so that the feet were planted firmly on the floor with a session aggregate activity time of 30 minutes three times a week for five weeks. The training was of moderate intensity and participants were asked to maintain a cadence of 1Hz (~60 revolutions per minute (RPM)). Participants were able to take short breaks during the training period if needed, but the aggregate time of 30 minutes remained the same. The arm cranks were adjusted to accommodate individual differences in range of motion on the more affected (MA) side. For participants with more severe weakness or spasticity, hand braces were used to ensure that the MA hand would stay on the handle.

Prior to and then repeated at five minute intervals throughout the 30 minute training sessions, participants were asked to rate their perceived exertion (RPE) using a 10 point scale. Heart rate was also assessed at five-minute intervals using a chest strap

heart rate monitor (PolarElectro, Quebec Canada). RPM were monitored throughout the training sessions to ensure that the target of ~60 was achieved.

The progressive training element of this study involved gradual and minimal increments of the workload over the course of the five weeks, similar to the approach used in other post-stroke training protocols (Zehr, 2011; Klarner *et al.*, 2016a, b). Participants were instructed to exercise at an intensity producing an RPE between 3 and 5, (i.e. 'moderate' activity) and workload was adjusted accordingly. This RPE corresponded to a target heart rate between 50-70% of HR max (Scherr *et al.*, 2013). With participants who used beta-blockers, adjustments were made to target heart rate goals (Tang *et al.*, 2006). The workload was increased across sessions to allow participants to maintain a consistent RPM of ~60 while maintaining a steady RPE. The minimum workload on the ergometer was 10 W. Two individuals were unable to cycle at this workload and instead trained on an arm cycle ergometer (Monark 871E arm ergometer) with no resistance. Blood pressure (BP) was obtained using a digital blood pressure cuff placed over the less affected arm before starting exercise and after its completion. BP was monitored until it returned to pre-exercise levels, at which point participants were allowed to leave the laboratory.

Baseline Control Procedures

As demonstrated in previous experiments, a multiple baseline within-participant control design took the place of a separate control group (Butefisch *et al.*, 1995; Klarner *et al.*, 2016a, b). This design has multiple benefits over a traditional control group design. Although this approach is more labour intensive and requires more time, the multiple baseline design has been used as a valid replacement to the design with a control group

and given high internal consistency of measures. It allowed participants to create a reliable pre-training baseline and enabled them to act as their own pre-intervention control. To evaluate single participant responses to arm cycling training, a 95% confidence interval (95%CI) of dependent variables was calculated from the 3 baseline tests. When the participant's post-test value was outside the 95% CI range, this participant was defined as having significant change. The direction of change was determined and identified as either an improvement or decrement for each participant. An additional benefit of this design is that no participants are relegated to a non-treatment group; therefore everyone receives the potential benefit of exercise. Also, between-participant variability is higher in chronic stroke populations and this design allows participants to be compared to their own variability, rather than the variability of others at baseline. Each participant completed three baseline sessions spread over three weeks prior to beginning the five weeks of training. Tests were completed at the same time each day and other environmental conditions such as lighting, participant position, noise and temperature were kept as consistent as possible (Zehr, 2002; Lagerquist *et al.*, 2006; Dragert & Zehr, 2013). Measures were comprised of three main categories: clinical, physical performance and neurophysiological integrity, previously shown to have high reliability across multiple baseline points (Klarner *et al.*, 2016a, b).

Clinical Measures

Walking measures included the 6 Minute Walk (Enright, 2003), the Timed Up and Go (TUG) (Podsiadlo & Richardson, 1991) and the Timed 10m Walk tests. Balance was assessed using the Berg Balance Scale. The Chedoke McMaster Stroke Assessment was used to evaluate the stage of upper and lower limb impairment on a 7 point scale

where 1 represents total assistance and 7 represents complete independence (Gowland *et al.*, 1993). The Modified Ashworth Scale was used to assess spasticity (Bakheit *et al.*, 2003; Pandyan *et al.*, 2003; Patrick & Ada, 2006), which was measured on the ankle, knee, wrist, elbow flexion and shoulder. The 6-point Functional Ambulation Categories Scale was used as a measure of the basic motor skills necessary for walking (Holden *et al.*, 1984). The ability to discern light touch and pressure was determined for the MA hand and foot using the 5-piece Semmes-Weinstein kit of calibrated monofilaments (Sammons Preston Rolyan, Cedarburg, WI, (Hage *et al.*, 1995)). All clinical measures were performed by the same licensed physiotherapist.

Physical Performance

Strength

Participants sat in a custom-fitted chair designed to minimize extraneous movements, with both feet securely fastened to plates on the floor (Dragert & Zehr, 2011, 2013; Klarner *et al.*, 2014; Klarner *et al.*, 2016a, b). Maximal voluntary isometric contractions (MVCs) for ankle dorsiflexion and plantarflexion were established via strain gauge (Omegadyne Ltd. Model 101-500) and converted to torque. For the upper limb, participants performed maximal isometric handgrip contractions using a commercially available handgrip dynamometer (Takei Scientific Instruments Company Ltd., Niigata, Japan). After being allowed a "test run" to ensure that the right movements were being produced, participants completed two separate trials of 5 s maximal contractions on both the LA and MA sides, for plantarflexion, dorsiflexion and handgrip. Maximum values were determined offline by taking the mean value of 500ms duration around the largest reading generated over the course of the two trials.

Electromyography (EMG)

Bipolar surface electrodes were placed bilaterally over the mid-muscle bellies of the soleus (SOL), tibialis anterior (TA) and anterior deltoid (AD), as well as the biceps brachii (BB), triceps brachii (TB) and the flexor carpi radialis (FCR) on the MA side only. Electrode positions were marked and recorded in relation to anatomical landmarks and placed by the same experimenter each day for consistency. To reduce variation in placement, anatomical landmarks and measurements taken from the first session were used on subsequent sessions. EMG signals from all muscles of interest were preamplified (x5000) and band pass filtered (100-300 Hz) (GRASS P511, AstroMed). This is consistent with previous experiments in this laboratory (Balter & Zehr, 2007; Zehr *et al.*, 2007; Vasudevan & Zehr, 2011; Zehr & Loadman, 2012; Zehr *et al.*, 2012; Klarner *et al.*, 2014; Klarner *et al.*, 2016a, b). After conversion to a digital signal, strength data were sampled at 2000 Hz and walking and arm cycling data were sampled at 1000 Hz using a custom built continuous acquisition software (LABVIEW, National Instruments, TX, USA). Data were low pass filtered at 100 Hz using a 4^{th} order Butterworth filter and full-wave rectified.

During cycling and walking, background electromyography (bEMG) amplitudes were calculated from unstimulated data broken into 8 phases of movement. Phasic bEMG were analyzed offline in three ways (Zehr *et al.*, 2012; Klarner *et al.*, 2014; Klarner *et al.*, 2016a, b): 1) the amplitude was calculated for each phase of the movement cycle (i.e. 1/8 of the movement cycle); 2) a modulation index (MI = [(EMGmax – EMGmin)/EMGmax] x 100) was calculated for each muscle across the movement cycle (Zehr & Haridas, 2003; Zehr & Loadman, 2012; Zehr *et al.*, 2012; Klarner *et al.*, 2014;

Klarner *et al.*, 2016a, b); and, 3) coactivation ratios were calculated for each phase of the movement cycle for the homologous muscles in the arms and legs (AD, TA and SOL), as well as for the antagonist muscles (BB/TB, TA/SOL) on the MA and LA sides. The coactivation ratios in the homologous muscles give an indication of the level of bilateral coordination after stroke, whereas the coactivation ratios in the antagonist muscles reveal the extent to which agonist/antagonist muscle pairs are coordinated during movement (Zehr *et al.*, 2012).

Arm Cycling

During the baseline and post-tests, participants performed arm cycling on an instrumented device that differed from the Sci-Fit used for training. They were seated in the same custom-fitted chair as was used for strength measurements and cycled on a custom made hydraulic arm ergometer (described in (Zehr *et al.*, 2003)) which was positioned directly in front of them. The handles of the ergometer moved together, yet 180 degrees out of phase. Participants were asked to hold the handles firmly, and when necessary, hand braces were provided to ensure the MA hand was securely attached. Depending on a participant's range of motion, the cranks could be adjusted for larger or smaller circular rotations. Prior work showed that asymmetrical changes in crank length were not associated with significant changes in cutaneous reflex modulation (Hundza & Zehr, 2006). The crank positions for individual participants were determined in the first baseline test and kept consistent throughout the study. Arm cycling was performed in a clockwise direction, with the 3 o'clock position (viewed from the right side of the body) being the position of maximal elbow extension and shoulder flexion. Participants cycled for about 4-6 minutes, which corresponded to 160 cycles for analysis. Continuously

acquired data were later broken into movement cycles in which the start and end was indicated by the MA arm at the 12 o'clock position. In order to compare across trials and participants, cycle time was normalized to 100%. Arm cycling phases are illustrated in figure 1B.

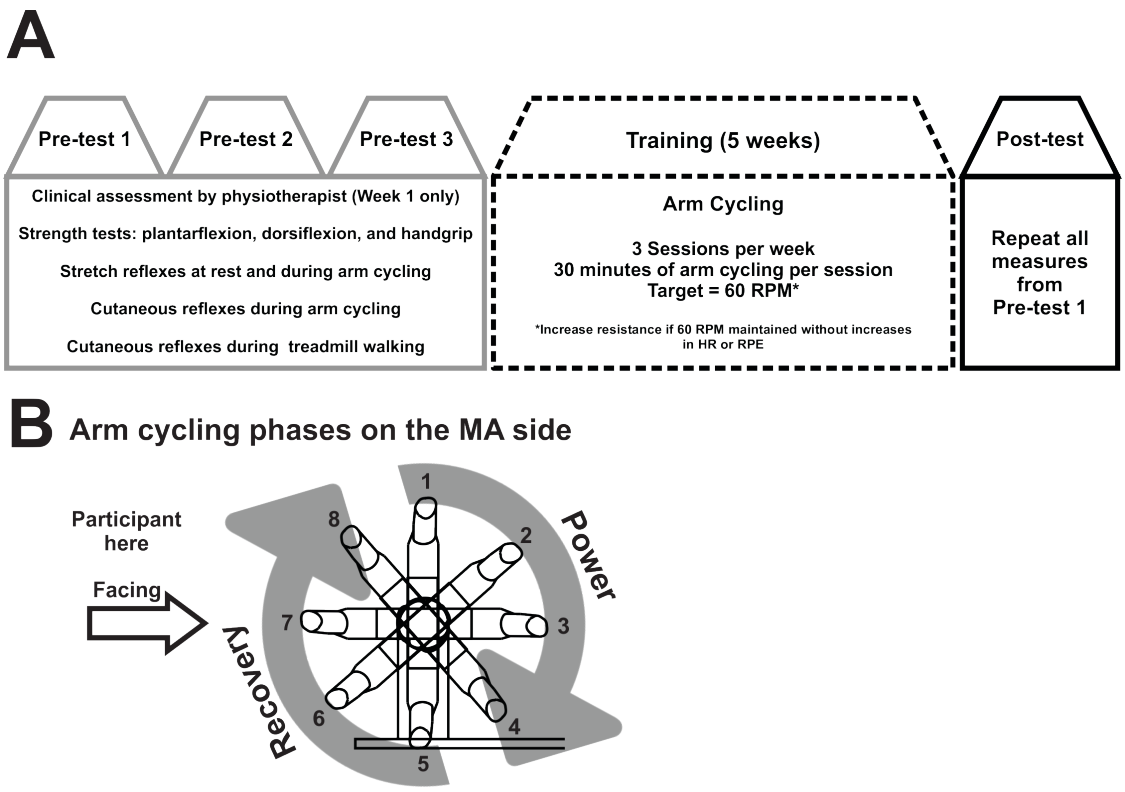

Figure 1. (A) A summary of the experimental timeline, which illustrates the pre- and post-test procedures, and the training parameters. A multiple baseline within-participant control design was used for this experiment. (B) On the left, a graphical summary of the arm cycling training position, and, on the right, labels for the phases of movement within the arm cycling task.

