A dissertation on nervous system control and interlimb coordination during rhythmic movement and on locomotor recovery after stroke

by

Taryn Klarner

BSc., University of Guelph, 2003-2007
MSc., University of British Columbia, 2008-2010

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

DOCTOR OF PHILOSOPHY

In the School of Exercise Science, Physical and Health Education

University of Victoria

Supervisory Committee

A dissertation on nervous system control and interlimb coordination during rhythmic movement and on locomotor recovery after stroke

by

Taryn Klarner

BSc., University of Guelph, 2003-2007
MSc., University of British Columbia, 2008-2010

Supervisory Committee

Dr. E. Paul Zehr, (School of Exercise Science, Physical & Health Education and Division of Medical Sciences)
Supervisor

Dr. Sandra Hundza, (School of Exercise Science, Physical & Health Education)
Departmental Member

Dr. Brian Christie, (Division of Medical Sciences)
Outside Member

Abstract

Supervisory Committee

Dr. E. Paul Zehr, (School of Exercise Science, Physical & Health Education and Division of Medical Sciences)
Supervisor

Dr. Sandra Hundza, (School of Exercise Science, Physical & Health Education)
Departmental Member

Dr. Brian Christie, (Division of Medical Sciences)
External Member

For those who have suffered a stroke, damage to the brain can result in a decreased ability to walk. The traditional therapy used for the recovery of walking, body weight supported treadmill training, has significant labour requirements that limit the availability of training to the larger stroke population. Thus, the conception and application of new, effective, and efficient rehabilitation therapies is required.

To approach this, an understating of the intricate neural control behind walking is needed to form the principled foundation upon which locomotor therapies are based. Due to observations that the arms and legs are connected in the nervous system during walking, and that nervous system control is the same across rhythmic tasks, arm and leg (A&L) cycling training could provide an effective means of locomotor rehabilitation.

Thus, the goal of this dissertation is focused upon exploring central nervous system control and interlimb coordination during rhythmic arm and leg movement and testing the extent to which A&L cycling training improves walking after stroke.

The first objective of this dissertation was to provide further evidence of central nervous system control of walking. Through a literature review in Chapter 1 and experimental evidence in Chapter 2 of common subcortical control across rhythmic locomotor tasks, evidence for the existence of central pattern generating networks in humans is given.

The second objective was to explore interlimb coordination during rhythmic movement. Results presented in Chapters 3 and 4 further our understanding of specific interlimb interactions during rhythmic arm and leg tasks.

The third objective was to evaluate the effects of an A&L cycling training intervention in a post-stroke population. To support this objective, it was shown in Chapter 5 that a multiple baseline design is appropriate for use in intervention studies. In Chapter 6, it was determined that A&L cycling training can be used to improve walking ability. And in Chapter 7, it was shown that training induced plasticity in interlimb reflex pathways.

Overall, results in this dissertation provide further knowledge on nervous system control and arm and leg interlimb interactions during rhythmic movements and their effect on locomotor recovery following a stroke.

Table of Contents

List of Tables

List of Figures

Acknowledgments

My greatest thanks are reserved for my parents, Linda and Rick, who have supported me throughout my entire educational journey. Special thanks also go to my sister Janine and Laurie. Many times, I needed the guidance, wisdom, and strength that only my family could provide. Mike, I could not have done it without you – your belief in me and your encouragement made this possible.

I would like to express my gratitude and appreciation for my committee members: Dr. E. Paul Zehr, Dr. Sandra Hundza, and Dr. Brian Christie who have been very generous and giving with their support and time.

I would also like to acknowledge the support and guidance I received from other faculty members, colleagues, friends, and family throughout the PhD process.

Finally, I cannot omit my recognition of the generous financial support received from the Heart and Stroke Foundation of Canada.

General Introduction

For those who have suffered a stroke, damage to the brain can result in a decreased ability to walk, thus decreasing quality of life in a significant way. Stroke is the first leading cause of disability in North America, as there are approximately 400,000 Canadians living with a stroke, and each year, that number will grow by 40-50,000 (Statistics Canada 2014). In addition, it is expected that the incidence of stroke will increase in the coming years as our population ages, because the risk of stroke doubles after the age of 55, and continues to double every 10 years (Michael and Shaughnessy 2006). For all of those who have had a stroke, 75% of them will have some impairment requiring rehabilitation (Pinter and Brainin 2012). Therefore it is imperative that as the number of people with a stroke increases, so too should the rigour with which we approach rehabilitation. Walking rehabilitation is an important part of recovery after a stroke and regaining locomotor function is a primary goal in stroke therapy (Zehr 2011; Hong 2015). To ultimately improve quality of life, exercise and rehabilitation are pivotal to enabling community participation and to maintaining independent mobility. Thus, the conception and application of new, effective, and efficient rehabilitation therapies are required.

To approach locomotor rehabilitation, an understating of the intricate neural control involved in coordinating the ‘simple’ task of walking is required. From studies in other animals, it has been identified that networks of neurons in the spinal cord, called central pattern generators (CPG), are responsible for generating the basic muscular rhythm associated with walking (Grillner and Wallén 1985; Duysens and Van De Crommert 1998; MacKay-Lyons 2002). After spinal cord transection, these remaining spinal pathways can be trained where treadmill walking facilitates positive use-dependent plasticity, corresponding to enhanced recovery of walking (Barbeau and Rossignol 1987). The same approach has been applied in humans with neurotrauma, where the remaining neural networks are strengthened with training, proposed to enable activation of spinal cord circuitry, to restore normal CPG function, and corresponding locomotor activity (Dobkin 2004; Langhorne et al. 2009).

The current therapy traditionally used for the recovery of walking after a stroke is body weight supported treadmill training therapy (Moseley et al. 2003; Duncan et al. 2011; Senthilvelkumar et al. 2015). For this therapy, participants practice walking on a motorized treadmill, with a harness system providing body weight support, where stepping is performed with the help of robotic interfaces or therapists. The arms are typically used for postural support on parallel bars, or hand rails, to help bear weight from the legs (Behrman and Harkema 2000).

Results from this therapy are positive, where training leads to improved walking for those with neurological injury (Dietz et al. 1998; Edgerton et al. 2001; Field-Fote 2001; Moseley et al. 2003; Dobkin 2004; Duncan et al. 2011), but, there are significant limitations that reduce access of training to the larger stroke population (Zehr et al. 2016). This therapy is typically only available in restricted environments such as in rehabilitation centres, has significant labour requirements, requires specialized equipment, and is expensive to administer. Given the expected increase in stroke incidence, a more accessible and cost-effective protocol, that could be more readily and easily used in therapy, would be of great benefit.

In addition to being easily implemented, an effective training intervention should exploit the neuronal and mechanical linkages between the arms and legs that are vital in normal human walking (Ferris et al. 2006; Dietz and Michel 2009; Zehr et al. 2009; Klimstra et al. 2009). Normal walking involves arm movement which is controlled by spinal CPG networks that are functionally connected to the legs. Data on interlimb responses obtained in persons with cervical spinal cord injury and stroke suggests that pathways mediating arm and leg interactions are conserved in humans, and remain accessible after neurological damage (Calancie 1991; Calancie et al. 1996; Zehr et al. 1998; Wirz et al. 2001; Zehr et al. 2009; Zehr and Loadman 2012). To optimize the benefits of task-dependent rehabilitation, given that the arms are linked to the legs during locomotion, it has been suggested that rehabilitation include arm movements (Behrman and Harkema 2000; Dietz 2002; Ferris et al. 2006; Zehr et al. 2009; Klimstra et al. 2009). With the current therapy, the lack of involvement with the arms only adds to the neural limitations that are already present due to the pathology, and impaired arm function may actually inhibit rhythmic stepping of the legs (Behrman and Harkema 2000).

Therefore a full body rhythmic task, such as arm and leg (A&L) cycling training, may usefully exploit interlimb connections, where rhythmic arm movement may lead to enhanced activation of the legs, to facilitate locomotor recovery. Another advantage of this therapy is that A&L cycling is similar to walking in terms of muscle activity, joint ranges of motion, and central nervous system control (Grillner and Wallén 1985; Zehr 2005; Zehr et al. 2007). This implies that A&L cycling training will activate and strengthen the interlimb and CPG networks that would also be activated with walking training (Zehr et al. 2007; Klimstra et al. 2009; Zehr et al. 2016).

Specific details on the control of arm and leg movements during rhythmic tasks, and on the extent to which A&L cycling training transfers to improvements in walking, remains untested. Thus, the goal of this dissertation is focused upon exploring central nervous system control and interlimb coordination during rhythmic arm and leg movement and the extent to which arm and leg (A&L) cycling training improves walking after stroke. To address this, there are three main objectives:

- The first objective is to support evidence on the existence of central pattern generating networks in humans.
- The second objective is to explore interlimb coordination during rhythmic arm and leg movement.
- The third objective is to evaluate the effects of an A&L cycling training intervention in a post-stroke population.

Overview of Research Study

To evaluate these objectives, a literature review which provides the background information upon which this dissertation is based is presented first. Experimental observations from both neurologically intact participants, and from participants with chronic stroke, also provide evidence supporting the main objectives. Seven papers, each in a separate chapter, will be presented as part of this dissertation.

To address the first objective, in Chapter 1, a literature review on central pattern generators explores how networks in the spinal cord form the foundation of walking activity. The structure and function of CPGs during locomotion is revealed from animal studies, mainly from the spinal cat, and indirect evidence supports their existence in humans. These data provide the principled foundation for the neural control of walking, upon which locomotor rehabilitation paradigms are based. Restoring normal CPG coupling and activity in interlimb networks is the goal for task-specific therapies for improving corresponding locomotor activity in those with neurological damage.

The basic pattern of arm and leg movement during rhythmic locomotor tasks is supported by common central neural control of CPG activity (Stoloff et al. 2007; Zehr et al. 2007), and in Chapter 2, a study is presented that explores if common control persists after stroke. Shared systems from interlimb cutaneous networks, facilitating arm and leg coordination, were investigated during A&L cycling and walking. If neural control is similar between A&L cycling and walking, there are translational implications for rehabilitation, where A&L cycling training could be usefully applied to improve walking function by training the same neural networks. Results presented from this study from stroke participants support the first objective, as there is evidence that a spinal mechanism is involved in common neural regulation across tasks.

For the second objective, to explore interlimb coordination during rhythmic arm and leg movement, a study investigating changes in arm to leg coupling, with slow walking, will be presented in Chapter 3. To further characterize the neural control behind the observation that the normal 1:1 arm:leg swing frequency ratio, that exists at normal walking speeds, switches to a 2:1 arm:leg swing ratio at slow walking speeds (Donker et al. 2001), modulation of cutaneous reflexes will be explored. Understanding the dynamic interactions between arm and leg coordination is important as it has been suggested that arm activity be integrated into locomotor rehabilitation.

Specific details of coupling between the arms and legs have been identified using interlimb reflex studies. To support the second objective, in Chapter 4 the effects of stimulation to discrete regions on the top of the foot on muscle activation and limb trajectory are evaluated.

It is expected, that along with observations from the bottom of the foot (Zehr et al. 2014), that these evoked effects would be topographically organized and involve interlimb coordination. These data will increase our understanding of how afferent feedback, from specific cutaneous locations on the foot dorsum, influences the mechanisms involved in stance and swing phase corrective interlimb responses. The results of this study may also provide potential rehabilitation means to restore normal corrective interlimb responses from foot dorsum stimulation, and to aid in enhancing functional interlimb modulation of gait, following neurological injury.

To explore the third objective, a study in Chapter 5 is presented which gives support for the use of multiple baseline designs as a valid alternative to the concept of a control group in intervention designs. Reliability of locomotion-related physiological measures, taken using a repeated test-retest protocol, is investigated. With this design, change in participant performance can be measured against their own baseline variability.

The extent to which A&L cycling training can lead to training adaptations, which transfer to improved walking function, remains untested, and is the third objective of this dissertation. The purpose of the study in Chapter 6 was to test the efficacy of A&L cycling training as a modality to improve locomotor function after stroke. If exploiting arm and leg connections with A&L cycling training improves walking after stroke, it will provide additional corroborative evidence that the arms should be included in locomotor rehabilitation.

As a result of training, plasticity in reflex pathways may be present, as has been observed after other training interventions (Whelan and Pearson 1997; Wirz et al. 2001; Zehr 2006). Chapter 7 explores the effects of A&L cycling training on plasticity in interlimb reflex pathways in stroke participants. Plasticity in reflex modulation and interlimb coordination, as a result of training, could be responsible for improvements in walking.

Conclusion

This dissertation makes a step further towards the development of improved recovery strategies for those who have suffered a stroke. Given the increasing age of Canadians, and subsequent increase in age-related diseases, maintaining independent mobility, achieved through exercise and rehabilitation, is pivotal to enabling community participation and to maintaining a high quality of life. Overall, results in this dissertation aim to provide further knowledge on nervous system control and arm and leg interlimb interactions during rhythmic movements, and aims to examine the application of these principles to the recovery of walking after a stroke.

References

Barbeau H, Rossignol S (1987) Recovery of locomotion after chronic spinalization in the adult cat. Brain Res 412:84–95. doi: 10.1016/0006-8993(87)91442-9

Behrman AL, Harkema SJ (2000) Locomotor training after human spinal cord injury: a series of case studies. Phys Ther 80:688–700.

Calancie B (1991) Interlimb reflexes following cervical spinal cord injury in man. Exp Brain Res. doi: 10.1007/BF00229423

Calancie B, Lutton S, Broton JG (1996) Central nervous system plasticity after spinal cord injury in man: interlimb reflexes and the influence of cutaneous stimulation. Electroencephalogr Clin Neurophysiol Mot Control 101:304–315. doi: 10.1016/0924-980X(96)95194-2

Dietz V (2002) Do human bipeds use quadrupedal coordination? Trends Neurosci 25:462–467. doi: 10.1016/S0166-2236(02)02229-4

Dietz V, Michel J (2009) Human bipeds use quadrupedal coordination during locomotion. Ann. N. Y. Acad. Sci. pp 97–103

Dietz V, Wirz M, Colombo G, Curt A (1998) Locomotor capacity and recovery of spinal cord function in paraplegic patients: A clinical and electrophysiological evaluation. Electroencephalogr Clin Neurophysiol - Electromyogr Mot Control 109:140–153. doi: 10.1016/S0924-980X(98)00002-2

Dobkin BH (2004) Strategies for stroke rehabilitation. Lancet Neurol 3:528–536. doi: 10.1016/S1474-4422(04)00851-8

Donker SF, Beek PJ, Wagenaar RC, Mulder T (2001) Coordination between arm and leg movements during locomotion. J Mot Behav 33:86–102. doi: 10.1080/00222890109601905

Duncan PW, Sullivan KJ, Behrman AL, et al. (2011) Body-weight-supported treadmill rehabilitation after stroke. N Engl J Med 364:2026–2036. doi: 10.1056/NEJMoa1010790

Duysens J, Van De Crommert HWAA (1998) Neural control of locomotion; Part 1: The central pattern generator from cats to humans. Gait Posture 7:131–141. doi: 10.1016/S0966-6362(97)00042-8

Edgerton VR, Leon RD, Harkema SJ, et al. (2001) Retraining the injured spinal cord. J Physiol 533:15–22. doi: 10.1111/j.1469-7793.2001.0015b.x

Ferris DP, Huang HJ, Kao PC (2006) Moving the arms to activate the legs. Exerc Sport Sci Rev 34:113–120. doi: 10.1249/00003677-200607000-00005

Field-Fote EC (2001) Combined use of body weight support, functional electric stimulation, and treadmill training to improve walking ability in individuals with chronic incomplete spinal cord injury. Arch Phys Med Rehabil 82:818–24. doi: 10.1053/apmr.2001.23752

Grillner S, Wallén P (1985) Central pattern generators for locomotion, with special reference to vertebrates. Annu Rev Neurosci 8:233–61. doi: 10.1146/annurev.ne.08.030185.001313

Hong E (2015) Comparison of quality of life according to community walking in stroke patients. J Phys Ther Sci 27:2391–3. doi: 10.1589/jpts.27.2391

Klimstra MD, Thomas E, Stoloff RH, et al. (2009) Neuromechanical considerations for incorporating rhythmic arm movement in the rehabilitation of walking. Chaos 19:026102. doi: 10.1063/1.3147404

Langhorne P, Langhorne P, Coupar F, et al. (2009) Motor recovery after stroke: a systematic review. Lancet Neurol 8:741–54. doi: 10.1016/S1474-4422(09)70150-4

MacKay-Lyons M (2002) Central pattern generation of locomotion: a review of the evidence. Phys Ther 82:69–83. doi: 10.1152/jn.00056.2009

Michael KM, Shaughnessy M (2006) Stroke prevention and management in older adults. J Cardiovasc Nurs 21:S21–S26.

Moseley AM, Stark A, Cameron ID, Pollock A (2003) Treadmill Training and Body Weight Support for Walking after Stroke. Stroke 34:3006. doi: 10.1161/01.STR.0000102415.43108.66

Pinter MM, Brainin M (2012) Rehabilitation after stroke in older people. Maturitas 71:104–108. doi: 10.1016/j.maturitas.2011.11.011

Senthilvelkumar T, Magimairaj H, Fletcher J, et al. (2015) Comparison of body weight-supported treadmill training versus body weight-supported overground training in people with incomplete tetraplegia: a pilot randomized trial. Clin Rehabil 29:42–49.

Statistics Canada (2014) Table 105-0501 - Health indicator profile, annual estimates, by age group and sex, Canada, provinces, territories, health regions (2013 boundaries) and peer groups, occasional. In: CANSIM (database).

Stoloff RH, Zehr EP, Ferris DP (2007) Recumbent stepping has similar but simpler neural control compared to walking. Exp Brain Res 178:427–38. doi: 10.1007/s00221-006-0745-7

Whelan PJ, Pearson KG (1997) Plasticity in reflex pathways controlling stepping in the cat. J Neurophysiol 78:1643–1650.

Wirz M, Colombo G, Dietz V (2001) Long term effects of locomotor training in spinal humans. J Neurol Neurosurg Psychiatry 71:93–96. doi: 10.1136/jnnp.71.1.93

Zehr E, Barss T, Dragert K, et al. (2016) Neuromechanical interactions between the limbs during human locomotion: an evolutionary perspective with translation to rehabilitation. Exp Brain Res. doi: 10.1007/s00221-016-4715-4

Zehr E, Nakajima T, Barss T, et al. (2014) Cutaneous stimulation of discrete regions of the sole during locomotion produces "sensory steering" of the foot. BMC Sport Sci. Med. Rehabil. 6:

Zehr EP (2005) Neural control of rhythmic human movement: the common core hypothesis. Exerc Sport Sci Rev 33:54–60.

Zehr EP (2011) Evidence-based risk assessment and recommendations for physical activity clearance: stroke and spinal cord injury. Appl Physiol Nutr Metab 1:214–31. doi: 10.1139/h11-055

Zehr EP (2006) Training-induced adaptive plasticity in human somatosensory reflex pathways. J Appl Physiol 101:1783–1794. doi: 10.1152/japplphysiol.00540.2006

Zehr EP, Balter JE, Ferris DP, et al. (2007) Neural regulation of rhythmic arm and leg movement is conserved across human locomotor tasks. J Physiol 582:209–27. doi: 10.1113/jphysiol.2007.133843

Zehr EP, Fujita K, Stein RB (1998) Reflexes from the superficial peroneal nerve during walking in stroke subjects. J Neurophysiol 79:848–58.

Zehr EP, Hundza SR, Vasudevan E V (2009) The quadrupedal nature of human bipedal locomotion. Exerc Sport Sci Rev 37:102–8. doi: 10.1097/JES.0b013e31819c2ed6

Zehr EP, Loadman PM (2012) Persistence of locomotor-related interlimb reflex networks during walking after stroke. Clin Neurophysiol 123:796–807. doi: 10.1016/j.clinph.2011.07.049

Chapter 1 Human locomotor central pattern generators: from Sherrington to Sherlock Holmes

Once you eliminate the impossible, whatever remains, no matter how improbable must be the truth.” Arthur Conan Doyle

Abstract

Based on evidence, first described years ago, the general concepts regarding the control of walking through spinal central pattern generators (CPG) have been made from studying reduced animal models. From these observations, we can speculate as to what should be observable in humans, where we must rely on indirect evidence and inferences to assess the role of CPGs in generating rhythmic movements. This review will present observations from humans, to test hypotheses posed from other animal studies, which supports the theory of CPG-mediated locomotion in humans.

Introduction

There is a wealth of data to support the existence of CPGs in other animals; however, there is less direct evidence for CPGs in humans. In animal models direct recordings of nervous system activity can be taken, giving indisputable evidence for the structure and function of CPGs in generating rhythmic movements. In humans, the experimental techniques needed to confirm this observation are unethical and unrealistic to perform therefore we must rely on indirect evidence and inferences to assess the role of CPGs in generating rhythmic movements.

By studying and understanding simple systems for the structure and function of CPGs, we can build up to understating walking control in humans. Using observations from other animal preparations as background, we can speculate and hypothesize about what should be observable if CPGs are activated to control rhythmic human movement. Evidence and observations from humans, to test hypotheses posed from animal studies, will support the theory of CPG-mediated locomotion in humans.

Finding supporting evidence to solve a perplexing puzzle may be “elementary” for Sherlock Holmes, a fictional character created by Arthur Conan Doyle, and in using his deductive reasoning approach, this review will present material to evaluate human locomotor CPGs. An understanding of the functioning of CPGs in humans, and in other animals, is important for the development of strategies for gait rehabilitation in individuals with spinal cord and brain injuries.

Central Pattern Generators in Animals

Over a century ago, Charles Sherrington discovered that rhythmic movements could be evoked in the nervous system, below the level of the brainstem (Sherrington 1906). In cats and dogs, made decerebrate by cutting the spinal cord at the level of the brain stem, electrical and mechanical stimulation of the skin elicited repetitive, stereotyped, and automatic hip and knee movements producing rudimentary stepping. Sherrington noted that the rhythm of the response is highly modifiable by peripheral feedback where, as stimulus intensity increased, movement amplitude increased, onset latency shortened, and the number of repetitions increased. From these observations, it was clear that the spinal cord is capable of producing a rhythm, without input from the brain, but Sherrington originally concluded that locomotor-like movements were of not of central origin. He thought that the crossed extension reflex, involving ipsilateral flexion and contralateral extension, could be responsible where one movement would elicit the next movement (Sherrington 1910).

It was Thomas Graham Brown, another pioneer in the field and a student of Sherrington’s, who made similar observations of rhythmic movement using decerebrate cats, that had undergone transection of the spinal cord at the thoracic level. These animals were also deafferented where proprioceptive feedback was removed by cutting the afferent nerves from the hind-limb muscles. While these animals were under general anesthesia and laying on one side, tonic electrical and mechanical stimulation of the limbs caused stepping movements in the hindlimbs to be spontaneously expressed. Brown recorded bursting in alternating pairs of antagonist muscles in the hind legs which occurred not only without higher level input from the brain, but without sensory input. From this observation, it was clear that something intrinsic, in the spinal cord itself, was responsible for generating patterned activity (Brown 1911). At this point, Brown extended Sherrington’s observations to state that locomotor rhythms are not of peripheral origin, but that a “mechanism confined to the lumbar part of the spinal cord is sufficient to determine in the hindlimbs an act of progression.” (Brown 1911, p. 308).

From these observations, Brown proposed and developed a widely accepted model called the ‘half center model’ (Brown 1911) (See Figure 1-1). This model can be used to describe the basic structural design of a CPG and how it oscillates to produce the basic rhythm and pattern for stepping. In this model, each half-center is constituted of two groups of spinal neurons that individually, have no rhythmogenic ability. Activity in the first group of neurons (e.g., extensor half-center) would send motor commands to motoneurons, exciting extensor muscles, and would simultaneously inhibit the reciprocal group of neurons (flexor half-center), via interneurons, preventing the excitation of antagonists, silencing flexor muscles. Brown proposed that with “fatigue” the firing in the active extensor half center slowed, releasing the opposing flexor half-centre from inhibition, and then the flexor half-center would predominate for a new phase of activity, and the pattern continues (Brown 1911).

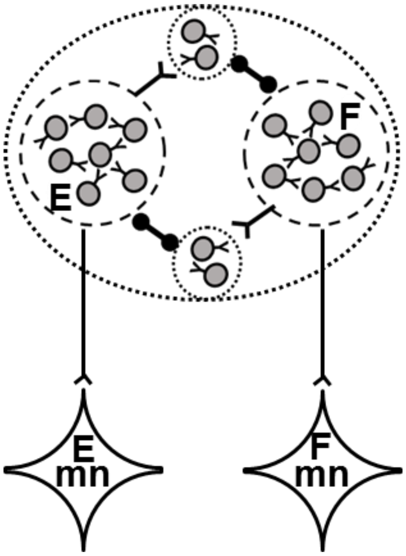


Figure 1-1: Half-center model. Pattern generating networks are contained within dotted circle. Each half-center for flexor (F) and extensor (E) activation is contained within the dashed circle, and each activates flexor (F) or extensor (E) motoneurons (mn). Inhibitory interneurons are also shown.

It was the early observations of Sherrington and Brown, at the beginning of the twentieth century, that opened a new line of research, and thinking about the spinal cord and its intrinsic capability of producing movement (Brown 1914). However, despite this corroborating evidence, there was a long pause, and decades passed before this work was investigated further. It wasn't until the 1960s and onwards where their seminal work would be supported by subsequent evidence with efforts to uncover the cellular and neural mechanisms involved in CPG activity.

It was with the development of intracellular recordings, in the 1960's, that showed the first evidence to support Brown's idea of half-center activity. By electrically stimulating high-threshold cutaneous and muscle afferents, short sequences of alternating rhythmic activity in flexor and extensor motoneurons were recorded (Jankowska et al. 1967) (see Figure 1-2). This revealed that spinal CPGs could serve as the basic building blocks of the circuitry required for locomotion. With this observation, half-centers are now generally referred to as the CPG.

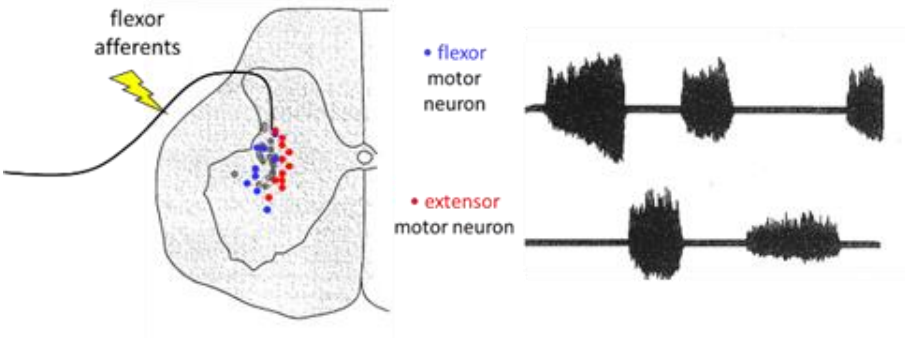


Figure 1-2: Elements of a spinal CPG. Adapted from Jankowska et al. 1967

Since the 1980's, with the advent of new technologies, various molecular, genetic, pharmacological, and imaging studies have been conducted to understand and further determine the localization and organization of the cellular and neural substrates for the locomotor CPG (Grillner 1975; Kiehn and Kjaerulff 1998; Kiehn and Butt 2003). To model locomotion, many researchers have relied on simpler vertebrate species, particularly the lamprey, to explain how an ensemble of spinal neural elements can elicit rhythmic motor patterns in the absence of supraspinal control or external feedback (Grillner et al. 1998a; Grillner et al. 1998b). The lamprey makes a good model because its nervous system has a simple structure with very few neurons, making measurement somewhat easier (Grillner 2006). In addition, when the spinal cord is removed intact, it can survive for days, where direct cellular measurements can be taken, and it can be made to produce motor outputs, indicative of a CPG (Grillner 2006).

To understand the cellular basis of rhythmic motor and locomotor patterns, studies on the sea slug, leech, cockroach, stick insect and crustacean have been conducted (Hughes and Wiersma 1960; Hooper and DiCaprio 2004; Friesen and Kristan 2007; Büschges et al. 2008). In addition, experiments in *in vitro* isolated neonatal rat and mouse preparations, and transected adult rat and mouse models, led to substantial advances in identification of the receptors and channels associated with locomotor rhythm-generation and modulation (reviewed in Guertin 2013).

Evidence from reduced animal preparations

Over fifty years of research has led to hundreds of experiments detailing the structure and function of locomotor CPGs across many species. From all these experiments, rhythmic motor patterns have been shown to anatomically originate from the spinal cord, generated via relatively small and autonomous neural networks. The existence of these neural networks, producing specific and rhythmic movements, is indisputable for practically all vertebrate mammalian species investigated (Grillner et al. 1985; Burke 2001; Hultborn and Nielsen 2007).

The most extensively characterized network in the spinal cord however is the CPG for locomotion in the walking spinal cat (Grillner et al. 1985). This model for studying locomotor CPGs is very advantageous because the spinal cord can be studied, in isolation without input from the brain, or sensory feedback, during actual movement. From these experiments several key observations have been made that gives indisputable evidence of the structure and function of locomotor CPGs. In the following is a summary of the most important findings to support spinal CPGs observable in spinal cats.

Pathways intrinsic to the spinal cord

The first piece of evidence to consider is that in a cat with a complete spinal cord transection, at T12-L1 between the forelimbs and hindlimbs, hind limb stepping recovers after 3-4 few weeks of intense daily treadmill training (Barbeau and Rossignol 1987). Initially after the injury, cats demonstrate a poorly organized hind-limb stepping pattern during treadmill walking, but after training they demonstrated a “near-normal” pattern (Barbeau and Rossignol 1987).

Electromyographical (EMG) recordings from hindlimb muscles in trained spinal cats are generally similar to those from intact cats and many of the normal muscle and skin reflex responses are apparent in the spinal preparation. Furthermore, by the end of training, the cats were able to adjust the locomotor cycle to adapt to varying treadmill speeds (Barbeau and Rossignol 1987). This evidence supports the notion that a central network of neurons is capable of producing motor patterns resembling walking, without input from the brain or brainstem. This evidence is good, however in this study of cats with a compete spinal cord transection, afferent connections from peripheral receptors below the level of the injury are intact, therefore these results highlight the importance of sensory afferent information in facilitating activity in CPGs. It has been found however, that these movements persist even if afferent input from the limbs has been removed, and movement-related feedback is no longer available (Grillner and Zangger 1984).

Movement related afferent input can be completely eliminated by stopping movement in a fictive locomotion model. This can be achieved by either injection of neuromuscular relaxants, or with transection of the efferent nerves at the ventral root, or at the muscle nerve level (Duysens and Van De Crommert 1998). In this model, tonic stimulation or drugs are applied to the spinal cord and rhythmic periods of activity can be recorded proximal to the cut efferent nerve. Rhythmic activity, recorded during fictive locomotion, is reciprocally organized between agonists and antagonists, and has been demonstrated in both cats' hindlimbs (Chandler et al. 1984; Fleshman et al. 1984), and forelimbs (Miller et al. 1975a; Amemiya and Yamaguchi 1984; Yamaguchi 1992). The demonstration of fictive locomotion is the most convincing evidence that neural networks in the isolated spinal cord are capable of generating rhythmic output, in absence of any signals from supraspinal centers, as well from movement-related afferent sources.

Indeed, with the goal of understanding how the central nervous system controls interlimb coordination during stepping, efferent discharges in muscle nerves of the four limbs were recorded simultaneously during spontaneous fictive locomotion in thalamic cats (Orsal et al. 1990). Distinct patterns of interlimb coordination exist during fictive locomotion which correspond to walking and trotting gaits. The results demonstrate that the central nervous system, deprived of phasic afferent inputs from the periphery, and most supraspinal inputs, has the capacity to generate most of the patterns of interlimb coordination which occur during real locomotion (Orsal et al. 1990).

Thus, to control rhythmic muscle output between forelimbs and hindlimbs, locomotor CPGs are organized as half-center modules residing in the cervical and lumbar region of the spinal cord. This has significant behavioral relevance during quadrupedal locomotion where it is necessary to control rhythmic movements of both forelimbs and hindlimbs. Figure 1-3 contains a simplistic view of a distributed spinal CPG network. A schematic diagram for the locomotor network for quadrupedal locomotion may also consist of at least one CPG for each limb, comprised of a CPG for each joint (at the ankle, knee, hip, shoulder, elbow joint). Interlimb communication between CPG modules between the left and right sides should also be included. Coordinated movements within a limb could be achieved through phase-dependent interactions of different CPGs controlling that limb (Duysens and Van De Crommert 1998; MacKay-Lyons 2002; McCrea and Rybak 2008). It has also been suggested that the CPG model is comprised of a timer layer which controls cadence and phase durations and a pattern formation layer which selects and modulates activation of motoneuronal outputs (Rybak, 2006).

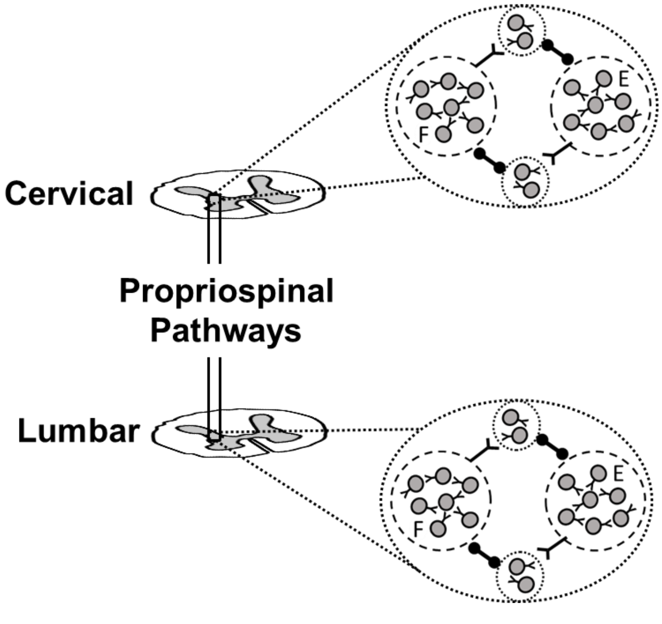


Figure 1-3: Distributed CPG model for interlimb coordination during locomotion.

Propriospinal pathways

Propriospinal pathways have been identified that allow for intersegmental connections in the spinal cord. As well as short pathways, long propriospinal pathways have been found between cervical and lumbar segments that could form the foundation of connections between segmental CPGs. Thus, coupling between forelimb and hindlimb locomotor networks could be achieved in deafferented cats by interconnecting propriospinal pathways, intrinsic to the spinal cord, that serve as linkages between cervical and lumbosacral spinal CPG networks (Gernandt and Megirian 1961; Miller et al. 1975a; Miller et al. 1975b; Skinner et al. 1980; Gandevia et al. 1986; Falgairolle et al. 2006) (See Figure 1-3). Indeed, an extensive network of propriospinal interlimb connections have been revealed in the spinal cat by direct cellular recordings (Gernandt and Megirian 1961; Miller et al. 1973; Skinner et al. 1980; Juvin et al. 2012).

Descending spinal linkages were shown following stimulation of the brachial plexus in the forelimb of a cat, which evoked response in the ventral roots of the lumbar spinal cord (Lloyd 1942). Descending pathways were also shown in the C1spinalized cat by identifying antidromic action potentials in the cervical enlargement following electrical stimulation of the spinal cord at the L2 level (Skinner et al. 1980). The cell bodies of these propriospinal neurons were found to terminate in the cervical enlargement. It was also found that mechanical manipulation of the skin, by moving the joints or by applying pressure to deep tissue, excites these descending pathways (Skinner et al. 1980). In cats, this idea is supported by the presence of long caudorostral propriospinal tracts that have been identified in spinal columns, and are presumed to participate in the hindlimb-forelimb coupling seen in locomotor (English et al. 1985). Also propriospinal neurons were found from C6 projecting to sacral segments (S1/S2) with collaterals branching to lumbar segments (L4) (Grottel et al. 1998). The existence of collaterals branching to segments of the lumbosacral enlargements raises the possibility that descending information can be relayed to several motor centers controlling different hindlimb muscles (Grottel et al. 1998).

Ascending pathways, with a great number of synapses, have also been identified in spinalized cats (Gernandt and Megirian 1961). Ascending connections were noted when cutaneous nerve stimulation of hindlimb nerves in spinalized cats evoked responses in the motoneurons associated with upper forelimb flexor muscles (Miller et al. 1973). Postsynaptic potentials increased in amplitude as stimulation intensity increased, and were greater in the ipsilateral motoneurons compared to contralateral ones. In normal cats, response in the forelimb motoneurons closely correspond to the pattern of hindlimb and forelimb stepping in normal cats (Miller et al. 1973). There is a demonstrable neural linkage between rhythmic hindlimb and forelimb movements resulting in coordinated movement in the cat (Miller et al. 1975a).

Sharing CPG networks across rhythmic tasks

Another concept about the function of spinal CPG's is that there is considerable "sharing" of neurons, via reorganization of synaptic activity, to produce different motor patterns within the same neural networks. Much evidence has been gathered for shared neurons in invertebrate species, such as the crayfish. The same neural circuits were found to be involved in the generation of rhythmic motor patterns for chewing, gastric mill, pylorus, and ventilation (Hooper and DiCaprio 2004). The same is true for locomotor patterns in invertebrate species, in that the behaviours CPGs generate consist of distinct spinal networks that are selectively activated in various rhythmic movements for specific control of joints or muscles (Marder and Calabrese 1996). Rhythmic pattern generation is altered by various neuromodulators that change activation and synaptic efficacy in various interneuronal pathways, and allow for the expression of different motor patterns with essentially the same neurons.

This concept of a "shared CPG" was first proposed as a hypothesis to explain rhythmic lamprey movement (Grillner et al. 1985). This model outlines a more complex network where spinal locomotor CPGs are distributed over several segments, each with an oscillatory unit, known as a 'unit burst generator' (Grillner and Matsumoto 1991). Recruited into a reciprocally-organized network, for a certain behaviour, a unit burst generator is maximally flexible with distributed organization throughout the spinal cord (Grillner et al. 1985). In vertebrates, using the shared CPG, evidence has shown that coordination of movements of the hindlimbs and forelimbs, in different forms of locomotion, including swimming, walking, and air stepping, is the same in normal and decerebrate cats (Miller et al. 1975a).

CPG needs sensory feedback

Despite the impressive capability of the isolated spinal cord in cats to generate rhythmic output via a CPG, afferent signals are an integral part of the overall motor control system, such that afferent input, and their associated reflexes, are essential for normal execution of locomotion (Grillner and Zangger 1984). Very early on the importance of sensory feedback in the control of locomotion was acknowledged for its ‘regulative’ role, rather than a ‘causative’ role (Brown 1911; Brown 1914). Brown demonstrated that these central oscillating mechanisms generate the basic stepping pattern, however he also acknowledged the role of sensory input in shaping the output. He commented that with respect to sensory input, “there can be no question of its importance nor its suitability to augment the central mechanism” (Brown 1911, p.318). These observations have subsequently been supported by ample evidence that CPGs require sensory feedback to modulate and adapt their rhythmic output.

The importance of the CPG is not only its ability to generate repetitive cycles, but also to receive, interpret, and predict the appropriate action at each part of the step cycle. If step cycle durations and muscle patterns were fixed, it would be impossible to adapt to changes in the external environments. To achieve this, afferent feedback acts directly on the CPG and contributes to the modulation of its output (Van de Crommert et al. 1998; Duysens and Van De Crommert 1998). In addition, afferent feedback is also connected to motoneurons via various reflex pathways and these pathways themselves are under the control of the CPG (Burke et al. 2001; Zehr et al. 2004a; Zehr 2005). This way, the CPG ensures that reflex activations are facilitated at appropriate times in the step cycle and supressed when not appropriate (phase-dependent modulation) (Duysens and Van De Crommert 1998) (see Figure 1-4).

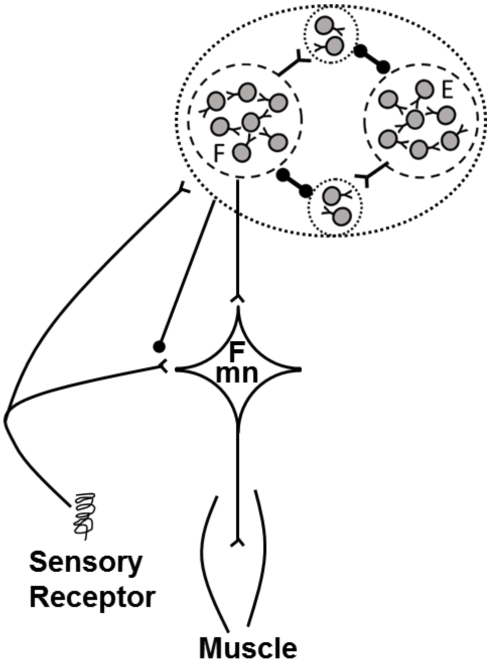


Figure 1-4: Schematic diagram of neural integration of sensory feedback and CPG.

There has been much speculation as to how phase-dependent modulation occurs during rhythmic tasks. One explanation is that reflexes could be involved in modulating other movement related feedback from muscle or joint receptors (Drew and Rossignol 1987; Misiaszek et al. 1998). That is, reflex input could lead to presynaptic inhibition to change the reflex gain from muscle spindle and golgi tendon organ pathways. However, phase-dependent modulation is present in the fictive cat model when movement is completely absent (Andersson et al. 1978; Schomburg and Behrends 1978). Intracellular postsynaptic potentials were recorded from motoneurons following electrical stimulation to the dorsum of the paw revealed both excitatory and inhibitory postsynaptic potentials found during the flexion and extension phases (Andersson et al. 1978). Characteristic phase-dependent modulation of cutaneous reflexes was also demonstrated during fictive locomotion in the hindlimb (Quevedo et al. 2005) and forelimb of the cat (Hishinuma and Yamaguchi 1989), done so by intracellular analysis of reflex pathways underlying the stumble corrective reflex. Given phase-dependent reflex modulation is observed in a fictive preparation, when actual movement is absent, reflex modulation has been ascribed, at least in part, to spinal CPG regulation (Andersson et al. 1978; LaBella et al. 1992). Convergence of information from locomotor CPGs onto segmental interneurons within cutaneous reflex pathways has been proposed as the source of the observed reflex modulation (Seki and

Yamaguchi 1997). Presynaptic inhibition from the CPG onto afferent reflex pathways is the proposed mechanism controlling phase-dependent modulation of the amplitude of primary afferent depolarization during fictive locomotion (Gossard et al., 1990; see diagram above).

Removing afferent input can also reveal its importance in modifying the locomotor CPG. This was initially evaluated in walking cats, and following denervation of foot pad afferents of a cat paw, no abnormalities were present (Sherrington 1910). However, it was shown later that indeed no walking problems were present, especially in the first few days after being tested, but clear abnormalities were observed under more difficult situations (Bouyer and Rossignol 2003). Cats were not able to step on rungs of a ladder, and had difficulty walking up and down inclines (Bouyer and Rossignol 2003). After spinalization of these same cats, sensory input was even more crucial for successful foot placement and normal weight bearing (Bouyer and Rossignol 2003). If afferent pathways were only partially removed, a substantial increase in recovery was observed, compared to the cats with full denervation of foot pad afferents. From these data, it is apparent that afferent input is crucially important for walking, especially in more challenging environments, and especially if supraspinal input is removed.

In the reverse experiment, afferent activity can be activated, rather than removed, and the same observation, of the importance of sensory feedback, is true. In cats with spinal transection resulting in reduced descending input, the spinal cord and specific sensory inputs can be examined. These studies revealed that sensory feedback is required to modify the level of patterned activity from CPGs and sensory feedback is required to cue phase transitions. Below, supporting evidence from cats will be given for these two observations.

Magnitude of activity

Sensory feedback is responsible for contributing to the generation and reinforcement of the magnitude of motor output of patterned CPG activity. In animals with reduced supraspinal control, the activation of sensory afferents from cutaneous receptors from the foot, had a dramatic effect on the locomotor cycle, and there is therefore little doubt that feedback pathways make direct connections with CPGs (Duysens and Pearson 1976; Duysens 1977). Sensory feedback's influence on stance and swing phase muscle activity will be evaluated below.

During the stance phase, high and low intensity stimulation from the skin of the pad of the foot (Duysens and Pearson 1976) and dorsum of the foot (Forssberg et al. 1975) acts to facilitate and prolong the extensor phase of locomotion. Stimulation of the cutaneous nerve supplying the dorsum of the foot, also enhanced extensor activity during the stance phase, in fictive locomotion of decerebrate-paralyzed cats (Guertin et al. 1995) or decerebrate cats with transected spinal cords (LaBella et al. 1992). Therefore, stimulation from skin areas during the stance phase, evoked prolongation of extension to delay the foot leaving the ground, by accessing excitation of the extensor half center of a CPG promoting ongoing and increased extensor activity (Pearson 2004). Electrical stimulation, at intensities that preferentially activate group I afferents, from knee and ankle extensors, also prolonged extensor activity, and delayed the following flexor burst associated with the onset of swing (Conway et al. 1987). Reinforcement of extensor activity from group 1b afferents on extensor motorneurons is made possible by a centrally-mediated switch from disynaptic inhibition, seen at rest to polysynaptic excitation via the CPG extensor half-center, seen during walking (McCrea et al. 1995; Pearson 1995; Quevedo et al. 2000). Inputs from Ia and Ib afferent fibers during locomotion and in fictive locomotion, were shown, in reduced animal models, to increase CPG-mediated extensor activity up to 50% (Guertin et al. 1995; Pearson 1995).

The same is observed in the swing phase where sensory feedback can access and enhance CPG activity. Activation of the skin on the plantar surface of the foot prolonged the swing phase with little effect on ipsilateral extensor activity (Duysens and Pearson 1976), and stimulation to the dorsum of the foot also prolonged the swing phase, and inhibited ipsilateral extensor activity (Forssberg et al. 1975). Stimulation of the cutaneous nerve supplying the dorsum of the foot, also enhanced flexor activity during the swing phase, in fictive locomotion of decerebrate-paralyzed cats (Guertin et al. 1995) or decerebrate cats with transected spinal cords (LaBella et al. 1992). Sensory feedback from flexor muscles can also access and modify CPG activity during the swing phase (Hiebert et al. 1996; Quevedo et al. 2000). Direct electrical stimulation of group I and II afferents, has been demonstrated to contribute to ongoing flexor muscle activity, and increase step cycle duration in decerebrate and fictive cats (Perreault et al. 1995; Lam and Pearson 2002; Stecina et al. 2005). Group II inputs from flexors may also promote deactivation of extensor half-centers, which corroborates an underlying mechanism of mutual inhibition mediated by CPG networks (Perreault et al. 1995).

Phase switching by sensory signals

Cued by peripheral signals, sensory feedback also has a role in acting directly on the CPG to initiate and facilitate phase transitions in rhythmic movements (Duysens and Pearson 1980; Conway et al. 1987). Sensory input from the limbs may truncate or extend individual phase durations where the timing of the motor output from a CPG can be modified. For example, if a limb that is swinging forward reaches the end of swing in less time than the current CPG-generated flexor phase duration, sensory input would cause the CPG timer to terminate swing and start the stance phase (Hiebert et al. 1996). Thus, sensory input is needed to adjust or override the CPG at stance-swing-stance transition (Donelan and Pearson 2004; Donelan et al. 2009) where peripheral cues provide critical signals regulating the duration of muscle activity to control phase transitions. In this way, it is ensured that a certain phase of the movement is not initiated until the appropriate biomechanical state of the moving part has been achieved. For example, to evaluate the contribution of proprioceptive information, arising from ankle extensors, onto the timing of CPG output, cats stepped on a trap door which opened into a hole while walking on the treadmill. During this simulated unloading, a 70% decrease in ankle extensor activity was experienced. In the same study, the hindquarters of cats were raised or lowered during treadmill walking, effectively unloading and loading the knee extensors, respectively. Raising the hindquarters during treadmill walking resulted in a strong reduction in knee extensor muscle activity; the opposite occurred when the hindquarters were lowered (Hiebert and Pearson, 1999). These data support the notion that sensory input acting on extensor motoneurons greatly affects the level and timing of activity in the leg extensors during stance.

When limb loading decreases, such as occurs at the end of the stance phase, the extensor reinforcing feedback is reduced, and the onset of swing is facilitated in the unloaded leg. Sensory feedback contributes to phase switching from flexion and extension where mechanical and electrical stimulation of sensory afferents in decerebrate cats have been shown to reset and entrain the locomotor rhythm. Termination of the stance phase is associated with a decrease in the discharge rate of load and length sensitive afferents from ankle extensor muscles from unloading of the leg (Pearson 1995). Mechanically loading or stretching the triceps surae results in a sudden disappearance of ankle flexor bursts in decerebrate cats during treadmill walking, and ankle flexor bursts only returned after stretching of the triceps surae stopped (Duysens and Pearson 1980). Therefore in order to initiate ankle flexion associated with swing, ankle unloading must occur first. High and low intensity stimulation from the skin of the pad of the foot (Duysens and Pearson 1976) and dorsum of the foot (Forssberg et al. 1975) also acts to delay the following flexion phase (Duysens and Pearson 1976).

Proprioceptive signals from hip flexors can also cue the termination of stance, initiation of swing, and are capable of resetting the locomotor rhythm (Hiebert et al. 1996). Hip extension activates the afferents arising from the muscle spindles of the elongated hip flexor muscles, thereby triggering the monosynaptic stretch reflex, which initiates a flexor burst near the end of the stance phase (Grillner and Rossignol 1978; Lam and Pearson 2001; McVea et al. 2005). In spinal and decerebrate cats, entrainment of the CPG was achieved by sinusoidal hip movements during fictive locomotion (Andersson and Grillner 1983). Manually imposing hip joint movement, to decrease leg extension, resets and entrains the locomotor rhythm. Stretching hip flexor muscles (iliopsoas) at the start of the stance phase, caused an early initiation of flexor activity in the ipsilateral leg and in the contralateral leg, flexor activity was shortened and extension occurred earlier (Hiebert et al. 1996). Activating hip flexor afferents from the sartorius muscle with electrical stimulation, also modulated CPG activity by resetting the locomotor rhythm from flexion to extension, and causes generation of flexor bursts in contralateral leg flexor muscles (Perreault et al. 1995). Flexor reflex afferents can also reset the step cycle to a new flexion (Jankowska et al. 1967; Schomburg et al. 1998). The critical role of hip joint afferents in the control of locomotion has been reinforced by evidence of sensory-evoked entrainment of the locomotor pattern in decerebrate cats during fictive locomotion (Kriellaars et al. 1994).

Input is needed to initiate CPG activity

Spinal CPGs can produce the basic rhythmic activity needed for walking, but input is required to initiate CPG activity. In intact animals, the commands for initiation and termination of spinal CPG activity are generally thought of as coming from descending drive from supraspinal centers (Van de Crommert et al. 1998; Jordan et al. 2008; Le Ray et al. 2011). Transection of the spinal cord results in most cats not being able to spontaneously initiate walking, implicating something above the level of the lesion in the initiation of locomotor activity. By varying the level of the transection, the regions for initiation of locomotion are found to be located in the brain stem (Whelan 1996). Therefore for volitional initiation of walking, areas of the brain, and in the brainstem, act to initiate the descending neural pathways that ultimately control and modulate CPG signals.

In the 1960's, when research on CPGs was re-initiated, details about inputs required to initiate and change the frequency of locomotion were garnered. It was found that locomotor command can be initiated by pathways that originate from nuclei in the mesencephalic locomotor region (MLR) of the caudal hindbrain. Repetitive electrical stimulation to this area in decerebrate cats induced locomotor-like activity (Shik et al. 1966a). Neural drive from the MLR was found to modify CPG output in a task dependent manner depending on simulation intensity. As the strength of stimulation increased, step cycle frequency increased, and the mode of locomotion changed from slow walking, to normal walking, to trotting, to galloping. And, when stimulation was stopped, locomotion was terminated (Shik et al. 1966a; Shik et al. 1966b; Shik et al. 1968). MLR pathways descend to the spinal cord, via the reticulospinal tract, to control and modulate CPG signals (Garcia-Rill and Skinner 1987). Interestingly, across many species, from lampreys to primates, the MLR has been implicated in initiating locomotion (Eidelberg et al. 1981; Skinner and Garcia-Rill 1984; Garcia-Rill and Skinner 1987; Dubuc et al. 2008).

For experimental purposes, descending drive can be mimicked in a number of ways (MacKay-Lyons 2002). By electrically activating these areas in the brainstem (MLR), rhythmic activity can be initiated. Descending drive can also be mimicked by applying exogenous excitatory neurotransmitters to the preparation. For example, L-DOPA can be used as a dopamine precursor to activate noradrenergic receptors in spinal cats and rabbits (Jankowska et al. 1967; Taylor et al. 1994). In addition, CPG activity can be activated by peripheral stimulation, especially in the perineal region, as a stimulus for triggering spontaneous locomotion in decerebrate preparations (Pearson, 2004).

Summary of evidence from reduced animal preparations

Experimental data obtained from non-human animals, particularly from decerebrate and fictive cat preparations, reveal a very intricate and detailed model for locomotion. From these experiments several key observations have been made to support the structure and function of locomotor CPG's. Below is a summary of the most important findings to support and describe spinal CPGs:

- The spinal cord can produce rhythmic movement, without descending supraspinal, or sensory feedback
- Treadmill training for cats that have undergone spinal transection induces recovery of stepping
- Spinal CPG networks are found in the spinal cord, particularly from the cervical and lumbar sections of the spinal cord, for forelimb and hindlimb control respectively, which are connected by propriospinal pathways
- The same spinal CPG networks are recruited for different rhythmic tasks
- Sensory feedback is needed by CPG networks to reinforce and modify rhythmic activity
- Supraspinal input is required to initiate CPG activity

Central Pattern Generators in Humans

In humans, since the same testing procedures cannot be applied, we must be led by what is observable in reduced animal preparations. Extrapolation of observations from the animal models of locomotion to humans should be possible because of the assumption that there are fundamental similarities in common principles of motor control across vertebrates and invertebrates (Pearson 1993; Duysens and Van De Crommert 1998; Zehr et al. 2016). Therefore evidence obtained from one species should be observable in another species. In view of the very extensive evidence for locomotor CPGs in other animals, it would be very surprising if in humans, there was a complete lack of a CPG network, and no evidence has been presented to support this (Duysens and Van De Crommert 1998; MacKay-Lyons 2002). In the following section it will be shown that there are indeed striking similarities between cats and humans, with respect to the neural control of locomotion.

Can the spinal cord produce stepping without brain or sensory feedback?

The first observation of CPGs from cats, to evaluate in humans, is that the spinal cord can produce rhythmic activity. While this cannot be directly shown in humans, indirect evidence of spinal CPGs can be gained. For example there is evidence from studying the stepping responses in those with spinal cord injury, from observations of air-stepping in healthy participants, an indirect observation of sleep related rhythmic leg movement, and in walking in human infants. These examples all have one thing in common; descending input from supraspinal centers is limited. These examples are some of the best when it comes to supporting CPGs in humans.

Evidenced in those with spinal cord injury

Perhaps the best examples of CPG-mediated locomotion in humans comes from studying rhythmic movement in those with spinal cord injury (SCI) (Bussel et al. 1988; Calancie et al. 1994; Dietz et al. 1994a; Harkema et al. 1997; Dimitrijevic et al. 1998). This is because in this ‘model’ we can better assess the role of the spinal cord, with reduced supraspinal control (Dietz et al. 1998). Following SCI, the spinal circuitry below the lesion site does not become silent rather it continues to maintain active and functional neuronal properties, although in a modified manner (Edgerton et al. 2001; de Leon et al. 2001).

In a case study of a patient with a complete cervical SCI, rhythmic, symmetrical and bilateral myoclonic movements of the trunk and lower limbs, resulting in hip and knee flexion-extension at about 0.6 Hz, were recorded when they were placed over a treadmill (Bussel et al. 1988). This observation demonstrated that, in man, rhythmic activity can be generated within the spinal cord, without supraspinal inputs (Bussel et al. 1988). It was also observed that periphral stimulation above the level of the spinal transection did not modify the myoclonus but stimualtion applied below the level of the transection, could induce, slow, or interrupt the rhythmic activity (Bussel et al. 1988). Electrical stimulation of flexor reflex afferents, from the sural nerve, also affected rhythmic activity. During extensor activation, stimulation of flexor reflex afferents induced a flexion reflex, that induced alternating flexor and extensor bursting activity which could be sustained for several cycles (Bussel et al. 1988). Similar activation of a spinal CPG by flexor reflex afferents is observed in cats (Jankowska et al. 1967; Duysens and Stein 1978; Pearson 1995; Seki and Yamaguchi 1997).

Other evidence comes from a patient with an incomplete injury of the cervical spinal cord (Calancie et al. 1994). Although they had no ability to generate voluntary lower leg muscle activity, involuntary lower-extremity stepping-like movements were expressed spontaneously when lying in a supine position. The movements were rhythmic where ‘forceful and patterned’ bursts of alternating activity were recorded from leg muscles bilaterally. Again, peripheral feedback modified the rhythm where movements were increased with dorsiflexion of the toes and were abolished by flexing the hips to 90 degrees, rolling over, or by sitting up or standing (Calancie et al. 1994). However due to the incompleteness of the lesion, this observation solicited further substantiation in patients with a complete spinal cord injury. And indeed, it was supported by observations of myoclonic rhythmic movements in six patients with complete spinal cord injury (Calancie 2006). And, these observations were corroborated by other evidence of spontaneous motor rhythms of the legs, resembling bipedal stepping, in another patient with complete spinal cord transection (Nadeau et al. 2010).

There is also evidence that treadmill training can improve some aspects of walking in individuals with clinically complete SCI. After training, patients who were otherwise unable to voluntarily produce muscle activation, were able to produce some locomotor activity (Dietz et al. 1995; Dietz et al. 1998; Maegele et al. 2002). Even individuals with clinically motor complete paralysis demonstrate modulated activity of distal leg muscles during assisted stepping with body weight support (Harkema et al. 1997; Dietz 2002). As training progressed, the levels of applied body weight support significantly decreased (Dietz et al. 1995) and activity in leg extensor muscles significant increased (Wirz et al. 2001). These improvements are thought to be a consequence of a re-activation of neural circuits located at the spinal level (Van de Crommert et al. 1998). Taken together, these findings support the idea that the stepping ability of clinically complete SCI subjects can improve in response to step training. However in all of these cases, some weight support assistance was provided, and patients did not recover enough for independent walking.

Observations of rhythmic movements are good evidence of a CPG in humans, but the best evidence has been provided by observations of rhythmic, locomotor-like movement of the lower limbs, in complete spinal cord injured patients lying in a bed, following epidural electrical stimulation of the spinal cord (Dimitrijevic et al. 1998). Conducted below the level of the injury, at the upper lumbar cord level (near L1–L3), tonic stimulation was found to trigger phasic bursts of rhythmic output in motoneurons for the legs. Increased stimulation amplitude resulted in increased EMG amplitudes and an increased frequency of rhythmic activity (Dimitrijevic et al. 1998). This evidence is good at showing that a spinal cord, without supraspinal input, can generate rhythmic movements, however there is still the case of the modulating effect of sensory feedback. To address this, in subsequent studies it has been shown that epidural stimulation can produce rhythmic EMG activities, even when the legs are stationary, producing diminished step-related sensory feedback (Minassian et al. 2004). While sensory feedback does influence features of the spinal rhythm, it seems that it is not required to produce CPG activity.

Evidence by restless leg syndromes

In another human model where supraspinal centers are functionally removed from the spinal cord, is the observation of restless leg syndromes found in individuals with SCI, multiple sclerosis, sleep disorders, and other neurological disorders (Guertin 2013). Either coming on spontaneously, or during sleep, restless leg syndrome presents as rhythmic flexion and extension of the ankle, toe, knee, and hip (Clardy and Connor 2010). In the case of those sleeping, restless leg syndromes could arise from a transient interruption in descending inhibition where spinal CPGs for locomotion are activated (Coleman et al. 1980; Chervin et al. 2003). In all cases, periodic leg movements of rhythmic activity, observed in one or both limbs, may be associated with abnormal and involuntary activation of spinal CPGs.

Evidenced during air-stepping

In intact humans, a way to activate and reveal rhythm generation via CPG circuits, is by using an “air-stepping” paradigm (Gurfinkel et al. 1998; Selionov et al. 2009; Gerasimenko et al. 2010; Sylos-Labini et al. 2014). With one leg horizontally suspended, and with subjects instructed to relax and not to intervene with the induced movement, it was shown that vibration of a muscle of the suspended leg could elicit cyclical hip and knee movements in both legs (Gurfinkel et al. 1998). Rhythmic EMG activity was reciprocally organized in the muscles around the hip joint with movement restricted to the hip and knee. The ankle joint was only involved if minimal loading forces were applied to the foot (Gurfinkel et al. 1998). It was also shown that cervical transcutaneous stimulation with vibration of the spinal cord significantly facilitated involuntary activation of the lumbosacral locomotor-related neuronal circuitry producing leg movements (Gorodnichev et al. 2012). The constant inflow of proprioceptive feedback, due to the vibration, is thought to have initiated and sustained activation of the spinal pattern generation circuitry (Solopova et al. 2015). One possible route for these trigger signals is through the intrinsic spinal pathways (propriospinal interneurons) linking cervical to lumbosacral regions in humans (Nathan et al. 1996). Vibration evoked rhythmic activity was not strong enough for body support and propulsion, but it does support the view that the basic rhythm underlying locomotion can be generated involuntarily in humans (Gurfinkel et al. 1998; Selionov et al. 2009; Gerasimenko et al. 2010; Sylos-Labini et al. 2014; Solopova et al. 2015).

Evidenced during infant walking

The final piece of indirect evidence for a locomotor CPG comes from studies of the automatic stepping response in human infants. Suspending an infant (who is unable to walk and bear weight on their own) over a treadmill can elicit rhythmic stepping movements (Yang et al. 1998). The fact that the descending pathways from the cerebellum and motor cortex are not fully mature in a human infant means less descending inputs would be observed (Khater-Boidin and Duron 1991; Yang et al. 2004). Stepping movements have also been observed in anencephalic infants, also suggesting that locomotor control centers could exist below the level of the brain stem (Forssberg 1992). In addition, coordinated movements are observed prenatally, long before brain development, where ultrasound recordings revealed in utero images of human fetuses producing alternating primitive step-like movements (Ianniruberto and Tajani 1981; Kozuma et al. 1997). These data support the notion that the onset of voluntary stepping precedes development and full myelination of descending pathways from the brain thus the infant stepping response is mediated by a spinal mechanism.

Stepping movements are also effected by sensory feedback where it was shown that limb loading is a powerful signals for regulating the stepping pattern (Yang et al. 1998; Pang and Yang 2000). Adding manual loading during the stance phase of gait, by pushing down on the hips, prolonged the stance phase (Pang and Yang 2000) and unloading the limb was an important cue for the transition into swing phase for forward, backward and sideways walking (Pang and Yang 2000; Pang and Yang 2001; Pang et al. 2002). In addition, infants also showed well organized and location-specific reflex responses to mechanical disturbances during walking in forward, backward and sideways walking (Lamb and Yang 2000; Pang and Yang 2000; Pang and Yang 2001). These results are consistent with what is found in spinal cats, that sensory feedback can access and entrain locomotor CPGs.

Together, all of these studies from human participants provide strong, indirect evidence that demonstrates that some of the basic neuronal circuitry for locomotion can function without supraspinal inputs. Thus human locomotion is controlled by CPG activity and sensory input interaction.

Does sensory feedback modulate CPG activity?

Although CPGs for locomotion can be inferred from humans with decreased descending drive, it is apparent that the human spinal cord is much more reliant on sensory feedback for the expression of locomotor activities. From the evidence in animals, sensory feedback, from load, muscle stretch, and cutaneous receptors, provides the information required by the CPG circuitry to generate functional and adaptive locomotion. In animals with reduced descending control from the brain, feedback along an afferent pathway is sufficient to affect CPG activity, however in intact animals manipulation of just a single type of sensory feedback is almost never sufficient to modulate rhythmic activity (Duysens and Stein 1978; Whelan and Pearson 1997).

Similar observations are true from human experiments were transient changes in reflex activity do increase muscle EMG amplitude, but do not change the duration of the step cycle (Stephens and Yang 1999). Therefore it has not been very clear to what extent CPGs and reflexes are integrated in the control of rhythmic movement in humans. There are no studies in humans, as there are in cats, which directly evaluate the exact contribution of sensory feedback to CPG output. However, indirect methodologies allow observations to be made in an intact nervous system to evaluate how the CPG regulates afferent feedback during rhythmic movement (Burke 1999; Zehr 2005). Reflexes, arising because of activation of afferent projections from receptors in skin and muscle, have been studied widely, and support the role of locomotor CPGs in the neural control of rhythmic human movement.

Testing reflex activity, and reflex modulation during rhythmic movement, can provide an indicator of CPG regulation. To approximate the input– output properties of neural control, stimulation of a given sensory input, and a record of the pattern of modulation of motor output during movement is used. Reflex responses, recorded at short latencies (< 150 ms), suggest that sensory feedback could be integrated at a propriospinal level. This approach has been used in the quadrupedal locomotor system (Burke et al. 2001), and is also effectively used in humans (Zehr et al. 2004a; Zehr and Duysens 2004). Examining the modulation of reflexes during rhythmic movement, as an indirect indicator of CPG regulation of afferent input, provides more data on which the concept of spinal CPGs in humans has been built.

Evidence by task- and phase-dependent modulation

The presence of task- and phase-dependent modulation has been used to infer the activity of CPGs in humans. Task and phase-dependent modulation of reflexes means that the reflex effect of the sensory input varies depending on the task and phase of the pattern in which it occurs (Yang and Stein 1990; Duysens et al. 1992; Van Wezel et al. 1997; Zehr et al. 1997; Zehr and Stein 1999). For example, H-reflexes are progressively inhibited from standing, to walking, to running (Stein and Capaday 1988). H-reflexes examined during walking also show phase-dependent modulation (Brooke et al. 1997; Zehr and Stein 1999). Over the course of the gait cycle, phasic modulation of Ia input has been demonstrated by changes in magnitude of H-reflexes and stretch reflexes (the mechanical analogue of the H-reflex) where inhibition of the reflex is greatest during the late stance phase (Yang and Whelan 1993). Attenuation of reflexes suggests an increased functional relevance of muscle stretch to end stance control due to an increase in excitability via facilitation along Ia reflex pathways (Capaday and Stein 1986; Verschueren et al. 2002).

Task- and phase-dependent modulation is also observed for modulation of cutaneous reflexes from receptors in the skin. In some cases, modulation is so powerful that a reflex response can even completely reverse in direction, observed as a phase-dependent reflex reversal. This is highlighted in the tibialis anterior where, in the same muscle, the “sign” of the reflex reverses from excitation to inhibition seen as activity is increased in the early swing phase, and inhibited in late swing, at the stance transition. This response is so powerful that it was apparent in all participants tested, and purposeful enough to alter joint kinematics during walking (Yang and Stein 1990; Duysens et al. 1992; Zehr et al. 1997; Haridas and Zehr 2003).

Phase dependency of reflexes acts to serve a functional role where highly integrated responses, allow the locomotor CPG pattern to progress smoothly to keep walking safe, by incorporating afferent information at appropriate times in the walking cycle. For example, during walking, resistance to the top of the swinging foot (ie by a horizontal stick or by electrical activation of cutaneous nerves) causes the foot to be lifted over the perturbation. However, the same input to the standing foot cannot cause the foot to lift or the person would collapse. Thus, depending on the phase, the same sensory input can cause the foot to be lifted higher or held firmly to the ground. Control of sensory input is so finely tuned and regulated, that even among functional synergist (eg. soleus, lateral gastrocnemius and medial gastrocnemius), the size of a reflex can vary throughout the step cycle and can reverse in sign (Zehr et al. 1997).

Locus of phase-dependent modulation

As in the cat, central pattern generators are likely responsible for regulating the overall strength of excitatory and inhibitory connections in the spinal cord, that allow sensory information to be incorporated in the control of motor tasks. Activity in CPG networks could explain these observations of task- and phase-dependence where reflex amplitude is modulated via premotoneuronal gating of afferent feedback (Andersson et al. 1978; Forssberg 1979; Abraham and Loeb 1985; Yang and Stein 1990; Duysens et al. 1990; Duysens et al. 1992; Van Wezel et al. 1997; Duysens and Van De Crommert 1998; Komiyama et al. 2000; Dietz et al. 2001; Dietz 2002; Zehr and Duysens 2004; Zehr et al. 2004b).

In static tasks there is a linear relationship between reflex and background activity while during walking, reflex responses do not always follow background activation (Yang and Stein 1990; Van Wezel et al. 1997; Zehr et al. 1997; Haridas and Zehr 2003). This observation suggests that modulation occurs at a pre-motoneuronal level (Matthews 1986). In the case of muscle afferent pathways, an increased reflex attenuation during tasks, that is independent of locomotor EMG, requiring a greater EMG output, implies a premotoneuronal mechanism (Stein and Capaday 1988). Most likely it is presynaptic inhibition of afferent transmission from CPGs, as a mechanism for inhibition, as presynaptic inhibition is a major mechanism influencing spinal cord excitability during interlimb locomotor activity (Capaday and Stein 1986; Crenna and Frigo 1987; Zehr 2006).

There is more evidence that spinal CPGs are responsible for modulation of cutaneous sensory feedback during locomotion. When a CPG for rhythmic movement is not active, as in passive movements, phase-dependent modulation is absent (Brooke et al. 1999; Carroll et al. 2005). Also, movement related afferent discharge, associated with the passive movement, does not modulate the cutaneous response. There was also no effect on cutaneous reflexes when activating muscle spindles of quadriceps muscles with patellar taps (Brooke et al. 1999). Considering the interaction of other reflex pathways has no effect on cutaneous reflexes, and that modulation does not occur with passive movement, a central mechanism is presumed to be responsible for phase-dependent modulation of cutaneous reflexes (Brooke et al. 1999).

The way in which CPG neurons transform cutaneous input, changes as a function of the locomotor cycle. The fact that cutaneous feedback during walking can cause a flexor response during the swing phase, and an extensor response during the stance phase, in the same muscle, suggests that parallel excitatory and inhibitory cutaneous pathways could exist between cutaneous receptors and motoneuron pools (Yang and Stein 1990). Indeed, the existence of parallel excitatory and inhibitory pathways to motoneurons was revealed by analysis with post-stimulus time histograms (PSTH) of single motor units from the tibialis anterior during walking (De Serres et al. 1995). With posterior tibial nerve stimulation, PSTH showed that the same motor unit was excited during the swing phase, and inhibited during the transition from swing to stance. The opening and closing of these parallel pathways depends on the phase of the rhythmic cycle where CPG's act to govern the overall strength of the excitatory and inhibitory connections in these parallel pathways (Yang and Stein 1990; Duysens et al. 1992).

