Exploiting evolutionarily conserved pathways to promote plasticity of human spinal circuits

by

Gregory EP Pearcey
Bachelor of Kinesiology (Honours), Memorial University of Newfoundland, 2012
Master of Science, Memorial University of Newfoundland, 2014

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

DOCTOR OF PHILOSOPHY

in the Division of Medical Sciences (Neuroscience)

University of Victoria

Supervisory Committee

Exploiting evolutionarily conserved pathways to promote plasticity of human spinal circuits

by

Gregory EP Pearcey

Bachelor of Kinesiology (Honours), Memorial University of Newfoundland, 2012
Master of Science (Exercise Physiology), Memorial University of Newfoundland, 2014

Supervisory Committee

Dr. E Paul Zehr, Supervisor
Division of Medical Sciences

Dr. Craig Brown, Departmental Member
Division of Medical Sciences

Dr. Olav Krigolson, Outside Member
School of Exercise Science, Physical and Health Education

Abstract

Supervisory Committee

Dr. E Paul Zehr, Supervisor
Division of Medical Sciences

Dr. Craig Brown, Departmental Member
Division of Medical Sciences

Dr. Olav Krigolson, Outside Member
School of Exercise Science, Physical and Health Education

Humans evolved from species that walked on all four limbs, which means that experiments in quadrupeds can guide and support experiments in humans. This is particularly helpful for neural rehabilitation because the central nervous system is plastic in nature, meaning that activities promoting central nervous system activity can alter subsequent output properties. This is known as neuroplasticity and can be measured as changes in spinal cord excitability through reflexes as a proxy. By targeting evolutionarily conserved pathways that act on similar interneurons within the spinal cord to either increase or decrease excitability, it may be possible to preferentially modulate spinal cord excitability based on a desirable outcome. For example, rhythmic movement reduces spinal cord excitability whereas brief sensory input to cutaneous afferents increases spinal cord excitability. Alterations in spinal cord excitability have been shown to outlast the activity duration, suggesting that neuroplasticity is not transient. This evidence suggests that both rhythmic movement and sensory input can induce acute neuroplasticity of spinal cord excitability. The overall purpose of this dissertation was two-fold; 1) to provide reviews of how evolutionarily conserved pathways are studied in humans and how they contribute to human rhythmic movement, and 2) experimentally examine how these conserved pathways, which converge onto similar interneuron circuitry, can be exploited to cause bidirectional changes in spinal cord excitability. Reviews indicate that humans have retained characteristics of quadrupedal locomotion and, in particular, activity of the arms affects the excitability of the legs, and vice versa. Cutaneous input is integrated throughout the body during locomotion, such that cutaneous sensations elicit neuromechanical responses that are nerve-specific and modulated according to the phase of movement. In experiment 1, there was increased spinal cord excitability following patterned stimulation of cutaneous afferents innervating the bottom of the foot. In experiment 2, stimulation to cutaneous afferents innervating both the top and bottom of the foot amplified voluntary plantar- and dorsiflexion. In experiment 3, cervicolumbar connections were exploited to amplify plasticity in spinal cord excitability induced by rhythmic movement. Finally, in experiment 4, there were interactions of rhythmic movement and fatigue, which both reduce spinal cord excitability, with cutaneous stimulation, which increases spinal cord excitability, such that reductions in spinal cord excitability associated with fatigue were mitigated by cutaneous stimulation. Taken together, these experiments suggest that cutaneous stimulation can increase spinal cord excitability, whereas quadrupedal locomotor activity can decrease spinal cord excitability. These conserved pathways can be exploited to intentionally modify spinal cord excitability in a bidirectional fashion, which provides fruitful information for the exploration of rehabilitation and sport performance practices.

Table of Contents

List of Tables

List of Figures

Acknowledgments

I must start by thanking my partner in life, Kirsten, for her unconditional love and support throughout this process. Following me from one end to the other of the second largest country in the world does not go unnoticed.

Secondly, I thank my sister, Laura, for always being a phone call away whenever I need her. Your support for me to pursue my passions has been and continues to be exceptional.

Third, I must acknowledge the incredible inspiration and support of my advisor, Paul, during my doctoral training. The countless hours of ‘rants’ provided me with numerous life lessons and unique learning experiences that I could never have imagined would be included in my doctoral degree. I will be forever grateful for the financial support to live and to attend prestigious conferences, the encouragement to travel and to maintain a personal life and overall exceptional mentorship received throughout my degree.

Thanks to Trevor for allowing me to share ideas over a beer whether relating to research or not. To Taryn for supporting my quest to understand the complexities of Matlab and LabVIEW. To Yao for showing me the culinary diversity that this world has to offer. To Chelsea, Steve, Hilary, Steph, Henry, Andrew, Bruno, Aimee, Hajer and Ben for being the greatest lab mates I could ever ask for.

To my Australian colleagues, Tim and Eva, I am very thankful that you took me into your lab and helped me learn new tools for my scientific toolbox in addition to helping me experience the Australian way of life. I promise I will repay the favor if you ever find yourself in Newfoundland.

To the entire Neuroscience Graduate Program faculty, thank-you for helping me learn about the many facets of neuroscience. A special thanks to both Craig and Olav for agreeing to be on my committee and giving up some of your precious time to help me through this process.

Finally, I must acknowledge the financial support I received from the Natural Sciences and Engineering Research Council of Canada, the International Collaboration on Repair Discoveries (ICORD), Endeavour Research Fellowships and the University of Victoria.

Dedication

I would like to dedicate this dissertation to both my late mother, Kerry Lorraine (Butt) Pearcey, who showed me that care and compassion have no limits, and my father, Edward Patrick Glenn Pearcey, who’s inspiration, drive and support has made me grow tremendously as a person. I must also add that his endless curiosity has rubbed off on me to promote the inquisitive scientific thinking I possess today.

Everyone you will ever meet knows something you don't

--Bill Nye

Chapter 1 - General Introduction

A fundamental feature of being an animal is the ability to move around and interact with the surroundings. Neural circuits play a crucial role in the production and regulation of locomotor behaviors, which allows an animal to move about. Animals use sensations to guide their movements and achieve the goals of their movement, whether those goals are to get from one place to another, move something, or search for food. Humans are animals too, but the similarities between neural circuitry in humans and other animals has frequently been debated. For the advancement of our society, it is important for us to place humans in evolution, and realize that we do have many similarities with other animals. Establishing neural circuits that have been conserved across species allows us to put animal and human physiological findings into context for guiding rehabilitation and sport performance practices.

Most of what is known about the neural control of human locomotion has been derived from inferences based on reduced animal preparations. Less invasive techniques are then used in humans to support the similarities or differences that exist in humans. Striking similarities in neural circuits exist between humans and other animals and these evolutionarily conserved pathways provide a unique window for inducing plasticity that have only recently been explored.

Neuroplasticity has certainly been conserved across species. Mechanisms of neuroplasticity have been predominantly studied in the brain, but have also been studied in the animal and human spinal cords (Leukel et al., 2012; Pockett and Figurov, 1993; Wolpaw and O'Keefe, 1984). One way of examining neuroplasticity in humans is measuring the excitability of the spinal cord with reflexes as a proxy. One such reflex, the muscle afferent reflex, can be evoked with rapid mechanical stretch or by applying transcutaneous stimulation to a mixed peripheral nerve (i.e. containing both sensory and motor axons), which will elicit the tendon and Hoffmann (H-) reflex, respectively (Knikou, 2008; Misiaszek, 2003; Pierrot-Deseilligny and Mazevet, 2000; Voerman et al., 2005; Zehr, 2002). These reflexes have a strong monosynaptic component arising from group Ia primary afferents onto alpha motoneurons providing an indication of motoneuron excitability, but are also subject to modulation from premotoneuronal processes (Knikou, 2008; Misiaszek, 2003; Pierrot-Deseilligny and Mazevet, 2000; Voerman et al., 2005; Zehr, 2002). Levels of pre-synaptic inhibition (PSI) can be modified via axo-axonal GABA-ergic synapses from inhibitory (Ia/Ib) interneurons (Rudomin, 1990), and levels of neurotransmitter release from Ia afferents can be modified in response to high frequency activation of the Ia afferents, known as post-activation depression or homosynaptic depression (Crone and Nielsen, 1989) (see figure 1-1 for a graphical summary of the Ia reflex arc and mechanisms that produce amplitude modulation). Descending commands, spinal locomotor networks and afferent feedback can all contribute to levels of PSI and dictate the level of spinal cord excitability.

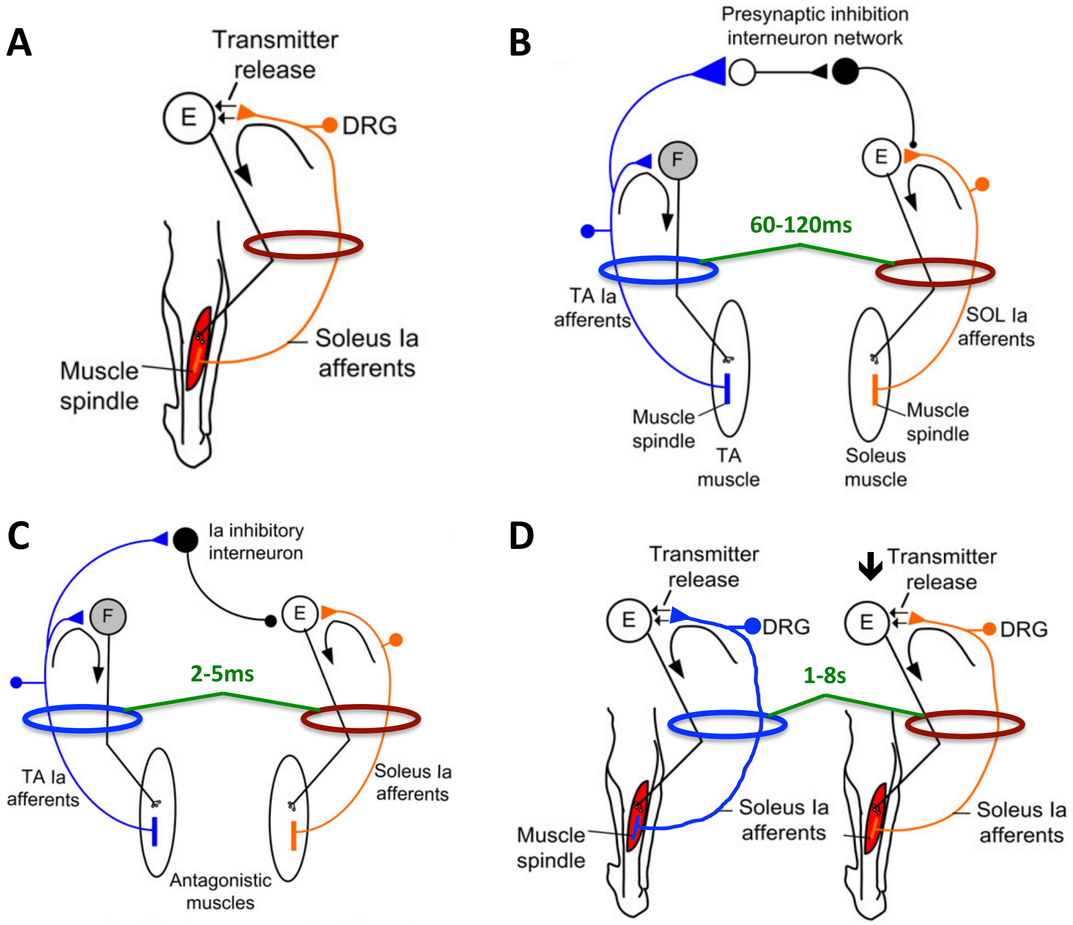

Figure 1-1: *Mechanisms responsible for H-reflex amplitude modulation. (A) The "simple" H-reflex pathway. Stimulation of the posterior tibial nerve at the popliteal fossa below motor threshold results in excitation of Ia afferents that largely induce monosynaptic*

excitation of homonymous motoneurons. (B) Presynaptic inhibition of Ia afferents. A conditioning afferent volley via common peroneal (CP) nerve stimulation at low intensities is delivered before (60-100ms) posterior tibial nerve stimulation to establish based on the amplitude of the conditioned soleus H-reflex the amount of presynaptic inhibition acting on soleus Ia afferent terminals. (C) Reciprocal Ia inhibition. This outlined spinal circuit designates the pathway of reciprocal inhibition exerted from ankle flexors following common peroneal (CP) nerve stimulation onto the soleus H-reflex. CP stimulation is applied at the TA motor threshold before (2-4ms) tibial nerve stimulation. Reciprocal inhibition involves the Ia inhibitory interneuron and is exerted at a postsynaptic level. (D) Homosynaptic (post-activation) depression. Repeated stimulation of the tibial nerve (innervating the soleus) causes a reduction of neurotransmitter to be released from the Ia afferent terminals, and therefore a reduction in test H-reflex amplitude. Red rings indicate test (tibial) nerve stimulation to evoke the soleus H-reflex and blue rings indicate conditioning stimuli. Blue lines indicate conditioning afferent volleys whereas orange lines indicate test afferent volleys of the soleus Ia afferents. Intervals between condition and test stimuli are indicated in green (modified from Knikou 2008).

Pathological spinal cord plasticity can cause neurological dysfunction. For example spasticity, which is one of the most common complications of individuals with stroke, MS, or SCI, involves a number of complex processes in the spinal cord and muscles (Dietz et al., 1986; Kurian et al., 2011; Nielsen et al., 2007). It is generally accepted that reductions in descending commands lead to hyperexcitability of the Ia reflex pathway (Levin and Hui-Chan, 1993), and this hyperexcitability is due to a reduction in pre-synaptic inhibitory mechanisms (Ashby and Verrier, 1976; Burke, 1988). Examining methods that can reduce spinal cord excitability through increases in PSI may provide guidance for the development and refinement of therapies for individuals with spasticity.

Although receiving little attention in clinical research, fatigue is also a common and debilitating consequence of neurological impairments such as multiple sclerosis (MS) (Chalah et al., 2015; Hameau et al., 2017; Tur, 2016), stroke (Knorr et al., 2012), spinal cord injury (SCI) (Papaiordanidou et al., 2014), and cerebral palsy (CP) (Neyroud et al., 2017). Stroke survivors characterize fatigue as a tremendous sense of tiredness, feeling of exhaustion, and lack of physical and mental energy that impede activities of daily living (De Groot et al., 2003). In fact, 46% of stroke patients rate fatigue as their most debilitating symptom (Michael et al., 2006). Neuromuscular fatigue of central origin almost certainly contributes to self-reported fatigue post-stroke (Knorr et al., 2012), in patients with MS (Hameau et al., 2017) and SCI (Papaiordanidou et al., 2014), which often interferes with the rehabilitation process. Therefore, mitigating neuromuscular fatigue represents a potential therapeutic target for reducing self-reported fatigue in the abovementioned neurologically impaired patients. Currently, though mitigating neuromuscular fatigue in a neurologically intact, let alone neurologically impaired, population is currently not well understood.

In both of the aforementioned situations (i.e. spasticity and fatigue), spinal cord excitability is altered from resting levels. For example, spasticity is accompanied by hyperexcitability, whereas fatigue is accompanied by hypoexcitability. Counteracting these alterations in spinal cord excitability would therefore represent a method of providing individualized therapeutic benefits for various individuals after neurological impairment (Thompson and Wolpaw, 2015).

Wolpaw and colleagues (Chen et al., 2002, 2014a, 2014b; X. Y. Chen et al., 2006a, 2006b; Y. Chen et al., 2006; Thompson et al., 2013a, 2013b; Wang et al., 2009) have done great work to highlight the plastic capacity of the spinal cord. Specifically, they have shown that operant conditioning in both rats and humans can both increase and decrease H-reflex amplitude. They have determined that down-conditioning acts by increasing Ia reciprocal inhibition, and can have beneficial effects on locomotion in spinal cord injured humans by decreasing spastic activity of extensor muscles (Manella et al., 2013; Thompson et al., 2013b), whereas up-conditioning improves locomotor muscle activity in extensor muscles which also improves walking in spinal cord injured rats (Chen et al., 2014a, 2014b). Locomotor training itself has also been shown to induce plastic changes of spinal reflex circuits in humans with SCI (Knikou et al., 2015; Knikou and Mummidisetty, 2014). Most recently, arm cycling and arm and leg (A&L) cycling has been shown to induce plastic changes of spinal reflex circuits in participants with chronic stroke (Kaupp et al., 2018; Klarner et al., 2016a, 2016b) and spinal cord injury (Zhou et al., 2018). Unfortunately, current rehabilitation practices lack the ability to maximize plastic changes in the spinal circuitry (Raineteau and Schwab, 2001; Thompson et al., 2009, 2006). Therefore, providing mechanistic insights to improve strategies to further enhance plastic changes of human spinal circuitry and improve limb function are warranted.