Walking

Participants walked on a motorized treadmill (Woodway US, Waukesha, WI) wearing an overhead safety harness (Pneu-weight, Pneumex Inc, Sandpoint, ID, USA) at a "comfortable" speed. Comfortable was defined to participants as the speed they would

normally comfortably walk. The body weight support feature of the harness was not used for any participants. All walked supporting their own body weight and the harness was used strictly for safety purposes in the event of a fall. An ankle foot orthosis was used only if participants required one for walking during daily activity. Participants were free to place their hands on the side or front railings with one individual requiring thier MA arm to be kept in an over the shoulder sling. Whatever their chosen hand positions were, they were noted and kept consistent across baseline and post-tests.

Neurophysiological Integrity

Cutaneous reflexes elicited during arm cycling and walking and arm cycling-induced modulation of stretch reflexes in the soleus were used to evaluate neurophysiological changes induced by arm cycling training.

Cutaneous Reflexes

Cutaneous reflexes evoked during walking and arm cycling were used to provide insight into the ability of arm cycling training to activate and modulate interlimb integrity over time. Reflexes were evoked via surface stimulation of the superficial radial nerve (SR; innervates the dorsum of the hand) on the LA side. Electrodes were placed just proximal to the radial head at the wrist in a bipolar configuration with the cathode proximal and the anode distal (Klarner *et al.*, 2014; Klarner *et al.*, 2016b). Prior to beginning a trial, perceptual (PT) and radiating (RT) thresholds were found for each participant. RT was determined, as the minimum stimulation intensity required causing radiating paresthesia into the entire innervation area of the nerve. To obtain RT, gradual increments in stimulation intensity were delivered to participants until the maximum area of paresthesia was found. This intensity was then determined as RT. Intensities were then

set to 3 x RT for the duration of the stimulation trials, providing it was tolerated by the participant. SR stimulation was delivered as trains of 5 x 1.0 ms pulses at 300Hz (P511 Astro-Med Grass Instrument) by a Grass S88 stimulator with SIU5 stimulus isolation and a CCUI constant current unit (Astro-Med Grass Instrument, West Warwick, RI). During arm cycling, participants received 160 stimulations pseudo-randomly with an inter-stimulus train interval of 1-5 seconds. During walking, stimulation was delivered in a similar manner, but yielding 120 stimulations.

All data were recorded using a custom-written LabVIEW (National Instruments, Austin, TX) and analyzed using custom written Matlab (version R2011b, Mathworks, Nantick, MA) applications. Stimulus artefact was removed from each reflex trace and data were low-pass filtered at 30 Hz using a dual-pass 4^{th} order Butterworth filter. Movement cycles were broken down into 8 equidistant phases. For each phase, the average non-stimulated "control" trace was subtracted from the average stimulated trace, producing a subtracted reflex trace. In order to account for the obscure phase-dependent modulation of net reflexes with cycling (Zehr *et al.*, 2001), we chose to include net reflexes along with the analysis of reflexes at given latencies. Cutaneous reflex amplitudes were quantified in three ways: subtracted peak amplitudes at 1) early (~50 - 80ms to peak) and 2) middle (~80 - 120ms to peak) latencies (Zehr & Loadman, 2012; Zehr *et al.*, 2012), and 3) the average cumulative reflex over 150 ms following stimulation ($ACRE_{150}$) within each phase (Klarner *et al.*, 2014; Klarner *et al.*, 2016b).

Stretch Reflexes

Stretch reflexes were evoked using an electrodynamic shaker with an attached accelerometer (ET-1126B; Labworks Inc) placed over the Achilles tendon similar to

procedures used previously in our lab (Palomino *et al.*, 2011; Mezzarane *et al.*, 2014; Klarner *et al.*, 2016b). Constant pressure was applied to the tendon, and the shaker was programmed to deliver a single sinusoidal pulse. Each participant completed six trials of stretch reflexes; three on the LA side and three on the MA side. The first trial consisted of a recruitment curve and participants received a series of pulses of increasing amplitudes until a maximal stretch reflex was found during quiet sitting. During the second trial, participants received 20 pulses at an amplitude that elicited ~70% of their maximal stretch reflex during quite sitting with their arms at rest (static) but at the "7 o'clock" position for the LA hand. During the third trial, reflexes were evoked during rhythmic arm cycling at 1 Hz (conditioned) when the LA hand was at the "7 o'clock" position. In order to evaluate the modulatory effect of arm cycling on stretch reflexes, the static amplitude was subtracted from the arm cycling conditioned amplitude and then expressed as a percentage of the static amplitude. A negative value indicates suppression and a positive value indicates a facilitation of stretch reflexes during cycling. In order to compare modulation of stretch reflexes between the LA and MA sides, the conditioned-static difference of the MA side was subtracted from the difference of the LA side. Negative and positive values indicate greater cycling-induced modulation on the LA and MA sides, respectively. Effects of homologous and heteronymous muscle activity was monitored and recorded from a 20ms prestimulus period. EMG data were normalized to the peak EMG recorded during either walking or arm cycling for each session and each muscle.

Statistics

Statistical procedures were performed using SPSS 18.0 (Chicago, Illinois). Two types of analysis were performed: single participant and group analyses.

For single participant comparisons, a 95% confidence interval (CI) was determined from the three pre-test values. Post-test values were then compared to the 95% CI established from the pre-tests. If the post-test fell outside of the 95% CI, it was considered statistically significant (Cummings, 2013). The total number of participants with significant changes is reported. All within-participant data are reported in appendix 1.

For pre- to post-training group comparisons, a repeated measure ANOVA was run to compare differences across the three pre-test sessions. If no differences were found, data were pooled together to form an average pre-test value for each measure, and compared to the post-test value using a paired samples *t*-test (Klarner *et al.*, 2016a, b). For phase-dependent modulatory effects of cycling and walking on bEMG and cutaneous reflexes irrespective of training effects, one-way (PHASE) repeated measures ANOVAs were performed. Significant effects of phase are reported as either significant (i.e. "*") or non-significant (i.e. "ns") in Table 4. Following the tests for phase-dependent modulation, multiple factor repeated measures ANOVAs were utilized to determine main and interaction effects of time point (i.e. pre- and post-training) and phase of movement (i.e. 8 phases of either arm cycling or walking). Assumptions for ANOVA and paired samples *t*-tests were evaluated as parametric tests for within-participants design. The observed effect for pre- to post-test differences are reported as Cohen's effect size (d), with $0.2 \leq d < 0.5$, $0.5 \leq d < 0.8$ and $d \geq 0.8$ corresponding to small, medium and large effects (Cohen, 1988), respectively. When direction of change was predicted because of

priori hypotheses, one-tailed paired samples t-tests were performed. In all cases, statistical significance was set at $p \leq 0.05$. Results are reported as means ± SD in text (SEM in figures).

Results

Arm Cycling Training

All participants completed 15 sessions of arm cycling training. Figure 2 shows the group means for the average HR, RPE, RPM and Workload recorded throughout each arm cycling training session. HR ($p = 0.79$) was maintained during each session and did not differ throughout the training sessions whereas RPM ($p < 0.001$) and workload ($p = 0.019$) increased over time and were significantly greater during session 15, compared to session 1. Despite the increased difficulty in arm cycling, perceived effort levels remained unchanged throughout the training program, evidenced by no change in RPE ($p = 0.15$).

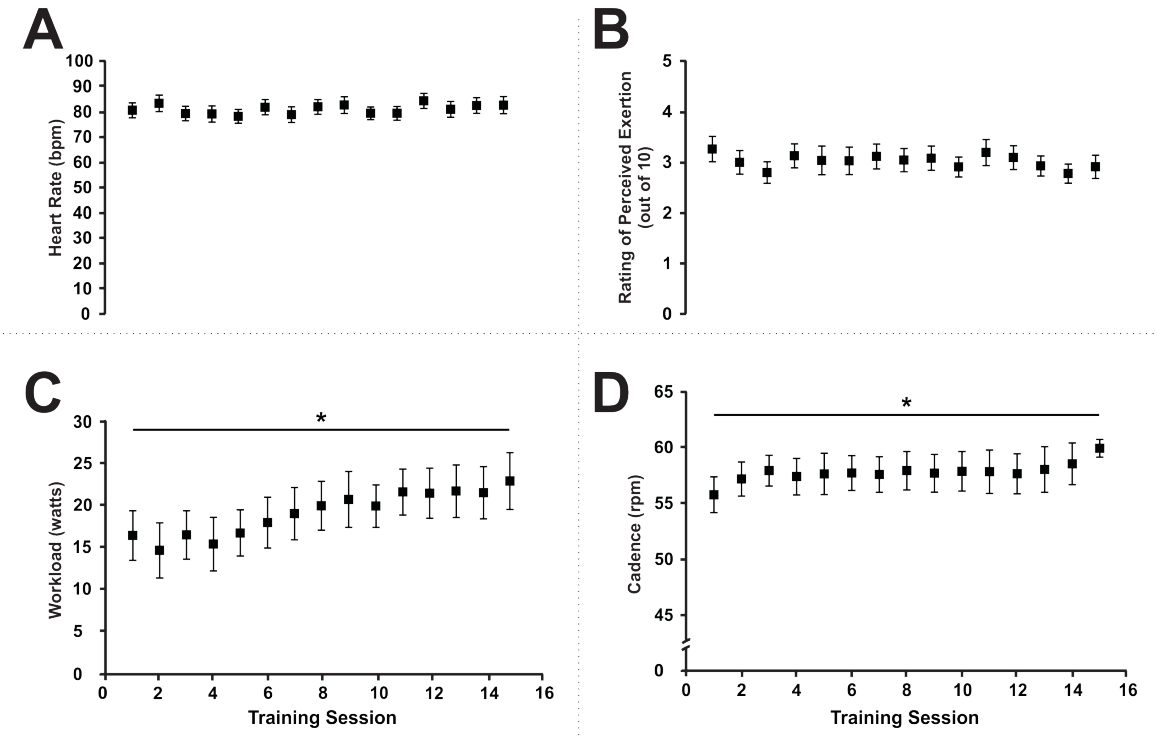

Figure 2. *Training data. Data recorded for training parameters of HR (A), RPE (B), Workload (C), and Cadence (D) throughout each training session. Data points are group (n = 19) means (± SEM) of an average of data recorded at 5-minute intervals. * indicates a significant ($p < 0.05$) difference between the first and last training session.*

Clinical Measures

Participants significantly improved their performance of the 6-Minute Walk, TUG, and 10 Meter Walk tests from pre- to post-training (Figure 3). For the 6 Minute Walk, participants walked an average of 245.1m initially which subsequently increased by 8.5% to 266.1m ($p = 0.011$, d = 0.46), and corresponds to a change greater than the 7.4m minimal detectable change for individuals after stroke (Perera *et al.*, 2006). Participants reduced their time to perform the Timed-Up and Go (TUG) by 28.9% ($p = 0.045$, d = 0.23) from 37.3 to 26.5s, which is greater than the 2.9s minimal detectable change for individuals after stroke (Flansbjer *et al.*, 2005). They furthermore reduced their 10 Meter Walk time by 15.1% ($p = 0.049$, d = 0.39) from and from 24.5 to 20.8s,

which is slightly less than the 3.7s minimal detectable change for individuals after stroke (Perera *et al.*, 2006). Balance, as assessed by the Berg Balance Scale, also improved (5.7%, $p = 0.014$, d = 0.3), from a score of 41.5 to 43.9, but this change was slightly less than the minimal detectable change of 2.5 for individuals after stroke (Liston & Brouwer, 1996).