The H-reflex and cutaneous reflexes behave very differently during passive movements. During passive stepping and cycling, there is distinct phase-dependent modulation of H-reflexes in humans (Brooke 1992, 1993, 1995). These results highlight the importance of cutaneous reflexes used to probe CPG rhythmicity as H-reflexes are more susceptible to afferent-induced modulation.

Examining task- and phase-dependent modulation of cutaneous reflexes during rhythmic movement provides an indirect indicator of CPG regulation of afferent input. In humans, these observations provide some of the main data on which the concept of spinal CPGs in humans has been built. There are several characteristics that reveal a central control mechanism in modulating sensory feedback for task- and phase-dependent modulation. These characteristics include the fact that reflex modulation is independent of changes in background EMG, the fact that modulation only occurs with active movement, and not passive movement, and the fact that modulation is not influenced by feedback in other sensory pathways. Together these observations are evidence that a spinal CPG is responsible for the fine-tuning of sensory feedback during rhythmic movement.

Are the arm and legs coordinated?

As outlined in the previous section, EMG and reflex studies support the role of locomotor CPGs in the neural control of rhythmic leg movement. However another observation about CPGs from cats to consider is that spinal CPGs are distributed along the spinal cord for functional integration between the forelimbs and hindlimbs. Given the potential for interspecies conservation, we would expect it is likely that in humans, CPG networks can be found in the cervical spinal cord and produce rhythmic activity for arm swing. We would also likely expect in humans that, reminiscent of what is demonstrated in quadruped locomotor studies; CPGs of various limbs are interconnected at a low level in the central nervous system.

Do CPGs contribute to the control of rhythmic arm movements?

To build from work in the cat, a question to consider is do cervical level CPGs contribute to the control of rhythmic arm movements? We will see in this section that cervical CPG activity does regulate arm swing, and in a similar way to how lumbar spinal circuits are believed to regulate rhythmic leg movement. One limitation to the extrapolation from cats to humans is that bipeds and quadrupeds use different modes to locomote, where quadrupeds use all four limbs to move around, and bipeds mainly only use two limbs. As humans, we can walk without moving our arms, and there is no obvious reason why humans need to move their arms when they walk, yet rhythmic movements, that are coordinated with the legs, naturally emerges while walking (Ford et al. 2007; Umberger 2008; Meyns et al. 2013).

Arm swing has been compared to a pendular motion, although arm swing is not a purely passive action (Ferris et al. 2006). Mechanically, arm swing acts to enhance stability where arm swing generates a horizontal torque at the upper trunk which may counteract pelvis rotation and leg progression to minimize angular momentum (Li et al. 2001; Park 2008; Umberger 2008). Arm swing movements may also be powered predominately by the legs, done so by forces being transferred to the arms between trunk and shoulder ligaments and muscles during walking (Pontzer et al. 2009). But it has long been believed that the natural arm movement during walking are not just a simple pendular movement resulting from leg motion, but neurally integrated into movement (Elftman 1939).

The arms, not only under neural control, seem to be modulated in a similar way to how the legs are modulated. Similarities between the legs and arms have been observed by examining coordination and electromyographic activity. Coordination patterns between the upper extremities are similar to that of the lower extremity in human bipedal locomotion, common with those of quadrupedal locomotion (Van Emmerik et al. 1998). As for EMG activity, in general, arm muscle activity is out-of-phase, and reciprocating, similar compared to that of the leg muscles, but with slightly more co-activation (Zehr and Kido 2001; Zehr et al. 2003b). In addition, within arm EMG activation patterns are coordinated with contralateral arm muscles (Zehr and Kido 2001) and with EMG activation in the legs (Zehr et al. 2003b).

Also as in the legs, task- and phase-dependent reflex modulation, of both cutaneous and H-reflex pathways, has been demonstrated during rhythmic arm movement (Zehr and Chua 2000; Zehr and Kido 2001; Zehr et al. 2003a). For example, cutaneous reflexes evoked with stimulation to either the median, ulnar, or radial nerve were of differing amplitude and sign during arm cycling, compared to static contractions at matched positions in the cycle (Zehr and Kido 2001). A full cutaneous reflex reversal can be seen in some arm muscles, in which reflexes may be excitatory during static contraction, but inhibitory during arm cycling. There is also extensive task- and phase-dependent modulation of cutaneous reflexes in arm muscles during the natural arm swing of walking, compared to static contractions in matched positions (Zehr et al. 2003b). Muscle afferent reflexes in arm muscles also showed task- and phase-dependent modulation. In the flexor carpi radialis, H-reflexes evoked during arm cycling were phase modulated, independent from bEMG, and not observed during static contractions (Zehr et al. 2003a). During movement, forearm reflexes were strongly inhibited compared to the amplitude expressed during static contraction (Zehr et al. 2003a). Indicating the influence of afferent feedback on this reflex pathway, H-reflex amplitudes were suppressed with both active and passive movement (Zehr et al. 2003a) as seen in the legs (Brooke et al. 1997).

Phase-dependent and task-dependent modulation of reflex amplitude in the arms, characteristic of CPG regulation, suggests equivalent neural control mechanism for the arms and legs during rhythmic movement (Dietz et al. 2001; Zehr and Kido 2001; Dietz 2002; Zehr et al. 2003b; Zehr et al. 2004a; Zehr and Duysens 2004; Balter and Zehr 2007; Zehr et al. 2007b). Supporting a central locus of control for rhythmic arm movement, as is seen in the legs (Brooke et al. 1999), cutaneous reflexes are not phase modulated during passive arm cycling (Carroll et al. 2006). These observations support the hypothesis that rhythmic arm movements are partly regulated by CPGs, just as documented for the leg (Jackson 1983; Dietz et al. 2001; Dietz 2002; Zehr and Duysens 2004).

Also similar to the legs, during static contractions, reflex amplitudes are highly correlated with background muscle activity, while during rhythmic tasks, this relationship is weak or absent, and reflex amplitudes in the arms are modulated in a manner that is independent of background EMG activity (Zehr and Kido 2001; Zehr et al. 2003a; Zehr et al. 2003b). These results highlight the different patterns of reflex modulation between static contractions, and rhythmic arm movement, reflecting the differences in their neural control, just as seen in the legs. This observation also suggests pre-motoneuronal gating of afferent feedback by spinal rhythm generating circuits in the arms, as seen in the legs (Dietz et al. 2001; Dietz 2002; Zehr et al. 2004a).

Other evidence supporting spinally-driven arm movements comes from measuring the size of motor-evoked potentials, in response to transcranial magnetic stimulation, during rhythmic arm movement (Carroll et al. 2006). It was found that motor-evoked potentials were reduced in size compared to those recorded with tonic, voluntary contraction (Carroll et al. 2006). This indicates a reduction in the corticospinal influence during rhythmic arm movement compared to voluntary movement. Thus, despite highly developed corticospinal projections to the human upper limb, subcortical regions contribute to the control of rhythmic arm movements.

The degree of coupling between the two legs compared to between the two arms is not as strong, perhaps due to their functional roles during bipedal walking. For the legs, reflex modulation on the ipsilateral side is dependent upon contralateral active or passive leg movement where a general suppressive effect was recorded in the ipsilateral leg (Collins et al. 1993; Cheng et al. 1998). In addition, reflex responses seem to follow the movement phase of the contralateral leg (Duysens et al. 1990; Tax et al. 1995). For the arms, modulation for both cutaneous and H-reflexes on the ipsilateral side is not dependent upon contralateral active or passive rhythmic arm movement (Delwaide et al. 1988; Zehr et al. 2003b; Carroll et al. 2005). Instead, reflex modulation in the arm is dependent on the activity state of the limb in which the reflex was evoked (Carroll et al. 2005; Hundza and Zehr 2006). These findings suggest that while coupling is strong between the CPGs for each leg, the CPGs for each arm seem to be less involved in gating crossed responses (Carroll et al. 2005). Comparatively stronger coupling between legs is likely from differences in the functional roles of the arms versus the legs in human bipedal walking. Although the arms can be free to act independently, it is essential to have strong leg coordination to maintain upright posture.

Interlimb coordination

Evidence suggests that there are CPGs for rhythmic arm movements, but to support interlimb coordination, significant connection between lumbar and cervical spinal cord CPGs, actuating individual limbs, is required (Swinnen and Duysens 2005). Indeed, during rhythmic arm and leg movements, arm activity contributes to the neural excitation of leg muscles, indicating neural coupling between upper and lower limbs in humans (Zehr et al. 2009b; Zehr et al. 2016), reminiscent of what is found in quadrupeds. In this section, as in previous parts of this review, evidence will be presented from human studies to show that indeed, the arms and legs are connected. The basic idea of all the experiments presented in this section is to detect coupling between the arms and legs of humans. However, again in humans, only indirect evidence exists for interlimb locomotor linkages to show that bipedal human locomotion is similar to quadrupedal neural coordination.

Providing solid evidence for the existence of interlimb connections regulating rhythmic activity of all limbs in humans, several categories of experimental evidence will be presented. Through observations of maintained coupling by changes in arm and leg mechanical interactions, and evidence of a widespread network of reflexes in leg or arm muscles, there is strong support for spinal pathways linking muscles in the arms and legs. Effects of remote rhythmic movement on motor output and reflex excitability in the opposite set of limbs also demonstrates interlimb coordination in humans. In this section we will review different modes of evidence that probe interlimb connections for the arms and legs through spinal CPGs in humans.

Evidenced by mechanical changes in interlimb coordination

Arm swing is coordinated to movement with the legs where the frequency of arm swing motion coordination depends on walking speed, and retains a phase relationship with the legs regardless of ambulatory velocity (Craik et al. 1976; Donker et al. 2002). At most walking speeds (normal walking, jogging, running) this pattern consists of a 1:1 frequency ratio with out-of-phase arm swing where each arm is paired with its contralateral leg and synchronized with stride frequency. Even across quadrupedal tasks such as walking, creeping, and swimming, arm and leg movements remain frequency locked with a fixed relationship. The characteristics of this coordination correspond to the observation that coordination be indicative of intrinsic spinal interconnections between the upper and lower spinal CPGs that are engaged in the locomotor function (Wannier et al. 2001).

To further uncover changes in interlimb coordination due to changes in interlimb kinetics, weights were added to the wrist or ankle (Donker et al. 2002). Adding a mass to the wrist during walking resulted in increased muscle activity in both arms, a decrease in the movement amplitude in only the loaded limb, but did nothing to change leg kinematics or cadence. Conversely, adding load to an ankle, produced increased muscle activity and movement in both arms (Donker and Beek 2002; Donker et al. 2002). These results indicate that during walking the loading of one of the limbs induces a general reorganization, involving all participating bodily segments, presumably to maintain balance while providing rhythm constancy.

Alterations in coupling patterns between upper and lower extremities associated with changes in walking speed implicate interaction among CPGs. Decreasing walking velocity, to relatively slow speeds at approximately less than 0.7-0.8 m/s, causes both arms to swing minimally and in-phase at twice the ipsilateral step frequency (producing a 2:1 frequency ratio) (Wagenaar and Van Emmerik 2000; Donker et al. 2001; Ford et al. 2007), resulting in a change in interlimb coordination between the arms and the legs. As humans change walking speed, their nervous systems adapt muscle activation patterns to modify arm swing to the appropriate frequency.

Coordination of arm and leg movements during human locomotion has also been evaluated by examining the effects of small leg perturbations during gait on leg and arm EMG activity (Dietz et al. 2001). With split-belt treadmill accelerations or decelerations, and stimulation of the distal tibial nerve, responses were observed in arm muscles that were small or absent during standing or walking, largest when the perturbation was applied to the stance phase, and correlated to compensatory responses in the TA (Dietz et al. 2001). These observations show that there is a task-dependent, flexible neuronal coupling between lower and upper limb muscles, as a residual function of quadrupedal locomotion (Dietz et al. 1994b; Dietz et al. 2001). Also using a split-belt paradigm, the use of four different combinations of left and right speed ratios, upper and lower limb coordination was revealed. Increasing the right side belt speed caused increased amplitude in the right limb, decreased amplitude in the left limb, but increased amplitude in both upper limbs. These observations show that CPGs for the upper and lower limb regulate full body movement to maintain the rhythmic locomotor pattern.

Adding support to a directional ascending bias in locomotor coupling, it was found that voluntary changes in leg cycling cadence modified arm cycling cadence, but voluntary changes in arm cycling cadence did not affect cadence in the legs (Sakamoto et al. 2007). This was evaluated using a combined arm and leg cycling task where arm and leg ergometers were mechanically independent. Not only are there changes in frequency coupling effects, and kinematic amplitudes as a result of altering interlimb coordination, but changes in EMG recordings also emerge. Active arm movement, in arm and leg recumbent stepping, has been found to significantly increase activation of the leg muscles (Huang and Ferris 2004; Ferris et al. 2006). This observation was only seen when the arms were active and disappeared when the legs were externally driven. The effect of changing arm movement frequency on interlimb coupling and leg activation was also examined during recumbent stepping (Kao and Ferris 2005). Fast upper limb movement facilitated neuromuscular recruitment of lower limb muscles (Kao and Ferris 2005) likely via spinal interlimb connections from propriospinal neural circuitry (Dietz 2002; Kao and Ferris 2005)

In a final paradigm revealing interlimb connections, participants laid horizontally on their side, with each leg suspended in an unloading exoskeleton, and locomotor-like leg movements were evoked by rhythmic arm movements (Sylos-Labini et al. 2014). Leg movements were accompanied by EMG activity in proximal leg muscles, that were modulated over each movement cycle, and displayed similar timing as that of normal locomotion (Sylos-Labini et al. 2014). In particular, movement of the shoulder joints led to increased activity of hip muscles and increased amplitude of hip and knee joint movements. Movement of the forearms and wrists had a similar facilitating effect as shoulder movement, but with a stronger influence on distal segments (Selionov et al. 2016).

Evidenced by interlimb reflexes

Another way to probe for neural interactions between the arms and legs is to study somatosensory linkages in the form of interlimb reflexes during rhythmic movement (Burke et al. 1991; Zehr et al. 2004a). For instance, inter-limb coupling in humans has previously been demonstrated by evoking reflexes in one limb and observing the extent to which the movement of another limb modulates reflex expression (Zehr et al. 2003b; Mezzarane et al. 2011; Massaad et al. 2014).

A possible route for these interlimb coordination signals is through intrinsic spinal pathways, made up of propriospinal interneurons, linking cervical to lumbosacral regions, as evidenced in humans with supraspinal lesions and spinal cord transections (Nathan et al. 1996). Evidence of pathways connecting spinal cord segments also comes from studying patients with spinal cord injury (Calancie et al. 1996). The location of the injury in these 15 subjects was at the cervical level sparing the propriospinal pathways linking the cervical and lumbar enlargements in the spinal cord. With stimulation of cutaneous nerves (distal tibial nerve) and muscle afferent nerves (tibial and median nerves) widespread responses in all limbs were evoked, identified with single motor unit recordings. Discharge of motor units was also evoked with light touch of the feet and individual hair movements (Calancie et al. 1996). Interlimb reflex properties were similar for both SCI and control groups for radial nerve stimulation, suggesting that the neural circuitry underlying these reflexes does not develop as a result of SCI (Butler et al. 2016).

In humans with intact nervous systems, there is also indirect evidence to suggest that these interlimb pathways exist. It was initially shown that with noxious, high intensity stimulation of tactile afferents in the median and sural nerves, distant motor nuclei undergo excitability changes (Delwaide et al. 1981). A reciprocal pattern of facilitation and suppression for the flexor and extensor reflexes in arm muscles emerged with sural nerve stimulation, suggesting a coordinated and functional interlimb linkage (Delwaide and Crenna 1984). A connection between the arms and legs was also recorded in human subjects where descending lumbosacral cord potentials were recorded intrathecally after stimulation of the median nerve at the elbow (Sarica and Ertekin 1985).

Examining cutaneous reflexes during static and rhythmic movement has revealed a widespread interlimb network, as there is an extensive distribution of reflexes across many muscles in both the arms and the legs, irrespective of which limb is directly stimulated (Zehr et al. 2001; Haridas and Zehr 2003; Zehr and Duysens 2004). In seated positions, strong, early latency interlimb cutaneous reflexes were documented in all limbs following stimulation to either the foot or the hand (Zehr et al. 2001). Responses were recorded from multiple ipsilateral and contralateral muscles, particularly from those that cross the ankle, wrist and shoulder joints. These connections provide a means for the direct relay of sensory information through the nervous system which could be used to increase co-ordination between the arms and legs for balance and movement.

During walking, interlimb effects are observed where wrist stimulation, at the superficial radial nerve, caused phase-dependent modulation in leg muscles, especially medial gastrocs, and foot stimulation, at the superficial peroneal nerve, caused phase depended modulation in arm muscles, especially posterior deltoid (Haridas and Zehr 2003). There were also coordinated changes in kinematics dependent on if stimulation was applied to the wrist or foot. It was seen that compensatory responses at the ankle were reversed, where stimulation at the foot caused ankle plantarflexion, and stimulation at the hand caused dorsiflexion (Haridas and Zehr 2003). Connections between the arms and legs have also been identified during other arm and leg movements including leg cycling (Sasada et al. 2010). Responses to superficial radial nerve stimulation in the arms were evaluated, and it was found that cutaneous reflexes in arm muscles were modulated by leg cycling, and further amplified with increased leg cycling frequency (Sasada et al. 2010).

In human locomotion, afferent signals related to specific arm movement are crucial signals to modify leg muscle activity through linked CPGs. Phase-dependent responses found in muscles of all four limbs during rhythmic movement, are modulated in a way suggestive of coupling between segmental spinal networks (Yang and Stein 1990; Duysens et al. 1992; Duysens et al. 1996; Zehr et al. 2003b; Haridas and Zehr 2003). For example, rhythmic arm movement significantly contributed to reflex expression in the legs (Balter and Zehr 2007). The largest effect was observed during the power phase of arm and leg cycling, which is at a comparable time to heel strike in walking. The contribution from the arm at this point, could be explained by a reliance on multisensory integration to ensure safe walking (Balter and Zehr 2007).

Evidenced by remote effects of rhythmic movement on interlimb coupling

Determining the specific locus mediating interlimb coordination is difficult because of the concurrent and interfering effects of rhythmic arm and leg activity. It may be possible that interlimb reflex effects, seen above, could be the result of afferent signals from the rhythmic movement, in modifying activity (Haridas and Zehr 2003). To mitigate these effects, interlimb coordination has been examined by evaluating the remote effects of rhythmic movement on stationary limbs (Frigon et al. 2004). An interaction between upper limb posturing and reflex transmission in the lower limb was first identified in humans, where changes in upper limb posture altered tendon reflexes in soleus, quadriceps, and biceps femoris muscles (Delwaide et al. 1977). In addition, passive flexion and extension movements at the elbow facilitated soleus H reflex amplitudes (Hiraoka and Nagata 1999) and conversely, arm swing movements reduced soleus H-reflex amplitudes (Hiraoka 2001).

An observation of interlimb effects has also been made by examining the effects of remote movement on H-reflex excitability (Frigon et al. 2004). In this paradigm, subjects performed rhythmic arm cycling while soleus H-reflexes were evoked and recorded. With arm cycling, soleus H-reflexes are significantly reduced, as compared to reflexes evoked when no arm movement was performed (Frigon et al. 2004). These results provided evidence of the existence of neuronal coupling between the arms and the legs (Dietz 2002; Frigon et al. 2004; Loadman and Zehr 2007; Zehr et al. 2007b; Hundza and Zehr 2009; Dragert and Zehr 2009; de Ruiter et al. 2010; Mezzarane et al. 2011; Hundza et al. 2012). And because the arm cycling effect on soleus H-reflex excitability are independent from background EMG changes in the soleus, a spinal processing prior to the motoneuron membrane has been suggested (Frigon et al. 2004). Likely, suppression is from increased segmental Ia PSI of afferent terminals from arm CPG circuits (Frigon et al. 2004).

Subsequent studies have been conducted to further evaluate and characterize this observation. It was found that there is phase-dependent modulation of soleus H-reflex amplitude, induced by rhythmic arm cycling, that displayed a bell-shaped modulation curve (de Ruiter et al. 2010). It was also found that soleus H-reflexes were suppressed for all arm, trunk or leg movements, but a distinct and marked reflex modulation occurred during locomotor-like anti-phase arm swing, and was maximally suppressed at a moment where the heel-strike would occur (Massaad et al. 2014). Changes in arm range of motion during cycling, resulting in muscle-length changes, did not alter soleus H-reflex suppression (Loadman and Zehr 2007), and an inhibitory effect was only observed with active, voluntary arm cycling, and not apparent during passive, externally driven arm movement (Hundza et al. 2012). Increased frequency of upper limb movement however, does increase the inhibitory effect of arm cycling on soleus H-reflex excitability (Hundza and Zehr 2009). There was no additional effect of varying arm cycling load or by adding vibration to the arm muscles (Hundza et al. 2012). After stroke, partial preservation of the descending modulatory effects of rhythmic arm cycling on lumbosacral spinal cord excitability can be seen where arm cycling modulates the soleus H-reflex (Barzi and Zehr 2008) and stretch reflex (Mezzarane et al. 2014).

In the reverse experiment, it was shown that leg cycling also leads to suppression of H-reflexes in stationary arm muscles, including the flexor carpi radialis (FCR) (Zehr et al. 2007b). Phase-dependent modulation of H-reflex amplitudes in arm muscles is not seen with leg cycling (Zehr et al. 2007b) however the temporal resolution, to detect phase-dependent modulation may not have been small enough. In walking, when analyzing sixteen bins, phase-dependent modulation of H-reflexes in FCR is evident (Domingo et al. 2014). Highlighting the importance of movement related feedback on modifying interlimb coordination, as opposed to load-related feedback, FCR H-reflex amplitudes were studied during robotic-assisted stepping with and without body weight support (Nakajima et al. 2011). Reflexes in the forearm muscles were supressed with stepping, and suppression was seen at all phases of stepping, irrespective of whether stepping was unloaded or if body weight support was provided (Nakajima et al. 2011).

Using this interlimb paradigm, observations to support the idea that the interlimb mechanics underlying arm and leg coupling are preferentially expressed during rhythmic tasks, comes from studying sub-threshold segmental Ia PSI conditioning (Nakajima et al. 2013). Conditioning the H-reflex with superficial radial nerve stimulation removes the suppression effect of leg cycling, and radial nerve stimulation amplifies suppression. When conditioning stimulation intensity is reduced, so that there is no post-synaptic effect, it was shown that conditioning re-emerges only during the rhythmic locomotor behaviour (Nakajima et al. 2013). Together, these results suggest that it is a central motor command, likely from the spinal cord, as the main source of regulation.

Is there a need for supraspinal input?

It is apparent that the human spinal cord is much more reliant on supraspinal control for the expression of locomotor activities, compared to other quadrupedal animals (Capaday 2002; Nielsen 2003; Yang 2006; Barthelemy et al. 2011; Petersen et al. 2012; Beloozerova et al. 2013). Of course, this is not surprising given the mechanical differences in quadrupedal versus bipedal gait.

Evidence exists in humans where brain imaging and non-invasive electrophysiological techniques have been adopted in order to better understand supraspinal control of walking in humans. Using functional MRI in healthy participants, it was shown that during imagined walking and running, areas in the brainstem (presumably from the mesencephalic locomotor region) are required to initiate walking (Jahn et al. 2008). These are the same locomotor centers shown in the cat to be involved in walking initiation (Nielsen 2003).

And again, there are several ways to mimic this experimentally. For example, transcutaneous electrical stimulation of the spinal cord can be used as a non-invasive tool for activation of locomotor circuitry in humans (Gorodnichev et al. 2012). Indirect activation can also be achieved with peripheral muscle vibration where step-like behaviour is generated in those with spinal cord injury (Field-Fote et al. 2012). The addition of chemicals can also be used to boost lumbar CPG activation and bipedal stepping expression (Guertin 2013).

Once initiated, under the normal conditions, walking is highly automated and requires few cognitive resources, however, under difficult conditions, cortical control is increased. For example, to significantly modulate muscle activity during walking adaptations (Nielsen 2003), and to steer around obstacles (Hess et al. 2003), intact corticospinal transmission is essential. Because of this observation, the engagement of supraspinal motor areas may be beneficial for gait recovery (van den Brand et al. 2012) where improvements in walking from those with SCI or stroke can be partially correlated with increased cortical and corticospinal activity (Dobkin et al. 2004; Winchester et al. 2005).

Is CPG control preserved across rhythmic tasks?

The final concept evidenced from cats, to find support for in humans, is that the pattern generating circuits for different rhythmic functions are not different, but are interconnected and overlap in the behaviours they generate. If shared circuitry for various rhythmic movements is also within the human spinal cord, it should be observed as characteristic of human reflex modulation. Indeed, as in the cat, in humans, it has been demonstrated that different full body rhythmic tasks (i.e. walking, combined arm-leg cycling and stepping, or arm and leg cycling) share common neural circuitry (Zehr et al. 2007a) which has been termed the common core hypothesis (Zehr 2005).

Cutaneous reflexes are phase-reversed when comparing between forward and backward treadmill walking (Duysens et al. 1996), leg cycling (Zehr et al. 2009a), and arm cycling (Zehr and Hundza 2005). Similarly in human infants, different directions of walking are ascribed to flexible use of common locomotor spinal circuits (Lamb and Yang 2000). A very similar coordination pattern between all four limbs during walking, creeping, and swimming motions, that are suggestive of similar CPG output in all activities, has also been shown (Wannier et al. 2001). In other quadrupedal tasks, cutaneous reflex amplitudes in arm and leg muscles were modulated in a similar way across level walking, incline walking, and stair climbing (Lamont and Zehr 2006). Commonalities in control are also seen across walking, arm and leg cycling and arm-assisted recumbent stepping, where similar phase-dependent modulation was observed despite differences in movement kinematics (Zehr et al. 2007a).

After a stroke common neural patterning from conserved subcortical regulation is seen, supporting the notion of a common core in locomotor tasks involving arm and leg movement

(Klarner et al. 2014). A contribution from subcortical and presumed spinal locomotor pattern generating networks is implicit in the observations in stroke, where networks for arm and leg coordination could reside in subcortical areas as damage to the brain following stroke does not seem to significantly affect common neural regulation (Klarner et al. 2014).

That neural control, evidenced by examining similarities in reflex modulation, during rhythmic movement is conserved across rhythmic arm and leg movements for different tasks, points to a common central control mechanism. That is, a central mechanism is likely responsible for regulating various types of rhythmic movement in a similar oscillatory fashion with a common core of subcortical elements expressing neural activity to produce the basic pattern of arm and leg movement (Zehr 2005; Zehr et al. 2007a; Klarner et al. 2014).

Application of CPG theories to locomotor training

An understanding of the functioning of CPGs in humans, and in other animals, is important for the development of strategies for gait rehabilitation in individuals with spinal cord and brain injuries. From studies in other animals, after spinal cord transection, remaining spinal pathways can be trained where treadmill walking facilitates positive use-dependent plasticity, corresponding to enhanced recovery of walking (Barbeau and Rossignol 1987). The same approach has been applied in humans with neurotrauma, where the remaining neural networks are strengthened with training, proposed to enable activation of spinal cord circuitry, to restore normal CPG function, and corresponding locomotor activity (Dobkin 2004; Langhorne et al. 2009).

The ability to step with training is replicated in humans with body weight supported treadmill training therapy (Moseley et al. 2003; Duncan et al. 2011; Senthilvelkumar et al. 2015). For this therapy, participants practice walking on a motorized treadmill, with a harness system providing body weight support, where stepping is performed with the help of robotic interfaces or therapists. The arms are typically used for postural support on parallel bars, or hand rails, to help bear weight from the legs (Behrman and Harkema 2000). Results from this therapy are positive, where training leads to improved walking for those with neurological injury (Dietz et al. 1998; Edgerton et al. 2001; Field-Fote 2001; Moseley et al. 2003; Dobkin 2004; Duncan et al.

2011), however body weight supported treadmill training does not fully exploit the neuronal and mechanical linkages between the arms and legs that are vital in normal human walking (Ferris et al. 2006; Dietz and Michel 2009; Zehr et al. 2009; Klimstra et al. 2009).

Normal walking involves arm movement which is controlled by spinal CPG networks that are functionally connected to the legs. With the current therapy, the lack of involvement with the arms only adds to the neural limitations that are already present due to the pathology, and impaired arm function may actually inhibit rhythmic stepping of the legs (Behrman and Harkema 2000). To optimize the benefits of task-dependent rehabilitation, given that the arms are linked to the legs during locomotion, it has been suggested that rehabilitation include arm movements (Behrman and Harkema 2000; Dietz 2002; Ferris et al. 2006; Zehr et al. 2009; Klimstra et al. 2009). Data on interlimb responses obtained in persons with cervical spinal cord injury and stroke suggests that pathways mediating arm and leg interactions are conserved in humans, and remain accessible after neurological damage (Calancie 1991; Calancie et al. 1996; Zehr et al. 1998; Wirz et al. 2001; Zehr et al. 2009; Zehr and Loadman 2012).

Indeed several studies have found benefits of incorporating arm movements in gait rehabilitation. In those with incomplete cervical spinal cord injury, when arm activity is incorporated with locomotor-like arm and leg movements, leg muscle activity is facilitated (Kawashima et al. 2008). Gait symmetry and a more normal presentation of EMG is also apparent in patients with spastic paresis when arm swings were incorporated with body weigh supported training (Visintin and Barbeau 1994). Recently, it has been shown that five weeks of arm and leg cycling exercise training improved clinical walking status, increased strength by ~25%, improved modulation of muscle activity, range of motion and stride frequency, and improved cutaneous reflex modulation during treadmill walking (Klarner et al. 2016a). Arm and leg cycling exercise also induced long-term plasticity in stretch reflex excitability where interlimb modulation was improved on the more affected side following training (Klarner et al., 2016b). Together these findings support the addition of rhythmic arm activity as a regular part of locomotor rehabilitation after neurotrauma. Allowing a normal simultaneous and reciprocating arm action during walking rehabilitation may facilitate stepping, and may be an important component needed to harness interlimb neural coupling, to help improve motor output for the legs (Ferris et al. 2006; Kawashima et al. 2008; Zehr et al. 2009; de Kam et al. 2013).

Conclusion

Using a deductive reasoning approach, made popular by the fictional character Sherlock Holmes, indirect evidence supporting observations made from animal studies, suggests that the foundation of rhythmic movements in humans comes from CPGs. Below is a summary of support for each of the key observations from animal studies. Figure 1-5 outlines this evidence in pictorial form.

- The spinal cord can produce rhythmic movement, without descending supraspinal, or sensory feedback

From animal studies it has been found that central pattern generators (CPG) are complex neural networks in the spinal cord that, in isolation, can produce the basic patterned motor outputs required for locomotion. Definitive evidence of a spinal CPG in humans would require the demonstration of locomotor-like rhythmic movements, in an isolated spinal cord, with no descending input, and no feedback from the periphery. This evidence, in the human spinal cord, is not fully available; however some indirect observations of rhythmic activity have come close to support the theory of CPGs sub serving human locomotion.

Observations of spontaneous rhythmic movements in the legs, particularly from those with complete SCI provide compelling evidence for the existence of a CPG for locomotion in humans. However, sensory feedback, below the level of the injury is still intact and cannot be discounted in modifying the locomotor rhythm. Also, the fact that the observation of spontaneous activity occurs more often in those with an incomplete SCI (Harkema 2008), suggesting to a stronger role for supraspinal input. Based on this, the observation in animals that the spinal cord in isolation can produce rhythmic activity via a CPG is not supported in humans.

- Treadmill training for cats that have undergone spinal transection induces recovery of stepping

Cats with complete spinal cord transections are able to recover walking function with body weight supported treadmill training. This is in contrast to findings in humans where those with a complete spinal cord injury are not able to recover walking. Although this observation from reduced animal preparations is not supported in humans, this is not to say that spinal CPGs do not exist, just that for walking recovery more is controlling human locomotion compared to a cat.

- Spinal CPG networks are found in cervical and lumbar sections of the spinal cord, for forelimb and hindlimb control respectively, which are connected by propriospinal pathways

Direct intracellular measurements have revealed that the CPG network in quadrupeds is distributed across the spinal cord, leading to a hypothesis that a distributed network should exist for humans too. Indeed, indirect observation from human studies support the observation that CPG networks are distributed in cervical and lumbar spinal cord areas and are interconnected for functional interlimb integration. Rhythmic forelimb movements are controlled by spinal CPGs, as evidenced during fictive locomotion in a cat. In humans, observations of rhythmic muscle activity and task-and phase-dependent modulation of reflexes suggests that the control of rhythmic arm movements, as they are in cat forelimbs, is from spinal CPGs. Control of rhythmic arm movements are similar to that of rhythmic leg movements, however coupling between the arms is not as strong as between the legs.

Despite the functional differences in usage of the upper limbs in humans compared to the cat, data suggest that interlimb coordination is apparent and contributes to the control of human locomotion. There is a measurable functional neural coupling between the arms and legs during rhythmic movement shown by flexible interactions between lower and upper limb muscles from mechanical changes in interlimb coordination, the presence of task-and phase-dependent interlimb reflex modulation, and shown by modulatory effects of remote rhythmic activity on local reflex excitability.

- The same spinal CPG networks are recruited for different rhythmic tasks

The circuits producing rhythm and pattern generation are the same across different functional tasks. If shared circuitry for various rhythmic movements is also within the human spinal cord, it should be observed as characteristic of human reflex modulation. Indeed, as in the cat, in humans, it has been demonstrated that different full body rhythmic tasks share common neural circuitry

- Sensory feedback is needed by CPG networks to reinforce and modify rhythmic activity

Although CPGs can function without sensory input, peripheral feedback signals, gained during locomotor movements, are strong modulators, capable of altering the activity of CPG networks exposing their flexibility and adaptability. In other animal studies, direct connections between sensory feedback and CPG activity have been shown where feedback entrains and modifies rhythmic CPG activity.

In humans, indirect methodologies of examining the modulation of reflexes during rhythmic movement are used as an indicator of CPG regulation of afferent input. Reflexes, arising because of activation of afferent projections from receptors in skin and muscle, have been studied widely, and support the role of locomotor CPGs in the neural control of rhythmic human movement. CPG activity allows for flexible transmission of sensory feedback during gait where signals are controlled and modulated by the motor task and phase of transmission. Because phase-dependent modulation is only apparent with voluntary movement, and not passive, is independent from background EMG, is not influenced by feedback in other sensory pathways, and is evident after supraspinal damage, provides the main data on which the concept of spinal CPGs in humans has been built. This allows for CPG output to not simply be a stereotyped pattern of flexor and extensor activity, but to have usefulness in a wide variety of situations, where sensory feedback provides the cues required to make CPGs respond in behaviorally appropriate ways. Therefore as in cats, humans possess a CPG for locomotion that is capable not only of rhythmic pattern generation, but also remarkable sensory feedback-induced adaptation.

- Supraspinal input is required to initiate CPG activity

In all animals, descending input from supraspinal areas is required to trigger and regulate the motor output of a CPG for stepping. Certainly, supraspinal inputs have a higher functional relevance to bipeds compared to quadrupeds, due to the increased need for balance control in bipedal human gait.

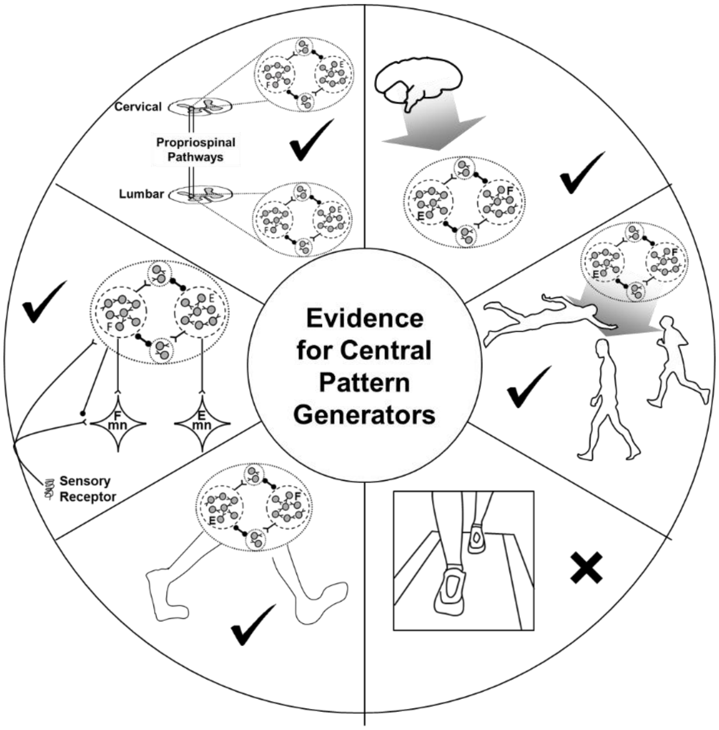


Figure 1-5: Summary of evidence for a spinal central pattern generator in humans.

While direct evidence from animal models supports the notion that a CPG is involved in the control of locomotion, there is only indirect evidence for a locomotor CPG in humans. In summary, all evidence, although indirect, points to CPG mediated locomotion in humans. Therefore, the general operational principles for the control of rhythmic movement found in cat preparations extend as well to humans.

References

Abraham LD, Loeb GE (1985) The distal hindlimb musculature of the cat. Exp Brain Res 58:580–593. doi: 10.1007/BF00235874

Amemiya M, Yamaguchi T (1984) Fictive locomotion of the forelimb evoked by stimulation of the mesencephalic locomotor region in the decerebrate cat. Neurosci Lett 50:91–96. doi: 10.1016/0304-3940(84)90468-3

Andersson O, Forssberg H, Grillner S, Lindquist M (1978) Phasic gain control of the transmission in cutaneous reflex pathways to motoneurones during “fictive” locomotion. Brain Res 149:503–507. doi: 0006-8993(78)90493-6 [pii]

Andersson O, Grillner S (1983) Peripheral control of the cat’s step cycle. II. Entrainment of the central pattern generators for locomotion by sinusoidal hip movements during “fictive locomotion.” Acta physiolgica Scand 118:229–239. doi: 10.1111/j.1748-1716.1983.tb07267.x

Balter JE, Zehr EP (2007) Neural coupling between the arms and legs during rhythmic locomotor-like cycling movement. J Neurophysiol 97:1809–18. doi: 10.1152/jn.01038.2006

Barbeau H, Rossignol S (1987) Recovery of locomotion after chronic spinalization in the adult cat. Brain Res 412:84–95. doi: 10.1016/0006-8993(87)91442-9

Barthelemy D, Grey MJ, Nielsen JB, Bouyer L (2011) Involvement of the corticospinal tract in the control of human gait. Prog Brain Res 192:181–197. doi: 10.1016/B978-0-444-53355-5.00012-9

Barzi Y, Zehr EP (2008) Rhythmic arm cycling suppresses hyperactive soleus H-reflex amplitude after stroke. Clin Neurophysiol 119:1443–52. doi: 10.1016/j.clinph.2008.02.016

Behrman AL, Harkema SJ (2000) Locomotor training after human spinal cord injury: a series of case studies. Phys Ther 80:688–700.

Beloozerova IN, Stout EE, Sirota MG (2013) Distinct Thalamo-Cortical Controls for Shoulder, Elbow, and Wrist during Locomotion. Front Comput Neurosci 7:62. doi: 10.3389/fncom.2013.00062

Bouyer LJG, Rossignol S (2003) Contribution of cutaneous inputs from the hindpaw to the control of locomotion. II. Spinal cats. J Neurophysiol 90:3640–53. doi: 10.1152/jn.00497.2003

van den Brand R, Heutschi J, Barraud Q, et al. (2012) Restoring voluntary control of locomotion after paralyzing spinal cord injury. Science 336:1182–5. doi: 10.1126/science.1217416

Brooke JD, Cheng J, Collins DF, et al. (1997) Sensori-sensory afferent conditioning with leg movement: gain control in spinal reflex and ascending paths. Prog Neurobiol 51:393–421.

Brooke JD, McIlroy WE, Staines WR, et al. (1999) Cutaneous reflexes of the human leg during passive movement. J Physiol 518:619–628. doi: 10.1111/j.1469-7793.1999.0619p.x

Brown TG (1911) The Intrinsic Factors in the Act of Progression in the Mammal. Proc R Soc B Biol Sci 84:308–319. doi: 10.1098/rspb.1911.0077

Brown TG (1914) On the nature of the fundamental activity of the nervous centres; together with an analysis of the conditioning of rhythmic activity in progression, and a theory of the evolution of function in the nervous system. J Physiol 48:18–46.

Burke D, Dickson HG, Skuse NF (1991) Task-dependent changes in the responses to low-threshold cutaneous afferent volleys in the human lower limb. J Physiol 432:445–58.

Burke RE (2001) The central pattern generator for locomotion in mammals. Adv Neurol 87:11–24.

Burke RE (1999) The use of state-dependent modulation of spinal reflexes as a tool to investigate the organization of spinal interneurons. Exp Brain Res 128:263–277. doi: 10.1007/s002210050847

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

Büschges A, Akay T, Gabriel JP, Schmidt J (2008) Organizing network action for locomotion: Insights from studying insect walking. Brain Res Rev 57:162–171. doi: 10.1016/j.brainresrev.2007.06.028

Bussel B, Roby-Brami a, Azouvi P, et al. (1988) Myoclonus in a patient with spinal cord transection. Possible involvement of the spinal stepping generator. Brain 111 (Pt 5:1235–1245. doi: 10.1093/brain/111.5.1235

Butler JE, Godfrey S, Thomas CK, et al. (2016) Interlimb Reflexes Induced by Electrical Stimulation of Cutaneous Nerves after Spinal Cord Injury. PLoS One 11:1–18.

Calancie B (1991) Interlimb reflexes following cervical spinal cord injury in man. Exp Brain Res. doi: 10.1007/BF00229423

Calancie B (2006) Spinal myoclonus after spinal cord injury. J Spinal Cord Med 29:413–24.

Calancie B, Lutton S, Broton JG (1996) Central nervous system plasticity after spinal cord injury in man: interlimb reflexes and the influence of cutaneous stimulation. Electroencephalogr Clin Neurophysiol Mot Control 101:304–315. doi: 10.1016/0924-980X(96)95194-2

Calancie B, Needham-Shropshire B, Jacobs P, et al. (1994) Involuntary stepping after chronic spinal cord injury. Evidence for a central rhythm generator for locomotion in man. Brain 117 (Pt 5:1143–1159. doi: 10.1093/brain/117.5.1143

Capaday C (2002) The special nature of human walking and its neural control. Trends Neurosci 25:370–376. doi: 10.1016/S0166-2236(02)02173-2

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

Carroll TJ, Baldwin ERL, Collins DF, Zehr EP (2006) Corticospinal excitability is lower during rhythmic arm movement than during tonic contraction. J Neurophysiol 95:914–21. doi: 10.1152/jn.00684.2005

Carroll TJ, Zehr EP, Collins DF (2005) Modulation of cutaneous reflexes in human upper limb muscles during arm cycling is independent of activity in the contralateral arm. Exp Brain Res 161:133–44. doi: 10.1007/s00221-004-2050-7

Chandler SH, Baker LL, Goldberg LJ (1984) Characterization of synaptic potentials in hindlimb extensor motoneurons during L-DOPA-induced fictive locomotion in acute and chronic spinal cats. Brain Res 303:91–100.

Cheng J, Brooke JD, Misiaszek JE, Staines WR (1998) Crossed inhibition of the soleus H reflex during passive pedalling movement. Brain Res 779:280–284. doi: 10.1016/S0006-8993(97)01168-2

Chervin RD, Consens FB, Kutluay E (2003) Alternating leg muscle activation during sleep and arousals: A new sleep-related motor phenomenon? Mov Disord 18:551–559. doi: 10.1002/mds.10397

Clardy SL, Connor JR (2010) Restless Leg Syndrome. Encycl. Neurosci. pp 131–135

Coleman RM, Pollak CP, Weitzman ED (1980) Periodic movements in sleep (nocturnal myoclonus): Relation to sleep disorders. Ann Neurol 8:416–421. doi: 10.1002/ana.410080413

Collins DF, McIlroy WE, Brooke JD (1993) Contralateral inhibition of soleus H reflexes with different velocities of passive movement of the opposite leg. Brain Res 603:96–101.

Conway BA, Hultborn H, Kiehn O (1987) Proprioceptive input resets central locomotor rhythm in the spinal cat. Exp Brain Res 68:643–656. doi: 10.1007/BF00249807

Craik R, Herman R, Finley F (1976) Human solutions for locomtoin. II. Interlimb coordination., In R.M. He.

Crenna P, Frigo C (1987) Excitability of the soleus H-reflex arc during walking and stepping in man. Exp Brain Res 66:49–60. doi: 10.1007/BF00236201

Van de Crommert HW, Mulder T, Duysens J (1998) Neural control of locomotion: sensory control of the central pattern generator and its relation to treadmill training. Gait Posture 7:251–263. doi: S0966636298000101 [pii]

Delwaide PJ, Crenna P (1984) Cutaneous nerve stimulation and motoneuronal excitability. II: Evidence for non-segmental influences. J Neurol Neurosurg Psychiatry 47:190–196. doi: 10.1136/jnnp.47.2.190

Delwaide PJ, Crenna P, Fleron MH (1981) Cutaneous nerve stimulation and motoneuronal excitability: I, soleus and tibialis anterior excitability after ipsilateral and contralateral sural nerve stimulation. J Neurol Neurosurg Psychiatry 44:699–707.

Delwaide PJ, Figiel C, Richelle C (1977) Effects of postural changes of the upper limb on reflex transmission in the lower limb: cervicolumbar reflex interactions in man. J Neurol Neurosurg Psychiatry 40:616–621.

Delwaide PJ, Sabatino M, Pepin JL, La Grutta V (1988) Reinforcement of reciprocal inhibition by contralateral movements in man. Exp Neurol 99:10–16. doi: 10.1016/0014-4886(88)90122-7

Dietz V (2002) Do human bipeds use quadrupedal coordination? Trends Neurosci 25:462–467. doi: 10.1016/S0166-2236(02)02229-4

Dietz V, Colombo G, Jensen L (1994a) Locomotor activity in spinal man. Lancet 344:1260–1263. doi: 10.1016/S0140-6736(94)90751-X

Dietz V, Michel J (2009) Human bipeds use quadrupedal coordination during locomotion. Ann. N. Y. Acad. Sci. pp 97–103

Dietz V, Colombo G, Jensen L, Baumgartner L (1995) Locomotor capacity of spinal cord in paraplegic patients. Ann Neurol 37:574–582. doi: 10.1002/ana.410370506

Dietz V, Fouad K, Bastiaanse CM (2001) Neuronal coordination of arm and leg movements during human locomotion. Eur J Neurosci 14:1906–1914. doi: 10.1046/j.0953-816x.2001.01813.x

Dietz V, Wirz M, Colombo G, Curt A (1998) Locomotor capacity and recovery of spinal cord function in paraplegic patients: A clinical and electrophysiological evaluation. Electroencephalogr Clin Neurophysiol - Electromyogr Mot Control 109:140–153. doi: 10.1016/S0924-980X(98)00002-2

Dietz V, Zijlstra W, Duysens J (1994b) Human neuronal interlimb coordination during split-belt locomotion. Exp Brain Res 101:513–520. doi: 10.1007/BF00227344

Dimitrijevic MR, Gerasimenko Y, Pinter MM (1998) Evidence for a spinal central pattern generator in humans. Ann. N. Y. Acad. Sci. pp 360–376

Dobkin BH, Firestine A, West M, et al. (2004) Ankle dorsiflexion as an fMRI paradigm to assay motor control for walking during rehabilitation. Neuroimage 23:370–381. doi: 10.1016/j.neuroimage.2004.06.008

Domingo A, Klimstra M, Nakajima T, et al. (2014) Walking phase modulates H-reflex amplitude in flexor carpi radialis. J Mot Behav 46:49–57. doi: 10.1080/00222895.2013.854731

Donelan JM, McVea D a, Pearson KG (2009) Force regulation of ankle extensor muscle activity in freely walking cats. J Neurophysiol 101:360–71. doi: 10.1152/jn.90918.2008

Donelan JM, Pearson KG (2004) Contribution of force feedback to ankle extensor activity in decerebrate walking cats. J Neurophysiol 92:2093–2104. doi: 10.1152/jn.00325.2004

Donker SF, Beek PJ (2002) Interlimb coordination in prosthetic walking: effects of asymmetry and walking velocity. Acta Psychol (Amst) 110:265–288. doi: 10.1016/S0001-6918(02)00037-9

Donker SF, Beek PJ, Wagenaar RC, Mulder T (2001) Coordination between arm and leg movements during locomotion. J Mot Behav 33:86–102. doi: 10.1080/00222890109601905

Donker SF, Mulder T, Nienhuis B, Duysens J (2002) Adaptations in arm movements for added mass to wrist or ankle during walking. Exp Brain Res 146:26–31. doi: 10.1007/s00221-002-1145-2

Dragert K, Zehr EP (2009) Rhythmic arm cycling modulates Hoffmann reflex excitability differentially in the ankle flexor and extensor muscles. Neurosci Lett 450:235–8. doi: 10.1016/j.neulet.2008.11.034

Drew T, Rossignol S (1987) A kinematic and electromyographic study of cutaneous reflexes evoked from the forelimb of unrestrained walking cats. J Neurophysiol 57:1160–1184.

Dubuc R, Brocard F, Antri M, et al. (2008) Initiation of locomotion in lampreys. Brain Res Rev

57:172–182. doi: 10.1016/j.brainresrev.2007.07.016

Duncan PW, Sullivan KJ, Behrman AL, et al. (2011) Body-weight-supported treadmill rehabilitation after stroke. N Engl J Med 364:2026–2036. doi: 10.1056/NEJMoa1010790

Duysens J (1977) Reflex control of locomotion as revealed by stimulation of cutaneous afferents in spontaneously walking premammillary cats. J Neurophysiol 40:737–751.

Duysens J, Van De Crommert HWAA (1998) Neural control of locomotion; Part 1: The central pattern generator from cats to humans. Gait Posture 7:131–141. doi: 10.1016/S0966-6362(97)00042-8

Duysens J, Pearson KG (1980) Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. Brain Res 187:321–332. doi: 10.1016/0006-8993(80)90206-1

Duysens J, Pearson KG (1976) The role of cutaneous afferents from the distal hindlimb in the regulation of the step cycle of thalamic cats. Exp Brain Res. doi: 10.1007/BF00235013

Duysens J, Stein RB (1978) Reflexes induced by nerve stimulation in walking cats with implanted cuff electrodes. Exp brain Res 32:213–24.

Duysens J, Tax AA, Murrer L, Dietz V (1996) Backward and forward walking use different patterns of phase-dependent modulation of cutaneous reflexes in humans. J Neurophysiol 76:301–310.

Duysens J, Tax AAM, Trippel M, Dietz V (1992) Phase-dependent reversal of reflexly induced movements during human gait. Exp Brain Res. doi: 10.1007/BF00227255

Duysens J, Trippel M, Horstmann GA, Dietz V (1990) Gating and reversal of reflexes in ankle muscles during human walking. Exp Brain Res. doi: 10.1007/BF00231254

Edgerton VR, Leon RD, Harkema SJ, et al. (2001) Retraining the injured spinal cord. J Physiol 533:15–22. doi: 10.1111/j.1469-7793.2001.0015b.x

Eidelberg E, Walden J, Nguyen L (1981) Locomotor control in macaque monkeys. Brain 104:

Van Emmerik REA, Wagenaar RC, Van Weegen E (1998) Interlimb coupling patterns in human locomotion: Are we bipeds or quadrupeds? Ann N Y Acad Sci 860:539–542.

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

Falgairolle M, de Seze M, Juvin L, et al. (2006) Coordinated network functioning in the spinal cord: An evolutionary perspective. J Physiol Paris 100:304–316. doi:

10.1016/j.jphysparis.2007.05.003

Ferris DP, Huang HJ, Kao PC (2006) Moving the arms to activate the legs. Exerc Sport Sci Rev 34:113–120. doi: 10.1249/00003677-200607000-00005

Field-Fote EC (2001) Combined use of body weight support, functional electric stimulation, and treadmill training to improve walking ability in individuals with chronic incomplete spinal cord injury. Arch Phys Med Rehabil 82:818–24. doi: 10.1053/apmr.2001.23752

Field-Fote E, Ness LL, Ionno M (2012) Vibration elicits involuntary, step-like behavior in individuals with spinal cord injury. Neurorehabil Neural Repair 26:861–9. doi: 10.1177/1545968311433603

Fleshman JW, Lev-Tov A, Burke RE (1984) Peripheral and central control of flexor digitorum longus and flexor hallucis longus motoneurons: The synaptic basis of functional diversity. Exp Brain Res 54:133–149. doi: 10.1007/BF00235825

Ford MP, Wagenaar RC, Newell KM (2007) Arm constraint and walking in healthy adults. Gait Posture 26:135–141. doi: 10.1016/j.gaitpost.2006.08.008

Forssberg H (1992) Evolution of plantigrade gait: is there a neuronal correlate. Dev Med Child Neurol 34:920–925.

Forssberg H (1979) Stumbling corrective reaction: a phase-dependent compensatory reaction during locomotion. J Neurophysiol 42:936–953.

Forssberg H, Grillner S, Rossignol S (1975) Phase dependent reflex reversal during walking in chronic spinal cats. Brain Res 85:103–107. doi: 10.1016/0006-8993(75)91013-6

Friesen WO, Kristan WB (2007) Leech locomotion: swimming, crawling, and decisions. Curr Opin Neurobiol 17:704–711. doi: 10.1016/j.conb.2008.01.006

Frigon A, Collins DF, Zehr EP (2004) Effect of rhythmic arm movement on reflexes in the legs: modulation of soleus H-reflexes and somatosensory conditioning. J Neurophysiol 91:1516–23. doi: 10.1152/jn.00695.2003

Gandevia SC, Miller S, Aniss AM, Burke D (1986) Reflex influences on muscle spindle activity in relaxed human leg muscles. J Neurophysiol 56:159–170.

Garcia-Rill E, Skinner RD (1987) The mesencephalic locomotor region. II. Projections to reticulospinal neurons. Brain Res 411:13–20. doi: 10.1016/0006-8993(87)90676-7

Gerasimenko Y, Gorodnichev R, Machueva E, et al. (2010) Novel and direct access to the human locomotor spinal circuitry. J Neurosci 30:3700–3708. doi:

10.1523/JNEUROSCI.4751-09.2010

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

Gorodnichev RM, Pivovarova EA, Pukhov A, et al. (2012) Transcutaneous electrical stimulation of the spinal cord: non-invasive tool for activation of locomotor circuitry in human. Fiziol Cheloveka 38:46–56. doi: 10.1134/S0362119712020065

Grillner S (2006) Neuronal networks in motion from ion channels to behaviour. An R Acad Nac Med 123:297–298.

Grillner S (1975) Locomotion in vertebrates: central mechanisms and reflex interaction. Physiol Rev 55:247–304.

Grillner S, Ekeberg Ö, El Manira A, et al. (1998a) Intrinsic function of a neuronal network - A vertebrate central pattern generator. Brain Res. Rev. pp 184–197

Grillner S, Matsumoto T (1991) The neural network underlying locomotion in lamprey--synaptic and cellular mechanisms. Neuron 7:1–15.

Grillner S, Parker D, El Manira A (1998b) Vertebrate locomotion - A lamprey perspective. Ann. N. Y. Acad. Sci. pp 1–18

Grillner S, Rossignol S (1978) On the initiation of the swing phase of locomotion in chronic spinal cats. Brain Res 146:269–277. doi: 10.1016/0006-8993(78)90973-3

Grillner S, Wallen P, Wallén P (1985) Central pattern generators for locomotion, with special reference to vertebrates. Annu Rev Neurosci 8:233–261. doi: 10.1146/annurev.ne.08.030185.001313

Grillner S, Zangger P (1984) The effect of dorsal root transection on the efferent motor pattern in the cat’s hindlimb during locomotion. Acta Physiol Scand 120:393–405. doi: 10.1111/j.1748-1716.1984.tb07400.x

Grottel K, Krutki P, Mrowczynski W (1998) Triple projections of neurones located in s1 and s2 segments of the cat spinal cord to the c6 segment, the cerebellum and the reticular formation. Exp Physiol 83:737–746.

Guertin P, Angel MJ, Perreault MC, McCrea DA (1995) Ankle extensor group I afferents excite extensors throughout the hindlimb during fictive locomotion in the cat. J Physiol 487:197–209.

Guertin PA (2013) Central pattern generator for locomotion: Anatomical, physiological, and pathophysiological considerations. Front Neurol 3:183. doi: 10.3389/fneur.2012.00183

Gurfinkel VS, Levik YS, Kazennikov O V, Selionov VA (1998) Locomotor-like movements evoked by leg muscle vibration in humans. Eur J Neurosci 10:1608–1612. doi: http://dx.doi.org/10.1046/j.1460-9568.1998.00179.x

Haridas C, Zehr EP (2003) Coordinated interlimb compensatory responses to electrical stimulation of cutaneous nerves in the hand and foot during walking. J Neurophysiol 90:2850–61. doi: 10.1152/jn.00531.2003

Harkema SJ (2008) Plasticity of interneuronal networks of the functionally isolated human spinal cord. Brain Res Rev 57:255–264. doi: 10.1016/j.brainresrev.2007.07.012

Harkema SJ, SL H, UK P, et al. (1997) Human lumbosacral spinal cord interprets loading during stepping. J Neurophysiol 77(2):797–811.

Hess F, Van Hedel HJA, Dietz V (2003) Obstacle avoidance during human walking: H-reflex modulation during motor learning. Exp Brain Res 151:82–89. doi: 10.1007/s00221-003-1415-7

Hiebert GW, Whelan P, Prochazka A, Pearson KG (1996) Contribution of hind limb flexor muscle afferents to the timing of phase transitions in the cat step cycle. J Neurophysiol 75:1126–1137.

Hiraoka K (2001) Phase-dependent modulation of the soleus H-reflex during rhythmical arm swing in humans. Electromyogr Clin Neurophysiol 41:43–47.

Hiraoka K, Nagata A (1999) Modulation of the soleus H reflex with different velocities of passive movement of the arm. Electromyogr Clin Neurophysiol 39:21–26.

Hishinuma M, Yamaguchi T (1989) Modulation of reflex responses during fictive locomotion in the forelimb of the cat. Brain Res 482:184–188. doi: 10.1016/0006-8993(89)90559-3

Hooper SL, DiCaprio RA (2004) Crustacean Motor Pattern Generator Networks. NeuroSignals 13:50–69. doi: 10.1159/000076158

Huang HJ, Ferris DP (2004) Neural coupling between upper and lower limbs during recumbent stepping. J Appl Physiol 97:1299–1308. doi: 10.1152/japplphysiol.01350.2003

Hughes GM, Wiersma CAG (1960) The Co-ordination of Swimmeret Movements in the Crayfish, Procambarus Clarkii (Girard). J Exp Biol 37:657.

Hultborn H, Nielsen JB (2007) Spinal control of locomotion - From cat to man. Acta Physiol. pp 111–121

Hundza SR, de Ruiter GC, Klimstra MD, Zehr EP (2012) Effect of afferent feedback and central motor commands on Soleus H-reflex suppression during arm cycling. J Neurophysiol 3049–3058. doi: 10.1152/jn.00485.2011

Hundza SR, Zehr EP (2006) Cutaneous reflexes during rhythmic arm cycling are insensitive to asymmetrical changes in crank length. Exp Brain Res 168:165–77. doi: 10.1007/s00221-005-0089-8

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

Ianniruberto A, Tajani E (1981) Ultrasonographic study of fetal movements. Semin Perinatol 5:175–181.

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

Jahn K, Deutschl??nder A, Stephan T, et al. (2008) Imaging human supraspinal locomotor centers in brainstem and cerebellum. Neuroimage 39:786–792. doi: 10.1016/j.neuroimage.2007.09.047

Jankowska E, Jukes MG, Lund S, Lundberg A (1967) The effect of DOPA on the spinal cord. 6. Half-centre organization of interneurones transmitting effects from the flexor reflex afferents. Acta Physiol Scand 70:389–402. doi: 10.1111/j.1748-1716.1967.tb03637.x

Jordan LM, Liu J, Hedlund PB, et al. (2008) Descending command systems for the initiation of locomotion in mammals. Brain Res Rev 57:183–191. doi: 10.1016/j.brainresrev.2007.07.019

Juvin L, Le Gal J-P, Simmers J, Morin D (2012) Cervicolumbar coordination in mammalian quadrupedal locomotion: role of spinal thoracic circuitry and limb sensory inputs. J Neurosci 32:953–965. doi: 10.1523/JNEUROSCI.4640-11.2012

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

de Kam D, Rijken H, Manintveld T, et al. (2013) Arm movements can increase leg muscle activity during submaximal recumbent stepping in neurologically intact individuals. J Appl Physiol 115:34–42. doi: 10.1152/japplphysiol.00510.2012

Kawashima N, Nozaki D, Abe MO, Nakazawa K (2008) Shaping appropriate locomotive motor output through interlimb neural pathway within spinal cord in humans. J Neurophysiol. doi:

10.1152/jn.00020.2008

Khater-Boidin J, Duron B (1991) Postnatal development of descending motor pathways studied in man by percutaneous stimulation of the motor cortex and the spinal cord. Int J Neurosci 9:15–26.

Kiehn O, Butt SJB (2003) Physiological, anatomical and genetic identification of CPG neurons in the developing mammalian spinal cord. Prog Neurobiol 70:347–361. doi: 10.1016/S0301-0082(03)00091-1

Kiehn O, Kjaerulff O (1998) Distribution of central pattern generators for rhythmic motor outputs in the spinal cord of limbed vertebrates. Ann N Y Acad Sci 16:110–129.

Klarner T, Barss T, Sun Y, et al. (2014) Preservation of common rhythmic locomotor control despite weakened supraspinal regulation after stroke. Front Integr Neurosci 8:1–9.

Klarner T, Barss T, Sun Y, et al. (2016a) Exploiting interlimb arm and leg connections for walking rehabilitation: a training intervention in stroke. Neural Plast 2016:1–19.

Klarner T, Barss T, Sun Y, et al. (2016b) Long term plasticity in reflex excitability after arm and leg cycling training in stroke. Brain Sciences. 6(4)54:1-17; doi:10.3390/brainsci6040054

Klimstra MD, Thomas E, Stoloff RH, et al. (2009) Neuromechanical considerations for incorporating rhythmic arm movement in the rehabilitation of walking. Chaos 19:026102. doi: 10.1063/1.3147404

Komiyama T, Zehr EP, Stein RB (2000) Absence of nerve specificity in human cutaneous reflexes during standing. Exp Brain Res 133:267–72.

Kozuma S, T. O, A. N, et al. (1997) Developmental sequence of human fetal body movements in the second half of pregnancy. Am J Perinatol 14:165–169. doi: 10.1055/s-2007-994120

Kriellaars DJ, Brownstone RM, Noga BR, Jordan LM (1994) Mechanical entrainment of fictive locomotion in the decerebrate cat. J Neurophysiol 71:2074–2086.

LaBella LA, Niechaj A, Rossignol S (1992) Low-threshold, short-latency cutaneous reflexes during fictive locomotion in the “semi-chronic” spinal cat. Exp Brain Res 91:236–248. doi: 10.1007/BF00231657

Lam T, Pearson KG (2001) Proprioceptive modulation of hip flexor activity during the swing phase of locomotion in decerebrate cats. J Neurophysiol 86:1321–1332.

Lam T, Pearson KG (2002) Sartorius muscle afferents influence the amplitude and timing of flexor activity in walking decerebrate cats. Exp Brain Res 147:175–185. doi:

10.1007/s00221-002-1236-0

Lamb T, Yang JF (2000) Could different directions of infant stepping be controlled by the same locomotor central pattern generator? J Neurophysiol 83:2814–2824.

Lamont E V, Zehr EP (2006) Task-specific modulation of cutaneous reflexes expressed at functionally relevant gait cycle phases during level and incline walking and stair climbing. Exp Brain Res 173:185–92. doi: 10.1007/s00221-006-0586-4

Langhorne P, Langhorne P, Coupar F, et al. (2009) Motor recovery after stroke: a systematic review. Lancet Neurol 8:741–54. doi: 10.1016/S1474-4422(09)70150-4de Leon RD, Roy RR, Edgerton VR (2001) Is the recovery of stepping following spinal cord injury mediated by modifying existing neural pathways or by generating new pathways? A perspective. Phys Ther 81:1904–1911.

Li Y, Wang W, Crompton R, Gunther M (2001) Free vertical moments and transverse forces in human walking and their role in relation to arm-swing. J Exp Biol 204:47–58.

Lloyd DPC (1942) Mediation of descending long spinal reflex activity. J Neurophysiol 5:435–458.

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

MacKay-Lyons M (2002) Central pattern generation of locomotion: a review of the evidence. Phys Ther 82:69–83. doi: 10.1152/jn.00056.2009

Maegele M, Müller S, Wernig a, et al. (2002) Recruitment of spinal motor pools during voluntary movements versus stepping after human spinal cord injury. J Neurotrauma 19:1217–29. doi: 10.1089/08977150260338010

Marder E, Calabrese RL (1996) Principles of rhythmic motor pattern generation. Physiol Rev 76:687–717. doi: 10.1007/s13398-014-0173-7.2

Massaad F, Levin O, Meyns P, et al. (2014) Arm sway holds sway: Locomotor-like modulation of leg reflexes when arms swing in alternation. Neuroscience 258:34–46. doi: 10.1016/j.neuroscience.2013.10.007

Matthews PB (1986) Observations on the automatic compensation of reflex gain on varying the pre-existing level of motor discharge in man. J Physiol 374:73–90.

McCrea D a, Shefchyk SJ, Stephens MJ, Pearson KG (1995) Disynaptic group I excitation of synergist ankle extensor motoneurones during fictive locomotion in the cat. J Physiol 487 (Pt 2:527–539.

McCrea DA, Rybak IA (2008) Organization of mammalian locomotor rhythm and pattern generation. Brain Res Rev 57:134–146. doi: 10.1016/j.brainresrev.2007.08.006

McVea DA, Donelan JM, Tachibana A, Pearson KG (2005) A role for hip position in initiating the swing-to-stance transition in walking cats. J Neurophysiol 94:3497–3508. doi: 10.1152/jn.00511.2005

Meyns P, Bruijn SM, Duysens J (2013) The how and why of arm swing during human walking. Gait Posture 38:555–562. doi: 10.1016/j.gaitpost.2013.02.006

Mezzarane R a, Klimstra M, Lewis A, et al. (2011) Interlimb coupling from the arms to legs is differentially specified for populations of motor units comprising the compound H-reflex during “reduced” human locomotion. Exp Brain Res 208:157–68. doi: 10.1007/s00221-010-2467-0

Mezzarane RA, Nakajima T, Zehr EP (2014) After stroke bidirectional modulation of soleus stretch reflex amplitude emerges during rhythmic arm cycling. Front Hum Neurosci 8:136. doi: 10.3389/fnhum.2014.00136

Miller S, Van der Burg J, van der Meché FGA (1975a) Coordination of movements of the hindlimbs and forelimbs in different forms of locomotion in normal and decerebrate cat. Brain Res 91:212–237.

Miller S, Van der Burg J, Van der Meché FGA (1975b) Locomotion in the cat: Basic programmes of movement. Brain Res 91:239–253. doi: 10.1016/0006-8993(75)90545-4

Miller S, Reitsma DJ, van der Meché FG, Meche FG (1973) Functional organization of long ascending propriospinal pathways linking lumbo-sacral and cervical segments in the cat. Brain Res 62:169–188.

Miller S, Ruit JB, van der Meche FG (1977) Reversal of sign of long spinal reflexes dependent on the phase of the step cycle in the high decerebrate cat. Brain Res 128:447–459.

Minassian K, Jilge B, Rattay F, et al. (2004) Stepping-like movements in humans with complete spinal cord injury induced by epidural stimulation of the lumbar cord: electromyographic study of compound muscle action potentials. Spinal Cord 401–416.

Misiaszek JE, Cheng J, Brooke JD, Staines WR (1998) Movement-induced modulation of soleus H reflexes with altered length of biarticular muscles. Brain Res 795:25–36. doi:

10.1016/S0006-8993(98)00246-7

Moseley AM, Stark A, Cameron ID, Pollock A (2003) Treadmill Training and Body Weight Support for Walking after Stroke. Stroke 34:3006. doi: 10.1161/01.STR.0000102415.43108.66

Nadeau S, Jacquemin G, Fournier C, et al. (2010) Spontaneous motor rhythms of the back and legs in a patient with a complete spinal cord transection. Neurorehabil Neural Repair 24:377–387.

Nakajima T, Kitamura T, Kamibayashi K, et al. (2011) Robotic-assisted stepping modulates monosynaptic reflexes in forearm muscles in the human. J Neurophysiol 106:1679–87. doi: 10.1152/jn.01049.2010

Nakajima T, Mezzarane RA, Klarner T, et al. (2013) Neural Mechanisms Influencing Interlimb Coordination during Locomotion in Humans: Presynaptic Modulation of Forearm H-Reflexes during Leg Cycling. PLoS One. doi: 10.1371/journal.pone.0076313

Nathan PW, Smith M, Deacon P (1996) Vestibulospinal, reticulospinal and descending propriospinal nerve fibres in man. Brain 119:1809–1833. doi: 10.1093/brain/119.6.1809

Nielsen JB (2003) How we walk: central control of muscle activity during human walking. Neurosci 9:195–204. doi: 10.1177/1073858403251978

Orsal D, Cabelguen JM, Perret C (1990) Interlimb coordination during fictive locomotion in the thalamic cat. Exp Brain Res 82:536–546. doi: 10.1007/BF00228795

Pang MY, Yang JF (2000) The initiation of the swing phase in human infant stepping: importance of hip position and leg loading. J Physiol 528 Pt 2:389–404. doi: 10.1111/j.1469-7793.2000.00389.x

Pang MYC, Yang JF (2001) Interlimb co-ordination in human infant stepping. J Physiol 533:617–625. doi: 10.1111/j.1469-7793.2001.0617a.x

Pang MYC, Yang JF, MY P, JF Y (2002) Sensory gating for the initiation of the swing phase in different directions of human infant stepping. J Neurosci 22:5734–5740. doi: 20026544

Park J (2008) Synthesis of natural arm swing motion in human bipedal walking. J Biomech 41:1417–1426. doi: 10.1016/j.jbiomech.2008.02.031

Pearson KG (1993) Common principles of motor control in vertebrates and invertebrates. Annu Rev Neurosci 16:265–297. doi: 10.1146/annurev.neuro.16.1.265

Pearson KG (2004) Generating the walking gait: Role of sensory feedback. Prog Brain Res

143:123–129. doi: 10.1016/S0079-6123(03)43012-4

Pearson KG (1995) Proprioceptive regulation of locomotion. Curr Opin Neurobiol 5:786–791. doi: 10.1016/0959-4388(95)80107-3

Perreault MC, Angel MJ, Guertin P, McCrea DA (1995) Effects of stimulation of hindlimb flexor group II afferents during fictive locomotion in the cat. J Physiol 487 (Pt 1:211–20.