Acute plasticity of spinal cord excitability can arise from movement and/or sensory stimulation. In the control of rhythmic movement, interactions between descending commands, sensory feedback and pattern generating networks ultimately dictate the motor output through the final common path (i.e. motoneurons). Since many pathologies reduce descending commands but often do not directly affect afferent feedback and pattern generating networks, increasing the activity of these interactions may provide the greatest potential for rehabilitation application. Thus, a likely candidate for inducing spinal cord plasticity are the interneuronal networks involved in the regulation of spinal reflex pathways during locomotion. Indeed, providing various sensory inputs (i.e. mechanical brushing, pressure on the foot sole, mechanical vibration, and electrical stimulation) to the limbs presents a feasible modality to elicit lasting changes in spinal cord excitability as well (Fujiwara et al., 2011; Levin and Hui-Chan, 1992; Perez et al., 2003; Winkler et al., 2010; Yamaguchi et al., 2016). These plastic changes in spinal cord excitability are likely due to activation of evolutionarily conserved pathways that were maintained through human evolution from quadrupedal to bipedal locomotion via spinal interneurons involved in the control of rhythmic movement.

Previous work in our lab has demonstrated that arm cycling can have suppressive effects on resting soleus (SOL) H-reflex amplitudes (i.e. a proxy of spinal cord excitability) that outlast exercise duration (Javan and Zehr, 2008). Similarly, other labs have identified that passive stepping (Nakajima et al., 2016), leg cycling (Motl and Dishman, 2003) and skilful leg cycling (Mazzocchio et al., 2006; Meunier et al., 2007) cause plastic changes in spinal cord excitability that outlast the activity duration. Short-term spinal cord plasticity induced by rhythmic movement has been hypothesized to result from changes in Ia PSI, which is likely due to increased activity of Ia inhibitory interneurons (Frigon et al., 2004). Acute stimulation to cutaneous afferents also acts on Ia inhibitory interneurons, but rather inhibits Ia inhibitory interneuron activity and thus decreases Ia PSI. However, a lack of understanding parameters of movement and sensory stimulation that specifically modulate spinal cord plasticity has left a void in recommendations for translation to rehabilitation settings.

In the chapters 2 and 3 of this dissertation, the goal is to provide reviews of 1) the study of locomotor circuits in humans, paying special attention to the similarities that we share with our quadrupedal cousins (i.e. cats), and 2) the integration of exteroceptive information through cutaneous pathways during human locomotion, again relating this to the reduced animal preparations of the cat. These reviews will lay the foundations for exploiting evolutionarily conserved pathways involved in human locomotion to promote plasticity in spinal circuits.

The purpose of experiments that follow is to determine whether exploiting two evolutionarily conserved pathways (i.e. locomotor circuits and cutaneous feedback pathways) can influence spinal cord excitability in a bi-directional manner. More specifically, experiment 1) will examine if cutaneous stimulation can alter spinal cord excitability (chapter 4), 2) will determine whether sensory enhancement can amplify force output (chapter 5), 3) will determine if exploiting cervicolumbar connections can amplify plasticity of spinal cord excitability induced by rhythmic movement (chapter 6), and 4) will explore if sensory enhancement can mitigate reductions in spinal cord excitability and performance associated with fatigue (chapter 7).

Based on experiments examining acute effects of cutaneous stimulation on spinal cord excitability, it is hypothesized that patterned stimulation to cutaneous afferents will increase spinal cord excitability, and this increased excitability will be attributable to reductions in Ia PSI (chapter 4). Since acute cutaneous stimulation causes widespread increases in reflex excitability throughout the body, it is hypothesized that sensory enhancement will amplify voluntary force output (chapter 5). Since exploiting cervicolumbar connections causes amplification of cutaneous reflex amplitudes, it is hypothesized that cycling with the arms and legs together will augment short term spinal cord plasticity when compared to using just the arms or just the legs (chapter 6). Finally, it is hypothesized that the increased spinal cord excitability resulting from cutaneous stimulation will mitigate the reductions in spinal cord excitability and cycling performance associated with fatigue (chapter 7). For a general overview of anatomical terms that will be frequently referred to, please see figure 1-2.

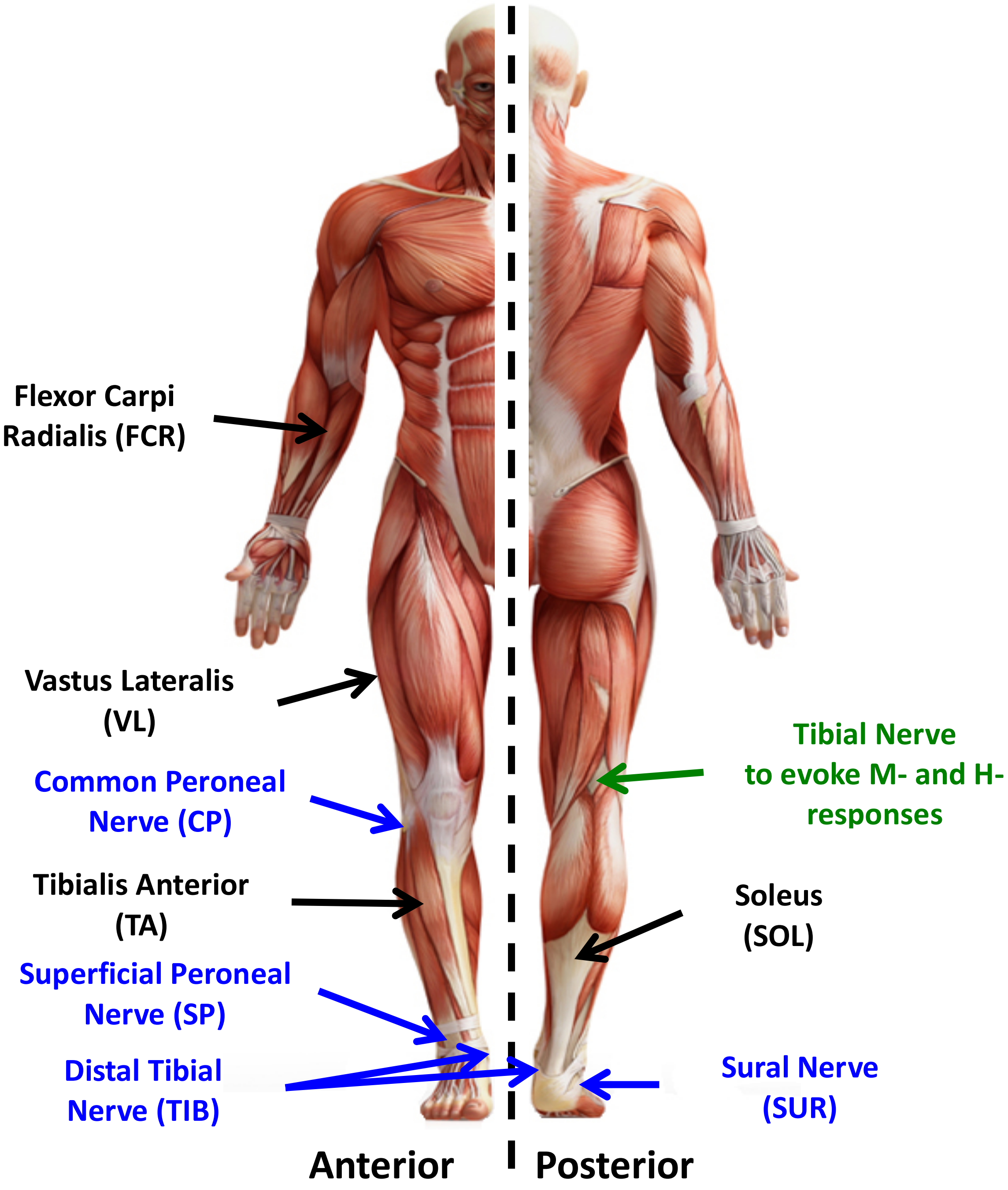

Figure 1-2: *Anterior (left) and Posterior (right) views of a human body. Muscle activity in the experiments within this dissertation will be from muscles labeled in black font, and arrows, sensory nerves stimulated to induce short-term spinal cord plasticity, provide sensory enhancement or to condition soleus H-reflexes are labeled with blue font and*

arrow, and tibial nerve stimulation to elicit H-reflex and M-waves is labeled with green font and arrows.

Conclusion

This dissertation is a step towards understanding how exploiting evolutionarily conserved pathways that contribute to locomotor control can influence spinal cord excitability. The results obtained from this work will help support and guide strategies used for targeted rehabilitation after neurological impairment. Given the void in recommendations to counteract maladaptive plasticity, promoting directional plasticity will allow rehabilitation to be tailored to the specific needs of a person with neurological impairment. Ultimately, considering the potential for bidirectional plasticity in spinal cord circuits will allow methods to both increase and decrease excitability as required.

References

Ashby, P., Verrier, M., 1976. Neurophysiologic changes in hemiplegia. Possible explanation for the initial disparity between muscle tone and tendon reflexes. Neurology 26, 1145–51.

Burke, D., 1988. Spasticity as an adaptation to pyramidal tract injury. Adv Neurol 47, 401–23.

Chalah, M.A., Riachi, N., Ahdab, R., Creange, A., Lefaucheur, J.P., Ayache, S.S., 2015. Fatigue in Multiple Sclerosis: Neural Correlates and the Role of Non-Invasive Brain Stimulation. Front Cell Neurosci 9, 460. https://doi.org/10.3389/fncel.2015.00460

Chen, X.Y., Carp, J.S., Chen, L., Wolpaw, J.R., 2006a. Sensorimotor cortex ablation prevents H-reflex up-conditioning and causes a paradoxical response to down-conditioning in rats. J Neurophysiol 96, 119–27. https://doi.org/10.1152/jn.01271.2005

Chen, X.Y., Carp, J.S., Chen, L., Wolpaw, J.R., 2002. Corticospinal tract transection prevents operantly conditioned H-reflex increase in rats. Exp Brain Res 144, 88–94. https://doi.org/10.1007/s00221-002-1026-8

Chen, X.Y., Chen, Y., Chen, L., Tennissen, A.M., Wolpaw, J.R., 2006b. Corticospinal tract transection permanently abolishes H-reflex down-conditioning in rats. J Neurotrauma 23, 1705–12. https://doi.org/10.1089/neu.2006.23.1705

Chen, Y., Chen, L., Liu, R., Wang, Y., Chen, X.Y., Wolpaw, J.R., 2014a. Locomotor impact of beneficial or nonbeneficial H-reflex conditioning after spinal cord injury. J Neurophysiol 111, 1249–58. https://doi.org/10.1152/jn.00756.2013

Chen, Y., Chen, L., Wang, Y., Wolpaw, J.R., Chen, X.Y., 2014b. Persistent beneficial impact of H-reflex conditioning in spinal cord-injured rats. J Neurophysiol 112, 2374–81. https://doi.org/10.1152/jn.00422.2014

Chen, Y., Chen, X.Y., Jakeman, L.B., Chen, L., Stokes, B.T., Wolpaw, J.R., 2006. Operant conditioning of H-reflex can correct a locomotor abnormality after spinal cord injury in rats. J Neurosci 26, 12537–43. https://doi.org/10.1523/JNEUROSCI.2198-06.2006

Crone, C., Nielsen, J., 1989. Methodological implications of the post activation depression of the soleus H-reflex in man. Exp Brain Res 78, 28–32.

De Groot, M.H., Phillips, S.J., Eskes, G.A., 2003. Fatigue associated with stroke and other neurologic conditions: Implications for stroke rehabilitation. Arch Phys Med Rehabil 84, 1714–20.

Dietz, V., Ketelsen, U.P., Berger, W., Quintern, J., 1986. Motor unit involvement in spastic paresis. Relationship between leg muscle activation and histochemistry. J Neurol Sci 75, 89–103.

Frigon, A., Collins, D.F., Zehr, E.P., 2004. Effect of rhythmic arm movement on reflexes in the legs: modulation of soleus H-reflexes and somatosensory conditioning. Journal of neurophysiology 91, 1516–23. https://doi.org/10.1152/jn.00695.2003

Fujiwara, T., Tsuji, T., Honaga, K., Hase, K., Ushiba, J., Liu, M., 2011. Transcranial direct current stimulation modulates the spinal plasticity induced with patterned electrical stimulation. Clin Neurophysiol 122, 1834–7. https://doi.org/10.1016/j.clinph.2011.02.002

Hameau, S., Zory, R., Latrille, C., Roche, N., Bensmail, D., 2017. Relationship between neuromuscular and perceived fatigue and locomotor performance in patients with multiple sclerosis. Eur J Phys Rehabil Med 53, 833–840. https://doi.org/10.23736/S1973-9087.16.04134-4

Javan, B., Zehr, E.P., 2008. Short-term plasticity of spinal reflex excitability induced by rhythmic arm movement. J Neurophysiol 99, 2000–5. https://doi.org/10.1152/jn.01315.2007

Kaupp, C., Pearcey, G.E.P., Klarner, T., Sun, Y., Cullen, H., Barss, T.S., Zehr, E.P., 2018. Rhythmic arm cycling training improves walking and neurophysiological integrity in chronic stroke: the arms can give legs a helping hand in rehabilitation. J Neurophysiol 119, 1095–1112. https://doi.org/10.1152/jn.00570.2017

Klarner, T., Barss, T.S., Sun, Y., Kaupp, C., Loadman, P.M., Zehr, E.P., 2016a. Exploiting Interlimb Arm and Leg Connections for Walking Rehabilitation: A Training Intervention in Stroke. Neural Plast 2016, 1517968. https://doi.org/10.1155/2016/1517968

Klarner, T., Barss, T.S., Sun, Y., Kaupp, C., Loadman, P.M., Zehr, E.P., 2016b. Long-Term Plasticity in Reflex Excitability Induced by Five Weeks of Arm and Leg Cycling Training after Stroke. Brain Sci 6. https://doi.org/10.3390/brainsci6040054

Knikou, M., 2008. The H-reflex as a probe: pathways and pitfalls. J Neurosci Methods 171, 1–12. https://doi.org/10.1016/j.jneumeth.2008.02.012

Knikou, M., Mummidisetty, C.K., 2014. Locomotor training improves premotoneuronal control after chronic spinal cord injury. J Neurophysiol 111, 2264–75. https://doi.org/10.1152/jn.00871.2013

Knikou, M., Smith, A.C., Mummidisetty, C.K., 2015. Locomotor training improves reciprocal and nonreciprocal inhibitory control of soleus motoneurons in human spinal cord injury. J Neurophysiol 113, 2447–60. https://doi.org/10.1152/jn.00872.2014

Knorr, S., Rice, C.L., Garland, S.J., 2012. Perspective on neuromuscular factors in poststroke fatigue. Disabil Rehabil 34, 2291–9. https://doi.org/10.3109/09638288.2012.683233

Kurian, M., Crook, S.M., Jung, R., 2011. Motoneuron model of self-sustained firing after spinal cord injury. J Comput Neurosci 31, 625–45. https://doi.org/10.1007/s10827-011-0324-1

Leukel, C., Taube, W., Beck, S., Schubert, M., 2012. Pathway-specific plasticity in the human spinal cord. Eur J Neurosci 35, 1622–9. https://doi.org/10.1111/j.1460-9568.2012.08067.x

Levin, M.F., Hui-Chan, C., 1993. Are H and stretch reflexes in hemiparesis reproducible and correlated with spasticity? J Neurol 240, 63–71.

Levin, M.F., Hui-Chan, C.W., 1992. Relief of hemiparetic spasticity by TENS is associated with improvement in reflex and voluntary motor functions. Electroencephalogr Clin Neurophysiol 85, 131–42.

Manella, K.J., Roach, K.E., Field-Fote, E.C., 2013. Operant conditioning to increase ankle control or decrease reflex excitability improves reflex modulation and walking function in chronic spinal cord injury. J. Neurophysiol. 109, 2666–2679. https://doi.org/10.1152/jn.01039.2011

Mazzocchio, R., Kitago, T., Liuzzi, G., Wolpaw, J.R., Cohen, L.G., 2006. Plastic changes in the human H-reflex pathway at rest following skillful cycling training. Clin Neurophysiol 117, 1682–91. https://doi.org/10.1016/j.clinph.2006.04.019

Meunier, S., Kwon, J., Russmann, H., Ravindran, S., Mazzocchio, R., Cohen, L., 2007. Spinal use-dependent plasticity of synaptic transmission in humans after a single cycling session. J Physiol 579, 375–88. https://doi.org/10.1113/jphysiol.2006.122911

Michael, K.M., Allen, J.K., Macko, R.F., 2006. Fatigue after stroke: relationship to mobility, fitness, ambulatory activity, social support, and falls efficacy. Rehabil Nurs 31, 210–7.