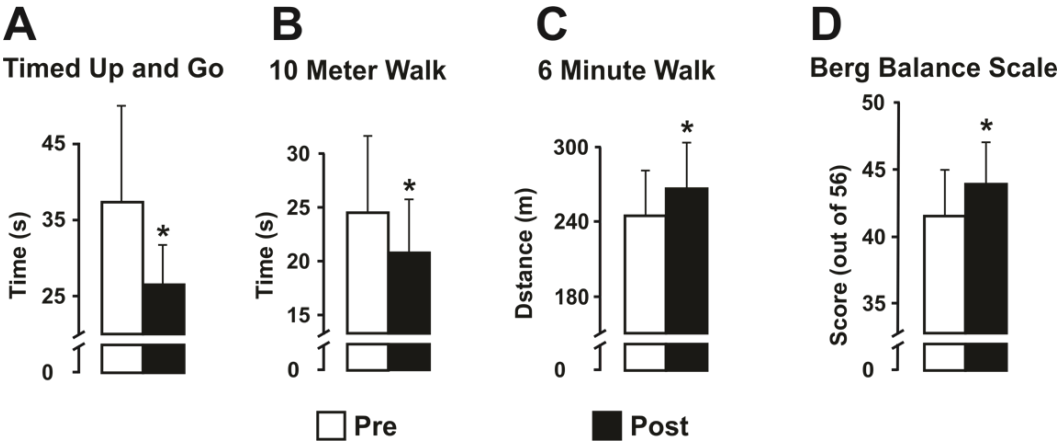

Figure 3. *Clinical assessments of walking and balance. Pre- (unfilled bars) and post-test (filled bars) group data for the Timed Up and Go (A), 10 Meter Walk (B), 6-minute Walk (C), and Berg Balance Scale (D). Bars are group (n = 18) means (± SEM) and * indicates a significant ($p < 0.05$) change from pre to post.*

Individual data for walking tests are shown in Table 2 and balance in Table 1. Chedoke-McMaster Stroke Assessment scores reflected on average, positive significant change in the Shoulder (3.1%, $p = 0.041$, d = 0.39), Hand (6.2%, $p = 0.01$, d = 0.46), Leg (3.5%, $p = 0.041$, d = 0.36) and Foot (5.6%, $p = 0.02$, d = 0.37) categories. Using the Modified Ashworth Scale, only a small number of participants (5) saw any positive change in spasticity in the ankle, knee, wrist or bicep. Individual scores are shown in Table 1. There was no meaningful change pre- to post-training of the ability to detect

light touch with the MA hand or foot, as measured with calibrated monofilaments (see table 1).

Table 2.

Summary of individual pre and post-training scores for the clinical assessments of walking ability

Participant	6 Minute Walk (m)			Timed Up and Go (s)			10 Meter Walk (s)		
	Pre	**Post**	***% Change***	**Pre**	**Post**	***% Change***	**Pre**	**Post**	***% Change***
1	217.8	273.3	*25.5*	21.1	18.1	*-14.2*	9.6	10.8	*12.4*
2	50.2	53.4	*6.4*	76	58.2	*-23.4*	67	57.6	*-14.1*
3	131.3	167.8	*27.8*	30.1	27.1	*-9.9*	23.1	19.1	*-17.6*
4	254.9	318.7	*25*	14.8	13.1	*-11.8*	11.8	10.7	*-9.2*
5	505.2	489.5	*-3.1*	7.3	7.6	*2.9*	6.5	6.7	*2.6*
6	370.3	366.9	*-0.9*	21	19.1	*-9.1*	11.9	10.1	*-15.3*
7	63.8	68.1	*6.8*	67.5	46.8	*-30.6*	51.3	44.2	*-13.9*
8	421.8	496.3	*17.7*	10.5	9.5	*-9.1*	7.4	6.9	*-6.7*
9	417.2	507	*21.5*	7.3	7.3	*0*	6	5.9	*-2*
10	412.6	412.2	*-0.1*	13.4	9.7	*-27.6*	9.4	7.6	*-19*
11	243.2	224	*-7.9*	15.9	16.6	*4.1*	11	12.1	*9.9*
12	39.5	37.9	*-4*	54.8	56.2	*2.6*	63.6	58.5	*-8.1*
13	60.8	86.9	*42.9*	80.8	56.7	*-29.8*	35.3	25.3	*-28.6*
14	222.2	242.5	*9.2*	14.4	14.5	*0.4*	12.2	11.5	*-5.1*
15	26.4	31.3	*18.6*	242	87.5	*-63.9*	127	77.9	*-38.7*
16	349.1	309.8	*-11.3*	10.5	11.9	*12.7*	7.8	6.3	*-20.3*
17	330.6	392	*18.6*	16.7	9.7	*-42*	10.6	5	*-52.8*
18		N/A			N/A			N/A	
19	298.6	316.4	*6*	16.5	12.7	*-22.8*	7.6	8.3	*9.1*
MEAN	**245.3**	**266.3**	***8.6***	**40**	**26.8**	***-33.1***	**26.6**	**21.3**	***-19.8***
SD	**153.86**	**162.93**	***14.52***	**55.89**	**23.6**	***19.08***	**32.01**	**22.34**	***16.72***

Note: Assessments include the 6-minute Walk (distance in meters), Timed Up and Go (time in seconds), and 10 Meter Walk (time in seconds).

Maximal isometric strength

Repeated measures ANOVA showed that there were no significant differences between baseline pre-test values for torque recorded during any MVCs (p ranged from 0.151-0.786) nor were there significant differences between baseline pre-test values for

EMG of any muscles measured during MVCs (p ranged from 0.187-0.903). Average pre- to post-training strength and muscle activity changes are summarized in Figure 4.

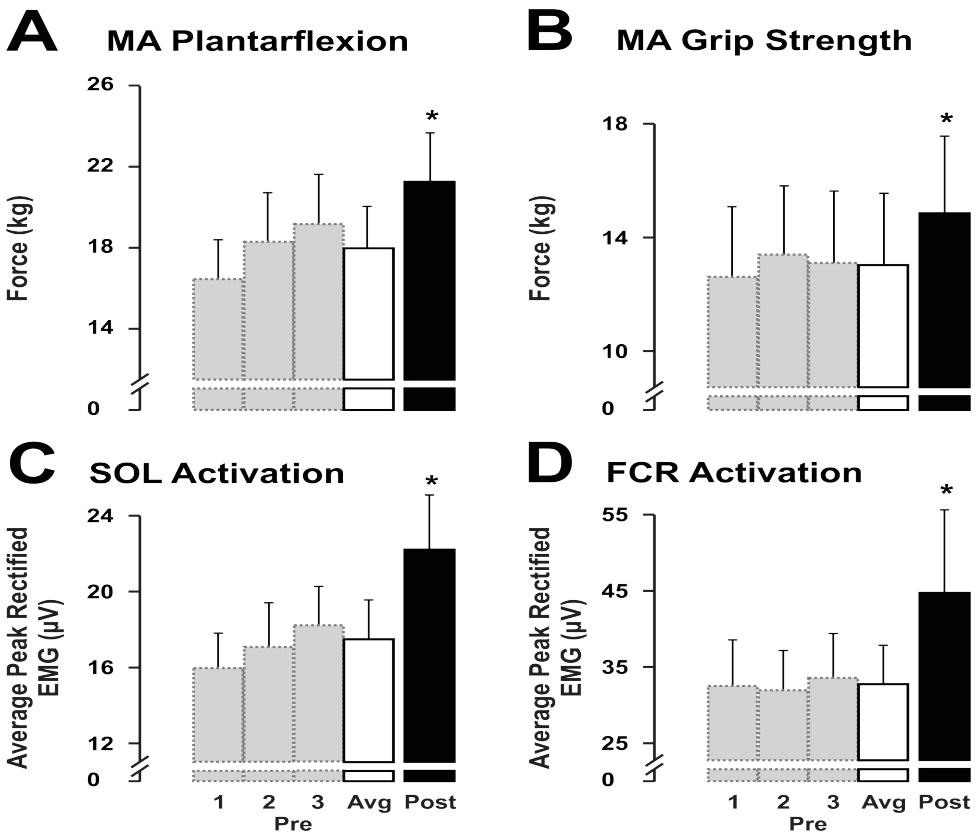

Figure 4. *Strength and muscle activity during isometric contractions. Pre 1, 2, and 3 data are displayed in gray, whereas pre- (unfilled bars) and post-test (filled bars) group data for MA Plantarflexion force (A), MA Grip Strength (B), MA SOL muscle activity during plantarflexion MVC (C), and MA FCR muscle activity during Handgrip MVC (D). Bars are group (n = 18) means (± SEM) and * indicates a significant ($p < 0.05$) change from pre average to post.*

From pre- to post-training, handgrip force increased by 13 ($p = 0.02$, d = 0.27) and 8.4% ($p < 0.001$, d = 0.39) on both the MA and LA sides, respectively. Peak EMG activity of the FCR measured on the MA side concurrently increased by 30% ($p = 0.04$, d = 0.32). Plantarflexion torque of the MA side increased by 20% ($p = 0.025$, d = 0.31),

while there were subsequent increases in bilateral SOL peak EMG during plantarflexion MVCs (MA: $p = 0.019$, d = 0.38, see figure 4B, LA: $p = 0.035$, d = 0.24). Dorsiflexion peak torque and subsequent TA peak EMG did not differ statistically pre- to post-arm cycling training (p ranged from 0.1 – 0.49). Individual participant data are summarized in Table 3.

Table 3.

Summary of the number of participants with post values for torque and EMG that were outside of the 95% CI established from their baseline measurements

	MA		LA	
	Torque	**EMG**	**Torque**	**EMG**
Handgrip	10	12 (FCR)	13	N/A
Plantarflexion	9	9 (SOL)	9	8 (SOL)
Dorsiflexion	6	7 (TA)	9	6 (TA)

Note: The EMG from a muscle of interest corresponding to handgrip, plantarflexion or dorsiflexion is indicated in parenthesis.

Muscle activity during arm cycling

Muscle activity across all 8 phases of movement during arm cycling did not differ between the three pre-tests for any muscle measured. Phase-dependent modulation of EMG during arm cycling was noted for the MA BB, MA TB and LA AD prior to training, however, following training the MA AD also showed phase-dependent modulation (see Table 4A). A two factor (Phase x Time) ANOVA revealed a significant interaction effect for the MA AD ($F_{(7,126)} = 6.325$, $p = 0.023$), MA BB ($F_{(7,126)} = 5.870$, $p = 0.006$) and LA AD ($F_{(7,126)} = 6.902$, $p = 0.001$). Compared to the pre-test average, EMG activity of the MA AD was significantly decreased during phases 2 ($p = 0.007$, $d = 0.5$), 3 ($p = 0.049$, $d = 0.3$), and 7 ($p = 0.037$, $d = 0.42$), which correspond to the late power (phase 2 and 3) and late recovery (phase 7) phase of cycling (see Figure 1B). EMG

activity of the LA AD was significantly increased at phases 1 through 4 (p ranged from < 0.001 to 0.015, d from 0.48 to 0.8) which correspond to the recovery and transition to power phase for that limb. Activity in the MA BB was increased at phase 4 (p = 0.033, d = 0.24), which corresponds to early recovery.

Table 4.