Petersen TH, Willerslev-Olsen M, Conway B a, Nielsen JB (2012) The motor cortex drives the muscles during walking in human subjects. J Physiol 590:2443–2452. doi: 10.1113/jphysiol.2012.227397

Pontzer H, Holloway JH, Raichlen D a, Lieberman DE (2009) Control and function of arm swing in human walking and running. J Exp Biol 212:523–534. doi: 10.1242/jeb.030478

Quevedo J, Fedirchuk B, Gosgnach S, McCrea D a (2000) Group I disynaptic excitation of cat hindlimb flexor and bifunctional motoneurones during fictive locomotion. J Physiol 525 Pt 2:549–564. doi: 10.1111/j.1469-7793.2000.t01-1-00549.x

Quevedo J, Stecina K, McCrea DA (2005) Intracellular analysis of reflex pathways underlying the stumbling corrective reaction during fictive locomotion in the cat. J Neurophysiol 94:2053–62. doi: 10.1152/jn.00176.2005

Le Ray D, Juvin L, Ryczko D, Dubuc R (2011) Supraspinal control of locomotion. Prog. Brain Res. pp 51–70

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

Rybak IA, Shevtsova NA, Lafreniere-Roula M, McCrea DA (2006) Modelling spinal circuitry involved in locomotor pattern generation: Insights from deletions during fictive locomotion. J Physiol 577:617-39

Sakamoto M, Tazoe T, Nakajima T, et al. (2007) Voluntary changes in leg cadence modulate arm cadence during simultaneous arm and leg cycling. Exp Brain Res 176:188–192. doi: 10.1007/s00221-006-0742-x

Sarica Y, Ertekin C (1985) Descending lumbosacral cord potentials (DLCP) evoked by stimulation of the median nerve. Brain Res 325:299–301. doi: 10.1016/0006-8993(85)90327-0

Sasada S, Tazoe T, Nakajima T, et al. (2010) Effects of leg pedaling on early latency cutaneous reflexes in upper limb muscles. J Neurophysiol 104:210–7. doi: 10.1152/jn.00774.2009

Schomburg ED, Behrends HB (1978) hasic control of the transmission in the excitatory and inhibitory reflex pathways from cutaneous afferents to alpha-motoneurones during fictive locomotion in cats. Neurosci Lett 8:277–282. doi: 0304-3940(78)90136-2 [pii]

Schomburg ED, Meinck HM, Haustein J, Roesler J (1978) Functional organization of the spinal reflex pathways from forelimb afferents to hindlimb motoneurones in the cat. Brain Res 139:21–33.

Schomburg ED, Petersen N, Barajon I, Hultborn H (1998) Flexor reflex afferents reset the step cycle during fictive locomotion in the cat. Exp Brain Res 122:339–350. doi: 10.1007/s002210050522

Seki K, Yamaguchi T (1997) Cutaneous reflex activity of the cat forelimb during fictive locomotion. Brain Res 753:56–62. doi: S0006-8993(96)01486-2 [pii]

Selionov V a, Ivanenko YP, Solopova I a, Gurfinkel VS (2009) Tonic central and sensory stimuli facilitate involuntary air-stepping in humans. J Neurophysiol 101:2847–58. doi: 10.1152/jn.90895.2008

Selionov VA, Solopova I, Zhvansky DS (2016) Activation of interlimb interactions increases the motor output in legs of healthy subjects: Study under the conditions of arm and leg unloading. Hum Physiol 42:43–53.

Senthilvelkumar T, Magimairaj H, Fletcher J, et al. (2015) Comparison of body weight-supported treadmill training versus body weight-supported overground training in people with incomplete tetraplegia: a pilot randomized trial. Clin Rehabil 29:42–49.

De Serres SJ, Yang JF, Patrick SK (1995) Mechanism for reflex reversal during walking in human tibialis anterior muscle revealed by single motor unit recording. J Physiol 488:249–258.

Sherrington CS (1906) Observations on the scratch-reflex in the spinal dog. J Physiol 34:1–50.

Sherrington CS (1910) Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. J Physiol 28–121.

Shik M, Orlovskii G, Severin F (1966a) Organization of locomotor synergism. Biofizika 11:879–886.

Shik M, Orlovskii G, Severin F (1968) Locomotion of the mesencephalic cat elicited by stimulation of the pyramids. Biophysics (Oxf) 13:143–152.

Shik ML, Severin F V., Orlovskii GN (1966b) Control of walking and running by means of electrical stimulation of the mid-brain. Biophysics (Oxf) 11:756–765.

Skinner RD, Garcia-Rill E (1984) The mesencephalic locomotor region (MLR) in the rat. Brain Res 323:385–389. doi: 10.1016/0006-8993(84)90319-6

Skinner RDD, Adams RJJ, Remmel RSS (1980) Responses of long descending propriospinal neurons to natural and electrical types of stimuli in cat. Brain Res 196:387–403. doi: 10.1016/0006-8993(80)90403-5

Solopova IA, Selionov VA, Sylos-Labini F, et al. (2015) Tapping into rhythm generation circuitry in humans during simulated weightlessness conditions. Front Syst Neurosci 9:14. doi: 10.3389/fnsys.2015.00014

Stecina K, Quevedo J, McCrea DA (2005) Parallel reflex pathways from flexor muscle afferents evoking resetting and flexion enhancement during fictive locomotion and scratch in the cat. J Physiol 569:275–90. doi: 10.1113/jphysiol.2005.095505

Stein RB, Capaday C (1988) The modulation of human reflexes during functional motor tasks. Trends Neurosci 11:328–332. doi: 10.1016/0166-2236(88)90097-5

Stephens MJ, Yang JF (1999) Loading during the stance phase of walking in humans increases the extensor EMG amplitude but does not change the duration of the step cycle. Exp Brain Res 124:363–370. doi: 10.1007/s002210050633

Swinnen S, Duysens J (2005) Neuro-Behavioral Determinants of Interlimb Coordination A Multidisciplinary Approach. Springer US. doi: 10.1007/978-1-4419-9056-3

Sylos-Labini F, Ivanenko YP, MacLellan MJ, et al. (2014) Locomotor-like leg movements evoked by rhythmic arm movements in humans. PLoS One. doi: 10.1371/journal.pone.0090775

Tax a a, Van Wezel BM, Dietz V, et al. (1995) Bipedal reflex coordination to tactile stimulation of the sural nerve during human running. J Neurophysiol 73:1947–1964.

Taylor LL, Sickles AE, Stehouwer DJ, Van Hartesveldt C (1994) Noradrenergic ??-1 and ??-2 antagonists block l-DOPA-induced air-stepping in neonatal rats. Dev Brain Res 79:242–248. doi: 10.1016/0165-3806(94)90128-7

Umberger BR (2008) Effects of suppressing arm swing on kinematics, kinetics, and energetics of human walking. J Biomech 41:2575–2580. doi: 10.1016/j.jbiomech.2008.05.024

Verschueren SM, Swinnen SP, Desloovere K, Duysens J (2002) Effects of tendon vibration on the spatiotemporal characteristics of human locomotion. Exp Brain Res 143:231–239. doi: 10.1007/s00221-001-0987-3

Visintin M, Barbeau H (1994) The effects of parallel bars, body weight support and speed on the modulation of the locomotor pattern of spastic paretic gait. A preliminary communication. Paraplegia 32:540–553.

Wagenaar RC, Van Emmerik REA (2000) Resonant frequencies of arms and legs identify different walking patterns. J Biomech 33:853–861. doi: 10.1016/S0021-9290(00)00020-8

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

Van Wezel BMH, Ottenhoff FAM, Duysens J (1997) Dynamic Control of Location-Specific Information in Tactile Cutaneous Reflexes from the Foot during Human Walking. J Neurosci 17:3804–3814.

Whelan PJ (1996) Control of locomotion in the decerebrate cat. Prog Neurobiol 49:481–515. doi: 10.1016/0301-0082(96)00028-7

Whelan PJ, Pearson KG (1997) Plasticity in reflex pathways controlling stepping in the cat. J Neurophysiol 78:1643–1650.

Winchester P, McColl R, Querry R, et al. (2005) Changes in supraspinal activation patterns following robotic locomotor therapy in motor-incomplete spinal cord injury. NeurorehabilNeural Repair 19:313–324. doi: 10.1177/1545968305281515

Wirz M, Colombo G, Dietz V (2001) Long term effects of locomotor training in spinal humans. J Neurol Neurosurg Psychiatry 71:93–96. doi: 10.1136/jnnp.71.1.93

Yamaguchi T (1992) Muscle activity during forelimb stepping in decerebrate cats. Jpn J Physiol 42:489–499.

Yang JF (2006) Spinal and Brain Control of Human Walking: Implications for Retraining of Walking. Neurosci 12:379–389. doi: 10.1177/1073858406292151

Yang JF, Lam T, Pang MYC, et al. (2004) Infant stepping: a window to the behaviour of the human pattern generator for walking. Can J Physiol Pharmacol 82:662–674.

Yang JF, Stein RB (1990) Phase-dependent reflex reversal in human leg muscles during walking. J Neurophysiol 63:1109–1117.

Yang JF, Stephens MJ, Vishram R (1998) Infant stepping: a method to study the sensory control of human walking. J Physiol 507:927–937. doi: 10.1111/j.1469-7793.1998.927bs.x

Yang JF, Whelan PJ (1993) Neural mechanisms that contribute to cyclical modulation of the soleus H-reflex in walking in humans. Exp Brain Res 95:547–556. doi: 10.1007/BF00227148

Zehr E, Barss T, Dragert K, et al. (2016) Neuromechanical interactions between the limbs during human locomotion: an evolutionary perspective with translation to rehabilitation. Exp Brain Res. doi: 10.1007/s00221-016-4715-4

Zehr EP, Fujita K, Stein RB (1998) Reflexes from the superficial peroneal nerve during walking in stroke subjects. J Neurophysiol 79:848–58.

Zehr EP (2006) Training-induced adaptive plasticity in human somatosensory reflex pathways. J Appl Physiol 101:1783–94. doi: 10.1152/japplphysiol.00540.2006

Zehr EP (2005) Neural control of rhythmic human movement: the common core hypothesis. Exerc Sport Sci Rev 33:54–60.

Zehr EP, Balter JE, Ferris DP, et al. (2007a) Neural regulation of rhythmic arm and leg movement is conserved across human locomotor tasks. J Physiol 582:209–27. doi: 10.1113/jphysiol.2007.133843

Zehr EP, Carroll TJ, Chua R, et al. (2004a) Possible contributions of CPG activity to the control of rhythmic human arm movement. Can J Physiol Pharmacol 82:556–68. doi: 10.1139/y04-056

Zehr EP, Chua R (2000) Modulation of human cutaneous reflexes during rhythmic cyclical arm movement. Exp Brain Res 135:241–250. doi: 10.1007/s002210000515

Zehr EP, Collins DF, Chua R (2001) Human interlimb reflexes evoked by electrical stimulation of cutaneous nerves innervating the hand and foot. Exp Brain Res 140:495–504. doi: 10.1007/s002210100857

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

Zehr EP, Duysens J (2004) Regulation of arm and leg movement during human locomotion. Neuroscientist 10:347–61. doi: 10.1177/1073858404264680

Zehr EP, Frigon A, Hoogenboom N, Collins DF (2004b) Facilitation of soleus H-reflex amplitude evoked by cutaneous nerve stimulation at the wrist is not suppressed by rhythmic arm movement. Exp Brain Res 159:382–8. doi: 10.1007/s00221-004-2092-x

Zehr EP, Haridas C, Zehr EP, et al. (2003b) Modulation of cutaneous reflexes in arm muscles during walking: further evidence of similar control mechanisms for rhythmic human arm and leg movements. Exp Brain Res 149:260–6. doi: 10.1007/s00221-003-1377-9

Zehr EP, Hundza SR, Balter JE, Loadman PM (2009a) Context-dependent modulation of cutaneous reflex amplitudes during forward and backward leg cycling. Motor Control 13:368–386.

Zehr EP, Hundza SR, Vasudevan E V (2009b) The quadrupedal nature of human bipedal locomotion. Exerc Sport Sci Rev 37:102–8. doi: 10.1097/JES.0b013e31819c2ed6

Zehr EP, Hundza SRS (2005) Forward and backward arm cycling are regulated by equivalent neural mechanisms. J Neurophysiol 93:633–40. doi: 10.1152/jn.00525.2004

Zehr EP, Kido a (2001) Neural control of rhythmic, cyclical human arm movement: task dependency, nerve specificity and phase modulation of cutaneous reflexes. J Physiol 537:1033–45. doi: 10.1111/j.1469-7793.2001.01033.x

Zehr EP, Klimstra M, Johnson E a, Carroll TJ (2007b) Rhythmic leg cycling modulates forearm muscle H-reflex amplitude and corticospinal tract excitability. Neurosci Lett 419:10–4. doi: 10.1016/j.neulet.2007.03.045

Zehr EP, Komiyama T, Stein RB (1997) Cutaneous reflexes during human gait: electromyographic and kinematic responses to electrical stimulation. J Neurophysiol 77:3311–25.

Zehr EP, Loadman PM (2012) Persistence of locomotor-related interlimb reflex networks during walking after stroke. Clin Neurophysiol 123:796–807. doi: 10.1016/j.clinph.2011.07.049

Zehr EP, Stein RB (1999) What functions do reflexes serve during human locomotion? Prog Neurobiol 58:185–205.

Chapter 2 Preservation of common rhythmic locomotor control despite weakened supraspinal regulation after stroke[1]

Abstract

The basic pattern of arm and leg movement during rhythmic locomotor tasks is supported by common central neural control from spinal and supraspinal centers in neurologically intact participants. The purpose of this study was to test the hypothesis that following a cerebrovascular accident, shared systems from interlimb cutaneous networks facilitating arm and leg coordination persist across locomotor tasks. Twelve stroke participants (>6 months post CVA) performed arm and leg (A&L) cycling using a stationary ergometer and walking on a motorized treadmill. In both tasks cutaneous reflexes were evoked via surface stimulation of the nerves innervating the dorsum of the hand (superficial radial; SR) and foot (superficial peroneal; SP) of the less affected limbs. Electromyographic (EMG) activity from the tibialis anterior, soleus, flexor carpi radialis, and posterior deltoid were recorded bilaterally with surface electrodes. Full-wave rectified and filtered EMG data were separated into eight equal parts or phases and aligned to begin with maximum knee extension for both walking and A&L cycling. At each phase of movement, background EMG data were quantified as the peak normalized response for each participant and cutaneous reflexes were quantified as the average cumulative reflex over 150 ms following stimulation. In general, background EMG was similar between walking and A&L cycling, seen especially in the distal leg muscles. Cutaneous reflexes were evident and modified in the less and more affected limbs during walking and A&L cycling and similar modulation patterns were observed suggesting activity in related control networks between tasks. After a stroke common neural patterning from conserved subcortical regulation is seen supporting the notion of a common core in locomotor tasks involving arm and leg movement. This has translational implications for rehabilitation where A&L cycling could be usefully applied to improve walking function.

Introduction

Supraspinal input, subcortical mechanisms and sensory feedback interact to coordinate limb movement during rhythmic locomotor tasks (Nielsen 2003; Zehr and Duysens 2004).

---

[1] Klarner T, Barss TS, Sun Y, Kaupp C, Zehr EP (2015) Preservation of common rhythmic locomotor control despite weakened supraspinal regulation after stroke. Front Integr Neurosci. 8:1–9.

Across different forms of rhythmic movement (e.g. swimming, walking, crawling, cycling etc.) similar coordination exists between these nervous system structures where common features of neural control facilitate the interactions between the arm and the legs (Dietz et al., 2001; Haridas and Zehr, 2003; Zehr, 2005). The relative contribution from various levels of control within the nervous system can be teased out with different experimental designs to determine which parts of the nervous system are important for controlling rhythmic movement. For example, volitional muscle activation (e.g. deliberate knee extension) reveals a shift towards strong supraspinal input whereas the same movement during a rhythmic task (e.g. knee extension during swing phase of walking) reveals a shift towards subcortical mechanisms (Zehr et al. 2007). Although different tasks rely more heavily on varying modes of control, all levels of the nervous system are required to fully support movement and are dynamically regulated.

This common nervous system control across rhythmic tasks can be determined by comparing the strength of connections during rhythmic activities probed during reflex studies. In neurologically intact (NI) participants these interactions can be seen in arm and leg muscles following a brief electrical pulse applied to a nerve in the hand or foot to evoke a reflex lasting at least 150ms in the ongoing background electromyographic (EMG) activity. For example, cutaneous reflex amplitudes in arm and leg muscles were modulated in a similar way across tasks of level walking, incline walking, and stair climbing (Lamont and Zehr, 2006). Commonalities in control are also seen across walking, arm and leg (A&L) cycling and arm-assisted recumbent stepping, where similar phase-dependent modulation was observed despite differences in movement kinematics (Zehr et al., 2007). Factor analysis revealed that across these tasks, four principal components explained 93% of variance in background EMG and cutaneous reflex amplitude. Commonalities in cutaneous reflex modulation across different forms of rhythmic arm and leg locomotion reveal common central nervous system control (Zehr et al., 2007).

Given that the arms and the legs are functionally linked during locomotion and are subjected to similar nervous system control across rhythmic tasks, incorporating rhythmic arm movement in the rehabilitation of walking after stroke should be considered (Klimstra et al. 2009). Currently, rehabilitation is commonly provided with body-weight-supported treadmill training. However arm and leg cycling, which is similar to walking in terms of muscle activity, joint ranges of motion, and the neural pathways activated, might potentially strengthen interlimb connections in a similar way to walking (Zehr 2005b; Balter and Zehr 2007). Therefore it would be useful to examine the extent of differences in neural control between A&L cycling and walking that may arise after stroke interrupts “normal” supraspinal regulation.

Following a stroke, decreased supraspinal input leads to alterations in muscle activation levels and patterns in locomotor tasks. Compared to NI participants, changes in burst durations, extent of co-contraction and amplitude modulations are observed during walking (Shiavi et al., 1987; Dimitrijević and Nathan, 1973; Zehr and Loadman, 2012; Burridge et al., 2001; Dimitrijevic and Nathan, 1970). Deficits in the regulation of walking are due to interruption of connectivity between supraspinal and subcortical areas occurring as a result of the stroke lesion.

Despite differences in background EMG activity following stroke compared to NI participants, cutaneous pathways remain accessible and part of the ‘intact’ regulation of sensory input still exits. For example, part of the stumble correction response, where stimulation to the top of the foot during the swing phase causes biceps femoris activation and tibialis anterior inhibition, normally observed in NI participants, was preserved in stroke participants (Zehr et al., 1998a). Interlimb connections have also been identified in stroke participants where cutaneous input can access reflex pathways in all four limbs, including the more affected (MA) limb, during rhythmic movement (Zehr and Loadman, 2012; Zehr et al., 2012). Interlimb reflexes were significantly phase-modulated and the depth of modulation for cutaneous reflexes was similar between stroke and NI participants (Zehr and Loadman 2012).

The extent to which common neural regulation from supraspinal and spinal centers is conserved between locomotor tasks after stroke however, remains uncertain. Thus, the purpose of this study was to test the hypothesis that with decreased supraspinal input in chronic stroke, shared reflex systems from cutaneous networks remain viable and accessible across locomotor tasks. Since rhythmic arm and leg cycling and walking rely on contribution from subcortical circuits (Carroll et al., 2006), it is hypothesized that there will be partial preservation of patterns of reflex modulation between the two tasks despite reduced supraspinal input after stroke.

Background EMG and reflex modulation serve as proxies for the commonalities in neural function and a difference in these variables between tasks will be determined. The evoked responses for each participant were analyzed for the net reflex effect with the use of the average cumulative reflex EMG after 150ms. This technique was employed because the major focus in this study is to determine the effect that reduced supraspinal regulation has on spinal cord and brainstem locomotor control centres (Komiyama et al., 2000; Zehr et al., 1998b). To probe arm and leg interactions, combined arm and leg stimulation was used as an index for arm and leg coupling where stimulation likely converges in shared reflex pathways (Nakajima et al., 2013).

Material and Methods

Participants

Twelve chronic stroke participants (≥six months post infarct), between 58 and 80 years old, participated with written informed consent in a protocol approved by the Human Research Ethics Board at the University of Victoria.

Experimental Protocol

To examine similarities in rhythmic locomotor tasks, participants performed two tasks: 1) level walking on a motorized treadmill belt with 0% body weight support (Woodway Desmo M, Waukesha, WI, USA) and 2) seated arm and leg (A&L) cycling using a coupled arm and leg cycle ergometer (SciFit Pro II, Tulsa, Oklahoma, USA). Participants were instructed to maintain A&L cycling at 1Hz and maintain walking at their self-selected walking speed.

Electromyography

Electromyographic (EMG) recordings were made from tibialis anterior (TA), soleus (Sol), posterior deltoid (PD), and flexor carpi radialis (FCR) from both the more (contralateral; MA) and less affected (ipsilateral; LA) limbs. Skin was cleaned with alcohol and 1cm surface EMG electrodes (Thought Technologies Ltd.) were applied in a bipolar configuration using a 2cm inter-electrode distance over the muscles of interest. Grounding electrodes were placed over the patella and medial epicondyle of the elbow. EMG signals were pre-amplified 5000x and band-pass filtered at 100-300Hz (P511 Grass Instrument, AstroMed, Inc.). Data were sampled at

1000Hz (A/D converter; National Instrument, Austin, TX), and stored to a computer for off-line analysis.

Nerve Stimulation

In both tasks cutaneous reflexes were evoked via simultaneous stimulation of the nerves innervating the dorsum of the hand (superficial radial; SR) and foot (superficial peroneal; SP). Electrodes for SR nerve stimulation were placed just proximal to the radial head and for SP nerve stimulation on the ankle of the LA limbs. Appropriate stimulation location was checked by ensuring that radiating paresthesia was evoked into the appropriate cutaneous innervation areas of the SR and SP nerves. Cutaneous reflexes were applied with trains of 5 x 1.0 ms pulses at 300Hz of isolated constant current stimulation (Grass S88 stimulator with SIU5 stimulus isolation and a CCU1 constant current unit AstroMed-Grass Inc., Canada). Stimulus intensity was set as multiples of the threshold for radiating paraesthesia (RT) at 2.2 x RT for the SR nerve, and 2.0 x RT for the SP nerve. Non-noxious stimulation intensities were found for each participant to ensure non-nociceptive pathways were stimulated. During both tasks, 120 stimulations were delivered pseudo-randomly with an inter-stimulus interval of 1-5 seconds.

Movement Timing

Timing events for arm and leg cycling were determined with custom-made optical encoders detecting position of the right arm crank throughout the movement cycle. Data were divided into cycles and aligned to begin with right arm top dead center. Walking cycle parameters (i.e. heel contact, toe-off) were obtained with the use of custom-made force sensors, located in the insole, and walking phases were divided to begin with LA heel strike.

For comparison of A&L cycling and walking, data were aligned to begin with maximum knee extension. A schematic diagram relating the phases of arm and leg movements for the tasks are shown in Figure 2-1 using the same functional phases as before (adapted from Zehr et al., 2007). Eight equally divided phases are shown at the top and functional locomotor phases are compared below.

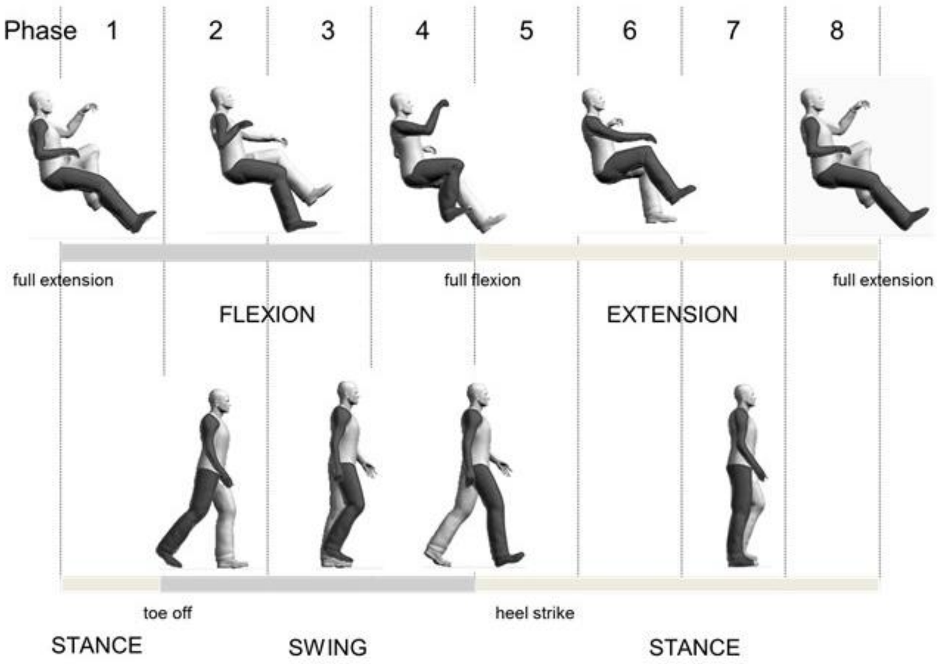


Figure 2-1: Overall schematic diagram for relating arm and leg cycling to walking.

Data Analysis

EMG data were analyzed for background amplitudes and reflexes using custom-written software programs (MATLAB, The Mathworks, Inc., Natick, MA). Background EMG was obtained from steps without stimulation and was determined as the average response within a phase normalized to the peak response for each task for each participant. Stimuli were then aligned to delivery within eight phases and within each phase, data were full-wave rectified, filtered, and averaged together The average trace from the non-stimulated data was subtracted from the average trace of the stimulated data to produce a subtracted EMG ‘reflex’ trace within each phase.. The stimulus artifact was removed from the subtracted reflex trace and data were then low-pass filtered at 30 Hz using a dual-pass, fourth order Butterworth filter.

Cutaneous reflexes were quantified as the average cumulative reflex over 150ms following stimulation. This value is determined as the integral obtained at 150ms divided by the time interval of integration to yield the overall reflex effect. If the value is positive, overall facilitation has occurred, if the value is negative, overall inhibition has occurred (Komiyama et al., 2000; Zehr et al., 1998b). This quantification method allows for interpretation of modulation of reflex pathways from spinal, brainstem and supraspinal centers where transcortical pathways have time to access and modify output from motoneurons during rhythmic activities and precedes any significant voluntary activation (Zehr et al., 1997). These values were normalized to the peak background EMG for each task for each participant.

Mathematical Analysis

To examine basic patterns in neural control, a principal components analysis (PCA) was performed on background EMG and reflex data separately for all arm and leg muscles, recorded during A&L cycling and walking (Zehr et al., 2007) (MATLAB princomp function). From an 8 x 8 correlation matrix, showing linear dependence between muscles, eigenvalues were determined first. To increase loading on each principal component, an orthogonal varimax rotation of the eigenvalues was performed which grouped variables with similar activity together (Ivanenko et al., 2004). The percentage of the total variance explained by each principal component was simultaneously calculated (MATLAB pcacov function).

Statistics

To compare between tasks, repeated measures analysis of variance (rmANOVA) was preformed separately for the variables of background EMG and net cutaneous reflex was used to determine significant differences (SPSS 18.0, Chicago, IL). The observed effect of each significant difference is also reported as the Cohen's effect size (d) where a small effect is $d \geq 0.2$, a medium effect is $d \geq 0.5$ and a large effect is $d \geq 0.8$ (Cohen, 2013). Cohen's d is useful for determining if any failure to observe significant differences was due to small sample sizes. Analyses were performed using the averaged normalized values for each subject. Using rmANOVA, differences in the pattern of response would be detected as a task-phase interaction indicating a difference in timing of peaks across phases between the two tasks. General amplitude differences in background EMG or net cutaneous reflex between tasks would be detected as a significant main effect of task. Any differences seen across phases, indicating phase dependent modulation of background EMG and net cutaneous reflex, would be seen as a significant main effect of phase. Taking a conservative approach and to examine all possible statistical differences, significant interaction and main effects tests were examined with paired samples t-tests to determine phase specific differences between tasks. Statistical significance was set at $p \leq 0.05$.

Results

Background EMG

Background EMG patterns for the Sol, TA, FCR and PD of the MA and LA limbs during SR and SP nerve stimulation for both A&L cycling and walking are shown as bar plots in Figure 2-2. Values for A&L cycling (black bars) and walking (gray bars) are normalized and expressed as percentages of the peak response for each task for each participant. Due to the varying capabilities of each stroke participant walking was maintained at 0.76 Hz and A&L cycling was maintained at 0.89 Hz and no significant differences (p=0.549) in frequency were found between tasks. This allows for comparisons between tasks without the confounding effects of movement frequency and to match movement parameters in Zehr et al., 2007.

In the legs, there were differences in the pattern of background amplitude as differences in timing of the peaks, as indicated by a task-phase interaction for the LA Sol and LA TA ($F_{(7,70)} = 4.951$, $p < 0.000$, $d = 0.994$ and $F_{(7,70)} = 9.211$, $p < 0.000$, $d = 0.999$ respectively). There was significant phase-dependent modulation for both tasks in LA TA (main effect of phase ($F_{(7,70)} = 7.519$, $p < 0.000$, $d = 0.997$)). In LA Sol and LA TA there was also a main effect of task ($F_{(1,10)} = 5.779$, $p = 0.037$, $d = 0.583$ and $F_{(1,10)} = 15.456$, $p = 0.003$, $d = 0.942$ respectively). Some significant post-hoc differences, between A&L cycling and walking, were observed for LA Sol and LA TA and there were no significant differences for MA Sol and MA TA (see * in Figure 2-2). The small number of differences can be better appreciated by considering the number of phases in which significant differences could have been observed, which is 32 [equal to the number of phases (8) x number of muscle recorded (4)]. In this context, there were 9 differences out of 32 for SR+SP stimulation trials. These few statistically significant differences between tasks indicate that the extent of background EMG amplitude modulation was similar across tasks.

In the arms, there were few differences in the pattern of background amplitude as differences in timing of the peaks (task-phase interaction for only the MA FCR ($F_{(7,70)} = 4.036$, $p = 0.001$, $d = 0.977$)). There was significant phase-dependent modulation for both tasks in the MA FCR and LA FCR seen as a significant main effect of phase ($F_{(7,70)} = 3.507$, $p = 0.003$, $d = 0.954$ and $F_{(7,70)} = 3.616$, $p = 0.002$, $d = 0.958$ respectively). Statistically significant differences between tasks were found in the MA FCR ($F_{(1,10)}$ = 13.941, p = 0.004, d = 0.919), LA FCR ($F_{(1,10)}$ = 6.909, p = 0.027, d = 0.649), and MA PD ($F_{(1,10)}$ = 7.382, p = 0.022, d = 0.688) but only a few significant post hoc differences were apparent between A&L cycling and walking for the MA FCR and LA FCR (see * in Figure 2-2). When the number of phases with significant differences is considered, as described for the arm muscles above, there were 3 differences out of 32 for SR+SP stimulation trials.

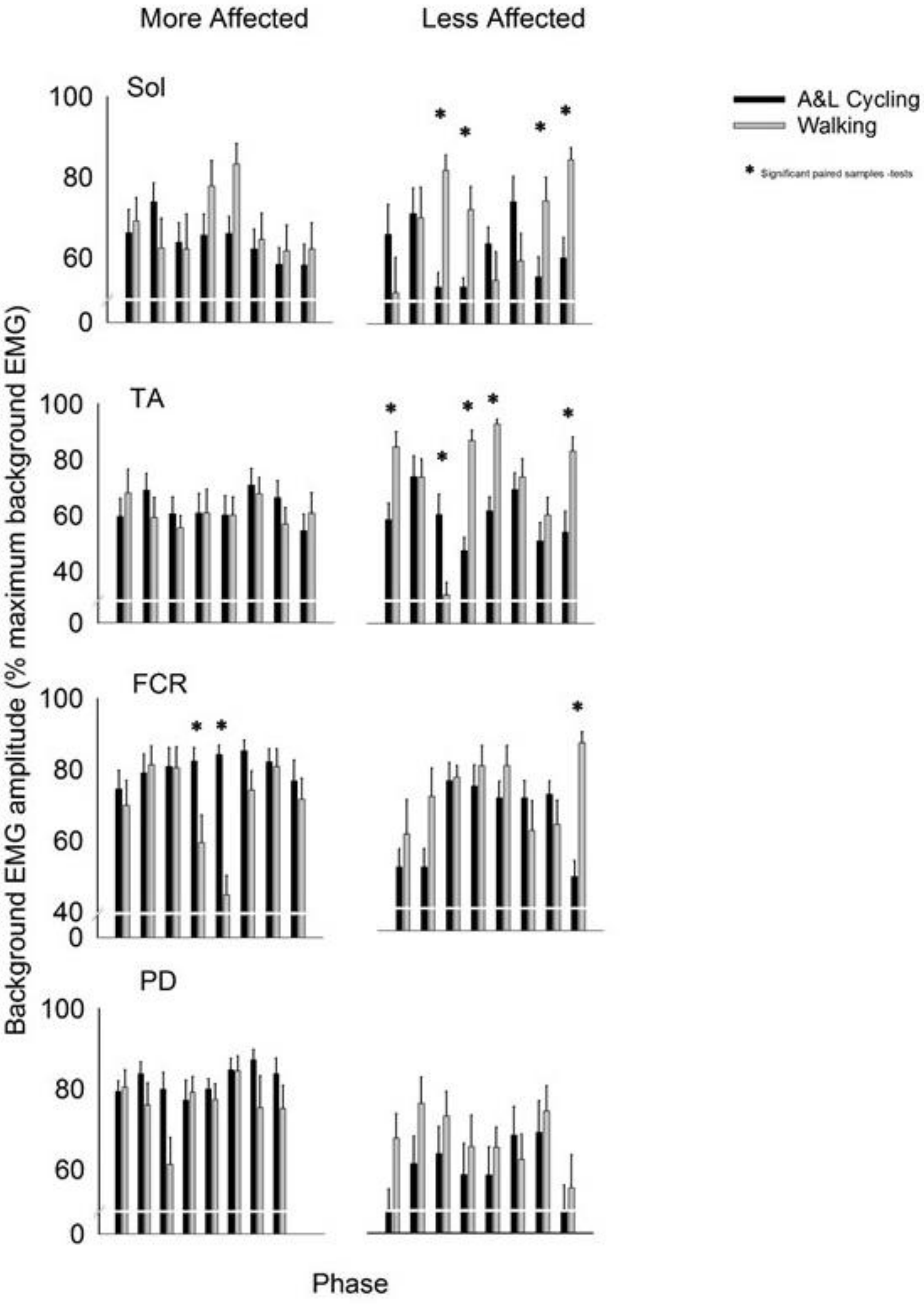


Figure 2-2: Background EMG amplitudes for muscles of the more and less affected arm and leg averaged across all participants. Black bars are for A&L cycling and gray bars are for walking tasks. The EMG amplitudes are means (± s.e.m) from all participants and are normalized to the peak control (i.e. background) EMG recorded in each task. Significant difference between tasks (*) were calculated with a paired samples t-test. Abbreviations are: Sol, soleus; TA, tibialis anterior; FCR, flexor carpi radialis; and PD, posterior deltoid.

Reflex Modulation

Figure 2-3 shows subtracted EMG traces for A&L cycling (black line) and walking (gray line) for MA TA taken from one participant during SP+SR nerve stimulation. The figure displays subtracted EMG traces for each phase moving top to bottom from flexion to extension. To the right of the subtracted traces control EMG for A&L cycling (black line) and walking (gray line) is plotted vertically. Data in this figure visually illustrates similarities for cutaneous reflexes between A&L cycling and walking across 8 phases of movement.

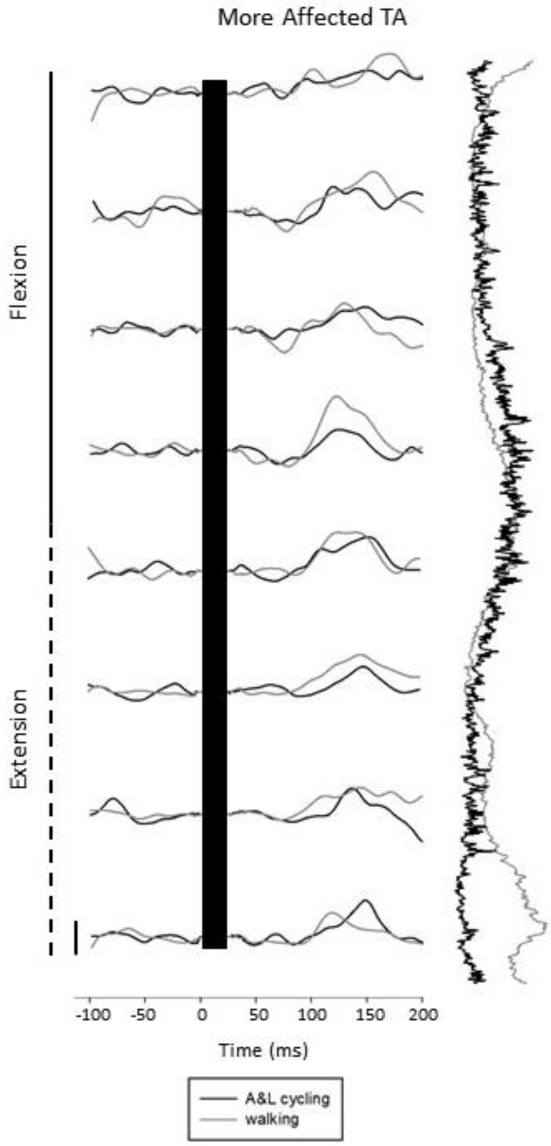


Figure 2-3: Subtracted electromyographic (EMG) traces of the more affected tibialis anterior (TA) from a representative participant evoked by superficial radial and superficial peroneal nerve stimulation during A&L cycling and walking. The stimulus artifact has been removed from each trace and replaced by a black bar extending from time 0 out to ~30 ms post stimulus. Background EMG during A&L cycling and walking is shown to the right of the trace plotted vertically. Calibration bar represents 10 µV.

General conservation in the pattern of reflexes between tasks can be seen in Figure 2-4 where grand average reflex traces from SP+SR nerve stimulation during A&L cycling (black line) and walking (gray line) are plotted. Although some expected differences in amplitude were observed, general patterns of modulation (i.e. sign of response) are similar. Between tasks facilitation was seen bilaterally in the arms and seen in the MA leg while in the LA leg suppression was observed.

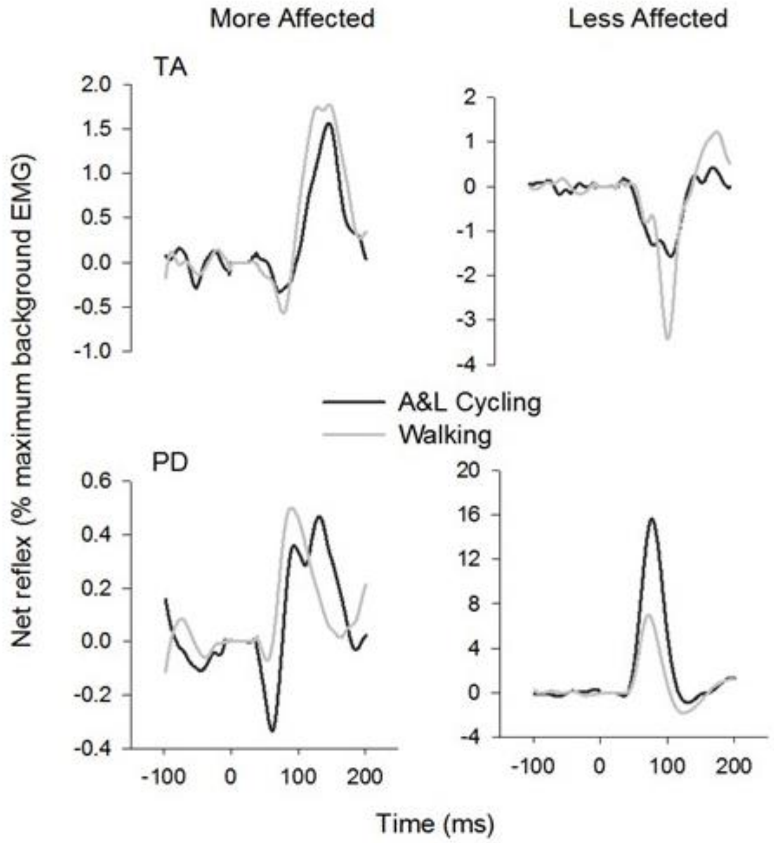


Figure 2-4: Ensemble grand average subtracted reflex traces from all phases and all subjects of A&L cycling and walking. There is a similar pattern of cutaneous reflex modulation across tasks. Note that despite some changes in amplitudes, the general pattern is conserved.

Net reflexes evoked in the legs and arms following SP+SR nerve stimulation for all participants are plotted as bars in Figure 2-5. Values for A&L cycling (black bars) and walking (gray bars) are normalized and expressed as percentages of the peak background value for each phase for each participant. In the legs, there were no significant main effects of phase or task for any muscle and there were no interaction effects indicating that the pattern and amplitude of reflexes was similar between A&L cycling and walking. In the arms, there was a significant main effect of task in the MA PD and LA PD ($F_{(1,10)} = 7.267$, $p = 0.022$, $d = 0.781$ and $F_{(1,10)} = 17.780$, $p = 0.002$, $d = 0.966$). While no significant differences in MA PD were detected by paired t-tests, there were significant differences for the LA PD between A&L cycling and walking across phases (see * in Figure 2-5).

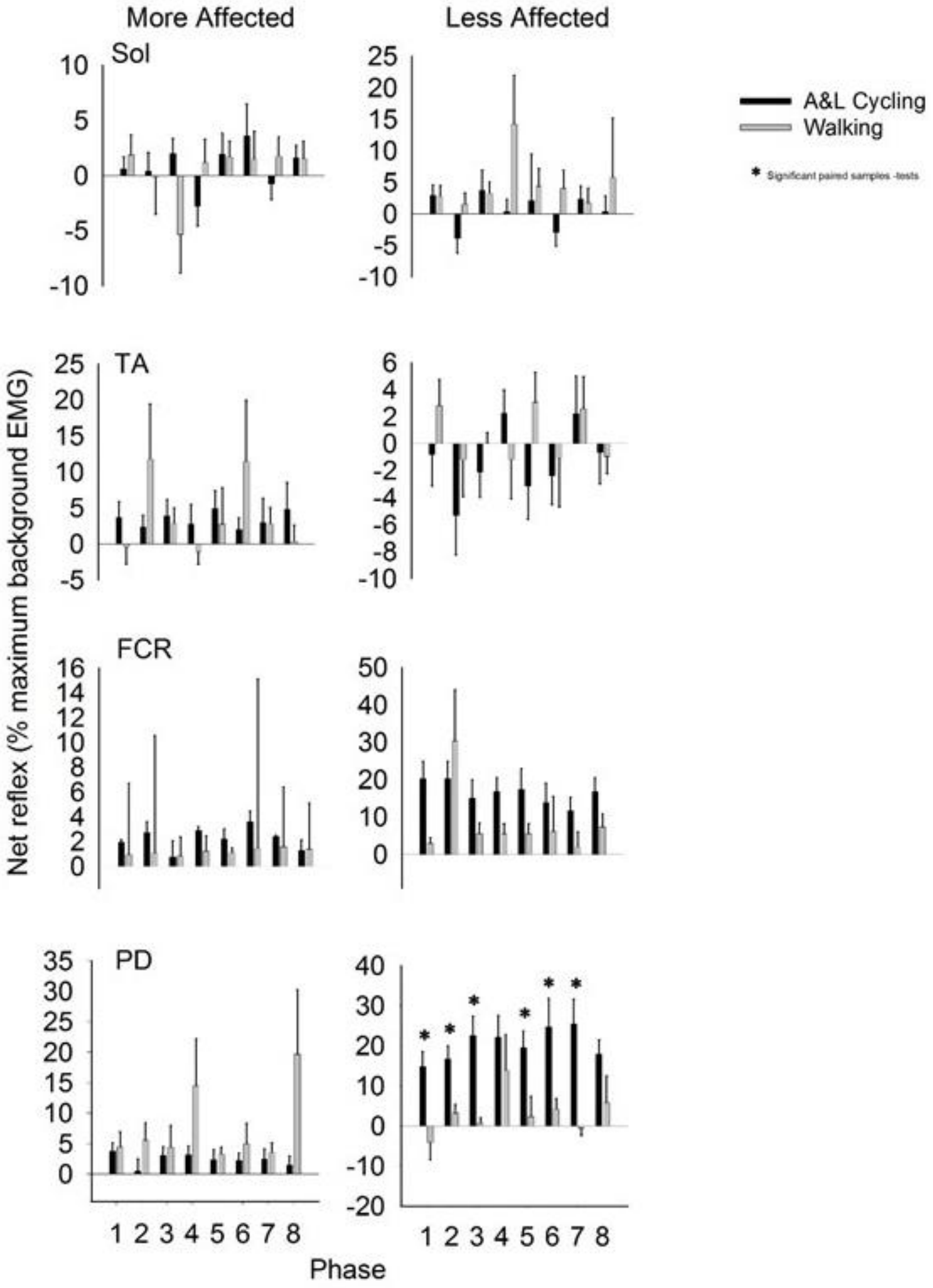


Figure 2-5: Net cutaneous reflex from SP+SR stimulation for muscles of the more and less affected arm and leg averaged across all participants. Black bars are for A&L cycling and gray bars are for walking tasks. Net cutaneous reflexes are means (± s.e.m) from all participants and are normalized to the peak control (i.e. background) EMG recorded in each task. Significant difference between tasks (*) were calculated with a paired samples t-test. Abbreviations are: Sol, soleus; TA, tibialis anterior; FCR, flexor carpi radialis; and PD, posterior deltoid.

Reflex amplitude is typically uncoupled from rhythmic background EMG amplitude in NI participants. To examine the extent to which reflex amplitudes were related to background EMG during A&L cycling and walking in stroke participants, Pearson's correlation coefficient was calculated. Across all eight muscles for each participant across all phases of A&L cycling and walking no significant correlations were found (see Table 2-1).

Table 2-1: Correlation coefficients between the net reflex response and background EMG during A&L cycling and walking tasks. The critical value for this 2-tailed comparison ($p < 0.05$) for 12 participants was 0.58. Abbreviations in text.

Muscle	A & L Cycling	Walking
MA SOL	0.052	0.053
LA SOL	0.251	0.035
MA TA	0.097	0.153
LA TA	0.254	0.103
MA FCR	0.047	0.120
LA FCR	0.269	0.097
MA PD	0.051	0.141
LA PD	0.133	0.071

Mathematical PCA

The summary for the principal components analysis for combined SP+SR nerve stimulation in A&L cycling and walking is shown in Figure 2-6. The subplot on the left is for the variance accounted for (%VAF) from each principal component of background EMG amplitudes and the subplot on the right is for %VAF of cutaneous responses. Across both tasks, five common factors explained more than 86% of the variance for background EMG and 90% of the variance for the cutaneous response. There was a substantial difference between A&L cycling and walking in the magnitude of variance accounted for by the first principal component of background EMG and reflex modulation with 40-69% in cycling and only 22-29% in walking.

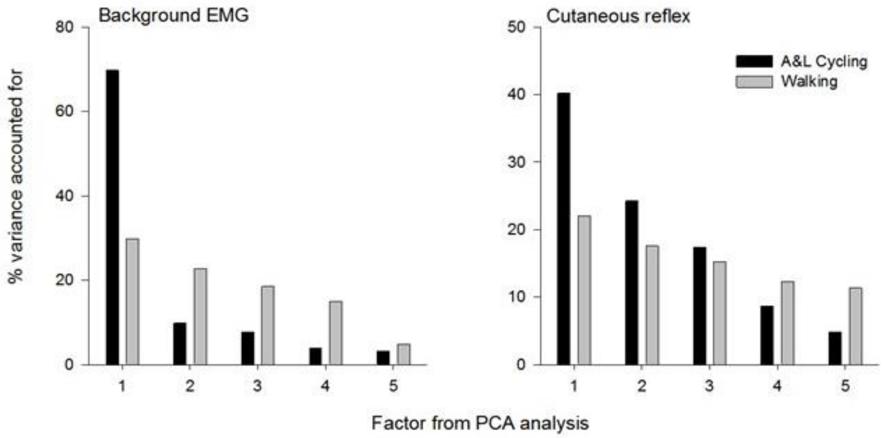


Figure 2-6: Summary of principal component analysis for background EMG and cutaneous response in A&L cycling and walking. Bars represent the variance accounted for by each factor.

Discussion

The purpose of this experiment was to evaluate the extent to which common neural regulation is conserved across locomotor tasks despite reduced supraspinal input after stroke. There were some but few significant differences between A&L cycling and walking for EMG amplitude modulation and net cutaneous reflex modulation (see Results) indicating that A&L cycling and walking have preserved modulation patterns after stroke. Some muscles displayed significant phase-dependent reflex modulation where no correlation to background EMG was present. Mathematical analysis revealed a dependence on five common factors explaining more than 86% of the variance for background EMG and 90% of the variance for the cutaneous reflex. These data suggest that after a stroke common neural patterning from conserved subcortical regulation in the arms and legs is conserved across locomotor tasks involving arm and leg movement. These results have translational implications for rehabilitation where A&L cycling could be usefully applied to recover walking function.

The role of supraspinal input

When comparing the results from this study of mathematical extraction of variance to results from a similar study by Zehr et al. (2007) in NI participants, some differences can be noted. Firstly, in this study, more principal components are required to explain less variance. Here, five components are required whereas only four principal components are required to explain 93% of the variance across tasks in a NI population (Zehr et al., 2007). We suggest the additional components could reflect the reduced extent of supraspinal regulation following stroke. Secondly, a larger division in the %VAF by the first principal component is seen when comparing NI participants and stroke participants. When comparing between walking and A&L cycling in NI participants, the largest difference in %VAF for the first principal component is approximately 30% whereas for stroke participants the largest difference is approximately 45% (see Figure 2-6). Again, this indirect observation may be ascribed to reduced supraspinal input following a stroke rendering integrations between the arms and legs more complex without the fine-tuning provided by an intact supraspinal system.

Within the framework of comparing muscle synergies in NI and to those present after stroke, alterations in the number of modules extracted is often observed. This is likely attributable to altered spinal cord excitability interacting with changes in descending motor regulation. Impaired upper limb function is associated with decreased number of reflex synergies (Trumbower et al., 2010, 2013) and reductions in voluntary synergy structures during isometric tasks (Roh et al., 2013), when compared to NI participants. During walking, changes in the modular organization of muscle synergies is also demonstrated post-stroke where there is a reduction in the number of synergies extracted (Clark et al., 2010) and modified recruitment organization (Gizzi et al., 2011). Our results are similar in that the components extracted are changed compared to NI participants, however, here we report instead an increases in the number of principal components is given. Diminished cortical modulation consequent to a stroke lesion could cause deficits in muscle synergy coordination leading to the observed functional impairments in locomotion following a stroke. These may be characterized during locomotion as an increased number of principal components, each accounting for a lower %VAF than is found in the intact nervous system.

Conservation in nervous system control across task has been previously ascribed to the action of locomotor central pattern generators (CPG) modulating transmission in cutaneous pathways by premotorneual gating. However differences between NI and stroke participants could arise from reduced supraspinal regulation of alphamotoneuronal and interneuronal activity caused by the stroke lesion (Dobkin, 2004, 2005). Descending supraspinal input can regulate reflex output through either modulation of excitability in the interneuron reflex pathways or through the internal networks that are part of the CPG itself (McCrea and Rybak, 2008; Zehr, 2005). Alterations in descending supraspinal regulation of interneuronal reflex pathways during rhythmic activity explains differences in neural conservation of locomotor tasks between stroke and NI participants. Some conservation of these mechanisms is still observed, thus implicating the spinal cord and subcortical areas in neural regulation across locomotor tasks.

Comparisons between the more and less affected limbs in stroke participants can reveal the effects of reduced supraspinal input on reflex modulation. Responses to stimulation in the tibialis anterior at approximately 80ms were small or absent during walking in stroke participants, in those with hereditary spastic paraparesis and in those with a spinal cord injury (Jones and Yang, 1994; Zehr et al., 1998a; Duysens 2003). Specifically, an absence of end-swing suppression in the TA (normally observed in NI participants) was noted however end-stance facilitations remained. This suggests that suppressions may be under the control of the cortex while facilitations are under the control of spinal CPGs (Duysens, et al., 2004). In the data presented here (see Figure 4), this fits nicely as in the MA TA (influenced by the lesioned cortex but still under the control of spinal CPGs) mainly facilitations are present and in the LA TA mainly suppressions are present. Therefore an intact cortex and corticospinal tract are required for full expression of the full range of reflex modulation during locomotion.

Evidence for conserved 'common core'

Common control across rhythmic movement tasks could be the result of a common core of subcortical elements expressing neural activity to produce the basic pattern of arm and leg movement (Zehr, 2005; Zehr et al., 2007, 1997). That is, a central mechanism is likely responsible for regulating various types of rhythmic movement in a similar oscillatory fashion. Measurements of muscle activity across various rhythmic tasks have shown a consistent frequency relationship between arm and leg movements for walking, cycling, creeping and swimming which could be indicative of spinal interconnections between the upper and lower spinal CPGs that are engaged in the locomotor function (Wannier et al., 2001). Indeed, propriospinal linkages between the fore and hindlimbs have been identified in the cat (Miller et al., 1973; Lloyd, 1942; Gernandt and Megirian, 1961) and data on interlimb responses obtained in persons with cervical spinal cord injury (Calancie, 1991; Calancie et al., 1996) suggests that quadrupedal links between forelimb and hindlimb coordination are conserved in humans (Zehr et al., 2009; Dietz et al., 2001; Wannier et al., 2001).

The main results of this experiment demonstrate the persistence and modulation of reflexes during A&L cycling and walking after stroke despite the interruption of some descending regulation of interneuronal excitability arising from the supraspinal lesion. The overall similarities in modulation patterns for background EMG and cutaneous reflexes provide insight into the status of neural control circuits in the damaged nervous system (Zehr and Duysens, 2004). A contribution from subcortical and presumed spinal locomotor pattern generating networks is implicit in the observations here where networks for arm and leg coordination could reside in subcortical areas as damage to the brain following stroke does not seem to significantly affect common neural regulation (Zehr et al., 2004).These results add to existing evidence that portions of the neural circuitry regulating rhythmic arm and leg movements remain accessible and intact after stroke (Ferris et al., 2006; Zehr and Duysens, 2004; Zehr and Loadman, 2012; Zehr et al., 2012).

Translational Applications

The neural similarities between A&L cycling and walking observed here have translational implications for rehabilitation where A&L cycling could be usefully applied to recover walking function. This can be achieved by activation of a set of similar residual neural pathways to strengthen interlimb neuronal coupling to improve walking performance after stroke (Klimstra et al., 2009; Zehr et al., 2007; Balter and Zehr, 2007; Zehr, 2005; Zehr and Loadman, 2012; Zehr et al., 2012, 2009; Ferris et al., 2006). In addition, A&L cycling is similar to walking in terms of muscle activity and joint ranges of motion (Zehr, 2005; Balter and Zehr, 2007).

Our experimental methods do not allow us to effectively delineate the specific locus of the observed reflex (intra- vs. interlimb) given we are using simultaneous stimulation of both the hand and foot (see Figure 5 in Nakajima et al., 2013). However, the presence of cutaneous reflexes seen here confirms that neuronal pathways linking the arms and the legs remain partially conserved in stroke providing a substrate for training induced plasticity to improve function. Combined arm and leg stimulation can be used as an index for arm and leg coupling where stimulation likely converges in reflex pathways from cutaneous inputs for the hand and foot to produce the responses observed (Nakajima et al., 2013). Cutaneous inputs and associated modulation of reflex amplitudes could serve as probes to monitor ensuing neuroplastic adaptations in interlimb pathways resulting from targeted rehabilitation (Zehr and Loadman, 2012; Wolpaw, 2010). In addition, the use of principal component analysis could provide a useful means of evaluating rehabilitation effects where reductions in the number of principal components and variance explained by each component could suggest improved control.

Conclusion

In general, background locomotor EMG was similar between A&L cycling and walking where similar phase dependent modulation patterns were observed. Modulation of cutaneous reflexes from hand and foot stimulation suggest a conserved ‘common core’ of subcortical regulation of locomotion despite altered descending supraspinal input from the stroke lesion. These results have translational implications for rehabilitation where A&L cycling could be usefully applied to improve walking function.

References

Balter, J. E., and Zehr, E. P. (2007). Neural coupling between the arms and legs during rhythmic locomotor-like cycling movement. *J. Neurophysiol.* 97, 1809–18.

Burridge, J. H., Wood, D. E., Taylor, P. N., and McLellan, D. L. (2001). Indices to describe different muscle activation patterns, identified during treadmill walking, in people with spastic drop-foot. *Med. Eng. Phys.* 23, 427–434.

Calancie, B. (1991). Interlimb reflexes following cervical spinal cord injury in man. *Exp. Brain Res.* 85.

Calancie, B., Lutton, S., and Broton, J. G. (1996). Central nervous system plasticity after spinal cord injury in man: interlimb reflexes and the influence of cutaneous stimulation. *Electroencephalogr. Clin. Neurophysiol. Mot. Control* 101, 304–315.

Carroll, T. J., Baldwin, E. R. L., Collins, D. F., and Zehr, E. P. (2006). Corticospinal excitability is lower during rhythmic arm movement than during tonic contraction. *J. Neurophysiol.* 95, 914–21.

Clark, D. J., Ting, L. H., Zajac, F. E., Neptune, R. R., and Kautz, S. A. (2010). Merging of healthy motor modules predicts reduced locomotor performance and muscle coordination complexity post-stroke. *J. Neurophysiol*. 103, 844–857.

Cohen, J. (2013). Statistical power analysis for the behavioral sciences. *Routledge Academic*.

Dietz, V., Fouad, K., and Bastiaanse, C. M. (2001). Neuronal coordination of arm and leg movements during human locomotion. *Eur. J. Neurosci.* 14, 1906–1914.

Dimitrijevic, M. R., and Nathan, P. W. (1970). Studies of spasticity in man. 4. Changes in flexion reflex with repetitive cutaneous stimulation in spinal man. *Brain* 93, 743–768.

Dimitrijević, M. R., and Nathan, P. W. (1973). Studies of spasticity in man. 6. Habituation, dishabituation and sensitization of tenson reflexes in spinal man. *Brain* 96, 337–54.

Dobkin, B. (2005). Clinical practice. Rehabilitation after stroke. *N. Engl. J. Med.* 352, 1677–1689.

Dobkin, B. H. (2004). Strategies for stroke rehabilitation. *Lancet Neurol.* 3, 528–536.

Duysens, J., Bastiaanse, C.M., Smits-Engelsman and B.C.M., Dietz, V. (2004). Gait acts as a gate for reflexes from the foot. *Can J Physiol Pharm*. 82, 715-222.

Duysens, J., Baken, B. C. M., Burgers, L., Plat, F. M., Den Otter, A. R., and Kremer, H. P. H. (2004). Cutaneous reflexes from the foot during gait in hereditary spastic paraparesis. *Clinical neurophys.* 115(5), 1057-1062.

Ferris, D. P., Huang, H. J., and Kao, P. C. (2006). Moving the arms to activate the legs. *Exerc. Sport Sci. Rev.* 34, 113–120.

Gernandt, B. E., and Megirian, D. (1961). Ascending Propriospinal Mechanisms. *J Neurophysiol* 24, 364–376.

Gizzi, L., Nielsen, J. F., Felici, F., Ivanenko, Y. P., and Farina, D. (2011). Impulses of activation but not motor modules are preserved in the locomotion of subacute stroke patients. *J. Neurophysiol*. 106, 202–210.

Haridas, C., and Zehr, E. P. (2003). Coordinated interlimb compensatory responses to electrical stimulation of cutaneous nerves in the hand and foot during walking. *J. Neurophysiol.* 90, 2850–61.

Ivanenko, Y. P., Poppele, R. E., and Lacquaniti, F. (2004). Five basic muscle activation patterns account for muscle activity during human locomotion. *J. Physiol.* 556, 267–282.

Jones, C. A., and Yang, J. F. (1994). Reflex behavior during walking in incomplete spinal-cord-injured subjects. *Experimental neurology*. 128(2), 239-248.

Klimstra, M. D., Thomas, E., Stoloff, R. H., Ferris, D. P., and Zehr, E. P. (2009). Neuromechanical considerations for incorporating rhythmic arm movement in the rehabilitation of walking. *Chaos* 19, 026102.

Komiyama, T., Zehr, E. P., and Stein, R. B. (2000). Absence of nerve specificity in human cutaneous reflexes during standing. *Exp. Brain Res.* 133, 267–72.

Lamont, E. V, and Zehr, E. P. (2006). Task-specific modulation of cutaneous reflexes expressed at functionally relevant gait cycle phases during level and incline walking and stair climbing. *Exp. Brain Res.* 173, 185–92.

Lloyd, D. P. C. (1942). Mediation of Descending Long Spinal Reflex Activity. *J Neurophysiol* 5, 435–458.

McCrea, D. A., and Rybak, I. A. (2008). Organization of mammalian locomotor rhythm and pattern generation. *Brain Res. Rev.* 57, 134–146.

Miller, S., Reitsma, D. J., and van der Meché, F. G. (1973). Functional organization of long ascending propriospinal pathways linking lumbo-sacral and cervical segments in the cat. *Brain Res.* 62, 169–88.

Nakajima, T., Barss, T., Klarner, T., Komiyama, T., and Zehr, E. P. (2013). Amplification of interlimb reflexes evoked by stimulating the hand simultaneously with conditioning from the foot during locomotion. *BMC Neurosci.* 14, 28.

Roh, J., Cheung, V. C., and Bizzi, E. (2011). Modules in the brain stem and spinal cord underlying motor behaviors. *J. Neurophysiol*. 106, 1363–1378.

Shiavi, R., Bugle, H. J., and Limbird, T. (1987). Electromyographic gait assessment, Part 2: Preliminary assessment of hemiparetic synergy patterns. *J. Rehabil. Res. Dev.* 24, 24–30.

Trumbower, R. D., Finley, J. M., Shemmell, J. B., Honeycutt, C. F., and Perreault, E. J. (2013). Bilateral impairments in task-dependent modulation of the long-latency stretch reflex following stroke. *Clin. Neurophysiol. 124*(7), 1373-1380.

Trumbower, R. D., Ravichandran, V. J., Krutky, M. A., and Perreault, E. J. (2010). Contributions of altered stretch reflex coordination to arm impairments following stroke. *J. Neurophysiol*. 104, 3612–3624.

Wannier, T., Bastiaanse, C., Colombo, G., and Dietz, V. (2001). Arm to leg coordination in humans during walking, creeping and swimming activities. *Exp. brain Res.* 141, 375–9.

Wolpaw, J. R. (2010). What can the spinal cord teach us about learning and memory? *Neuroscientist* 16, 532–549.

Zehr, E. P., Balter, J. E., Ferris, D. P., Hundza, S. R., Loadman, P. M., and Stoloff, R. H. (2007). Neural regulation of rhythmic arm and leg movement is conserved across human locomotor tasks. *J. Physiol.* 582, 209–27.

Zehr, E. P., Carroll, T. J., Chua, R., Collins, D. F., Frigon, A., Haridas, C., Hundza, S. R., and Thompson, A. K. (2004). Possible contributions of CPG activity to the control of rhythmic human arm movement. *Can. J. Physiol. Pharmacol.* 82, 556–68.

Zehr, E. P., and Duysens, J. (2004). Regulation of arm and leg movement during human locomotion. *Neuroscientist* 10, 347–61.

Zehr, E. P., Fujita, K., and Stein, R. B. (1998a). Reflexes from the superficial peroneal nerve during walking in stroke subjects. *J. Neurophysiol.* 79, 848–58.

Zehr, E. P., Hundza, S. R., and Vasudevan, E. V (2009). The quadrupedal nature of human bipedal locomotion. *Exerc. Sport Sci. Rev.* 37, 102–8.

Zehr, E. P., Komiyama, T., and Stein, R. B. (1997). Cutaneous reflexes during human gait: electromyographic and kinematic responses to electrical stimulation. *J. Neurophysiol.* 77, 3311–25.

Zehr, E. P., and Loadman, P. M. (2012). Persistence of locomotor-related interlimb reflex networks during walking after stroke. *Clin. Neurophysiol.* 123, 796–807.

Zehr, E. P., Loadman, P. M., and Hundza, S. R. (2012). Neural control of rhythmic arm cycling after stroke. *J. Neurophysiol.* 108, 891–905.

Zehr, E. P., Stein, R. B., and Komiyama, T. (1998b). Function of sural nerve reflexes during human walking. *J. Physiol*. 507 Pt 1, 305–14.

Zehr, E. P. (2005). Neural control of rhythmic human movement: the common core hypothesis. *Exerc. Sport Sci. Rev.* 33, 54–60.

Chapter 3 Changing coupling between the arms and legs with slow walking speeds alters neural control

Abstract

Understanding the dynamic interactions between arm and leg coordination is important as it has been suggested that arm activity be integrated into locomotor rehabilitation where rhythmic activities in the arm may alter leg activity via interlimb spinal pathways. The normal 1:1 frequency ratio that exists at normal walking speeds changes to a 2:1 ratio at slow walking speeds. It is hypothesized that this change in interlimb frequency results in a change in nervous system control and thus the purpose of this study was to evaluate the effects of changing walking speed and interlimb coordination on the modulation of cutaneous reflexes. Participants walked in four combinations of walking speed (normal, slow) and interlimb coordination (1:1, and 2:1) and cutaneous reflexes and background muscle activity were evaluated following stimulation applied to the superficial peroneal nerve at the ankle and superficial radial nerve at the wrist. Results show that neural control is dependent on interlimb coordination and walking speed and effects are largest in the swing phase when walking is unstable. A directional coupling of arm to leg interlimb control was also observed where arm perturbations had a greater effect on leg activity instead of the reverse. These results continue to elucidate spinal connections between the arms and legs and their role during walking. Understanding the underlying neural mechanisms for the organization of rhythmic arm movement, and its coordination with the legs, can give insight into pathological walking, and will enable development of effective strategies for rehabilitation of walking.

Introduction

Understanding the dynamic interactions between arm and leg coordination is important and timely as it has been suggested that arm movement be integrated into locomotor rehabilitation (Ferris et al. 2006; Dietz and Michel 2009; Zehr et al. 2009; Klimstra et al. 2009; Guertin 2012; de Kam et al. 2013; Zehr et al. 2016). In animals, tight coupling between cervical and lumbar central pattern generators (CPG) networks exists, where rhythmic activity of one CPG influences activity in the other CPG, via propriospinal interlimb connections between the hind- and forelimbs (Gernandt and Megirian 1961; Miller et al. 1973; Skinner et al. 1980; Akay et al. 2006; Juvin et al. 2012; Thibaudier and Frigon 2014).

In humans, despite the fact that the arms do not directly generate propulsion during walking, neuronal connections between the cervical and lumbar pattern generators are apparent, and likely have a role in coordinating whole-body responses to perturbations during gait (Haridas et al. 2006; Ford et al. 2007a; Umberger 2008; Meyns et al. 2013; Zehr et al. 2016). Arm swing is coordinated to movement with the legs, where the frequency of arm swing motion coordination depends on walking speed and retains a phase relationship with the legs regardless of ambulatory velocity (Craik et al. 1976; Donker et al. 2002). At most walking speeds (normal walking, jogging, running) this pattern consists of a 1:1 arm:leg frequency ratio with out-of-phase arm swing where each arm is paired with its contralateral leg and synchronized with stride frequency.

Across other rhythmic arm and leg tasks, such as creeping and swimming, arm and leg movements remain frequency locked, with a fixed relationship (Wannier et al. 2001), revealing tight neural coupling between upper and lower limbs (Zehr et al. 2009; Zehr et al. 2016). A change in coordination between the arms and the legs also comes into effect at relatively slower walking speeds. Walking at approximately less than 0.7-0.8 m/s causes both arms to swing minimally, and in-phase, at twice the ipsilateral step frequency producing a 2:1 arm:leg frequency ratio (Wagenaar and Van Emmerik 2000; Donker et al. 2001; Ford et al. 2007a). These highly coordinated arm and leg movement patterns represent the nervous system`s ability to maintain stability at slow walking velocities (Donker et al. 2001; Tester et al. 2012).

Investigating the neural interactions between arm and leg coordination in neurologically intact participants can give insight into pathological walking and lead to the development of strategies for walking rehabilitation. The current study furthers the characterization of arm and leg interactions by evaluating nervous system control during slow walking. Neural interactions between the limbs may be changed as relative phasing is changed with slow walking. To evaluate this, modulation of interlimb cutaneous reflexes, that might accompany the 2:1 frequency coordination between arm and leg movements that occurs at low walking velocities, is examined. Due to previously recorded changes in reflex modulation as a result of changing interlimb coordination, it is hypothesize that changes in walking speed and interlimb frequency ratios will influence the depth of modulation of interlimb cutaneous reflexes.

Material and Methods

Participants

Twelve participants (6 males and 6 females; 23.4 (±2.9) years old, 68.7 (±14.9) kg, 169.2 (±11.6) cm), who gave written informed consent in a protocol approved by the Human Research Ethics Board at the University of Victoria, were involved in this study.

Experimental Protocol

To examine changes in cutaneous reflex modulation as a result of changes in walking speed and interlimb coordination, participants walked in four combinations of conditions of walking speed (normal, slow) and interlimb coordination (1:1, and 2:1). Walking was performed on a motorized treadmill belt (Woodway Desmo M, Waukesha, WI, USA) and participants were given time to familiarize themselves and to find their comfortable walking speed. For the slow walking conditions, belt speed was decreased incrementally until the 2:1 arm:leg interlimb coordination pattern emerged. In all of our participants, this pattern emerged after a few minutes of slow treadmill walking. The order of the trials was randomized and breaks were provided between each trial in order to avoid fatigue.