Misiaszek, J.E., 2003. The H-reflex as a tool in neurophysiology: its limitations and uses in understanding nervous system function. Muscle Nerve 28, 144–60. https://doi.org/10.1002/mus.10372

Motl, R.W., Dishman, R.K., 2003. Acute leg-cycling exercise attenuates the H-reflex recorded in soleus but not flexor carpi radialis. Muscle Nerve 28, 609–14. https://doi.org/10.1002/mus.10479

Nakajima, T., Kamibayashi, K., Kitamura, T., Komiyama, T., Zehr, E.P., Nakazawa, K., 2016. Short-Term Plasticity in a Monosynaptic Reflex Pathway to Forearm Muscles after Continuous Robot-Assisted Passive Stepping. Front Hum Neurosci 10, 368. https://doi.org/10.3389/fnhum.2016.00368

Neyroud, D., Armand, S., De Coulon, G., Sarah, R.D.D.S., Maffiuletti, N.A., Kayser, B., Place, N., 2017. Plantar flexor muscle weakness and fatigue in spastic cerebral palsy patients. Res Dev Disabil 61, 66–76. https://doi.org/10.1016/j.ridd.2016.12.015

Nielsen, J.B., Crone, C., Hultborn, H., 2007. The spinal pathophysiology of spasticity--from a basic science point of view. Acta Physiol (Oxf) 189, 171–80. https://doi.org/10.1111/j.1748-1716.2006.01652.x

Papaiordanidou, M., Varray, A., Fattal, C., Guiraud, D., 2014. Neural and muscular mechanisms of electrically induced fatigue in patients with spinal cord injury. Spinal Cord 52, 246–50. https://doi.org/10.1038/sc.2013.172

Perez, M.A., Field-Fote, E.C., Floeter, M.K., 2003. Patterned sensory stimulation induces plasticity in reciprocal ia inhibition in humans. J Neurosci 23, 2014–8.

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

Pockett, S., Figurov, A., 1993. Long-term potentiation and depression in the ventral horn of rat spinal cord in vitro. Neuroreport 4, 97–9.

Raineteau, O., Schwab, M.E., 2001. Plasticity of motor systems after incomplete spinal cord injury. Nat Rev Neurosci 2, 263–73. https://doi.org/10.1038/35067570

Rudomin, P., 1990. Presynaptic inhibition of muscle spindle and tendon organ afferents in the mammalian spinal cord. Trends Neurosci 13, 499–505.

Thompson, A.K., Chen, X.Y., Wolpaw, J.R., 2013a. Soleus H-reflex operant conditioning changes the H-reflex recruitment curve. Muscle Nerve 47, 539–44. https://doi.org/10.1002/mus.23620

Thompson, A.K., Doran, B., Stein, R.B., 2006. Short-term effects of functional electrical stimulation on spinal excitatory and inhibitory reflexes in ankle extensor and flexor muscles. Exp Brain Res 170, 216–26. https://doi.org/10.1007/s00221-005-0203-y

Thompson, A.K., Estabrooks, K.L., Chong, S., Stein, R.B., 2009. Spinal reflexes in ankle flexor and extensor muscles after chronic central nervous system lesions and functional electrical stimulation. Neurorehabil Neural Repair 23, 133–42. https://doi.org/10.1177/1545968308321067

Thompson, A.K., Pomerantz, F.R., Wolpaw, J.R., 2013b. Operant conditioning of a spinal reflex can improve locomotion after spinal cord injury in humans. J Neurosci 33, 2365–75. https://doi.org/10.1523/JNEUROSCI.3968-12.2013

Thompson, A.K., Wolpaw, J.R., 2015. Targeted neuroplasticity for rehabilitation. Prog Brain Res 218, 157–72. https://doi.org/10.1016/bs.pbr.2015.02.002

Tur, C., 2016. Fatigue Management in Multiple Sclerosis. Curr Treat Options Neurol 18, 26. https://doi.org/10.1007/s11940-016-0411-8

Voerman, G.E., Gregoric, M., Hermens, H.J., 2005. Neurophysiological methods for the assessment of spasticity: the Hoffmann reflex, the tendon reflex, and the stretch reflex. Disabil Rehabil 27, 33–68.

Wang, Y., Pillai, S., Wolpaw, J.R., Chen, X.Y., 2009. H-reflex down-conditioning greatly increases the number of identifiable GABAergic interneurons in rat ventral horn. Neurosci Lett 452, 124–9. https://doi.org/10.1016/j.neulet.2009.01.054

Winkler, T., Hering, P., Straube, A., 2010. Spinal DC stimulation in humans modulates post-activation depression of the H-reflex depending on current polarity. Clin Neurophysiol 121, 957–61. https://doi.org/10.1016/j.clinph.2010.01.014

Wolpaw, J.R., O’Keefe, J.A., 1984. Adaptive plasticity in the primate spinal stretch reflex: evidence for a two-phase process. J Neurosci 4, 2718–24.

Yamaguchi, T., Fujiwara, T., Tsai, Y.A., Tang, S.C., Kawakami, M., Mizuno, K., Kodama, M., Masakado, Y., Liu, M., 2016. The effects of anodal transcranial direct current stimulation and patterned electrical stimulation on spinal inhibitory interneurons and motor function in patients with spinal cord injury. Exp Brain Res 234, 1469–78. https://doi.org/10.1007/s00221-016-4561-4

Zehr, E.P., 2002. Considerations for use of the Hoffmann reflex in exercise studies. Eur J Appl Physiol 86, 455–68. https://doi.org/10.1007/s00421-002-0577-5

Zhou, R., Alvarado, L., Ogilvie, R., Chong, S.L., Shaw, O., Mushahwar, V.K., 2018. Non-gait-specific intervention for the rehabilitation of walking after SCI: role of the arms. J Neurophysiol 119, 2194–2211. https://doi.org/10.1152/jn.00569.2017

Chapter 2 – What lies beneath the brain: studying neural circuits involved in human locomotion

Abstract

A key feature of studies utilizing humans to understand neural circuitry involved in locomotor behavior is the innate dependence on inferences. In this chapter, we provide a brief history of the inferences that have been drawn from other animals and how advances in technology have progressed our understanding of the neural control of locomotion in humans.

Pearcey G.E. and Zehr E.P. (2019). What lies beneath the brain: studying neural circuits involved in human locomotion. Will be published as chapter in: Neural Control of Movement: Model Systems for Examining Motor Function. Edited by Sharples SA and Whelan P.

Introduction

"*I should be writing a third paper on the Nerves, but I cannot proceed without making some experiments, which are so unpleasant to make that I defer them. You may think me silly, but I cannot perfectly convince myself that I am authorized in nature, or religion, to do these cruelties—for what?—for anything else less than a little egotism or self-aggrandizement…"* ***Letter to his brother on 1 July 1822; in Letters of Sir Charles Bell, K.H., F.R.S.L. & E. Selected from his Correspondence with his Brother, George Joseph Bell.*** (1870)

The challenges associated with understanding human circuits for locomotion have endured many centuries of intellectual and technological advancement, yet empirical evidence for these circuits is still not available. This is due to the invasive, damaging, and unethical procedures that would be required to directly examine the underlying neuroanatomical structures and their functions involved in the control of locomotion. Instead, we must rely on assumptions and inferences relating human locomotor circuitry to that of other animals. This approach is quite fruitful because after all, humans are animals too.

Controversy suggesting that human neural architecture differs from that of other animals was probably inspired by the natural theological beliefs of Sir Charles Bell (1774-1842), whose work in the late 18th and early 19th centuries was influential and notable. For instance, he was involved in the discoveries that showed functional differences between the ventral and dorsal roots of the spinal cord (Cranefield, 1974). Scientific and natural theological beliefs coexisted in Bell's time and his inadequacies and misdirection resided in his agreements with those before him, such as Johann Kasper Lavater (1741-1801), who believed that "of all terrestrial beings man is the most perfect, the most replete with life" (Lavater, 1804). Bell believed that humans were the work of a divine creator, and argued that facial muscles that are present in humans, but absent in other animals, were designed to expand our range of expression (Hughes and Gardner-Thorpe, 2018). An incredibly notable student of Bell's would later reject his theological framework. This student's name was Charles Darwin (1809-1882), and he went on to conceptualize Natural Selection with the publication of “On the Origin of Species” in 1859 (Darwin, 1859). Darwin strongly asserted that non-human animals, too, could show facial expressions of emotion (Darwin, 1872).

Since the time of Bell and Darwin, many advances in science have revealed striking similarities between humans and other animals. Even with the simple example of facial expression mentioned above, Langford and colleagues (2010) were able to code facial expressions of pain in the mouse with high accuracy and reliability. Higher cortical function required for self-recognition in a mirror, once thought only to exist in primates, has also been shown in the bottlenose dolphin, suggesting that these neural processes are not specific to primates but, rather can be attributed to high degrees of encephalization (Reiss and Marino, 2001).

So what exactly differentiates the neural control of human locomotion from other animals? The proposed theories to answer this question tend to have many shortcomings, yet the existence of spinal central pattern generators (CPGs) in humans is often questioned. We suggest the argument is typically addressed backwards. That is, seeking evidence to support the existence of human locomotor CPGs. Instead, from an evolutionary perspective we should more appropriately search for evidence that refutes spinal CPGs in humans. Indeed, the plethora of evidence to support the existence of CPGs in humans far outweighs the evidence refuting their existence. In the following, we summarize the use of inferences guided by experiments in non-human animals that have been used to further our understanding of the neural control of human locomotion.

Neural control of locomotion in non-human animals

It is beyond the scope here to provide a comprehensive review of locomotor circuits in non-human animals, for that please refer to (Burke, 2001; Duysens and Van de Crommert, 1998; Frigon, 2012; Grillner, 2011a; Grillner and Wallén, 1985; Hultborn and Nielsen, 2007), rather we reiterate some characteristics of non-human locomotor control that have motivated experiments in humans. More specifically, we identify that a complex interplay between spinal CPGs, somatosensory feedback and supraspinal commands (i.e. both cortical and sub-cortical structures) underlies the innate ability for non-human animals to functionally locomote throughout diverse environments.

Seminal work by Sherrington (Sherrington, 1906) showed that cats and dogs with complete cervical transections could produce basic rhythmic stepping patterns in response to electrical and mechanical stimulation. He noted that these movements were adjustable by peripheral feedback and suggested that locomotor-like movements were driven from peripheral afferent activity, an idea largely influenced by Sechenov's reflex chain hypothesis (Clower, 1998). Sherrington's student, Thomas Graham Brown, further explored the production of locomotor-like movements in the absence of descending drive by using a thoracic transection in the cat, except he also transected the afferents of the hindlimb (Brown, 1911). Using this model, Brown concluded that alternating bursts of flexor and extensor muscle activity could be produced in the absence of both supraspinal input and peripheral afferent feedback. These findings would contribute to the "half-centre" model (Brown, 1911) which paved the way for thinking that the spinal cord could intrinsically produce rhythmic limb movement (Brown, 1914). The properties and characteristics of spinal CPGs in mammals have been extensively reviewed elsewhere (for examples, see (Burke, 2001; Duysens and Van de Crommert, 1998; Frigon, 2012; Grillner, 2011a; Grillner and Wallén, 1985; Hultborn and Nielsen, 2007)), but some important characteristics relevant to the study of human locomotion should be pointed out. Spinal CPGs are capable of producing and maintaining rhythmic motor output in isolation, modify sensory feedback based on the task or timing within the task to ensure output meets the environmental demands, are distributed throughout the spinal cord and interconnected through commissural and propriospinal pathways, and contribute common interneuronal circuitry to the production of multiple rhythmic activities (Klarner and Zehr, 2018; Zehr, 2005).

While the capacity for spinally mediated rhythmic movement in isolation has been well-established (Burke, 2001; Duysens and Van de Crommert, 1998; Frigon, 2012; Grillner, 2011a; Grillner and Wallén, 1985; Hultborn and Nielsen, 2007), contributions of afferent feedback and supraspinal commands to functional locomotor behavior are critical. Sensory feedback plays a critical regulatory role during stepping that assists with positioning of the feet, responding to obstacles or perturbations, altering ongoing muscle activity, and initiating phase transitions at discrete phases of the step cycle (Rossignol et al., 2006). This is particularly revealed in challenging scenarios. For example, if cutaneous afferents supplying the paw are transected, cats cannot walk on a horizontal ladder and increase time spent in double support (Bouyer and Rossignol, 2003). In response to unexpected contact to the dorsum of the paw during swing, cats exhibit a stumbling corrective reaction, which is characterized by increased hip, knee and ankle flexion (Forssberg, 1979). Such reactions to cutaneous input enable obstacle avoidance during locomotion. Stimulation to cutaneous afferents innervating the plantar surface of the foot during swing prolongs the phase (Duysens and Pearson, 1976) whereas, during stance stimulation of the plantar surface of the paw (Duysens and Pearson, 1976; Guertin et al., 1995) or loading of the extensor muscles of the hindlimbs (Duysens and Pearson, 1980; Fouad and Pearson, 1997; Pearson and Collins, 1993) enhances ankle extensor muscle activity. Phase transitions are mediated by both hip position and positive force feedback from the ankle extensors. Hip flexion initiates the swing-to-stance transition (McVea et al., 2005) whereas reductions in extensor activity during unloading decreases positive force feedback and initiates swing (Pearson et al., 1998). In all of the aforementioned cases, sensory feedback provides adjustments in the ongoing muscle activity, however, it is important to note that this feedback is modulated based on the phase of movement as the cat walks. This allows for functional adaptations to the environmental demands, such that spinal CPGs can either supress or facilitate the responses to given sensory feedback to ensure the outcome that will result in maintenance of forward progression (Duysens and Van de Crommert, 1998). Phase-dependent modulation of sensory feedback as assessed by reflexes has become increasingly important in the study of human locomotion.

Initial experiments on the neural control of locomotion in the decerebrate cat showed that increasing current, injected to the mesencephalic locomotor region (MLR), causes stepping frequency to increase (i.e. from a slow walk, to a trot, to a gallop) (Shik et al., 1966; Shik and Orlovsky, 1976). This general brainstem region has since been suggested as the command center to initiate locomotor behavior by tonic descending control to spinal CPGs via reticulospinal pathways (Garcia-Rill and Skinner, 1987). The basal ganglia contributes to locomotion by exerting tonic inhibition to the MLR (Garcia-Rill et al., 1990). With removal of the bilateral caudate nuclei, cats are seemingly unable to terminate the pursuit of any object that is seen (Villablanca et al., 1976).

The motor cortex is not required for non-human locomotion, but its importance is emphasized in demanding environments. After a lesion to the motor cortex, a cat can walk with only modest impairments during unobstructed locomotion (i.e. slight dragging of the paw during the swing phase) but if required to navigate difficult terrain (i.e. walk on the rungs of a horizontal ladder or step over obstacles) cats suffer more severe impairments (see (Drew et al., 1996) for review). The cerebellum is not required for non-human locomotion either, but does support the timing, rate and amount of rhythmic muscle activity (Schwartz et al., 1987; Udo et al., 1979, 1976), is responsible for postural tone (Sprague and Chambers, 1953) and contributes to both short- (Matsukawa et al., 1982) and long-term adaptations during locomotion (Yanagihara and Kondo, 1996) (for a more extensive review on cerebellar function during locomotion see Morton and Bastian (2007, 2004)). The relative importance of these supraspinal inputs is surely larger in humans, but the neural structures involved and their general role appears to persist across species.

Characteristics of human gait

As with all forms of animal locomotion, human walking shares the common purpose of moving the body from one location to another. To achieve this goal, a number of functions must interact to provide propulsion of our center of mass in an upright/erect posture. They include the generation of force to produce/maintain velocity, the generation of force to provide shock absorption, stability, and deceleration, the maintenance of upright posture and balance of the entire body, and the control of foot trajectory to avoid stumbling and ensure gentle and safe heel or toe landing (Winter, 1987). Clinical studies tend to focus on temporal and stride measures such as stance and swing times, as well as stride length, cadence, and velocity (Winter, 1987). Biomechanists have focused more on the kinematic, kinetic and energetic outcomes of walking, which provide more information about the cause of the gait pattern, rather than simply describing the movements (Winter, 1987).

In an attempt to understand the neural control of human locomotion, investigators have focused on surface electromyography (EMG). Based on methodological restraints accompanying human participants, EMG provides the best general-purpose measure of the neural output in humans during locomotion. Early work with EMG showed reciprocal activation of the lower limb flexor and extensor muscles that coincide with the general phases of human gait (see figure 2-1 – phases of gait with representative muscle activity from multiple muscles). Those phases include heel strike (i.e. swing to stance transition), stance, toe off (i.e. stance to swing transition), and swing. Of particular importance is the activity of the ankle extensor (soleus and medial and lateral gastrocnemius) and flexor (tibialis anterior) muscles. Ankle extensor muscles provide the majority of propulsion in the late stance to toe off phase, whereas the ankle flexors play an essentially important role in both ensuring toe clearance during the swing phase and the acceptance of weight during heel contact. Abnormalities in the gait pattern become particularly evident in the activity of these muscles when a lesion occurs (Awai and Curt, 2015; Beyaert et al., 2015; Comber et al., 2017; Dimitrijevic et al., 2015; Fouad and Pearson, 2004; McDonald and Sadowsky, 2002; Wirz and van Hedel, 2018). Incorporation of clinical, biomechanical and motor control outcomes provides considerable insight on the neural control of human locomotion in health and disease.