Summary of significant main effects during a one factor RM ANOVA across all phases of movement for arm cycling (A) and walking (B)

A) Arm cycling	***bEMG***		***ELR***		***MLR***		***ACRE150***	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
MA AD	ns	*	*	*	ns	ns	ns	ns
MA BB	*	*	ns	ns	ns	ns	ns	ns
MA TB	*	*	ns	ns	ns	ns	ns	ns
MA FCR	ns	ns	ns	ns	ns	ns	ns	ns
LA AD	*	*	*	*	*	*	*	*

B) Walking	***bEMG***		***ELR***		***MLR***		***ACRE150***	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
MA SOL	*	*	ns	ns	ns	ns	ns	ns
MA TA	ns	*	ns	*	ns	ns	ns	ns
LA SOL	*	*	*	*	ns	ns	ns	ns
LA TA	ns	*	ns	ns	ns	*	ns	ns
MA AD	ns	*	ns	ns	ns	ns	ns	ns
LA AD	*	*	ns	ns	ns	ns	ns	ns

*Note: * indicates a significant main effect of phase (i.e. phase-dependent modulation of EMG or reflex), whereas 'ns' indicates no main effect of phase was found.*

Individual participant analysis revealed that there was increased modulation of muscle activity during arm cycling in about half of the participants for most muscles (see table 5). As a group, the modulation index of bilateral AD muscle activity was altered following arm cycling training (see figure 5A). On the MA side, the MI of AD EMG increased by 18% (p = 0.013, d = 0.56) over the entire arm cycling movement. The MI of the LA AD EMG, inversely, decreased by 20% (p < 0.001, d = 0.87).

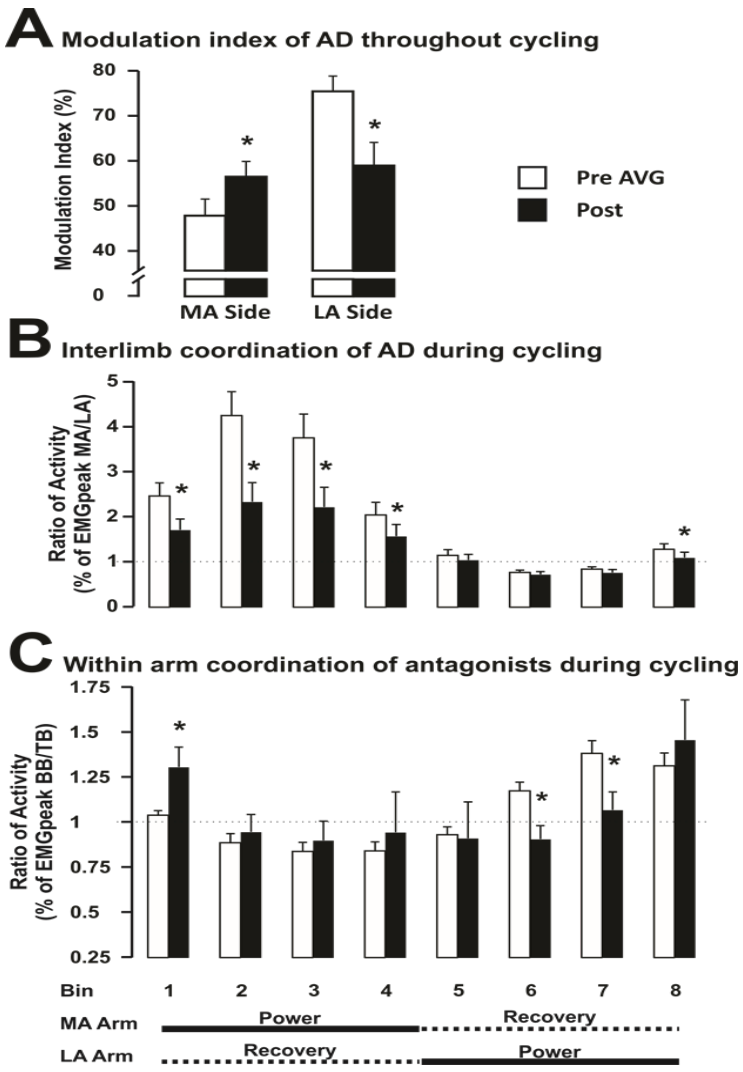

Figure 5 *Muscle activity during arm cycling. The modulation index for both the MA and LA AD during arm cycling is shown in (A). The ratio of normalized muscle activity of the MA divided by LA AD throughout arm cycling is displayed in (B). The ratio of normalized muscle activity of the BB divided by TB on the MA side throughout arm cycling is displayed in (C). For panels (B) and (C), phases of movement are indicated at the bottom for both the MA and LA arms. In all panels, unfilled are the pre average and filled bars are the post values. All bars are group (n = 18) means (± SEM) and * indicates a significant ($p < 0.05$) change from pre average to post.*

Coordination of AD muscle activity from the MA to LA side was altered following arm cycling training (see figure 5B). The ratio of MA AD to LA AD activity was decreased at phase 1 ($p < 0.001$, $d = 0.62$), 2 ($p < 0.001$, $d = 0.86$), 3 ($p = 0.002$, $d =$

0.68), 4 ($p = 0.012$, $d = 0.3$) and 8 ($p = 0.011$, $d = 0.39$) corresponding to the power phase of the MA limb, at which point there should be more activity in the LA AD and inhibition of the MA AD to perform a coordinated movement. Within arm coordination of the MA BB and TB was also altered following arm cycling training (see figure 5C). During phases 6 ($p = 0.008$, $d = 0.99$) and 7 ($p = 0.043$, $d = 0.84$), the BB/TB ratio was decreased, whereas, during phase 1 ($p = 0.041$, $d = 0.77$), the BB/TB ratio was increased, compared to pre-test values.

Table 5

Summary of the number of participants with arm cycling bEMG modulation index (MI) post values for that were outside of the 95% CI established from their baseline measurements.

bEMG Modulation Index During Arm Cycling	
Measure	Count
MA AD	12/19
MA BB	6/19
MA TB	11/19
MA FCR	11/19
LA AD	12/19

Muscle activity during walking

Muscle activity during all 8 phases of walking did not differ between the three pre-tests for any muscle measured. Phase-dependent modulation of EMG during walking was noted for the MA and LA SOL, and LA AD prior to training. However, after training the MA and LA TA, and MA AD also showed phase-dependent modulation (see Table 4B). A two factor (Phase x Time) ANOVA revealed a significant interaction effect for the MA TA ($F_{(7,126)} = 6.372$, $p = 0.002$), LA TA ($F_{(7,126)} = 3.613$, $p = 0.029$), MA SOL

($F_{(7,126)} = 8.363$, $p = 0.002$), LA AD ($F_{(7,126)} = 7.647$, $p < 0.001$), and MA AD ($F_{(7,126)} = 2.677$, $p = 0.013$). Following arm cycling training, the most notable changes in muscle activity during walking were in the MA TA (see figure 6A). EMG of the MA TA was increased at phases 2 ($p = 0.004$, $d = 0.64$), 3 ($p = 0.002$, $d = 0.72$) and 4 ($p = 0.037$, $d = 0.4$), whereas it was decreased at phases 6 ($p = 0.046$, $d = 0.39$), 7 ($p = 0.027$, $d = 0.53$) and 8 ($p = 0.04$, $d = 0.55$), which correspond to swing and stance of the MA limb, respectively. There was also a decrease in MA SOL EMG during phase 2 ($p = 0.049$, $d = 0.37$) compared to pre-test, which corresponds to early swing. On the LA side, there was a significant increase in TA activity at phase 8 ($p = 0.024$, $d = 0.57$), which corresponds to late swing of the LA limb. In the upper limbs, there was increased AD activity at phase 2 ($p = 0.022$, $d = 0.36$) and 8 ($p = 0.015$, $d = 0.38$) for the MA and LA sides, respectively, which both correspond to phases of movement that contain forward arm swing.

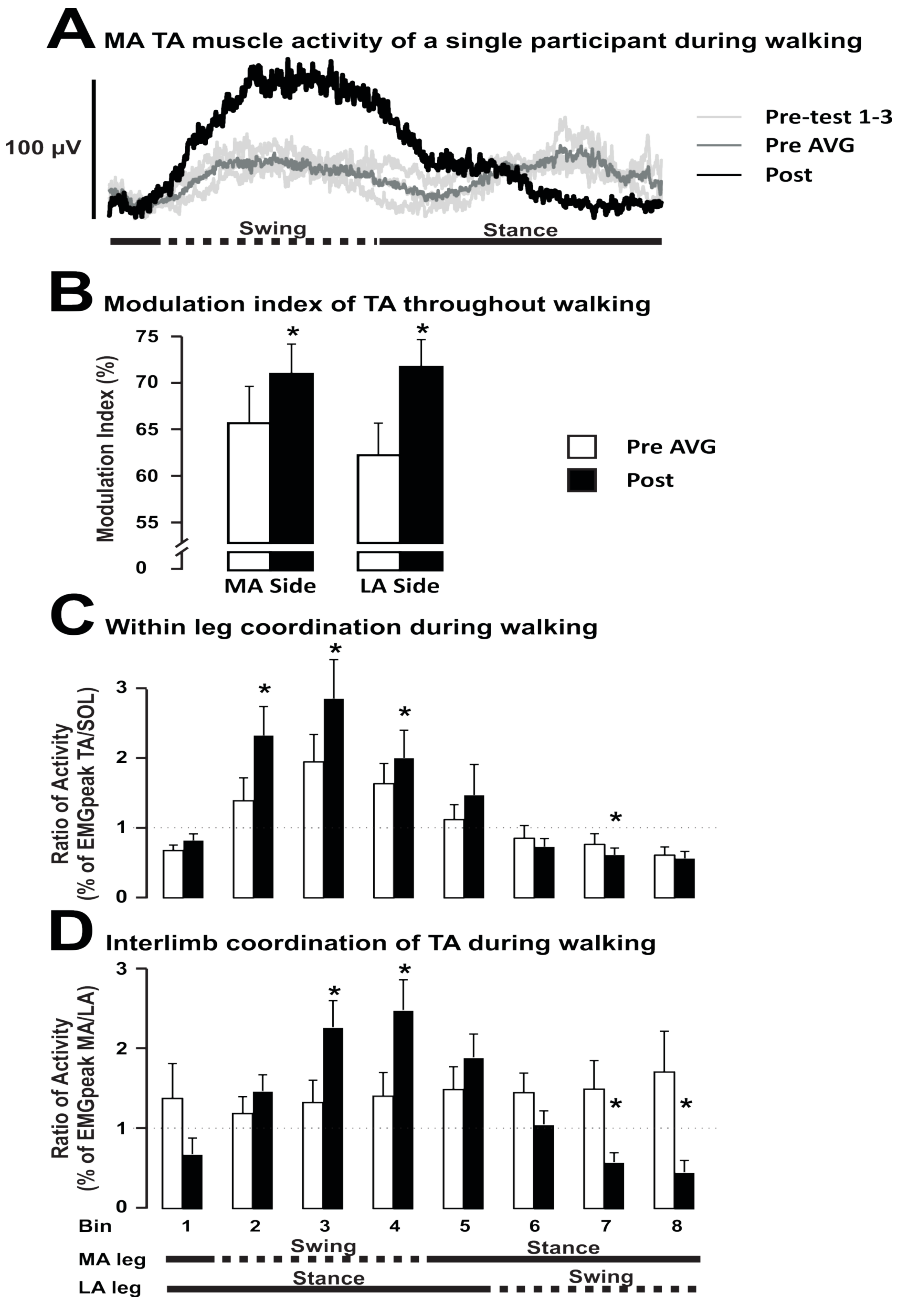

Figure 6. *Muscle activity during walking. An individual's raw EMG recording of the MA TA is shown in (A). Lighter gray traces are pre-test recordings, whereas the dark gray trace indicates the pre average and the black trace is the post-test recording. The modulation index for both the MA and LA TA during walking is shown in (B). The ratio of normalized muscle activity of the TA divided by SOL on the MA side throughout walking is displayed in (C). The ratio of normalized muscle activity of the MA divided by LA TA during walking is displayed in (D) For panels (C) and (D), phases of movement are*

*indicated at the bottom for both the MA and LA legs. In panels (B), (C), and (D), unfilled are the pre average and filled bars are the post values. All bars are group (n = 18) means (± SEM) and * indicates a significant ($p < 0.05$) change from pre average to post.*

Individual participant analysis revealed that there was increased modulation of muscle activity during walking in less than half of the participants for most muscles (see Table 6). As a group, the MI of muscle activity throughout the walking cycle was altered in the TA bilaterally following arm cycling training (see figure 6B). The MI increased by 8 ($p = 0.03$, $d = 0.26$) and 15.2% ($p = 0.002$, $d = 0.53$) for the MA and LA TA, respectively.