Electromyography

Electromyographic (EMG) recordings were made from the right medial gastrocnemius (MG), soleus (SOL), tibialis anterior (TA), vastus lateralis (VL), biceps femoris (BF), anterior deltoid (AD), posterior deltoid (PD), and biceps brachaii (BB). The skin over the muscles of interest was cleaned with alcohol and 1cm surface EMG electrodes (Thought Technologies Ltd.) were applied in a bipolar configuration using a 2cm inter-electrode distance. Grounding electrodes were placed over the patella and medial epicondyle of the elbow. EMG signals were pre-amplified 5000x and band-pass filtered at 100-300Hz (P511 Grass Instrument, AstroMed, Inc.). Data were sampled at 1000Hz (A/D converter; National Instrument, Austin, TX), and stored to a computer for off-line analysis.

Force Sensing Resistors and Joint Kinematics

Force sensing resistors (FSRs) were firmly attached to the insole of the participant's right shoe at the heel, head of the first metatarsal, and distal end of the fifth metatarsal. Throughout the experiment, force signals were pre-amplified and recorded for offline analysis. Angular positions of the ankle (planter/dorsiflexion), knee (flexion/extension), elbow (flexion/extension), and shoulder (flexion/extension) were measured with electrogoniometers (Biometrics Ltd., Gwent, UK) positioned over the saggital planes at these joints. These devices were calibrated and output in degrees was recorded. As with similar studies (Zehr et al. 1997; Haridas et al. 2006; Lamont and Zehr 2007; Zehr and Loadman 2012), all data were sampled at 1000 Hz with a 12 bit A/D converter connected to a computer running custom-written LabVIEW (National Instruments Corp., Austin, Texas, USA) acquisition software and stored for off-line analysis.

Nerve Stimulation

The pattern of cutaneous reflex modulation during walking was used to assess the changes arising from alterations in interlimb coordination. In separate trials for each condition, cutaneous reflexes were evoked via stimulation to the nerves innervating the dorsum of the foot (superficial peroneal; SP) and of the hand (superficial radial; SR). Electrodes for SR nerve stimulation were placed just proximal to the radial head and for SP nerve stimulation on the ankle of the LA limbs. Appropriate stimulation location was checked by ensuring that radiating paresthesia was evoked into the appropriate cutaneous innervation areas of the SR and SP nerves. Cutaneous reflexes were applied with trains of 5 x 1.0 ms pulses at 300Hz of isolated constant current stimulation (Grass S88 stimulator with SIU5 stimulus isolation and a CCU1 constant current unit AstroMed-Grass Inc., Canada). Stimulus intensity was set as multiples of the threshold for radiating paraesthesia (RT) at 2.0 x RT for the SR nerve, and 3.0 x RT for the SP nerve. Non-noxious stimulation intensities were found for each participant to ensure non-nociceptive pathways were stimulated. During all trials, 120 stimulations were delivered pseudo-randomly with an inter-stimulus interval of 1-5 seconds.

Data Analysis

All data were analyzed with custom written software (MATLAB, MathWorks, Inc., Natick, MA, USA). Background EMG data were full-wave rectified and low pass filtered at a cut-off frequency of 100Hz with a 4th order Butterworth filter and kinematic data were low pass filtered at a cut-off frequency of 6Hz with a 4th order Butterworth filter. FSR signals from the foot sole were summed and used to establish step cycle parameters; heel contact, periods of stance, toe-off and swing based on methods previously described (Zehr et al. 1997). Stride frequency was determined as the average number of strides taken in one second.

EMG and kinematic data were normalized to 100% of the gait cycle and averaged within a step. For comparisons between participants EMG data of each muscle from each trial were normalized to the peak average value of that muscle's activation pattern. EMG and kinematic data were aligned to begin with heel contact.

To examine cutaneous reflexes, the step cycle was divided into 8 separate equally-timed phases, beginning with heel contact and ending with the subsequent heel contact at the swing to stance transition with phases 1–5 representing stance and phases 6–8 representing swing for walking (Zehr et al. 2014).

The average trace from the non-stimulated data was subtracted from the average trace of the stimulated data to produce a subtracted EMG 'reflex' trace within each phase. Stimuli (n=~10-20 responses per phase) where then aligned to delivery within eight phases, were full-wave rectified, filtered, and averaged together. The stimulus artifact was removed from the subtracted reflex trace and data were then low-pass filtered at 30 Hz using a dual-pass, fourth order Butterworth filter. For evaluation of the effect of stimulation, the subtracted data traces were analyzed in all instances.

Background EMG was obtained from steps without stimulation and was determined as the average response within a phase normalized to the peak response for each trial for each participant. Cutaneous reflexes were quantified as the average cumulative reflex activity occurring 150 ms after stimulation (ACRE150). As with previous studies (Zehr et al. 1997; Zehr et al. 1998; Klarner et al. 2014), this measure involved calculating a subtracted reflex (see above) then cumulatively summing the signal over a post-stimulus period of 150 ms and dividing by the time interval of integration to measure an overall reflex effect. Net reflex values were normalized to peak control EMG amplitude. This value yields the overall reflex effect where if the value is positive, overall facilitation has occurred, and if the value is negative, overall inhibition has occurred (Komiyama et al., 2000; Zehr et al., 1998). Kinematic data were quantified by determining the angular excursion in degrees recorded in each phase through the stride cycle. Interlimb ratio`s between arm and leg movement were determined as the ratio between number of peak events for Heel FSR and elbow signals. Changes in kinematic data due to stimulation were analyzed within a 140-220 ms window post stimulus (Zehr et al. 1997; Klimstra et al. 2011). Responses were considered significant if they exceeded a 2-SD band of the mean value of the subtracted pre-stimulus level of the ongoing kinematic parameter at each phase.

Statistics

Standard statistical software was used for all statistical evaluations (SPSS 11.0, SPSS, Chicago, IL). In all instances, analysis was conducted on averaged data from each phase of the step cycle for each participant. To compare between trials, repeated measures analysis of variance (rmANOVA) was used to determine significant differences in stride frequencies, background EMG level, net cutaneous reflexes, kinematic excursions, and changes in kinematic excursions due to stimulation (SPSS 18.0, Chicago, IL). Each variable was analyzed separately for each nerve stimulation site, as was each phase of walking, in order to determine if changes in walking speed or interlimb coordination have a discernible effect on cutaneous reflex modulation.

To test the hypothesis that modifying walking speed and interlimb coordination will change cutaneous reflex modulation, the initial approach for all data was to conduct omnibus 8 (phase) x 2 (walking speed) x 2 (interlimb ratio) repeated measures ANOVAs. Effect of walking speed and interlimb ratio were further investigated at each phase of walking, thus a 2 (walking speed) x 2 (interlimb ratio) repeated measures ANOVA was completed at each phase. Differences in the pattern of response between trials would be detected as a speed-ratio interaction, and general amplitude differences in speed and ratio will be seen as a significant main effects. Pearson’s correlation coefficients (r) were calculated between several variables and tested for significance. All statistical tests were 2-tailed and significance was accepted at $p \leq 0.05$.

Results

Arm and leg coordination between conditions

Figure 3-1 shows heel FSR, kinematic data from the elbow, and walking speed averaged across all participants to illustrate interlimb coordination across experimental conditions. Each panel represents an experimental condition representing one of four combinations of walking speed (normal and slow) and interlimb ratio (1:1 and 2:1 arm to leg ratio). In each panel, elbow and heel FSR data are shown for SP and SR nerve stimulation conditions.

For all participants, treadmill belt speed was set to 1.82 (±0.48) mph for the normal walking speed conditions and 0.71 (±0.04) mph for the slow walking conditions. Stride frequencies were altered across experimental conditions, regardless of interlimb ratio between normal and slow walking. For SP nerve stimulation conditions, there was a significant main effect of walking speed ($F_{(1,11)} = 67.858$, $p=0.000$) and for SR nerve stimulation conditions, there was a significant main effect of walking speed ($F_{(1,11)} = 54.092$, $p=0.000$). Interlimb ratio`s between arm and leg movement were quantified using peak amplitudes from Heel FSR and elbow signals and were found to be maintained at 1:1 and 2:1 across all participants regardless of walking speed.

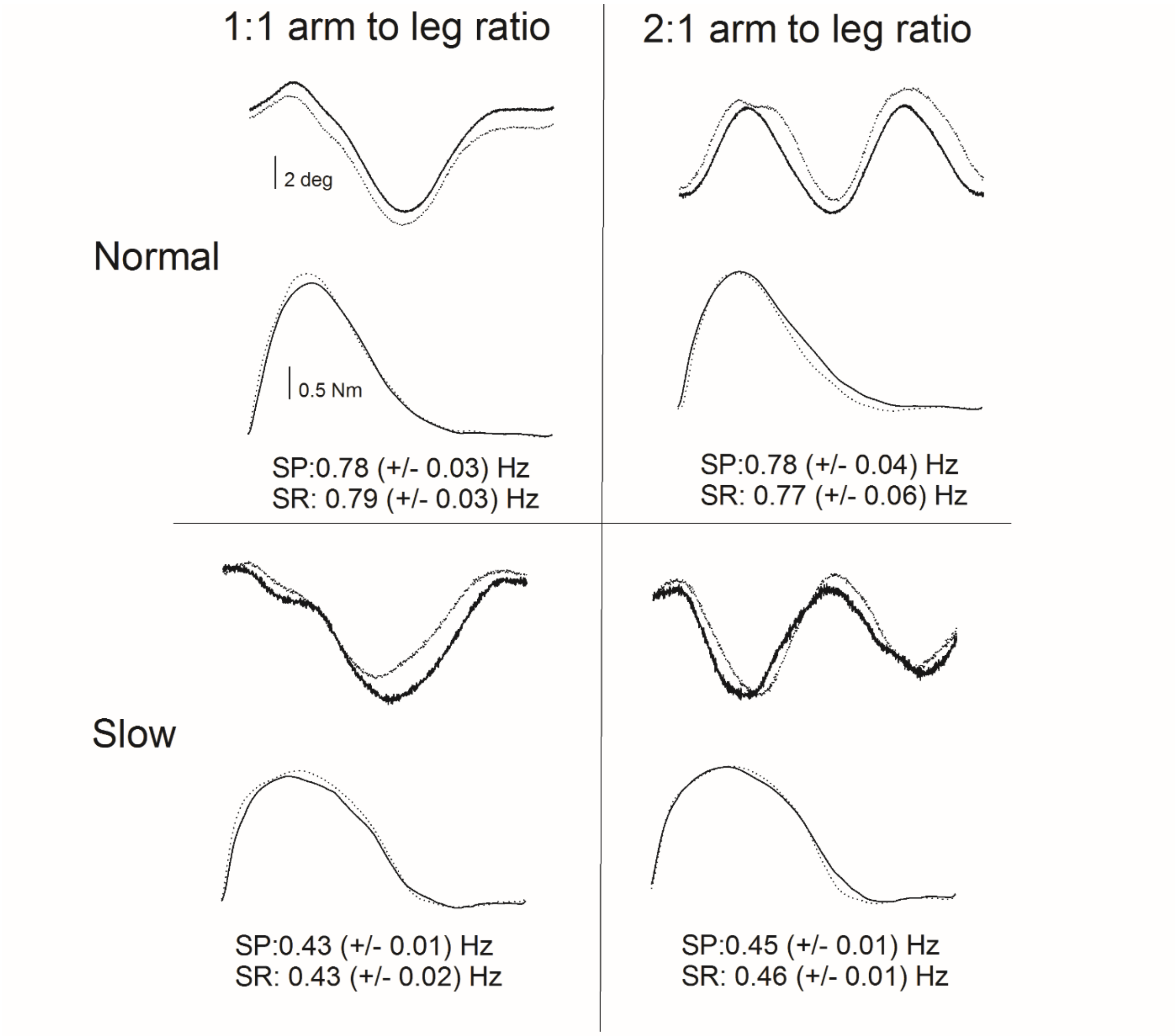


Figure 3-1: elbow excursions (upper traces) and heel FSR (lower traces) for four conditions of walking speed (normal and slow) and interlimb ratio (1:1 ratio and 2:1 ratio). The dark lines are from superficial radial nerve stimulation and light lines are from superficial peroneal nerve stimulation. Stride frequencies, averaged across all participants, are listed for all conditions

Cutaneous Reflexes with superficial peroneal nerve stimulation

Figure 3-2 shows reflex amplitudes (bars) for 8 phases of walking averaged across all participants for all muscles from superficial peroneal nerve stimulation. Omnibus rmANOVA`s for all phases, walking speed, and interlimb ratio conditions revealed significant main effects and interaction effects for several of the muscles tested. For the MG, a main effect of speed was recorded ($F_{(1,11)}$=5.004, p=0.047) where across all phases, reflexes in the normal walking condition show increased inhibition compared to reflexes recorded in the slow walking conditions. For the SOL, there was a significant phase by walking speed by interlimb ratio interaction ($F_{(7,77)}$=3.035, p=0.007) indicating an overall change in reflex modulation across conditions. For the TA, there was a significant interaction of phase by speed ($F_{(7,77)}$=3.161, p=0.005). For the VL, there was a significant phase by speed interaction ($F_{(7,77)}$=2.212, p=0.042) and a significant phase by ratio interaction ($F_{(7,77)}$=2.968, p=0.008) where the pattern of reflexes in the VL varied across walking phases for both speed and interlimb ratio. In BF, there was a significant phase by speed interaction ($F_{(7,77)}$=2.304, p=0.035) and a significant phase by speed by ratio interaction ($F_{(7,77)=}$2.164, p=0.048). When examining reflexes at each phase, rmANOVA revealed several significant interaction and main effects of walking speed and interlimb ratio, denoted on Figure 3-2 with a dollar sign, asterisk and hashtag, respectively. Of note are the significant effects on reflexes in the TA which show increased inhibition in the slow walking condition compared to the normal walking conditions and which varies as a function of the walking phase.

Figure 3-2 shows normalized background EMG (lines) for 8 phases of walking averaged across all participants for all muscles from superficial peroneal nerve stimulation. For background data at each phase, omnibus rmANOVA revealed several significant main effects and interaction effects. For the MG, there was a significant phase by walking speed interaction ($F_{(7,77)}$=6.262, p=0.000. For the SOL, there was a significant phase by walking speed interaction ($F_{(7,77)}$=5.008, p=0.000), and a significant phase by walking speed by interlimb ratio interaction ($F_{(7,77)}$=204.571, p=0.029). For the TA, there was a significant phase by walking speed interaction ($F_{(7,77)}$=6.432, p=0.000), speed by interlimb ratio interaction ($F_{(1,11)}$=21.739, p=0.001), and a phase by walking speed by interlimb ratio interaction ($F_{(7,77)}$=8.901, p=0.000). For the VL, there was a significant main effect of phase ($F_{(7,77)}$=16.192, p=0.000) and speed ($F_{(1,11)}$=4.752, p=0.025). For the BF, there was a significant phase by walking speed interaction ($F_{(7,77)}$=2.394, p=0.029), and a phase by interlimb ratio interaction ($F_{(7,77)}$=4.108, p=0.001). For the AD, there was a significant phase by walking speed interaction ($F_{(7,77)}$=3.836, p=0.001), phase by interlimb ratio interaction ($F_{(7,77)}$=9.542,p=0.000), and a phase by walking speed by interlimb ratio ($F_{(7,77)}$=7.241, p=0.000). For the PD, where was a significant phase by walking speed interaction ($F_{(7,77)}$=11.514, p=0.000), walking speed by interlimb ratio interaction ($F_{(1,11)}$=8.694, p=0.013), and a phase by walking speed by interlimb ratio interaction ($F_{(7,77)}$=6.119, p=0.000). For the BB, there was a significant phase by walking speed interaction ($F_{(7,77)}$=3.278, p=0.004), phase by interlimb ratio interaction ($F_{(7,77)}$=6.262, p=0.000), and a significant phase by walking speed by interlimb ratio interaction ($F_{(7,77)}$=5.290, p=0.000). At each bin, rmANOVA revealed several significant interaction and main effects of walking speed and interlimb ratio, denoted on Figure 3-2 with a dollar sign, asterisk and hashtag, respectively.

Correlational analysis of cutaneous reflexes with bEMG was performed for phases of interest. In the TA, correlational analyses were performed for phase 5,7, and 8 and no significant correlations were observed.

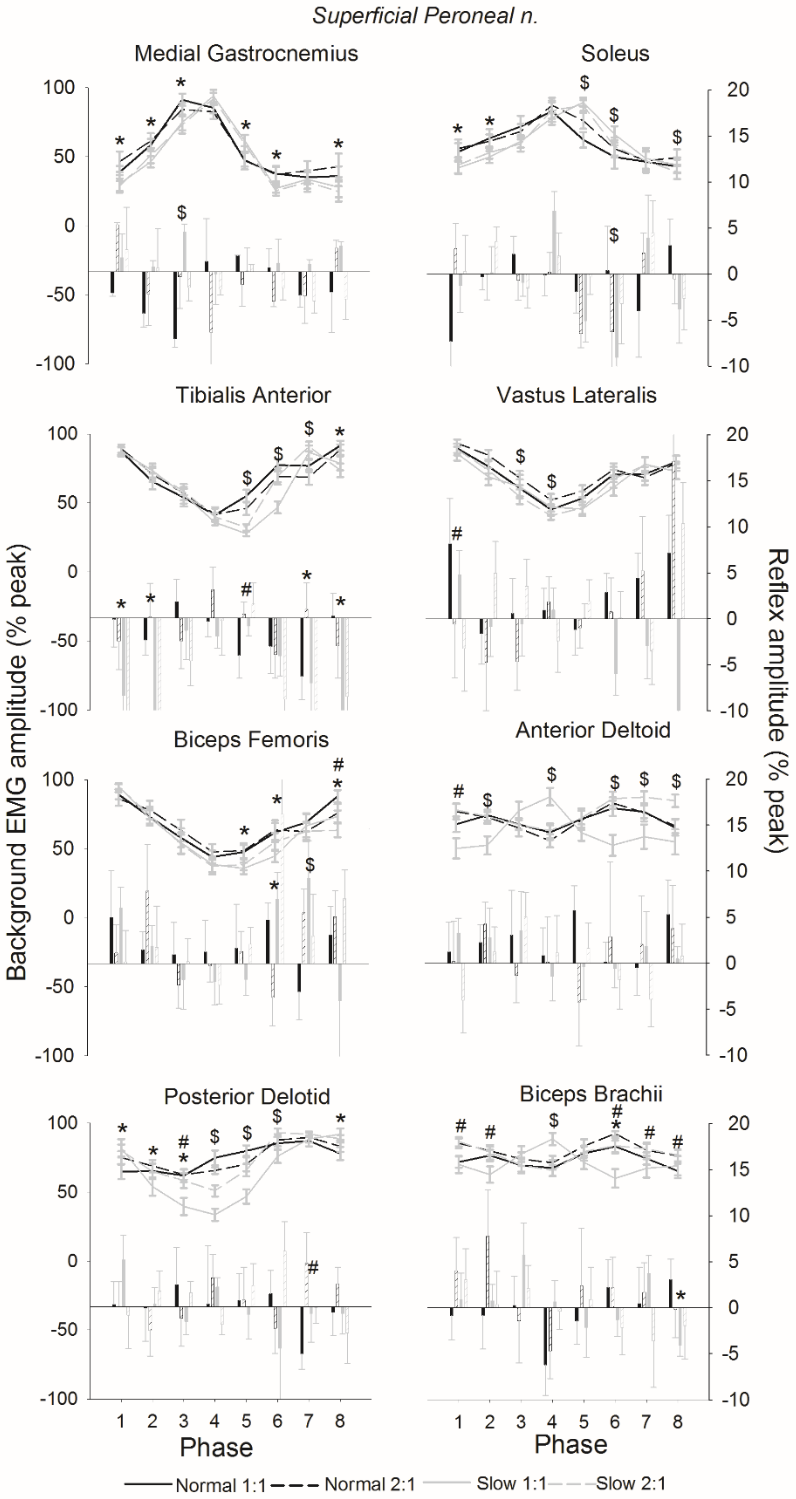


Figure 3-2: Normalized background EMG and reflex amplitudes for superficial peroneal nerve stimulation. Background EMG is shown in line plots and reflex amplitude is shown in bar plots. Values are means (±standard

error) averaged across all participants and normalized to the peak undisturbed EMG during walking for each condition. Significant differences between conditions are indicated with * for main effect of walking speed, # for main effect of interlimb ratio, and $ for interaction.

Cutaneous Reflexes with superficial radial nerve stimulation

Figure 3-3 shows reflex amplitudes (bars) for 8 phases of walking averaged across all participants for all muscles from superficial radial nerve stimulation. Omnibus rmANOVA`s for all phases, walking speed, and interlimb ratio conditions revealed significant main effects and interaction effects for several of the muscles tested. For the SOL, there was a significant phase by walking speed by interlimb ratio interaction ($F_{(7,77)}$=2.350, p=0.031) indicating an overall change in reflex modulation across conditions. For the TA, there was a significant main effect of phase ($F_{(1,11)}$=3.334, p=0.004). For the VL, there was a significant phase by ratio interaction ($F_{(7,77)}$=2.186, p=0.048) where the pattern of reflexes in the VL varied across walking phases for both speed and interlimb ratio. For the AD, there was a significant main effect of walking speed ($F_{(1,11)}$= 4.601, p=0.042) and a significant main effect of interlimb ratio ($F_{(1,11)}$= 5.977, p = 0.033). Reflexes in AD were smallest with slow walking and largest with normal walking condition and larger with the 2:1 interlimb ratio compared to the 1:1 interlimb ratio. For the PD, there was a significant main effect of speed ($F_{(7,77)}$ = 2.311, p=0.034) where reflexes were largest with the slow walking speed. When examining reflexes at each phase, rmANOVA revealed several significant interaction and main effects of walking speed and interlimb ratio, denoted on Figure 3-3 with a dollar sign, asterisk and hashtag, respectively.

Figure 3-3 shows normalized background EMG (lines) for 8 phases of walking averaged across all participants for all muscles from superficial radial nerve stimulation. For background data at each phase, omnibus rmANOVA revealed several significant main effects and interaction effects. For the MG, there was a significant phase by walking speed interaction ($F_{(7,77)}$=5.110, p=0.000). For the SOL, there was a significant phase by walking speed interaction ($F_{(7,77)}$=6.193, p=0.000), and a significant phase by walking speed by interlimb ratio ($F_{(7,77)}$=473.673, p=0.000). For the TA, there was a significant phase by walking speed interaction ($F_{(7,77)}$=4.979, p=0.000), phase by interlimb ratio interaction ($F_{(1,11)}$=2.395, p=0.029), and a phase by walking speed by interlimb ratio interaction ($F_{(7,77)}$=4.165, p=0.001). For the VL, there was a significant main effect of phase ($F_{(7,77)}$=14.389, p=0.000) and speed ($F_{(1,11)}$=8.250, p=0.015). For the BF, there was a significant phase by interlimb ratio interaction ($F_{(7,77)}$=3.276, p=0.004), and a phase by walking speed by interlimb ratio interaction ($F_{(7,77)}$=2.290, p=0.036). For the AD, there was a significant phase by walking speed interaction ($F_{(7,77)}$=6.896, p=0.000), phase by interlimb ratio interaction ($F_{(7,77)}$=15.307,p=0.000), walking speed by interlimb ratio interaction ($F_{(1,11)}$=8.977, p=0.012), and a phase by walking speed by interlimb ratio ($F_{(7,77)}$=9.494, p=0.000). For the PD, where was a significant phase by walking speed interaction ($F_{(7,77)}$=5.257, p=0.000), walking speed by interlimb ratio interaction ($F_{(1,11)}$=10.113, p=0.009), and a phase by walking speed by interlimb ratio interaction ($F_{(7,77)}$=5.360, p=0.000). For the BB, there was a significant phase by walking speed interaction ($F_{(7,77)}$=5.367, p=0.000), phase by interlimb ratio interaction ($F_{(7,77)}$=4.562, p=0.000), and a significant phase by walking speed by interlimb ratio interaction ($F_{(7,77)}$=6.474, p=0.000). At each bin, rmANOVA revealed several significant interaction and main effects of walking speed and interlimb ratio, denoted on Figure 3-3 with a dollar sign, asterisk and hashtag, respectively.

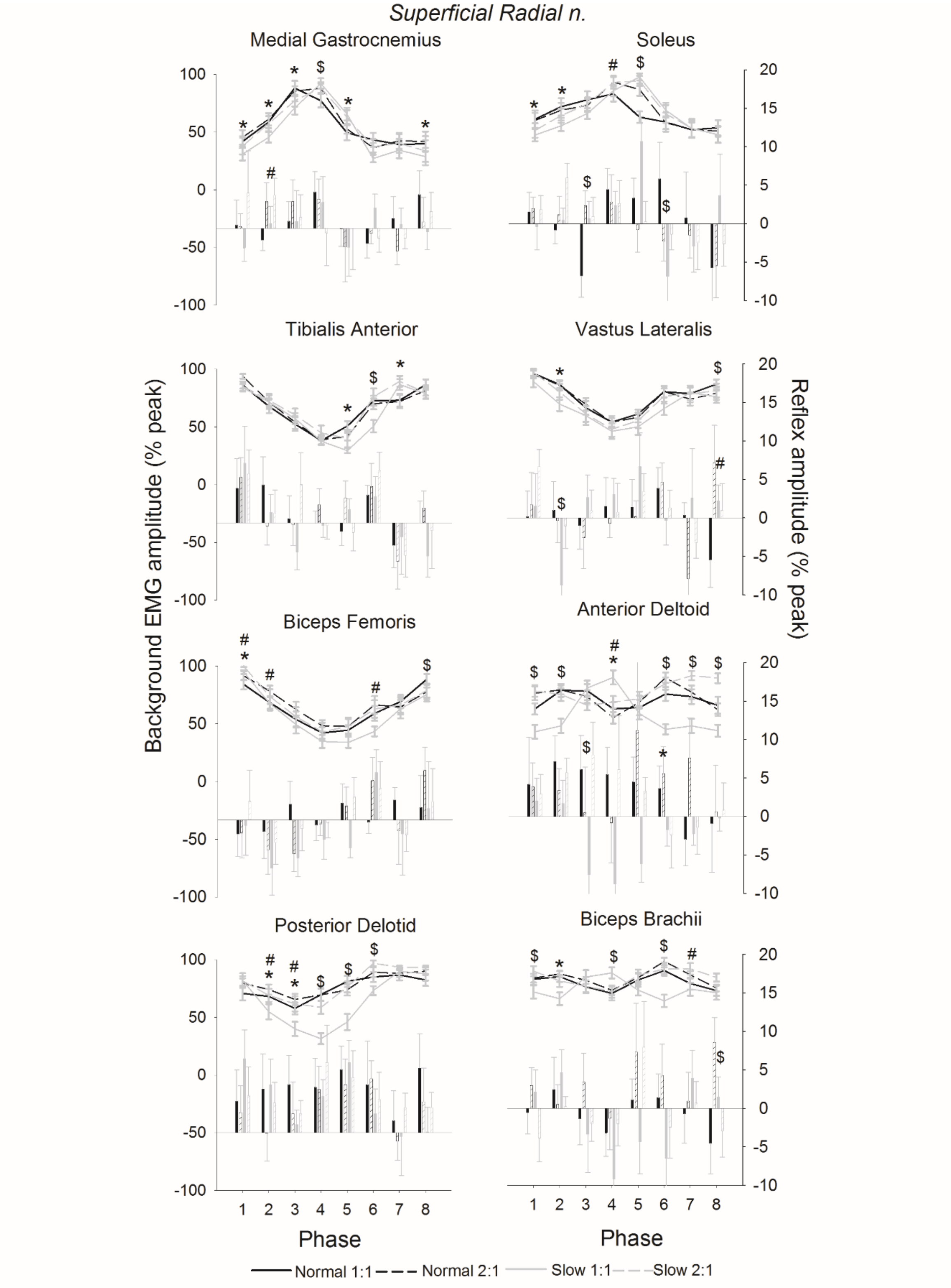


Figure 3-3: Normalized background EMG and reflex amplitudes for superficial radial nerve stimulation**.** Background EMG is shown in line plots and reflex amplitude is shown in bar plots. Values are means (±standard error) averaged across all participants and normalized to the peak undisturbed EMG during walking for each condition. Significant differences between conditions are indicated with * for main effect of walking speed, # for main effect of interlimb ratio, and $ for interaction.

Kinematic responses with superficial peroneal nerve stimulation

Figure 3-4 shows subtracted kinematic and FSR values (bars) for 8 phases of walking averaged across all participants from superficial peroneal nerve stimulation. Omnibus rmANOVA`s for all phases, walking speed, and interlimb ratio conditions revealed significant main effects and interaction effects for several of the variables tested. For the ankle, there was a significant phase by walking speed interaction ($F_{(7,77)}$=3.816, p=0.001). For the knee, there was a significant main effect of phase ($F_{(7,77)}$=3.389, p=0.003). For the heel FSR, there is a significant main effect of phase ($F_{(7,77)}$=2.334, p=0.032).

Figure 3-4 shows the mean undistributed value (lines) for 8 phases of walking averaged across all participants for all kinematic and FSR variables during superficial peroneal nerve stimulation. For background data at each phase, omnibus rmANOVA revealed several significant main effects and interaction effects. For the ankle, there was a significant phase by walking speed interaction ($F_{(7,77)}$=9.868, p=0.000). For the knee, there was a significant phase by walking speed interaction ($F_{(7,77)}$=2.534, p=0.021), and a phase by interlimb ratio interaction ($F_{(7,77)}$=3.808, p=0.001). For the elbow, there was a significant phase by walking speed ($F_{(7,77)}$=6.946, p=0.000) and phase by interlimb ratio ($F_{(7,77)}$=15.692, p=0.000) interaction, and a significant phase by walking speed by interlimb ratio interaction ($F_{(7,77)}$=5.742, p=0.000). For the shoulder, there was a significant phase by walking speed (F(7,77)=2.201, p=0.035) and phase by interlimb ratio (F(7,77)=5.366, p=0.000) interaction. In the heel FSR, there was a significant phase by walking speed interaction ($F_{(7,77)}$=4.684, p=0.000). In the medial FSR, there was a significant phase by walking speed ($F_{(7,77)}$=2.979, p=0.008) and phase by interlimb ratio ($F_{(7,77)}$=3.237, p=0.005) interaction. For the lateral FSR, there was a significant phase by walking speed ($F_{(7,77)}$=4.597, p=0.000) and phase by interlimb ratio ($F_{(7,77)}$=5.947, p=0.000) interaction. At each bin, rmANOVA revealed several significant interaction and main effects of walking speed and interlimb ratio, denoted on Figure 3-4 with a dollar sign, asterisk and hashtag, respectively.

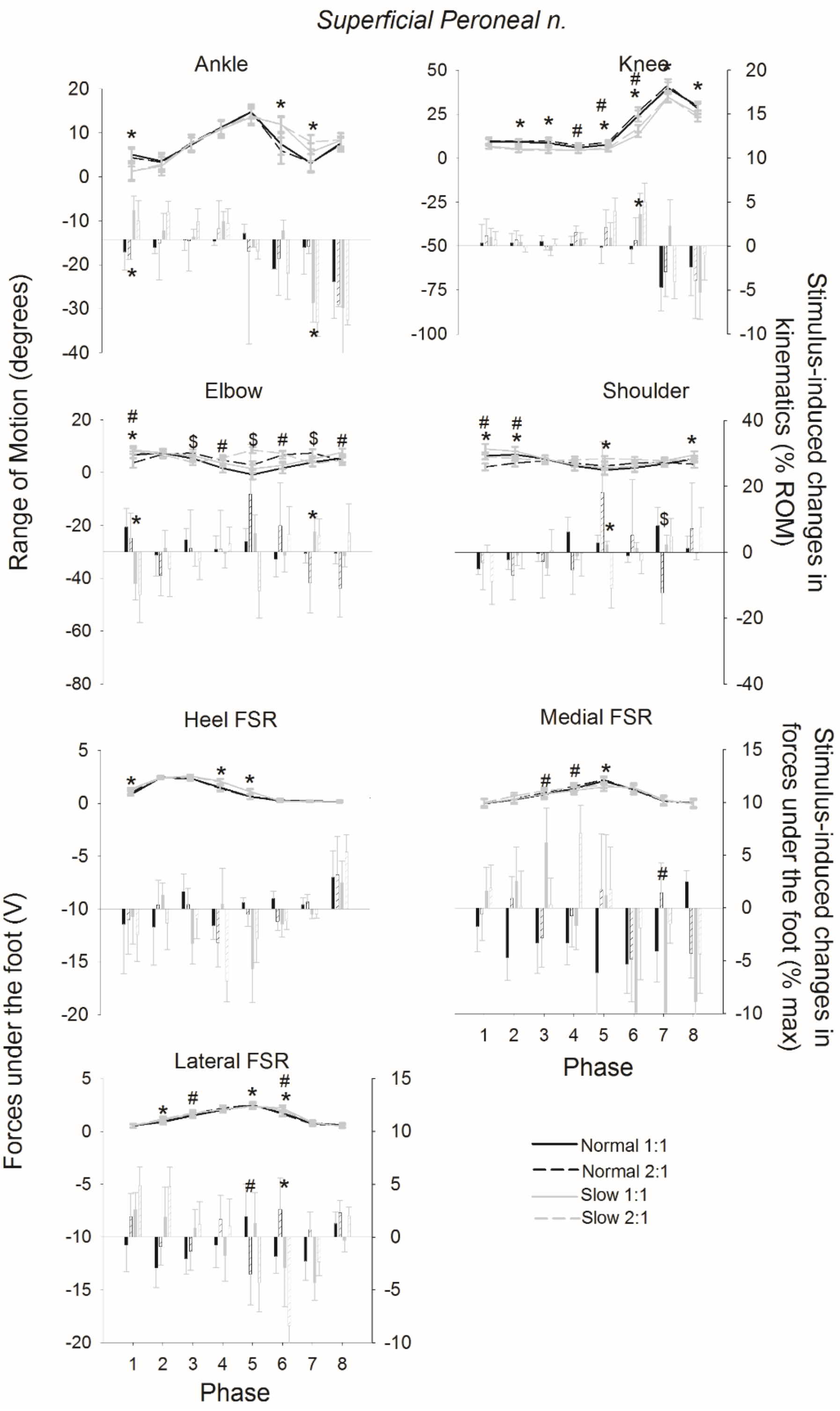


Figure 3-4: Stimulus induced changes in kinematics and force under foot for superficial peroneal nerve stimulation. Background data is shown in line plots and reflex amplitude is shown in bar plots. Values are means (±standard error) averaged across all participants and normalized to the peak undisturbed value during walking for each condition. Significant differences between conditions are indicated with * for main effect of walking speed, # for main effect of interlimb ratio, and $ for interaction.

Kinematic responses with superficial radial nerve stimulation

Figure 3-5 shows subtracted kinematic and FSR values (bars) for 8 phases of walking averaged across all participants from superficial radial nerve stimulation. Omnibus rmANOVA`s for all phases, walking speed, and interlimb ratio conditions revealed significant main effects and interaction effects for several of the variables tested. For the ankle, there was a significant phase by interlimb ratio interaction ($F_{(7,77)}$ =3.408, p=0.003). For the knee, there was a significant main effect of phase ($F_{(7,77)}$=5.121, p=0.000). For the elbow, there was a significant walking speed by interlimb ratio interaction ($F_{(1,11)}$=6.509, p=0.027). For the heel FSR, there is a significant main effect of phase ($F_{(7,77)}$ =4.710, p=0.000) and interlimb ratio ($F_{(1,11)}$=5.150, p=0.044). For the medial FSR, there is a significant main effect of phase ($F_{(7,77)}$ =4.512, p=0.000). For the lateral FSR, there was a significant main effect of phase ($F_{(7,77)}$ =2.839, p=0.011).

Figure 3-5 shows the mean undistributed value (lines) for 8 phases of walking averaged across all participants for all kinematic and FSR variables during superficial radial nerve stimulation. For background data at each phase, omnibus rmANOVA revealed several significant main effects and interaction effects. For the ankle, there was a significant phase by walking speed interaction ($F_{(7,77)}$=3.364, p=0.003). For the knee, there was a significant phase by walking speed interaction ($F_{(7,77)}$=2.377, p=0.030), and a phase by interlimb ratio interaction ($F_{(7,77)}$=2.707, p=0.043). For the elbow, there was a significant phase by walking speed ($F_{(7,77)}$=6.666, p=0.000) and phase by interlimb ratio ($F_{(7,77)}$=11.420, p=0.000) interaction, and a significant phase by walking speed by interlimb ratio interaction ($F_{(7,77)}$=5.848, p=0.000). For the shoulder, there was a significant phase by walking speed ($F_{(7,77)}$=3.276, p=0.004) and phase by interlimb ratio ($F_{(7,77)}$=10.105, p=0.000) interaction. In the heel FSR, there was a significant phase by walking speed interaction ($F_{(7,77)}$=9.212, p=0.000). In the medial FSR, there was a significant phase by walking speed interaction ($F_{(7,77)}$=2.963, p=0.008). For the lateral FSR, there was a significant phase by walking speed interaction ($F_{(7,77)}$=5.947, p=0.000).

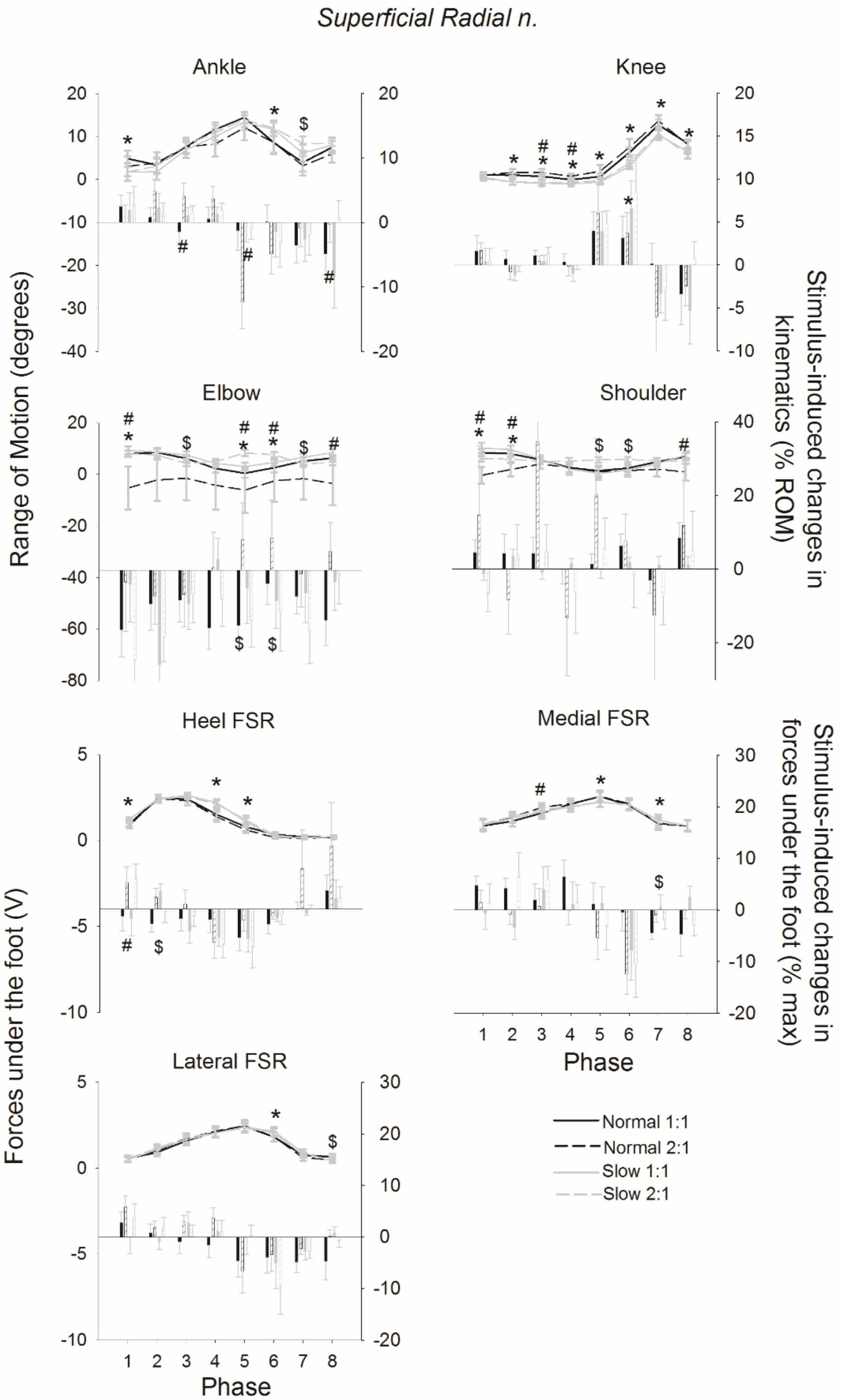


Figure 3-5: Stimulus induced changes in kinematics and force under foot for superficial radial nerve stimulation. Background data is shown in line plots and reflex amplitude is shown in bar plots. Values are means (±standard error) averaged across all participants and normalized to the peak undisturbed value during walking for each condition. Significant differences between conditions are indicated with * for main effect of walking speed, # for main effect of interlimb ratio, and $ for interaction.

Discussion

The purpose of this study was to evaluate the change in neural control that might accompany the 2:1 frequency ratio between arm and leg movements that occurs at low walking velocities. Understanding the underlying neural mechanisms on the organization of rhythmic arm movement and its coordination with the legs could enable development of effective strategies for rehabilitation of pathological walking. To test for changes in neural coordination, intralimb and interlimb cutaneous reflexes from stimulation to the superficial peroneal nerve in the foot and superficial radial nerve in the hand were examined. It was found that manipulating interlimb coordination and walking speed resulted in some changes in reflex modulation, especially for the tibialis anterior muscle. Stimulation effects from the arm to the leg was larger compared to stimulation effects from the leg to the arm. Taken together, slow walking, and the accompanying changes in interlimb ratio, does significantly modulate neural control. This observation could be mediated by possible changes in interlimb coordination of central pattern generating networks, controlling arm and leg swing and interlimb coupling, and has implications for the recovery of walking after neurological damage affecting arm and leg interactions.

Responses from superficial peroneal nerve stimulation

To assess the changes arising from alterations in interlimb coordination as a result of slow walking, the pattern of cutaneous reflex modulation during walking was evaluated. Stimulation to the superficial peroneal nerve generally produced reflex effects during the swing phase and reflexes were mostly affect by walking speed, regardless of interlimb ratio.

Electrical stimulation to the superficial peroneal nerve, innervating the dorsum of the foot, generally causes inhibition of the tibialis anterior during the swing phase leading to reduced dorsiflexion (Van Wezel et al. 1997; Zehr et al. 1997) significantly correlated with ankle plantarflexion (Zehr et al. 1997). This response seems appropriate to help the foot clear a perturbation by removing it with plantarflexion. Here, we see corroborating evidence of tibialis anterior inhibition during the swing phase from superficial peroneal nerve stimulation. We extend this observation however because we have found that tibialis anterior inhibition is also dependent upon the speed of walking, regardless of interlimb ratio. During slow walking, inhibition is increased from stimulation, especially during the late swing phase.

At the transition into the stance phase, there was also an increased modulatory effect of the normally observed inhibition in the tibialis anterior (Van Wezel et al. 1997). This inhibitory effect was modulated by walking speed where inhibition was increased with slow walking, compared to normal walking across both conditions of interlimb coordination. We also see an associated increase in plantarflexion of the ankle at the swing to stance transition that was amplified for the slow walking condition regardless of interlimb ratio.

With superficial peroneal nerve simulation, there was also an increase in knee flexion in the swing phase which was seen alongside an increase in BF reflex activity, further amplified for slow walking regardless of interlimb ratio. Facilitation of the biceps femoris and semitendinosus has previously been seen to also occur with superficial peroneal stimulation to cause knee flexion and hip extension to clear the encountered obstacle (Duysens et al. 1990).

Responses from superficial radial nerve stimulation

Stimulation to the superficial radial nerve produced facilitatory reflex effects in the posterior deltoid that were facilitatory regardless of the walking speed or interlimb coordination condition. This observation supports previous observations of facilitation from superficial radial nerve stimulation during walking (Haridas and Zehr 2003). In the anterior deltoid and biceps brachii, the pattern of reflex modulation interacted between interlimb coordination and walking speed.

As for an interlimb effect, stimulation to the wrist, caused facilitation in the tibialis anterior, similar to previous observations (Haridas and Zehr 2003). It can also be seen that stimulation caused more reflex effects in the leg muscles than reflex effects seen in the arm muscles from leg stimulation. The presence of interlimb reflexes supports the notion of connected interlimb coordination and a difference of effects suggests a directional preference for this coupling. Other studies on reduced locomotor movements, such as with arm and leg stepping or cycling, have shown that arm movement contributes to support reflexive activity in the legs

(Balter and Zehr 2007). This corresponds to similar effects from forelimb to hindlimb in the cat (Miller et al. 1975; Miller and Van Der Meche 1975). Directional coupling between the arms and legs serves a functional role in bipedal locomotion to help ensure the swinging limb is guided safely to the ground for proper foot placement and balance dependent on interactions with the arms.

Background EMG

For both nerve stimulation protocols, there were also changes in background EMG across conditions of walking speed and interlimb ratio. For the arm muscles, there were changes in background activity, that were not surprising given the changes in arm mechanics expected when the interlimb ratio changes from a 1:1 to a 2:1 coordination ratio. For the leg muscles, changes were also observed in background muscle activity. For the medial gastrocnemius generally activity was decreased in the slow walking conditions for the stance phase. There also seemed to be a shift in timing of medial gastrocnemius inhibition, where for slow walking, there was a concurrent increase in stance phase heel FSR activity causing stance to be longer in duration. In the tibialis anterior there were also changes in background activity where the general pattern of activity interacted between conditions of walking speed and interlimb ratio. Changes in reflex were also seen at this moment in the walking cycle however no correlation was found between background EMG and reflexes in the tibialis anterior. Therefore the changes in background activity, and accompanying changes in gain scaling of motoneuronal pool excitability (Duysens et al. 1993), cannot be implicated a significant source of modulation for cutaneous reflexes in the tibialis anterior.

Task organization

A consideration for the interpretation of the changes in the interlimb reflex effects seen here is to consider the functional and behavioural context of the conditions. How the nervous system controls a movement is strongly dependent upon the task (Zehr and Stein 1999), and changing interlimb coupling between the arms and the legs with slow walking changes the task. As a result, the neural control structure is modified, and as seen here, interlimb reflex control depends upon walking speed and interlimb ratio.

For arm cycling, it is interesting that changing tasks with backwards and forwards arm cycling does not modulate neural control (Zehr and Hundza 2005) and neither does changing crank length (Hundza and Zehr 2006). However when arm swing changes threaten posture and balance, changes in neural control can occur. When the arms were crossed in front of the body during an unstable walking task, a general facilitation of interlimb reflexes in the arms were observed (Haridas et al. 2005). Holding a hand rail also caused global changes in reflex thresholds across the body and facilitatory reflexes in the posterior deltoid and triceps brachii that emerged only during the swing phase and only when subjects held a rail. In addition, this facilitatory reflex was further amplified during the more challenging tasks of incline walking and stair climbing (Lamont and Zehr 2007; Forero and Misiaszek 2015). Seen here, when arm and leg interlimb coordination changed, with slow walking, changes in neural control were observed and were preferentially expressed at an unstable point in the walking cycle. This reveals a general role for interlimb reflexes that emerge to assist in interlimb communication during locomotion.

Interlimb coordination from a trans-species comparison

It has been established that arm movements during human gait affect leg activity due to neural coupling between arms and legs (Zehr et al. 2009). While human bipedal locomotor networks evolved over time, many preserved features from our early quadrupedal ancestors, remain intact, and it has been suggested that we use vestigial quadrupedal mechanisms of coordination (Dietz et al. 2001; Dietz 2002; Zehr et al. 2009; Zehr et al. 2016). Therefore, as in the cat fore-limbs (Miller et al. 1973), arm swing may be generated by CPG networks via a linkage between networks in the lumbar and cervical spinal cord (Eke-Okoro, 1991; Yang and Pang; 2000). Interactions between the formelimbs and hindlimbs have also been evidenced during transverse split-belt locomotion in intact cats (Thibaudier et al. 2014). When the back limbs step slower than the front limbs, they can adjust by an increase in stepping cadence resulting in a 2:1 fore-hind step relationship (Thibaudier et al. 2014). Even after spinal hemisection, this phasing between forelimb and hindlimb movements is maintained with the front belt moving at twice the speed of the back belt (Thibaudier et al. 2016). These data presented here, as it is in other animals, support the observation that arm swing is integrated into the neural control of locomotor movement.

The locus of control is difficult to determine although interlinked CPG networks likely have a probably involvement in the coordination. Other factors changing interlimb coordination could be due to changes in mechanical linkages and subsequent effects on afferent feedback. There could also be increased descending drive or cognitive control in the atypical tasks. It is likely that both central and peripheral mechanisms contribute to the observed results. Empirical evidence to rule out possible explanations are needed and until then, all possible mechanisms should be considered. Although the contribution from a central or peripheral mechanism is unknown, it does not diminish the possible clinical application as it functionally matters not why there might be improved motor control.

Clinical Translation

Studies evaluating arm and leg interactions are important as evidence suggests that arm integration during rhythmic activities may alter leg movement via interlimb spinal pathways connecting the arms and legs (Ferris et al. 2006; Dietz and Michel 2009; Zehr et al. 2009; Klimstra et al. 2009; de Kam et al. 2013; Zehr et al. 2016). After neurological injury, interlimb coordination is compromised as a result of disrupted neural regulation. Arm and leg interlimb coordination can be affected following stroke (Wagenaar and van Emmerik 1994; Ford et al. 2007b), incomplete spinal cord injury (Tester et al. 2012) and in early onset Parkinson's disease patients (Dietz and Colombo 1998).

In participants with spinal cord injury, the more stable 1:1 arm to leg ratio is always adopted, regardless of walking speed, in order to maintain balance and stability (Tester et al. 2012 Donker and Beek 2002). The speed-dependent transition from a 2:1 to a 1:1 frequency ratio normally observed in controls is absent in these patients which may indicate an inability of individuals with spinal cord injury to make adaptations outside of their reproducible movement patterns (Tester et al. 2012). In addition, the location of the spinal cord injury, for some of the participants, could have disrupted interlimb communication and prevented modifications in nervous system control.

The stability and adaptability of interlimb coordination is reduced in those with neurological injury. Restoring normal interlimb coordination may be enhanced by training to walk at varying velocities. Perhaps training at slow speeds, which is usually the case for those with neurological damage, and allowing the arms to swing freely and naturally would allow the natural arm coordination to emerge, perhaps facilitating rehabilitation of impaired neural networks. Understanding the underlying neural mechanism on the control of interlimb coordination at slow walking speeds in participants without neurological injury, as was done here, could enable development of effective strategies for rehabilitation of pathological walking. Devising training protocols, that make use of the bidirectional influences of the cervical and lumbar locomotor pattern generators, could strengthen interlimb coordination, and promote locomotor recovery (Thibaudier et al. 2016).

Conclusion

The purpose of this study was to evaluate the effects of altered interlimb coordination on neural control. Coordination between the arms and legs was naturally changed from a 1:1 to a 2:1 arm to leg swing frequency with a slow treadmill velocity. Results show that neural control is dependent on interlimb coordination and walking speed and effects are largest in the swing phase when walking is unstable. A directional coupling of arm to leg interlimb control was also observed where arm activity had a greater effect on leg activity compared to the reverse. Understanding the underlying neural mechanisms for the organization of rhythmic arm movement, and its coordination with the legs, can give insight into pathological walking, and will enable development of effective strategies for rehabilitation of walking.

References

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

Collins SH, Adamczyk PG, Kuo AD (2009) Dynamic arm swinging in human walking. Proc Biol Sci 276:3679–88. doi: 10.1098/rspb.2009.0664

Craik R, Herman R, Finley F (1976) Human solutions for locomtoin. II. Interlimb coordination., In R.M. He.

Dietz V (2002) Do human bipeds use quadrupedal coordination? Trends Neurosci 25:462–467. doi: 10.1016/S0166-2236(02)02229-4

Dietz V, Colombo G (1998) Influence of body load on the gait pattern in Parkinson's disease. Mov Disord 13:255–261. doi: 10.1002/mds.870130210

Dietz V, Fouad K, Bastiaanse CM (2001) Neuronal coordination of arm and leg movements during human locomotion. Eur J Neurosci 14:1906–1914. doi: 10.1046/j.0953-816x.2001.01813.x

Dietz V, Michel J (2009) Human bipeds use quadrupedal coordination during locomotion. Ann. N. Y. Acad. Sci. pp 97–103

Donker SF, Beek PJ (2002) Interlimb coordination in prosthetic walking: effects of asymmetry and walking velocity. Acta Psychol (Amst) 110:265–288. doi: 10.1016/S0001-6918(02)00037-9

Donker SF, Beek PJ, Wagenaar RC, Mulder T (2001) Coordination between arm and leg movements during locomotion. J Mot Behav 33:86–102. doi: 10.1080/00222890109601905

Donker SF, Mulder T, Nienhuis B, Duysens J (2002) Adaptations in arm movements for added mass to wrist or ankle during walking. Exp Brain Res 146:26–31. doi: 10.1007/s00221-002-1145-2

Duysens J, Tax AAM, Trippel M, Dietz V (1993) Increased amplitude of cutaneous reflexes during human running as compared to standing. Brain Res 613:230–238. doi: 10.1016/0006-8993(93)90903-Z

Duysens J, Trippel M, Horstmann GA, Dietz V (1990) Gating and reversal of reflexes in ankle muscles during human walking. Exp Brain Res. doi: 10.1007/BF00231254

Ferris DP, Huang HJ, Kao PC (2006) Moving the arms to activate the legs. Exerc Sport Sci Rev 34:113–120. doi: 10.1249/00003677-200607000-00005

Ford MP, Wagenaar RC, Newell KM (2007a) Arm constraint and walking in healthy adults. Gait Posture 26:135–141. doi: 10.1016/j.gaitpost.2006.08.008

Ford MP, Wagenaar RC, Newell KM (2007b) The effects of auditory rhythms and instruction on walking patterns in individuals post stroke. Gait Posture 26:150–155. doi: 10.1016/j.gaitpost.2006.08.007

Forero J, Misiaszek JE (2015) The amplitude of interlimb cutaneous reflexes in the leg is influenced by fingertip touch and vision during treadmill locomotion. Exp Brain Res 233:1773–1782. doi: 10.1007/s00221-015-4250-8

Gernandt B, Megirian D (1961) Ascending propriospinal mechanisms. J Neurophysiol 24:364–376.

Guertin P a (2012) Central pattern generator for locomotion: anatomical, physiological, and pathophysiological considerations. Front Neurol 3:183. doi: 10.3389/fneur.2012.00183

Haridas C, Zehr EP (2003) Coordinated interlimb compensatory responses to electrical stimulation of cutaneous nerves in the hand and foot during walking. J Neurophysiol 90:2850–61. doi: 10.1152/jn.00531.2003

Haridas C, Zehr EP, Misiaszek JE (2006) Context-dependent modulation of interlimb cutaneous reflexes in arm muscles as a function of stability threat during walking. J Neurophysiol 96:3096–103. doi: 10.1152/jn.00746.2006

Haridas C, Zehr EP, Misiaszek JE (2005) Postural uncertainty leads to dynamic control of cutaneous reflexes from the foot during human walking. Brain Res 1062:48–62. doi: 10.1016/j.brainres.2005.09.003

Huang HJ, Ferris DP (1985) Neural coupling between upper and lower limbs during recumbent stepping. J Appl Physiol 97:1299–1308. doi: 10.1152/japplphysiol.01350.2003

Hundza SR, Zehr EP (2006) Cutaneous reflexes during rhythmic arm cycling are insensitive to asymmetrical changes in crank length. Exp Brain Res 168:165–77. doi: 10.1007/s00221-005-0089-8

Juvin L, Le Gal J-P, Simmers J, Morin D (2012) Cervicolumbar coordination in mammalian quadrupedal locomotion: role of spinal thoracic circuitry and limb sensory inputs. J Neurosci 32:953–965. doi: 10.1523/JNEUROSCI.4640-11.2012 de Kam D, Rijken H, Manintveld T, et al. (2013) Arm movements can increase leg muscle activity during submaximal recumbent stepping in neurologically intact individuals. J Appl Physiol 115:34–42. doi: 10.1152/japplphysiol.00510.2012

Klarner T, Barss T, Sun Y, et al. (2014) Preservation of common rhythmic locomotor control despite weakened supraspinal regulation after stroke. Front Integr Neurosci 8:1–9.

Klimstra MD, Thomas E, Stoloff RH, et al. (2009) Neuromechanical considerations for incorporating rhythmic arm movement in the rehabilitation of walking. Chaos 19:026102. doi: 10.1063/1.3147404

Klimstra MD, Thomas E, Zehr EP (2011) Biomechanical outcomes and neural correlates of cutaneous reflexes evoked during rhythmic arm cycling. J Biomech 44:802–9. doi: 10.1016/j.jbiomech.2010.12.017

Kubo M, Wagenaar RC, Saltzman E, Holt KG (2004) Biomechanical mechanism for transitions in phase and frequency of arm and leg swing during walking. Biol Cybern 91:91–98. doi: 10.1007/s00422-004-0503-5

Lamont E V, Zehr EP (2007) Earth-referenced handrail contact facilitates interlimb cutaneous reflexes during locomotion. J Neurophysiol 98:433–42. doi: 10.1152/jn.00002.2007

Li Y, Wang W, Crompton R, Gunther M (2001) Free vertical moments and transverse forces in human walking and their role in relation to arm-swing. J Exp Biol 204:47–58.

Meyns P, Bruijn SM, Duysens J (2013) The how and why of arm swing during human walking. Gait Posture 38:555–562. doi: 10.1016/j.gaitpost.2013.02.006

Meyns P, Van De Walle P, Hoogkamer W, et al. (2014) Coordinating arms and legs on a hybrid rehabilitation tricycle: The metabolic benefit of asymmetrical compared to symmetrical arm movements. Eur J Appl Physiol 114:743–750. doi: 10.1007/s00421-013-2814-5

Miller S, Reitsma DJ, Meche FG (1973) Functional organization of long ascending propriospinal pathways linking lumbo-sacral and cervical segments in the cat. Brain Res 62:169–188.

Ortega JD, Fehlman LA, Farley CT (2008) Effects of aging and arm swing on the metabolic cost of stability in human walking. J Biomech 41:3303–3308. doi: 10.1016/j.jbiomech.2008.06.039

Park J (2008) Synthesis of natural arm swing motion in human bipedal walking. J Biomech 41:1417–1426. doi: 10.1016/j.jbiomech.2008.02.031

Pontzer H, Holloway JH, Raichlen D a, Lieberman DE (2009) Control and function of arm swing in human walking and running. J Exp Biol 212:523–534. doi: 10.1242/jeb.030478

Sakamoto M, Tazoe T, Nakajima T, et al. (2007) Voluntary changes in leg cadence modulate arm cadence during simultaneous arm and leg cycling. Exp Brain Res 176:188–192. doi: 10.1007/s00221-006-0742-x

Skinner RD, Adams RJ, Remmel RS (1980) Responses of long descending propriospinal neurons to natural and electrical types of stimuli in cat. Brain Res 196:387–403. doi: 10.1016/0006-8993(80)90403-5

Tester NJ, Barbeau H, Howland DR, et al. (2012) Arm and leg coordination during treadmill walking in individuals with motor incomplete spinal cord injury: a preliminary study. Gait Posture 36:49–55. doi: 10.1016/j.gaitpost.2012.01.004

Thibaudier Y, Frigon A (2014) Spatiotemporal control of interlimb coordination during transverse split-belt locomotion with 1:1 or 2:1 coupling patterns in intact adult cats. J Neurophysiol 2006–2018. doi: 10.1152/jn.00236.2014

Thibaudier Y, Hurteau M-F, Dambreville C, et al. (2016) Interlimb Coordination During Tied-Belt and Transverse Split-Belt Locomotion Before and After an Incomplete Spinal Cord Injury. J. Neurotrauma

Umberger BR (2008) Effects of suppressing arm swing on kinematics, kinetics, and energetics of human walking. J Biomech 41:2575–2580. doi: 10.1016/j.jbiomech.2008.05.024

Wagenaar RC, Van Emmerik REA (2000) Resonant frequencies of arms and legs identify different walking patterns. J Biomech 33:853–861. doi: 10.1016/S0021-9290(00)00020-8

Wagenaar RC, van Emmerik REA (1994) Dynamics of pathological gait. Hum Mov Sci 13:441–471. doi: 10.1016/0167-9457(94)90049-3

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

Van Wezel BMH, Ottenhoff FAM, Duysens J (1997) Dynamic Control of Location-Specific Information in Tactile Cutaneous Reflexes from the Foot during Human Walking. J Neurosci 17:3804–3814.

Zehr E, Barss T, Dragert K, et al. (2016) Neuromechanical interactions between the limbs during human locomotion: an evolutionary perspective with translation to rehabilitation. Exp Brain Res. doi: 10.1007/s00221-016-4715-4

Zehr E, Nakajima T, Barss T, et al. (2014) Cutaneous stimulation of discrete regions of the sole during locomotion produces “sensory steering” of the foot. BMC Sport Sci. Med. Rehabil. 6:

Zehr EP, Fujita K, Stein RB (1998) Reflexes from the superficial peroneal nerve during walking in stroke subjects. J Neurophysiol 79:848–58.

Zehr EP, Hundza SR (2005) Forward and backward arm cycling are regulated by equivalent neural mechanisms. J Neurophysiol 93:633–40. doi: 10.1152/jn.00525.2004

Zehr EP, Hundza SR, Vasudevan E V (2009) The quadrupedal nature of human bipedal locomotion. Exerc Sport Sci Rev 37:102–8. doi: 10.1097/JES.0b013e31819c2ed6

Zehr EP, Komiyama T, Stein RB (1997) Cutaneous reflexes during human gait: electromyographic and kinematic responses to electrical stimulation. J Neurophysiol 77:3311–25.

Zehr EP, Loadman PM (2012) Persistence of locomotor-related interlimb reflex networks during walking after stroke. Clin Neurophysiol 123:796–807. doi: 10.1016/j.clinph.2011.07.049

Zehr EP, Stein RB (1999) What functions do reflexes serve during human locomotion? Prog Neurobiol 58:185–205.

Chapter 4 Cutaneous reflexes evoked from discrete sites on the foot dorsum have a topographical organization that shapes muscle activation and limb trajectory during locomotion[2]

Abstract

Stimulating the main nerve innervating the foot dorsum produces a functional stumble corrective response that is phase-dependently modulated. We speculated that effects evoked by activation of discrete skin regions on the foot dorsum would be topographically organized, as with the foot sole. Non-noxious electrical stimulation was delivered to five discrete locations on the dorsal surface of the foot during treadmill walking. Muscle activity from muscles acting at the ankle, knee, hip and shoulder were recorded along with ankle, knee and hip kinematics and kinetic information from forces under the foot. All data were sorted based on stimulus occurrence in twelve step-cycle phases, before being averaged together within a phase for subsequent analysis. Results reveal statistically significant dynamic changes in reflex amplitudes and kinematics that are site-specific and phase-dependent. Most responses from discrete sites on the foot dorsum were seen in the swing phase and results suggest that responses functionally served to conform foot trajectory to maintain stability of the swinging limb. In general, responses from lateral stimulation differed from medial stimulation and effects were most prominent from stimulation at the distal end of the foot at the metatarsals. Thus, in anatomical locations where actual impact with an object in the environment is most likely. Responses to stimulation extend to include muscles at the hip and shoulder. These data increase our understanding of how afferent feedback from specific cutaneous locations on the foot dorsum influences the mechanisms involved in stance and swing phase corrective responses. This may provide potential rehabilitative application to restore these normal corrective responses following neurological injury and for the development of footwear that serves to facilitate normal action in these pathways.

---

[2] Klarner T, Pearcey G, Sun Y, Barss T, Munro B, Frank N, and Zehr EP (2016) Cutaneous reflexes evoked from discrete sites have a topographical organization. Under Revision. MSSE-S-16-01404

Introduction

Walking requires complex neurological interaction between supraspinal and spinal mechanisms. In addition to central nervous system processing, afferent feedback is required to effectively regulate rhythmic locomotor activity in accordance with environmental demands. Feedback from nociceptors, muscle and skeletal mechanoreceptors as well as cutaneous and subcutaneous mechanoreceptors dynamically interact to sculpt locomotor output (Zehr and Stein 1999; Kennedy and Inglis 2002; Rossignol et al. 2006). Although all types of feedback (and their related reflexes) are important, exteroceptive information from cutaneous receptors permeating the skin are particularly important in the fine regulation of walking (Zehr and Stein 1999).

Response to somatosensory feedback is not stereotyped and instead reflex sign and amplitude depends on the context of the movement and is preferentially expressed according to the phase of movement. Reflexes are gated differently depending on the location (e.g. nerve) of the stimulus, a characteristic known as 'local sign' (Sherrington 1947). The area of the foot that is mechanically touched or electrically stimulated affects the type of response observed where touch to different areas signals different types of problems that require different corrective responses (Van Wezel et al. 1997; Zehr et al. 1997; Zehr et al. 1998b). Stimulation to the foot sole produces a response that is different from stimulation to the foot dorsum and these responses also depend on the phase of walking.

During stance, receptors in the foot sole are required to perceive the ground during locomotion and indicate destabilization of posture producing responses to counteract uneven terrain through ankle joint stabilization (Nakajima et al. 2006; Nakajima et al. 2009; Zehr et al. 2014). In standing, forefoot stimulation caused inhibition in the soleus and medial gastrocnemius but excitation in tibialis anterior and the opposite occurred following heel stimulation (Nakajima et al. 2006). In walking, increased resolution obtained by stimulation at five discrete areas on the foot sole revealed site-specific topographical organization of cutaneous reflexes (Zehr et al. 2014). Activation under the foot sole produced a kind of tuned 'sensory steering' response directed at guiding the foot away from or around a perceived obstacle (Zehr et al. 2014). This suggests, somatosensory feedback from the foot sole acts in a functional and location-specific manner to maintain stability and balance.

During the swing phase, corrections are ready to compensate for a perturbation which obstructs the forward progression of the limb if we hit an unexpected obstacle on the top of the foot. The rapid correction needed to prevent a trip has been termed a ‘stumbling corrective reaction’ (Forssberg et al. 1975) and has been observed in decerebrate and intact cats as well as human infants and adults (Prochazka et al. 1978; Forssberg 1979; Wand et al. 1980; Drew and Rossignol 1987; Buford and Smith 1993; Eng et al. 1994; Schillings et al. 1996; Van Wezel et al. 1997; Zehr et al. 1997; Lam et al. 2003). In human adults, the stumble corrective reaction consists of increased knee flexion and a decrease in ankle dorsiflexion during the swing phase allowing for smooth forward progression as the leg is lifted over an obstacle to preserve balance during walking. This response is observed with both mechanical and electrical stimulation to the foot dorsum (Forssberg 1979; Schillings et al. 1996) and is carefully controlled by the nervous system in a phase-dependent manner such that the same touch during the stance phase of walking, likely not hindering walking, elicits a less consistent response. Task specificity of the corrective stumble response also applies where a flexion response is observed during forward and backward walking (Buford and Smith 1993; Duysens et al. 1996) and the response is amplified and spreads to the arms when the arms are crossed, producing instability, compared to the arms swinging freely (Haridas et al. 2006).

We have seen that cutaneous responses are dictated by the location of stimulation on the plantar foot surface, and we expect the same fine tuning of location-specific responses for foot dorsum stimulation. The purpose of the current study was to examine cutaneous reflexes evoked by stimulation at discrete foot dorsum regions during locomotion. Additionally, we simultaneously looked to determine the neuromechanical effect of cutaneous inputs from discrete sites during walking. The two main hypotheses tested in this study were: 1) that stimulation at discrete skin locations on the foot dorsum would evoke topographically discrete cutaneous reflexes during walking; and, 2) that cutaneous reflexes would have mechanical correlates detected as changes in kinematics of the stimulated leg.

Methods

Participants

Fifteen neurologically intact volunteers (7 males and 8 females) participated in the study. Participants were 22.8 ± 2.9 years old, with an average height of 170.8 ± 10.3 cm and weight of 68.2 ± 14.1 kg. Informed, written consent was obtained from all participants prior to the experiment under a protocol approved by the University of Victoria Human Research Ethics Committee and performed in accordance with the Declaration of Helsinki.

Experimental protocol

To improve procedural standardization and consistency, all participants were fitted with the same make and model of running shoe (Nike Free 4.0 Flyknit) and the same sock (Nike Elite Dri-Fit Sock). Participants walked on a motorized treadmill (Woodway USA, Waukesha, WI) at a self-selected, comfortable walking pace (0.8± 0.13 m/s) that remained constant throughout the experiment. During each trial, participants walked continuously while electrical stimulation was delivered to one of the five stimulation sites for five separate trials. Background data was collected simultaneously for each trial. The order of presentation for each of the five trials was randomized.

Cutaneous stimulation

Stimulating electrodes (Grass, 10 mm Gold Cup-Disk Electrodes) were filled with electrode conductive gel (Spectra 360 Electrode Gel, Parker Laboratories, INC, Orange, NJ) and taped onto the 5 different sites on the dorsal surface of each participant's right foot with the cathode positioned distally. The five sets of electrode pairs were located at the: 1) distal end of the 1st metatarsal (red); 2) distal end of the 4th metatarsal (orange); 3) middle medial skin surface (yellow); 4) middle lateral skin surface (green); and, 5) ankle crease (blue). These locations can be seen in each figure legend. Electrodes and lead wires were adhered to the surface of the skin with flexible tape (BSN Medical, Hypafix Tape) and the sock and show were then placed over the electrodes. Stimulation was provided by a Digitimer Constant Current High Voltage Stimulator (Model DS7AH) with trains of 5 x 1.0 ms pulses at 300 Hz. During each trial, a total of 160 randomly-timed stimulations (1 – 3 seconds) were delivered throughout the step cycle.

Immediately prior to each trial, perceptual threshold (PT) was determined for each stimulation site. PT was defined as the stimulus intensity found to evoke a detectable tactile sensation at the lowest intensity possible. Participants remained standing while stimulation intensities were gradually decreased by the researchers until the participant could barely discern the stimulus (identified as PT). The stimulation intensity delivered to the foot dorsum was set to approximately three times PT. The stimulation intensity of 3 x PT was chosen in order to evoke a non-noxious cutaneous sensation during each trial by activating cutaneous afferents immediately under the electrodes and to provide the same relative activation at all stimulation sites. Participants described this in self-report as a fluttering, buzzing, or prickly sensation.