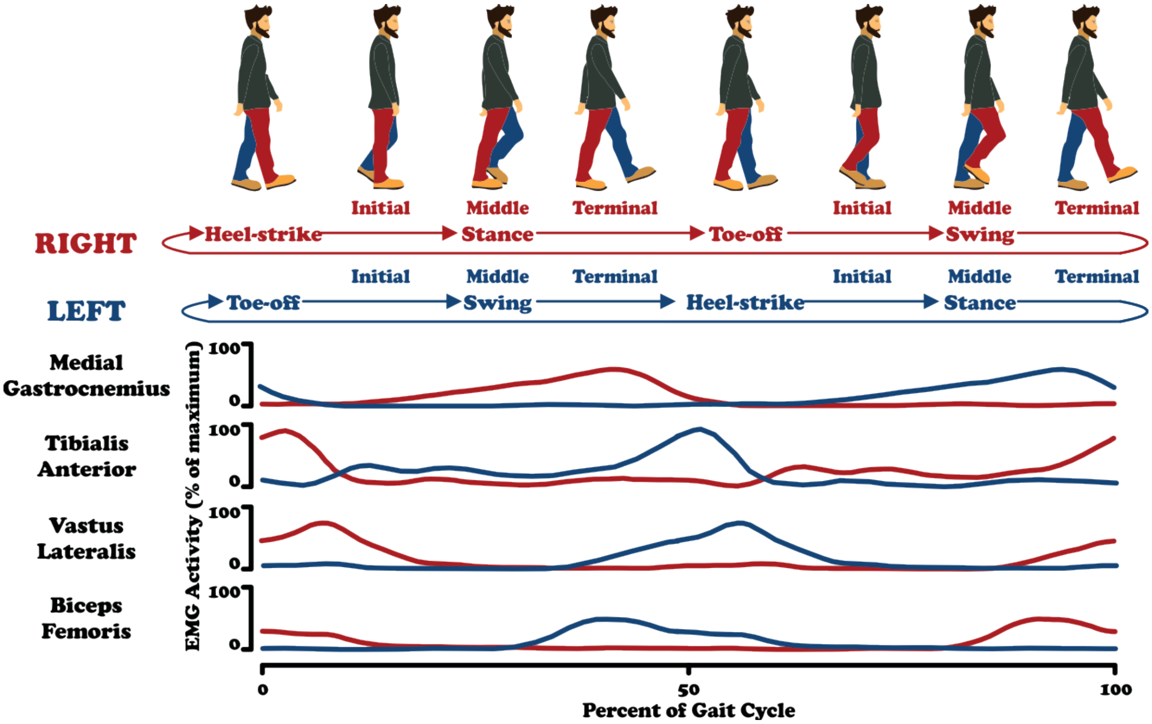

Figure 2-1*: A typical neurologically intact person's gait cycle is shown with important phases emphasized in the underlying text. Below is typical phase-averaged electromyographic data bilaterally from ankle plantar flexors (medial gastrocnemius) and dorsiflexors (tibialis anterior), and knee extensors (vastus lateralis) and flexors (biceps femoris) from 200 steps of normal walking. Data has been full-wave rectified and low-pass*

filtered to create a linear envelope. Red and blue correspond to the right and left legs, respectively, for the phases of gait and electromyograms.

Is bipedalism a defining feature in human evolution?

Bipedalism in our species probably emerged some 2.5-3 million years ago (Lovejoy, 1988), as shown in the timeline human ancestral lineage depicted in figure 2-2, but bipedalism is not unique to humans and our ancestors. Some flightless birds also use bipedal locomotion and it can be taught to monkeys, but our upright walking with erect posture does set us apart. Upright bipedal locomotion probably provided a survival advantage by allowing extensive viewing distances and for the arms to be freed for tasks such as signaling, carrying and throwing. The differences between primates and other quadrupeds highlights the evolution to bipedal gait, as primate locomotion relies more predominantly on the hindlimbs for propulsion than that of other quadrupeds who rely on a "front-steering—front-driving" system (Kimura et al., 1979). Primates likely shift weight to their hindlimbs during locomotion because of the ill-equipped forelimbs for accepting compressive loads (Reynolds, 1985). To achieve these reductions in compressive forces on the forelimbs, primates must activate substantially more musculature than that used in other quadrupeds. This probably explains why bipedal locomotion is also substantially more cost efficient than quadrupedal or bipedal gait of our closest cousins, the chimpanzee (Sockol et al., 2007). Muscle activity during walking in human toddlers is strikingly similar to that of rats, cats and monkeys (Dominici et al., 2011), and only differs slightly in adulthood to accommodate for the unique characteristics of bipedal gait including heel-strike well in front of the center of mass (Grillner, 2011b). Despite the evolution to bipedalism, there seems to be no evidence to suggest that the circuitry used to control locomotion in our ancestors has been abandoned in humans.

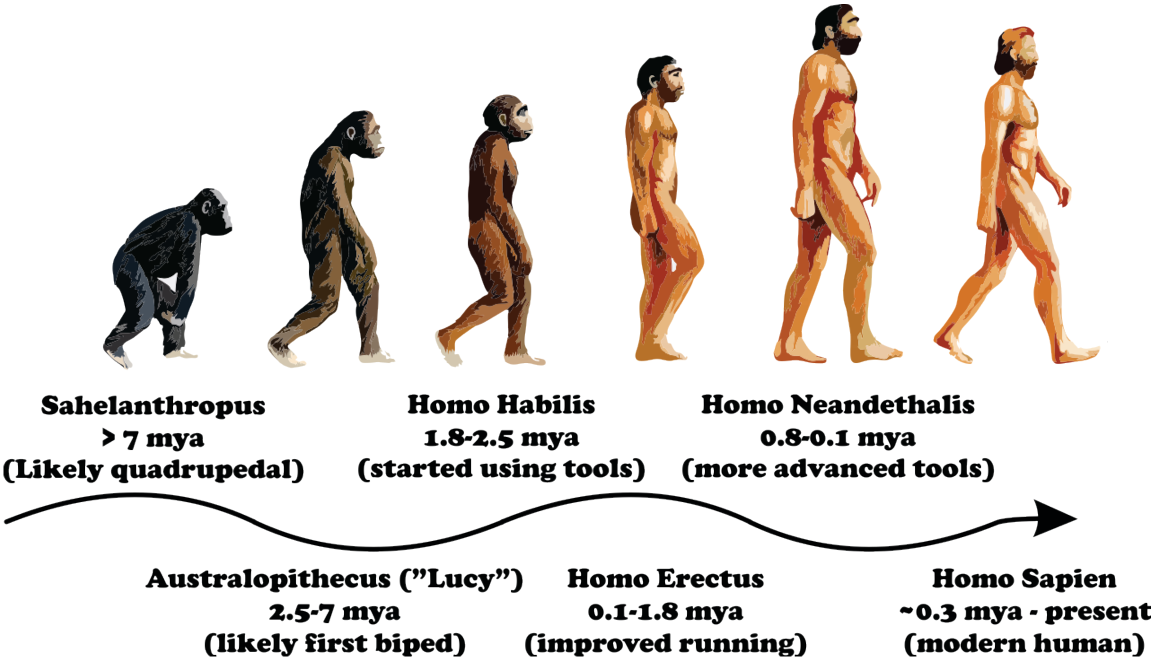

Figure 2-2*: An estimate of the ancestral lineage of present day humans based on a synthesis of morphological and genetic findings (Brunet et al., 2002; Gruss et al., 2017; Haile-Selassie, 2001; Kimbel William H. and Villmoare Brian, 2016; Ko, 2015; Lovejoy, 1988; Shields, 2000; Stringer Chris, 2016; Suwa et al., 2007; White et al., 2009, 2006). It is important to note that dates are estimated ranges, and sometimes overlap, indicating that there was possibly coexistence. Furthermore, to reinforce the message that these dates are estimations, the timeline has been represented with a squiggled, rather than straight, line for emphasis. Million years ago has been abbreviated to* ***mya****.*

Reflexes as a probe to understand the neural control of rhythmic movement

Unlike the measurement in reduced animal preparations, we cannot safely make intracellular recordings in uninjured human participants. Instead, we must rely on inferences from non-invasive methodologies to inform us on the topic of neural control of locomotion. Spinal reflexes provide great utility for this purpose since they can provide an estimate of the probable contributions of CPG activity on afferent feedback during rhythmic movement (Zehr, 2005). In particular, reflexes can be elicited by activation of afferents innervating muscle spindle receptors (i.e. Hoffmann (H-) and stretch reflexes) and tactile mechanoreceptors (i.e. cutaneous reflexes) (Zehr, 2006). By measuring these reflex pathways, considerable insights can be gained about the effect of different motor tasks (task-dependent modulation) and different phases within a rhythmic movement (phase-dependent modulation) on afferent feedback transmission (Duysens et al., 1992; Van Wezel et al., 1997; Yang and Stein, 1990; Zehr et al., 1997; Zehr and Stein, 1999). Task-dependent modulation is manifested with changes in posture from lying to standing, from standing to walking and from walking to running, which causes a progressive decline in soleus H-reflex excitability (Angulo-Kinzler et al., 1998; Capaday and Stein, 1986; Crenna and Frigo, 1987; Koceja et al., 1995, 1993; Mynark and Koceja, 1997; Stein and Capaday, 1988). The decrease in H-reflex excitability results from increases in Ia presynaptic inhibition and has been shown during rhythmic leg (Brooke et al., 1997; Capaday and Stein, 1986; Crenna and Frigo, 1987) , arm (Frigon et al., 2004) and wrist movements (Aimonetti et al., 2000a, 2000b, 1999; Brooke et al., 2000). Similarly, cutaneous reflexes are altered when compared between standing and walking or running (Duysens et al., 1993; Komiyama et al., 2000). Interestingly, there is an absence of nerve-specificity of cutaneous reflexes during standing, which are generally suppressive and correlated with EMG amplitude. During walking cutaneous reflexes are suppressive or facilitatory but are modulated independently from muscle activity and tied closely to the events occurring in the step cycle (Komiyama et al., 2000).

Phase-dependent modulation of muscle afferent reflexes is thought to reflect the transient effects of spinal CPG gating of Ia presynaptic inhibition. During static contractions, the soleus H-reflex is smallest during swing and largest during late stance, even when participants are trained to walk with altered EMG patterns, such that they contracted their tibialis anterior during stance or soleus during swing (Yang and Whelan, 1993), suggesting that the modulation cannot simply be due to the levels of agonist and antagonist muscle activity. Instead, the functional capacity of spinal CPGs to influence reductions in plantarflexion during swing, and maintain plantarflexion during stance is revealed (Capaday and Stein, 1986; Verschueren et al., 2002).

Phase-dependent modulation of cutaneous reflexes also reflects the CPGs ability to gate sensory feedback to create functionally relevant responses that ensure the maintenance of forward progression (Duysens et al., 2004; Zehr and Stein, 1999). In the cat, reflexes evoked from the paw dorsum (Forssberg, 1979; Forssberg et al., 1977) and plantar surface of the paw (Forssberg et al., 1975) are phase-dependently modulated. Likewise, either mechanical or electrical stimulation of the human foot dorsum cause stumble correction reactions (i.e. decreased dorsiflexion and increased hip and knee flexion) during swing (Schillings et al., 1996; Van Wezel et al., 1997; Zehr et al., 1997), which allows the limb to move past a perturbation and proceed with forward progression. During stance, stimulation to the foot dorsum prolongs the stance phase but does not result in knee or hip flexion, as this would perturb stability. Stimulation of the distal tibial nerve also undergoes phase-dependent modulation in the human tibialis anterior muscle. During the swing to stance transition, distal tibial stimulation causes suppression that resembles a placing reaction. Reflex reversal occurs when distal tibial stimulation is applied during the stance to swing transition causing facilitation of the tibialis anterior, which resembles a withdrawal reaction (Duysens et al., 1990; Nakajima, 2016; Zehr et al., 1997). Since phase-dependent modulation of cutaneous reflexes is absent during passive rhythmic movement, CPG activity likely plays a strong role in the gating of sensory feedback to produce functionally relevant responses.

Coordinating activity between the legs

To successfully locomote on two limbs body weight must always be supported by one leg during stance, while the other leg swings to produce forward progression. This makes the coupling of muscle activity from one leg to the other a vital component of human locomotion. Indeed, the activity of one leg strongly influences the presynaptic control of reflexes in the opposite leg. Movement in one leg causes a general suppression of H-reflexes in the other leg (Cheng et al., 1998; Collins et al., 1993; McIlroy et al., 1992; Misiaszek et al., 1998). Furthermore, humans are capable of maintaining coordination between the limbs when walking on split-belt treadmills with belts running at different speeds between the legs (Erni and Dietz, 2001; Prokop et al., 1995). Humans accommodate these discrepancies between belt speeds by adjusting stance times. The foot on the slower belt has longer stance times, whereas the foot on the faster belt has shorter stance times and longer swing times (Prokop et al., 1995). This strategy helps maintain an equal number of steps between the legs and is similar to the strategy used by spinally transected cats (Frigon et al., 2013), suggesting that this process is mediated at a spinal level, most likely via asymmetric sensory feedback (Frigon et al., 2015). Humans are also capable of coordinating both of the legs by moving them in opposite directions (Choi and Bastian, 2007). During these circumstances, swing phases never overlap, indicating tight coupling of activity between the legs. Similar to cats (Forssberg et al., 1977) is the fact that swing initiation in humans has profound effects on the contralateral limb, such that early swing initiation (via rapid stretch) in one limb causes a truncation of swing of the opposite limb (Berger et al., 1984; Dietz et al., 1984). Bilateral coordination of the legs also occurs in response to perturbations during the swing phase (Dietz et al., 1986; Eng et al., 1994; Schillings et al., 1996). These responses ensure forward progression without compromising upright postural stability (Zehr et al., 2016).

Coordinating activity between the arms

Comparable neural control of the forelimbs to that of their hindlimbs has been demonstrated in habitual quadrupeds (Yamaguchi, 2004). Accumulating evidence suggests that the arms are controlled by similar neural networks as the legs during rhythmic movements in humans (Zehr and Duysens, 2004). For instance, cutaneous reflexes in the arms are phase-modulated in a pattern that cannot be attributable to background muscle activity (Zehr and Haridas, 2003). The coupling between the limbs is weaker than that of the legs during locomotion, however, since rhythmic activity of one arm has little effect on the H-reflex excitability reflexes in the contralateral stationary arm (Zehr et al., 2003) and rhythmic activity of one arm has little effect on cutaneous reflex amplitudes of the opposite arm (Carroll et al., 2005). The phase-modulation of reflexes in the arms depends on the state of the ipsilateral limb (Carroll et al., 2005; Hundza and Zehr, 2006; Vasudevan and Zehr, 2011), unlike the legs, which also depend on the functional state of the contralateral limb (Zehr et al., 2016). It should be noted, however, that rhythmic movement of the arms during locomotion is not entirely passive. Even when they are physically restrained, rhythmic muscle activity is present during walking (Ballesteros et al., 1965; Kuhtz-Buschbeck and Jing, 2012). Thus, it is likely that humans have retained the networks responsible for the coordination of rhythmic activity of the arms, but these networks have been suppressed during the process of evolution to bipedal walking, subsequently freeing the arms for other tasks (Dietz, 2002; Zehr et al., 2016). Development of direct cortico-motoneuronal connections likely contribute to the increases in dexterity and fine motor control seen in humans and other primates (Lemon, 2008). These strong and direct connections probably predominate during volitional actions (Nicolas et al., 2001) however, activation of motoneurons via propriospinal neurons in humans is still possible (Pierrot-Deseilligny, 1996), and likely contributes to rhythmic motor output of the arms (Dietz, 2002).

Coordinating activity between the arms and legs

Bidirectional coupling between the locomotor centers within the cervical and lumbar spinal cord has been demonstrated in rats and cats and are attributed to propriospinal pathways between the limb girdles (Gernandt and Megirian, 1961; Gernandt and Shimamura, 1961; Juvin et al., 2005; Thibaudier et al., 2013; Thibaudier and Frigon, 2014). There is significant evidence to support the preservation of cervicolumbar connections in human locomotion, despite the fact that the arms do not contribute directly to propulsion during walking (Dietz, 2002; Zehr et al., 2016). For instance, on an uncoupled arm and leg cycle ergometer, voluntary changes in leg cycling cadence alters arm cycling cadence, however, voluntary changes in arm cycling cadence do not influence leg cycling cadence (Sakamoto et al., 2007). Active engagement of the arms facilitates activity of the legs during recumbent arm and leg stepping (Ferris et al., 2006; Huang and Ferris, 2004). Furthermore, rhythmic arm movement modulates reflexes evoked in stationary legs (de Ruiter et al., 2010; Dragert and Zehr, 2009; Frigon et al., 2004; Hundza et al., 2012; Hundza and Zehr, 2009; Loadman and Zehr, 2007) and rhythmic leg movement causes modulation of reflexes evoked in stationary arms (Carroll et al., 2005). When actively engaged in arm and leg rhythmic movement cutaneous reflexes are facilitated based on the number of rhythmically active limbs (Nakajima et al., 2014). This facilitation is greater than the algebraic sum of the two reflexes alone, suggesting a facilitation effect from descending drive and/or CPG related activity that is dependent upon the number of rhythmically active limbs. Interlimb coupling between the arms and legs is also evident from studies examining the neuromechanical outcome of cutaneous reflexes. For example, stimulation to the wrist causes ankle dorsiflexion during the stance to swing transition, which probably acts to reduce forward progression in response to a perturbation encountered by arm swing (Haridas and Zehr, 2003). When the arms are crossed in front of the body (i.e. the participant is unstable), stimulation to the foot dorsum results in facilitated reflexes in upper limb muscles (Haridas et al., 2005). This general facilitation of responses in the arms is interpreted as a preparedness response since the arms do not have the capability to directly interact with the ground and promote stability and forward progression. When the hand is in contact with an earth-referenced handrail and can directly contribute to stability during walking, cutaneous reflexes in response to stimulation of the foot are enhanced in arm muscles (Lamont and Zehr, 2007, 2006). Exploiting these cervicolumbar connections could be used to enhance neurorehabilitation (Dietz, 2002; Ferris et al., 2006; Zehr et al., 2009b; Zehr, 2016). Indeed, experiments in those with chronic stroke (Kaupp et al., 2018; Klarner et al., 2016; Klarner, 2016) or spinal cord injury (Zhou et al., 2018) have demonstrated the capacity of these connections (depicted in figure 2-3) to improve neurological integrity and locomotor ability in those with neurological impairment.