Within the MA limb (see figure 6C), the TA/SOL coactivation ratio was increased during MA swing phases 2 ($p = 0.042$, $d = 0.51$), 3 ($p = 0.049$, $d = 0.4$), and 4 ($p = 0.029$, $d = 0.21$) whereas it was decreased at MA stance phase 7 ($p = 0.027$, $d = 0.26$). Interlimb coordination of the TA muscle was significantly altered following arm cycling training (see figure 6D). The MA/LA ratio of TA activity increased during MA swing phases 3 ($p = 0.036$, $d = 0.8$) and 4 ($p = 0.014$, $d = 0.84$), whereas it was significantly decreased during LA swing phases 7 ($p = 0.03$, $d = 0.56$) and 8 ($p = 0.028$, $d = 0.44$).

Table 6

Summary of the number of participants with walking bEMG modulation index (MI) post-training values that exceeded the 95% CI established from baseline measurements

bEMG Modulation Index During Walking	
Measure	Count
MA SOL	4/19
MA TA	5/19
LA SOL	12/19
LA TA	7/19
MA AD	6/19
MA BB	9/19
MA TB	9/19
MA FCR	4/19
LA AD	7/19

Neurophysiological Integrity

Cutaneous reflexes during arm cycling

Reflexes evoked by stimulation of the LA arm (i.e. SR nerve) resulted in significant phase-dependent modulation in the MA and LA AD of early latency reflexes (see figure 7A, top and bottom panel), but only in the LA AD for middle latency reflexes and $ACRE_{150}$ (see bottom panel of figure 7B) during arm cycling (see Table 4A).

Group averaged early latency reflexes are plotted in figure 7A. There was sign reversals in the MA TB and BB, a general reduction in the reflex amplitude in the MA FCR, and a trend for increased modulation of reflexes in the LA AD. Interaction effects (phase x time) of early latency reflexes were revealed on the MA side for the BB ($F_{(7,126)} = 5.280$, $p = 0.034$), TB ($F_{(7,126)} = 7.683$, $p = 0.013$) and FCR ($F_{(7,126)} = 3.477$, $p = 0.014$) muscles, and also in the LA AD ($F_{(7,126)} = 6.372$, $p = 0.002$). Following arm cycling training, early latency reflexes in the MA TB were significantly reduced during the

transition from recovery to power phase (phase 8, $p = 0.044$, d = 0.37) and majority of the power phase (phase 1, $p = 0.039$, d = 0.34, phase 2, $p = 0.022$, d = 0.37, phase 3, $p = 0.03$, d = 0.37), including kinematic phase-reversals for phases 2 and 3. In the MA BB, early latency reflexes were reduced from 4.13 to -4.51% of peak bEMG during the transition from power to recovery phase (phase 4, $p = 0.008$, d = 0.43), a kinematic phase-reversal. In the MA FCR, early latency reflexes were generally reduced, which was significant during phases 1 ($p = 0.033$, d = 0.64), 5 ($p = 0.023$, d = 0.54), and 6 ($p = 0.019$, d = 0.51). In the LA AD, early latency reflexes were reversed from 1.91 to -2.7% of peak bEMG during the late recovery phase (phase 3, $p = 0.007$, d = 0.53), and reduced in mid to late-power (phase 6, $p = 0.038$, d = 0.53, phase 7, $p = 0.049$, d = 0.46).

Interaction effects (phase x time) of middle latency reflexes were revealed on the MA side for the BB ($F_{(7,126)} = 5.363$, $p = 0.033$) and TB ($F_{(7,126)} = 5.079$, $p = 0.037$). Compared to pre-training, middle latency reflexes in the MA TB were reversed from excitatory to inhibitory during the mid to late-power (phase 2, $p = 0.021$, d = 0.51, phase 3, $p = 0.01$, d = 0.5). Furthermore, middle latency reflexes were reduced in the MA BB during late recovery (phase 7, $p = 0.049$, d = 0.35) and transition to the power phase (phase 8, $p = 0.025$, d = 0.46). No other significant training effects of middle latency effects were observed.

Group averaged net reflexes (i.e. $ACRE_{150}$) are plotted in figure 7B. General blunting of reflex modulation is observed in the MA TB, BB and FCR prior to training, however there are changes in reflex amplitudes that suggest more modulation of reflexes throughout the phases of arm cycling. Interaction effects (phase x time) of net reflexes were revealed for all upper limb muscles measured in this experiment (MA AD: $F_{(7,126)} =$

3.877, $p = 0.04$, MA BB: $F_{(7,126)} = 7.318$, $p = 0.014$, MA TB: $F_{(7,126)} = 13.799$, $p = 0.002$, MA FCR: $F_{(7,126)} = 5.237$, $p = 0.006$, LA AD: $F_{(7,126)} = 3.612$, $p = 0.015$). In general, compared to pre-training, $ACRE_{150}$ amplitudes were less facilitatory post-training. In the MA AD, $ACRE_{150}$ was decreased in the early power phase (phase 1, $p = 0.03$, d = 0.39). In the MA TB, $ACRE_{150}$ was reduced during early power (phase 1, $p = 0.016$, d = 0.62) and functionally reversed through mid-power (phase 2, $p = 0.021$, d = 0.51, phase 3, $p = 0.024$, d = 0.59). In the MA BB, $ACRE_{150}$ was reduced during mid-power (phase 3, $p = 0.04$, d = 0.61) and the transition to recovery (phase 4, $p = 0.03$, d = 0.45). In the MA FCR, there were general reductions in the ACRE150 but this measure was only significantly reduced during early power (phase 1, $p = 0.045$, d = 0.59). On the LA side in the AD, $ACRE_{150}$ became more inhibitory during mid-power (phase 6, $p = 0.022$, d = 0.7).

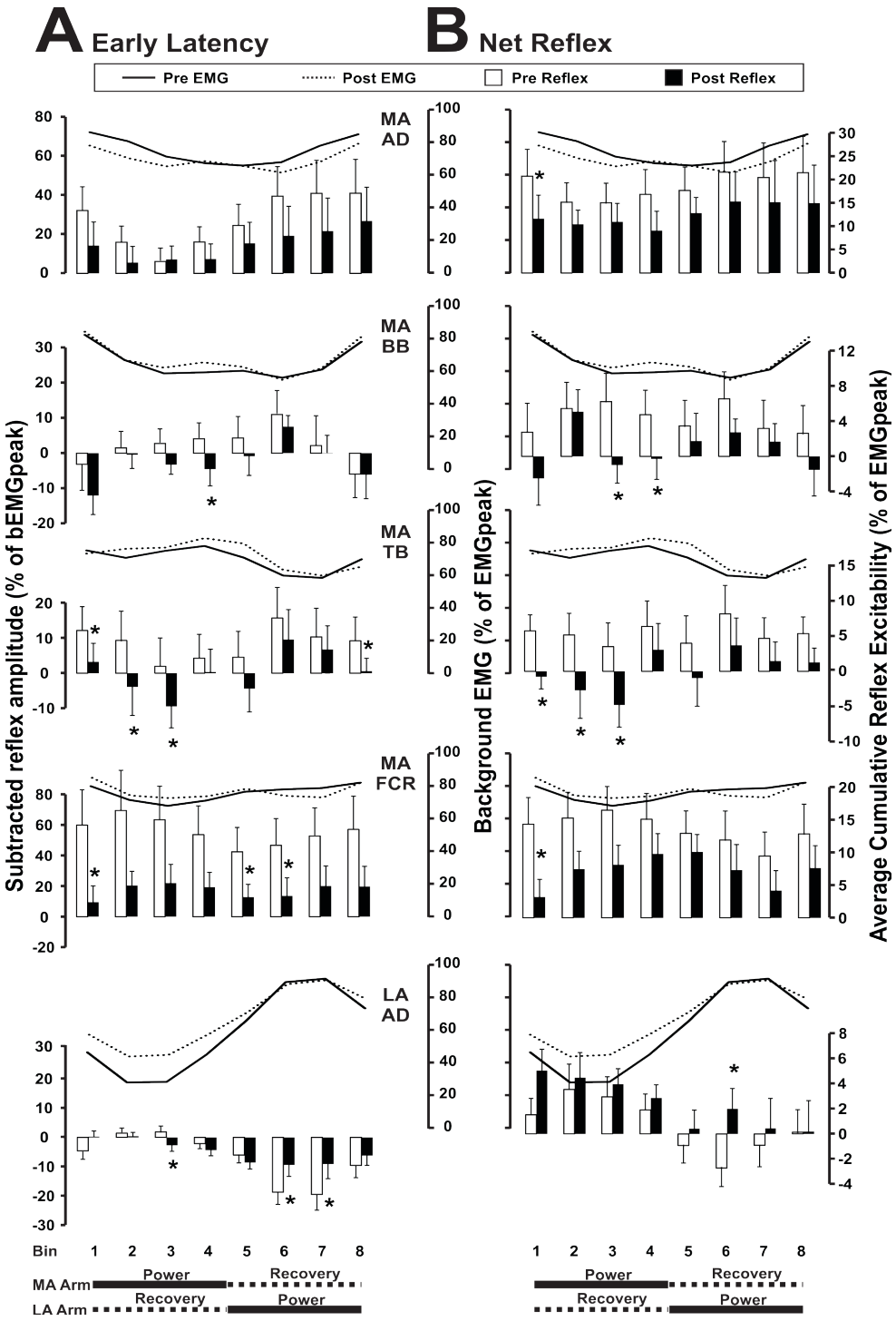

Figure 7. *Cutaneous reflexes during arm cycling. Early latency (A) and net reflexes (i.e. $ACRE_{150}$,(B)) during eight phases of arm cycling are shown for the MA AD (top), MA BB (second from top), MA TB (third from top), MA FCR (fourth from top) and LA AD (bottom). Unfilled are the pre average and filled bars are the post values for reflexes. Secondary axis (right for (A) and Left for (B)) values indicate EMG amplitude as a*

*percentage of the peak EMG and are displayed as line graphs in each panel. The solid line is the pre average whereas the broken line is the post value. All bars are group (n = 18) means (± SEM) and * indicates a significant (p < 0.05) change from pre average to post.*

Cutaneous reflexes during walking

Training induced plasticity of cutaneous reflexes transferred to walking but was modest compared with the changes observed above during arm cycling. Reflexes evoked by stimulation of the LA arm (i.e. SR nerve) resulted in significant phase-dependent modulation of early latency reflexes in the LA SOL pre- and post-training and in the MA TA post-training only. Furthermore, there was significant phase-dependent modulation of middle latency reflexes in the LA TA post-training only. No other phase-dependent modulation of early or middle latency and net reflexes was observed (see Table 4B).

No significant interaction effects were revealed for early latency reflexes during walking.

Interaction effects (phase x time) of middle latency reflexes were revealed for the LA TA ($F_{(7,126)} = 2.573$, $p = 0.016$). There were kinematic reversals from inhibitory to facilitatory during early stance (phase 2, $p = 0.014$, d = 0.59) and from facilitatory to inhibitory during late swing (phase 7, $p = 0.037$, d = 0.70).

Group averaged net reflexes (i.e. $ACRE_{150}$) are plotted in figure 8. General blunting of reflex modulation is observed, especially in the LA and MA AD muscles, prior to training. After training, there are changes in reflex amplitudes that suggest more modulation of reflexes at specific phases of walking. Interaction effects (phase x time) of net reflexes (ACRE150) were revealed for the LA TA ($F_{(7,126)} = 2.868$, $p = 0.025$) and MA AD ($F_{(7,126)} = 2.573$, $p = 0.016$). Compared to pre-training, LA TA ACRE150 was decreased during early stance (phase 2, $p = 0.044$, d = 0.50). In the MA AD, ACRE150

was decreased during transition to backswing (phase 4, $p = 0.017$, d = 0.47) and during mid-backswing (phase 6, $p = 0.03$, d = 0.37, phase 7, $p = 0.014$, d = 0.45). No other interaction effects were revealed.