Electromyography

Once the skin was cleaned with alcohol wipes, disposable surface electromyography (EMG) electrodes were placed on the skin over muscles in the upper and lower leg and shoulder. All EMG recordings were ipsilateral to the site of stimulation (right side). Muscles included tibialis anterior (TA), medial gastrocnemius (MG), peroneus longus (PL), vastus lateralis (VL), biceps femoris (BF), gracilis (GR), gluteus medius (GM) and posterior deltoid (PD). These muscles were chosen so that comparisons could be made to previous research (Zehr et al. 2014). Ground electrodes were placed over electrically neutral tissue at the knee cap and medial olecranon of the elbow. EMG signals were amplified at 5000 times and filtered from 100-300 Hz (Grass P511, Astro-Med Grass Inc.).

Force sensing resistors and kinematics

Force sensing resistors (FSRs) were firmly attached to the insole of the participant's right shoe at the heel, head of the first metatarsal, and distal end of the fifth metatarsal. Validity of FSRs has been previously shown (Zehr et al. 1995) and this measure has been useful in determining changes in under-foot kinetics (Zehr et al. 2014). Throughout the experiment, force signals were pre-amplified and recorded for offline analysis. Angular positions of the hip (flexion/extension), knee (flexion/extension), and ankle (inversion/eversion and plantar/dorsiflexion) were measured with electrogoniometers (Biometrics Ltd., Gwent, UK) positioned over the sagittal planes of the ankle, hip and knee. These devices were calibrated and output in degrees was recorded. As with similar studies (Zehr et al. 1997; Haridas et al. 2006;

Lamont and Zehr 2007; Zehr and Loadman 2012), all data were sampled at 1000 Hz with a 12 bit A/D converter connected to a computer running custom-written LabVIEW (National Instruments Corp., Austin, Texas, USA) acquisition software and stored for off-line analysis.

Data analysis

All data were analyzed with custom written software (MATLAB, MathWorks, Inc., Natick, MA, USA). Background EMG data were full-wave rectified and low pass filtered at a cut-off frequency of 100 Hz with a 4th order Butterworth filter, kinematic data were low pass filtered at a cut-off frequency of 6 Hz with a 4th order Butterworth filter and mechanical data were low pass filtered at a cut-off frequency of 20 Hz using a 4th order Butterworth filter. FSR signals from the foot sole were summed and used to establish step cycle parameters; heel contact (HC), periods of stance, toe-off (TO) and swing based on methods previously described (Zehr et al. 1997). For each step in each condition, EMG, FSR and kinematic data were normalized to 100% of the gait cycle and averaged together to create a representative step. For comparisons between participants EMG data of each muscle from each dorsal stimulation site condition were normalized to the peak average value of that muscle's activation pattern for that trial for each participant.

To examine cutaneous reflexes, the step cycle was divided into 12 separate equally-timed phases, beginning with Phase 1 (early stance) at HC, through to Phase 7 (swing transition), Phase 8 (early swing), and ending with Phase 12 (stance transition) at the subsequent HC (Zehr et al. 2014). Responses for all data for each stimulus condition occurring in the same phase of the step cycle (n=~10-20 responses per phase) were aligned to stimulus delivery and averaged together. Averages from the same phase of walking during unstimulated cycles ("control" EMG n=~50–60 per phase) were then subtracted from each of the corresponding 12 averages after stimulation yielding subtracted traces of reflex EMG, and stimulation-induced changes in kinematics. For evaluation of the effect of stimulation, the subtracted data traces were analyzed in all instances.

Cutaneous reflexes were quantified as the average cumulative reflex occurring 150 ms after stimulation (ACRE150). This quantification method may not accurately capture the cases where after stimulation the EMG is initially inhibited and then excited, but it is useful in determining if overall excitation or inhibition is changed between conditions. As with previous studies (Zehr et al. 1997; Zehr et al. 1998a; Klarner et al. 2014), this measure involved calculating a subtracted reflex (see above) then cumulatively summing the signal over a post-stimulus period of 150 ms and dividing by the time interval of integration to measure an overall reflex effect. Net reflex values were normalized to peak control EMG amplitude. Changes in kinematic and FSR data due to stimulation were analyzed within a 140-220 ms window post stimulus (Zehr et al. 1997; Klimstra et al. 2011). Responses were considered significant if they exceeded a 2-SD band of the mean value of the subtracted pre-stimulus level of the ongoing kinematic or mechanical parameter at each phase.

Statistics

All statistical analyses were completed using SPSS version 18 (Chicago, IL). In all instances, analysis was conducted on averaged data from each part of the step cycle for each participant. Each variable was analyzed separately, as was each phase of walking, in order to determine if the site of stimulation at a specific location on the foot dorsum and during a particular part of the step cycle had a discernible effect.

To test our first hypothesis that stimulation at discrete locations on the foot dorsum would evoke topographically distinct cutaneous reflexes, the initial approach for all data was to conduct omnibus 12 (phase) x 5 (dorsal sites of stimulation) repeated measures ANOVAs. To address our hypothesis of site-dependency of dorsal skin site simulation, and using an approach applied in prior work (Lamont and Zehr 2006; Lamont and Zehr 2007), as a follow up to significant main effects for site, each phase of walking was further investigated. Thus a 1 (phase) x 5 (dorsal sites of stimulation) repeated measures ANOVA was completed. Note that all significant differences indicated on the figures showed a significant 1 x 5 ANOVA main effect. Fisher's LSD post-hoc tests were used to determine site specific differences.

All statistical tests were 2-tailed and significance was accepted at $p < 0.05$. Thus, all data described in the following text or shown in the accompanying figures and described as “significant” or indicated with an * were determined as a main effect or interaction from the Omnibus and from the RM ANOVA.

Results

Background EMG and kinematics

Figure 4-1 shows the grand average background data (without stimulation) across all participants from all stimuli given across the entire step cycle (that is, averaged together and normalized). Background data for all muscles collected and kinematics averaged across all participants showed no differences between foot dorsum site conditions. Therefore, differences from stimulation in EMG or kinematics between dorsal foot sites are due to stimulation and not due to changes in background data. There were also no correlations between the amplitude of the response and with the amplitude of muscle activation across phases of walking.

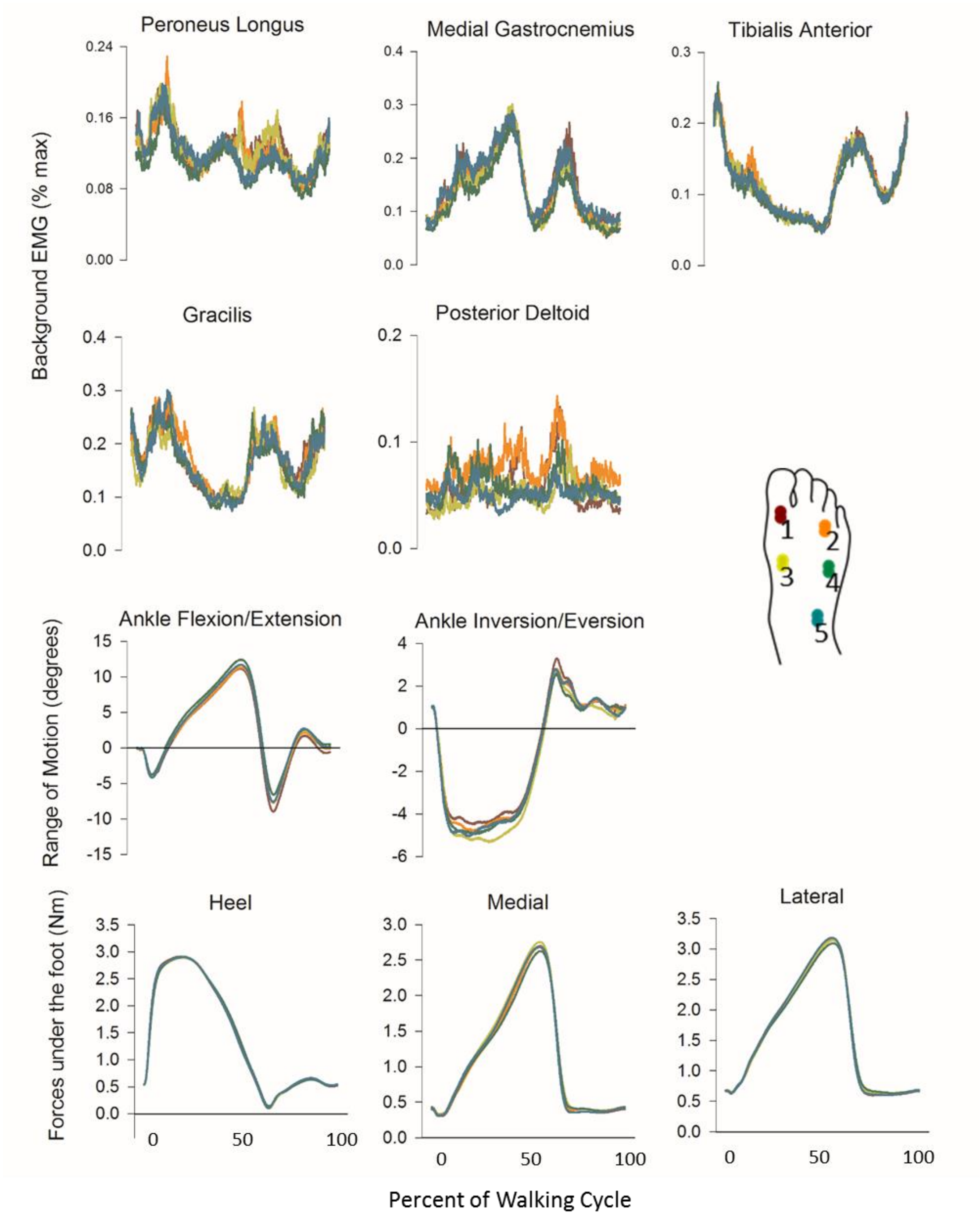


Figure 4-1: Background amplitudes for muscles, ankle kinematics, and FSR data averaged across all participants for each condition. The cartoon foot diagram containing five pairs of coloured dots refers to dorsal foot skin stimulation sites.

Cutaneous reflexes

Main effects of foot dorsum stimulation site emerged for several of the muscles tested including PL, MG, TA, GR and PD (statistical details can be found beneath each variable on the following figures). Given there were no statistical effects of site for VL, BF and GM these muscles will not be discussed further and the focus will remain on muscles with significant site-specific main effects. In several instances (for PL, TA, PD muscles) significant main effects of phase are noted by statistical analysis with the omnibus repeated measures ANOVA. At phases of the walking cycle with prominent and important location-specific effects, data are highlighted with a black outline.

Quantified data for each phase are shown for PL, MG, and TA in Figure 4-2. As indicated on Figure 4-2, PL reflexes showed significant main effects for site and phase. When examining site dependence at specific phases, several stimulation conditions influenced the sign of the evoked response. In PL (a muscle functioning as both an ankle evertor and plantar flexor) significant site-dependence of foot dorsum stimulation was found in 2 of the 12 step cycle phases: at the swing transition (phase 7) and early swing (phase 8). At these phases, there was predominately facilitation except at the middle medial skin surface for phase 7 and at the distal end of the 4th metatarsal for phase 8. Additionally, when considering the sign of the response, in the stance phases, there was a predominately inhibitory effect for phases 3-5 while during swing there was a predominately facilitatory effect (for phase 11).

Reflexes in MG muscle, a plantar flexor and ankle evertor, showed a significant main effect for site. Figure 4-2 shows quantified data for each phase in walking and 3 out of the 12 phases showed significant site-dependence. At early stance (phase 2) stimulation to the middle medial skin surface showed facilitation and stimulation to the middle lateral skin surface showed inhibition. In general, however, stimulation during the stance phase was predominately facilitatory. At the swing transition (phase 7), all stimulation sites showed facilitation except for the ankle crease site while at early swing (phase 8) stimulation at the lateral skin surface showed increased activation compared to other stimulation sites. In general, swing phases (phases 9, 11 and 12) showed facilitation.

In TA muscle, an ankle dorsiflexor and invertor, cutaneous reflexes showed a significant main effect for phase and site. Quantified reflexes at each phase for TA are shown in Figure 4-2. There was general inhibition evident for swing (phases 8-12) and at phase 9, inhibition was largest for the distal end of the 1st metatarsal skin site compared to all other dorsal skin sites.

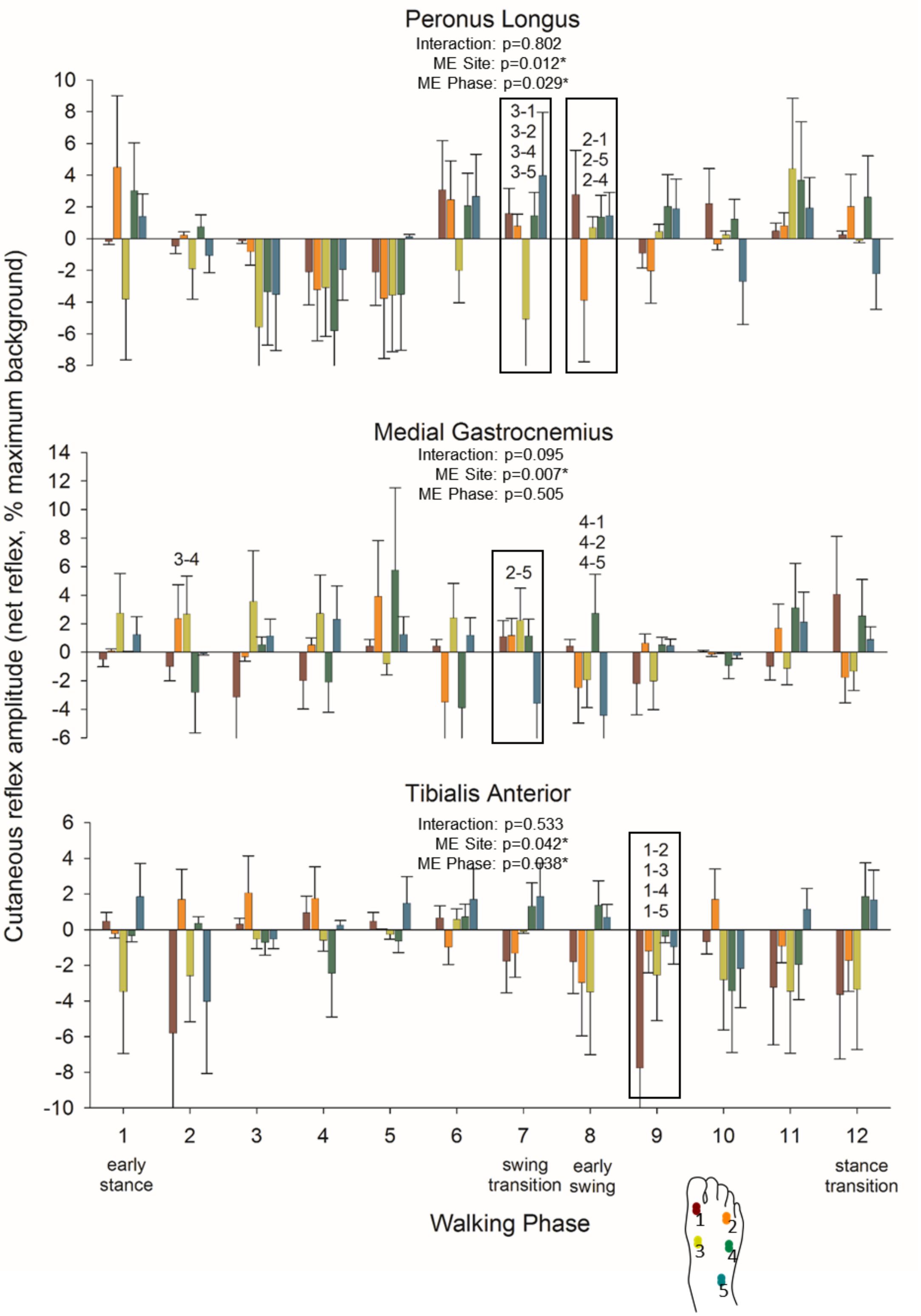


Figure 4-2: Average quantified net (ACRE150) cutaneous reflexes across all 12 phases of the step cycle for peroneus longus (PL), medial gastrocnemius (MG), and tibialis anterior (TA). Data are percentages normalized to maximum background EMG measured across all phases of walking. Negative values indicate overall suppression and positive values overall facilitation of muscle activity. * indicates statistical differences at p<0.05 between

stimulation conditions within a phase. The cartoon foot diagram containing five pairs of coloured dots refers to dorsal foot skin stimulation sites.

Site dependence was also evident for GR, a hip adductor and flexor and a knee flexor, and quantified reflexes are shown for each phase in Figure 4-3. Significant site-dependence is mainly observed during the swing (phase 10) and at the transition from swing to stance (phase 12). At phase 10, medial foot dorsum stimulation, specifically at the 1st metatarsal and at the middle medial skin surface, caused GR inhibition. Whereas lateral dorsal skin site stimulation shows facilitation. At the stance transition, stimulation at the 1st metatarsal produces GR inhibition compared to stimulation at the 4th metatarsal and at the middle medial dorsal skin surface.

PD muscle, a muscle contributing to shoulder abduction and extension, showed a significant main effect of phase and site. Quantified reflexes (see Figure 4-3) reveal that in stance (phase 3) facilitation from middle lateral stimulation was higher than for medial stimulation at the 4th metatarsal and during stance (phases 5 and 6) there was an overall facilitation of PD from all stimulation sites. At end stance (phase 6), stimulation at the 1st metatarsal causes increased facilitation. During swing facilitations were seen. At the swing phase (phase 9), stimulation to the 1st metatarsal and ankle crease evoked excitation compared to other stimulation sites. At the swing to stance transition (phase 12), inhibition was evoked by stimulation at the 1st metatarsal compared to stimulation at other sites.

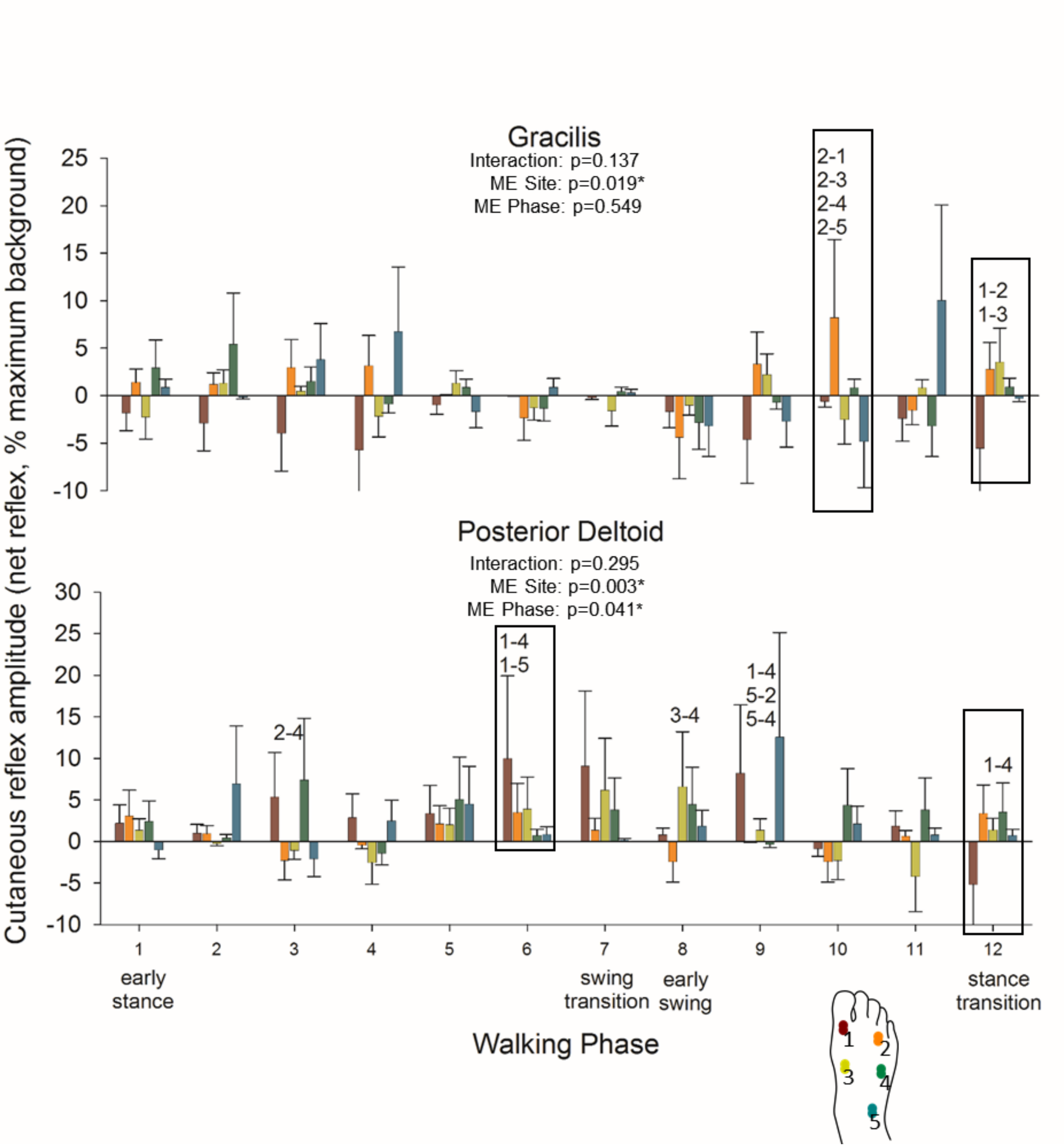


Figure 4-3: Average quantified net (ACRE$_{150}$) cutaneous reflexes across all 12 phases of the step cycle for gracilis (GR) and posterior deltoid (PD). Data are percentages normalized to maximum background EMG measured across all phases of walking. Negative values indicate overall suppression and positive values overall facilitation of muscle activity. * indicates statistical differences at $p<0.05$ between stimulation conditions within a phase. The cartoon foot diagram containing five pairs of coloured dots refers to dorsal foot skin stimulation sites.

Kinematics

Reflex-induced changes in kinematics were also inspected for main effects of phase and site and for interaction between phase and site. Omnibus ANOVA revealed a significant effect of site for ankle inversion/eversion (I/V) ($F_{(4,56)} = 10.071$, p=0.000) and ankle plantar/dorsiflexion

(P/D) ($F_{(4,56)} = 3.58$, p=0.037). There were no significant main effects in the knee or hip data, therefore these data are not plotted or discussed further.

Ankle plantar/dorsiflexion

As stated above, statistical analysis of ankle plantar/dorsiflexion revealed a main effect for site. Data for plantar/dorsiflexion along with summary results from the statistical tests at each phase are plotted in Figure 4. During stance phase, reduced dorsiflexion was observed. At the end of stance (phase 6), stimulation at the ankle crease caused dorsiflexion while sites on the lateral margin of the foot caused plantar flexion (by reduced dorsiflexion). In the swing phase, reduced dorsiflexion is evident for most phases at most sites.

Ankle inversion-eversion

Data for stimulus-induced changes in ankle inversion-eversion across phases of the step cycle are shown in Figure 4. Significant site-dependent changes in kinematics were detected at the ankle for inversion and eversion during stance (phase 2) and throughout swing (phases 7-11). During stance stimulation to the dorsal foot surface caused predominately inversion at the ankle. At early stance (phase 2), inversion was noted for all stimulation sites except for stimulation at the 1st metatarsal where stimulation reduces inversion. During swing, all phases showed significant effects of stimulation site. At the swing transition and in early swing, stimulation to the medial dorsal skin surface (at the 1st metatarsal and in the middle of the foot) reduced inversion, while stimulation to the lateral part of the foot (at the 4th metatarsal and in the middle of the foot) increased inversion. This trend was generally seen for phases 8-11. At the swing to stance transition, all stimulation sites reduced inversion and at the distal end of the foot, the reduction was largest. In general, stimulation to the medial side of the foot dorsum caused eversion and stimulation to the lateral foot dorsum caused inversion.

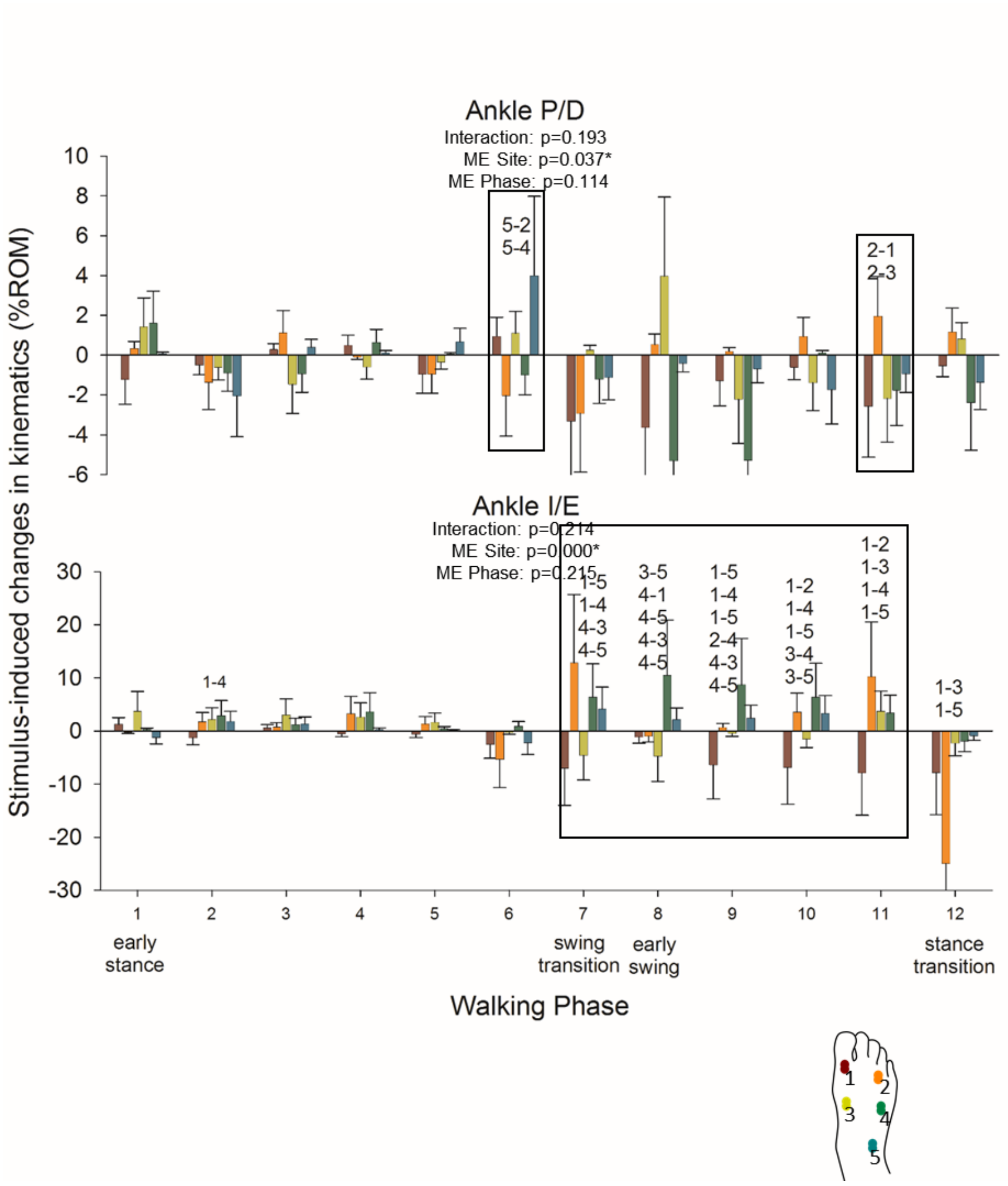


Figure 4-4: Stimulation-induced average changes in ankle joint kinematics for plantar flexion/dorsiflexion (P/D) (dorsiflexion = up) and ankle inversion/eversion (I/V) (inversion = up). Data are percentages normalized to maximum range of motion across all phases of walking. * indicates statistical differences at $p<0.05$ between stimulation conditions within a phase. The cartoon foot diagram containing five pairs of coloured dots refers to dorsal foot skin stimulation sites.

Kinetics

Stimulus-induced changes in kinetics were also inspected for main effects of phase and site and for interaction between phase and site. Omnibus ANOVA revealed a significant effect of site for the heel FSR ($F_{(4,56)} = 2.642$, $p=0.038$), a main effect of site and phase for the medial FSR

($F_{(4,56)}$ = 1.490, p=0.042 and $F_{(4,56)}$ = 2.469, p=0.007, respectively) and a main effect of site for the lateral FSR ($F_{(4,56)}$ = 2.575, p=0.047).

Heel FSR

Data from the heel FSR along with results from the statistical tests at each phase are plotted in Figure 5. During early stance, medial stimulation at the 1st metatarsal and medial skin surface tended to produce an increase in force at the heel FSR compared to lateral metatarsal simulation and ankle crease stimulation. In mid stance, all skin stimulation sites increased force at the heel FSR. At the stance transition most stimulation sites produced increased force at the heel FSR with stimulation at the lateral foot site producing the greatest amount of force.

Medial FSR

Data from the medial FSR along with results from the statistical tests at each phase are plotted in Figure 5. Medial FSR showed site dependence at early (phase 2) and mid to late stance (phase 4, 5, and 6). At early stance, stimulation at the 1st metatarsal increased medial FSR pressure compared to ankle crease stimulation. In mid stance (phase 4) a general reduction in medial FSR force was observed with stimulation at the ankle crease producing an increased reduction in force. At end stance (phase 5 and 6), stimulation at the lateral dorsal skin surface tended to increase medal FSR force compared to stimulation at the medial skin surface and ankle crease. At the stance transition (phase 12) stimulation to all dorsal skin sites tended to increase medial FSR force.

Lateral FSR

Data from the lateral FSR along with results from the statistical tests at each phase are plotted in Figure 5. Site dependence of dorsal skin site stimulation was observed in mid stance (phase 3) where stimulation to the 4th metatarsal produced increased lateral FSR force compared to stimulation at the lateral skin site. At the stance transition (phase 12) stimulation to all skin sites tended to increase lateral FSR force.

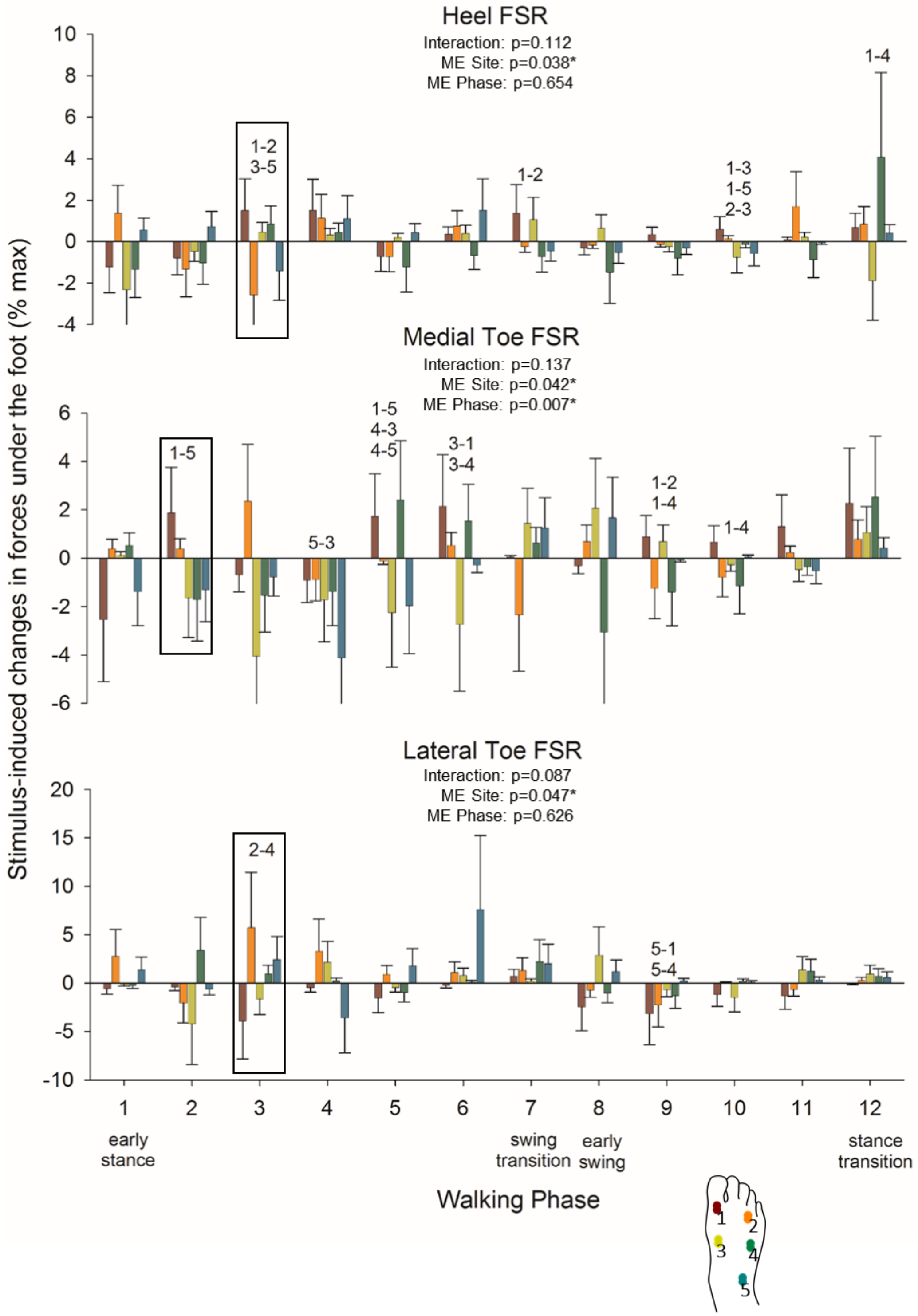


Figure 4-5: Stimulation-induced average changes in forces under the foot detected by FSRs at the heel, medial foot, and lateral foot. Data are percentages normalized to maximum FSR load detected in the stance phase of walking. * indicates statistical differences at $p<0.05$ between stimulation conditions within a phase. The cartoon foot diagram containing five pairs of coloured dots refers to dorsal foot skin stimulation sites.

Discussion

In this paper we examined the site and phase-dependency of gait adaptations in response to non-noxious cutaneous stimulation at five discrete sites on the dorsal foot surface. We evaluated reflex and neuromechanical effects in order to explore the topographical organization of cutaneous afferents innervating the foot dorsum. Our results indicate that cutaneous inputs from discrete regions on the foot dorsum evoke location-specific reflexes in muscles acting at the ankle. As well, the topographic distribution of responses in muscle, skin stimulation produced changes in ankle kinematics.

In particular, in the stance phase topographic effects are prominent in forces under the foot, and in the swing phase topographic effects are prominent in ankle muscle and kinematic data. In general, medial and lateral stimulation evoke increased medial and lateral forces in the stance phase and in the swing phase cause ankle eversion and inversion, respectively. Functionally these responses serves to tilt the foot away from the perturbation. As with stimulation of whole cutaneous nerves innervating the foot dorsum from the superficial peroneal nerve, changes were found in muscles distant from the ankle. As discussed below, responses at each phase of walking, and their functional interpretations, are given.

Topographical organization in each phase of locomotion

Interpretations of the integrated neuromechanical responses from stimulation at discrete foot dorsum regions are organized within the functional phases of walking: stance, stance to swing transition, swing, and swing to stance transition. Figure 6 includes a diagram of the functional effects of dorsal skin surface stimulation during the stance and swing phases of walking. In some cases, the functional effects of stimulation are proposed.

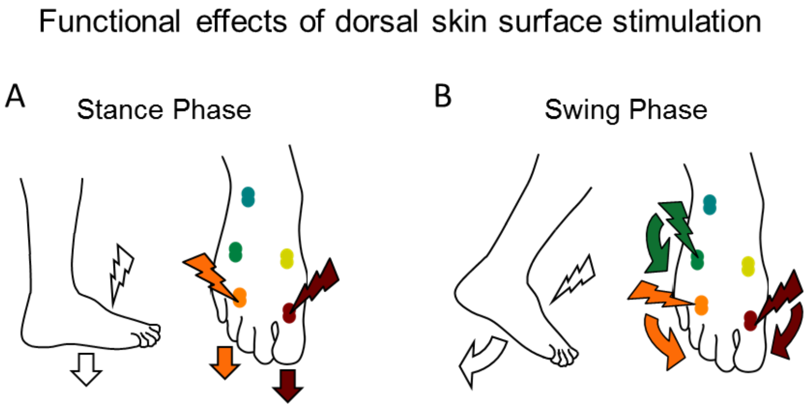


Figure 4-6: Functional diagram of effects of foot dorsum skin surface stimulation.Panel A and B show functional effects in the Stance and Swing Phases, respectively. The foot outline on the left of each panel shows the overall functional effect of stimulation. The foot outline on the right of each panel shows the location-specific functional effects of stimulation.

Stance phase

Generally during the stance phase, responses to superficial peroneal nerve stimulation are small or absent in ankle muscles, with no response evoked in TA muscle (Eng et al. 1994; Van Wezel et al. 1997; Zehr et al. 1997). For the responses that are present, a general extensor response is observed (Duysens and Stein 1978; Abraham and Loeb 1985; Drew and Rossignol 1987; Zehr et al. 1997; Zehr et al. 1998b). Stimulation mainly caused inversion of the ankle except during early stance where stimulation to the medial side of the foot reduced inversion. This could serve to quickly place the foot on the ground for safe weight acceptance. At the end of the stance phase, before the transition into the swing phase, stimulation on the lateral side of the foot decreased dorsiflexion. This is consistent with previous observations of plantar flexion facilitation generally evoked with superficial peroneal nerve stimulation at the end of the stance phase (Van Wezel et al. 1997).

Stimulation at discrete dorsal skin foot sites showed site dependency for force produced at the heel, medial and lateral FSR's. In general, stimulation to the lateral side of the foot increased lateral force and stimulation to the medial side of the foot increased medial force (see Figure 6). This is opposite to the effect seen with stimulation to the plantar surface of the foot where stimulation to the lateral foot margin tended to decrease lateral FSR force and stimulation to the medial foot margin decreased medial FSR force (Zehr et al. 2014). The difference in force patterns observed between plantar and dorsal surface skin stimulation was expected given the functional differences expected with stimulation to these regions.

In previous experiments superficial peroneal nerve stimulation also significantly increases activity in the biceps femoris and semitendinosus producing knee flexion which could be incorporated to facilitate flexor activity at the end of the stance phase, aiding the transition into the swing phase (Duysens et al. 1998). We therefore speculated a similar effect with these data, however this was not seen as there were no effects on knee kinematics. Perhaps this occurred because stimulation was not substantial enough to require a whole leg correction.

For interlimb effects, evaluated with responses obtained from the PD muscle, stimulation to the foot dorsum facilitated muscle activity. Functionally, this would facilitate abduction of the arms to maintain stability to compensate for the potential destabilization caused by dorsal foot stimulation. At the end of the stance phase, a location specific effect was observed where stimulation to the 1st metatarsal further increased PD facilitation for balance maintenance prior to the unstable transition into swing. In other studies, a large facilitatory reflex in the posterior deltoid muscle in the shoulder was produced to stabilize the upper body as the leg leaves the ground (Haridas and Zehr 2003).

Swing transition

The dominant location specific effects of stimulation applied during the swing transition were changes in PL and MG muscle activity. Seen previously at the stance to swing transition, stimulation of the superficial peroneal nerve in the foot produces plantar flexion and forward progression by medial gastrocnemius facilitation to clear an encountered obstacle (Haridas and Zehr 2003). Here the general response in MG is facilitation. However, when stimulation is applied to the ankle, facilitation is reduced. Functionally, this may reduce plantar flexion to avoid driving the foot into the destabilizing perturbation. Also showing a location specific effect, activity in PL muscle is facilitated except when stimulation is applied to middle medial foot dorsum. Functionally, this may reduce plantar flexion and eversion to slow the initiation of the swinging leg and reduce push-off force.

Swing phase and stance transition

Stimulation to the foot dorsum had the largest effect to muscle activity and ankle kinematics during the swing phase. During this phase, the swinging foot is particularly vulnerable to encountering an unexpected obstacle which may cause a fall, thus necessitating flexible control. Location specific effects were seen for the TA muscle. Here, TA muscle activity shows predominately inhibition following foot dorsum stimulation and when stimulation is applied to the 1st metatarsal, inhibition is largest compared to all other stimulation sites. Stimulation to the superficial peroneal nerve, innervating the foot dorsum, also causes inhibition of TA muscle activity, as a defining feature of a stumbling corrective response (Yang and Stein 1990; Van Wezel et al. 1997; Zehr et al. 1997; Haridas et al. 2008).

Correlated with this reduction in TA, ankle dorsiflexion is generally reduced with superficial peroneal nerve stimulation (Van Wezel et al. 1997; Zehr et al. 1997). This is similar to results here as dorsal skin stimulation also generally causes reduced ankle dorsiflexion. However, at the end of the swing phase, stimulation at the 4th metatarsal increased dorsiflexion, revealing topographical organization. The general reduction in dorsiflexion, seen at most phases in swing for most sites, may not be safe at this phase of the gait cycle as the heel prepares to strike the ground.

For the plantar flexor muscles, PL and MG, dorsal foot stimulation generally increased activity. However in early swing, PL muscle activity was reduced with stimulation at the 4th metatarsal. Functionally, this may be helpful in reducing plantar flexion and push off force to avoid pushing the swinging foot into a perturbation.

For ankle inversion/eversion there were many significant effects during the swing phase. Stimulation to the 1st metatarsal caused reduced inversion at all points in the swing phase especially compared to sites on the lateral side of the foot. Conversely, stimulation to the lateral side of the foot increased ankle inversion when compared to medial stimulation sites. Functionally this response serves to tilt the foot away from the perturbation where lateral stimulation inverts the foot and medial stimulation everts the foot (see Figure 6).

Swing phase stimulation effects were also seen in GR muscle. In early swing, general inhibition is seen which functionally may decrease hip flexion to slow the swinging leg as it enters swing phase to avoid a perturbation. Increased hip extension following superficial peroneal nerve stimulation has been previously documented by a facilitation of the biceps femoris and semitendinosus (Duysens et al. 1998). In mid swing, stimulation to the lateral side of foot increases GR activity to perhaps promote hip and knee flexion whereas stimulation to medial side of foot reduced GR activity to decease hip and knee flexion. Location specific effects were also seen for the GR at the stance transition, where stimulation to the 1st metatarsal inhibited activity compared to facilitation seen from stimulation at the other sites. Functionally, this could translate into reduced hip and knee flexion which would allow the foot to get to the ground quickly to avoid a potentially dangerous perturbation at the toe to maintain balance.

As a means of inspecting interlimb effects, the PD muscle was investigated. It was found that stimulation to the 1st metatarsal also had a significant inhibitory effect compared to other stimulation sites where PD muscle is facilitated. At this point in the gait cycle, it could be dangerous to lift the arms, causing destabilization, as the body transitions into stance.

Topographic organization from discrete activation of the foot dorsum during walking

Location-specific effects are a feature of cutaneous reflexes during locomotion in humans (Van Wezel et al. 1997). Increased resolution of effects can be seen here where stimulation to discrete foot dorsum skin sites reveal topographic organization of responses in muscle activity and in ankle kinematics. The swing phase is considerably more unstable compared to the stance phase of walking, when the foot is safely on the ground without much potential for adjustment, therefore responses to dorsal foot stimulation were expected to be more prominent during this time. Indeed, there were increased responses to stimulation from discrete foot dorsum skin site stimulation in the swing phase compared to the stance phase. Swing phase disturbances to the dorsal surface of the foot resulted in a response to withdraw the foot away from the perturbation. In general, responses from lateral stimulation differs from medial stimulation and effects seem most prominent from stimulation at the distal end of the foot at the 1st and 4th metatarsals. Given the flexible nature of the swing phase, responses to stimulation go beyond the muscles that control the ankle to include muscles at the hip and at the shoulder. This was expected given the strong interlimb reflexes evoked in muscles across the body by cutaneous stimulation of the superficial peroneal nerve (Zehr et al. 2001; Haridas and Zehr 2003).

Neuronal networks underlying swing phase corrections from foot dorsum stimulation

The neuronal networks underlying the stumbling corrective and preventive reactions from foot dorsum stimulation are expected to lay within the spinal cord. Cutaneous afferents are especially important for evoking a corrective stumble reaction because the normal response is abolished by anesthesia of the skin of the foot (Forssberg et al. 1977; Wand et al. 1980). In a fictive cat model, where supraspinal and afferent input is removed, excitatory and inhibitory postsynaptic potentials, reminiscent of the stumble correction response are recorded following stimulation of the superficial peroneal nerve (Quevedo et al. 2005). Human infants also demonstrate well-organized responses to touch of the foot dorsum during the swing phase. These responses appear prior to the full maturation of descending pathways from supraspinal centers (Lam et al. 2003; Yang et al. 2004). In adults, observations of reflex effects, as seen here, causing brief kinematic changes also supports the notion that stumbling corrective reflexes are evoked mainly by activation of cutaneous afferents in the superficial peroneal nerve and integrated within the spinal cord.

With the results presented here, it can be seen that activation of these presumably spinal pathways continues with activation of local populations of afferents, as opposed to whole nerve trunks (as in superficial peroneal nerve stimulation). The number of afferents activated, and subtypes recruited could differ at each site, and it is not fully known how each type of afferent influences muscle activity. It is also possible that activation of these afferents might signal different skin stretch or compression depending on stimulus location.

This results of this study provide important information that increase our understanding of how afferent feedback from specific cutaneous locations on the foot dorsum influences the mechanisms involved in locomotor output. This information may also have potential use in the design of rehabilitation strategies for those with impaired gait, such as in those arising after neurological damage. Site specific stimulation may be applied to aid in enhancing functional modulation of muscle activity. With a better understanding of how each receptive site on the dorsal foot contributes to locomotion, researchers may be able to harness the intrinsic effects of cutaneous reflexes. This study provides a better understanding of the behavioral relevancy, and potential rehabilitative use, of cutaneous input from specific regions on the dorsal foot during locomotion.

Conclusion

The results of this study further support suggestions that cutaneous nerves of the foot dorsum produce location-specific responses that are highly organized and produce topographic reflex effects. Both site and phase dependence were observed in the muscular, kinematic, and kinetic responses to discrete foot dorsum stimulation and these responses serve to maintain ongoing locomotion. This information is of importance to increase our understanding of how afferent feedback from specific cutaneous locations on the foot dorsum influences the mechanisms involved in stance and swing phase corrective responses.

References

Abraham LD, Loeb GE (1985) The distal hindlimb musculature of the cat. Exp Brain Res 58:580–593. doi: 10.1007/BF00235874

Buford JA, Smith JL (1993) Adaptive control for backward quadrupedal walking. III. Stumbling corrective reactions and cutaneous reflex sensitivity. J Neurophysiol 70:1102–1114.

Drew T, Rossignol S (1987) A kinematic and electromyographic study of cutaneous reflexes evoked from the forelimb of unrestrained walking cats. J Neurophysiol 57:1160–1184.

Duysens J, Stein RB (1978) Reflexes induced by nerve stimulation in walking cats with implanted cuff electrodes. Exp brain Res 32:213–24.

Duysens J, Tax AA, Murrer L, Dietz V (1996) Backward and forward walking use different patterns of phase-dependent modulation of cutaneous reflexes in humans. J Neurophysiol 76:301–310.

Duysens J, van Wezel BMHM, van de Crommert HWAAW, et al. (1998) The role of afferent feedback in the control of hamstrings activity during human gait. Eur J Morphol 36:293–299. doi: 10.1076/ejom.36.4.293.5820

Eng JJ, Winter DA, Patla AE (1994) Strategies for recovery from a trip in early and late swing during human walking. Exp Brain Res 102:339–349. doi: 10.1007/BF00227520

Forssberg H (1979) Stumbling corrective reaction: a phase-dependent compensatory reaction during locomotion. J Neurophysiol 42:936–953.

Forssberg H, Grillner S, Rossignol S (1975) Phase dependent reflex reversal during walking in chronic spinal cats. Brain Res 85:103–107. doi: 10.1016/0006-8993(75)91013-6

Forssberg H, Grillner S, Rossignol S (1977) Phasic gain control of relexes from the dorsum of the paw during spinal locomotion. Brain Res 132:121–139. doi: 10.1016/0006-8993(77)90710-7

Haridas C, Zehr EP (2003) Coordinated interlimb compensatory responses to electrical stimulation of cutaneous nerves in the hand and foot during walking. J Neurophysiol 90:2850–61. doi: 10.1152/jn.00531.2003

Haridas C, Zehr EP, Misiaszek JE (2006) Context-dependent modulation of interlimb cutaneous reflexes in arm muscles as a function of stability threat during walking. J Neurophysiol 96:3096–103. doi: 10.1152/jn.00746.2006

Haridas C, Zehr EP, Misiaszek JE (2008) Adaptation of cutaneous stumble correction when tripping is part of the locomotor environment. J Neurophysiol 99:2789–97. doi: 10.1152/jn.00487.2007

Kennedy PM, Inglis JT (2002) Distribution and behaviour of glabrous cutaneous receptors in the human foot sole. J Physiol 538:995–1002. doi: 10.1113/jphysiol.2001.013087

Klarner T, Barss T, Sun Y, et al. (2014) Preservation of common rhythmic locomotor control despite weakened supraspinal regulation after stroke. Front Integr Neurosci 8:1–9.

Klimstra MD, Thomas E, Zehr EP (2011) Biomechanical outcomes and neural correlates of cutaneous reflexes evoked during rhythmic arm cycling. J Biomech 44:802–9. doi: 10.1016/j.jbiomech.2010.12.017

Lam T, Wolstenholme C, van der Linden M, et al. (2003) Stumbling corrective responses during treadmill-elicited stepping in human infants. J Physiol 553:319–31. doi: 10.1113/jphysiol.2003.043984

Lamont E V, Zehr EP (2007) Earth-referenced handrail contact facilitates interlimb cutaneous reflexes during locomotion. J Neurophysiol 98:433–42. doi: 10.1152/jn.00002.2007

Lamont E V, Zehr EP (2006) Task-specific modulation of cutaneous reflexes expressed at functionally relevant gait cycle phases during level and incline walking and stair climbing. Exp Brain Res 173:185–92. doi: 10.1007/s00221-006-0586-4

Nakajima T, Sakamoto M, Tazoe T, et al. (2006) Location specificity of plantar cutaneous reflexes involving lower limb muscles in humans. Exp Brain Res 175:514–525. doi: 10.1007/s00221-006-0568-6

Nakajima T, Sakamoto M, Tazoe T, et al. (2009) Location-specific modulations of plantar cutaneous reflexes in human (peroneus longus muscle) are dependent on co-activation of ankle muscles. Exp brain Res 195:403–12. doi: 10.1007/s00221-009-1802-9

Prochazka A, Sontag K-H, Wand P (1978) Motor reactions to perturbations of gait: proprioceptive and somesthetic involvement. Neurosci Lett 7:35–39. doi: 10.1016/0304-3940(78)90109-X

Quevedo J, Stecina K, Gosgnach S, McCrea DA (2005) Stumbling corrective reaction during fictive locomotion in the cat. J Neurophysiol 94:2045–2052. doi: 10.1152/jn.00176.2005

Rossignol S, Dubuc R, Gossard J-P (2006) Dynamic sensorimotor interactions in locomotion. Physiol Rev 86:89–154. doi: 10.1152/physrev.00028.2005

Schillings AM, Van Wezel BMH, Duysens J (1996) Mechanically induced stumbling during human treadmill walking. J Neurosci Methods 67:11–17. doi: 10.1016/0165-0270(95)00149-2

Sherrington CS (1947) Integrative Actions of the Nervous System. Cambridge University Press, Cambridge, UK

Wand P, Prochazka A, Sontag K-H (1980) Neuromuscular responses to gait perturbations in freely moving cats. Exp Brain Res. doi: 10.1007/BF00237937

Van Wezel BMH, Ottenhoff FAM, Duysens J (1997) Dynamic Control of Location-Specific Information in Tactile Cutaneous Reflexes from the Foot during Human Walking. J Neurosci 17:3804–3814.

Yang JF, Lam T, Pang MYC, et al. (2004) Infant stepping: a window to the behaviour of the human pattern generator for walking. Can J Physiol Pharmacol 82:662–674.

Yang JF, Stein RB (1990) Phase-dependent reflex reversal in human leg muscles during walking. J Neurophysiol 63:1109–1117.

Zehr E, Nakajima T, Barss T, et al. (2014) Cutaneous stimulation of discrete regions of the sole during locomotion produces “sensory steering” of the foot. BMC Sport Sci. Med. Rehabil. 6:

Zehr EP, Collins DF, Chua R (2001) Human interlimb reflexes evoked by electrical stimulation of cutaneous nerves innervating the hand and foot. Exp Brain Res 140:495–504. doi: 10.1007/s002210100857

Zehr EP, Fujita K, Stein RB (1998a) Reflexes from the superficial peroneal nerve during walking in stroke subjects. J Neurophysiol 79:848–58.

Zehr EP, Komiyama T, Stein RB (1997) Cutaneous reflexes during human gait: electromyographic and kinematic responses to electrical stimulation. J Neurophysiol

77:3311–25.

Zehr EP, Loadman PM (2012) Persistence of locomotor-related interlimb reflex networks during walking after stroke. Clin Neurophysiol 123:796–807. doi: 10.1016/j.clinph.2011.07.049

Zehr EP, Stein RB (1999) What functions do reflexes serve during human locomotion? Prog Neurobiol 58:185–205.

Zehr EP, Stein RB, Komiyama T (1998b) Function of sural nerve reflexes during human walking. J Physiol 507 (Pt 1:305–14.

Zehr EP, Stein RB, Komiyama T, Kenwell Z (1995) Linearization of force sensing resistors (FSR's) for forcemeasurement during gait. Proc 17th Int Conf Eng Med Biol Soc. doi: 10.1109/IEMBS.1995.579833

Chapter 5 Reliability of multiple baseline measures for locomotor retraining after stroke[3]

Abstract

The present work investigated the reliability of locomotion-related physiological measures taken using a repeated test-retest protocol in stroke participants. Data were collected across 3 testing sessions. Measurements of muscle activity and force during maximum isometric dorsiflexion, plantarflexion and hand grip contractions were taken for both the less and more affected limbs. Cardiovascular measures and stretch reflex amplitudes in soleus muscle were evaluated at rest. Background EMG activity and amplitudes of cutaneous reflexes (following stimulation of the superficial peroneal and superficial radial nerves) were evaluated during walking. Intraclass correlation coefficients (ICC) and results from repeated measures ANOVA revealed no significant differences across and between testing sessions and high absolute agreement (ICC = 0.665 to 0.998) of measures within a participant. Our data support the suggestion that multiple baseline measures obtained from the same participants should be considered a valid alternative to the concept of a control group in intervention studies.

Introduction

Training and rehabilitative interventions require valid assessment of outcomes that can be ascribed to the intervention. In turn, the assessment itself must have low method error and good reliability if it is to be usefully applied. Additionally, intervention studies have often used reference untrained "control" groups to compare against the intervention or treatment groups. In clinical studies, this can mean relegating committed volunteer participants to the role of control participants. Accordingly, some researchers have instead opted for the "multiple baseline" model where the participants who will be trained serve as their own control (Butefisch et al., 1995).

The purpose of the present work was to investigate the reliability of multiple baseline measures using a repeated test protocol in stroke participants. Locomotion-related physiological measures will be tested including isometric strength, cardiovascular measures, and muscle and reflex modulation during treadmill walking. Previous researchers have found high reliability in

---

[3] Similar to: Klarner T, Barss T, Sun Y, Kaupp C, Beattie S, Zehr EP (2014) Reliability of multiple baseline measures for locomotor retraining after stroke. In: Jensen W, Andersen OK, Akay M (eds) Replace, repair, restore, relieve—bridging clinical and engineering solutions in neurorehabilitation. Springer, Heidelberg, pp 479–486

other baseline measures in neurologically intact and in stroke participants (Butefisch et al., 1995; Mong et al., 2010; Collen et al., 1990; Holden et al., 1984) and we predict high association across test sessions. Results of this study have potential implications for any future studies evaluating plasticity arising from training or rehabilitative interventions.

Materials and methods

Participants

Twelve chronic stroke subjects (at least 6 months post infarct), between 58 and 80 y old, participated with written informed consent in a protocol approved by the Human Research Ethics Board at the University of Victoria.

Experimental Protocol

Repeated measurements were obtained from participants in three experimental sessions over a period of three to four weeks, with at least six days between sessions. At these sessions, the same tests were performed in the same order and environmental conditions (i.e. temperature, noise, lighting, subject position) and session time of day were kept as constant as possible (Lagerquist et al., 2006; Zehr 2002).

For strength measures, stretch reflex testing, and resting cardiovascular measures, participants were assessed while seated in a custom-fit chair designed to minimize movement. Locomotor parameters and cutaneous reflex amplitudes were evaluated while participants performed level walking on a motorized treadmill belt with 0% body weight support (Woodway Desmo M, Waukesha, WI, USA).

Electromyographic (EMG) data from the soleus (SOL), tibialis anterior (TA), flexor carpi radialis (FCR), and posterior deltoid (PD), from the more (MA) and less (LA) limbs, were collected with surface electrodes placed in bipolar configuration over the muscle bellies of interest. To control for variation in positioning of electrodes across days, we used anatomical landmarks, measurements, and pictures taken at the first session, and the electrodes were placed by the same experimenter each time.

Recordings of MVIC's

Maximal voluntary isometric contractions (MVIC's) were performed for ankle dorsiflexion, plantarflexion and hand grip for both the LA and MA limbs. Following a brief warm-up, participants were given two attempts for achieving a maximum contraction. In 10 second trials, following a silent period of 5 s, contractions were held for each limb separately. Maximum values were taken as the greatest reading generated over two trials.

Maximum forces produced during dorsiflexion and plantarflexion contractions were established via strain gauge (Omegadyne Ltd. Model 101-500) and converted to torque using a moment arm length of 0.15 m (measured from the heel block to the center of the strain gauge). Hand grip was performed with a hand grip dynamometer (Takei Scientific Instruments Company Ltd., Niigata, Japan). EMG signals were amplified (GRASS P511, AstroMed) and band pass filtered from 100 to 300 Hz and all signals were sampled at 2000 Hz using custom-written continuous acquisition software (LABVIEW, National Instruments, TX, USA). Using custom-written software programs (Matlab, The Mathworks, Inc., MA, USA) maximum contractions were quantified offline by obtaining the mean value over 500 ms when force and EMG signals were highest. EMG data were rectified and low pass filtered at 100 Hz using a 4th order Butterworth filter. Values are reported as maximum torque in Newton-meters (Nm) for ankle dorsi- and plantarflexion, kilograms (kg) for hand grip and EMG in microvolts (µV).

Soleus Stretch Reflex

Soleus stretch reflexes were evoked using an electrodynamic shaker (ET-1126B; Labworks Inc), placed over the more and less affected triceps surae tendons, in separate trials (Palomino et al., 2011) Constant pressure was applied against the tendon and the shaker was programmed to deliver a single sinusoidal pulse at a frequency of 100Hz, 10 ms duration. A total of 30 pulses were delivered pseudo-randomly between 3 and 5 seconds. As a proxy for the intensity of the pulse between days, an accelerometer (ADXL193; Analog devices), mounted to the tip of the shaker, ensured consistent stimulation amplitude. Maximum soleus stretch reflexes were determined as the average of the three largest peak to peak amplitude values of the reflex sweep, measured and reported in millivolts (mV).

Cardiovascular Measures

These values were obtained with a digital blood pressure cuff placed over the less affected arm. Values are reported in beats per minute (bpm) and millimeters of mercury (mmHg) for systolic and diastolic pressure.

Nerve Stimulation

During treadmill walking, cutaneous reflexes were evoked via combined surface stimulation of the nerves innervating the dorsum of the hand (superficial radial; SR) and foot (superficial peroneal; SP). Electrodes for SR and SP nerve stimulation were placed just proximal to the radial head and on the crease of the ankle, respectively, on the LA limbs. A Grass S88 stimulator with SIU5 stimulus isolation and a CCU1 constant current unit (AstroMed-Grass Inc., Canada) was used to deliver stimulation in trains of 5 x 1.0 ms pulses at 300Hz. Perceptual and radiating thresholds (RT) were determined and non-noxious intensities were found for each participant. Stimulation intensities were set to 2.2 x RT for the SR nerve, and 2.0 x RT for the SP nerve. During treadmill walking, 120 stimulations were delivered pseudo-randomly with an interstimulus interval of 1-5 seconds.

EMG data were sampled at 1000 Hz and analyzed offline for background amplitudes and cutaneous reflexes. Background amplitudes were obtained from steps without stimulation and quantified as the average peak response within a step. A step was identified with custom-made force sensing resistors and determined as the time between ipsilateral heel-strikes of the LA leg. Peak amplitudes are reported in microvolts (μV).

To analyze cutaneous reflexes, stimuli were aligned to delivery and averaged together. The average trace from the non-stimulated data was subtracted from the stimulated average trace to produce a subtracted EMG ‘reflex’ trace. The stimulus artifact was removed from the subtracted reflex trace and data were then low-pass filtered at 30 Hz using a dual-pass, fourth order Butterworth filter. Cutaneous reflexes were quantified as the average cumulative reflex over 150 ms following stimulation.

Statistics

Using commercially available software (SPSS 18.0, Chicago, IL) reliability of measures was determined with intraclass correlation coefficients (ICC) to examine absolute agreement in measures across time. A repeated measures ANOVA was also used to examine differences across testing sessions. Statistical significance was set at $p \leq 0.05$.

Results

Data averaged across all participants from the three testing sessions are shown in Table 5-1. To assess reliability, intraclass correlation coefficients and p-values from RM ANOVA were calculated and are reported in Table 5-1. Asterisks indicate significant correlations. Figure 5-1 shows background EMG and cutaneous reflexes for muscles of the LA and MA sides. Quantified levels of background EMG and reflex activity averaged across all participants are reported in the bottom half of Table 5-1. In general, moderate to high correlations were observed for all variables with a range of 0.665 to 0.998 for significant intraclass correlation coefficients. Although some variables (e.g. cutaneous reflexes from LA Sol and MA PD) do not have significant correlation across testing sessions, no significant differences were observed for any variable following RM ANOVA and overall reliability of measures was supported.

Table 5-1: Group data of baseline measures

Measure	Test 1	Test 2	Test 3	ICC	p
EMG During MVC (µV, standard deviation (sd))					
LA SOL	27.7, 14.6	23.3, 8.3	29.9, 12.0	0.564*	0.417
MA SOL	15.2, 11.7	11.5, 5.7	12.9, 8.1	0.886*	0.318
LA TA	81.2 ,33.0	82.4, 39.4	91.1, 47.2	0.930*	0.459
MA TA	36.2, 31.9	27.0, 13.0	27.4, 17.9	0.665	0.511
LA FCR	31.1, 12.9	30.3, 7.4	28.9, 9.6	0.869*	0.756
MAFCR	10.4, 9.4	12.9, 10.2	10.9, 8.1	0.950*	0.316
Maximum Plantarflexion and Dorsiflexion Torque (Nm, sd)					
LA DF	10.6, 8.6	12.5, 9.1	11.4, 9.5	0.968*	0.343
LA PF	15.3, 11.0	18.6, 8.7	17.4, 10.2	0.875*	0.484
MA DF	6.5, 3.9	5.1, 4.3	6.3, 4.9	0.714*	0.401
MA PF	10.2, 5.7	9.9, 10.0	10.6, 5.4	0.736*	0.973
Maximum Hand Grip (kg, sd)					
LA GRIP	32.3, 7.9	33.2, 8.1	32.6, 8.7	0.970*	0.753
MA GRIP	11.3, 14.7	11.0, 15.2	10.7, 14.7	0.998*	0.634

Stretch Reflex (mV, sd)					
LA	0.64, 0.37	0.55, 0.47	0.51, 0.25	0.932*	0.183
MA	0.59, 0.35	0.56, 0.32	0.67, 0.22	0.714*	0.567
Cardiovascular Measures (beats/min & mmHG, sd)					
HR	74.2, 8.9	73.1, 5.9	74.1, 9.9	0.949*	0.757
Systolic BP	132.5, 10.7	134.6, 11.5	126.3, 14.6	0.775*	0.136
DiastolicBP	85.0, 8.8	81.7, 7.5	80.2,12.1	0.704*	0.115
Locomotor Background EMG Value (μV, sd)					
LA SOL	18.9, 6.8	22.6, 11.9	18.7, 9.2	0.819*	0.144
LA TA	17.8, 9.5	20.3, 11.6	21.9, 13.2	0.904*	0.219
LA FCR	16.1, 7.7	16.7, 6.1	15.6, 4.3	0.813*	0.702
LA PD	17.7, 7.2	20.2, 21.2	19.3, 17.1	0.852*	0.825
MA SOL	13.3, 18.5	9.2, 2.5	11.9, 6.7	0.525	0.595
MA TA	16.4, 14.0	14.0, 10.7	11.9, 7.6	0.581	0.975
MA FCR	5.8, 2.6	5.4, 1.7	5.2, 1.6	0.840*	0.578
MA PD	5.9, 1.4	5.5, 2.0	5.6, 2.6	0.865*	0.561
Subtracted Cutaneous Reflex Amplitude (μV, sd)					
LA SOL	-0.15, 3.3	-0.22, 3.4	0.71, 1.9	0.626	0.617
LA TA	-1.4, 4.8	0.80, 7.5	0.88, 4.0	0.691*	0.342
LA FCR	7.3, 8.4	5.8, 7.3	4.1, 7.4	0.874*	0.287
LA PD	1.8, 3.6	3.4, 5.2	3.2, 4.5	0.873*	0.223
MA SOL	0.63, 1.2	0.30, 1.2	0.82, 0.9	0.700*	0.351
MA TA	0.04, 1.7	0.92, 1.9	-0.29, 1.9	0.680*	0.109
MA FCR	0.05, 0.5	0.34, 0.4	0.00, 0.2	0.792*	0.214
MA PD	0.08, 0.2	0.26, 0.4	0.23, 0.2	0.413	0.233

* indicates significant intraclass correlation. p-values from RM ANOVA are also reported. Values are means for the less (LA) and more (MA) affected limbs for the soleus (SOL), tibialis anterior (TA), flexor carpi radialis (FCR) and posterior deltoid (PD).

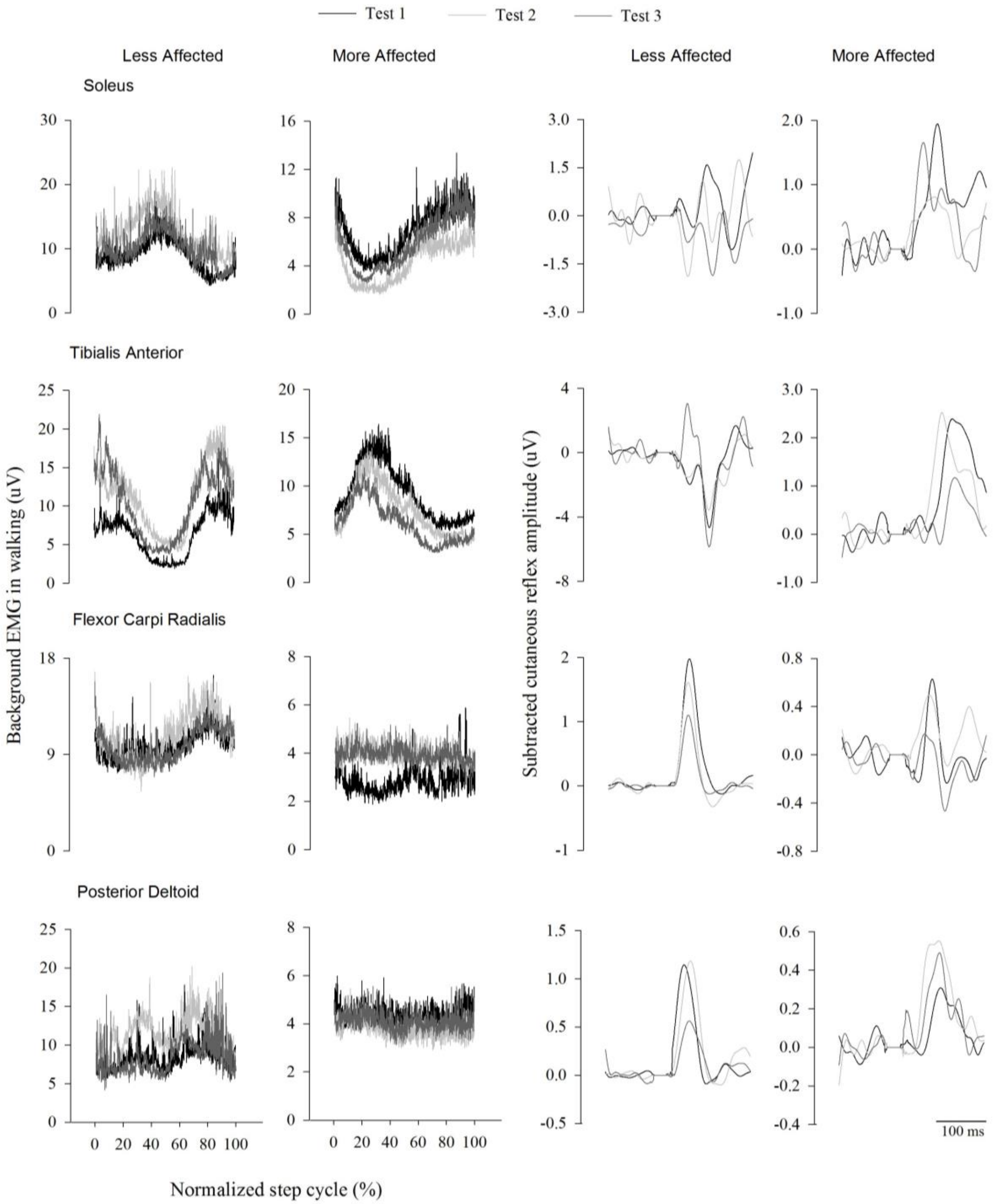


Figure 5-1: Background EMG and cutaneous reflexes during treadmill walking for three testing sessions averaged across all stroke participants. Test session 1 is in black, test session 2 is in light grey and test session 3 is dark grey. Plots on the left show averaged EMG acivity plotted as a function of the less affected locomotor cycle. Plots on the right show the average cutaneous reflexes following combined stimulation of the SP and SR nerves during walking. Data are plotted as a function of time with 100 ms before stimulation and 200 ms post.