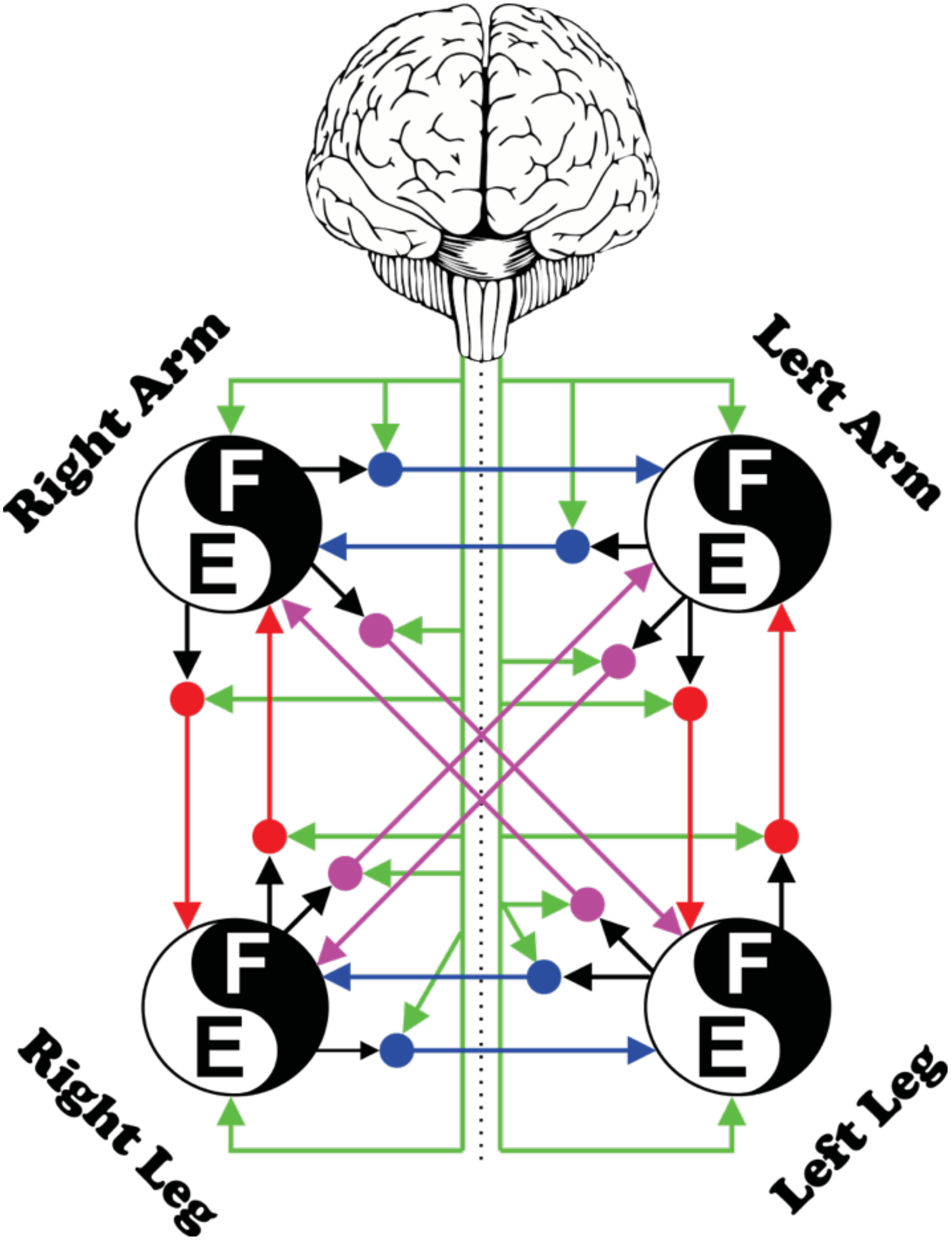

***Figure 2-3**: A schematic representation of the interlimb connections in the spinal cord with reference to the review of Frigon* (2017). *Yin yangs represent a CPG for each limb. Green lines represent connections from supraspinal centers that exert influence over all interneurons and each CPG. Blue, red and magenta lines represent commissural, homolateral propriospinal and diagonal propriospinal pathways, respectively.*

Involuntary stepping in neurologically intact humans

Tonic input, either from sensory afferent activity or direct stimulation to the spinal cord, can elicit involuntary movements that resemble stepping in neurologically intact humans. Typically, gravitational forces and sensory feedback associated with standing and maintaining an upright posture inhibits the activation of rhythmic movements in response to tonic sensory input or spinal cord stimulation (Gurfinkel et al., 1998). Thus, simulated weightlessness has been used to examine the capacity of the human spinal cord to generate involuntary rhythmic motor output in response to tonic input (see (Solopova et al., 2015) for an extensive review). To do this, a person lays on their side with the weight of their arms and legs suspended with either long ropes to the ceiling or an exoskeleton and instructed to avoid intervening with any induced movements. Tonic frequencies (20-60Hz) of vibration (~1mm depth) to the quadriceps or biceps femoris can evoke rhythmic stepping that has coordinated flexion and extension with reciprocal alternation between the legs (Gurfinkel et al., 1998; Selionov et al., 2009). Similar stepping can be evoked with constant (60Hz) and continuous stimulation (2 times the perceptual threshold) of nerves containing afferents which innervate mechanoreceptors of the foot. The involuntary stepping movements evoked by tonic inputs produce movements that are strikingly similar to voluntary stepping in the same experimental set-up, and if participants engage in mental arithmetic (i.e. counting backwards) the involuntary stepping is either unaltered or increases in intensity, suggesting that they are not produced by volitional commands (Solopova et al., 2015). Attention should be drawn to the fact that, in these paradigms of involuntary stepping, very little ankle movement or muscle activity of the extensors and flexors of the ankle is observed. The lack of ankle movements resembles that of fictive locomotion in the cat (Grillner, 2006a), and suggests that sensory feedback is required to mold the functional movements at the ankle, however the intrinsic circuitry of the spinal cord in capable of generating the basic patterns required for rhythmic motor output (Solopova et al., 2015). However, when a small load (4-8N) is applied to the foot, ankle movements with corresponding muscle activity emerge (Selionov et al., 2009).

Non-invasive but direct spinal cord stimulation techniques can also evoke involuntary stepping (Gerasimenko et al., 2015a, 2015b, 2010; Gorodnichev et al., 2012). Similar to the seminal work of Shik (1966) in the cat, it appears that a general command can induce stepping in humans too. Like the cat, increases in the strength of electromagnetic stimulation causes increases the amplitude and frequency of movements (Gerasimenko et al., 2010). Electromagnetic stimulation of various frequencies (1-20Hz) and strengths (1.06-1.82 tesla) evokes coordinated involuntary stepping immediately, and can be altered in pattern (but not rhythm) by vibration. Similarly, transcutaneous spinal cord stimulation applied at tonic frequencies (4-50Hz) augments amplitude of stepping movements, but has little effect on cycle duration (Gorodnichev et al., 2012). Direct stimulation of the spinal cord probably activates the spinal CPG circuitry though the dorsal roots and is usually applied over T11-T12 vertebrae (Gerasimenko et al., 2015b, 2010; Gorodnichev et al., 2012; Solopova et al., 2015). It is important to note that direct stimulation of the lumbar spinal cord causes more robust stepping patterns than that of sensory stimulation (Gerasimenko et al., 2010) suggesting that the circuitry to produce rhythmic output lies within the spinal cord, rather than peripherally.

Rhythmic stepping in humans with spinal cord injury

Spinal cord injury may allow for the study of spinal cord mediated rhythmic motor output in humans without the influence of descending control. Years of evidence, including spontaneous rhythmic stepping and rhythmic stepping in response to tonic stimulation and/or pharmacological agents, suggest that under the right conditions the human spinal cord can produce and maintain rhythmic muscle activity in the absence of descending control.

Spinal myoclonus is the slow rhythmic and involuntary activation of muscles below the level of a spinal cord injury and was first described and initially observed a century ago (Lhermitte, 1919). More comprehensive study of this phenomenon has lead clinicians and scientists to believe that the spinal cord is capable of producing rhythmic motor output in the absence of supraspinal influences, likely through the activation of spinal stepping generators (Bussel et al., 1988). This is supported by the fact that sensory feedback can induce rhythmic myoclonic activity (i.e. by twisting the toes or unilateral hip flexion), slow the frequency of the rhythmic myoclonic activity (i.e. with tactile stimulation or by rubbing an ice cube on the lower back) and completely abolish rhythmic myoclonic activity (i.e. by bilateral hip flexion). In a person with incomplete spinal cord injury, but no volitional control of lower leg muscles, forceful, alternating and rhythmic movements of the legs can be evoked when they lay supine with the hips extended (Calancie et al., 1994). These movements were completely involuntary and emerged after intensive locomotor training. They were abolished with bilateral hip flexion or facilitated with dorsiflexion of the toes, indicating that sensory feedback has access to the networks responsible for the movements and modify the output (Calancie et al., 1994). In a similar case of incomplete spinal cord injury (Dobkin et al., 1995) involuntary stepping patterns diminished shortly after locomotor training ended. The rare expression of these involuntary movements has been reported in complete spinal cord injury (Calancie, 2006; Nadeau et al., 2010), however it has been suggested that the pattern generating circuits in humans do rely more heavily on supraspinal influences since spontaneous movements are more often reported in those with incomplete injuries (Harkema, 2008).

Iwahara and colleagues (1992) found that stimulation over the cervical enlargement could elicit coordinated stepping in all four limbs, whereas stimulation over the lumbosacral enlargement could elicit hindlimb stepping in decerebrate cats. The stimulation was a tonic input with low current and low frequency, suggesting that central pattern generators in the spinal cord are capable of producing rhythmic stepping in response to tonic input. Subsequently, epidural stimulation in humans has shed considerable light on the capacity of the human spinal cord to generate rhythmic outputs in response to tonic input. Initial reports (Dimitrijevic et al., 1998; Gerasimenko et al., 1997; Shapkova et al., 1997) showed that facilitation of the lumbar spinal cord (L1-L3) via epidural stimulation could elicit locomotor-like stepping in both children and adults with motor complete spinal cord injury.

During locomotor-like rhythmic activation induced by epidural stimulation, soleus H-reflexes are suppressed (Gerasimenko et al., 1997) and the frequency and amplitude of muscle activity increases with stimulation strength (Dimitrijevic et al., 1998), suggesting that activation of lumbar circuits can gate sensory feedback and convert tonic input to rhythmic output in the absence of descending control from supraspinal structures. More recent examinations (Danner et al., 2015; Minassian et al., 2007, 2004; Shapkova, 2004) of epidural stimulation-induced stepping in humans with spinal cord injury has shown that the parameters of stimulation can influence the stepping characteristics. For example, the frequency of stimulation does not dictate the frequency of stepping, rather it is dictated by the strength of stimulation (Shapkova, 2004), similar to that of the MLR locomotor-like activity (Shik et al., 1966). Furthermore, increases in epidural stimulation-induced stepping frequencies are achieved by reducing the stance phase duration (Danner et al., 2015), as it is during walking of intact humans (Murray, 1967) and cats (Frigon, 2012), suggesting that the control of asymmetric modulation between swing and stance phases during walking at various speeds in humans, at least partially, lies within the lumbar spinal CPG (Minassian et al., 2017).

Neurotransmitters such as glutamate, noradrenaline, dopamine, and serotonin are involved in the initiation of locomotion (Jordan et al., 2008). Many drug therapies have been used to improve locomotor outcomes in humans with incomplete spinal cord injuries for some time now, but their effects have been largely attributed to reductions in spasticity (Barbeau et al., 1999; Fung et al., 1990; Wainberg et al., 1990). More recently, oral doses of buspirone (i.e. a serotonin receptor agonist) can facilitate percutaneous stimulated stepping in humans with spinal cord injury (Moshonkina et al., 2016). In the spinalized mouse, however, Spinalon, a concoction of levodopa (noradrenergic/dopaminergic precursor) combined with carbidopa (decarboxylase inhibitor) and buspirone, can induce weight bearing stepping without any other form of assistance or training (Guertin et al., 2011, 2010). This work has recently been translated to humans. A large-scale clinical trial has identified that this same drug (i.e. Spinalon) can induce spontaneous rhythmic muscle contractions in humans with motor complete spinal cord injuries (Radhakrishna et al., 2017). These stepping patterns are presented as significant muscle activity with visually detectable movements and appear shortly after (15-120 minutes) oral administration of the drug. The muscle activity is rhythmic and alternating activity between flexors and extensors of the left and right legs. Although this activity was only observed in about one third of the participants who received the drug, it does provide substantial evidence for the human spinal CPG to produce rhythmic activity via pharmacological intervention.

Infant stepping

Infants within the first year of life provide a convenient model to study the spinal circuitry of an intact spinal cord in the absence of developmentally complete descending control (Yang et al., 2004). Axon diameters and consequent conduction velocities are substantially lower in infants compared to adults, probably because myelin is not well developed until about 2 years of age (Yang et al., 2004). Stepping patterns develop prior to birth, however, as evidenced by in utero stepping that can be seen with ultrasound (de Vries et al., 1986). After birth, patterned stepping can be evoked by lifting the infant above a surface and leaning them forward (Yang et al., 2004). Weight support and hip extension are both important contributors to the facilitation of infant stepping (Yang et al., 2004), similar to that of the cat (Pearson, 2004). Likewise, increases in arousal (i.e. when the infant is crying) can cause increases in infant stepping (Forssberg, 1985; Thelen et al., 1982; Yang et al., 2004), suggesting that neuromodulatory drive may be an important contributor. Infant stepping is characterized by rhythmic alterations in flexor and extensor activity about the ankle, but co-contraction of thigh muscles throughout (Yang et al., 1998). General features that differentiate infant stepping from typical adult locomotion include toe stepping rather than heel contact, the hip, knee and ankle flex and extend in unison rather than independently, and toe drag that is very common (perhaps due to the lack of descending control of the ankle flexors) (Forssberg, 1985; Okamoto et al., 2003, 2001; Yang et al., 2004).

Infants can step in all directions by simply changing their orientation above a treadmill. For example, a belt moving in an anterior-posterior direction causes forward stepping, whereas a belt moving from right to left, causes sideway stepping, and a belt running in a posterior-anterior direction induces backward stepping (Lamb and Yang, 2000). Although infants can accommodate a wide range of stepping speeds, they do not possess the intralimb coordination required to change their gait pattern from a walk to a run with increasing speeds, rather they perform the same patterns at a higher rate (Vasudevan et al., 2016). The two legs show impressive coordination though. The effects of hip stretch on eliciting the swing phase are dependent on the position of the contralateral limb (Pang and Yang, 2000), similar to that of cats and adult walking. Disturbances to stance caused by pulling cardboard from beneath the foot is walking direction dependent. For example, pulling the cardboard backwards (i.e. causing hip extension) during forward walking initiates swing, whereas lateral movement of the cardboard initiates swing during sideways walking (Pang and Yang, 2002). Stumbling corrective reactions, characterized by hip, knee and ankle flexion in response to mechanical stimulation of the foot are location specific and modulated by phase in infants as well (Lam et al., 2003). Touch to the foot dorsum during swing of forward stepping causes the abovementioned stumbling corrective reaction, but stance is prolonged with touch of the dorsum during the stance phase. Touch to the side of the foot does not elicit any kinematic change during forward stepping but does cause increased flexion during sideways stepping. Infants also possess the incredible ability to step over a split-belt treadmill with belts running at different speeds and maintain stepping with one belt moving up to 5 times the speed of the other without swing occurring simultaneously between the legs (Yang et al., 2004).