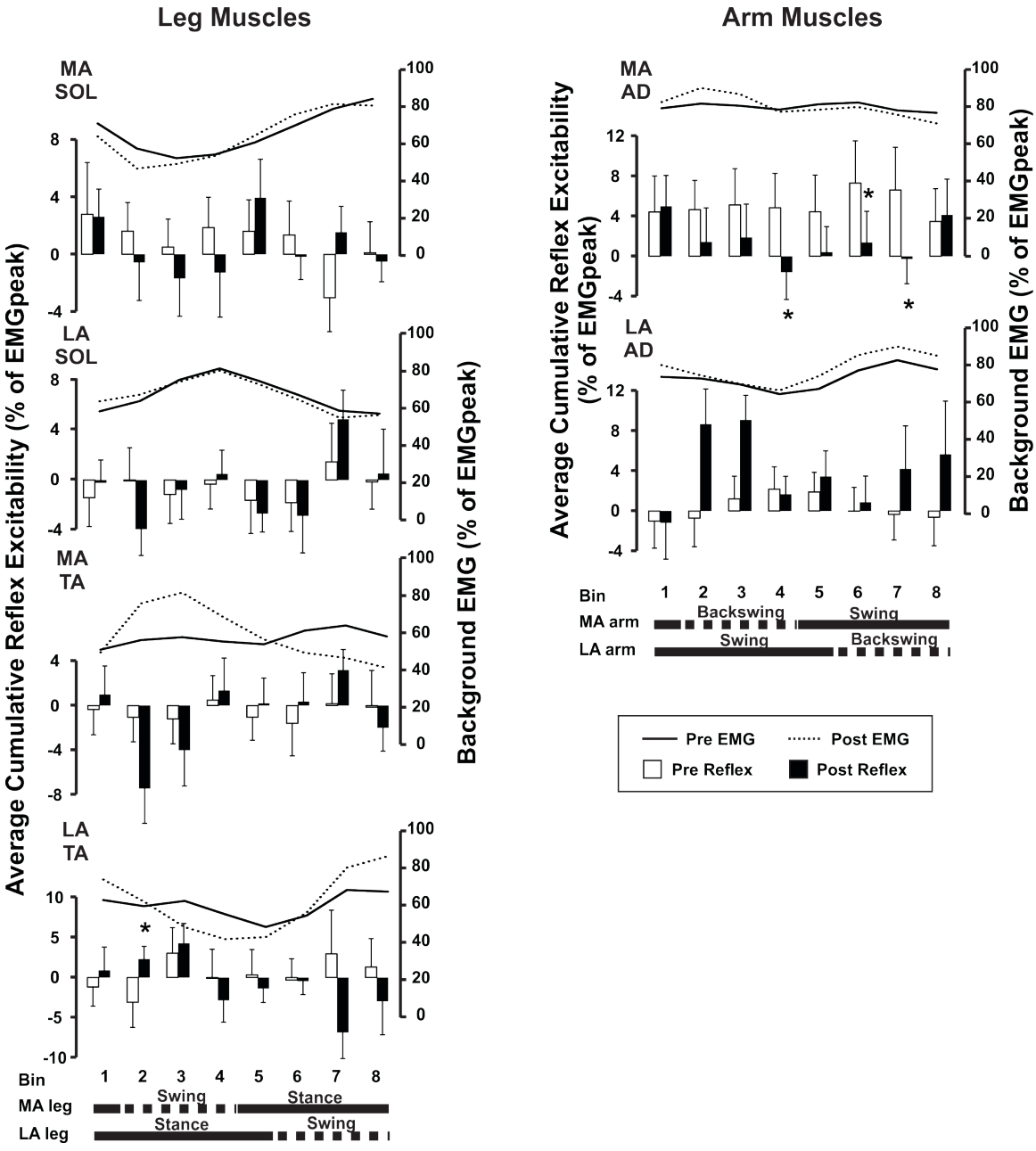

Figure 8. *Cutaneous reflexes during walking. Net reflex ($ACRE_{150}$) amplitudes during eight phases of walking for leg muscles (left) and arm muscles (right). Unfilled bars are the pre average and filled bars are the post values for reflexes. Secondary y-axis (right) values indicate EMG amplitude as a percentage of the peak EMG during walking and are displayed as line graphs in each panel. The solid line is the pre average whereas the broken line is the post value. All bars are group means (± SEM) and * indicates a significant ($p < 0.05$) change from pre average to post for reflexes. For clarity of display, differences of reflexes between phase and any differences in EMG are omitted.*

Arm cycling interlimb modulation of stretch reflexes at the ankle

The number of participants reported in the stretch reflex (n=15) data is less than other measures. Two participants had stretch reflexes that could not be reliably elicited each day on one or both sides of the body. Of particular note, one participant who lacked a stretch reflex (even with manual attempt at elicitation) prior to training was able to produce a small stretch reflex on both sides following the intervention. Two further participants were excluded from analysis due to inconsistencies in the shaker acceleration during the post-test measurements.

A three factor (Time x Side x Condition) repeated measures ANOVA showed that there were no significant differences in the displacement amplitude of the shaker between the pre-tests or post-test (effect of time: $p = 0.631$), between limbs (effect of side: $p = 0.910$) or between static and cycling (effect of condition: $p = 0.252$).

Arm cycling modulation of stretch reflexes is shown in Figure 9. A two factor (Time x Side) repeated measures ANOVA showed that there were no significant differences between pre-tests or between sides, however there was an interaction effect ($F_{(1,42)} = 3.192$, $p = 0.036$). Paired sample *t*-tests determined that prior to arm cycling training, the modulation of stretch reflexes was 93.5% greater on the LA side than the MA side ($p = 0.014$, d = 0.86). After training, there was no significant difference in arm

cycling modulation of stretch reflexes between sides ($p = 0.36$). Furthermore, arm cycling modulation of stretch reflexes on the MA side increased nearly ten-fold (1.37 to -13.89%, $p = 0.026$, d = 0.57). In terms of the ratio of modulation between sides (see figure 9B), a one factor (time) repeated measures ANOVA revealed a significant main effect of time ($F_{(1,42)} = 3.184$, $p = 0.037$). Pairwise comparisons revealed that, following arm cycling training, the difference between the arm cycling modulation of stretch reflexes between the LA and MA side was significantly reduced ($p = 0.026$, d = 0.69). Using individual statistical analysis, 8 out of 15 participants showed a significant increase in arm cycling modulation of stretch reflexes on the MA side, whereas 3 participants showed a decrease and 2 participants showed an increase in arm cycling modulation of stretch reflexes on the LA after training, compared to the baseline pre-tests.

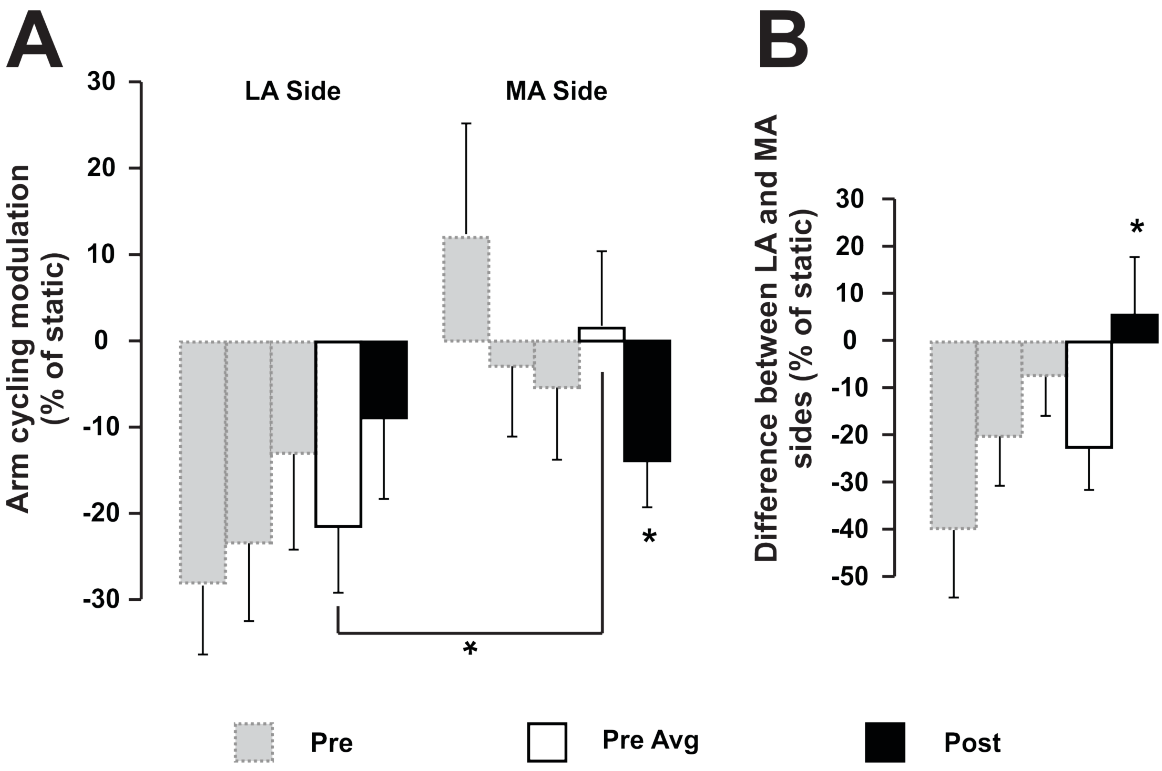

Figure 9. *Arm cycling-induced modulation of stretch reflexes. The difference between SOL stretch reflexes recorded at rest and during arm cycling on the LA (left) and MA (right) side are shown in (A). The difference between the LA and MA sides is shown in (B). Pre 1, 2, and 3 data are displayed in gray, whereas pre- and post-test group data are displayed with unfilled and filled bars, respectively. Bars are group (n = 14) means (± SEM), * indicates a significant (p < 0.05) change from pre average to post, and * with a line indicates a significant (p < 0.05) difference between LA and MA sides.*

Discussion

Arm cycling training can produce neuroplasticity and improve walking after stroke. Following 30 minutes of arm cycling training 3 times per week for 5-weeks at a moderate intensity, there were significant improvements in clinical assessments of walking and modest improvements in balance, along with strength and muscle activity measured at the hands and ankles during isometric contractions. Neurophysiological integrity, as assessed through phasic modulation of muscle activity, rhythmic modulation of cutaneous reflexes and arm cycling-induced modulation of stretch reflexes, displayed significant plasticity following arm cycling training. Many of the training adaptations from arm cycling correspond with those previously reported from a combined arm and leg cycling training intervention (Klarner *et al.*, 2016a, b). This suggests that at least some effects observed previously are related to rhythmic training of the upper limbs.

Functional Improvements

In general, arm cycling training caused improvements in walking ability and balance that are similar to our previous report of arm and leg cycling training (Klarner *et al.*, 2016a). Similar to our previous experiment, we examined individual participant data because group data rarely provides a clear indication of improvements of clinical tests. We observed meaningful improvements from individual data in 9 of 18 participants for the 6 Minute Walk test (i.e. > 6.7 m increase (Perera *et al.*, 2006)), 8 of 18 for the Timed

Up and Go (i.e. > 2.9 s decrease (Flansbjer *et al.*, 2005)), 11 of 18 for the 10 Meter Walk Test (i.e. > 0.5 m/s increase (Perera *et al.*, 2006)), and 5 of 18 for the Berg Balance Scores (i.e. > 2.5 point increase (Liston & Brouwer, 1996)). Although the range of improvements is diverse in this population, certain participants were especially responsive to the arm cycling training intervention. For example, one participant was able to walk 91.5m further in 6 minutes following the intervention, compared to baseline. Positive changes in the Chedoke-McMaster Stroke Assessment scores reflect less deficiency of movement following training and were noted for 11 of 18 participants.