Discussion

Here we investigated the reliability of multiple baseline measures using a repeated test protocol in stroke participants. Reliability analysis revealed significant statistical correlation amongst most variables with no significant differences when comparing across multiple time points.

Multiple baseline measures should be considered a valid alternative or replacement to the concept of a control group, given high internal consistency of measures. In this way individual participant data contributes to a meaningful set of baseline data with which to compare measures following an intervention, allowing each participant to act as their own control. Indeed, several previous investigators have successfully used a multiple baseline design to show improvement in motor function for stroke participants. Butefisch and colleagues (1995) showed an improvement in hand function following training and more recently Dragert and Zehr (2013) showed that unilateral dorsiflexion training can increase strength compared to a participants' baseline control values.

The robust nature of these baseline measures in stroke participants highlights their value as assessment tools for intervention studies. These results were expected as others have also found high association between baseline measures in stroke participants. For example, high ICCs were found for the amount of time taken during five repetitions of the sit-to-stand test (Mong et al., 2010), time taken on the 10-m walk test (Collen et al., 1990), and in other temporal gait measures (Holden et al., 1984). Although we found several measures to have a significant ICC value, many clinical researches argue that a higher standard needs to be achieved for clinical measures. Generally the standard required is 0.9 to ensure reasonable reliability for clinical measures (Cicchetti, 1994). Nevertheless, there were still several variables from these data that would be considered to be reliable.

Here we expand current literature on test reliability of baseline values in stroke participants. Measures of strength and cardiovascular resting state test physiological baselines and are often directly targeted by training interventions. While reflex measures may not be the target of training itself, these measures can be used to test underlying changes in overall nervous system plasticity. For example, the strength of transmission between the arms and the legs can be tested by remote movement conditioning of reflex amplitudes. In addition, the soleus stretch reflex can be used as a clinical evaluation tool and for the assessment of changes in spasticity (Voerman et al., 2005).

Differences in absolute values between the more and less affected sides observed here corroborate previous findings of asymmetry following stroke. However, the general presence of interlimb cutaneous reflexes confirms that neuronal pathways linking the arms and the legs remain at least partially conserved (Zehr and Loadman, 2012) providing a substrate for training induced plasticity to improve function after stroke.

Conclusion

Reliability of measures was supported with observations of high consistency in values obtained across multiple baseline time points in stroke participants. These results support the idea that multiple measures could provide valid, reliable and meaningful baseline data useful for comparing results of an intervention study where participants act as their own control.

References

Butefisch C, Hummelsheim H, Densler P, Mauritz K. (1995) Repetitive training of isolated movements improves the outcome of motor rehabilitation of the centrally paretic hand. J Neurol Sci 130:59–68.

Colleen F, Wade D, Bradshaw C (1990) Mobility after stroke: Reliability of measures of impairment and disability. Disabil Rehabil 12:6–9. doi: 10.3109/03790799009166594

Dragert K, Zehr EP (2013) High-intensity unilateral dorsiflexor resistance training results in bilateral neuromuscular plasticity after stroke. Exp Brain Res 225:93–104. doi: 10.1007/s00221-012-3351-x

Holden MK, Gill KM, Magliozzi MR, et al. (1984) Clinical gait assessment in the neurologically impaired. Reliability and meaningfulness. Phys Ther 64:35–40.

Lagerquist O, Zehr EP, Baldwin ERL, et al. (2006) Diurnal changes in the amplitude of the Hoffmann reflex in the human soleus but not in the flexor carpi radialis muscle. Exp Brain Res 170:1–6. doi: 10.1007/s00221-005-0172-1

Mong Y, Teo TW, Ng SS (2010) 5-Repetition Sit-to-Stand Test in Subjects With Chronic Stroke: Reliability and Validity. Arch Phys Med Rehabil 91:407–413. doi: 10.1016/j.apmr.2009.10.030

Palomino AF, Hundza SR, Zehr EP (2011) Rhythmic arm cycling differentially modulates stretch and H-reflex amplitudes in soleus muscle. Exp Brain Res 214:529–37. doi: 10.1007/s00221-011-2851-4

Cicchetti DV (1994) Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. Psychological Assessment. 6(4):284–290.

Voerman GE, Gregoric M, Hermens HJ (2005) Neurophysiological methods for the assessment of spasticity: the Hoffmann reflex, the tendon reflex, and the stretch reflex. Disabil Rehabil 27:33–68. doi: 10.1080/09638280400014600

Zehr EP (2002) Considerations for use of the Hoffmann reflex in exercise studies. Eur J Appl Physiol 86:455–68. doi: 10.1007/s00421-002-0577-5

Zehr EP, Loadman PM (2012) Persistence of locomotor-related interlimb reflex networks during

Chapter 6 Exploiting interlimb arm and leg connections for walking rehabilitation: a training intervention in stroke[4]

Abstract

Rhythmic arm and leg (A&L) movements share common elements of neural control. The extent to which A&L cycling training can lead to training adaptations which transfer to improved walking function remains untested. The purpose of this study was to test the efficacy of A&L cycling training as a modality to improve locomotor function after stroke. Nineteen chronic stroke (>six months) participants were recruited and performed 30 minutes of A&L cycling training three times a week for five weeks. Changes in walking function were assessed with: 1) clinical tests; 2) strength during isometric contractions; and, 3) treadmill walking performance and cutaneous reflex modulation. A multiple baseline (3 pre-tests) within-subject control design was used. Data show that A&L cycling training improved clinical walking status, increased strength by ~25%, improved modulation of muscle activity by ~25%, increased range of motion by ~20%, decreased stride duration and increased frequency during treadmill walking. Cutaneous reflexes showed improved modulation during walking after A&L cycling training. On most variables, the majority of participants showed a significant improvement. In this study A&L cycling training improves walking ability after stroke. These results suggest that exploiting arm and leg connections with A&L cycling training, an accessible and cost-effective training modality, could be used to improve walking ability after stroke.

Introduction

Body weight supported treadmill training therapy can be used for the recovery of walking after neurological damage. In this rehabilitation paradigm participants walk on a motorized treadmill with a harness system allowing the weakened leg muscles to be freed from the necessity of body weight support and stepping is performed with the help of robotic interfaces or therapists. This protocol was initially utilized after spinal cord injury and may be equally beneficial for recovery of walking after stroke (Dietz et al. 1998; Moseley et al. 2003; Wirz et al. 2005; Mehrholz et al. 2008; Duncan et al. 2011).

---

[4] Klarner T, Barss TS, Kaupp C, Sun Y, Loadman PM, Zehr EP (2016) Exploiting interlimb arm and leg connections for walking rehabilitation: a training intervention in stroke. Neural Plasticity 2016; 2016:1-19.

Results from this therapy are positive, but there are significant limitations that limit access for the broader stroke population. Body weight supported treadmill training therapy has significant labour requirements, requires specialized equipment, and is typically only available in restricted environments such as in rehabilitation centres (Ferris et al. 2006; Høyer et al. 2012). In addition, body weight supported treadmill training offers no additional benefit over conventional physical therapy, as demonstrated in a large randomized clinical trial (Duncan et al. 2011). A more cost-effective and generally accessible protocol based upon a device (eg. arm and leg ergometer or a recumbent stepper) that could be more readily used in therapy would be of great benefit where less training is required for physical therapists to supervise training and participants may be more likely to comply with a community-based training regimen (Huang and Ferris 2004; Duncan et al. 2011).

In addition to finding a rehabilitation program that is widely accessible, exploiting the neural and mechanical linkages between the arms and legs that are inherent parts of human locomotion could enhance the recovery of walking (Ferris et al. 2006; Zehr et al. 2009; Klimstra et al. 2009). Therefore incorporating rhythmic arm movement paradigms for locomotor rehabilitation, such as with arm and leg (A&L) cycling, could be very beneficial to stroke locomotor recovery. Although there are differences in kinematics, balance requirements, and loading of the arms between walking and A&L cycling, this type of training activates similar neural networks as are engaged during walking (Zehr et al. 2007a). We have recently shown that even following a stroke, neural commonalities between A&L cycling and walking persist, despite altered descending supraspinal input from the stroke lesion (Klarner et al. 2014a). Given that A&L cycling and walking share common neural elements, and that this persists following stroke, there is a reasonable basis for expectation of training transfer to improve walking.

The extent to which A&L cycling training can lead to training adaptations which transfer to improved walking function remains untested. Thus the objective of this project was to test the efficacy of A&L cycling training to enhance walking after stroke. Given that A&L cycling and walking share a common core of subcortical regulation, it is hypothesized that A&L cycling training will transfer to an improvement in walking. Improvements in walking function were gauged by changes in clinical walking status, strength, and walking performance. If indirect training with A&L cycling does improve walking function, this adjunct therapy could be used as an additional modality to improve walking ability after stroke.

Materials and Methods

Participants

Participant recruitment occurred through community stroke support groups, posters in medical offices/hospitals, and newspaper articles. As inclusion criteria, participants were required to be a minimum of six months post-infarct, after spontaneous post-stroke changes are thought to have occurred (Cramer 2008), and able to stand free without assistive devices. Participants were screened with the Physical Activity Readiness Questionnaire to determine eligibility to participate in physical activity. If a response of ‘yes’ was given for any of the questions in the Questionnaire, indicating the presence of bone or joint problems or dizziness, written medical permission was obtained for that participant. A list of current medications was also obtained for each participant. Exclusion criteria included: medications affecting muscle tone less than three months prior, and self-report of any cardiovascular, musculoskeletal, respiratory, or other chronic diseases. A sample size of twenty five participants were recruited, in line with statistical reports that a sample size of 25 will specify a power of 0.80 at a large effect size and criterion value of $p=0.05$ (Cohen 1992). Sample size was based on previous studies of locomotor studies after stroke and other interventions yielding strength gains after stroke (Dragert and Zehr 2013; Andersen et al. 2014).

To assist with determining a participant’s functional status, clinical assessments were performed by a licensed physical therapist. Muscle tone was measured using the Modified Ashworth Scale (5 points) at the ankle and knee for the lower limb (Bohannon and Smith 1987; Lee et al. 1989). This is a graded rating of spasticity scored from 0 to 4, with 0 being flaccid and 4 being rigid. A measure of the basic motor skills necessary for functional ambulation was derived using the 6-point Functional Ambulation Categories Scale where a level 1 indicates that a patient is non-ambulatory and a level 6 indicates a patient is fully independent (Holden et al. 1984). To measure general physical impairment, the Chedoke-McMaster Stroke Assessment (Gowland et al. 1993) was used. Impairment at the arm (A), hand (H), leg (L), and foot (F) were determined using the 7-point activity scale where a score of 1 represents complete independence and a score of 7 represents total assistance. Using the 5-piece Semmes-Weinstein kit of calibrated monofilaments (Sammons Preston Roylan, Cedarburg WI), ability to discern light touch and pressure was measured in the more affected hand and foot (Hage et al. 1995). Reflexes obtained using a reflex hammer were graded on a 0 to 4+ scale where 0 means a reflex is absent and 4+ represents a hyperactive reflex with clonus for hip flexion (L1) and ankle plantarflexion (S1) (Walker 1990).

Ethics Statement

The authors confirm that all ongoing and related trials for this intervention are registered (ClinicalTrials.gov: D015017-2011-2015). Informed written consent from each participant was obtained for a protocol approved by the University of Victoria Human Research Ethics Committee (Protocol number: 07-480-04d) and performed according to the Declaration of Helsinki. The study protocol that was registered was the same as the study protocol approved by the University of Victoria Human Research Ethics Committee prior to subject enrollment.

Training Protocol

Participants performed training three times a week, with 30 minutes of aggregate activity time per session, for a total duration of five weeks. Most participants completed training on Monday, Wednesday and Friday. All experimental and training sessions took place in the Rehabilitation Neuroscience Laboratory at the University of Victoria.

For training, an arm and leg cycling ergometer with coupled upper and lower cranks was used (Sci-Fit Pro 2 ergometer). Dependent motion of the cranks for the arms and legs allow for passive assistance of weaker limbs during training. Mechanical modifications were made to the cycle ergometer to ensure a customized and comfortable fit for each training session. The cranks of the arm and leg ergometer were individually adjusted to the range of motion for each limb of each stroke participant and hand braces were worn as needed to ensure grip on the handle with the more affected (MA) hand. During each session, participants were allowed to take short 1-2 minute breaks during training if required, but the aggregate time for each session was always met. In fact, few participants took breaks and those that did, only required them in the early days of training. Participants were expected to tolerate the protocol very well as this was a modification of a previous protocol where chronic stroke participants performed four trials of six minute bouts (totalling 24 minutes) of active A&L cycling (Andersen et al. 2014).

To evaluate the physiological cost of training activity, heart rate (HR), rating of perceived exertion (RPE) and revolutions per minute (RPM) were collected every five minutes. Heart rate was monitored with a chest strap heart rate monitor (PolarElectro, Quebec Canada) and recorded in beats per minute (bpm). The rating of perceived exertion was self-reported using the 10-pt scale (Borg and Löllgen 2001). A&L cycle ergometer RPM were recorded visually from the digital display on the cycle ergometer as participants used this signal as a source of visual feedback for maintaining cadence. A single value for each variable was created each session by individually averaging the HR, RPE, RPM, and Watts over the 30 minutes of training and differences between the first and last training session were inspected.

Participants were encouraged to exercise at a moderate level sufficient to report a RPE value between three and five, corresponding to a target heart rate between 50-70% HR_{max} (Scherr et al. 2013). Target heart rate training zones were calculated with the Karvonen Formula taking into account heart rate reserve, and if a participant reported being on beta blockers, adjustments to target heart rate goals were made (Tang et al. 2006). The progressive element of this steady state training included increasing the resistance of the ergometer over the five weeks in order to maintain the same relative RPE. This is in line with many other post-stroke treadmill training protocols where training volume was increased (Zehr 2011). Increases in resistance were only required for 6 out of the 19 participants and generally increases were made in 5W increments to a maximum of 40W. During the training and testing time, participants were also encouraged to maintain their normal activity levels, but not participate in additional research programs or interventions.

All exercise sessions were supervised by a Certified Exercise Physiologist with the Canadian Society for Exercise Physiology, as well as several laboratory assistants to ensure appropriate monitoring. Exercise sessions were not initiated if a participant’s blood pressure exceeded 140/90 mmHg in accordance with Canada’s Physical Activity Guidelines (Warburton et al. 2010). Exercise was terminated if HR exceeded 85% of the age-predicted maximum, if blood pressure exceeded 200/110 mmHg, if the participant felt dizzy, nervous, or pains in the chest. Upon completion of the 30 minutes in each training session, participants were given three to five minutes to cool down, and remained in the laboratory until blood pressure returned to pre-exercise values. All blood pressure values were obtained with a digital blood pressure cuff placed over the less affected arm.

Multiple Baseline and Post-test Measures

A multiple baseline within-subject control design was used for this study (Butefisch et al. 1995; Klarner et al. 2014b). Figure 6-1 illustrates the testing and training protocol. A multiple baseline design allowed for the creation of a reliable and consistent pre-test measure, allowed for inspection of spontaneous recovery effects, and provided baseline data against which changes were evaluated. In this design, the control group is the experimental group. Multiple baseline measurements were obtained from participants in three baseline experimental sessions over a period of three to four weeks, with at least six days between baseline sessions. The post-test following training was performed within three days following training. As it was impossible to blind participants in this trial, several things were done to help control for potential sources of bias. At experimental sessions, the same tests were performed in the same order and environmental conditions (i.e. temperature, noise, lighting, participant position) and session time of day were kept as consistent as possible (Zehr 2002; Lagerquist et al. 2006; Dragert and Zehr 2013). These measures have been previously shown to have high reliability across multiple baseline points (Klarner et al. 2014b). The project manager, who was in charge of participant recruitment and scheduling, did not take part in the assessment of outcomes, nor did the exercise supervisors. Analysis of data was mainly performed by laboratory assistants who were not involved in the design or interpretation of results.

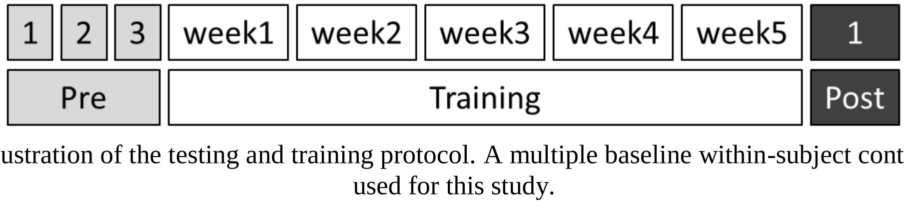


Figure 6-1: Illustration of the testing and training protocol. A multiple baseline within-subject control design was used for this study.

Clinical Walking and Balance Measures

Clinical assessment of walking was performed by the same licensed physical therapist who was not involved in the study both pre- and post-intervention. Tests included the Timed Up and Go Test (Podsiadlo and Richardson 1991), Timed 10 m Walk Test (Multiple 2013), and the 6 minute Walking test (Enright 2003). These clinical walking tests assessed over-ground walking mobility, speed and endurance. Balance was also assessed pre- and post-intervention with the Berg Balance Scale (Berg et al. 2009).

Strength and muscle activation (EMG)

Maximal voluntary isometric contractions were assessed for ankle dorsiflexion and plantarflexion and for handgrip, measured bilaterally. Similar to previous studies (Dragert and Zehr 2011; Dragert and Zehr 2013), participants were assessed while seated in a custom-fit chair designed to minimize movement. Maximum forces produced during dorsiflexion and plantarflexion contractions were established via strain gauge (Omegadyne Ltd. Model 101-500) and converted to torque using a moment arm length of 0.15 m (measured from the heel block to the center of the strain gauge). Hand grip was performed with a commercial dynamometer (Takei Scientific Instruments Company Ltd., Niigata, Japan). In 10 second trials, following a silent period of 5 seconds, contractions were held for each limb separately. Following a brief warm-up, participants were given two attempts for achieving a maximum contraction.

Electromyographic (EMG) data from the soleus (SOL), tibialis anterior (TA), flexor carpi radialis (FCR), and posterior deltoid (PD), from the more affected (MA) and less affected (LA) limbs, were collected with surface electrodes placed in bipolar configuration over the muscle bellies of interest. Electrodes were placed on the skin and oriented longitudinally along the fibre direction, in accordance with SENIAM procedures (Hermens et al. 2000). Electrodes on the upper and lower limbs were placed in the same positions at each testing session. This was accomplished by recording cathode and anode electrode distances from anatomical landmarks and with pictures taken at the first session and the electrodes were placed by the same experimenter each time. As with other studies from this laboratory (Zehr and Hundza 2005; Zehr et al. 2007a; Zehr et al. 2007b; Vasudevan and Zehr 2011; Zehr and Loadman 2012; Zehr et al.

2012), EMG signals were pre-amplified (×5000) and band-pass filtered (100–300 Hz) (GRASS P511, AstroMed). Data were converted to a digital signal, sampled at 1000 Hz using custom built continuous acquisition software (LABVIEW, National Instruments, TX, USA) and stored to a PC for offline analysis. Using custom-written software programs (Matlab, The Mathworks, Inc., MA, USA) EMG data were full-wave rectified and low-pass filtered at 6Hz using a 4th order Butterworth filter to obtain the liner envelope. Maximum values were taken as the greatest reading generated over two trials by obtaining the mean value over 500ms when force and EMG signals were highest.

Walking

Similar to previously reported methods (Zehr and Loadman 2012), participants walked at a self-selected (‘‘comfortable’’) speed on a motorized treadmill (Woodway USA, Waukesha, WI) while wearing an overhead safety harness (Pneu-weight, Pneumex Inc, Sandpoint, ID, USA). All participants wore the safety harness without body weight support both pre- and post-intervention and none wore an ankle foot orthosis. Participants were free to use hand-held railings in front or beside them during the trial and arm position did not change between pre-tests and the post-test. The self-selected treadmill speed (0.51 ± 0.32 mph) was held constant for that participant for baseline and post training tests to control for the effects of change in treadmill speed with changes in EMG (Hof et al. 2002).

EMG data for walking were collected in a similar manner as for strength but were normalized to maximal EMG recorded during walking. To quantify the rhythmic activation of muscles during walking, a modulation index ($MI = [(EMGmax - EMGmin)/EMGmax] \times 100$) was calculated for each muscle across each movement cycle and averaged. This measure provides a means of comparing the extent to which muscles varied between phasic bursts of activity to alternatively tonic activity throughout the movement cycle (Zehr and Chua 2000; Zehr and Haridas 2003; Zehr and Loadman 2012; Zehr et al. 2012). This measure provides an index of overall amplitude modulation across the movement cycle. Higher values, closer to 100%, indicate a larger range of modulation for a muscle with periods of contraction and periods of relaxation, while a lower value indicates that a muscle’s activity is more constant (Zehr and Chua 2000).

To detect joint kinematics, goniometers (Biometrics Inc, Ladysmith, VA) were used for both the LA and MA ankle (dorsi- plantarflexion) and knee (flexion/extension). These devices were calibrated, output in degrees was determined, and were sampled at 1000Hz. Kinematic data were low-pass filtered at a cut off frequency of 6Hz with a fourth order dual-pass Butterworth filter and were quantified by determining the range of motion by calculating the maximum and minimum angular excursions recorded through the stride cycle.

Similar to other studies (Zehr et al. 1995; Zehr et al. 1997; Lamont and Zehr 2006; Lamont and Zehr 2007; Zehr and Loadman 2012), custom-made force sensing resistors (FSR) (model 1027-1001- ND, Digi-Key, Thief River Falls, MN, USA) were inserted into both shoes under the heel and first metatarsal head of each foot. Heel-contact could not be precisely determined as there was some impairment in heel-strike for these participants therefore FSR signals from the foot sole were summed and used to define stride cycles as periods of stance (foot contact) and swing (no foot contact). The average duration between the start of ipsilateral foot-contacts, duration of stance, and duration of swing were determined. Stride frequency was determined as the average number of strides taken in one second. EMG and kinematic data for the LA and MA sides were aligned to begin with foot contact for that respective side.

Cutaneous Reflexes

The pattern of cutaneous reflex modulation during walking was used to assess the strength of adaptation arising from A&L cycling training. Cutaneous reflexes were evoked via combined surface stimulation of the nerves innervating the dorsum of the hand (superficial radial; SR) and foot (superficial peroneal; SP). Electrodes for SR and SP nerve stimulation were placed just proximal to the radial head and on the crease of the ankle, respectively, on the LA limbs. A Grass S88 stimulator with SIU5 stimulus isolation and a CCU1 constant current unit (Astro-Med Grass Instrument, West Warwick, RI) were used to deliver stimulation in trains of 5 x 1.0ms pulses at 300Hz (P511 Astro-Med Grass Instrument). Perceptual and radiating thresholds (RT) were determined and non-noxious intensities were found for each participant. Stimulation intensities were set to 2.2 x RT for the SR nerve, and 2.0 x RT for the SP nerve.

During treadmill walking, 120 stimulations were delivered pseudo-randomly with an inter-stimulus interval of 1-5 seconds.

All data were sampled at 1 kHz with a 12-bit A/D converter connected to a computer running custom-written LabVIEW (National Instruments, Austin, TX) virtual instrument applications. Evoked reflexes in all muscles tested were aligned to delivery and averaged together. The stimulus artefact was removed from the reflex trace and data were then low-pass filtered at 30 Hz using a dual-pass, fourth order Butterworth filter. To investigate phase-dependent modulation within each movement cycle, data were broken apart into 8 equally timed phases with phases 1-5 representing LA stance and phases 6-8 representing LA swing for walking (Zehr et al. 2007a). For reflexes within each phase, the average trace from the non-stimulated data was subtracted from the stimulated average trace to produce a subtracted EMG reflex trace. Cutaneous reflexes were quantified as the average cumulative reflex over 150 ms following stimulation within each of the 8 phases (Zehr et al. 1997; Zehr et al. 1998b). Background EMG levels between tests were also compared to inspect for a possible scaling effect on reflex activity. A modulation index (MI) for change in reflexes relative to maximum background activity (bEMG) across phases for each muscle was also calculated (MI = $[(\text{Reflex}_{max} – \text{Reflex}_{min})/ \text{bEMG}_{max}] \times 100$).

Statistics

Using commercially available software (SPSS 18.0, Chicago, IL) pre-test and post-test data were compared. To evaluate the extent to which arm and leg cycling training altered walking ability, post-test data were compared to the 95% confidence interval (CI) created from three pre-test sessions and compared to a pre-test average for each participant. To establish the 95% CI for each measure, variability was computed from 3 pre-test sessions and used to create a data range with which the post-test value was compared. If the post-test value fell outside the 95%CI range in the predicted direction, it was considered significant for that participant. The total number of participants with a significant test is reported and dichotomous scores (1 representing a post-test score outside of the 95%CI range and 2 representing a score within the 95%CI range) for each participant for each measure were compared with the chi-squares test statistic to examine association.

For pretest data a repeated-measures ANOVA was performed to examine difference across the three pre-test sessions. If no difference was found, data were pooled together to create an average pre-test value and compared to post-tests values with a paired-samples t-test (t). For each test, the degrees of freedom are reported in subscript. Assumptions for ANOVA and paired-samples t-tests were evaluated for parametric tests for a within-subject design. Pearson's correlation coefficients (r) were calculated between several variables and tested for significance. The observed effect for post-test differences for clinical measures, strength and walking parameters is also reported as the Cohen's effect size (d) where a small effect is $d = 0.2$, a medium effect is $d = 0.5$ and a large effect is $d = 0.8$ (Cohen 2013). For a priori hypotheses where direction of change was predicted, a one tailed paired samples t-test was performed. Statistical significance was set at $p \leq 0.05$.

Results

A total of 25 participants were recruited. Six participants were excluded because of self-withdrawal (n = 1), change in physical activity patterns (n = 1), and not meeting minimum inclusion criteria or were already participating in A&L cycling exercise (n = 4). Baseline and demographic data are reported for the remaining 19 participants (see Table 6-1). All participants contributed data to each measure.

Table 6-1: Participant Data and Clinical Assessment Parameters

N	Sex/ Age/ MA	Modified Ashworth (ankle/knee)	FAC (/6)	Chedoke-McMaster (A/H/L/F)	Monofilament (hand/foot)	Reflexes (S1 / L1)	Years since stroke
1	M/74/R	3 / 1+	4	2 / 2 / 3 / 2	J 4.31 / J 4.31	3+ / 1+	5
2	F/70/R	0 / 0	5	7 / 5 / 7 / 7	J 4.31 / J 4.31	2+ / 2+	2
3	F/45/R	1 / 0	5	5 / 5 / 6 / 5	F 3.61 / J 4.31	0 / 0	7
4	M/59/R	2 / 0	5	2 / 2 / 4 / 2	T 6.65 / J 4.31	3+ / 3+	3
5	M/82/R	0 / 1	3	4 / 6 / 6 / 5	UTF / UTF	3+ / 0	3
6	M/86/L	1+ / 0	5	7 / 7 / 6 / 5	J 4.31 / T 6.65	0 / 0	4
7	F/80/R	0 / 0	5	3 / 5 / 5 / 5	J 4.31 / J 4.31	0 / 0	6
8	M/59/R	2 / 1	5	5 / 5 / 5 / 4	T 6.65 / T 6.65	3+ / 4+	11
9	M/74/R	1 / 1	5	6 / 5 / 6 / 5	J 4.31 / F 3.61	3+ / 2+	6
10	M/47/L	4 / 2	4	2 / 1 / 2 / 2	T 6.65 / T 6.65	4+ / 3+	6
11	M/69/L	2 / 3	4	2 / 2 / 3 / 2	T 6.65 / T 6.65	3+ / 3+	5
12	F/72/R	2 / 2	3	2 / 3 / 2 / 3	UTF / J 4.31	1+ / 3+	6

13	M/59/L	1 / 1	6	6 / 6 / 6 / 4	J 4.31 / J 4.31	3+ / 2+	5
14	M/56/L	1 / 1	5	1 / 1 / 4 / 2	T 6.65 / T 6.65	3+ / 3+	8
15	M/77/L	2 / 2	3	4 / 5 / 5 / 3	UTF / T 6.65	3+ / 3+	8
16	F/63/L	1 / 2	5	2 / 2 / 3 / 4	T 6.65 / K 4.56	3+ / 1+	13
17	M/71/R	1 / 2	4	3 / 2 / 4 / 4	F 3.61 / J 4.31	2+ / 3+	6
18	M/62/R	1+ / 2	4	4 / 3 / 4 / 5	D 2.83 / D 2.83	3+ / 3+	8
19	M/78/L	3 / 1+	4	3 / 3 / 4 / 4	T 6.65 / T 6.65	0 / 0	29

Abbreviations: MA, more affected; M, male; F, female; L, left; R, right; FAC, Functional Ambulation Category; A, arm; H, hand; L, leg; F, foot; UTF, unable to feel; S1, 1st sacral vertebrae and L1, 1st lumbar vertebrae.

Training Results

All participants completed the 15 sessions of A&L cycling training. Figure 6-2 shows the average HR, RPE, RPM and Work in each of the 15 training sessions averaged across all participants. Within a session, HR increased between minute 5 and minute 30 from 76.0 ± 1.9 bpm to 98.9 ± 3.1 bpm and there was no significant difference between the first training session and the last training session. Across sessions, while there was no change in HR and RPE, there was a significant increase in RPM ($t_{(18)}$ = 2.399, p=0.014, Figure 2 subplot C) and Work ($t_{(18)}$ = 6.475, p=0.000, Figure 2 subplot D) between the first and last training session. Despite increases in RPM and work, the same relative RPE was maintained.

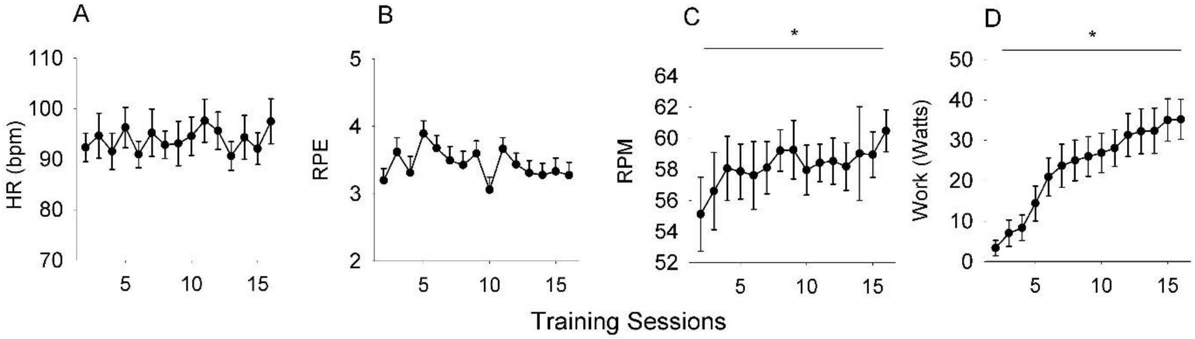


Figure 6-2: Training parameters for HR, RPE, RPM and Work over 15 training sessions. Data are means (±sem) averaged across all participants for all training sessions. * indicates a significant difference between first and last training sessions.

Clinical Measures

A paired t-test revealed that there was a significant decrease (14.4% change ($t_{(18)}$ = 2.100, p=0.025, d=0.350) in the time taken for the Timed Up and Go test where participants completed the test in 29.33 ± 25.83 seconds before training and 25.12 ± 22.14 seconds after training . Time taken for the 10m walk test also significantly decreased where participants completed the test at 0.45 ± 0.50 m/sec before training and 0.51 ± 0.48 m/sec after training indicating a 13.3% improvement ($t_{(18)}$ = 2.342, p=0.015, d=0.192). The number of steps taken for the 10m walk test also significantly decreased with 27.17 ± 12.44 steps before training and 25.69 ± 12.50 steps after training indicating a 5.45% improvement ($t_{(18)}$ = 2.140, p=0.023, d=0.239). The total distance covered in the 6 minute walk test significantly increased between the pre and post-test from 217.41 ± 107.67 feet to 252.43 ± 138.38 feet indicating a 16.10% improvement ($t_{(18)}$ = 3.586, p=0.001, d=0.564). The total score from the Berg Balance Scale significantly increased following A&L cycling training from a mean score of 42.04 ±10.48 to a mean score of 45.06 ± 2.38 (median scores of 45 to 48 post-training) indicating a 4.94% improvement ($t_{(18)}$ = 2.825, p=0.005, d=0.528).

Table 6-2 summarizes results from the single-participants statistical tests that are discussed below. The number of participants with a significant post-test value is reported for each variable in the Table. For most variables, the majority of participants did show a significant post-test change.

Table 6-2: Single-Subject Analysis

Measure	Number of participants (out of 19) with significant changes after training
Strength Increases	
LA Plantarflexion	10
LA SOL	10
MA Plantarflexion	10
MA SOL	8
LA Dorsiflexion	11
LA TA	7
MA Dorsiflexion	12
MA TA	11
LA Grip	14
LA FCR	7
MA Grip	17
MA FCR	8
Walking bEMG Modulation Index	
MA SOL	13
MA TA	9

LA SOL	10
LA TA	12
MA FCR	12
MA PD	11
LA FCR	13
LA PD	12
Walking Kinematics	
LA Ankle	9
LA Knee	9
MA Ankle	10
MA Knee	11
Walking Parameters	
LA Stride Duration	8
MA Stride Duration	9
LA Stance Duration	11
MA Stance Duration	10
LA Swing Duration	14
MA Swing Duration	10
LA Stride Frequency	8
MA Stride Frequency	8
Walking Cutaneous Reflex Modulation Index	
MA SOL	13
MA TA	10
LA SOL	13
LA TA	12
MA FCR	13
MA PD	12
LA FCR	9
LA PD	12

Abbreviations: MA, more affected; LA, less affected; SOL, soleus; TA, tibialis anterior; FCR, flexor carpi radialis; PD, posterior deltoid; bEMG, background electromyography.

Strength and EMG

Figure 6-3 shows peak EMG activity and force during plantarflexion, dorsiflexion, and handgrip averaged across all participants for three pretests and the post-test conditions. No significant differences were found for pre-test baseline data. Following training, plantarflexion force was significantly increased on the LA side by 15.48% and on the MA side by 44.93% ($t_{(18)}$=2.061, p=0.029, d=0.437, Figure 6-3 subplot B and $t_{(18)}$=2.073, p=0.029, d=0.439, Figure 6-3 subplot D for the LA and MA sides, respectively). Maximal soleus EMG on the LA side also increased by 27.14% ($t_{(18)} = 2.154$, p=0.025, d=0.453, Figure 6-3 subplot A). The increase in plantarflexion force and SOL EMG on the LA side were significantly correlated (r=0.499, p=0.045). For dorsiflexion, LA force significantly increased by 16.61% and MA force significantly increased by 34.93% ($t_{(18)} = 1.821$, p=0.045, d=0.394, Figure 6-3 subplot F and $t_{(18)} = 2.244$, p=0.021, d=0.568, d=1.057, Figure 6-3 subplot H for the LA and MA sides, respectively). Peak tibialis anterior EMG also significantly increased on the MA side by 27.91% ($t_{(18)} = 1.946$, p=0.036, d=0.417, Figure 6-3 subplot G). The increase in MA dorsiflexion force and MA TA EMG activity was significantly related (r=0.742, p=0.001). Handgrip strength significantly increased on the LA side by 16.74% and on the MA side by 44.78% ($t_{(18)} = 4.010$, p=0.001, d=0.687, Figure 6-3 subplot J and $t_{(18)} = 5.026$, p=0.000, d=0.764, Figure 6-3 subplot L for the LA and MA sides, respectively). There was an association between the likelihood of a significant increase in LA strength and MA strength ($\chi^2_{(1)}=23.768$, $p<0.0001$).

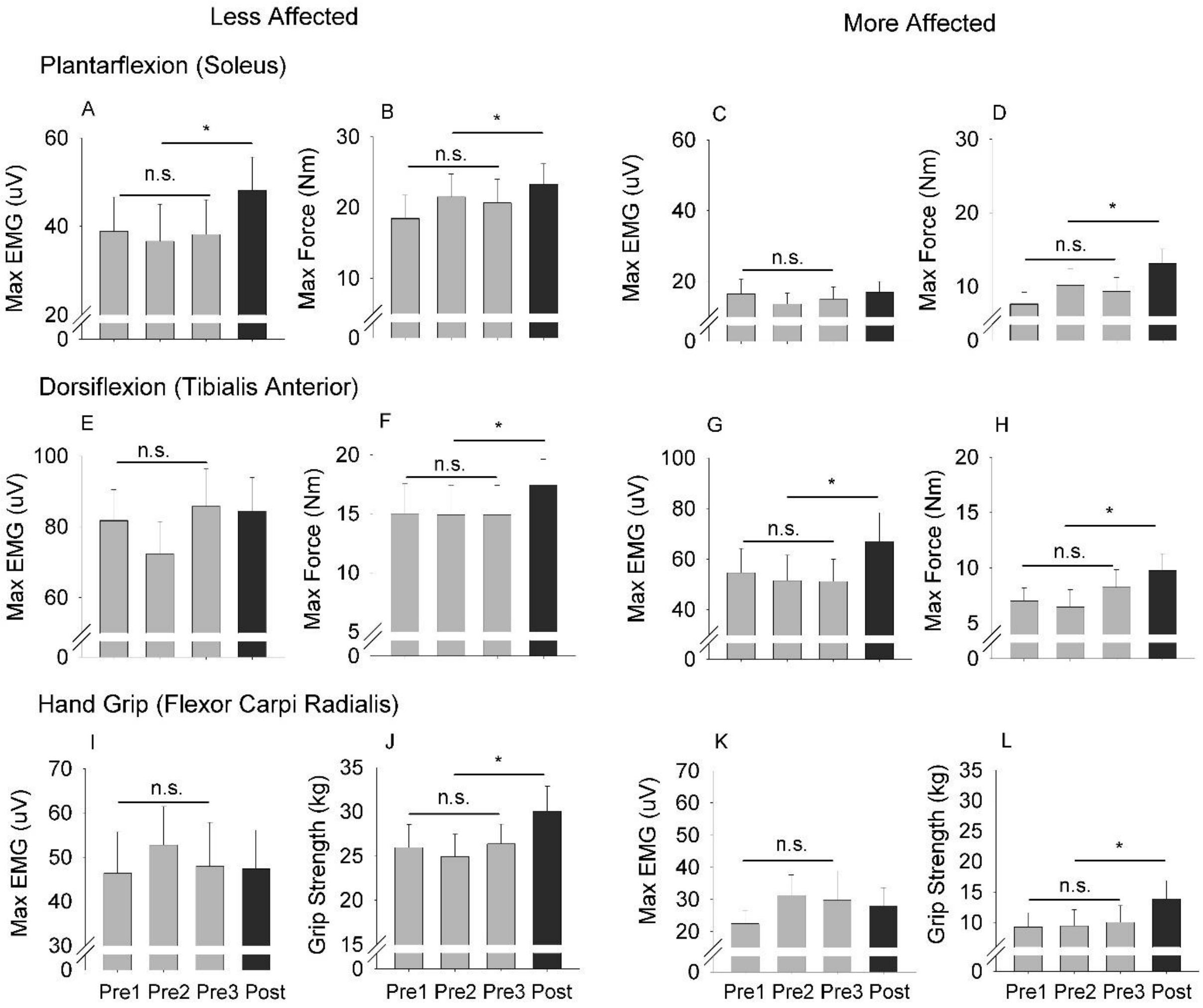


Figure 6-3: Plantarflexion, Dorsiflexion, and Hand Grip strength and muscle activation. Bar graphs are means (±sem) for EMG and force during isometric strength tests averaged across all participants. * indicates significant differences between the pre-test average and the post-test value and n.s. indicates a non-significant difference for the three baseline measures.

Walking

Figure 6-4 shows EMG for the muscles of the LA and MA limbs averaged across all participants for three pretests and for post-test values during walking. Line graphs are data expressed as a percentage of the gait cycle where 0% indicates foot contact for that side. Bar graphs are background EMG modulation indices across muscles averaged for all participants. No significant pre-test differences were found for any muscles. Following training, for the LA TA, there was a significant decrease ($t_{(18)}$ = 1.875, p=0.041, d=0.398, Figure 6-4 subplot F) in modulation by 6.4%. In the MA FCR, modulation significantly increased ($t_{(18)}$ = 2.134, p=0.027, d=0.496, Figure 6-4 subplot K) by 34.7% and modulation also significantly increased for both the LA and MA PD by 12.1% and 28.9% ($t_{(18)}$ = 2.975, p=0.004, d=0.827, Figure 6-4 subplot N and $t_{(18)}$ = 2.259, p=0.021, d=0.649, Figure 6-4 subplot P for the LA and MA PD, respectively). When comparing the ratio of modulation between the LA and MA sides for each muscle, there was a significant decreases of 49.2% in ratio for the PD ($t_{(18)}$ = 3.085, p=0.009, d=0.423).

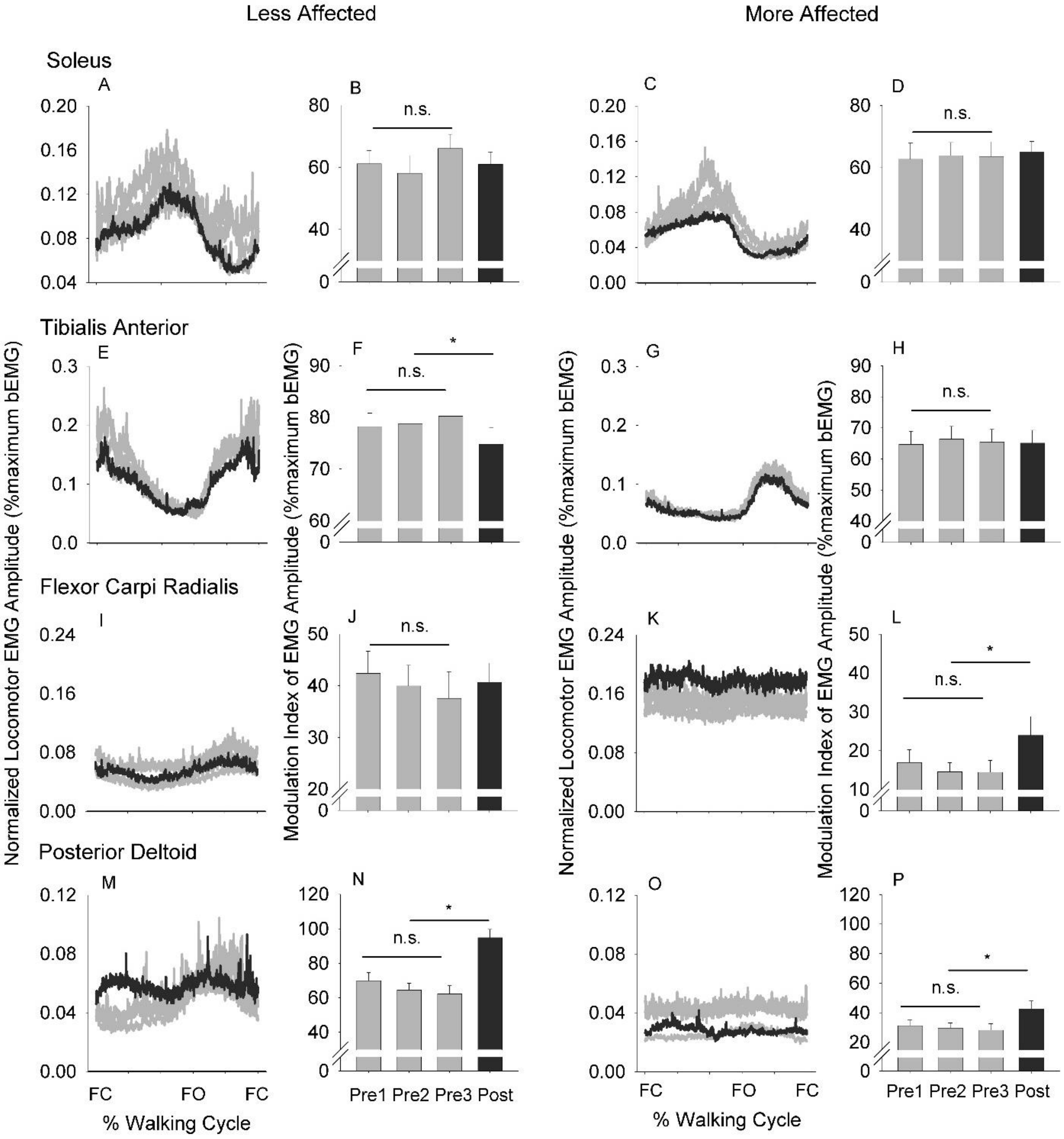


Figure 6-4: Background EMG during walking. Line graphs are normalized and averaged EMG for the walking cycle for three baseline tests (light gray lines) and for the post-test (dark gray lines). Foot contact (FC) and foot off (FO) times are indicated. Bar graphs are mean (± sem) modulation indices for all muscles averaged across all participants.

* indicates significant differences between the pre-test average and the post-test value and n.s. indicates a non-significant difference for the three baseline measures.

Figure 6-5 shows kinematic data for the LA and MA ankle and knee averaged across all participants for three pretests and for post-test values during walking. Line graphs are from a representative participant aligned to begin at foot contact and bar graphs are ROM values averaged across all participants. No pre-test differences were found for any kinematic variables. Following training, all variables showed statistically significant increases in ROM for post-test compared to pretest values (LA ankle $t_{(18)} = 2.970$, p=0.004 d=0.558, Figure 6-5 subplot B, MA ankle $t_{(18)} = 2.078$, p=0.027, d=0.426, Figure 6-5 subplot D, LA knee $t_{(18)} = 2.561$, p=0.010, d=0.382, Figure 6-5 subplot F, and MA knee $t_{(18)} = 3.404$, p=0.002, d=0.476, Figure 6-5 subplot H). For the ankle, there was a 25.51% increase in ROM for the LA side and a 21.73% increase in ROM for the MA side. For the knee, there was a 19.37% increase in ROM for the LA side and a 22.21% increase in ROM for the MA side. There was a significant association between a change in LA and MA kinematics ($\chi^2_{(1)}$=3.979, p=0.046).

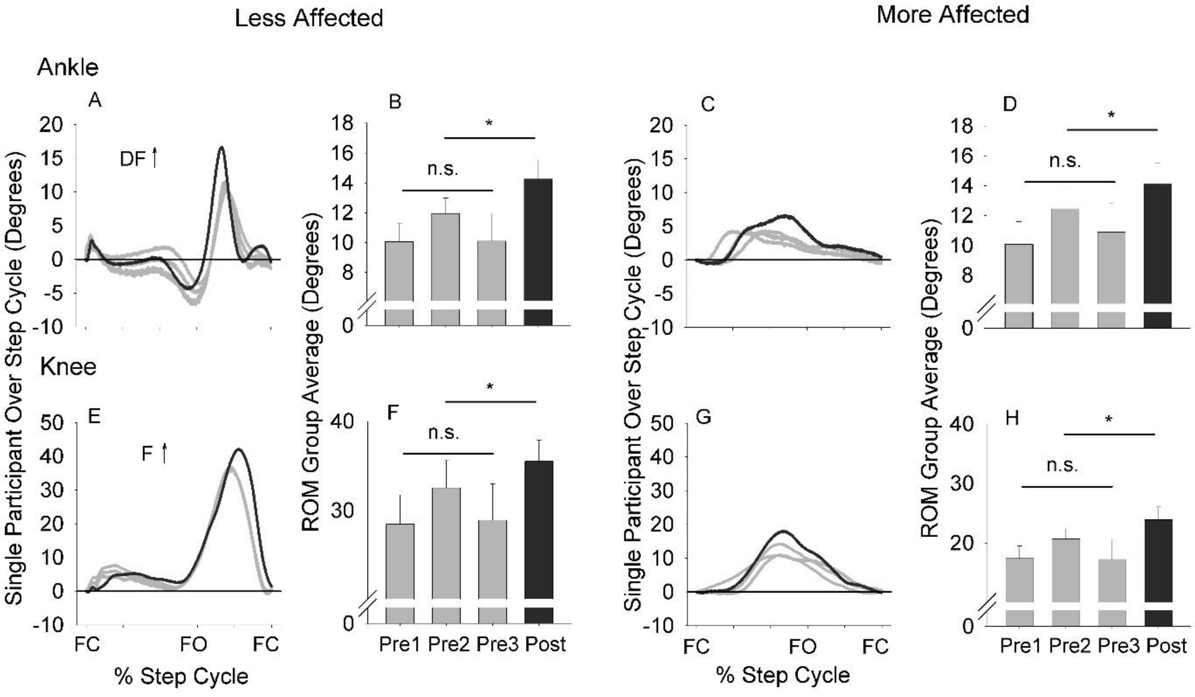


Figure 6-5: Kinematics during walking. Line graphs are single participant kinematics for the walking cycle for three baseline tests (light gray lines) and for the post-test (dark gray lines). Foot contact (FC) and foot off (FO) times are indicated. Dorsiflexion (DF) and flexion (F) increases are positive. Bar graphs are mean (± sem) range of motion values averaged across all participants. * indicates significant differences between the pre-test average and the post-test value and n.s. indicates a non-significant difference for the three baseline measures.

Walking parameters including average stride, stance and swing durations and stride frequencies from the LA and MA sides averaged across all participants for the three pre-tests and for post-test are shown in Figure 6-6. No baseline differences were detected for any walking parameter data. Following training, there was a significant decrease in stride duration on the LA and MA sides ($t_{(18)}$ = 2.448, p=0.013, d=0.500, Figure 6-6 subplot A and $t_{(18)}$ = 3.077, p=0.003 d=0.587, Figure 6-6 subplot B for the LA and MA sides, respectively) with a 5.25% and 8.74% decrease in LA and MA stride duration. Stance duration for the LA side significantly decreased ($t_{(18)}$ = 2.457, p=0.013, d=0.501, Figure 6-6 subplot C) by 12.53% while swing duration increased ($t_{(18)}$ = 1.837, p=0.042, d=0.397, Figure 6-6 subplot E) by 11.29% following A&L cycling training. There were also significant increases in stride frequency compared to the pre-test values for both the LA and MA sides ($t_{(18)}$ = -1.961, p=0.033, d=0.419, Figure 6-6 subplot G and $t_{(18)}$ = -2.114, p=0.025, d=0.446, Figure 6-6 subplot H, for the LA and MA sides, respectively). Stride frequency increased by 3.82% for the LA side and 4.07% for the MA side. Percentage change in stride duration is significantly correlated to percentage change in stride frequency for both the LA (r=-0.989, p=0.000) and MA (r=-0.702, p=0.001) sides. There was a significant association between a change in MA and LA walking parameters ($\chi^2_{(1)}$=30.728, p=0.000). These changes in walking parameters following training were independent of changes in speed as treadmill speed was held constant across all testing sessions.

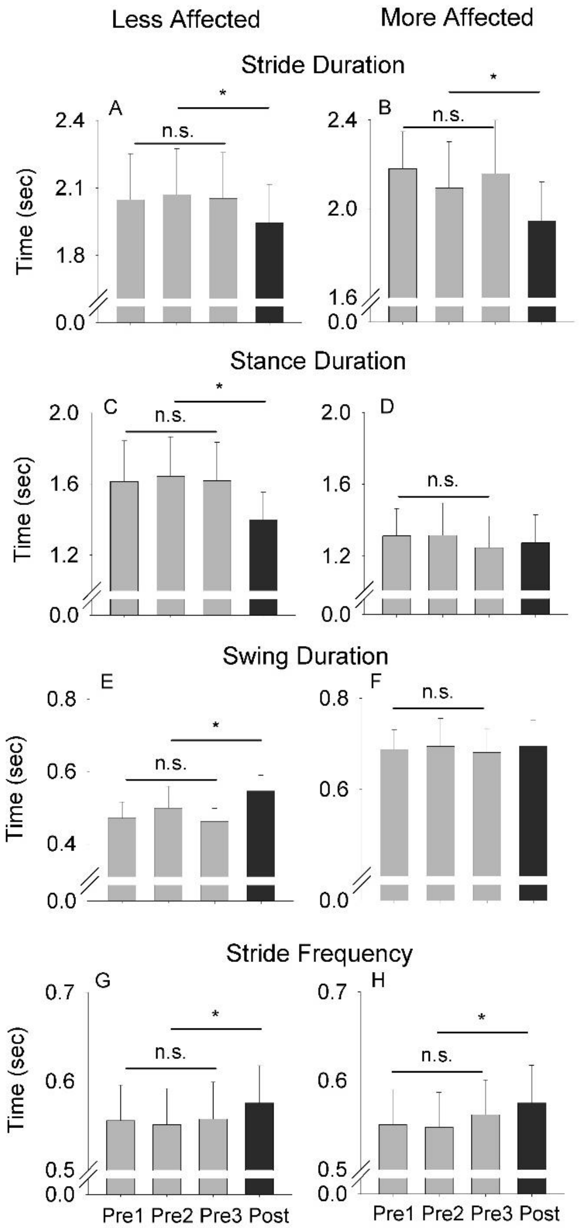


Figure 6-6: Temporal parameters of walking. Bar graphs are mean (±sem) values for stride duration, stance duration, swing duration and stride frequency for three baseline tests and the post-test averaged across all participants. * indicates significant differences between the pre-test average and the post-test value and n.s. indicates a non-significant difference for the three baseline measures.

Cutaneous Reflexes

Figure 6-7 shows data for all reflexes evoked during treadmill walking averaged across all participants. This process reveals the general trend in evoked responses but obscures phase-modulation. To quantify overall modulation of reflexes, a modulation index was quantified for the muscles on the LA and MA sides and shown as bar graphs on Figure 6-7. Following training, modulation was significantly decreased for the LA SOL ($t_{(18)} = 2.217$, p=0.045, d=0.355, Figure 6-7 subplot B) by 29.3%. For the LA TA modulation significantly increased by 44.6% ($t_{(18)} = 3.493$, p=0.004 d=0.378, Figure 6-7 subplot F) and for the LA PD modulation increased by 80.4% ($t_{(18)} = 2.197$, p=0.047 d=0.386, Figure 6-7 subplot N, respectively). There was a significant association between a change in MA and LA cutaneous reflex modulation ($\chi^2_{(1)}$=5.793, p=0.016).

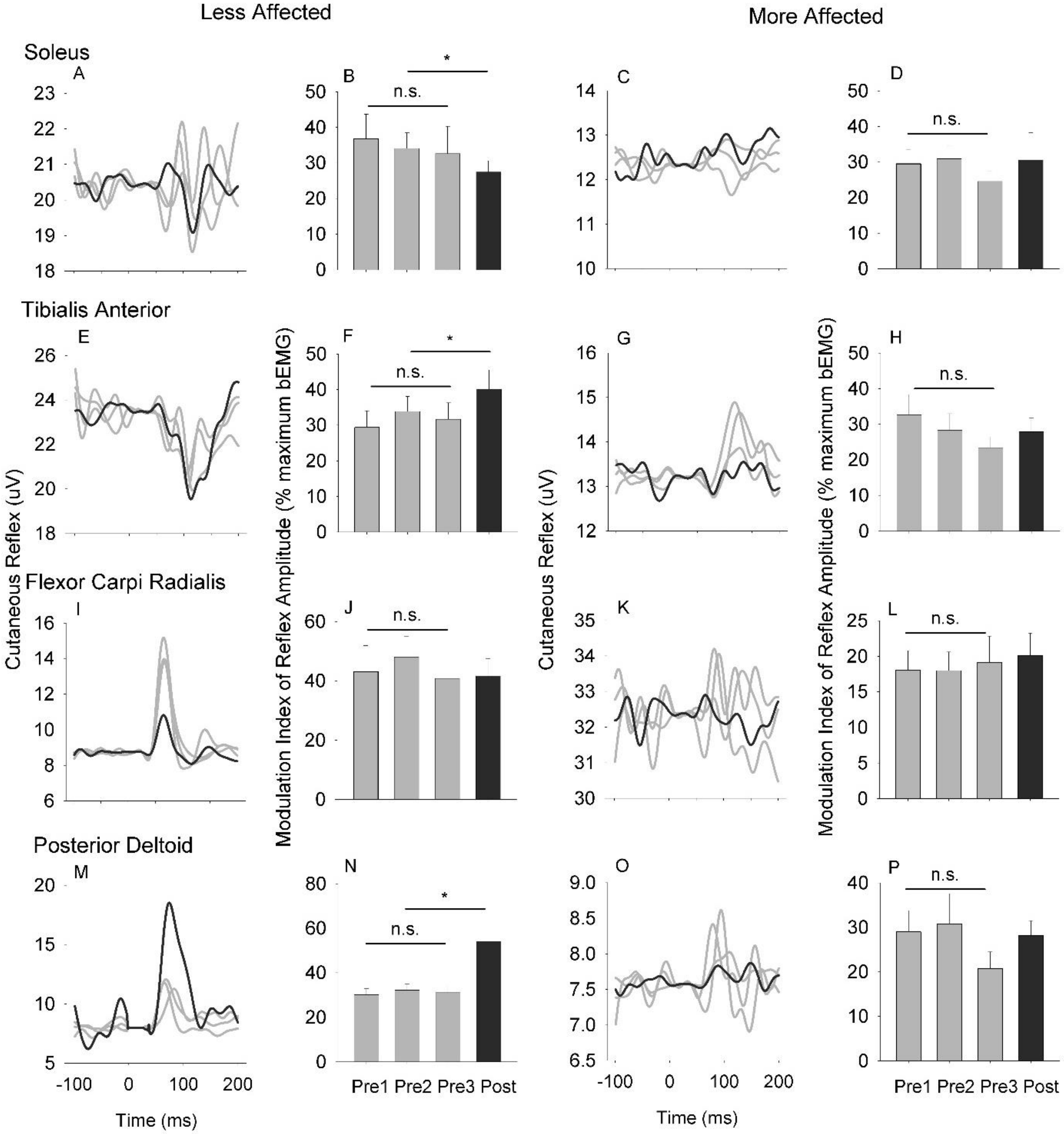


Figure 6-7: Cutaneous reflexes during walking. Line graphs are averages across all participants for three baseline

tests (light gray lines) and for the post-test (dark gray line). The stimulus artefact beginning at time 0 has been blanked out and replaced by a flat line. Stimulation was applied to the superficial radial nerve of the hand and the superficial peroneal nerve of the foot on the LA side. Bar graphs are means (±standard error) averaged across all participants for baseline and post-test values. * indicates significant differences between the pre-test average and the post-test value and n.s. indicates a non-significant difference for the three baseline measures.

Figure 6-8 shows cutaneous reflexes (bars) during walking at all phases. Since there were no significant differences between the pretest data, for simplification the average value across the three tests is shown. For reflex amplitudes there are significant differences between baseline and post-test values for several muscles including the LA SOL, LA and MA TA, and for the LA and MA FCR. For the LA SOL, there was a significant change in reflex amplitude for phase 2 ($t_{(18)}$=2.207, p=0.046) and phase 7 ($t_{(18)}$=2.271, p=0.021). For the LA TA phase 1 and phase 8 showed significant differences in post-test values compared to the baseline average (for phase 1 $t_{(18)}$=2.271, p=0.041 and for phase 8 $t_{(18)}$=1.871, p=0.042). For the MA TA a significant post-test difference was found for phase 1 ($t_{(18)}$=2.660, p=0.012). For the LA FCR, phase 5 showed a significant post-test difference ($t_{(18)}$=2.718, p=0.018) and for the MA FCR, phase 1 showed a significant post-test difference ($t_{(18)}$=2.660, p=0.012).

Investigating background EMG levels between tests allows for comparison of reflex amplitudes that cannot be explained by scaling with background EMG. Figure 6-8 shows bEMG (lines) during waking at all phases. For bEMG at specific phases of walking, there are significant differences between baseline and post-test values for the LA and MA FCR and LA PD muscles. For the MA FCR, significant differences were found for phase 2 ($t_{(18)}$=2.227, p=0.036), 3 ($t_{(18)}$=2.142, p=0.044), and 4 ($t_{(18)}$=2.406, p=0.033). For the LA FCR, significant differences were found for phase 7 ($t_{(18)}$=3.578, p=0.004). For the LA PD, significant differences were found for phases 1 and 2 ($t_{(18)}$=2.407, p=0.033 and $t_{(18)}$=2.754, p=0.017, respectively).

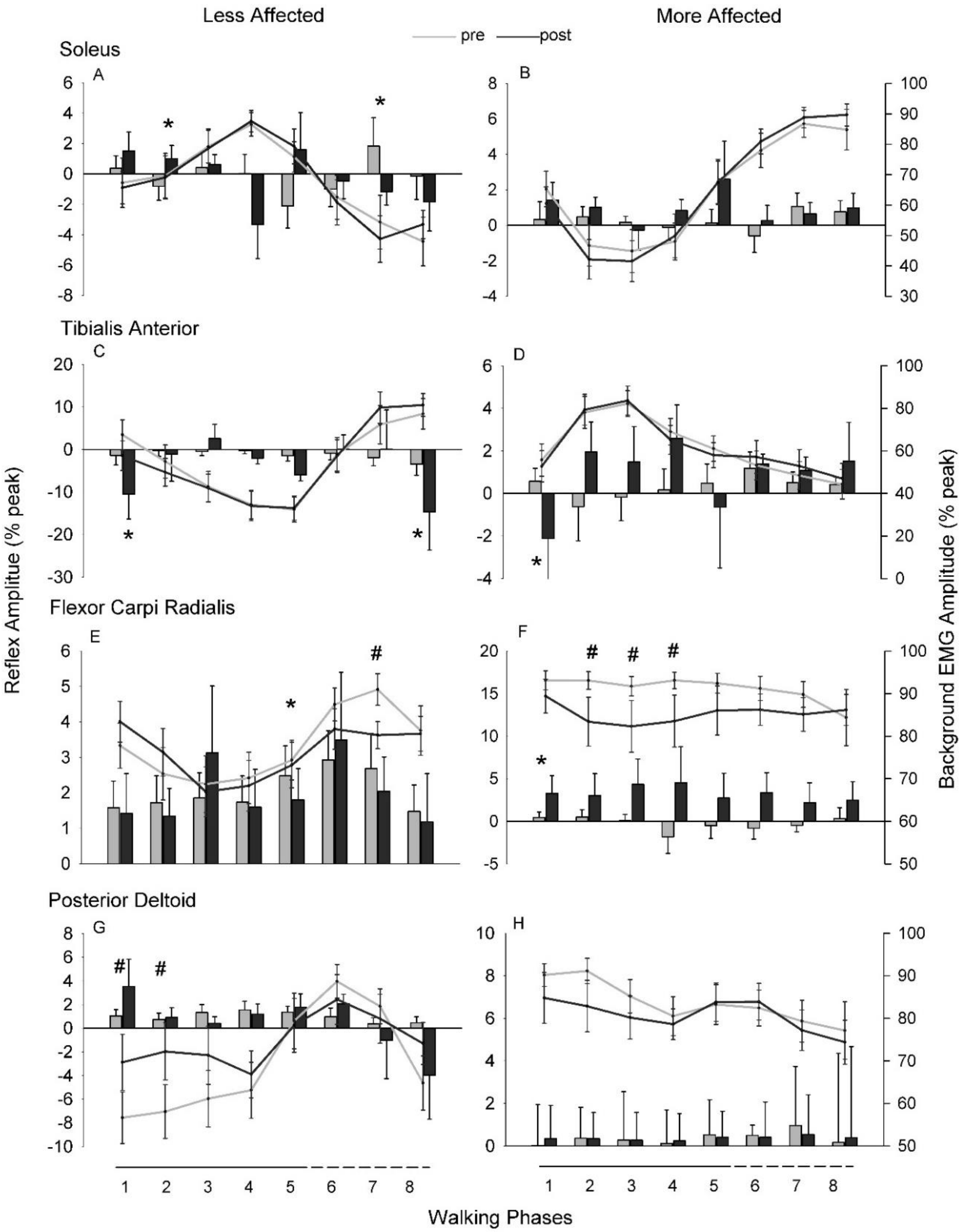


Figure 6-8: Normalized background EMG and reflex amplitudes during walking**.** Background EMG is shown in line plots and reflex amplitude is shown in bar plots. Values are means (±standard error) averaged across all participants and normalized to the peak undisturbed EMG during walking. The horizontal bars below the y-axes represent the stance (solid line) and swing (dotted line) phase of walking. Significant differences between the pre-test average and the post-test value are indicated with # for background EMG and * for reflex amplitude.

Figures 6-9 shows cutaneous reflexes during walking at specific phases of interest. Reflex modulation for the LA and MA TA and FCR are shown for specific phases of interest. At these phases, there are significant effects of training on post-test values and no significant differences in bEMG. Line graphs are of the subtracted reflex averaged across all participants for that phase.

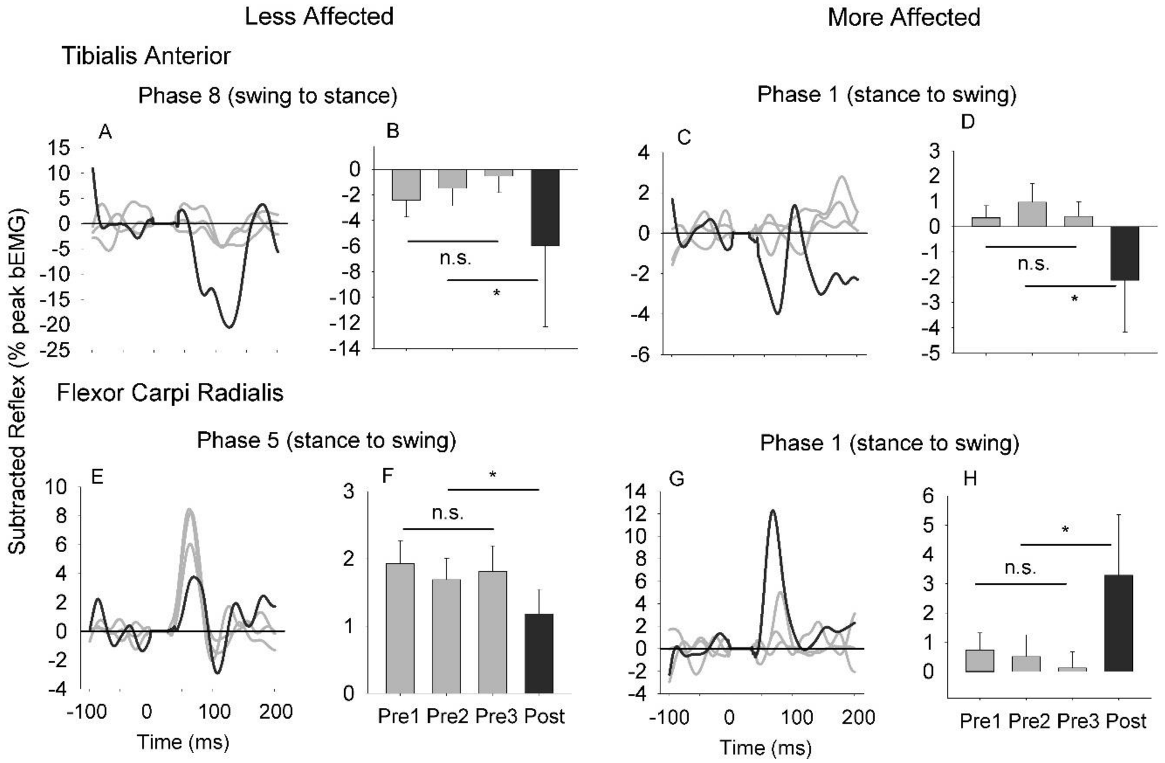


Figure 6-9: Normalized background EMG and reflex amplitudes at specific phase of interest during walking. Bar graphs are means (±standard error) for reflex amplitude averaged across all participants for baseline and post-test values. Line graphs are means (±standard error) for bEMG. * indicates significant differences between the pre-test average and the post-test value. There were no significant differences for bEMG.

Discussion

This project tested the efficacy of A&L cycling training for improving walking ability after stroke. Participants performed A&L cycling three times per week for five weeks for 30 minutes of exercise time each session. This aggregate 450 minutes of activity, performed at a moderate level, improved walking after stroke. A&L cycling training improved clinical walking status, increased strength, range of motion, temporal parameters of treadmill walking and improved modulation of muscle activity and cutaneous reflexes. These results demonstrate that maximizing activity in inherent arm and leg connections spared after a stroke, with A&L cycling, could facilitate motor recovery. A&L cycling could be used as a novel rehabilitation modality to maximize functional motor recovery and improve walking ability after stroke.

A&L cycling training produced global changes in clinical status. There was a 4.19 second improvement in the Timed Up and Go test. This corresponds to a noticeable change in ability as the minimal detectable change for chronic stroke participants is 2.9 seconds (Flansbjer et al. 2005). Although there was an improvement in time taken for this test, values still fall below normative values for community-dwelling elderly people who finish the test in approximately 9-12 seconds (Steffen et al. 2002). For the 10-m Walk Test, speed increased by 0.06 m/s indicating a small but meaningful change (Perera et al. 2006). Normative data for the stroke population (n = 48, age 68, with reduced muscle strength and walking capacity) is 0.84 ± 0.30 m/s. For the 6 Minute Walk Test, participants improved by walking an additional 114.87ft which is above a minimal detectable change of 112.76ft for stroke participants (Eng et al. 2004). Therefore in summary, the walking tests showed minimal changes outside of error that reflect a true change between baseline tests and post-test values. For the balance test, scores on the Berg Balance Scale improved by 2.12 points which is just below the 2.5 point minimal detectable change criterion difference for a chronic stroke population (Liston and Brouwer 1996). We consider this change significant given that A&L cycling may not require the same trunk and pelvic control that walking does yet still improves balance after training.

Strength during isometric contractions increased for both the LA side and the MA side for plantarflexion and dorsiflexion following A&L cycling training. For the LA SOL and the MA TA, the increase in force was correlated to an increase in EMG. Handgrip strength also increased for both hands following training. A concomitant increase in EMG with force was not surprising as there is a linear correlation between the amplitude of EMG and the force produced during isometric contractions (Perry and Bekey 1981). It is surprising however that no increase in EMG was recorded for the LA and MA FCR to match the increase in grip strength. Co-contraction of adjacent muscle groups, not being recorded from could account for this difference. Alternatively, perhaps no increase in EMG activity of these forearm muscles was observed because in some cases we used a hand brace to secure the weakened hand to the ergometer handle during training.

Nevertheless, these results show an overall increase in strength resulting from A&L cycling training. A number of other studies also report improvements in strength following treadmill training interventions in those with spinal cord injury (Dietz et al. 1998; Behrman and Harkema 2000; Field-Fote 2001; Senthilvelkumar et al. 2015). Similar observations have been made in stroke and there is a positive correlation between strength gains and walking speed (Richards and Olney 1996; Kim and Eng 2003). Strength gains, an indirect result of A&L cycling training, likely contributes to the increase in walking ability seen here.