Whether the neural control of infant stepping is integrated into control strategies later in life was a controversial point for quite some time. In an elegant experiment, infant stepping was elicited over a table and activity was recorded from 24 muscles, simultaneously (Dominici et al., 2011). Nonnegative-matrix-factorization was used to derive basic patterns from averaged muscle activity from infants, toddlers, preschoolers, adults and non-human animals. They identified two basic patterns of sinusoidal modulation of muscle activity within the step cycle in infants. These patterns were coactivation of the leg flexors during swing and coactivation of the extensors during stance. The patterns were accompanied by a flexed posture, high swing height of the foot, and a lack of shift in pressure from the heel to toe during stance. There was also an absence of appropriate activity that contributed to transition phases (i.e. heel contact and toe off). This basic oscillation of flexors and extensors is similar to the reports of fictive locomotion in the cat (Grillner, 2006b). It is not until later in development that new basic patterns are integrated in the control of locomotion, which reflects the integration of supraspinal and sensory control (Kiehn, 2011). For example, new patterns of muscle activity that correspond to weight acceptance and propulsion emerge in toddlers, whereas it is not until adulthood that further refinements in patterns of muscle activity exploit the inverted pendulum and optimize muscle activity to support and propel the body, while appropriately shifting the center of pressure under the foot from heel to toe (Dominici et al., 2011). Taken together, these data suggest that the circuitry required to produce rhythmic and coordinated stepping movements in multiple directions, and the circuitry required to modulate responses to afferent feedback are available before the onset of independent walking, but the development of supraspinal and sensory control refines walking as we age.

Supraspinal control in human locomotion

Although the capacity of the human spinal cord to produce and modulate rhythmic stepping is clearly evident, this occurs in correspondence with the essential role of supraspinal commands to produce meaningful human locomotion. Supraspinal control seems of increasing importance in primates when compared to other animals (Barthélemy et al., 2011; Capaday, 2002; Nielsen, 2003; Solopova et al., 2015; Yang and Gorassini, 2006). Indeed, the role of intact descending control has been stressed in primates because MLR evoked locomotor-like activity is much more pronounced than locomotor-like activity induced by pharmacological agents or electrical stimulation of dorsal roots after spinal transection (Eidelberg et al., 1981; Fedirchuk et al., 1998). Unlike cats (Drew et al., 1996), humans cannot walk after complete spinal lesions under normal circumstances (Yang and Gorassini, 2006), thus highlighting the vital importance of descending control during human locomotion. Yet when provided with assistance in partial body weight support that compensates for the additional limb loading inherent in bipedal locomotion, locomotor stepping can occur (Dietz, 2009). Our knowledge on the role of supraspinal contributions to human locomotion has relied primarily on deductive reasoning from those with lesions, neuroimaging techniques and non-invasive neurostimulation, some of which are outlined below.

By using functional magnetic resonance imaging in humans during imagery of lying, standing, walking and running, Jahn and colleagues (2008b, 2008a) have demonstrated that the MLR is highly active during imagined walking and running when compared to lying or standing. Activation of this area underscores the similarity of the initiation center that has been identified in cats (Shik et al., 1966; Shik and Orlovsky, 1976). Patients with midbrain lesions (i.e. the area where the MLR is localized) have been reported to have difficulties with the initiation of gait (Hathout and Bhidayasiri, 2005; Masdeu et al., 1994) further supporting the existence of the MLR in humans. Although the basal ganglia has been shown to indirectly control output from the MLR in the mouse (Roseberry et al., 2016), less evidence is available in the human (see Ryczko and Duboc (2017) for current review). However, deep brain stimulation in patients with Parkinson’s disease (Plaha and Gill, 2005), which activates the subthalamic nucleus (for example see (Holiga et al., 2015)) and sends glutamatergic drive to the pedunculopontine nucleus (Neagu et al.,

2013) has shown potential for improving gait dysfunctions associated with basal ganglia disorders (see Ryczko and Duboc (2017)).

Lesions to the motor cortex of the cat cause only minor impairments (i.e. slight dragging of the paw during swing) to walking under facile conditions (i.e. on a treadmill). In demanding conditions, however, the motor cortex becomes more essential (Drew et al., 1996). In humans, the motor cortex appears to play a more dominant role in normal locomotor output (Barthélemy et al., 2011; Capaday, 2002; Nielsen, 2003; Solopova et al., 2015; Yang and Gorassini, 2006). Neuroimaging studies, that include single-photon emission computed tomography (SPECT; (Fukuyama et al., 1997)), positron emission tomography (PET; (Mishina et al., 1999)), and near-infrared spectroscopy (NIRS; (Miyai et al., 2001)), have shown that there are high levels of activity in the somatosensory cortices during walking. To account for the effects of sensory feedback Christensen et al (2000) used PET to examine differences between voluntary and passive cycling. When activity during passive cycling was subtracted from the voluntary cycling, high levels of activation was still observed in the primary motor cortex, highlighting the role in the cortical control of muscle activity during rhythmic motor output.

There is general phase-dependent modulation of motor evoked potentials measured in the arm (Barthelemy and Nielsen, 2010) and both proximal (Bonnard et al., 2002) and distal (Capaday et al., 1999; Schubert et al., 1997) leg muscles during walking, but amplitudes are generally increased under demanding conditions. For example, visually guided gait (Schubert et al., 1997) and walking with resistance from elastic bands (Bonnard et al., 2002) both increase motor evoked potential amplitudes when compared to unrestrained walking. Similarly, motor evoked potentials recorded from the both proximal (biceps femoris and rectus femoris) and distal (tibialis anterior) leg muscles are increased during voluntary, compared to vibration-induced, air-stepping (Solopova et al., 2014). These findings suggest that the motor cortex does in fact contribute to the excitability during skilled stepping in humans as it does in cats.

Accumulating evidence suggests that the motor cortex also contributes to the ongoing control of muscle activity during walking in facile conditions. For example, weak transcranial magnetic stimulation applied to the cortex, which causes intracortical inhibition (Davey et al., 1994), causes suppression of ongoing TA muscle activity during the first half of the swing phase (Petersen et al., 2001). Since this inhibition is not evident during electrical stimulation of the cortex, which bypasses the motor cortex and activates the axons directly (Edgley et al., 1990), the authors suggested that the motor cortex plays a role in the maintenance of tibialis anterior muscle activity during walking. Similar work has also shown the likelihood of the motor cortex in the maintenance of muscle activity in the arms during walking (Barthelemy and Nielsen, 2010). Coherence between tibialis anterior muscle activity and electroencephalography recorded from Cz shows synchrony between 600ms prior to and 200ms after heel strike (Petersen et al., 2012) with peak synchrony occurring between 500 and 300ms prior heel strike, illustrating the role that the motor cortex likely plays in the ongoing muscle activity of the tibialis anterior during the swing phase.

Further support of the cortical contributions to the muscle activity of the tibialis anterior has been derived from intramuscular coherence analysis during the swing phase of human walking (Halliday et al., 2003). High frequency (~8-30Hz) synchronization of two independent recording sites over the same tibialis anterior during walking suggest that this synchrony comes from a common presynaptic drive (Jensen et al., 2018). Indeed, this is supported by the work in the cat that shows dragging of the paw after lesions in the motor cortex (Drew et al., 1996). The contributions of the motor cortex to the extensor muscles (i.e. soleus, medial and lateral gastrocnemius, etc.) remains elusive, which is probably accounted by the fact that there is substantially less monosynaptic corticospinal projections to the extensors compared to flexors of the lower leg (Brouwer and Ashby, 1992). Indeed, the issue of intermuscular differences in corticospinal projections between the flexors and extensors during human locomotor output has been raised before (Power et al., 2018).

Advancing technologies enhance our understanding of the extent that the motor cortex plays during facile walking. For example, subdural recordings from the motor cortex of two individuals implanted with electrocorticography grids for epilepsy surgery evaluation were recently acquired during walking (McCrimmon et al., 2018). There is generalized gamma-band synchronization throughout the entire gait cycle, but gamma-band movement-related power is significantly elevated around the stance-to-swing and swing-to-stance transitions. This suggests that the motor cortex primarily everts high level control during locomotion (i.e. gait initiation, duration, speed, transitions, perturbations, etc.). The motor cortex likely interacts with subcortical networks (i.e. MLR and spinal CPGs) to determine appropriate muscle activation and movement trajectories during normal human walking (McCrimmon et al., 2018).

The motor cortex also contributes to adaptations during walking. In particular, fast adaptations to force fields are likely driven by the motor cortex. Motor evoked potentials are increased during walking with resistive loads and decreased during walking with an assistive load (Barthélemy et al., 2012). Repetitive transcranial magnetic stimulation at subthreshold intensities and applied over the motor cortex is sufficient to abolish any adaptations that occur during resistive ankle perturbations, suggesting that the corticospinal system is indeed contributing to fast adaptations to walking against resistive loads.

Together with the posterior parietal cortex, the motor cortex aids with obstacle avoidance (Drew and Marigold, 2015; Marigold, 2019). In the cat, posterior parietal cortical activity is modulated when hindlimb stepping over an obstacle is delayed, suggesting that information about the obstacle is stored in the working memory and used later to avoid an obstacle. Humans can also delay the stepping with our trailing leg over an obstacle by ~2 minutes (Lajoie et al., 2012). If vision is obstructed, and the lead leg is moved passively over an obstacle (i.e. efference copy removed), there are increases in trail leg variability and clearance as a person steps over the obstacle. If vision is reintroduced, the variability and clearance return to normal, suggesting that vision and the posterior parietal cortex play a role in guiding the output from our motor cortex to avoid obstacles. Indeed, lesions in the area of the posterior parietal cortex cause impairments in visually guided foot placements (Evans et al., 2013).

The cerebellum plays a role in adaptations during walking too, but also maintains postural control and supports the timing, rate and amount of locomotor muscle activity (Morton and Bastian, 2007, 2004). Much of what we know about the contributions of the cerebellum to human locomotion comes from clinical presentations of those with cerebellar lesions or degeneration. For example, lesions or atrophy to the cerebellum cause gait ataxia, which resembles drunken gait (Morton and Bastian, 2007). Patients with cerebellar lesions have significant variability in their step timing, length and direction, the trajectory of the swing limb is quite irregular, and the intra- and interlimb coordination is compromised (Earhart and Bastian, 2001; Palliyath et al., 1998). Lesions to the anterior vermis specifically affects balance during tandem walking, but does not particularly affect regular walking, standing or hopping (Bastian et al., 1998). Cerebellar degeneration causes perturbations in anticipation of gait initiation, but not reactive postural perturbations (Timmann and Horak, 1998). Cerebellar lesions do not completely compromise the ability to compensate for a perturbation (i.e. sudden slowing and then re-acceleration of a treadmill), however the adaptation of a gait pattern that better prepares a person to respond to gait perturbations is less than a control participant (Rand et al., 1998). Cerebellar dysfunction also weakens predictive feedforward locomotor adaptability but does not appear to affect reactive feedback-driven locomotor adaptability (Morton and Bastian, 2006). Although the aforementioned studies highlight what the nervous system cannot do in the absence of the cerebellar structures, they cannot completely elucidate the role of the cerebellum in the neurologically intact nervous system (Morton and Bastian, 2006).

The ’common core’ – similar neural control during many rhythmic behaviors

The neural control of rhythmic movement in humans is not limited to walking, rather flexible access to central neural control mechanisms produce many rhythmic behaviors, including crawling, walking, running, cycling, swimming, and others. This flexible access to common neural elements has been suggested in the cat, because the both forward and backward walking are produced with common flexor-extensor muscle synergies (Buford and Smith, 1990). Reversals in phase-dependent reflex modulation and muscle activity compared during forward and backward walking (Duysens et al., 1996; Thorstensson, 1986; Winter et al., 1989) and cycling (Zehr et al., 2009a; Zehr and Hundza, 2005) show commonalities in humans. Phase-dependent reflex modulation also shares similarities between level walking, incline walking and stair climbing (Lamont and Zehr, 2006), and between walking, arm and leg cycling, and recumbent stepping (Zehr et al., 2007). The sharing of common elements of neural control for many rhythmic behaviors has previously been coined as the ‘common core hypothesis’, which suggests that, regardless of the motor task, rhythmic motor timing is common across many motor tasks (i.e. walking, crawling, running, cycling, swimming, etc.). Spinal interneurons are influenced by sensory feedback that assists with the maintenance and modulation of motoneuronal excitability required for each specific type of rhythmic task, whereas supraspinal commands oversee the activity of spinal CPGs according the overarching goals of the task (i.e. start/stop, speed up/slow down, etc.). The sublime interactions between spinal CPGs, sensory feedback and supraspinal control (see figure 2-4) form a flexible control strategy that can be adjusted based on the environmental constraints and goal of the behavior, which allows humans to move around many environments to achieve many goals.

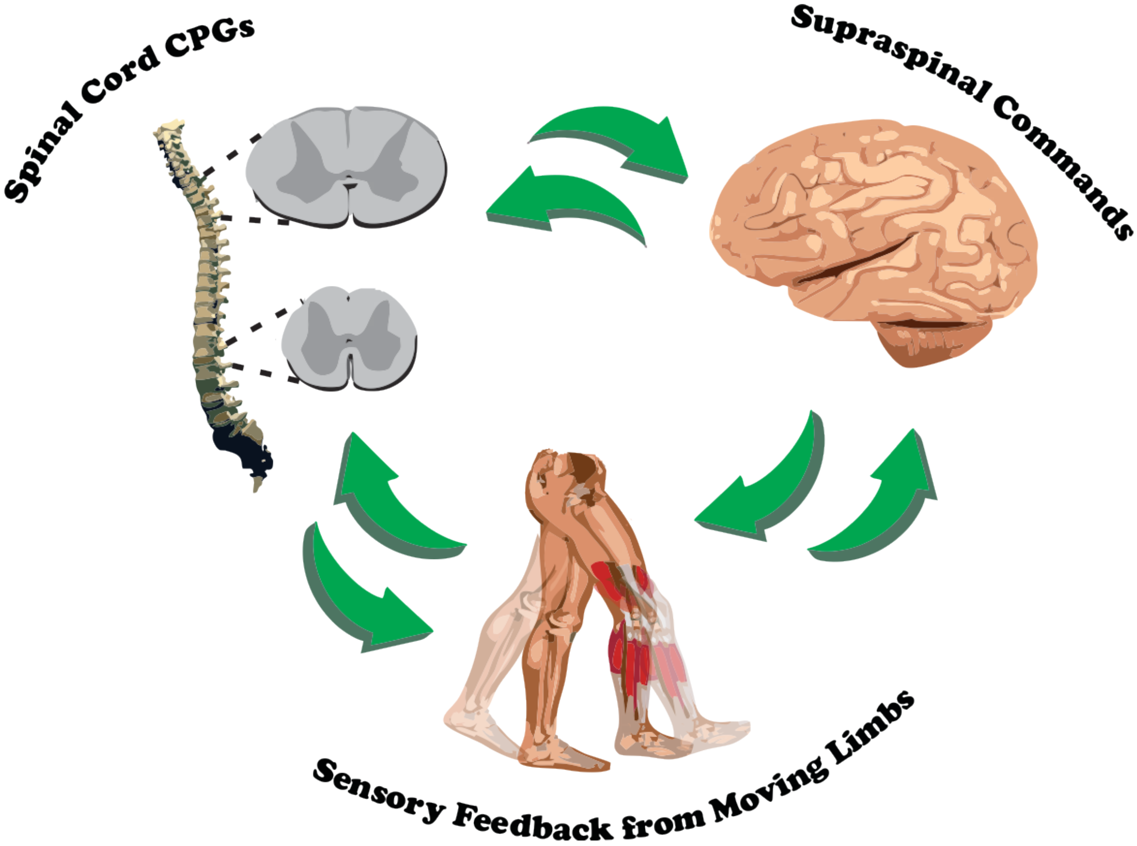

Figure 2-4*: A depiction of the nonhierarchical 3-part system that controls rhythmic behavior in humans. Sensory feedback from the moving limbs primarily arises from afferents innervating the muscles and skin. Supraspinal commands includes inputs from the brainstem, cerebellum, basal ganglia and cortex. Spinal CPGs refers to the networks of spinal interneurons that contribute to rhythmic motor output.*

Conclusion

Humans share many characteristics with other animals, which can be attributed to the evolution of our species from our quadrupedal ancestors. Although the upright nature of bipedal walking is quite unique to humans, the control of our locomotor behavior is indeed controlled by similar structures. Supraspinal commands from the motor cortex, basal ganglia, cerebellum and brainstem contribute to the initiation and overarching control of locomotor behaviors. Central pattern generators in the spinal cord generate rhythmic and alternating locomotor output from the descending input. Sensory feedback from the moving limbs provides important information for phase transitions, regulation of muscle activity and avoiding perturbations to both the spinal cord and supraspinal centers. Sensory feedback from the limbs is shared through interneuronal pathways allowing for impressive coordination between the limbs. Together, the sublime interactions between these neural pathways allow humans to perform complicated motor behaviors with relative ease and little cognitive effort.

References

Aimonetti, J.M., Schmied, A., Vedel, J.P., Pagni, S., 1999. Ia presynaptic inhibition in human wrist extensor muscles: effects of motor task and cutaneous afferent activity. J. Physiol. Paris 93, 395–401.

Aimonetti, J.M., Vedel, J.P., Schmied, A., Pagni, S., 2000a. Mechanical cutaneous stimulation alters Ia presynaptic inhibition in human wrist extensor muscles: a single motor unit study. J Physiol 522 Pt 1, 137–45.