Following training, there were improvements in strength of muscles at the wrist and ankle. There were bilateral increases in grip strength that was accompanied by increased muscle activation in the wrist flexors (i.e. MA FCR). Interestingly, on the MA side, there was increased plantarflexion force, which was accompanied by increased SOL activation. On the LA side, SOL muscle activity was increased, but was not accompanied by increases in plantarflexion force. Although these results suggest arm cycling training improves the strength generating capacity of both the upper and lower limbs during isometric contractions, the changes are not as robust compared to during arm and leg cycling (Klarner *et al.*, 2016a, b), however this is not surprising given the differences in lower limb movement across the two exercise tasks. Nonetheless, as we suggested previously (Klarner *et al.*, 2016a), the fact that positive correlations have been drawn between strength gains and walking speeds in chronic stroke (Richards, 1996; Kim & Eng, 2003) suggests that any intervention that improves strength should be considered beneficial.

Neurophysiological function of arm CPGs

Although the primary objective of this study was to determine whether training the arms transfers to improvements in walking, the results of surface EMG during arm cycling provide some informative evidence of training-induced changes in muscle coordination during the training task itself. Phase-dependent modulation of muscle activity and reflexes is a hallmark of rhythmic movement (Burke, 1999; Zehr *et al.*, 2004a) that can be attributed to activity of spinal CPG networks (Zehr *et al.*, 2003; Zehr *et al.*, 2004a; Zehr & Duysens, 2004; Zehr, 2005, 2016; Frigon, 2017). Although there is persistence of CPG activity following stroke (Ferris *et al.*, 2006; Zehr & Loadman, 2012; Zehr *et al.*, 2012; Klarner *et al.*, 2014; Zehr, 2016), there can be reductions in the amount of phase-dependent modulation. This "blunting" of modulation (Zehr *et al.*, 2012) is seen as reductions in the modulation of muscle activity due to more tonic activity of muscles, predominantly in the MA limb. Prior to training here, participants had very little modulation of their MA AD muscle activity, a main contributor of rhythmic movement during arm cycling. After arm cycling training, the MA AD modulation index was increased, illustrating that the MA arm had more phasic muscle activity than prior to training. Interestingly, the modulation index of the LA AD decreased, suggesting that the muscle activity was less phasic. This may be a consequence of more equal distribution of power output from both the MA and LA arms rather than relying solely on the LA arm to drive the ergometer to the same extent post-training, compared to pre-training.

Arm cycling training normalized coactivation between the MA and LA AD muscles (decreased throughout the majority of phases of cycling), therefore suggesting less tonic activity of the MA side. Furthermore, prior to training, there were high levels of coactivation between the antagonist BB and TB of the MA arm. Following training there

were reductions in the amount of coactivation at various phases of movement. This is likely attributed to reductions in flexor activity, which is typically excessive in hemiparetic participants following stroke (Kline *et al.*, 2007).

We previously reported on cutaneous reflexes from SR nerve stimulation during arm cycling (Zehr *et al.*, 2012) and walking (Zehr & Loadman, 2012) in chronic stroke participants and determined that, although circuits regulating interlimb coordination of rhythmic movement remain accessible, they are somewhat blunted compared to neurologically intact participants. Here, it appears that spinal circuits are severely blunted compared to previous reports. There was very little evidence for phase-dependent modulation of upper limb muscles on the MA side during arm cycling. In fact, there was only a significant effect for phase in the MA AD early latency reflexes, and that was not changed with arm cycling training.

The LA AD was more similar to neurologically intact participants, with significant phase-dependent modulation during arm cycling for early latency, middle latency and net reflexes, both prior to and following arm cycling training. This suggests that the interlimb linkages from the LA to the MA side in the participants of this experiment are deficient. However, arm cycling training did induce plasticity of the interlimb reflexes pre- to post-training at certain phases of arm cycling so that they more closely resemble those of neurologically intact participants. For example, early latency reflexes recorded from the contralateral FCR of neurologically intact participants are typically inhibitory (i.e. negative sign) throughout arm cycling. Prior to training, reflexes in FCR measured on the MA side displayed strong facilitation, but this facilitation was substantially reduced throughout the movement cycle following training. Similarly, early

latency reflexes measured in the contralateral BB and TB during the late power and early recovery phases of neurologically intact participants are typically small and/or inhibitory (Zehr *et al.*, 2012). Here, there was training induced plasticity of cutaneous reflexes from facilitation to inhibition in both the MA BB and TB, suggesting that arm cycling training 'normalized' reflex control.

Taken together, these findings suggest that the rhythmic movement (i.e. arm cycling training) has induced adaptations to the neural control of movement that more closely resemble characteristics of neurologically intact participants (Zehr *et al.*, 2012).

Enhanced interlimb connectivity of cervicolumbar CPG networks

Control of limb movements during human locomotion is enhanced through interlimb linkages, which can be observed in the form of 'interlimb reflexes' (Dietz *et al.*, 2001; Haridas & Zehr, 2003; Lamont & Zehr, 2007; Zehr, 2016; Frigon, 2017) and neural coupling between the arms and legs (Dietz, 2002; Zehr *et al.*, 2007; Mezzarane *et al.*, 2011; Nakajima *et al.*, 2013a; Nakajima *et al.*, 2013b; Nakajima *et al.*, 2014; Zehr, 2016; Frigon, 2017). Similar to muscle activity, cutaneous reflexes undergo phase-dependent modulation in all limbs, regardless of the limb that is stimulated (Haridas & Zehr, 2003).

During walking, training effects of arm cycling on interlimb reflexes were relatively modest, but there was evidence for training induced plasticity. For example, although phase dependent modulation of early and middle latency reflexes was absent in the MA and LA TA, respectively, prior to arm cycling training, following training there were significant main effects for phase. This suggests that the arm cycling training activated interlimb networks that contribute to the coordination of rhythmic walking through arm cycling training. Stimulation of the LA wrist prior to training caused

facilitation of the MA AD throughout the gait cycle, however, after training this facilitation was reduced and even reversed in some phases. In fact, the modulation of the reflexes seemed to increase, suggesting improved interlimb coordination during walking. It therefore appears that arm cycling training can improve interlimb coordination of reflexes, similar to training the arms and legs together (Klarner *et al.*, 2016b).

Modulation of reflexes in the stationary legs during arm movement provides convincing evidence for the existence of neuronal linkages between the arms and legs that are active during locomotor tasks (Dietz, 2002; Zehr, 2016). Experiments in neurologically intact (Frigon *et al.*, 2004; Zehr *et al.*, 2004b; Loadman & Zehr, 2007; Dragert & Zehr, 2009; Hundza & Zehr, 2009) and stroke participants (Barzi & Zehr, 2008; Mezzarane *et al.*, 2014; Klarner *et al.*, 2016b) demonstrate that arm cycling can cause modulation of the Ia reflex pathway in the legs, and this neuronal linkage remains accessible after stroke. Modulation of Hoffmann (H-) and stretch reflexes has been attributed to group Ia presynaptic inhibition (Frigon *et al.*, 2004) and it has been postulated that a loss of descending commands to spinal interneurons is a key contributor to the hyperactive Ia reflex pathway after neurotrauma (including stroke, SCI, MS, etc.), which is correlated with the presence of spasticity (Stein *et al.*, 1993). A single session experiment showed that, unlike H-reflex suppression with arm cycling in stroke participants (Barzi & Zehr, 2008), there is bidirectional modulation of stretch reflexes (Mezzarane *et al.*, 2014). Although our recent arm and leg cycling training intervention suggests that rhythmic training of the arms and legs together can influence arm cycling modulation of stretch reflexes to become more suppressive in the MA limb (Klarner *et al.*, 2016b).

Here, we observed that prior to arm cycling training, arm cycling-induced modulation of stretch reflexes was suppressive for the LA limb but was more variable between participants for the MA limb, such that some participants had very little suppression of stretch reflexes and some had facilitation. Pre-training variability observed for stretch reflex modulation might be attributed to the differences in lesion location and size (Calautti & Baron, 2003). However, following arm cycling training, there was an increase in arm cycling-induced suppression on the MA side. Modulation increased post-training so that it was no longer different from the LA side, suggesting an increase in symmetry between the LA and MA sides. It is likely that arm cycling caused training induced plasticity to the corticospinal projections to leg muscles influencing the modulation of EMG and reflexes during cycling and walking. Zhou *et al.* (2017) recently showed that corticospinal excitability to the TA is facilitated with 12 weeks of arm and leg cycling, compared to leg only cycling, in those with incomplete spinal cord injury.

Although suppression induced by arm cycling was the main interest for us in this experiment, an interesting finding was that one participant who had experienced multiple strokes and was lacking a functional stretch reflex (even through manual elicitation), was able to produce a reliable elicited reflex on both sides following the training period. The findings of the current experiment therefore suggest that arm cycling improves arm to leg linkages that are more typical of neurologically intact participants. A graphical summary of how arm cycling training might affect the neural control of rhythmic movement after stroke is provided in figure 10.

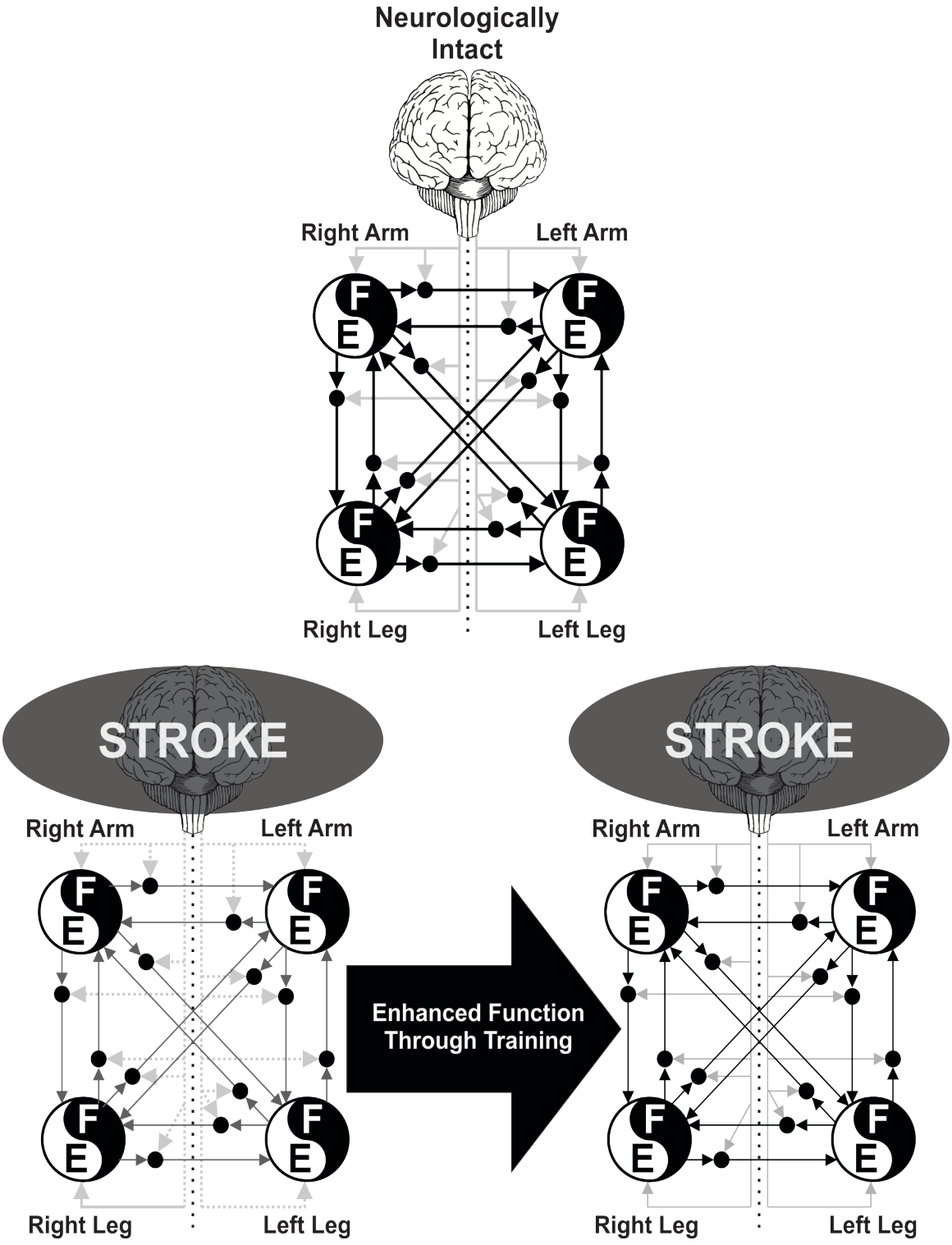
Neurologically
Intact
Right Arm
Left Arm
F
E
Right Leg
Left Leg
STROKE
Right Arm
Left Arm
Right Leg
Left Leg
Enhanced Function
Through Training
STROKE
Right Arm
Left Arm
Right Leg
Left Leg