Changes in several variables measured during walking gauge training transfer effects following A&L cycling training. Variables include EMG modulation, kinematics, and stride parameters including duration, stance percentages, and frequency. For depth of modulation following A&L cycling training, the LA TA showed decreased modulation representing a smoothing out of dorsiflexor activity. Increased control to eliminate unwanted dorsiflexor activity is required to increase walking endurance in people with hemiplegia after stroke (Ng and Hui-Chan 2012). For the arms, in general the arm muscles showed increased depth of modulation after training. An increased depth of modulation, indicating an increase in the amount of phasic activity, more closely represents what is found in neurologically intact participants (Zehr and Loadman 2012), could have been due to changes in weight support borne through the arms after training. The significant changes in modulation indices and the pattern of EMG activity for FCR and PD muscles appears different between the MA and LA sides. This may be important for the A&L-induced walking improvements observed here where increased modulation of these muscles may decrease exaggerated interlimb neural coupling producing a “flexor synergy” that has been previously reported post-stroke (Kline et al. 2007).

Walking kinematics for all joints tested increased range of motion following training with an average increase of 22%. The transfer from A&L cycling training to improve walking kinematics is particularly interesting given kinematics are constrained on the A&L cycle ergometer (Zehr et al. 2007a). Several variables related to walking parameters were also changed by A&L cycling training. Stride duration was decreased following training related to an increase in stride frequency. Within a stride for the LA side, it was found that stance duration decreased while swing duration increased. These changes in swing and stance duration represent a more normal gait pattern (von Schroeder et al. 1995). Treadmill belt speed between pre-tests and the post-test was held constant for that participant and cannot be implicated as a source of the change in walking parameters seen here.

Changes in cutaneous reflex modulation were taken as a proxy of spinal plasticity arising from the A&L cycling training. Overall reflexes showed some improved modulation patterns following training. Cutaneous stimulation produced reflex effects in all muscles tested and are modulated during walking in a similar way to that found in neurologically intact participants (Haridas and Zehr 2003). By using an index of modulation it is possible to see how the depth of reflex modulation changed with A&L cycling training. In the LA TA and LA PD, reflex modulation increased, representing an overall increase in the depth of modulation, perhaps due to increased access to these interlimb networks following training.

When examining the grand average reflex traces from cutaneous stimulation, activity in the LA TA is mainly suppressive, while the MA TA shows mainly facilitations (see Figure 6-7). This is in line with previous observations of cutaneous responses in the TA following stroke (Jones and Yang 1994; Zehr et al. 1998a; Duysens et al. 2004) where on the MA side, the decreased influence of the corticospinal tract on reflex excitability, as a result of the stroke lesion, fails to produce the appropriate suppressions associated with normal reflex activity (Duysens et al. 2004). When examining specific functional phases for walking, adaptive plasticity was seen following training. Responses in the LA TA at phase 8, representing the swing to stance transition, showed increased inhibition. In the MA TA at phase 1, reflexes turned from facilitation to suppression following training. Normally at these phases, in neurologically intact participants, inhibitory responses are observed in the TA to aid with safe footfall allowing passive plantarflexion (Yang and Stein 1990; Duysens et al. 1990; Eng et al. 1994; Van Wezel et al. 1997; Zehr et al. 1997) and the re-emergence of end-swing suppressions following training reveals the normalization of reflexes as a result of A&L cycling training. In the arm muscles, stimulation following training produced decreased facilitation on the LA side and increased facilitation on the MA side, again representing a return to what one normally observes in modulation in these interlimb networks (Zehr and Loadman 2012; Zehr et al. 2012).

Together these results demonstrate that adaptive plasticity in interlimb spinal networks is possible following rehabilitative training. It is unknown, however, how long these results persist and their functional implications. Further investigation of chronic plasticity in somatosensory pathways is warranted in order to fully understand motor adaptation to maximize functional recovery after neurological injury.

Task Transfer and Asymmetry of Changes between Sides

A bias between the observed training transfer effects between the LA and MA sides existed following A&L cycling training. A larger effect of strength gains following A&L cycling training was observed for the MA side for ankle dorsiflexion, plantarflexion and for handgrip. However, following training, an asymmetry was still observed between sides where MA post-test values were still below LA pre-test values. An asymmetry in strength gains was also observed following treadmill aerobic exercise in patients with chronic hemiparesis following stroke where the greatest relative strength gains were seen in the MA limbs (Smith et al. 1998). Asymmetry was also observed for EMG modulation following A&L cycling training where modulation was greater for the MA PD. The bias towards greater improvement on the MA side following A&L cycling training likely results from the increased potential for improvements on the MA side due to the higher degree of impairment (Langhorne et al. 2011). Despite increased range of motion, alignment of walking kinematics between the LA and MA sides did not appear to improve after A&L cycling training. Therefore, although A&L cycling training does appear to result in a positive task transfer to improved walking, it does not produce a return to symmetry as kinematics on the MA side are still quite different from kinematics on the LA side.

A&L cycling training was an indirect training paradigm where walking was not the target of the training. The improvements in the trained task of A&L cycling transferred to improvements in the untrained task of walking. A few studies in stroke suggest partial transfer of a trained task on improving walking. For example, fitness training, high-intensity therapy and repetitive task training all show beneficial improvements to walking after stroke (Kwakkel et al. 2004; Morris et al. 2004; Ada et al. 2006; Langhorne et al. 2009).

The success of training transfer depended on how similar A&L cycling and walking are to each other and indeed, all forms of rhythmic human movement share common neural elements (Zehr 2005). The “common core” controlling cyclical limb movements is predicated upon multi-segmental central pattern generating networks reinforced by somatosensory feedback regulated by supraspinal inputs (Grillner and Dubuc 1988; Duysens and Van De Crommert 1998; Zehr 2005; McCrea and Rybak 2008). Common neural elements are seen across different forms of walking (level, incline, and stair climbing; (Lamont and Zehr 2007)), between different modes of rhythmic arm movement (Carroll et al. 2005; Klimstra et al. 2009), and between different modes of arm and leg coordination during recumbent stepping, cycling, and walking (Balter and Zehr 2007; Mezzarane et al. 2011). The neuronal activity associated with generating rhythmic A&L cycling contains about 60% of what is found during treadmill walking (Zehr et al. 2007a) implying that rhythmic arm and leg movement performed in a task such as cycling could activate common locomotor networks.

Improvements in the temporal parameters of walking, kinematics, muscle modulation during walking, and clinical assessments of walking all demonstrate a positive transfer of A&L cycling training to enhanced walking function. The locus of task-transfer is unknown, but could originate from shared neural elements between the two tasks of A&L cycling and walking. More research on which physiological systems are affected by A&L cycling training is warranted.

Study Limitations

The observed improvement in walking could have been due to enhancements in cardiopulmonary fitness following A&L cycling training, a regular, prescribed fitness program. However, the level of training intensity for A&L cycling was quite low with little change in HR observed over a training session. The level of aerobic activity required to increase cardiopulmonary fitness in individuals with stroke is more intense than the level of exercise here (Gordon et al. 2004; Pang et al. 2006). Future studies could, however, measure changes in cardiovascular function as a result of A&L cycling training. Another limitation of this study also has to do with the change in the intended sample. Although some participants did withdraw, significant effects were seen for many of the dependent variables indicating sufficient power. Additionally, intervention studies have often used reference untrained “control” groups to compare against the intervention or treatment groups. We have instead opted for the “multiple baseline” model where each participant serves as their own control and no committed volunteer participants are relegated to the role of an untrained control participant. In addition, in studying a patient population there tends to be a large degree of between-subject variability as there is a wide range of abilities across participants. However using a multiple baseline approach, we are able to mitigate this limitation as participants are instead compared against their own individual variability generated over multiple baseline sessions. We believe that multiple baseline measures should be considered a valid alternative or replacement to the concept of a control group.

Clinical Translation

Transfer of improvements following A&L cycling training to enhance walking could open the way to the development of a new approach for the rehabilitation of stroke patients. Current therapies for walking do not fully exploit the neuronal and mechanical linkages between the arms and legs that are inherent parts of human locomotion (Ferris et al. 2006; Zehr et al. 2007a; Zehr et al. 2009; Klimstra et al. 2009). We have shown here that A&L cycling improves walking ability after stroke and suggest that A&L cycling be used as an additional training modality for locomotor recovery. A&L cycling is a safe and low-stress activity and the linked cranks allow for physical assistance to the weakened limbs to encourage rhythmic co-ordination. In addition, A&L cycle ergometers are widely available in most gyms and recreation centers and are relatively cheap to access. This type of community based exercise allows for equalization of opportunity for training with increased equipment access outside of major rehabilitation centers. Increasing the ease of training based upon a device that could be more readily used in therapy would directly impact the health and quality of life for those who have suffered a stroke. Given that other types of training, such as strength training or treadmill training also improve walking, we do not suggest that A&L cycling training be used to replace these therapies. Instead we suggest that this therapy be used as an adjunct modality to improve walking ability after stroke and may be particularly valuable as a bridging approach for those who initially lack strength and balance control for independent walking. To fully understand the relative benefits of A&L cycling training to other therapies, a randomized controlled trial should be conducted. In addition, given the link between the arms and the legs, to examine the benefits of just arm cycling training on enhancing walking ability after stroke should also be conducted.

Conclusion

A&L cycling training improves walking ability after stroke. Results showed improved clinical walking status, increased strength, improved physical performance on the untrained task of walking and improved reflex modulation especially in the leg muscles. These results suggest that A&L cycling training, an accessible and cost-effective training modality, could be used to improve walking ability after stroke.

References

Ada L, Dorsch S, Canning CG (2006) Strengthening interventions increase strength and improve activity after stroke: a systematic review. Aust J Physiother 52:241–248. doi: 10.1016/S0004-9514(06)70003-4

Andersen OK, Klimstra M, Thomas E, et al. (2014) Human cutaneous reflexes evoked with simultaneous multiple nerve stimulation during rhythmic locomotor-like arm and leg cycling in stroke subjects. Replace Repair, Restore, Reli Bridg Clin Eng Solut Neurorehabil 1:255–261.

Balter JE, Zehr EP (2007) Neural coupling between the arms and legs during rhythmic locomotor-like cycling movement. J Neurophysiol 97:1809–18. doi: 10.1152/jn.01038.2006

Behrman AL, Harkema SJ (2000) Locomotor training after human spinal cord injury: a series of case studies. Phys Ther 80:688–700.

Berg T, Scale B, Bbs T, Score T (2009) Berg Balance Scale. Arch Phys Med Rehabil 73:2–5. doi: 10.1111/j.1532-950X.2008.00478.x

Bohannon RW, Smith MB (1987) Interrater reliability of a modified Ashworth scale of muscle spasticity. Phys Ther 67:206–207.

Borg G, Löllgen H (2001) Borg's perceived exertion and pain scales. Dtsch Z Sportmed 52:252. doi: 10.1097/00005768-199809000-00018

Butefisch C, Hummelsheim H, Densler P, Mauritz K. (1995) Repetitive training of isolated movements improves the outcome of motor rehabilitation of the centrally paretic hand. J Neurol Sci 130:59–68.

Carroll TJ, Zehr EP, Collins DF (2005) Modulation of cutaneous reflexes in human upper limb muscles during arm cycling is independent of activity in the contralateral arm. Exp Brain Res 161:133–44. doi: 10.1007/s00221-004-2050-7

Cohen J (1992) A power primer. Psychol Bull 112:155–159. doi: 10.1037/0033-2909.112.1.155

Cohen J (2013) Statistical power analysis for the behavioral sciences. Stat Power Anal Behav Sci. doi: 10.1234/12345678

Cramer SC (2008) Repairing the human brain after stroke: I. Mechanisms of spontaneous recovery. Ann Neurol 63:272–287. doi: 10.1002/ana.21393

Dietz V, Wirz M, Colombo G, Curt A (1998) Locomotor capacity and recovery of spinal cord function in paraplegic patients: A clinical and electrophysiological evaluation.

Electroencephalogr Clin Neurophysiol - Electromyogr Mot Control 109:140–153. doi: 10.1016/S0924-980X(98)00002-2

Dragert K, Zehr EP (2013) High-intensity unilateral dorsiflexor resistance training results in bilateral neuromuscular plasticity after stroke. Exp Brain Res 225:93–104. doi: 10.1007/s00221-012-3351-x

Dragert K, Zehr EP (2011) Bilateral neuromuscular plasticity from unilateral training of the ankle dorsiflexors. Exp Brain Res 208:217–27. doi: 10.1007/s00221-010-2472-3

Duncan PW, Sullivan KJ, Behrman AL, et al. (2011) Body-weight-supported treadmill rehabilitation after stroke. N Engl J Med 364:2026–2036. doi: 10.1056/NEJMoa1010790

Duysens J, Baken BCM, Burgers L, et al. (2004) Cutaneous reflexes from the foot during gait in hereditary spastic paraparesis. Clin Neurophysiol 115:1057–1062. doi: 10.1016/j.clinph.2003.12.006

Duysens J, Van De Crommert HWAA (1998) Neural control of locomotion; Part 1: The central pattern generator from cats to humans. Gait Posture 7:131–141. doi: 10.1016/S0966-6362(97)00042-8

Duysens J, Trippel M, Horstmann GA, Dietz V (1990) Gating and reversal of reflexes in ankle muscles during human walking. Exp Brain Res. doi: 10.1007/BF00231254

Eng J, Winter D, Patla A (1994) Strategies for recovery from a trip in early and late swing during human walking. Exp Brain Res. doi: 10.1007/BF00227520

Eng JJ, Dawson AS, Chu KS (2004) Submaximal Exercise in Persons with Stroke: Test-Retest Reliability and Concurrent Validity with Maximal Oxygen Consumption. Arch Phys Med Rehabil 85:113–118. doi: 10.1016/S0003-9993(03)00436-2

Enright PL (2003) The six-minute walk test. Respir Care 48:783–785.

Ferris DP, Huang HJ, Kao PC (2006) Moving the arms to activate the legs. Exerc Sport Sci Rev 34:113–120. doi: 10.1249/00003677-200607000-00005

Field-Fote EC (2001) Combined use of body weight support, functional electric stimulation, and treadmill training to improve walking ability in individuals with chronic incomplete spinal cord injury. Arch Phys Med Rehabil 82:818–24. doi: 10.1053/apmr.2001.23752

Flansbjer UB, Holmbäck AM, Downham D, et al. (2005) Reliability of gait performance tests in men and women with hemiparesis after stroke. J Rehabil Med 37:75–82. doi: 10.1080/16501970410017215

Gordon NF, Gulanick M, Costa F, Fletcher G (2004) Physical Activity and Exercise Recommendations for Stroke Survivors. Circulation 109:2031–2041. doi: 10.1161/01.STR.0000127303.19261.19

Gowland C, Stratford P, Ward M, et al. (1993) Measuring physical impairment and disability with the Chedoke-McMaster Stroke Assessment. Stroke 24:58–63. doi: 10.1161/01.STR.24.1.58

Grillner S, Dubuc R (1988) Control of locomotion in vertebrates: Spinal and supraspinal mechanisms. Adv Neurol Funct Recover Neurol Dis 47:425–453.

Hage JJ, Van der Steen LPE, De Groot PJM (1995) Difference in sensibility between the dominant and nondominant index finger as tested using the Semmes-Weinstein monofilaments pressure aesthesiometer. J Hand Surg Am 20:227–229. doi: 10.1016/S0363-5023(05)80012-7

Haridas C, Zehr EP (2003) Coordinated interlimb compensatory responses to electrical stimulation of cutaneous nerves in the hand and foot during walking. J Neurophysiol 90:2850–61. doi: 10.1152/jn.00531.2003

Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G (2000) Development of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kinesiol 10:361–374. doi: 10.1016/S1050-6411(00)00027-4

Hof a. L, Elzinga H, Grimmius W, Halbertsma JPK (2002) Speed dependence of averaged EMG profiles in walking. Gait Posture 16:78–86. doi: 10.1016/S0966-6362(01)00206-5

Holden MK, Gill KM, Magliozzi MR, et al. (1984) Clinical gait assessment in the neurologically impaired. Reliability and meaningfulness. Phys Ther 64:35–40.

Høyer E, Jahnsen R, Stanghelle JK, Strand LI (2012) Body weight supported treadmill training versus traditional training in patients dependent on walking assistance after stroke: a randomized controlled trial. Disabil Rehabil 34:210–219. doi: 10.3109/09638288.2011.593681

Huang HJ, Ferris DP (2004) Neural coupling between upper and lower limbs during recumbent stepping. J Appl Physiol 97:1299–1308. doi: 10.1152/japplphysiol.01350.2003

Jones CA, Yang JF (1994) Reflex behavior during walking in incomplete spinal-cord-injured subjects. Exp Neurol 128:239–248. doi: 10.1006/exnr.1994.1133

Kim CM, Eng JJ (2003) The relationship of lower-extremity muscle torque to locomotor performance in people with stroke. Phys Ther 83:49–57.

Klarner T, Barss T, Sun Y, et al. (2014a) Preservation of common rhythmic locomotor control despite weakened supraspinal regulation after stroke. Front Integr Neurosci 8:1–9.

Klarner T, Barss T, Sun Y, et al. (2014b) Reliability of Multiple Baseline Measures for Locomotor Retraining after Stroke. Replace Repair, Restore, Reli Bridg Clin Eng Solut Neurorehabilitation 7:479–486.

Klimstra MD, Thomas E, Stoloff RH, et al. (2009) Neuromechanical considerations for incorporating rhythmic arm movement in the rehabilitation of walking. Chaos 19:026102. doi: 10.1063/1.3147404

Kline TL, Schmit BD, Kamper DG (2007) Exaggerated interlimb neural coupling following stroke. Brain 130:159–169. doi: 10.1093/brain/awl278

Kwakkel G, Van Peppen R, Wagenaar RC, et al. (2004) Effects of augmented exercise therapy time after stroke: A meta-analysis. Stroke. pp 2529–2536

Lagerquist O, Zehr EP, Baldwin ERL, et al. (2006) Diurnal changes in the amplitude of the Hoffmann reflex in the human soleus but not in the flexor carpi radialis muscle. Exp Brain Res 170:1–6. doi: 10.1007/s00221-005-0172-1

Lamont E V, Zehr EP (2006) Task-specific modulation of cutaneous reflexes expressed at functionally relevant gait cycle phases during level and incline walking and stair climbing. Exp Brain Res 173:185–92. doi: 10.1007/s00221-006-0586-4

Lamont E V, Zehr EP (2007) Earth-referenced handrail contact facilitates interlimb cutaneous reflexes during locomotion. J Neurophysiol 98:433–42. doi: 10.1152/jn.00002.2007

Langhorne P, Bernhardt J, Kwakkel G (2011) Stroke rehabilitation. Lancet 377:1693–702. doi: 10.1016/S0140-6736(11)60325-5

Langhorne P, Langhorne P, Coupar F, et al. (2009) Motor recovery after stroke: a systematic review. Lancet Neurol 8:741–54. doi: 10.1016/S1474-4422(09)70150-4

Lee K., Carson L, Kinnin E, Patterson V (1989) The Ashworth Scale: a reliable and reproducible method of measuring spasticity. Neuro Rehabiliat Neural Repair 3:205–209.

Liston RAL, Brouwer BJ (1996) Reliability and validity of measures obtained from stroke patients using the balance master. Arch Phys Med Rehabil 77:425–430. doi: 10.1016/S0003-9993(96)90028-3

McCrea DA, Rybak IA (2008) Organization of mammalian locomotor rhythm and pattern generation. Brain Res Rev 57:134–146. doi: 10.1016/j.brainresrev.2007.08.006

Mehrholz J, Kugler J, Pohl M (2008) Locomotor training for walking after spinal cord injury. Cochrane Database Syst Rev 16:1–24. doi: 10.1002/14651858.CD006676.pub2

Mezzarane R a, Klimstra M, Lewis A, et al. (2011) Interlimb coupling from the arms to legs is differentially specified for populations of motor units comprising the compound H-reflex during "reduced" human locomotion. Exp Brain Res 208:157–68. doi: 10.1007/s00221-010-2467-0

Morris SL, Dodd KJ, Morris ME (2004) Outcomes of progressive resistance strength training following stroke: a systematic review. Clin Rehabil 18:27–39. doi: 10.1191/0269215504cr699oa

Moseley AM, Stark A, Cameron ID, Pollock A (2003) Treadmill Training and Body Weight Support for Walking after Stroke. Stroke 34:3006. doi: 10.1161/01.STR.0000102415.43108.66

Multiple (2013) Timed 10-Meter Walk Test. Instr - 10MWT 10–12.

Ng SS, Hui-Chan CW (2012) Contribution of ankle dorsiflexor strength to walking endurance in people with spastic hemiplegia after stroke. Arch Phys Med Rehabil 93:1046–1051. doi: 10.1016/j.apmr.2011.12.016

Pang MYC, Eng JJ, Dawson AS, Gylfadóttir S (2006) The use of aerobic exercise training in improving aerobic capacity in individuals with stroke: a meta-analysis. Clin Rehabil 20:97–111. doi: 10.1191/0269215506cr926oa

Perera S, Mody SH, Woodman RC, Studenski SA (2006) Meaningful change and responsiveness in common physical performance measures in older adults. J Am Geriatr Soc 54:743–749. doi: 10.1111/j.1532-5415.2006.00701.x

Perry J, Bekey G a (1981) EMG-force relationships in skeletal muscle. Crit Rev Biomed Eng 7:1–22.

Podsiadlo D, Richardson S (1991) The timed "Up & Go": a test of basic functional mobility for frail elderly persons. J Am Geriatr Soc 39:142–148. doi: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1991946

Richards CL, Olney SJ (1996) Hemiparetic gait following stroke. Part II: Recovery and physical therapy. Gait Posture 4:149–162. doi: 10.1016/0966-6362(96)01064-8

Scherr J, Wolfarth B, Christle JW, et al. (2013) Associations between Borg’s rating of perceived exertion and physiological measures of exercise intensity. Eur J Appl Physiol 113:147–155. doi: 10.1007/s00421-012-2421-x

von Schroeder HP, Coutts RD, Lyden PD, et al. (1995) Gait parameters following stroke: a practical assessment. J Rehabil Res Dev 32:25–31.

Senthilvelkumar T, Magimairaj H, Fletcher J, et al. (2015) Comparison of body weight-supported treadmill training versus body weight-supported overground training in people with incomplete tetraplegia: a pilot randomized trial. Clin Rehabil 29:42–49.

Smith G V., Macko RF, Silver KHC, Goldberg AP (1998) Treadmill Aerobic Exercise Improves Quadriceps Strength in Patients with Chronic Hemiparesis Following Stroke: A Preliminary Report. Neurorehabil Neural Repair 12:111–118. doi: 10.1177/154596839801200306

Steffen TM, Hacker TA, Mollinger L (2002) Age- and gender-related test performance in community-dwelling elderly people: Six-Minute Walk Test, Berg Balance Scale, Timed Up & Go Test, and gait speeds. Phys Ther 82:128–137.

Tang A, Sibley KM, Thomas SG, et al. (2006) Maximal Exercise Test Results in Subacute Stroke. Arch Phys Med Rehabil 87:1100–1105. doi: 10.1016/j.apmr.2006.04.016

Vasudevan EVL, Zehr EP (2011) Multi-frequency arm cycling reveals bilateral locomotor coupling to increase movement symmetry. Exp Brain Res 211:299–312. doi: 10.1007/s00221-011-2687-y

Walker HK (1990) Clinical Methods: The History, Physical, and Laboratory Examinations. Boston : Butterworths

Warburton DE, Charlesworth S, Ivey A, et al. (2010) A Systematic Review of the Evidence for Canada’s Physical Activity Guidelines. Int J Behav Nutr Phys Act 7:39. doi: 10.1186/1479-5868-7-39

Van Wezel BMH, Ottenhoff FAM, Duysens J (1997) Dynamic Control of Location-Specific Information in Tactile Cutaneous Reflexes from the Foot during Human Walking. J Neurosci 17:3804–3814.

Wirz M, Zemon DH, Rupp R, et al. (2005) Effectiveness of automated locomotor training in patients with chronic incomplete spinal cord injury: A multicenter trial. Arch Phys Med Rehabil 86:672–680. doi: 10.1016/j.apmr.2004.08.004

Yang JF, Stein RB (1990) Phase-dependent reflex reversal in human leg muscles during walking.

J Neurophysiol 63:1109–1117.

Zehr EP (2002) Considerations for use of the Hoffmann reflex in exercise studies. Eur J Appl Physiol 86:455–68. doi: 10.1007/s00421-002-0577-5

Zehr EP (2005) Neural control of rhythmic human movement: the common core hypothesis. Exerc Sport Sci Rev 33:54–60.

Zehr EP (2011) Evidence-based risk assessment and recommendations for physical activity clearance: stroke and spinal cord injury. Appl Physiol Nutr Metab 1:214–31. doi: 10.1139/h11-055

Zehr EP, Balter JE, Ferris DP, et al. (2007a) Neural regulation of rhythmic arm and leg movement is conserved across human locomotor tasks. J Physiol 582:209–27. doi: 10.1113/jphysiol.2007.133843

Zehr EP, Chua R (2000) Modulation of human cutaneous reflexes during rhythmic cyclical arm movement. Exp Brain Res 135:241–250. doi: 10.1007/s002210000515

Zehr EP, Fujita K, Stein RB (1998a) Reflexes from the superficial peroneal nerve during walking in stroke subjects. J Neurophysiol 79:848–58.

Zehr EP, Haridas C (2003) Modulation of cutaneous reflexes in arm muscles during walking: further evidence of similar control mechanisms for rhythmic human arm and leg movements. Exp Brain Res 149:260–6. doi: 10.1007/s00221-003-1377-9

Zehr EP, Hundza SR (2005) Forward and backward arm cycling are regulated by equivalent neural mechanisms. J Neurophysiol 93:633–40. doi: 10.1152/jn.00525.2004

Zehr EP, Hundza SR, Vasudevan E V (2009) The quadrupedal nature of human bipedal locomotion. Exerc Sport Sci Rev 37:102–8. doi: 10.1097/JES.0b013e31819c2ed6

Zehr EP, Klimstra M, Dragert K, et al. (2007b) Enhancement of arm and leg locomotor coupling with augmented cutaneous feedback from the hand. J Neurophysiol 98:1810–4. doi: 10.1152/jn.00562.2007

Zehr EP, Komiyama T, Stein RB (1997) Cutaneous reflexes during human gait: electromyographic and kinematic responses to electrical stimulation. J Neurophysiol 77:3311–25.

Zehr EP, Loadman PM (2012) Persistence of locomotor-related interlimb reflex networks during walking after stroke. Clin Neurophysiol 123:796–807. doi: 10.1016/j.clinph.2011.07.049

Zehr EP, Loadman PM, Hundza SR (2012) Neural control of rhythmic arm cycling after stroke. J

Neurophysiol 108:891–905. doi: 10.1152/jn.01152.2011

Zehr EP, Stein RB, Komiyama T (1998b) Function of sural nerve reflexes during human walking. J Physiol 507 (Pt 1:305–14.

Zehr EP, Stein RB, Komiyama T, Kenwell Z (1995) Linearization of force sensing resistors (FSR's) for forcemeasurement during gait. Proc 17th Int Conf Eng Med Biol Soc. doi: 10.1109/IEMBS.1995.579833

Chapter 7 Long term plasticity in reflex excitability induced by 5 weeks of arm and leg cycling training after stroke[5]

Abstract

Neural connections remain partially viable after stroke and access to these residual connections provides a substrate for training induced plasticity. The objective of this project was to test if reflex excitability could be modified with arm and leg (A&L) cycling training. Nineteen individuals with chronic stroke (>six months post-lesion) performed 30 minutes of A&L cycling training three times a week for five weeks. Changes in reflex excitability were inferred from modulation of cutaneous and stretch reflexes. A multiple baseline (3 pre-tests) within-subject control design was used. Plasticity in reflex excitability was determined as an increase in the conditioning effect of arm cycling on soleus stretch reflex amplitude on the more affected side, by the index of modulation, and by the modulation ratio between sides for cutaneous reflexes. In general, A&L cycling training modifies reflex excitability after stroke. Plasticity in spinal networks occurs following rehabilitative locomotor training after stroke.

Introduction

The arms and the legs are coupled in the human nervous system such that activity in the arms affects activity in the legs and vice versa. In quadrupeds, forelimb-hindlimb coordination is well documented and has been attributed to propriospinal linkages between cervical and lumbosacral spinal central pattern generating networks (Skinner et al. 1980; Dietz 2002; Frigon et al. 2004; Zehr and Duysens 2004; Zehr et al. 2009; Thibaudier et al. 2013). Bipedal human locomotion, likely built upon elements of quadrupedal coordination (Zehr et al. 2009; Dietz 2002), also involves coordination of all four limbs however only indirect evidence for locomotor linkages exists.

The modulation of reflex amplitudes can be used to probe for changes in interlimb neural activity (Burke et al. 1991; Zehr et al. 2004). Investigations of soleus stretch and H-reflex modulation during rhythmic arm movement provide evidence of neuronal coupling between the arms and the legs (Dietz 2002; Frigon et al. 2004; Zehr et al. 2007c; Dragert and Zehr 2009;

---

[5] Klarner T, Barss TS, Kaupp C, Sun Y, Loadman PM, Zehr EP (2016) Long term plasticity in reflex excitability after arm and leg cycling training in stroke. Brain Sci., *6*(4)54:1-17; doi:10.3390/brainsci6040054

Mezzarane et al. 2011). Examining cutaneous reflexes during rhythmic movement can also probe for interactions between the limbs. In this context, a widespread interlimb network is revealed by the extensive distribution of reflexes across many muscles in both the arms and the legs regardless of which limb is directly stimulated (Zehr et al. 2001; Haridas and Zehr 2003; Zehr and Duysens 2004). In addition, phase dependent modulation found in muscles of all four limbs during rhythmic movement is suggestive of coupling between segmental spinal networks (Yang and Stein 1990; Duysens et al. 1992, 1996; Haridas and Zehr 2003; Zehr and Haridas 2003; Sasada et al. 2016). Regulation of rhythmic arm and leg movement is supported by somatosensory linkages in the form of interlimb reflexes (Dietz et al. 2001; Haridas and Zehr 2003; Lamont and Zehr 2007) and neural coupling between lumbar and cervical spinal cord networks (Zehr et al. 2007a, 2007b; Mezzarane et al. 2011; Nakajima et al. 2013).

Exploiting the neural and mechanical linkages between the arms and legs that are inherent parts of human locomotion could enhance the recovery of walking for those who have suffered neurological damage such as a stroke (Dietz 2002; Ferris et al. 2006; Klimstra et al. 2009; Zehr et al. 2009). Incorporating paradigms for locomotor rehabilitation that include rhythmic arm movements, as with arm and leg (A&L) cycling, may enhance leg activity (Behrman and Harkema 2000; Dietz 2002; Ferris et al. 2006; Zehr et al. 2009). Only using the arms for postural and weight-bearing activity (e.g. on parallel bars or handrails), as is commonly applied in traditional locomotor rehabilitation, may actually inhibit rhythmic stepping with the legs (Behrman and Harkema 2000). Conversely, when arm activity is facilitated with locomotor-like arm and leg movements in those with incomplete cervical spinal cord injury, leg muscle activity is facilitated (Kawashima et al. 2008). Allowing a normal simultaneous and reciprocating arm action may facilitate stepping and may be an important component needed to harness neural coupling to help improve motor output for the legs during walking (Ferris et al. 2006; de Kam et al. 2013).

A complication of stroke is alterations in interneuronal pathways stemming from damage to supraspinal centers from the stroke lesion which disrupts some of the descending regulation of alphamotoneuronal and interneuronal excitability (Dobkin, 2004, 2005). The decreased influence of the corticospinal tract fails to produce the appropriate suppressions associated with normal reflex activity (Duysens et al. 2004). It is this abnormal neural integration that contributes to reduced walking ability. However, connections between the arms and legs remain partially viable despite the fact that stroke typically presents with hemiparesis resulting in a more affected (MA) and less affected (LA) side (Thilmann et al. 1990; Zehr and Loadman 2012; Zehr et al. 2012). Partial preservation of the descending modulatory effects of rhythmic arm cycling on lumbosacral spinal cord excitability can be seen after stroke where arm cycling modulates the soleus H-reflex (Barzi and Zehr 2008) and stretch reflex (Mezzarane et al. 2014). In addition, the protective stumbling corrective response ordinarily observed in healthy participants during walking remains partially intact in stroke participants (Zehr et al. 1998a; Zehr and Loadman 2012). Altered neural connectivity following stroke produces impairments in limb function with a stereotypical bias of arm flexor and leg extensor activity emerges, showing excessive activation coupling between the upper and lower extremities (Kline et al. 2006).

Although neural pathways are corrupted bilaterally after stroke, residual connectivity in spinal networks provides a substrate for training induced plasticity arising from A&L cycling training (Zehr 2006). The extent to which A&L cycling training in stroke could modify plasticity in reflex excitability remains unknown. Thus the objective of this project was to test if neurophysiological changes in reflex excitability are sensitive to A&L cycling training. It is hypothesized that A&L cycling training would improve interlimb reflex excitability as assessed by changes in stretch and cutaneous reflex amplitudes. Recently we have shown that A&L cycling training successfully improves walking after stroke (Klarner et al. 2016). Results from this study will have implications for the mechanistic understanding of plasticity and training transfer following rehabilitative locomotor training in clinical populations.

Materials and Methods

Participants

Nineteen individuals with chronic stroke (more than six months post infarct) were enrolled in the study. These are the same participants as in Chapter 6 of this dissertation. To assist with determining a participant's functional status and the clinical features of this population, clinical assessments were performed by a licensed physical therapist before and after A&L cycling training (see Table 7-1). Muscle tone was measured using the Modified Ashworth

Scale (5 points) at the ankle and knee for the lower limb (Bohannon and Smith 1987; Lee et al. 1989) with a graded rating of spasticity scored from 0 to 4, with 0 being flaccid and 4 being rigid. A measure of the basic motor skills necessary for functional ambulation was derived using the 6-point Functional Ambulation Categories Scale where a level 1 indicates that a patient is non-ambulatory and a level 6 indicates a patient is fully independent (Holden et al. 1984). To measure general physical impairment, the Chedoke-McMaster Stroke Assessment (Gowland et al. 1993) was used. Impairment at the arm (A), hand (H), leg (L), and foot (F) were determined using the 7-point activity scale where score of 1 represents complete independence and a score of 7 represents total assistance. Using the 5-piece Semmes-Weinstein kit of calibrated monofilaments (Sammons Preston Roylan, Cedarburg WI), ability to discern light touch and pressure was measured in the more affected hand and foot (Hage et al. 1995). Reflexes obtained using a reflex hammer were graded on a 0 to 4+ scale where 0 means a reflex is absent and 4+ represents a hyperactive reflex with clonus for knee jerk (L1) and ankle plantarflexion jerk (S1) (Walker 1990). Table 7-1 outlines participant demographic information and the clinical features of the population as assessed above taken before and after A&L cycling training. Informed written consent was obtained for a protocol approved by the University of Victoria Human Research Ethics Committee and performed according to the Declaration of Helsinki.

Table 7-1: Summary of participant demographics and results from tests assessing clinical status including a test for muscle tone (Modified Ashworth), functional ambulation (FAC), physical impairment (Chedoke-McMaster scale), touch discrimination (Monofilament test) and reflex function for stroke participants before and after A&L cycling training.

N	Sex/Age/MA Side/Years Since Stroke	Modified Ashworth (ankle/knee)		FAC (/ 6)		Chedoke-McMaster (A/H/L/F)		Monofilament (hand/foot)		Reflexes (L1 / S1)	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	M/74/R/5	3/1+	3/1+	4	4	2/2/3/2	2/2/2/3	J4.31/J4.31	J4.31/J4.31	3+/1+	3+/1+
2	F/70/R/2	0/0	0/0	5	5	7/5/7/7	7/7/7/7	J4.31/J4.31	J4.31/J4.31	2+/2+	2+/2+
3	F/45/R/7	1/0	1/0	5	5	5/5/6/5	5/5/6/4	F3.61/J4.31	D2.83/F3.61	0/0	1+/1+
4	M/59/R/3	2/0	2/0	5	5	2/2/4/2	2/2/4/2	T6.65/J4.31	K4.56/J4.31	3+/3+	3+/3+
5	M/82/R/3	0/1	0/1	3	3	4/6/6/5	5/6/6/5	UTF/UTF	UTF/UTF	3+/0	3+/0
6	M/86/L/4	1+/0	1+/0	5	5	7/7/6/5	7/7/6/6	J4.31/T6.65	J4.31/T6.65	0/0	0/0
7	F/80/R/6	0/0	0/0	5	5	3/5/5/5	3/5/5/6	J4.31/J4.31	F3.61/J4.31	0/0	0/0
8	M/59/R/11	1/1	2/1	5	5	5/5/5/4	5/6/6/4	T6.65/T6.65	T6.65/T6.65	3+/4+	3+/3+
9	M/74/R/6	1/0	1/1	5	5	6/5/6/5	7/7/6/5	J4.31/F3.61	F3.61/D2.83	3+/2+	3+/2+
10	M/47/L/6	4/2	2/2	4	4	2/1/2/2	2/2/2/2	T6.65/T6.65	T6.65/T6.65	4+/3+	4+/3+
11	M/69/L/5	2/3	1+/2	4	4	2/2/3/2	2/2/3/3	T6.65/T6.65	T6.65/T6.65	3+/3+	3+/3+
12	F/72/R/6	2/2	2/2	3	6	2/3/2/3	3/3/3/3	UTF/J4.31	T6.65/J4.31	1+/3+	2+/2+
13	M/59/L/5	1/1	1/0	6	5	6/6/6/4	7/6/6/6	J4.31/J4.31	J4.31/J4.31	3+/2+	3+/2+
14	M/56/L/8	1/1	0/1	5	4	1/1/4/2	1/1/4/2	T6.65/T6.65	D2.83/K4.56	3+/3+	3+/3+
15	M/77/L/8	2/2	2/2	3	5	4/5/5/3	5/5/5/3	UTF/T6.65	T6.65/T6.65	3+/3+	3+/3+
16	F/63/L/13	1/2	1/2	5	4	2/2/3/4	2/2/5/5	T6.65/K4.56	D2.83/D2.83	3+/1+	3+/1+
17	M/71/R/6	1/2	1/2	4	4	3/2/4/4	4/2/4/4	F3.61/J4.31	F3.61/F3.61	2+/3+	2+/2+
18	M/62/R/8	1+/2	1/2	4	5	4/3/4/5	4/3/5/5	D2.83/D2.83	D2.83/D2.83	3+/3+	3+/2+
19	M/78/L/29	3/1	2/1+	4	4	3/3/4/4	3/4/4/4	T6.65/T6.65	J4.31/F3.61	0/0	0/1

Abbreviations: MA, more affected; M, male; F, female; L, left; R, right; FAC, Functional Ambulation Category; A, arm; H,

A&L Cycling Training

Participants performed A&L cycling training (Sci-Fit Pro 2 ergometer, see Figure 7-1) three times a week, with 30 minutes of aggregate activity time per session, for a total duration of five weeks. Most participants completed training on Monday, Wednesday and Friday. For training, an arm and leg cycling ergometer with coupled upper and lower cranks was used. Linked motion of the cranks for the arms and legs enabled assistance for the weaker limbs where regardless of deficit, all limbs could be moving, allowing for interlimb coordination during training. Mechanical modifications were made to the cycle ergometer to ensure a customized and comfortable fit for each training session. The cranks of the arm and leg ergometer were individually adjusted to the range of motion for each limb of each stroke participant and hand braces were worn as needed to ensure grip on the handle with the MA hand. During each session, participants were allowed to take short breaks during training but the aggregate time each session was always met. Participants were expected to tolerate the protocol very well as this was a modification of a previous protocol where chronic stroke participants performed four trials of six minute bouts (totalling 24 minutes) of active A&L cycling (Andersen et al. 2014).

The progressive element of this steady state training included increasing the resistance of the ergometer over the five weeks in order to maintain the same relative rating of perceived exertion (RPE) score. This is in line with many other post-stroke treadmill training protocols where training volume was increased (Zehr 2011). Participants were encouraged to exercise at a level sufficient to report a RPE value between three and five, corresponding to a target heart rate (HR) between 50-70% of their maximum HR (Scherr et al. 2013). If a participant reported being on beta blockers, adjustments to target heart rate goals were made (Tang et al. 2006). During the training and testing time, participants were also encouraged to maintain their normal activity levels, but not participate in additional research programs or interventions.

All exercise sessions were supervised by a CSEP (Canadian Society for Exercise Physiology) Certified Exercise Physiologist, as well as several laboratory assistants to ensure appropriate monitoring. Exercise sessions were not initiated if a participant’s blood pressure (BP), measured with a digital blood pressure cuff placed over the LA arm, exceeded 140/90 mmHg in accordance with Canada’s Physical Activity Guidelines (Warburton et al. 2010).

Exercise was terminated if HR exceeded 85% of the age-predicted maximum, if BP exceeded 200/110 mmHg, if the participant felt dizzy, nervous, or pains in the chest. Upon completion of the 30 minutes in each training session, participants were given three to five minutes to cool down, and remained in the laboratory until BP returned to pre-exercise values.

Multiple Baseline and Post-Test Measures

A multiple baseline within-subject control design was used for this study (Butefisch et al. 1995; Klarner et al. 2014a). Figure 7-1 illustrates the testing and training protocol. Multiple baseline measurements were obtained from participants in three baseline experimental sessions over a period of three to four weeks, with at least six days between baseline sessions. The post-test following training was performed within three days of the last exercise session. At these sessions, the same tests were performed in the same order and environmental conditions (i.e. temperature, noise, lighting, participant position) and session time of day were kept as consistent as possible (Zehr 2002; Lagerquist et al. 2006; Dragert and Zehr 2013). This design allowed for the creation of a reliable and consistent pre-test measure that allowed for inspection of spontaneous recovery effects, and provided baseline data against which changes were evaluated. These measures have been previously shown to have high reliability across multiple baseline points (Klarner et al. 2014a).

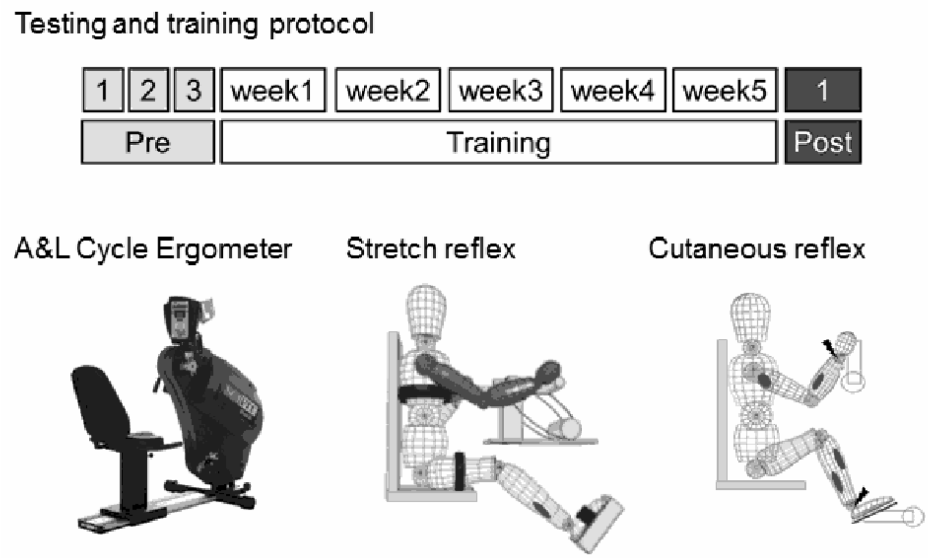


Figure 7-1: Illustration of the testing and training protocols. A multiple baseline within-subject control design was used for this study. An A&L cycle ergometer (Sci-Fit Pro 2) was used for training. The setups for stretch reflex and cutaneous reflex testing are shown. Muscles of interest are shown with a grey oval and electrical stimulation is shown with a black lightning bolt.

Stretch Reflexes

Soleus stretch reflexes were evoked using an electrodynamic shaker (ET-1126B; Labworks Inc), placed over the triceps surae tendons of the LA and MA legs, in separate trials (Palomino et al. 2011; Mezzarane et al. 2014). Constant pressure was applied as best as possible against the tendon and the shaker was programmed to deliver a single sinusoidal pulse at a frequency of 100Hz, 10 ms duration. A total of 20 pulses were delivered pseudo-randomly with an inter-stimulus interval between three and five seconds. Figure 7-2 illustrates the stretch reflex setup and position of stimulus delivery.

Stretch reflexes were collected under two conditions: 1) with the arms and legs not moving (static) and 2) with the arms cycling rhythmically at 1Hz (conditioned) while the legs remained static. Arm cycling frequency was set to 1Hz and participants maintained cycling frequency with the use of visual feedback. All reflexes were evoked at the ‘7 o’clock’ position for the LA hand for both the static and conditioned trials. This position was previously shown to have the largest modulatory effect on H-reflex amplitude (de Ruiter et al. 2010). The same procedure was then repeated for the contralateral leg after repositioning the shaker. Each procedure lasted about 2 minutes and rest periods of 5 minutes between them were allowed. Background EMG activity in the ipsilateral TA and contralateral SOL and TA, were also monitored. In both conditions, SOL stretch reflexes were recorded while the participant was relaxed and instructed not to generate any muscle activity.

As a proxy for the intensity of the pulse between conditions, an accelerometer (ADXL193; Analog devices), mounted to the tip of the shaker, recorded stimulation amplitude, as in previous studies (Burke et al. 1981; Fornari and Kohn 2008; Mezzarane et al. 2014). The peak-to-peak value from the accelerometer signal was obtained based upon the sinusoidal displacement of the shaker tip. Using a standard equation for peak sinusoidal motion, displacement was calculated as:

$$D = \frac{GA}{2\pi^2F^2}$$

Where ‘D’ is the peak to peak displacement of the tip of the shaker in contact with the tendon, ‘G’ is a constant, the acceleration due to gravity, ‘A’ is the acceleration measured by the accelerometer in units of gravity, and ‘F’ is the frequency of the sinusoid (100 Hz).

Modulation of stretch reflexes due to arm cycling was evaluated by calculating the index of modulation as the change in stretch reflex peak-to-peak amplitude between the static and conditioned trials and then expressed as a percentage (Modulation = $[(\text{StretchReflex}_{\text{ArmCycle}} - \text{StretchReflex}_{\text{Static}})/ \text{StretchReflex}_{\text{Static}}] \times 100$). Negative values indicate a decrease in reflex amplitude during the arm cycling conditioned trial compared to the static trial. To compare modulation between the LA and MA sides, the difference in stretch reflex amplitudes was calculated by subtracting values for arm cycling from static control amplitudes. A negative value indicates a greater degree of modulation on the LA side and a positive value indicates a greater degree of modulation on the MA side. Background EMG (bEMG) was assessed to monitor possible effects of heteronymous and contralateral muscle activity on reflex amplitudes. For the contralateral SOL and TA bilaterally, bEMG was calculated as the average value of background activity from a 20ms pre-stimulus period. Data were normalized to the peak EMG recorded during A&L cycling for each muscle for each session.

Cutaneous Reflexes

The pattern of cutaneous reflex modulation involving combined simultaneous superficial radial (SR) and superficial peroneal (SP) nerve stimulation during A&L cycling was used to assess neurophysiological changes in reflex excitability arising from locomotor training. Cutaneous reflexes were evoked via simultaneous combined surface stimulation of the nerves innervating the dorsum of the hand (SR) and foot (SP) (Klarner et al. 2014b). Electrodes for SR and SP nerve stimulation were placed just proximal to the radial head and on the crease of the ankle, respectively, on the LA limbs. Similar to previous studies (Zehr and Hundza 2005; Zehr et al. 2007b, 2012; Vasudevan and Zehr 2011; Zehr and Loadman 2012) a Grass S88 stimulator with SIU5 stimulus isolation and a CCU1 constant current unit (Astro-Med Grass Instrument, West Warwick, RI) were used to deliver stimulation in trains of 5 x 1.0ms pulses at 300Hz (P511 Astro-Med Grass Instrument). Perceptual and radiating thresholds (RT) were determined as the point at which nerve stimulation produces a perceptible stimulation and the point at which a stimulation produced radiating parasthaesia in the entire cutaneous receptive field of that nerve, respectively. Non-noxious intensities were found for each participant and stimulation intensities for the SR nerve were set to 2.2±0.1, 2.0±0.1, 2.3±0.2, and 2.1±0.1 x RT for pretest 1, 2, 3 and the post test, respectively and for the SP nerve stimulation intensities were set to 1.9±0.2, 2.1±0.1, 2±0.1, and 2±0.1 x RT for pretest 1, 2, 3 and the post test, respectively. No significant differences were found in stimulation intensity across test sessions.

Electromyographic (EMG) data from the soleus (SOL), tibialis anterior (TA), flexor carpi radialis (FCR), and posterior deltoid (PD), from the LA and MA limbs, were collected with surface electrodes placed in bipolar configuration over the muscle bellies of interest. Muscles from all four limbs bilaterally that have been previously associated with interlimb reflex effects were chosen (Zehr and Hundza 2005; Zehr et al. 2007b, 2012; Vasudevan and Zehr 2011; Zehr and Loadman 2012). Electrodes were placed on the skin, oriented longitudinally along the fibre direction, in accordance with SENIAM procedures (Hermens et al. 2000). Electrodes on the upper and lower limbs were placed in the same position at each testing session. This was accomplished by recording cathode and anode electrode distances from anatomical landmarks, using pictures taken at the first session, and by placement of the electrodes by the same experimenter each time. EMG signals were pre-amplified (×5000), band-pass filtered (100–300 Hz), were converted to a digital signal (GRASS P511, AstroMed) and sampled at 1000 Hz using custom built continuous acquisition software (LabVIEW, National Instruments, TX, USA). Using custom-written software programs (Matlab, The Mathworks, Inc., MA, USA) EMG data were full-wave rectified and low-pass filtered at 100 Hz using a 4th order Butterworth filter.

Participants performed A&L cycling on the same cycle ergometer used for training. After establishing a consistent steady pace for A&L cycling (55.2 ± 9.2 rpm), data were collected over a four to six minute trial providing approximately 160 stimulations delivered pseudo-randomly with an inter-stimulus interval of 1-5 seconds. Continuous data for A&L cycling were broken into movement cycles with the vertical position of the LA arm indicating the start and end of a cycle. For comparisons between participants, cycle time was normalized to 100%.

To investigate phase-dependent modulation within each movement cycle, data were broken apart into 8 equidistant phases. Phases one to four represent the arm and leg power phase, corresponding to the LA arm at top dead center (0 deg) to full extension of the arm and leg (180 deg) (Zehr et al. 2007a). Evoked reflexes in all muscles tested were aligned to delivery and averaged together. The stimulus artefact was removed from the reflex trace and data were then low-pass filtered at 30 Hz using a dual-pass, fourth order Butterworth filter. For reflexes within each phase, the average trace from the non-stimulated data was subtracted from the stimulated average trace to produce a subtracted EMG reflex trace. Cutaneous reflexes were quantified as the average cumulative reflex over 150ms following stimulation within each of the 8 phases (Zehr et al. 1997, 1998b). A positive value indicates overall facilitation while a negative value (only revealed with background activity) indicates overall inhibition (Komiyama et al. 2000) Duysens 1977; Zehr et al. 2007b). This process reveals the general trend in evoked responses in the muscles tested (Zehr et al. 2014). However, it does reduce the ability to identify reflex reversals as this method mixes facilitations and suppressions losing some of the temporal and spatial characteristics of the response (Brooke et al. 1997; Komiyama et al. 2000). Background EMG (bEMG) levels were investigated for a corresponding comparison of reflex amplitudes between tests. A modulation index for change in bEMG across phases for each muscle was calculated (Modulation = $[(\text{bEMG}_{\text{max}} – \text{bEMG}_{\text{min}})/ \text{bEMG}_{\text{max}}] \times 100$) and this measure provides an index of overall amplitude modulation independent of the pattern of modulation across A&L cycling phases (Zehr and Loadman 2012). Cutaneous reflex amplitudes and background EMG for each subject were normalized to the peak value of the unstimulated EMG for that muscle across the movement cycle for A&L cycling. A modulation index for change in reflexes across phases for each muscle was calculated (Modulation = $[(\text{Reflex}_{\text{max}} – \text{Reflex}_{\text{min}})/ \text{bEMG}_{\text{max}}] \times 100$). The ratio between the LA and MA modulation index for each muscle was also determined.

Statistics

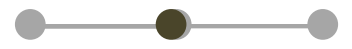
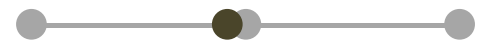
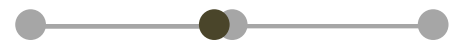
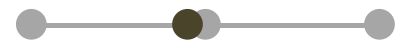
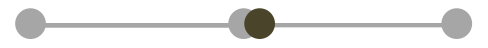
Using commercially available software (SPSS 18.0, Chicago, IL) pre-test and post-test data were compared within-subjects and between-subjects to evaluate the extent to which arm and leg cycling training altered reflex modulation. With within-subject analyses, post-test data were compared to the 95% confidence interval (CI) created from three pre-test sessions and compared to a pre-test average for each participant. To establish the 95% CI for each measure, variability was computed from 3 pre-test sessions and used to create a data range with which the post-test value was compared. If the post-test value fell outside the 95% CI range, it was considered statistically significant for that participant (Cummings, 2013). A graph illustrating the 95% CI across pre-tests sessions and the post-test value is included for each measure. Using group data, we used repeated-measures ANOVA to evaluate the extent to which arm and leg cycling training altered reflex modulation. For stretch reflex and modulation index parameters, a 1 x 4 (time; test sessions) repeated-measures ANOVA was used. For cutaneous reflex parameters a 8 (phase) x 4 (time; test sessions) repeated-measure ANOVA was used and only main effects and interaction effects for time are reported and considered. For comparison between baseline and post-test data, a repeated-measures ANOVA was first performed in a planned contrast to examine differences across the three pre-test sessions. If no difference was found data were pooled together to create an average pre-test value which was compared to the post-test. Assumptions for ANOVA were evaluated for parametric tests for a within-subject design with statistical significance set at $p \leq 0.05$. As an additional measure of the magnitude of any differences between pre-post, the observed effect size for post-test differences for stretch and cutaneous reflex parameters is also reported using Cohen's d. In this calculation we used the conventional small effect as $d = 0.2$, a medium effect as $d = 0.5$ and a large effect as $d = 0.8$ (Cohen, 2013).

Results

NOTE: Table 7-2 summarizes results from the single-participants statistical tests that are discussed below. The number of participants with a significant post-test value is reported for each variable in the Table. A graph illustrating the average and 95% CI across three pre-tests sessions (grey circles) and the post-test value (black circle) is included for each measure. For stretch reflex modulation for the MA SOL and for the ratio of stretch reflex modulation between the LA and MA sides, the average post-test value fell outside of the 95% CI from the pre-test sessions, indicating a significant training effect. For cutaneous reflexes, the modulation index for the MA FCR and the modulation index ratio for the SOL and TA have a post-test value that fell outside of the 95% CI band from the pre-test sessions. For bEMG, the modulation index for the

MA TA, MA FCR and LA PD, and modulation index ratio for the TA, the average post-test value fell outside of the 95% CI from the pre-test sessions. In the text below, the percent change in each significant measure, and the number of participants showing a significant post-test effect is reported along with the results from repeated measures ANOVA.

Table 7-2: Summary of results from within-subject analyses

Measure	Participants (/19) with significant changes after training	Average pretest with 95% Confidence Interval and post test score
Stretch Reflex		
LA SOL	10	
MA SOL	15	
Ratio	10	
bEMG with arm cycling		
LA iTA	2	
LA cSOL	3	
LA cTA	1	
MA iTA	3	
MA cSOL	4	
MA cTA	1	
Cutaneous Reflex Modulation Index		
MA SOL	7	
MA TA	8	
LA SOL	5	
LA TA	8	
MA FCR	11	
MA PD	8	
LA FCR	10	
LA PD	10	
Cutaneous Reflex Modulation Index Ratio		
SOL	12	
TA	10	
FCR	5	
PD	8	
bEMG Modulation Index		
MA SOL	7	
MA TA	9	
LA SOL	9	
LA TA	10	
MA FCR	8	

MA PD	7	
LA FCR	10	
LA PD	8	
bEMG Modulation Index Ratio		
SOL	4	
TA	7	
FCR	10	
PD	8	

Abbreviations: MA, more affected; LA, less affected; i, ipsilateral; c, contralateral SOL, soleus; TA, tibialis anterior; FCR, flexor carpi radialis; PD, posterior deltoid; bEMG, background electromyography

Stretch Reflexes

Figure 7-2 shows example representative traces for the shaker trigger, the accelerometer input, and for the soleus stretch reflex for the static and conditioned trial from a representative participant. Suppression due to arm cycling can be seen for the soleus stretch reflex amplitude with a constant trigger input.

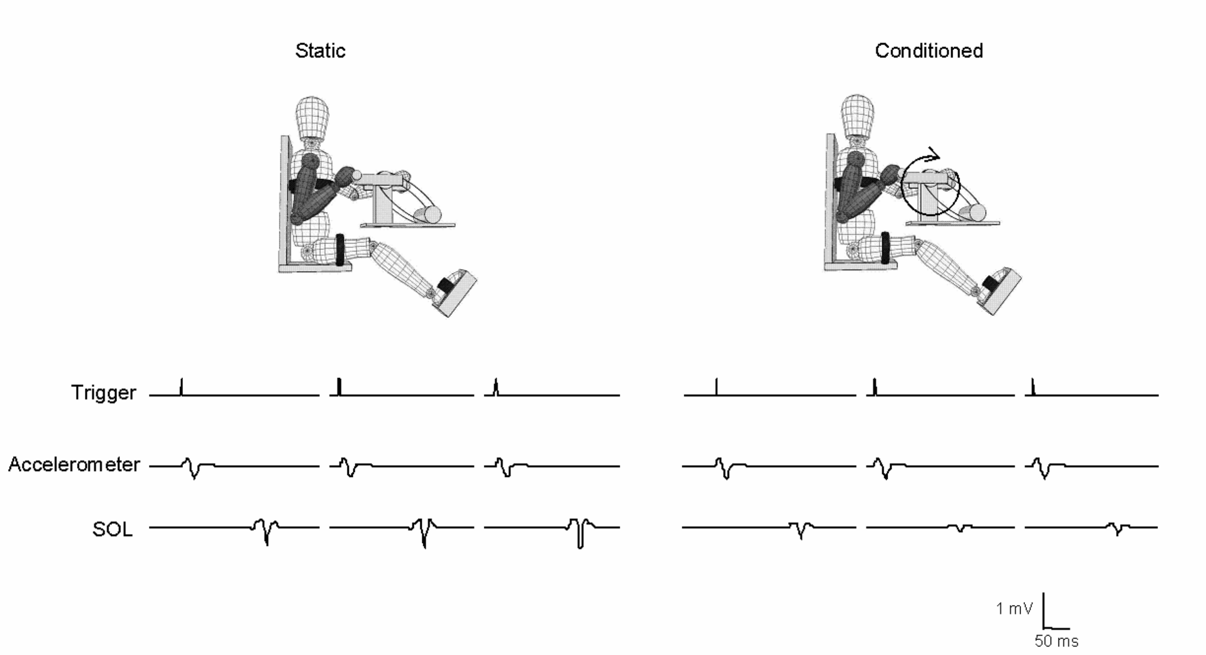


Figure 7-2: Representative traces in static and conditioned trials Trigger, accelerometer and soleus stretch reflex amplitudes from a single subject showing suppression of reflex amplitude in the conditioned arm cycling trial.

No significant differences for input trigger amplitude between the static and conditioned trials for the pretests and the post-test were found. A constant displacement of the pulse was maintained during all stretch reflex trials within each experimental session. For the LA side the shaker displacement was 0.51±0.23, 0.52±0.15, 0.47±0.13, and 0.52±0.12mm for pre1, pre2, pre2 and post-tests, respectively. For the MA side the shaker displacement was 0.48±0.17, 0.49±0.11, 0.44±0.13, and 0.48±0.16mm for pre1, pre2, pre2 and post-tests, respectively. There was no significant difference between shaker displacement between the LA and MA sides. Therefore differences in stretch reflex amplitude were not due to changes in input size and modulation due to arm cycling could be directly compared between trials.

Figure 7-3 shows the percent change in the conditioned soleus stretch during arm cycling compared to static control for the baseline and post-tests, and the difference in amplitude modulation between the LA and MA sides averaged across all participants. Soleus stretch reflex modulation on the LA side increased by 19.9% in 10 of the 19 participants from the within-subject analysis but there was no significant main effect of time or difference in the pre-test average and post-test value across participants. For all tests, the LA soleus stretch reflex was reduced with arm cycling compared to static. For the MA SOL, there was a significant main effect of time ($F_{(3,54)}$=3.497, p=0.025, d=0.733). When comparing the baseline average with the post-test, the MA SOL shows an 80.8% increase in the inhibitory effect of arm cycling on soleus stretch reflex excitability in 15 of the 19 participants ($F_{(1,18)}$=8.983, p=0.011, d=1.034) after training.

At the pre-test sessions there was a significantly larger degree of modulation on the LA side compared to the MA side ($F_{(1,18)}$=9.558, p=0.008, d=1.012) where pre-test stretch reflex amplitudes for all participants were reduced with arm cycling by 38.4% for the LA side compared to 10.9% for the MA side. After training the post-test revealed there was no longer a significant difference between the sides in stretch reflex inhibitory modulation where now both LA and MA stretch reflexes for all participants are reduced by 36.4%. When comparing the absolute difference in the degree of modulation between the LA and MA sides after training, there was a significant main effect of time across all sessions ($F_{(3,54)}$=3.770, p=0.020, d=0.763). With the baseline average and post-test values there was a significant effect ($F_{(1,18)}$=10.190, p=0.007, d=0.839) such that the relative effect of modulation during arm cycling for the pre-tests was 27.8% stronger for the LA side and for the post-test 14.2% stronger on the MA side in 10 of the 19 participants.

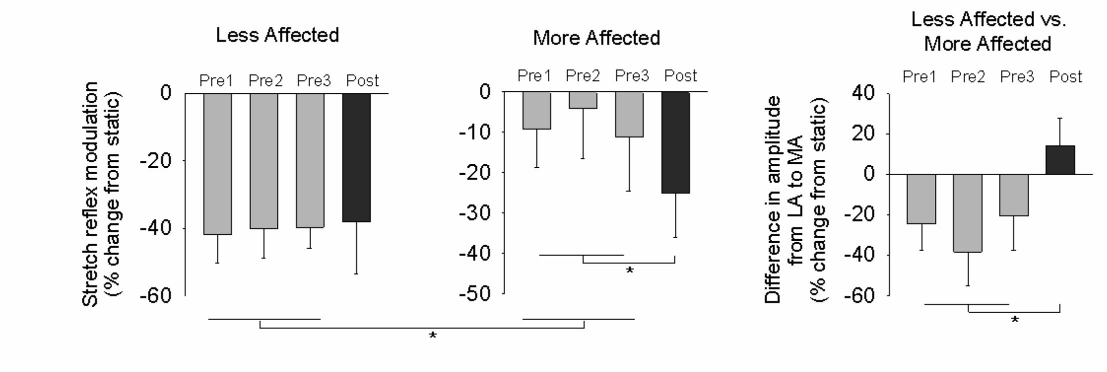


Figure 7-3: Modulation of stretch reflex by arm cycling. Bar graphs are means (±sem) averaged across all participants for baseline and post-test values. * indicates significant differences between the pre-test average and the post-test value. For the LA side, MA side, and difference in amplitude modulation between the LA and MA sides.

Figure 7-4 shows background EMG activity during testing of soleus stretch reflex amplitude modulation by arm cycling in the LA and MA sides. No significant differences were found for background EMG between the static and conditioned trials for the pre-tests or the post-test.

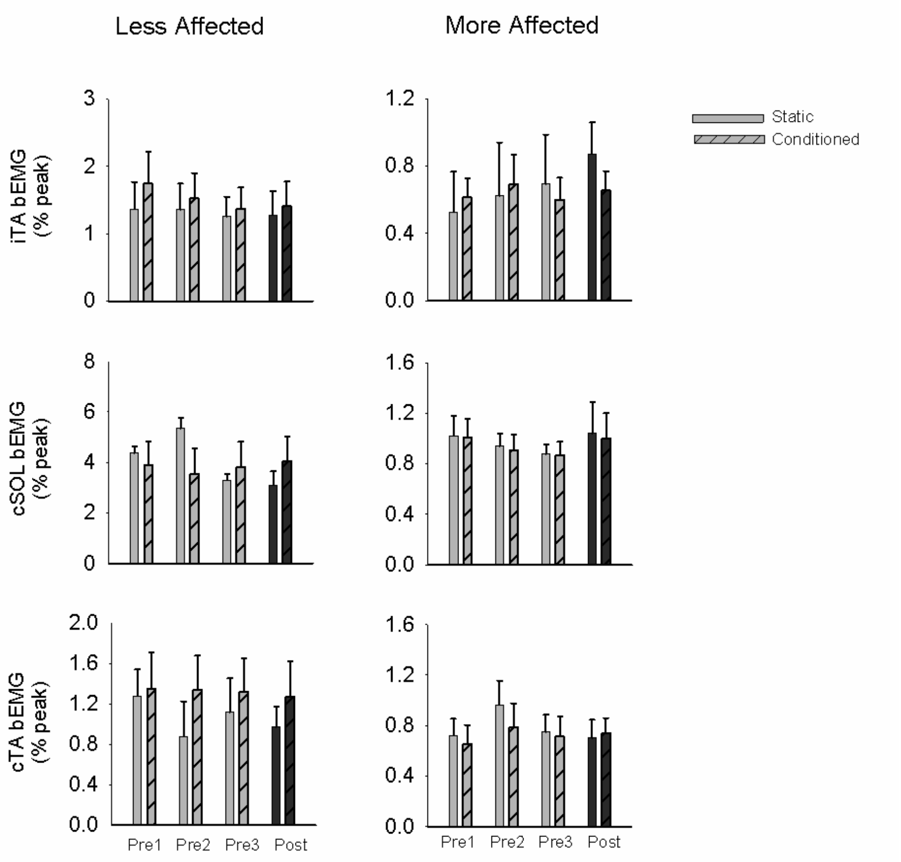


Figure 7-4: Background EMG during stretch reflex testing. Bar graphs are means (±sem) averaged across all participants for static and conditioned stretch reflexes on the less affected and more affected sides. Background EMG values are reported in %peak. For ipsilateral (i) TA and contralateral (c) SOL and TA.

Cutaneous Reflexes

Figure 7-5 shows grand average data across all phases for all participants for reflexes evoked during A&L cycling. Stimulation was applied to the superficial radial nerve of the hand and the superficial peroneal nerve of the foot on the LA side. Line graphs are averages across all participants for three baseline tests (light gray lines) and for the post-test (dark gray line). This representation is useful for showing the general trends in the evoked responses independent of the phase of the step cycle (Klarner et al. 2014; Zehr et al. 2014). Such grand averages taken irrespective of phase provide only an "at a glance" qualitative assessment of changes and provides a visual representation of the strongest effects. However, things like phase dependent reflex reversals, which occur only at a small number of phases, actually are obscured by this process and, in fact, may appear as "noise". Cutaneous reflexes are observed in all muscles tested bilaterally from LA hand and foot stimulation.

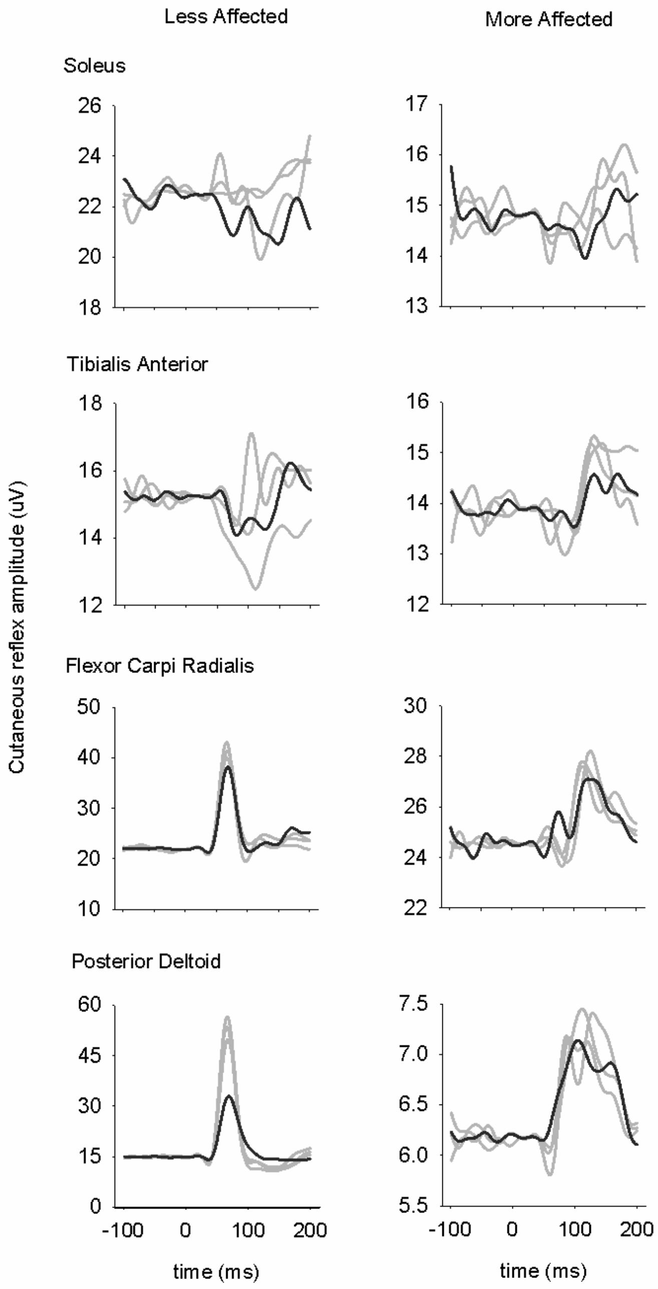


Figure 7-5: Grand average cutaneous reflex traces for all phases for A&L cycling. Line graphs are averages across all participants for three baseline tests (light gray lines) and for the post-test (dark gray line). The stimulus artefact beginning at time 0 has been blanked out and replaced by a flat line. Stimulation was applied to the superficial radial nerve of the hand and the superficial peroneal nerve of the foot on the LA side.

Figure 7-6 shows normalized background EMG (lines) and reflex amplitudes (bars) during A&L cycling. The horizontal bars below the y-axes represent the power (solid line) and recovery (dotted line) phases of A&L cycling. As there were no significant differences between pre-test data samples, for simplification the average value across the three tests is shown.