Aimonetti, J.M., Vedel, J.P., Schmied, A., Pagni, S., 2000b. Task dependence of Ia presynaptic inhibition in human wrist extensor muscles: a single motor unit study. Clin Neurophysiol 111, 1165–1174.

Angulo-Kinzler, R.M., Mynark, R.G., Koceja, D.M., 1998. Soleus H-reflex gain in elderly and young adults: modulation due to body position. J. Gerontol. A Biol. Sci. Med. Sci. 53, M120-125.

Awai, L., Curt, A., 2015. Chapter 1 - Comprehensive assessment of walking function after human spinal cord injury, in: Dancause, N., Nadeau, S., Rossignol, S. (Eds.), Progress in Brain Research, Sensorimotor Rehabilitation. Elsevier, pp. 1–14. https://doi.org/10.1016/bs.pbr.2014.12.004

Ballesteros, M.L., Buchthal, F., Rosenfalck, P., 1965. The Pattern of Muscular Activity during the Arm Swing of Natural Walking. Acta Physiol Scand 63, 296–310. https://doi.org/10.1111/j.1748-1716.1965.tb04069.x

Barbeau, H., Ladouceur, M., Norman, K.E., Pépin, A., Leroux, A., 1999. Walking after spinal cord injury: evaluation, treatment, and functional recovery. Arch Phys Med Rehabil 80, 225–235.

Barthélemy, D., Alain, S., Grey, M.J., Nielsen, J.B., Bouyer, L.J., 2012. Rapid changes in corticospinal excitability during force field adaptation of human walking. Exp Brain Res 217, 99–115. https://doi.org/10.1007/s00221-011-2977-4

Barthélemy, D., Grey, M.J., Nielsen, J.B., Bouyer, L., 2011. Involvement of the corticospinal tract in the control of human gait. Prog. Brain Res. 192, 181–197. https://doi.org/10.1016/B978-0-444-53355-5.00012-9

Barthelemy, D., Nielsen, J.B., 2010. Corticospinal contribution to arm muscle activity during human walking. J. Physiol. (Lond.) 588, 967–979. https://doi.org/10.1113/jphysiol.2009.185520

Bastian, A.J., Mink, J.W., Kaufman, B.A., Thach, W.T., 1998. Posterior vermal split syndrome. Ann. Neurol. 44, 601–610. https://doi.org/10.1002/ana.410440405

Berger, W., Dietz, V., Quintern, J., 1984. Corrective reactions to stumbling in man: neuronal co-ordination of bilateral leg muscle activity during gait. J. Physiol. (Lond.) 357, 109–125.

Beyaert, C., Vasa, R., Frykberg, G.E., 2015. Gait post-stroke: Pathophysiology and rehabilitation strategies. Neurophysiol Clin 45, 335–355. https://doi.org/10.1016/j.neucli.2015.09.005

Bonnard, M., Camus, M., Coyle, T., Pailhous, J., 2002. Task-induced modulation of motor evoked potentials in upper-leg muscles during human gait: a TMS study. Eur. J. Neurosci. 16, 2225–2230.

Bouyer, L.J., Rossignol, S., 2003. Contribution of cutaneous inputs from the hindpaw to the control of locomotion. I. Intact cats. J Neurophysiol 90, 3625–39. https://doi.org/10.1152/jn.00496.2003

Brooke, J.D., Cheng, J., Collins, D.F., McIlroy, W.E., Misiaszek, J.E., Staines, W.R., 1997. Sensori-sensory afferent conditioning with leg movement: gain control in spinal reflex and ascending paths. Prog Neurobiol 51, 393–421.

Brooke, J.D., Peritore, G., Staines, W.R., McIlroy, W.E., Nelson, A., 2000. Upper limb H reflexes and somatosensory evoked potentials modulated by movement. J Electromyogr Kinesiol 10, 211–215.

Brouwer, B., Ashby, P., 1992. Corticospinal projections to lower limb motoneurons in man. Exp Brain Res 89, 649–654.

Brown, T.G., 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. (Lond.) 48, 18–46.

Brown, T.G., 1911. The intrinsic factors in the act of progression in the mammal. Proceedings of the Royal Society 84, 308–319.

Brunet, M., Guy, F., Pilbeam, D., Mackaye, H.T., Likius, A., Ahounta, D., Beauvilain, A., Blondel, C., Bocherens, H., Boisserie, J.-R., Bonis, L.D., Coppens, Y., Dejax, J., Denys, C., Duringer, P., Eisenmann, V., Fanone, G., Fronty, P., Geraads, D., Lehmann, T., Lihoreau, F., Louchart, A., Mahamat, A., Merceron, G., Mouchelin, G., Otero, O., Campomanes, P.P., Leon, M.P.D., Rage, J.-C., Sapanet, M., Schuster, M., Sudre, J., Tassy, P., Valentin, X., Vignaud, P., Viriot, L., Zazzo, A., Zollikofer, C., 2002. A new hominid from the Upper Miocene of Chad, Central Africa. Nature 418, 145. https://doi.org/10.1038/nature00879

Buford, J.A., Smith, J.L., 1990. Adaptive control for backward quadrupedal walking. II. Hindlimb muscle synergies. Journal of Neurophysiology 64, 756–766. https://doi.org/10.1152/jn.1990.64.3.756

Burke, R.E., 2001. The central pattern generator for locomotion in mammals. Adv Neurol 87, 11–24.

Bussel, B., Roby-Brami, A., Azouvi, P., Biraben, A., Yakovleff, A., Held, J.P., 1988. Myoclonus in a patient with spinal cord transection. Possible involvement of the spinal stepping generator. Brain 111 (Pt 5), 1235–1245.

Calancie, B., 2006. Spinal Myoclonus After Spinal Cord Injury. J Spinal Cord Med 29, 413–424.

Calancie, B., Needham-Shropshire, B., Jacobs, P., Willer, K., Zych, G., Green, B.A., 1994. Involuntary stepping after chronic spinal cord injury: Evidence for a central rhythm generator for locomotion in man. Brain 117, 1143–1159. https://doi.org/10.1093/brain/117.5.1143

Capaday, C., 2002. The special nature of human walking and its neural control. Trends Neurosci. 25, 370–376.

Capaday, C., Lavoie, B.A., Barbeau, H., Schneider, C., Bonnard, M., 1999. Studies on the Corticospinal Control of Human Walking. I. Responses to Focal Transcranial Magnetic Stimulation of the Motor Cortex. Journal of Neurophysiology 81, 129–139. https://doi.org/10.1152/jn.1999.81.1.129

Capaday, C., Stein, R.B., 1986. Amplitude modulation of the soleus H-reflex in the human during walking and standing. J Neurosci 6, 1308–13.

Carroll, T.J., Zehr, E.P., Collins, D.F., 2005. Modulation of cutaneous reflexes in human upper limb muscles during arm cycling is independent of activity in the contralateral arm. Experimental brain research 161, 133–44. https://doi.org/10.1007/s00221-004-2050-7

Cheng, J., Brooke, J.D., Misiaszek, J.E., Staines, W.R., 1998. Crossed inhibition of the soleus H reflex during passive pedalling movement. Brain Res. 779, 280–284.

Choi, J.T., Bastian, A.J., 2007. Adaptation reveals independent control networks for human walking. Nat. Neurosci. 10, 1055–1062. https://doi.org/10.1038/nn1930

Christensen, L.O., Johannsen, P., Sinkjaer, T., Petersen, N., Pyndt, H.S., Nielsen, J.B., 2000. Cerebral activation during bicycle movements in man. Exp Brain Res 135, 66–72.

Clower, W.T., 1998. Early Contributions to the Reflex Chain Hypothesis. Journal of the History of the Neurosciences 7, 32–42. https://doi.org/10.1076/jhin.7.1.32.13099

Collins, D.F., McIlroy, W.E., Brooke, J.D., 1993. Contralateral inhibition of soleus H reflexes with different velocities of passive movement of the opposite leg. Brain Res 603, 96–101.

Comber, L., Galvin, R., Coote, S., 2017. Gait deficits in people with multiple sclerosis: A systematic review and meta-analysis. Gait Posture 51, 25–35. https://doi.org/10.1016/j.gaitpost.2016.09.026

Cranefield, P.F., 1974. The Way in and the way out: François Magendie, Charles Bell, and the roots of the spinal nerves : with a facsim. of Charles Bell's annotated copy of ... of the brain (The History of medicine), The History of Medicine. Futura Pub. Co.

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

Danner, S.M., Hofstoetter, U.S., Freundl, B., Binder, H., Mayr, W., Rattay, F., Minassian, K., 2015. Human spinal locomotor control is based on flexibly organized burst generators. Brain 138, 577–588. https://doi.org/10.1093/brain/awu372

Darwin, C., 1872. The expression of emotions in man and animals. John Murray, London.

Darwin, C., 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life, first edition. ed. John Murray, London.

Davey, N.J., Romaiguère, P., Maskill, D.W., Ellaway, P.H., 1994. Suppression of voluntary motor activity revealed using transcranial magnetic stimulation of the motor cortex in man. J. Physiol. (Lond.) 477, 223–235.

de Ruiter, G.C., Hundza, S.R., Zehr, E.P., 2010. Phase-dependent modulation of soleus H-reflex amplitude induced by rhythmic arm cycling. Neurosci Lett 475, 7–11. https://doi.org/10.1016/j.neulet.2010.03.025

de Vries, J.I.P., Visser, G.H.A., Prechtl, H.F.R., 1986. Fetal behaviour in early pregnancy. European Journal of Obstetrics & Gynecology and Reproductive Biology, Van Deventer Symposium 21, 271–276. https://doi.org/10.1016/0028-2243(86)90003-1

Dietz, V., 2009. Body weight supported gait training: From laboratory to clinical setting. Brain Research Bulletin, Special Issue: Gait recovery after spinal cord injury. From bench to bed 78, I–VI. https://doi.org/10.1016/S0361-9230(08)00410-3

Dietz, V., 2002. Do human bipeds use quadrupedal coordination? Trends Neurosci 25, 462–7.

Dietz, V., Quintern, J., Berger, W., 1986. Stumbling reactions in man: release of a ballistic movement pattern. Brain Res. 362, 355–357.

Dietz, V., Quintern, J., Berger, W., 1984. Corrective reactions to stumbling in man: functional significance of spinal and transcortical reflexes. Neurosci Lett 44, 131–5.

Dimitrijevic, M.R., Danner, S.M., Mayr, W., 2015. Neurocontrol of Movement in Humans With Spinal Cord Injury. Artificial Organs 39, 823–833. https://doi.org/10.1111/aor.12614

Dimitrijevic, M.R., Gerasimenko, Y., Pinter, M.M., 1998. Evidence for a spinal central pattern generator in humans. Ann. N. Y. Acad. Sci. 860, 360–376.

Dobkin, B.H., Harkema, S., Requejo, P., Edgerton, V.R., 1995. Modulation of locomotor-like EMG activity in subjects with complete and incomplete spinal cord injury. Neurorehabil. Neural Repair 9, 183–190.

Dominici, N., Ivanenko, Y.P., Cappellini, G., d'Avella, A., Mondì, V., Cicchese, M., Fabiano, A., Silei, T., Di Paolo, A., Giannini, C., Poppele, R.E., Lacquaniti, F., 2011. Locomotor primitives in newborn babies and their development. Science 334, 997–999. https://doi.org/10.1126/science.1210617

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

Drew, T., Jiang, W., Kably, B., Lavoie, S., 1996. Role of the motor cortex in the control of visually triggered gait modifications. Can. J. Physiol. Pharmacol. 74, 426–442.

Drew, T., Marigold, D.S., 2015. Taking the next step: cortical contributions to the control of locomotion. Curr. Opin. Neurobiol. 33, 25–33. https://doi.org/10.1016/j.conb.2015.01.011

Duysens, J., Bastiaanse, C.M., Smits-Engelsman, B.C., Dietz, V., 2004. Gait acts as a gate for reflexes from the foot. Can J Physiol Pharmacol 82, 715–22. https://doi.org/10.1139/y04-071

Duysens, J., Pearson, K.G., 1980. Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. Brain Res. 187, 321–332.

Duysens, J., Pearson, K.G., 1976. The role of cutaneous afferents from the distal hindlimb in the regulation of the step cycle of thalamic cats. Exp Brain Res 24, 245–55.

Duysens, J., Tax, A.A., Murrer, L., Dietz, V., 1996. Backward and forward walking use different patterns of phase-dependent modulation of cutaneous reflexes in humans. J Neurophysiol 76, 301–10.

Duysens, J., Tax, A.A., Trippel, M., Dietz, V., 1993. Increased amplitude of cutaneous reflexes during human running as compared to standing. Brain Res 613, 230–8.

Duysens, J., Tax, A.A., Trippel, M., Dietz, V., 1992. Phase-dependent reversal of reflexly induced movements during human gait. Exp Brain Res 90, 404–14.

Duysens, J., Trippel, M., Horstmann, G.A., Dietz, V., 1990. Gating and reversal of reflexes in ankle muscles during human walking. Exp Brain Res 82, 351–358.

Duysens, J., Van de Crommert, H.W., 1998. Neural control of locomotion; The central pattern generator from cats to humans. Gait Posture 7, 131–141.

Earhart, G.M., Bastian, A.J., 2001. Selection and coordination of human locomotor forms following cerebellar damage. J. Neurophysiol. 85, 759–769. https://doi.org/10.1152/jn.2001.85.2.759

Edgley, S.A., Eyre, J.A., Lemon, R.N., Miller, S., 1990. Excitation of the corticospinal tract by electromagnetic and electrical stimulation of the scalp in the macaque monkey. J. Physiol. (Lond.) 425, 301–320.

Eidelberg, E., Walden, J.G., Nguyen, L.H., 1981. Locomotor control in macaque monkeys. Brain 104, 647–663.

Eng, J.J., Winter, D.A., Patla, A.E., 1994. Strategies for recovery from a trip in early and late swing during human walking. Exp Brain Res 102, 339–349.

Erni, T., Dietz, V., 2001. Obstacle avoidance during human walking: learning rate and cross-modal transfer. J. Physiol. (Lond.) 534, 303–312.

Evans, C., Milner, A.D., Humphreys, G.W., Cavina-Pratesi, C., 2013. Optic ataxia affects the lower limbs: Evidence from a single case study. Cortex 49, 1229–1240. https://doi.org/10.1016/j.cortex.2012.07.008

Fedirchuk, B., Nielsen, J., Petersen, N., Hultborn, H., 1998. Pharmacologically evoked fictive motor patterns in the acutely spinalized marmoset monkey (Callithrix jacchus). Exp Brain Res 122, 351–361.

Ferris, D.P., Huang, H.J., Kao, P.C., 2006. Moving the arms to activate the legs. Exerc Sport Sci Rev 34, 113–20.

Forssberg, H., 1985. Ontogeny of human locomotor control. I. Infant stepping, supported locomotion and transition to independent locomotion. Exp Brain Res 57, 480–493.

Forssberg, H., 1979. Stumbling corrective reaction: a phase-dependent compensatory reaction during locomotion. J Neurophysiol 42, 936–53.

Forssberg, H., Grillner, S., Rossignol, S., 1977. Phasic gain control of reflexes from the dorsum of the paw during spinal locomotion. Brain Res 132, 121–39.

Forssberg, H., Grillner, S., Rossignol, S., 1975. Phase dependent reflex reversal during walking in chronic spinal cats. Brain Res 85, 103–7.

Fouad, K., Pearson, K., 2004. Restoring walking after spinal cord injury. Progress in Neurobiology 73, 107–126. https://doi.org/10.1016/j.pneurobio.2004.04.003

Fouad, K., Pearson, K.G., 1997. Effects of extensor muscle afferents on the timing of locomotor activity during walking in adult rats. Brain Res. 749, 320–328. https://doi.org/10.1016/S0006-8993(96)01328-5

Frigon, A., 2017. The neural control of interlimb coordination during mammalian locomotion. J Neurophysiol 117, 2224–2241. https://doi.org/10.1152/jn.00978.2016

Frigon, A., 2012. Central pattern generators of the mammalian spinal cord. Neuroscientist 18, 56–69. https://doi.org/10.1177/1073858410396101

Frigon, A., Collins, D.F., Zehr, E.P., 2004. Effect of rhythmic arm movement on reflexes in the legs: modulation of soleus H-reflexes and somatosensory conditioning. J Neurophysiol 91, 1516–23. https://doi.org/10.1152/jn.00695.2003

Frigon, A., Hurteau, M.-F., Thibaudier, Y., Leblond, H., Telonio, A., D'Angelo, G., 2013. Split-belt walking alters the relationship between locomotor phases and cycle duration across speeds in intact and chronic spinalized adult cats. J. Neurosci. 33, 8559–8566. https://doi.org/10.1523/JNEUROSCI.3931-12.2013

Frigon, A., Thibaudier, Y., Hurteau, M.-F., 2015. Modulation of forelimb and hindlimb muscle activity during quadrupedal tied-belt and split-belt locomotion in intact cats. Neuroscience 290, 266–278. https://doi.org/10.1016/j.neuroscience.2014.12.084

Fukuyama, H., Ouchi, Y., Matsuzaki, S., Nagahama, Y., Yamauchi, H., Ogawa, M., Kimura, J., Shibasaki, H., 1997. Brain functional activity during gait in normal subjects: a SPECT study. Neurosci. Lett. 228, 183–186.