Figure 10. *A schematic representation of the interlimb pathways that could contribute to the control of human walking in chronic stroke (left) and chronic stroke after training (right). Pathways are drawn with reference to Frigon et al. (2017), however for ease of display, sensory feedback from the limbs is not depicted. The yin/yang cartoons represent a central pattern generator (CPG) for each limb. Arrows represent neuronal connections and can be either excitatory or inhibitory. Broken lines from supraspinal centers in the chronic stroke represents the dysfunctional commands that can have influences in any location of the spinal cord due to variability in lesion type, location and size. Decreased thickness in the lines connecting CPGs represents decreased strength of connectivity. Although not back to the level of the neurologically intact nervous system, after training, solidified lines from supraspinal centers and thickened lines within the spinal cord compared to chronic stroke represent improved connectivity from supraspinal centers and within the spinal cord resulting in a 'normalization' of rhythmic output.*

Transfer of neuroplasticity from arm training to walking function

As with arm cycling, walking is, at least partially, controlled by activity from locomotor CPGs (Dietz, 2002; Zehr & Duysens, 2004; Zehr, 2005, 2016; Frigon, 2017) and phasic modulation of muscle activity is therefore important for successful, coordinated locomotion. As mentioned previously, these networks remain intact following stroke (Zehr & Loadman, 2012) and based on findings from arm and leg cycling training (Klarner *et al.*, 2016a, b), adaptations transfer from one rhythmic task to another (i.e. from arm and leg cycling to walking). Nonetheless, there are significant deficits that have been reported in the neural control of walking in stroke participants in not only the MA side, but also the LA side (Zehr & Loadman, 2012). Typically, there are increases in co-contraction during stance (Shiavi *et al.*, 1987) and reduced modulation of muscle activity throughout the gait cycle (Burridge *et al.*, 2001). Of particular interest in this experiment are the substantial changes in TA activity during walking that occurred as a result of arm cycling training. This is functionally very important because after stroke, the TA is heavily impacted, and an inability to activate the TA of the MA side leads to foot drop, which is associated with toe drag, stumbling, and an increased fall rate (Zehr &

Loadman, 2012). Prior to training, participants in this experiment had very little TA activity bilaterally, as seen in the individual trace of figure 6A. Furthermore, prior to arm cycling training, the modulation index of both the MA and LA TA throughout the gait cycle was lower than that reported for neurologically intact participants in previous experiments (Zehr *et al.*, 1998; Zehr & Loadman, 2012). Following the cycling intervention, however, there were not only functionally relevant changes in TA EMG bilaterally, but there were also bilateral increases to the modulation index of the TA muscles during walking, suggesting that the participants were better able to activate their TA in a phasic and functionally relevant manner.

Furthermore, the coordination of agonist-antagonist within the MA leg showed training-induced changes that included increased TA/SOL activity during the swing phase and decreased TA/SOL during the stance phase. Further support for the 'normalization' of TA coordination arises from the comparison of the MA/LA TA muscle activity. Prior to training, there appears to be tonic activation throughout the gait cycle that is higher on the MA side compared to the LA side. Following training, however, this interlimb coordination became more phasic, such that the MA side was far more active during the swing phase of the MA leg, whereas the LA side was far more active during the swing phase of the LA leg. Overall, the results of the EMG analysis during walking indicate changes to the activation patterns of the TA muscle, a muscle that is often subject to irregularity of activation following stroke. Hence, rhythmic arm cycling training appears to induce a 'normalization' of TA activity, especially on the MA side, during walking.

During walking, there were only modest changes to muscle activity in the upper limbs measured in the current experiment. There was increased AD activity for the MA and LA sides during forward arm swing. Although methodological restraints make it difficult to conclude, it appears that training rhythmic movements of the arms (via arm cycling training) has transferred to increased arm swing activity during walking. This may be complicated because different participants required adapted set ups for their arms during walking. Some participants required an arm sling, and very few had confidence to walk without gripping the parallel bars or front bar, even though their risk of falling was negated by the use of an overhead harness. Efforts were made to keep the setup the same within the experiment for each participant. These results echo previous studies that show that even when bound, a rhythmic pattern of activation can be determined in the upper limbs during treadmill walking (Ballesteros *et al.*, 1965). It seems as though this phenomenon is at least partially preserved in stroke.

Clinical Translation

The results of the current experiment suggest that rhythmic arm training can assist with rehabilitation of walking in chronic stroke (Ferris *et al.*, 2006; Zehr, 2016). Often in rehabilitation, participants are trained at treadmill walking with the arms holding stationary parallel bars. This has been shown to be less effective than therapies such as the body weight support treadmill training, where the arms are free to swing during walking training (Tester *et al.*, 2011). Sometimes body weight supported treadmill training is out of the question, as it can be quite expensive, and ambulation is not possible, even for short periods of time. In such cases, rehabilitation practices should turn

to combined arm and leg cycling or recumbent stepping, both of which have been shown to influence interlimb neural connections (Ferris *et al.*, 2006; de Kam *et al.*, 2013).

Study Limitations

Often in chronic stroke, the impairments brought on by the initial injury have been compounded by years of disuse. While some individuals maintain their fitness following a stroke, many do not. This leads to the compounding of secondary complications over time. It has been observed that simply participating in cardiovascular activity can provide benefits by targeting the disuse related effects on individuals after stroke. Indeed, it is possible that some of the effects seen in this study could be attributed to such mechanisms. However, this remains unlikely because the training itself was of moderate intensity, at the very most, as neither heart rate nor rate of perceived exertion increased over the course of the training. The level of aerobic activity required was even less than that in our previous combined arm and leg cycling study (Klarner *et al.*, 2016a, b), which itself fell below the level required to increase cardiovascular fitness for individuals after stroke (Gordon *et al.*, 2004; Pang *et al.*, 2006). Furthermore, we did not include a group of participants who would have come to the lab for an equal number of sessions as the training group. Therefore, it is possible that there were increases in activity of this group of individuals, which is typically decreased following stroke (Mayo *et al.*, 2002; Gadidi *et al.*, 2011). Thus, a commute to and from the laboratory for testing and training sessions may act as a training stimulus on its own. To draw this conclusion, we would have also needed a second group, coming to the laboratory for only the testing sessions. This would result in two groups of participants that would have been deprived of the almost certain benefits of performing exercise (i.e. arm cycling). In an attempt to

ensure all participants received benefits of exercise, we chose to avoid the traditional control groups altogether and use the more time consuming procedures of multiple baseline control. Additionally, in a previous experiment (Dragert & Zehr, 2013), we had stroke participants come to the laboratory for strength training and this commute to the laboratory did not cause improvements in walking and balance which are noted in the current experiment. This suggests that the arm cycling training did provide additional improvements in walking and balance that were not provided to participants that simply commuted to the laboratory for training and testing.

A second limitation is the relatively small sample size, however since this is a proof of principle investigation rather than a clinical trial, the sample size is sufficient to draw initial insights on the mechanisms and guide future planning.

A third limitation is that rhythmic activation of the leg muscles could have occurred during training sessions. Although participants were instructed to keep their legs at rest during each training session, and investigators monitored the legs to ensure there was no apparent movement, it is possible that slight activation of the lower limbs took place during the training sessions. If rhythmic activation in the legs occurred, such activation would have been involuntary but could contribute to some of the improvements in leg function noted with training. However, the limited and non-specific nature of this activation would not account for many of the specific training induced changes highlighted in this experiment.

Broader Context and Future Directions

Given and indeed despite the limitations of this study, the results observed provide several important contributions to the broader field of human locomotion, as well

as insights into rehabilitation potential within the chronic stroke population. While several studies have shown that activity in the arms can produce measurable short term changes in neural plasticity in the legs, this study provides direct evidence that training the arms over a longer period of time produces long term changes in neural plasticity of the legs which actually results in functionally relevant improvements. These changes are not limited to the trained task, but also translate to an untrained rhythmic task, walking. These observations build on previous findings from this lab, which indicate the importance the arms play in human locomotion and lend evidence to the fact that the arms play an active rather than passive role in driving activity in the legs.

Observing these findings within a post-stroke population as opposed to a neurologically intact group is important for a number of reasons. First, the nervous system post-stroke differs from that of the neurologically intact system. Depending on the lesion size and location, descending surpraspinal control of movement is altered or in some cases non-existent. As mentioned in the limitations discussion, this altered descending control is compounded by years of disuse and deconditioning. As such, the nervous system in a post-stroke population is not functioning at optimal levels. The finding from this study that activity in the arms can drive changes in the legs despite the altered supraspinal inputs and years of disuse provides evidence that underlying spinal networks are still functional in a post-stroke population and still capable of driving rhythmic movement.

This study also shows the relative ease at which these networks can be accessed and taken advantage of in order to promote meaningful, functional change. The arm cycling protocol was not particularly taxing, as evidenced by the steady rate of perceived

exertion, and does not require a particularly high level of function to take part in. This has important ramifications for those post-stoke individuals who are functionally unable to participate in walking rehabilitation. Arm cycling alone could be utilized to boost function to a level where one is able to participate in more demanding training, such as combined arm and leg cycling, recumbent stepping and eventually walking. While the effects observed in this study are limited compared to those seen in the aforementioned therapies, they may be enough to progress activity until the individual is ready to receive the full benefits of moving all four limbs together. Even with the relatively small sample size observed in this study, a number of participants saw significant gains that translated to functional improvements across a variety of parameters. When speaking in terms of rehabilitation practices, functional changes are the changes the participant will value above all else.

This study also helps to debunk the "six month myth", or the idea perpetuating the health care field that functional improvements are limited beyond the first 6 months post stroke (Sun et al., 2015). With relative ease of activity and only five weeks of training, participants who were many years post-stroke were able to make functional gains and improvements across a variety of physical performance and neurophysiological parameters. These observations highlight the fact that stroke recovery is a long process, and should not be limited to the first six months after the stroke occurs.

Future studies may build on these observations by investigating how long the changes persist after the completion of the study. One, three or six months post training would be interesting time points to evaluate the level of preservation of the produced changes. It would also be of value to note any changes the participants made to their daily

routines following the improvements gained from training, and whether training resulted in their being able to begin participating in any other forms of fitness.

Conclusion

Arm cycling training improves walking, physical performance, and neurophysiological integrity after stroke. Although improvements in walking may not be as robust as those from other training modalities, they do highlight the integral role that training the arms can have on rehabilitation of human locomotion. The positive changes in clinical assessments, strength and reflex control suggest that the arms do in fact give the legs a helping hand in rehabilitation, even years after neurological injury.

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