The results of omnibus ANOVA for all phases and tests reveal main effects of time and phase. As main effects of phase are not of interest, we only addressed main effects of time. Results revealed that there were main effects of time for the MA TA ($F_{(3,54)}$= 3.947, p = 0.001, d = 0.976), LA TA ($F_{(3,54)}$= 2.720, p = 0.045, d = 0.768), and MA FCR ($F_{(3,54)}$= 2.620, p = 0.016, d = 0.873. For the LA PD there was a significant interaction for phase and time ($F_{(21,273)}$= 1.699, p = 0.031, d = 0.966). When looking at specific phases for reflex amplitudes there are significant differences between the baseline average and post-test values for muscles with significant main effects. For the MA TA significant effects were found for phase 2 and 3 ($F_{(1,18)}$=8.561, p=0.012, d=0.435 and $F_{(1,18)}$=5.049, p=0.043, d=0.400, respectively). For the LA TA, significant effects were found for phase 3 and 5 ($F_{(1,18)}$=5.813, p=0.031, d=0.254 and $F_{(1,18)}$=4.486, p=0.045, d=0.362, respectively). For the MA FCR, there were significant differences found when comparing the baseline average and post-test values for phase 6 ($F_{(1,18)}$=5.094, p=0.040, d=0.204) and 7 ($F_{(1,18)}$=4.601, p=0.042, d=0.215). For the LA PD, significant effects were found for phase 2 ($F_{(1,18)}$=3.561, p=0.041, d=0.379).

Investigating background EMG levels between conditions allows for comparison of reflex amplitudes that cannot be explained by simple gain scaling with motoneuronal pool excitability. The results of omnibus ANOVA for all phases and test-sessions reveal main effects of time for background EMG in the LA TA ($F_{(3,54)}$ = 3.149, p = 0.037, d = 0.682). When comparing the pre-test average to post-test values for bEMG at specific phases of A&L cycling, there were significant differences between baseline and post-test values for the MA TA, MA FCR and LA PD muscles. For MA TA, A&L cycling training increased activity for phase 2 ($F_{(1,18)}$=5.373, p=0.039, d=0.555), 3 ($F_{(1,18)}$=5.871, p=0.032, d=0.494) and 6 ($F_{(1,18)}$=5.044, p=0.044, d=0.564). For the MA FCR, bEMG was decreased for phase 2 following training ($F_{(1,18)}$=5.956, p=0.046, d=0.371) and for the LA PD bEMG was increased for phase 7 following training ($F_{(1,18)}$=5.063, p=0.043, d=0.464).

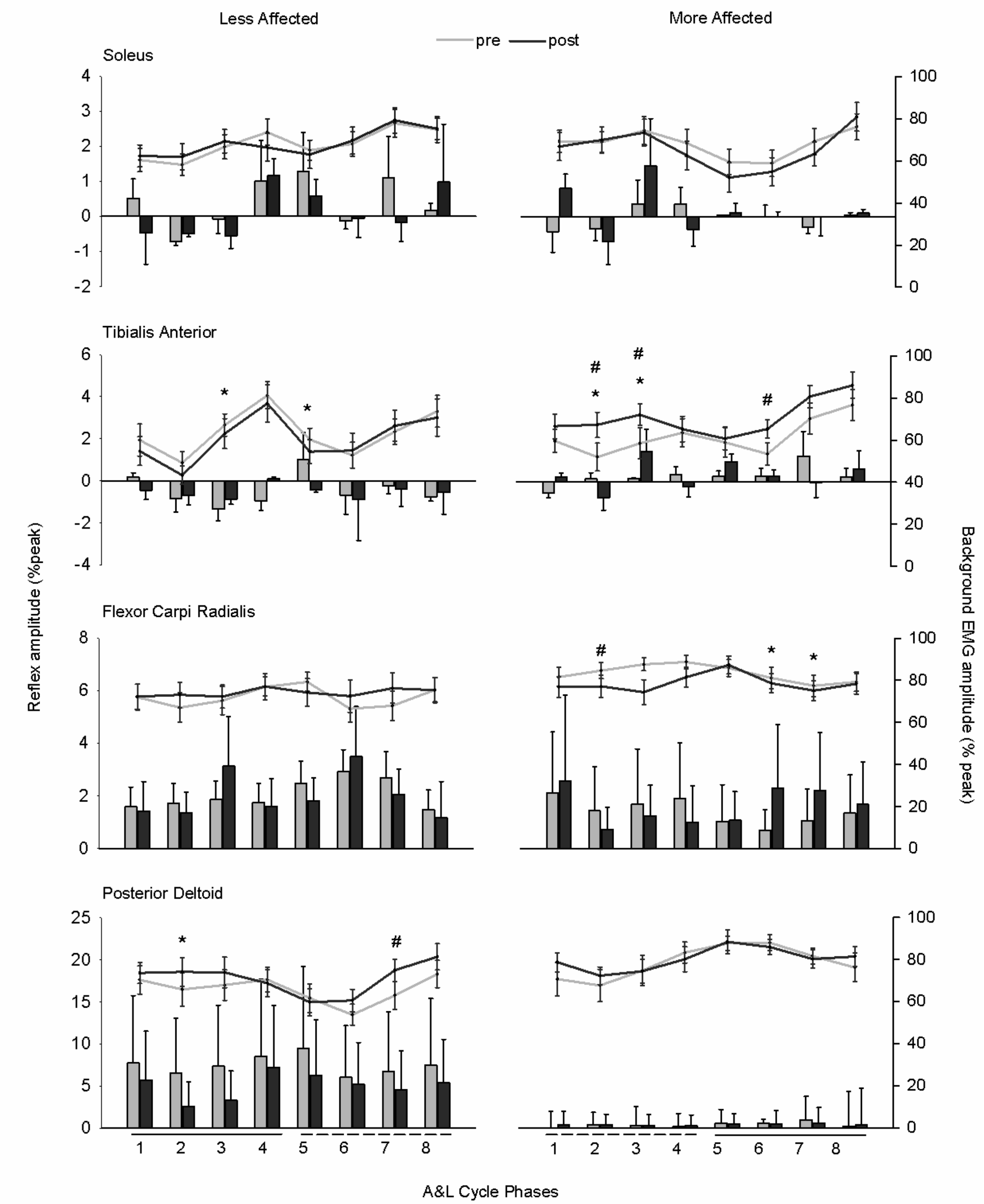


Figure 7-6: Normalized background EMG and reflex amplitudes during A&L cycling. Background EMG is shown in line plots and reflex amplitude is shown in bar plots. Values are means (±standard error) averaged across all participants and normalized to the peak undisturbed EMG during A&L cycling. The horizontal bars below the y-axes represent the power (solid line) and recovery (dotted line) phase of A&L cycling. Significant differences between the pre-test average and the post-test value are indicated with # for background EMG and * for reflex amplitude.

Figure 7-7 shows specific phases of interest for which there were significant effects of training on reflex amplitude. Phase 5 is shown for the LA TA and phase 2 is shown for the LA PD muscle. For the MA FCR, phases 6 and 7 are shown. For bEMG data in Figure 7-7, there were no significant effects of training on modulation of these values.

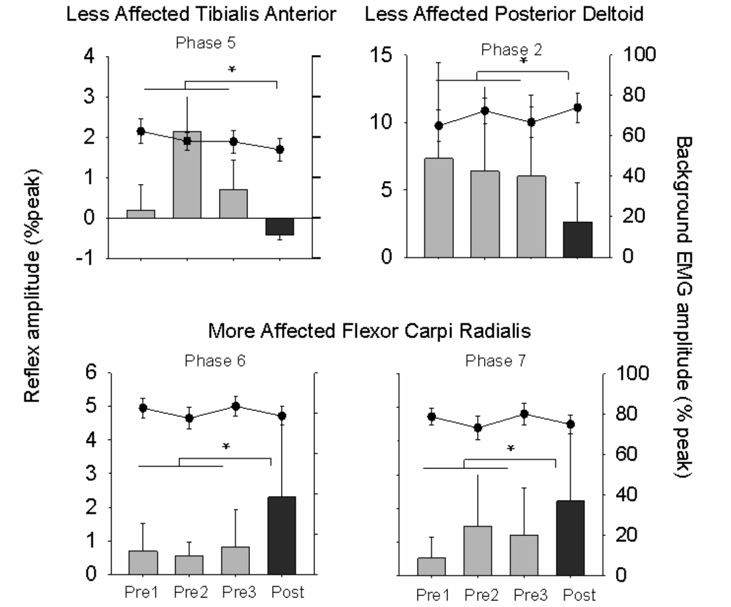


Figure 7-7: Normalized background EMG and reflex amplitudes at specific phase of interest during A&L cycling. Bar graphs are means (±standard error) for reflex amplitude averaged across all participants for baseline and post-test values. Line graphs are means (±standard error) for bEMG. * indicates significant differences between the pre-test average and the post-test value. There were no significant differences for bEMG.

Modulation Index

Figure 7-8 shows the modulation index for the muscles on the LA and MA side and the ratio between LA and MA modulation for cutaneous reflexes and bEMG. Results from omnibus ANOVA reveal that there was a significant main effect of time for the MA FCR ($F_{(3,54)}$= 3.809, p=0.025, d=0.631). When comparing the pre-test average to the post-test, it was found that modulation increased by 30.3% in 11 out of 19 participants for the MA FCR ($F_{(1,18)}$=5.026, p=0.039, d=0.771) following training. Although no significant main effect was found, we did see a 9.8% increase in 8 out of 19 participants ($F_{(1,18)}$=4.999, p=0.040, d=0.359) for the LA TA when comparing with the pre-test average. There was a significant main effect of time for the ratio of modulation for the TA ($F_{(3,54)}$=3.889, p=0.014, d=0.631). Following training, modulation was decreased by 54.6% in 10 out of 19 participants ($F_{(1,18)}$=5.345, p=0.034, d=0.613). When comparing the pretest average to the post-test it was found that modulation was also decreased by 14.2% in 12 out of 19 participants for the SOL ($F_{(1,18)}$=4.566, p=0.048, d=0.317).

For bEMG, there were significant main effects of time for the MA TA ($F_{(3,54)}$=4.095, p=0.013, d=0.804), LA PD ($F_{(3,54)}$=3.656, p=0.043, d=0.731) and MA FCR ($F_{(3,54)}$=3.396, p=0.028, d=0.719). When comparing the pretest average to the post test, it was found that modulation decreased by 23.1% in 9 out of 19 participants following training in the MA TA ($F_{(1,18)}$=8.372, p=0.013, d=0.732) and decreased by 20.2% in 8 out of 19 participants in the LA PD ($F_{(1,18)}$=5.219, p=0.041, d=0.518). For the ratio of modulation for background EMG, there was a significant main effect of time for the TA ($F_{(3,54)}$=3.311, p=0.046, d=0.697) and the FCR ($F_{(3,54)}$=3.150, p=0.037, d=0.789). When comparing the pretest average to the post test, it was found that the ratio increased by 21.2% in 7 out of 19 participants for the TA ($F_{(1,18)}$=5.685, p=0.034, d=0.617) and decreased by 20.2% in 10 out of 19 participants for the FCR ($F_{(1,18)}$=7.988, p=0.015, d=0.737) following training.

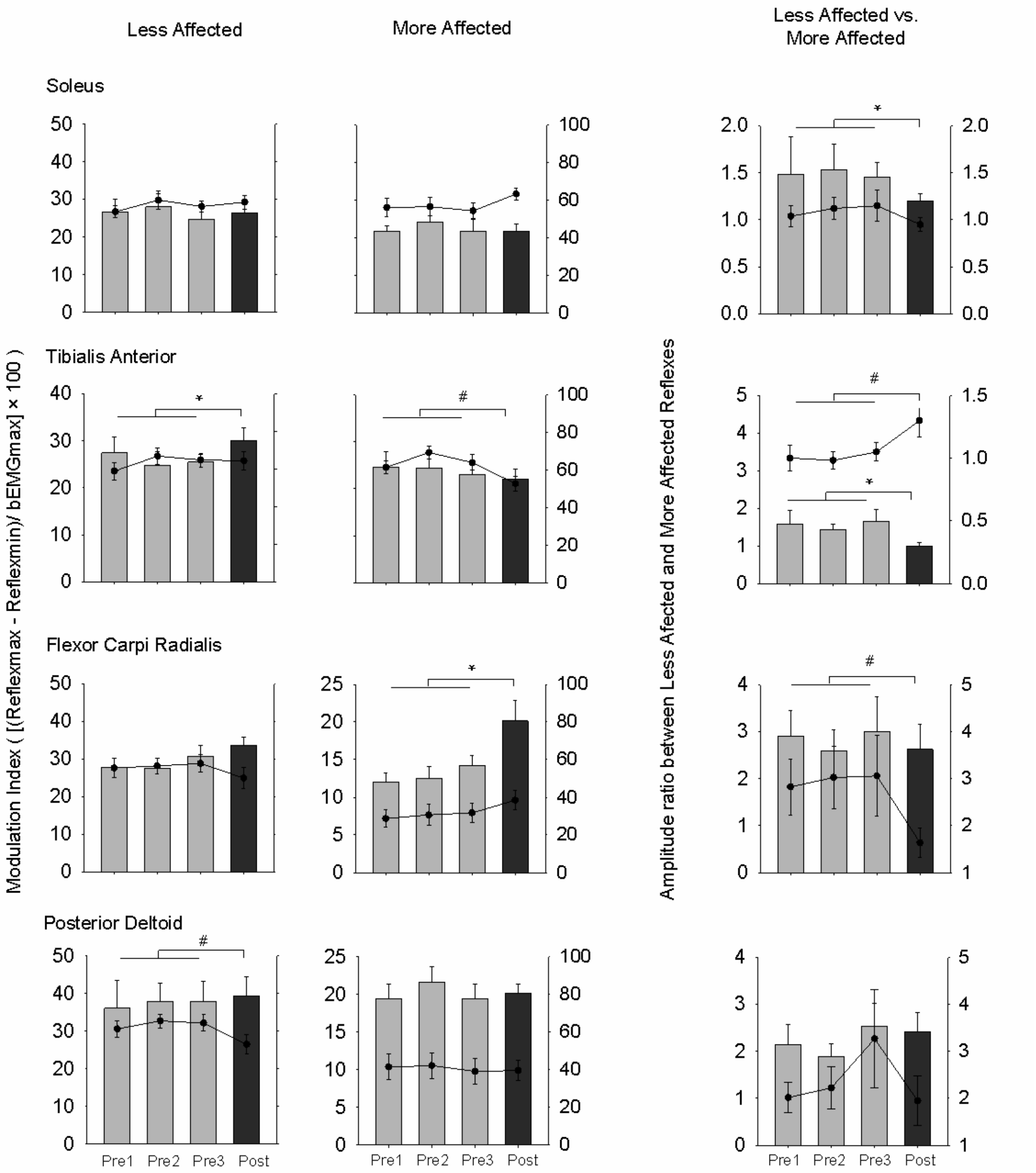


Figure 7-8: Modulation indices and amplitude ratios for reflexes for all muscles during A&L cycling. Bar graphs are means (±standard error) averaged across all participants for baseline and post-test values. Line graphs are means (±standard error) for bEMG * for cutaneous reflex and # for bEMG indicates significant differences between the pre-test average and the post-test value.

Discussion

Here we demonstrate that A&L cycling training can alter reflex excitability after stroke. This is seen as differences in stretch reflex and cutaneous reflex activity prior to and after five weeks of A&L cycling training. These results reveal that plasticity in spinal networks is possible following rehabilitative locomotor training for stroke participants. Testing for plasticity in reflex pathways may be used to probe changes in neural connectivity as a result of rehabilitation interventions for patient populations.

Plasticity in stretch reflex modulation

Examining changes in arm and leg coupling effects on soleus stretch reflexes shows plasticity following A&L cycling training. Results from this study showed plasticity in stretch reflex pathways for the MA side producing increased modulatory effects of arm cycling following training. This observation was supported by both the results of a within-participant and repeated measures analysis. Since heteronymous and contralateral muscle activity influences stretch reflex excitability via the soleus Ia pathway (Hultborn et al. 1987; Crone and Nielsen 1994; Morita et al. 1998; Pierrot-Deseilligny and Mazevet 2000), bEMG activity was recorded to monitor possible effects on the soleus stretch amplitude. The contralateral SOL and ipsilateral and contralateral TA muscles showed no statistically significant differences between the static and conditioned trials. Therefore, the bEMG activity of the above muscles cannot be implicated as a source of the significant suppression seen on the MA stretch reflex amplitude with arm cycling.

A&L cycling training could have altered Group Ia PSI to cause the difference in modulatory effects of arm cycling on MA SOL stretch reflex excitability seen after training here. Modulation of Ia PSI by rhythmic activity in cervical spinal cord oscillators is an important mechanism for reflex modulation in the legs in response to arm cycling (Frigon et al. 2004; Nakajima et al. 2011). This mechanism appears partially preserved after stroke (Barzi and Zehr 2008; Mezzarane et al. 2014), although participants with stroke have difficulty in modulating the excitability of inhibitory pathways in different motor tasks (Morita et al. 2001; Kagamihara and Masakado 2005; Nielsen et al. 2005). However, given the involvement of the fusimotor system on excitability in the stretch reflex pathway (Zehr and Stein 1999; Rossi-Durand 2002), changes in gamma bias related to changes in supraspinal regulation, could also have contributed to changes in MA SOL stretch reflex excitability. Another possible source of the improved reflex modulation is that sensory function was restored after training allowing better transmission of afferent signals.

The A&L cycling training produced a change in modulation of stretch reflex amplitude for the LA and MA sides. The amount of suppression on the MA side was restored to the relative levels seen on the LA side, and showed an increased depth of modulation after A&L cycling training. Initial differences between sides in stretch reflex modulation during arm cycling could be due to asymmetrical differences of presynaptic regulation following stroke. Arm and leg muscles show reduced Ia PSI (Lamy et al. 2009) and only higher threshold motor units can be modulated on the MA side (Barzi and Zehr 2008). Therefore there is a larger potential for plastic modulation on the MA side compared to the less impaired LA side.

Plasticity in cutaneous reflex modulation

Responses to cutaneous stimulation showed altered modulation patterns following training. When examining specific functional phases for A&L cycling, reflexes in the LA SOL, LA TA, MA TA, MA FCR and LA PD are differentially modulated following A&L cycling training. There were also some instances where there were differences in background EMG as a result of training, which could contribute to changes in reflex activity, see for example MA TA. And in a few cases the background changes were in line with changes in reflex activity in the LA TA, MA FCR and LA PD for example. Thus for these muscles only phases where there were no concomitant changes in bEMG were considered further.

In some cases, the modulation following training represents a return to what one “normally” observes in these networks. For example, cutaneous reflex activity in the LA TA is mainly suppressive during locomotion after stroke (Zehr et al. 1998a) or hereditary spastic paraparesis (Duysens et al. 2004). Here in the LA TA, modulation switches from excitation, seen during all three pre-tests, to inhibition following A&L cycling training. In the LA PD, stimulation following training produced decreased facilitation which also represents a “return” to what is typically observed in neurologically intact subjects (Zehr and Loadman 2012; Zehr et al. 2012). These results suggest that neurophysiological reflex excitability can be modified by A&L cycling training and in some cases can help restore cutaneous reflex transmission.

In the wrist flexor FCR muscle on the MA side, reflexes were facilitated following A&L cycling training, seen with both the within-participant and repeated measures analysis. This is evidence for increased response to stimulation from the contralateral (LA) side. Increased reflex activity from contralateral stimulation could represent an increase in crossed responses following training where access is to be best gained by stimulating cutaneous fields in the LA hand (Zehr and Loadman 2012). Measuring reflexes in contralateral muscles gives information on crossed responses and can provide an index of the participation of the contralateral side of the body. By using an index of modulation it is also possible to see how the depth of reflex modulation changed with A&L cycling training. In the LA TA and MA FCR, modulation increased representing an increased depth of modulation following training. When examining the grand average reflex traces from cutaneous stimulation, reflexes on the MA side were lower in amplitude than reflexes on the LA side. This was not surprising given stimulation was applied to the LA side. However, after training, both within-participant and repeated measures analysis revealed that reflexes in the leg muscles had a decreased amplitude ratio, representing a normalization of response amplitudes between the LA and MA sides.

Methodological Considerations

These results should be interpreted in light of several methodological considerations that must be acknowledged. Concomitant changes in bEMG, as a result of the training, could affect both stretch and cutaneous reflex amplitudes between tests. As a result, only trials and phases without changes in bEMG were considered in detail. In addition, evoked responses may vary somewhat from one session to the next making comparison across multiple tests difficult. However the measures used here have been previously shown to have high reliability across multiple baseline points (Klarner et al. 2014a), and for the phases with functionally significant changes, no differences in reflex modulation were seen across several pretest sessions. Lastly, statistical design for multiple baseline designs is limited and little consensus exists across the literature on the best statistical design to use. Therefore we offer two types of statistical assessments: a within-participant design whereby we use the confidence interval from three pretest sessions to compare the post-test value and a design using repeated measures ANOVA to evaluate significant post-test effects with a planned contrast. For many of our outcome variables, results from both statistical designs support the same conclusion, therefore we are confident in our observations.

Plasticity and locomotor rehabilitation

In response to motor training, neural circuits have the plastic ability to alter their structure and function (Wolpaw and Tennissen 2001; Adkins et al. 2006; Wolpaw 2007, 2010). Plasticity in somatosensory reflex pathways has been well documented in humans where rapid, short-term and persistent chronic changes are observed (Zehr 2006). Improvements in locomotor ability and walking, as a result of chronic adaptation in neural circuits, is the goal of locomotor rehabilitation. Improvements in walking observed following locomotor rehabilitation for those with spinal cord injury and stroke (Dietz et al. 1998; Field-Fote 2001; Moseley et al. 2003; Dobkin 2004; Duncan et al. 2011) could be due to plasticity in neural networks. Locomotor training improves premotoneuronal control as seen by the reversal of presynaptic facilitation to presynaptic inhibition of soleus monosynaptic motoneurons at rest and phase-dependent modulation of presynaptic inhibition during walking (Knikou 2013; Knikou and Mummidisetty 2014; Smith et al. 2015) and an improvement in soleus H-reflexes modulation where after 10 days of locomotor training in those with cerebral palsy, reflexes were almost completely supressed during the swing phase, showing a return to what is normally exhibited by healthy subjects (Hodapp et al. 2009). Plasticity in reflex pathways could reflect an improvement in reflex transmission leading to more functional reflex modulation and potentially enhancing walking. However, it may not always be beneficial to simply enhance neural coupling, as this may exaggerate abnormal post-stroke effects, but to induce a beneficial adaptation with targeted training aimed at improving function. For example, operant down-conditioning of H-reflexes can be used to control exaggerated responses leading to enhanced recovery of function and improved walking after incomplete spinal cord injury (Thompson et al. 2013).

Here we have evidence of neural plasticity in the portion of neural linkages that remain accessible after stroke arising from A&L cycling training affecting muscle afferent and cutaneous reflex pathway excitability after stroke. Exactly how these changes in reflex excitability are associated with the recovery of motor function should be the focus of future research. We have recently shown that A&L cycling does indeed transfer to improved walking ability after stroke (Klarner et al. 2016) and the changes in reflex plasticity seen here could be related to this observation. The development of new and targeted therapeutic innovations will further the functional improvements for individuals with neurological disorder.

Conclusion

A&L cycling training for stroke participants modifies reflex excitability. This was determined as an increase in the conditioning effect of arm cycling on soleus stretch reflex excitability on the MA side and as a change in modulation, a change in the index of modulation, and a change in the modulation ratio between the LA and MA sides of cutaneous reflexes. Testing for plasticity in reflex pathways may be used to probe changes in neural connectivity as a result of rehabilitation interventions for clinical populations.

References

Adkins DL, Boychuk J, Remple MS, Kleim JA (2006) Motor training induces experience-specific patterns of plasticity across motor cortex and spinal cord. J Appl Physiol 101:1776–1782. doi: 10.1152/japplphysiol.00515.2006

Andersen OK, Klimstra M, Thomas E, et al. (2014) Human cutaneous reflexes evoked with simultaneous multiple nerve stimulation during rhythmic locomotor-like arm and leg cycling in stroke subjects. Replace Repair, Restore, Reli Bridg Clin Eng Solut Neurorehabil 1:255–261.

Barzi Y, Zehr EP (2008) Rhythmic arm cycling suppresses hyperactive soleus H-reflex amplitude after stroke. Clin Neurophysiol 119:1443–52. doi: 10.1016/j.clinph.2008.02.016

Behrman AL, Harkema SJ (2000) Locomotor training after human spinal cord injury: a series of case studies. Phys Ther 80:688–700.

Bohannon RW, Smith MB (1987) Interrater reliability of a modified Ashworth scale of muscle spasticity. Phys Ther 67:206–207.

Brooke JD, Cheng J, Collins DF, et al. (1997) Sensori-sensory afferent conditioning with leg movement: gain control in spinal reflex and ascending paths. Prog Neurobiol 51:393–421.

Burke D, Dickson HG, Skuse NF (1991) Task-dependent changes in the responses to low-threshold cutaneous afferent volleys in the human lower limb. J Physiol 432:445–58.

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

Butefisch C, Hummelsheim H, Densler P, Mauritz K. (1995) Repetitive training of isolated movements improves the outcome of motor rehabilitation of the centrally paretic hand. J Neurol Sci 130:59–68.

Cohen J (2013) Statistical power analysis for the behavioral sciences. Stat Power Anal Behav Sci. doi: 10.1234/12345678

Crone C, Nielsen J (1994) Central control of disynaptic reciprocal inhibition in humans. Acta Physiol Scand 152:351–363. doi: 10.1111/j.1748-1716.1994.tb09817.x

Cummings G (2013) Understanding the new statistics. Effect sizes, confidence intervals, and meta-analysis.

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

doi: 10.1016/S0166-2236(02)02229-4

Dietz V, Fouad K, Bastiaanse CM (2001) Neuronal coordination of arm and leg movements during human locomotion. Eur J Neurosci 14:1906–1914. doi: 10.1046/j.0953-816x.2001.01813.x

Dietz V, Wirz M, Colombo G, Curt A (1998) Locomotor capacity and recovery of spinal cord function in paraplegic patients: A clinical and electrophysiological evaluation. Electroencephalogr Clin Neurophysiol - Electromyogr Mot Control 109:140–153. doi: 10.1016/S0924-980X(98)00002-2

Dobkin BH (2004) Strategies for stroke rehabilitation. Lancet Neurol 3:528–536. doi: 10.1016/S1474-4422(04)00851-8

Dragert K, Zehr EP (2009) Rhythmic arm cycling modulates Hoffmann reflex excitability differentially in the ankle flexor and extensor muscles. Neurosci Lett 450:235–8. doi: 10.1016/j.neulet.2008.11.034

Dragert K, Zehr EP (2013) High-intensity unilateral dorsiflexor resistance training results in bilateral neuromuscular plasticity after stroke. Exp Brain Res 225:93–104. doi: 10.1007/s00221-012-3351-x

Duncan PW, Sullivan KJ, Behrman AL, et al. (2011) Body-weight-supported treadmill rehabilitation after stroke. N Engl J Med 364:2026–2036. doi: 10.1056/NEJMoa1010790

Duysens J, Baken BCM, Burgers L, et al. (2004) Cutaneous reflexes from the foot during gait in hereditary spastic paraparesis. Clin Neurophysiol 115:1057–1062. doi: 10.1016/j.clinph.2003.12.006

Duysens J, Tax AA, Murrer L, Dietz V (1996) Backward and forward walking use different patterns of phase-dependent modulation of cutaneous reflexes in humans. J Neurophysiol 76:301–310.

Duysens J, Tax AAM, Trippel M, Dietz V (1992) Phase-dependent reversal of reflexly induced movements during human gait. Exp Brain Res. doi: 10.1007/BF00227255

Ferris DP, Huang HJ, Kao PC (2006) Moving the arms to activate the legs. Exerc Sport Sci Rev 34:113–120. doi: 10.1249/00003677-200607000-00005

Field-Fote EC (2001) Combined use of body weight support, functional electric stimulation, and treadmill training to improve walking ability in individuals with chronic incomplete spinal cord injury. Arch Phys Med Rehabil 82:818–24. doi: 10.1053/apmr.2001.23752

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

Frigon A, Collins DF, Zehr EP (2004) Effect of rhythmic arm movement on reflexes in the legs: modulation of soleus H-reflexes and somatosensory conditioning. J Neurophysiol 91:1516–23. doi: 10.1152/jn.00695.2003

Gowland C, Stratford P, Ward M, et al. (1993) Measuring physical impairment and disability with the Chedoke-McMaster Stroke Assessment. Stroke 24:58–63. doi: 10.1161/01.STR.24.1.58

Hage JJ, Van der Steen LPE, De Groot PJM (1995) Difference in sensibility between the dominant and nondominant index finger as tested using the Semmes-Weinstein monofilaments pressure aesthesiometer. J Hand Surg Am 20:227–229. doi: 10.1016/S0363-5023(05)80012-7

Haridas C, Zehr EP (2003) Coordinated interlimb compensatory responses to electrical stimulation of cutaneous nerves in the hand and foot during walking. J Neurophysiol 90:2850–61. doi: 10.1152/jn.00531.2003

Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G (2000) Development of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kinesiol 10:361–374. doi: 10.1016/S1050-6411(00)00027-4

Holden MK, Gill KM, Magliozzi MR, et al. (1984) Clinical gait assessment in the neurologically impaired. Reliability and meaningfulness. Phys Ther 64:35–40.

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

Kagamihara Y, Masakado Y (2005) Excitability of spinal inhibitory circuits in patients with spasticity. J Clin Neurophysiol 22:136–147. doi: 10.1097/01.WNP.0000158948.00901.4E

de Kam D, Rijken H, Manintveld T, et al. (2013) Arm movements can increase leg muscle activity during submaximal recumbent stepping in neurologically intact individuals. J Appl Physiol 115:34–42. doi: 10.1152/japplphysiol.00510.2012

Kawashima N, Nozaki D, Abe MO, Nakazawa K (2008) Shaping appropriate locomotive motor output through interlimb neural pathway within spinal cord in humans. J Neurophysiol. doi: 10.1152/jn.00020.2008

Klarner T, Barss T, Sun Y, et al. (2014a) Preservation of common rhythmic locomotor control despite weakened supraspinal regulation after stroke. Front Integr Neurosci 8:1–9.

Klarner T, Barss T, Sun Y, et al. (2014b) Reliability of Multiple Baseline Measures for Locomotor Retraining after Stroke. Replace Repair, Restore, Reli Bridg Clin Eng Solut Neurorehabilitation 7:479–486.

Klarner T, Barss T, Sun Y, et al. (2016) Exploiting interlimb arm and leg connections for walking rehabilitation: a training intervention in stroke. Neural Plast 2016:1–19.

Klimstra MD, Thomas E, Stoloff RH, et al. (2009) Neuromechanical considerations for incorporating rhythmic arm movement in the rehabilitation of walking. Chaos 19:26102. doi: 10.1063/1.3147404

Knikou M (2013) Functional reorganization of soleus H-reflex modulation during stepping after robotic-assisted step training in people with complete and incomplete spinal cord injury. Exp Brain Res 228:279–296. doi: 10.1007/s00221-013-3560-y

Knikou M, Mummidisetty CK (2014) Locomotor Training Improves Premotoneuronal Control after Chronic Spinal Cord Injury. J Neurophysiol. doi: 10.1152/jn.00871.2013

Komiyama T, Zehr EP, Stein RB (2000) Absence of nerve specificity in human cutaneous reflexes during standing. Exp Brain Res 133:267–72.

Lagerquist O, Zehr EP, Baldwin ERL, et al. (2006) Diurnal changes in the amplitude of the Hoffmann reflex in the human soleus but not in the flexor carpi radialis muscle. Exp Brain Res 170:1–6. doi: 10.1007/s00221-005-0172-1

Lamont E V, Zehr EP (2007) Earth-referenced handrail contact facilitates interlimb cutaneous reflexes during locomotion. J Neurophysiol 98:433–42. doi: 10.1152/jn.00002.2007

Lamy JC, Wargon I, Mazevet D, et al. (2009) Impaired efficacy of spinal presynaptic mechanisms in spastic stroke patients. Brain 132:734–748. doi: 10.1093/brain/awn310

Lee K., Carson L, Kinnin E, Patterson V (1989) The Ashworth Scale: a reliable and reproducible method of measuring spasticity. Neuro Rehabiliat Neural Repair 3:205–209.

Mezzarane R a, Klimstra M, Lewis A, et al. (2011) Interlimb coupling from the arms to legs is differentially specified for populations of motor units comprising the compound H-reflex during “reduced” human locomotion. Exp Brain Res 208:157–68. doi: 10.1007/s00221-010-2467-0

Mezzarane RA, Nakajima T, Zehr EP (2014) After stroke bidirectional modulation of soleus stretch reflex amplitude emerges during rhythmic arm cycling. Front Hum Neurosci 8:136.

doi: 10.3389/fnhum.2014.00136

Morita H, Crone C, Christenhuis D, et al. (2001) Modulation of presynaptic inhibition and disynaptic reciprocal Ia inhibition during voluntary movement in spasticity. Brain. doi: 10.1093/brain/124.4.826

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

Moseley AM, Stark A, Cameron ID, Pollock A (2003) Treadmill Training and Body Weight Support for Walking after Stroke. Stroke 34:3006. doi: 10.1161/01.STR.0000102415.43108.66

Nakajima T, Kitamura T, Kamibayashi K, et al. (2011) Robotic-assisted stepping modulates monosynaptic reflexes in forearm muscles in the human. J Neurophysiol 106:1679–87. doi: 10.1152/jn.01049.2010

Nielsen JB, Petersen NT, Crone C, Sinkjaer T (2005) Stretch reflex regulation in healthy subjects and patients with spasticity. Neuromodulation 8:49–57. doi: 10.1111/j.1094-7159.2005.05220.x

Pierrot-Deseilligny E, Mazevet D (2000) The monosynaptic reflex: A tool to investigate motor control in humans. Interest and limits. Neurophysiol Clin 30:67–80. doi: 10.1016/S0987-7053(00)00062-9

Rossi-Durand C (2002) The influence of increased muscle spindle sensitivity on Achilles tendon jerk and H-reflex in relaxed human subjects. Somatosens Mot Res 19:286–295. doi: 10.1080/0899022021000037755

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

Sasada S, Tazoe T, Nakajima T, et al. (2016) A common neural element receiving rhythmic arm and leg activity as assessed by reflex modulation in arm muscles. J Neurophysiol 115:2065–2075.

Scherr J, Wolfarth B, Christle JW, et al. (2013) Associations between Borg’s rating of perceived exertion and physiological measures of exercise intensity. Eur J Appl Physiol 113:147–155. doi: 10.1007/s00421-012-2421-x

Schneider C, Capaday C (2003) Progressive adaptation of the soleus H-reflex with daily training at walking backward. J Neurophysiol 89:648–656. doi: 10.1152/jn.00403.2002

Smith A, Rymer W, Knikou M (2015) Locomotor training modifies soleus monosynaptic motoneuron responses in human spinal cord injury. Exp Brain Res 233:89–103.

Tang A, Sibley KM, Thomas SG, et al. (2006) Maximal Exercise Test Results in Subacute Stroke. Arch Phys Med Rehabil 87:1100–1105. doi: 10.1016/j.apmr.2006.04.016

Thilmann AF, Fellows SJ, Garms E (1990) Pathological stretch reflexes on the “good” side of hemiparetic patients. J Neurol Neurosurg Psychiatry 53:208–214. doi: 10.1136/jnnp.53.3.208

Thompson AK, Pomerantz FR, Wolpaw JR (2013) Operant conditioning of a spinal reflex can improve locomotion after spinal cord injury in humans. J Neurosci 33:2365–2375. doi: 10.1523/JNEUROSCI.3968-12.2013

Vasudevan EVL, Zehr EP (2011) Multi-frequency arm cycling reveals bilateral locomotor coupling to increase movement symmetry. Exp Brain Res 211:299–312. doi: 10.1007/s00221-011-2687-y

Walker HK (1990) Clinical Methods: The History, Physical, and Laboratory Examinations. Boston : Butterworths

Warburton DE, Charlesworth S, Ivey A, et al. (2010) A Systematic Review of the Evidence for Canada’s Physical Activity Guidelines. Int J Behav Nutr Phys Act 7:39. doi: 10.1186/1479-5868-7-39

Wolpaw JR (2007) Spinal cord plasticity in acquisition and maintenance of motor skills. Acta Physiol. pp 155–169

Wolpaw JR (2010) What can the spinal cord teach us about learning and memory? Neuroscientist 16:532–549. doi: 10.1177/1073858410368314

Yang JF, Stein RB (1990) Phase-dependent reflex reversal in human leg muscles during walking. J Neurophysiol 63:1109–1117.

Zehr E, Nakajima T, Barss T, et al. (2014) Cutaneous stimulation of discrete regions of the sole during locomotion produces “sensory steering” of the foot. BMC Sport Sci. Med. Rehabil. 6:

Zehr EP (2006) Training-induced adaptive plasticity in human somatosensory reflex pathways. J Appl Physiol 101:1783–94. doi: 10.1152/japplphysiol.00540.2006

Zehr EP (2011) Evidence-based risk assessment and recommendations for physical activity clearance: stroke and spinal cord injury. Appl Physiol Nutr Metab 36 Suppl 1:S214-31. doi: 10.1139/h11-055

Zehr EP, Balter JE, Ferris DP, et al. (2007a) Neural regulation of rhythmic arm and leg movement is conserved across human locomotor tasks. J Physiol 582:209–27. doi: 10.1113/jphysiol.2007.133843

Zehr EP, Carroll TJ, Chua R, et al. (2004) Possible contributions of CPG activity to the control of rhythmic human arm movement. Can J Physiol Pharmacol 82:556–68. doi: 10.1139/y04-056

Zehr EP, Collins DF, Chua R (2001) Human interlimb reflexes evoked by electrical stimulation of cutaneous nerves innervating the hand and foot. Exp Brain Res 140:495–504. doi: 10.1007/s002210100857

Zehr EP, Duysens J (2004) Regulation of arm and leg movement during human locomotion. Neuroscientist 10:347–61. doi: 10.1177/1073858404264680

Zehr EP, Fujita K, Stein RB (1998a) Reflexes from the superficial peroneal nerve during walking in stroke subjects. J Neurophysiol 79:848–58.

Zehr EP, Haridas C, Zehr EP, et al. (2003) Modulation of cutaneous reflexes in arm muscles during walking: further evidence of similar control mechanisms for rhythmic human arm and leg movements. Exp Brain Res 149:260–6. doi: 10.1007/s00221-003-1377-9

Zehr EP, Hundza SR, Vasudevan E V (2009) The quadrupedal nature of human bipedal locomotion. Exerc Sport Sci Rev 37:102–8. doi: 10.1097/JES.0b013e31819c2ed6

Zehr EP, Hundza SRS (2005) Forward and backward arm cycling are regulated by equivalent neural mechanisms. J Neurophysiol 93:633–40. doi: 10.1152/jn.00525.2004

Zehr EP, Klimstra M, Dragert K, et al. (2007b) Enhancement of arm and leg locomotor coupling with augmented cutaneous feedback from the hand. J Neurophysiol 98:1810–4. doi: 10.1152/jn.00562.2007

Zehr EP, Klimstra M, Johnson E a, Carroll TJ (2007c) Rhythmic leg cycling modulates forearm muscle H-reflex amplitude and corticospinal tract excitability. Neurosci Lett 419:10–4. doi: 10.1016/j.neulet.2007.03.045

Zehr EP, Komiyama T, Stein RB (1997) Cutaneous reflexes during human gait: electromyographic and kinematic responses to electrical stimulation. J Neurophysiol 77:3311–25.

Zehr EP, Loadman PM (2012) Persistence of locomotor-related interlimb reflex networks during walking after stroke. Clin Neurophysiol 123:796–807. doi: 10.1016/j.clinph.2011.07.049

Zehr EP, Loadman PM, Hundza SR (2012) Neural control of rhythmic arm cycling after stroke. J Neurophysiol 108:891–905. doi: 10.1152/jn.01152.2011

Zehr EP, Stein RB, Komiyama T (1998b) Function of sural nerve reflexes during human walking. J Physiol 507(1):305–14.

General Conclusions

This dissertation focused upon exploring central nervous system control and interlimb coordination during rhythmic arm and leg movement and the extent to which arm and leg (A&L) cycling training improves walking after stroke. To address this, three objectives were posed:

- The first objective was to provide further evidence of central nervous system control of walking. Through a literature review in Chapter 1 and experimental evidence in Chapter 2, of common subcortical control across rhythmic locomotor tasks, evidence for the existence of central pattern generating (CPG) networks in humans is given.
- The second objective was to explore interlimb coordination during rhythmic arm and leg movement. Results presented in Chapters 3 and 4 further our understanding of specific interlimb interactions during rhythmic arm and leg tasks.
- The third objective was to evaluate the effects of an A&L cycling training intervention in a post-stroke population. To support this objective, it was shown in Chapter 5 that a multiple baseline design is appropriate for use in intervention studies. In Chapter 6, it was determined that A&L cycling training can be used to improve walking ability. And in Chapter 7, it was shown that training induced plasticity in interlimb reflex pathways.

Overall, results in this dissertation provide further knowledge on CPG control of walking, on arm and leg interlimb interactions during rhythmic movements, and on the effect of A&L cycling training for locomotor recovery. The following is a summary of how the main findings from each chapter support the three main objectives. Future research to expand on measures, explore the efficacy of A&L cycling training, and to introduce training as a mainstream community based program are proposed.

Objective 1: Central pattern generating networks in humans

Evidence on the existence of CPGs supporting walking in humans is given in Chapter 1. Based on evidence, first described years ago, the general concepts regarding the control of walking through spinal central pattern generators (CPG) have been made from studying reduced animal models. From these observations, we can speculate as to what should be observable in humans, where we must rely on indirect evidence and inferences to assess the role of CPGs in generating rhythmic movements. The literature review in Chapter 1 presents observations from humans, to test hypotheses posed from other animal studies, which supports the theory of CPG-mediated locomotion in humans. An understating of the neural control for walking and interlimb coordination is necessary in determining the conditions required to successfully rehabilitate walking (Zehr et al. 2016).

The basic pattern of arm and leg movement during rhythmic locomotor tasks is supported by common central neural control from spinal centers in neurologically intact participants. The main results from Chapter 2 demonstrates that following stroke common regulation of modulation of interlimb reflexes persists between two rhythmic tasks; A&L cycling and walking. In both the less and more affected limbs, cutaneous reflexes were evident, and modulated in a similar way between tasks. Background muscle activity was also similar between walking and arm and leg cycling tasks, especially in the distal leg muscles. Commonality of neural control across rhythmic tasks was also observed across walking, arm and leg cycling, and arm-assisted recumbent stepping, where similar phase-dependent modulation was seen despite differences in movement kinematics (Zehr et al. 2007). Cutaneous reflex amplitudes in arm and leg muscles were also modulated in a similar way across tasks of level walking, incline walking, and stair climbing (Lamont and Zehr 2006). Commonality in control among rhythmic movement tasks could be the result of a common core of subcortical elements expressing neural activity to produce the basic pattern of arm and leg movement (Zehr 2005; Zehr et al. 2007). A central mechanism is likely responsible for regulating various types of rhythmic movement in a similar oscillatory fashion.

The status of neural control circuits in the damaged nervous system, provide insight into the locus of control of common neural patterning (Zehr and Duysens 2004). The overall similarities in modulation patterns for background EMG and cutaneous reflexes, observed after stroke (Chapter 2), supports the notion of a common core in locomotor tasks from conserved subcortical regulation, despite the interruption of some descending regulation arising from the supraspinal lesion. As damage to the brain following stroke does not seem to significantly affect common neural regulation, a contribution from subcortical, and spinal CPG networks, is presumed.

An important consideration in applying locomotor retraining in rehabilitation is the extent to which the training task transfers to the intended functional task (Zehr et al. 2016). The neural similarities between A&L cycling and walking, observed in Chapter 2, have translational implications for rehabilitation, where there is a reasonable basis for expectation of training transfer of A&L cycling to improve walking. This can be achieved by activation of a set of similar residual neural pathways to strengthen interlimb neuronal coupling to improve walking after stroke (Zehr 2005; Ferris et al. 2006; Balter and Zehr 2007; Zehr et al. 2007; Zehr et al. 2009; Klimstra et al. 2009; Zehr and Loadman 2012; Zehr et al. 2012).

Objective 2: Interlimb coordination during rhythmic movement

Experimental evidence presented in Chapters 3 and 4 revealed interlimb neural coupling during rhythmic arm and leg tasks. These observations extend knowledge on the specific interactions between neural control and mechanical action during rhythmic movements (Dietz et al. 1994; Dietz et al. 2001; Frigon et al. 2004; Haridas et al. 2006; Zehr et al. 2009; Zehr et al. 2016). Understanding the underlying neural mechanisms organizing rhythmic arm movement, and its coordination with the legs, helps inform development of effective strategies for rehabilitation of pathological walking.

Data presented in Chapter 3 show how changes in interlimb coordination during rhythmic movement, induced with slow walking, modify reflex modulation and neural control. Cutaneous reflex modulation was found to be dependent upon interlimb coordination and walking speed, with large effects in the tibialis anterior, and during the swing phase when walking is most unstable (Zehr et al. 1998; Haridas and Zehr 2003; Ruff et al. 2014). When arm swing changes threaten posture and balance, changes in neural control are also revealed (Haridas et al. 2005; (Lamont and Zehr 2007; Forero and Misiaszek 2015). This underscores a general role for interlimb reflexes that emerge to assist in the maintenance of postural stability during locomotion (Zehr et al. 2016).

A directional coupling of arm to leg interlimb control was also observed in Chapter 3, where arm activity had a greater effect on leg activity, instead of the reverse. Directional coupling of interlimb coordination was also observed in other studies on reduced locomotor movements, such as with arm and leg stepping or cycling, that have shown that arm movement contributes to support reflexive activity in the legs (Balter and Zehr 2007). This corresponds to similar effects from forelimb to hindlimb in the cat (Miller et al. 1973; Orsal et al. 1990). Directional coupling between the arms and legs serves a functional role in bipedal locomotion to help ensure the swinging limb is guided safely to the ground for proper foot placement and balance, dependent on interactions with the arms (Haridas and Zehr 2003; Zehr et al. 2016). These results further understanding of the underlying neural mechanism on the control of interlimb coordination. Given interlimb coordination, arm integration in locomotor rehabilitation of pathological walking is warranted.

As in Chapter 3, Chapter 4 contains data which reveal the intricate properties of interlimb coordination during rhythmic movement. These details were gleaned by evaluating the effects of cutaneous stimulation at discrete regions of the foot dorsum. Results reveal dynamic changes in reflex amplitudes, kinematics, and kinetics, which are site-specific, phase-dependent, and seen in muscles from both the arms and legs. Also as in Chapter 3, most responses from stimulation were seen in the swing phase, and were most prominent from stimulation at the distal end of the foot, at the 1st and 4th metatarsals. This is at an anatomical location where actual impact with an object in the environment is most likely, necessitating a corrective response. In this case, and in other cases, cutaneous and kinematic responses functionally served to conform foot trajectory to maintain stability of the swinging limb (Zehr et al. 1997; Haridas and Zehr 2003; Haridas et al. 2008).

Responses to stimulation go beyond the muscles that control the ankle to include muscles at the hip and at the shoulder joints (Chapter 4). This extends further observations of strong interlimb reflexes evoked in muscles across the body by cutaneous stimulation of the superficial peroneal nerve (Zehr et al. 2001; Haridas and Zehr 2003). Presented in Chapter 4, stimulation to the foot dorsum facilitated activity in the posterior deltoid (PD) muscle in the shoulder. Functionally this would facilitate abduction of the arms to maintain stability to compensate for the potential destabilization caused by dorsal foot stimulation. Showing phase-dependent modulation, at the end of the stance phase, stimulation to the 1st metatarsal further increased PD facilitation, likely for balance maintenance prior to the unstable transition into swing. In other studies, a large facilitatory reflex in the PD muscle was also produced to stabilize the upper body as the leg leaves the ground (Haridas and Zehr 2003).

To support the second objective, results from Chapters 3 and 4, further our understanding of specific interlimb interactions during rhythmic arm and leg movement tasks. Given that the arms and legs are functionally connected during rhythmic locomotor tasks, A&L cycling could be usefully applied to recover walking function.

Objective 3: A&L cycling training after stroke

The third objective was to evaluate the effects of A&L cycling training in a post-stroke population. It was shown in Chapter 5 that there is high reliability of multiple baseline measures, in Chapter 6 that A&L cycling training does improve walking, and in Chapter 7 that training induces plasticity in arm and leg interlimb reflex excitability.

Reliability of measures tested across multiple baseline sessions, using a repeated test protocol, in stroke participants was confirmed in Chapter 5. Reliability analysis revealed strong correlation amongst most variables, where no significant differences were found when comparing across multiple time points. Given high internal consistency of measures, multiple baseline measures should be considered a valid alternative, or replacement, to the concept of a control group. In this design, multiple baseline measures are conducted to develop a meaningful set of baseline data, so that each subject acts as their own control (Butefisch et al. 1995). Post-test data can then be compared against the participant's own pre-test variability. Therefore to test the effects of A&L cycling training, a multiple baseline (3 pre-tests) within-subject control design was used.

Chapter 6 is the first evidence available that exploiting interlimb arm and leg connections, with A&L cycling training, on a stationary ergometer, improves walking after stroke. Nineteen individuals with chronic stroke (>six months post-lesion) performed 30 minutes of A&L cycling training three times a week for five weeks. Data show that A&L cycling training improved clinical walking status, increased strength by ~25%, improved modulation of muscle activity by ~25%, increased range of motion by ~20%, decreased stride duration and increased frequency during treadmill walking. After A&L cycling training, cutaneous reflexes also showed improved modulation during walking. On most variables, the majority of participants showed a significant improvement. Exploiting arm and leg connections, with A&L cycling training, an accessible and cost-effective training modality, could be used to improve walking ability after stroke.

These findings are in line with other studies that have found benefits of incorporating arm movements in gait rehabilitation. In those with incomplete cervical spinal cord injury, when arm activity is incorporated with locomotor-like arm and leg movements, leg muscle activity is facilitated (Kawashima et al. 2008). Gait symmetry and a more normal presentation of EMG is also apparent in patients with spastic paresis when arm swings were incorporated with body weigh supported training (Visintin and Barbeau 1994). Together these findings, as well as the ones presented in Chapter 6, support the addition of rhythmic arm activity as a regular part of locomotor rehabilitation after neurotrauma. Allowing a normal simultaneous and reciprocating arm action during walking rehabilitation may facilitate stepping, and may be an important component needed to harness interlimb neural coupling, to help improve motor output for the legs (Ferris et al. 2006; Kawashima et al. 2008; Zehr et al. 2009; de Kam et al. 2013).

The findings of Chapters 2, 6 and 7 also extends the observation that interlimb coordination and neural control is present in stroke, despite interruption of connectivity between supraspinal and subcortical areas, occurring as a result of the stroke lesion. The presence of interlimb reflex effects, from cutaneous stimulation, reveals that pathways remain intact and accessible, and the presence of phase-dependent modulation of sensory inputs reveals that some of the normal regulation still exits after stroke. Past evidence also supports the observation of preserved neural control after stroke. For example, part of the stumble correction response, where stimulation to the top of the foot during the swing phase, normally observed in NI participants, was preserved in stroke participants (Zehr et al. 1998). Somatosensory interlimb reflex connections have also been identified in stroke participants where cutaneous input can access reflex pathways in all four limbs, including the more affected limb, during rhythmic movement (Zehr and Loadman 2012; Zehr et al. 2012). Interlimb cutaneous reflexes were phase-dependently modulated, and the depth of modulation was similar for stroke and neurologically intact participants (Zehr and Loadman 2012). Partial preservation of the descending modulatory effects of rhythmic arm cycling on lumbosacral spinal cord excitability can also be seen after stroke where arm cycling modulates the soleus H-reflex (Barzi and Zehr 2008) and stretch reflex (Mezzarane et al. 2014). Evidence for the existence of portions of the neural circuitry regulating rhythmic interlimb arm and leg movements, that remain accessible and intact after stroke (Chapters 2, 6, and 7), provides a substrate for training induced plasticity to improve function (Zehr et al. 2016).

Indeed, presented in Chapter 7, it was shown that 5 weeks of arm and leg cycling training induced long term plasticity in reflex excitability. Changes in plasticity of reflex excitability were inferred from modulation of cutaneous and stretch reflexes. Plasticity was determined as an increase in the conditioning effect of arm cycling on soleus stretch reflex amplitude on the more affected side, by the index of modulation, and by the modulation ratio between sides for cutaneous reflexes. This observation supports the notion that in response to motor training, neural circuits have the ability to alter their structure and function (Wolpaw and Tennissen 2001; Adkins et al. 2006; Wolpaw 2007; Wolpaw 2010).

Few studies have demonstrated functional plasticity of the spinal cord after changes in supraspinal or peripheral inputs. However, there is some evidence in the cat model which suggests reorganization of spinal networks after step training (Côté and Gossard 2004). Spinalization in untrained cats produced exaggerated reflex effects which were decreased after stepping training. This suggested a normalization of cutaneous transmission, which was part of the plastic adaptations resulting in recovery of foot placement for successful locomotion in the cat (Côté and Gossard 2004). In another study, locomotion of the hindlimbs following a neurectomy of the ankle flexors in intact and spinal cats, revealed plastic changes that may have taken place in spinal circuitry to maintain locomotion following peripheral nerve lesion (Carrier et al. 1997). These results suggest that in the cat, plasticity in the spinal cord, and in somatosensory reflex pathways, leads to improved function and performance.

There is clear behavioural evidence suggesting that spinal locomotor networks in humans undergo adaptations and plastic changes following a stroke. Use-dependent plasticity in spinal pathways, involved in locomotor generation, are trained with walking rehabilitation and result in improved walking function (Dietz et al. 1998; Field-Fote 2001; Moseley et al. 2003; Dobkin 2004; Duncan et al. 2011). Enhancements in walking could be the result of plasticity in spinal centers and reflex pathways, leading to an improvement in neural transmission and more functional modulation and control. Constant rhythmic movement, achieved with the A&L cycle ergometer, cyclically activates afferent reflexes which can trigger, modulate, and reset CPG activity, as shown in animal models (Conway et al. 1987; Schomburg et al. 1998; Lam and Pearson 2002; Guertin 2012). Therefore, improvement in walking may be attributed largely to modulation of simple local reflex arcs, and pattern generating centers, activated cyclically during training, and inducing plasticity overtime.

Short-term plasticity in somatosensory reflex pathways has been documented in humans where rapid changes are observed (Zehr 2006). However, there is currently little data available in humans to show persistent chronic changes in reflex pathways and spinal CPGs after training. Operant conditioning protocols have shown that, over 30 sessions, soleus H-reflexes decreased and remained smaller several months after testing (Thompson et al. 2013), indicating that activity in specific spinal cord pathways can be modified. Here, in Chapter 7, there is also evidence of neural plasticity arising from A&L cycling training, affecting muscle afferent and cutaneous reflex pathway excitability, after stroke. It is unknown however if these changes are responsible for the improvements in walking, observed in Chapter 6.

Future Directions

Understanding interlimb coordination after neurological injury, and the effects of rhythmic arm activity on locomotor rehabilitation, is starting to advance. Work must continue to refine our understanding of the central neural control of locomotion and mechanisms of interlimb coupling before this information can be properly implemented in locomotor rehabilitation. Increasing evidence on methods to test plasticity after training, to maximize the efficacy of A&L cycling training, and to introduce training as a mainstream community-based program are proposed.

Testing for plasticity after training

Testing for plasticity of reflex pathways can be used to probe changes in neural connectivity as a result of rehabilitation interventions for patient populations. For example, testing the conditioning effects of arm cycling on the soleus stretch reflex could be used as a proxy for measuring the change in strength of excitability in neural connections between the arms and the legs. Stretch reflex testing, a protocol based on a relatively simple procedure (a tendon tap), might be useful for application in stroke populations, compared to testing the electrically evoked H-reflex pathway, as allodynia is not uncommon in this group. In addition, hyperreflexia, as measured by stretch reflexes, may be closer to the clinical manifestation of spasticity than measured with the H reflex (Grey et al. 2008), and would more clearly reveal reflex modulation associated with the fusimotor system, as stretch reflex testing does not bypass muscle spindles compared to H-reflex testing (Rossi-Durand 2002). Therefore, the soleus stretch reflex could be used as a clinical evaluation tool and for the assessment of changes in spasticity (Voerman et al. 2005), and for plasticity in response to training and exercise.

Testing cutaneous reflex connections in A&L cycling could also be used to evaluate changes in neural coordination. Measuring reflexes in the MA limb, from stimulation of the LA limbs, gives information on crossed responses, and could be used to provide an index of the participation of the contralateral side of the body. Methodologies that could be widely incorporated in the stroke population are of increased use to researchers and clinicians engaged in the conception and refinement of rehabilitative procedures.

Maximize the efficacy of A&L cycling training

Transfer of improvements following A&L cycling training to enhance walking (Chapter 6) could open the way to the development of a new approach for the rehabilitation of stroke patients. Therefore, future research should be focused on optimizing efficiency and effectiveness of rhythmic A&L cycling training strategies for stroke, and other aging, or neurologically injured populations. Defining the optimal training parameters, including dose, intensity, and frequency, is key in maximizing efficacy and effectiveness of A&L cycling training. However, to fully understand the benefits of A&L cycling training relative to other conventional forms of rehabilitation, a randomized controlled trial should be conducted.

Given that other types of training, such as strength training or treadmill training, also improve walking (Dobkin 2004; Gordon et al. 2004; Morris et al. 2004), A&L cycling training should not be used to replace these therapies, but instead be used as an adjunct modality for improving walking ability after stroke. This therapy may be particularly valuable as an initial training tool for participants who initially lack strength and balance control for independent walking. Given the linked cranks, using A&L cycling as the training modality allows for physical assistance to the weakened limbs to encourage rhythmic interlimb coordination across the entire body.

Community based training program

Given the increasing age of Canadians, and subsequent increase in age-related diseases, maintaining independent mobility, achieved through exercise and rehabilitation, is pivotal to enabling community participation and to maintaining a high quality of life. Therefore, it is also of great importance to increase the ease of accessibility, and integration into the community, of locomotor rehabilitation training programs.

Individuals living with spinal cord injury or stroke show significant improvements following a formal rehabilitation program. But these improvements can diminish because of decreased participation once the patient leaves the hospital or rehabilitation setting, leading to a decline in health status, and an increased need to access the healthcare system (Pinter and Brainin 2012). It is essential that novel, low cost strategies, such as A&L cycling training, implemented to help maintain strength and fitness.

Community-based exercise programs for individuals with neurological conditions, as well as for members of the general aging population, could help to improve health status for Canadians. A&L cycling is a safe and low-stress activity that, has been shown to improve walking after stroke (Chapter 6), and could be integrated as a community-based training program. A&L cycle ergometers are widely available in most gyms and recreation centers that are relatively cheap and easy to access. This type of community based exercise allows for equalization of opportunity for training, with increased equipment access outside of major rehabilitation centers.

Future research should aim to examine feasibility of A&L cycling as a community-based exercise program. Increasing the ease of training, based upon a device that could be more readily used in therapy, would directly impact the health and quality of life for those people who have suffered a stroke.

Conclusion

Overall, results in this dissertation provide further knowledge on nervous system control and arm and leg interlimb interactions during rhythmic movements. This dissertation also attempts to make a step further towards the development of improved recovery strategies for those who have suffered a stroke by exploiting the principles of nervous system control with A&L cycling training. This work directly impacts the health and quality of life for those who have suffered a stroke, and is considered very important as the demand to develop successful rehabilitation strategies is increased as our population continues to age.

References

Adkins DL, Boychuk J, Remple MS, Kleim JA (2006) Motor training induces experience-specific patterns of plasticity across motor cortex and spinal cord. J Appl Physiol 101:1776–1782. doi: 10.1152/japplphysiol.00515.2006

Balter JE, Zehr EP (2007) Neural coupling between the arms and legs during rhythmic locomotor-like cycling movement. J Neurophysiol 97:1809–18. doi: 10.1152/jn.01038.2006

Barzi Y, Zehr EP (2008) Rhythmic arm cycling suppresses hyperactive soleus H-reflex amplitude after stroke. Clin Neurophysiol 119:1443–52. doi: 10.1016/j.clinph.2008.02.016

Butefisch C, Hummelsheim H, Densler P, Mauritz K. (1995) Repetitive training of isolated movements improves the outcome of motor rehabilitation of the centrally paretic hand. J Neurol Sci 130:59–68.

Carrier L, Brustein E, Rossignol S (1997) Locomotion of the hindlimbs following a neurectomy of the ankle flexors in intact and spinal cats: model for the study of locomotor plasticity. J Neurophysiol 77:1979–1993.

Conway BA, Hultborn H, Kiehn O (1987) Proprioceptive input resets central locomotor rhythm in the spinal cat. Exp Brain Res 68:643–656. doi: 10.1007/BF00249807

Côté M-P, Gossard J-P (2004) Step training-dependent plasticity in spinal cutaneous pathways. J Neurosci 24:11317–11327. doi: 10.1523/JNEUROSCI.1486-04.2004

Dietz V, Fouad K, Bastiaanse CM (2001) Neuronal coordination of arm and leg movements during human locomotion. Eur J Neurosci 14:1906–1914. doi: 10.1046/j.0953-816X.2001.01813.x

Dietz V, Wirz M, Colombo G, Curt A (1998) Locomotor capacity and recovery of spinal cord function in paraplegic patients: A clinical and electrophysiological evaluation. Electroencephalogr Clin Neurophysiol - Electromyogr Mot Control 109:140–153. doi: 10.1016/S0924-980X(98)00002-2

Dietz V, Zijlstra W, Duysens J (1994) Human neuronal interlimb coordination during split-belt locomotion. Exp Brain Res 101:513–520. doi: 10.1007/BF00227344

Dobkin BH (2004) Strategies for stroke rehabilitation. Lancet Neurol 3:528–536. doi: 10.1016/S1474-4422(04)00851-8

Duncan PW, Sullivan KJ, Behrman AL, et al. (2011) Body-weight-supported treadmill rehabilitation after stroke. N Engl J Med 364:2026–2036. doi: 10.1056/NEJMoa1010790

Ferris DP, Huang HJ, Kao PC (2006) Moving the arms to activate the legs. Exerc Sport Sci Rev 34:113–120. doi: 10.1249/00003677-200607000-00005

Field-Fote EC (2001) Combined use of body weight support, functional electric stimulation, and treadmill training to improve walking ability in individuals with chronic incomplete spinal cord injury. Arch Phys Med Rehabil 82:818–24. doi: 10.1053/apmr.2001.23752

Frigon A, Collins DF, Zehr EP (2004) Effect of rhythmic arm movement on reflexes in the legs: modulation of soleus H-reflexes and somatosensory conditioning. J Neurophysiol 91:1516–23. doi: 10.1152/jn.00695.2003

Gordon NF, Gulanick M, Costa F, Fletcher G (2004) Physical Activity and Exercise Recommendations for Stroke Survivors. Circulation 109:2031–2041. doi: 10.1161/01.STR.0000127303.19261.19

Grey MJ, Klinge K, Crone C, et al. (2008) Post-activation depression of Soleus stretch reflexes in healthy and spastic humans. Exp Brain Res 185:189–197. doi: 10.1007/s00221-007-1142-6

Guertin P a (2012) Central pattern generator for locomotion: anatomical, physiological, and pathophysiological considerations. Front Neurol 3:183. doi: 10.3389/fneur.2012.00183

Haridas C, Zehr EP (2003) Coordinated interlimb compensatory responses to electrical stimulation of cutaneous nerves in the hand and foot during walking. J Neurophysiol 90:2850–61. doi: 10.1152/jn.00531.2003

Haridas C, Zehr EP, Misiaszek JE (2006) Context-dependent modulation of interlimb cutaneous reflexes in arm muscles as a function of stability threat during walking. J Neurophysiol 96:3096–103. doi: 10.1152/jn.00746.2006

Haridas C, Zehr EP, Misiaszek JE (2008) Adaptation of cutaneous stumble correction when tripping is part of the locomotor environment. J Neurophysiol 99:2789–97. doi: 10.1152/jn.00487.2007

de Kam D, Rijken H, Manintveld T, et al. (2013) Arm movements can increase leg muscle activity during submaximal recumbent stepping in neurologically intact individuals. J Appl Physiol 115:34–42. doi: 10.1152/japplphysiol.00510.2012

Kawashima N, Nozaki D, Abe MO, Nakazawa K (2008) Shaping appropriate locomotive motor output through interlimb neural pathway within spinal cord in humans. J Neurophysiol. doi: 10.1152/jn.00020.2008

Klimstra MD, Thomas E, Stoloff RH, et al. (2009) Neuromechanical considerations for incorporating rhythmic arm movement in the rehabilitation of walking. Chaos 19:026102. doi: 10.1063/1.3147404

Lam T, Pearson KG (2002) Sartorius muscle afferents influence the amplitude and timing of flexor activity in walking decerebrate cats. Exp Brain Res 147:175–185. doi: 10.1007/s00221-002-1236-0

Lamont E V, Zehr EP (2006) Task-specific modulation of cutaneous reflexes expressed at functionally relevant gait cycle phases during level and incline walking and stair climbing. Exp Brain Res 173:185–92. doi: 10.1007/s00221-006-0586-4

Mezzarane RA, Nakajima T, Zehr EP (2014) After stroke bidirectional modulation of soleus stretch reflex amplitude emerges during rhythmic arm cycling. Front Hum Neurosci 8:136. doi: 10.3389/fnhum.2014.00136

Miller S, Reitsma DJ, van der Meché FG (1973) Functional organization of long ascending propriospinal pathways linking lumbo-sacral and cervical segments in the cat. Brain Res 62:169–88.

Morris SL, Dodd KJ, Morris ME (2004) Outcomes of progressive resistance strength training following stroke: a systematic review. Clin Rehabil 18:27–39. doi: 10.1191/0269215504cr699oa

Moseley AM, Stark A, Cameron ID, Pollock A (2003) Treadmill Training and Body Weight Support for Walking after Stroke. Stroke 34:3006. doi: 10.1161/01.STR.0000102415.43108.66

Orsal D, Cabelguen JM, Perret C (1990) Interlimb coordination during fictive locomotion in the thalamic cat. Exp Brain Res 82:536–546. doi: 10.1007/BF00228795

Pinter MM, Brainin M (2012) Rehabilitation after stroke in older people. Maturitas 71:104–108. doi: 10.1016/j.maturitas.2011.11.011

Rossi-Durand C (2002) The influence of increased muscle spindle sensitivity on Achilles tendon jerk and H-reflex in relaxed human subjects. Somatosens Mot Res 19:286–295. doi: 10.1080/0899022021000037755

Ruff CR, Miller AB, Delva ML, et al. (2014) Modification of cutaneous reflexes during visually guided walking. J Neurophysiol 111:379–93. doi: 10.1152/jn.01076.2012

Schomburg ED, Petersen N, Barajon I, Hultborn H (1998) Flexor reflex afferents reset the step cycle during fictive locomotion in the cat. Exp Brain Res 122:339–350. doi: 10.1007/s002210050522

Thompson AK, Pomerantz FR, Wolpaw JR (2013) Operant conditioning of a spinal reflex can improve locomotion after spinal cord injury in humans. J Neurosci 33:2365–75. doi: 10.1523/JNEUROSCI.3968-12.2013

Visintin M, Barbeau H (1994) The effects of parallel bars, body weight support and speed on the modulation of the locomotor pattern of spastic paretic gait. A preliminary communication. Paraplegia 32:540–553.

Voerman GE, Gregoric M, Hermens HJ (2005) Neurophysiological methods for the assessment of spasticity: the Hoffmann reflex, the tendon reflex, and the stretch reflex. Disabil Rehabil 27:33–68. doi: 10.1080/09638280400014600

Wolpaw JR (2007) Spinal cord plasticity in acquisition and maintenance of motor skills. Acta Physiol. pp 155–169

Wolpaw JR (2010) What can the spinal cord teach us about learning and memory? Neuroscientist 16:532–549. doi: 10.1177/1073858410368314

Wolpaw JR, Tennissen AM (2001) Activity-dependent spinal cord plasticity in health and disease. Annu Rev Neurosci 24:807–843. doi: 10.1146/annurev.neuro.24.1.807

Zehr E, Barss T, Dragert K, et al. (2016) Neuromechanical interactions between the limbs during human locomotion: an evolutionary perspective with translation to rehabilitation. Exp Brain Res. doi: 10.1007/s00221-016-4715-4

Zehr EP (2005) Neural control of rhythmic human movement: the common core hypothesis. Exerc Sport Sci Rev 33:54–60.

Zehr EP (2006) Training-induced adaptive plasticity in human somatosensory reflex pathways. J Appl Physiol 101:1783–1794. doi: 10.1152/japplphysiol.00540.2006

Zehr EP, Balter JE, Ferris DP, et al. (2007) Neural regulation of rhythmic arm and leg movement is conserved across human locomotor tasks. J Physiol 582:209–27. doi: 10.1113/jphysiol.2007.133843

Zehr EP, Collins DF, Chua R (2001) Human interlimb reflexes evoked by electrical stimulation of cutaneous nerves innervating the hand and foot. Exp Brain Res 140:495–504. doi: 10.1007/s002210100857

Zehr EP, Duysens J (2004) Regulation of arm and leg movement during human locomotion.

Neuroscientist 10:347–61. doi: 10.1177/1073858404264680

Zehr EP, Fujita K, Stein RB (1998) Reflexes from the superficial peroneal nerve during walking in stroke subjects. J Neurophysiol 79:848–58.

Zehr EP, Hundza SR, Vasudevan E V (2009) The quadrupedal nature of human bipedal locomotion. Exerc Sport Sci Rev 37:102–8. doi: 10.1097/JES.0b013e31819c2ed6

Zehr EP, Komiyama T, Stein RB (1997) Cutaneous reflexes during human gait: electromyographic and kinematic responses to electrical stimulation. J Neurophysiol 77:3311–25.

Zehr EP, Loadman PM (2012) Persistence of locomotor-related interlimb reflex networks during walking after stroke. Clin Neurophysiol 123:796–807. doi: 10.1016/j.clinph.2011.07.049

Zehr EP, Loadman PM, Hundza SR (2012) Neural control of rhythmic arm cycling after stroke. J Neurophysiol 108:891–905. doi: 10.1152/jn.01152.2011