Fung, J., Stewart, J.E., Barbeau, H., 1990. The combined effects of clonidine and cyproheptadine with interactive training on the modulation of locomotion in spinal cord injured subjects. J. Neurol. Sci. 100, 85–93.

Garcia-Rill, E., Kinjo, N., Atsuta, Y., Ishikawa, Y., Webber, M., Skinner, R.D., 1990. Posterior midbrain-induced locomotion. Brain Research Bulletin 24, 499–508. https://doi.org/10.1016/0361-9230(90)90103-7

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

Gerasimenko, Y., Avelev, V., Anissimova, N., Dubrovin, V., Makaravsky, A., Nikitin, O., 1997. Motor control functions of the spinal cord in paraplegic patients and in cats with various lesions in the CNS, in: Gurfinkel, V.S., Levik, Y.S. (Eds.), Proceedings of the International Symposium on Brain and Movement. Moscow, p. 79.

Gerasimenko, Y., Gorodnichev, R., Machueva, E., Pivovarova, E., Semyenov, D., Savochin, A., Roy, R.R., Edgerton, V.R., 2010. Novel and direct access to the human locomotor spinal circuitry. J. Neurosci. 30, 3700–3708. https://doi.org/10.1523/JNEUROSCI.4751-09.2010

Gerasimenko, Y., Gorodnichev, R., Moshonkina, T., Sayenko, D., Gad, P., Reggie Edgerton, V., 2015a. Transcutaneous electrical spinal-cord stimulation in humans. Ann Phys Rehabil Med 58, 225–231. https://doi.org/10.1016/j.rehab.2015.05.003

Gerasimenko, Y., Gorodnichev, R., Puhov, A., Moshonkina, T., Savochin, A., Selionov, V., Roy, R.R., Lu, D.C., Edgerton, V.R., 2015b. Initiation and modulation of locomotor circuitry output with multisite transcutaneous electrical stimulation of the spinal cord in noninjured humans. J. Neurophysiol. 113, 834–842. https://doi.org/10.1152/jn.00609.2014

Gernandt, B.E., Megirian, D., 1961. Ascending propriospinal mechanisms. J. Neurophysiol. 24, 364–376. https://doi.org/10.1152/jn.1961.24.4.364

Gernandt, B.E., Shimamura, M., 1961. Mechanisms of interlimb reflexes in cat. J. Neurophysiol. 24, 665–676. https://doi.org/10.1152/jn.1961.24.6.665

Gorodnichev, R.M., Pivovarova, E.A., Pukhov, A., Moiseev, S.A., Savokhin, A.A., Moshonkina, T.R., Shcherbakova, N.A., Kilimnik, V.A., Selionov, V.A., Kozlovskaia, I.B., Edgerton, V.R., Gerasimenko, I.P., 2012. [Transcutaneous electrical stimulation of the spinal cord: non-invasive tool for activation of locomotor circuitry in human]. Fiziol Cheloveka 38, 46–56.

Grillner, S., 2011a. Control of Locomotion in Bipeds, Tetrapods, and Fish, in: Comprehensive Physiology. American Cancer Society, pp. 1179–1236. https://doi.org/10.1002/cphy.cp010226

Grillner, S., 2011b. Human Locomotor Circuits Conform. Science 334, 912–913. https://doi.org/10.1126/science.1214778

Grillner, S., 2006a. Biological pattern generation: the cellular and computational logic of networks in motion. Neuron 52, 751–766. https://doi.org/10.1016/j.neuron.2006.11.008

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

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

Gruss, L.T., Gruss, R., Schmitt, D., 2017. Pelvic Breadth and Locomotor Kinematics in Human Evolution. The Anatomical Record 300, 739–751. https://doi.org/10.1002/ar.23550

Guertin, P., Angel, M.J., Perreault, M.C., McCrea, D.A., 1995. Ankle extensor group I afferents excite extensors throughout the hindlimb during fictive locomotion in the cat. J. Physiol. (Lond.) 487, 197–209.

Guertin, P.A., Ung, R.-V., Rouleau, P., 2010. Oral administration of a tri-therapy for central pattern generator activation in paraplegic mice: proof-of-concept of efficacy. Biotechnol J 5, 421–426. https://doi.org/10.1002/biot.200900278

Guertin, P.A., Ung, R.-V., Rouleau, P., Steuer, I., 2011. Effects on locomotion, muscle, bone, and blood induced by a combination therapy eliciting weight-bearing stepping in nonassisted spinal cord-transected mice. Neurorehabil Neural Repair 25, 234–242. https://doi.org/10.1177/1545968310378753

Gurfinkel, V.S., Levik, Y.S., Kazennikov, O.V., Selionov, V.A., 1998. Locomotor-like movements evoked by leg muscle vibration in humans. European Journal of Neuroscience 10, 1608–1612. https://doi.org/10.1046/j.1460-9568.1998.00179.x

Haile-Selassie, Y., 2001. Late Miocene hominids from the Middle Awash, Ethiopia. Nature 412, 178. https://doi.org/10.1038/35084063

Halliday, D.M., Conway, B.A., Christensen, L.O.D., Hansen, N.L., Petersen, N.P., Nielsen, J.B., 2003. Functional Coupling of Motor Units Is Modulated During Walking in Human Subjects. Journal of Neurophysiology 89, 960–968. https://doi.org/10.1152/jn.00844.2002

Haridas, C., Zehr, E.P., 2003. Coordinated interlimb compensatory responses to electrical stimulation of cutaneous nerves in the hand and foot during walking. Journal of neurophysiology 90, 2850–61. https://doi.org/10.1152/jn.00531.2003

Haridas, C., Zehr, E.P., Misiaszek, J.E., 2005. Postural uncertainty leads to dynamic control of cutaneous reflexes from the foot during human walking. Brain research 1062, 48–62. https://doi.org/10.1016/j.brainres.2005.09.003

Harkema, S.J., 2008. Plasticity of interneuronal networks of the functionally isolated human spinal cord. Brain Res Rev 57, 255–264. https://doi.org/10.1016/j.brainresrev.2007.07.012

Hathout, G.M., Bhidayasiri, R., 2005. Midbrain ataxia: an introduction to the mesencephalic locomotor region and the pedunculopontine nucleus. AJR Am J Roentgenol 184, 953–956. https://doi.org/10.2214/ajr.184.3.01840953

Holiga, Š., Mueller, K., Möller, H.E., Urgošík, D., Růžička, E., Schroeter, M.L., Jech, R., 2015. Resting-state functional magnetic resonance imaging of the subthalamic microlesion and stimulation effects in Parkinson's disease: Indications of a principal role of the brainstem. Neuroimage Clin 9, 264–274. https://doi.org/10.1016/j.nicl.2015.08.008

Huang, H.J., Ferris, D.P., 2004. Neural coupling between upper and lower limbs during recumbent stepping. J Appl Physiol (1985) 97, 1299–308. https://doi.org/10.1152/japplphysiol.01350.2003

Hughes, S., Gardner-Thorpe, C., 2018. Charles Bell (1774-1842) and Natural Theology. J Med Biogr 967772018790736. https://doi.org/10.1177/0967772018790736

Hultborn, H., Nielsen, J.B., 2007. Spinal control of locomotion--from cat to man. Acta Physiol (Oxf) 189, 111–121. https://doi.org/10.1111/j.1748-1716.2006.01651.x

Hundza, S.R., de Ruiter, G.C., Klimstra, M., Zehr, E.P., 2012. Effect of afferent feedback and central motor commands on soleus H-reflex suppression during arm cycling. J Neurophysiol 108, 3049–58. https://doi.org/10.1152/jn.00485.2011

Hundza, S.R., Zehr, E.P., 2009. Suppression of soleus H-reflex amplitude is graded with frequency of rhythmic arm cycling. Exp Brain Res 193, 297–306. https://doi.org/10.1007/s00221-008-1625-0

Hundza, S.R., Zehr, E.P., 2006. Cutaneous reflexes during rhythmic arm cycling are insensitive to asymmetrical changes in crank length. Experimental brain research 168, 165–77. https://doi.org/10.1007/s00221-005-0089-8

Iwahara, T., Atsuta, Y., Garcia-Rill, E., Skinner, R.D., 1992. Spinal cord stimulation-induced locomotion in the adult cat. Brain Res. Bull. 28, 99–105.

Jahn, K., Deutschländer, A., Stephan, T., Kalla, R., Hüfner, K., Wagner, J., Strupp, M., Brandt, T., 2008a. Supraspinal locomotor control in quadrupeds and humans. Prog. Brain Res. 171, 353–362. https://doi.org/10.1016/S0079-6123(08)00652-3

Jahn, K., Deutschländer, A., Stephan, T., Kalla, R., Wiesmann, M., Strupp, M., Brandt, T., 2008b. Imaging human supraspinal locomotor centers in brainstem and cerebellum. Neuroimage 39, 786–792. https://doi.org/10.1016/j.neuroimage.2007.09.047

Jensen, P., Jensen, N.J., Terkildsen, C.U., Choi, J.T., Nielsen, J.B., Geertsen, S.S., 2018. Increased central common drive to ankle plantar flexor and dorsiflexor muscles during visually guided gait. Physiol Rep 6. https://doi.org/10.14814/phy2.13598

Jordan, L.M., Liu, J., Hedlund, P.B., Akay, T., Pearson, K.G., 2008. Descending command systems for the initiation of locomotion in mammals. Brain Res Rev 57, 183–191. https://doi.org/10.1016/j.brainresrev.2007.07.019

Juvin, L., Simmers, J., Morin, D., 2005. Propriospinal circuitry underlying interlimb coordination in mammalian quadrupedal locomotion. J. Neurosci. 25, 6025–6035. https://doi.org/10.1523/JNEUROSCI.0696-05.2005

Kaupp, C., Pearcey, G.E.P., Klarner, T., Sun, Y., Cullen, H., Barss, T.S., Zehr, E.P., 2018. Rhythmic arm cycling training improves walking and neurophysiological integrity in chronic stroke: the arms can give legs a helping hand in rehabilitation. J Neurophysiol 119, 1095–1112. https://doi.org/10.1152/jn.00570.2017

Kiehn, O., 2011. Development and functional organization of spinal locomotor circuits. Curr. Opin. Neurobiol. 21, 100–109. https://doi.org/10.1016/j.conb.2010.09.004

Kimbel William H., Villmoare Brian, 2016. From Australopithecus to Homo: the transition that wasn't†. Philosophical Transactions of the Royal Society B: Biological Sciences 371, 20150248. https://doi.org/10.1098/rstb.2015.0248

Kimura, T., Okada, M., Ishida, H., 1979. Kinesiological characteristics of primate walking: Its significance in human walking, in: Morbeck, M.E., Preuschoft, H., Gomberg, N. (Eds.), Environment, Behavior, and Morphology: Dynamic Interactions in Primates. Gustav Fischer, New York, pp. 297–311.

Klarner, T., Barss, T.S., Sun, Y., Kaupp, C., Loadman, P.M., Zehr, E.P., 2016. Long-Term Plasticity in Reflex Excitability Induced by Five Weeks of Arm and Leg Cycling Training after Stroke. Brain Sci 6. https://doi.org/10.3390/brainsci6040054

Klarner, T., Barss, T.S...Sun, Y...Kaupp, C...Loadman, P.M...Zehr, E.P., 2016. Exploiting interlimb arm and leg connections for walking rehabilitation: a training intervention in stroke. Neural Plasticity In Press.

Klarner, T., Zehr, E.P., 2018. Sherlock Holmes and the curious case of the human locomotor central pattern generator. J. Neurophysiol. 120, 53–77. https://doi.org/10.1152/jn.00554.2017

Ko, K.H., 2015. Origins of Bipedalism. Brazilian Archives of Biology and Technology 58, 929–934. https://doi.org/10.1590/S1516-89132015060399

Koceja, D.M., Markus, C.A., Trimble, M.H., 1995. Postural modulation of the soleus H reflex in young and old subjects. Electroencephalogr Clin Neurophysiol 97, 387–393.

Koceja, D.M., Trimble, M.H., Earles, D.R., 1993. Inhibition of the soleus H-reflex in standing man. Brain Res. 629, 155–158.

Komiyama, T., Zehr, E.P., Stein, R.B., 2000. Absence of nerve specificity in human cutaneous reflexes during standing. Experimental brain research 133, 267–72.

Kuhtz-Buschbeck, J.P., Jing, B., 2012. Activity of upper limb muscles during human walking. J Electromyogr Kinesiol 22, 199–206. https://doi.org/10.1016/j.jelekin.2011.08.014

Lajoie, K., Bloomfield, L.W., Nelson, F.J., Suh, J.J., Marigold, D.S., 2012. The contribution of vision, proprioception, and efference copy in storing a neural representation for guiding trail leg trajectory over an obstacle. J. Neurophysiol. 107, 2283–2293. https://doi.org/10.1152/jn.00756.2011

Lam, T., Wolstenholme, C., van der Linden, M., Pang, M.Y., Yang, J.F., 2003. Stumbling corrective responses during treadmill-elicited stepping in human infants. J Physiol 553, 319–31. https://doi.org/10.1113/jphysiol.2003.043984

Lamb, T., Yang, J.F., 2000. Could different directions of infant stepping be controlled by the same locomotor central pattern generator? J Neurophysiol 83, 2814–24.

Lamont, E.V., Zehr, E.P., 2007. Earth-referenced handrail contact facilitates interlimb cutaneous reflexes during locomotion. Journal of neurophysiology 98, 433–42. https://doi.org/10.1152/jn.00002.2007

Lamont, E.V., 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. https://doi.org/10.1007/s00221-006-0586-4

Langford, D.J., Bailey, A.L., Chanda, M.L., Clarke, S.E., Drummond, T.E., Echols, S., Glick, S., Ingrao, J., Klassen-Ross, T., LaCroix-Fralish, M.L., Matsumiya, L., Sorge, R.E., Sotocinal, S.G., Tabaka, J.M., Wong, D., van den Maagdenberg, A.M.J.M., Ferrari, M.D., Craig, K.D., Mogil, J.S., 2010. Coding of facial expressions of pain in the laboratory mouse. Nature Methods 7, 447–449. https://doi.org/10.1038/nmeth.1455

Lavater, J.K., 1804. Essays on physiognomy; for the promotion of the knowledge and the love of mankind. printed by C. Whittingham, for H.D. Symonds.

Lemon, R.N., 2008. Descending pathways in motor control. Annu. Rev. Neurosci. 31, 195–218. https://doi.org/10.1146/annurev.neuro.31.060407.125547

Lhermitte, J., 1919. La section totale de la moelle dorsale,. Imprimerie Vve Tardy-Pigelet et fils, Bourges.

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

Lovejoy, C.O., 1988. Evolution of human walking. Sci. Am. 259, 118–125.

Marigold, D.S., 2019. Working Memory: Why You Didn’t Trip on that Rock. Curr. Biol. 29, R25–R27. https://doi.org/10.1016/j.cub.2018.09.046

Masdeu, J.C., Alampur, U., Cavaliere, R., Tavoulareas, G., 1994. Astasia and gait failure with damage of the pontomesencephalic locomotor region. Annals of Neurology 35, 619–621. https://doi.org/10.1002/ana.410350517

Matsukawa, K., Kamei, H., Minoda, K., Udo, M., 1982. Interlimb coordination in cat locomotion investigated with perturbation. I. Behavioral and electromyographic study on symmetric limbs of decerebrate and awake walking cats. Exp Brain Res 46, 425–437.

McCrimmon, C.M., Wang, P.T., Heydari, P., Nguyen, A., Shaw, S.J., Gong, H., Chui, L.A., Liu, C.Y., Nenadic, Z., Do, A.H., 2018. Electrocorticographic Encoding of Human Gait in the Leg Primary Motor Cortex. Cerebral Cortex 28, 2752–2762. https://doi.org/10.1093/cercor/bhx155

McDonald, J.W., Sadowsky, C., 2002. Spinal-cord injury. The Lancet 359, 417–425. https://doi.org/10.1016/S0140-6736(02)07603-1

McIlroy, W.E., Collins, D.F., Brooke, J.D., 1992. Movement features and H-reflex modulation. II. Passive rotation, movement velocity and single leg movement. Brain Res 582, 85–93.

McVea, D.A., Donelan, J.M., Tachibana, A., Pearson, K.G., 2005. A Role for Hip Position in Initiating the Swing-to-Stance Transition in Walking Cats. Journal of Neurophysiology 94, 3497–3508. https://doi.org/10.1152/jn.00511.2005

Minassian, K., Hofstoetter, U.S., Dzeladini, F., Guertin, P.A., Ijspeert, A., 2017. The Human Central Pattern Generator for Locomotion: Does It Exist and Contribute to Walking? Neuroscientist 23, 649–663. https://doi.org/10.1177/1073858417699790

Minassian, K., Jilge, B., Rattay, F., Pinter, M.M., Binder, H., Gerstenbrand, F., Dimitrijevic, M.R., 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 42, 401–416. https://doi.org/10.1038/sj.sc.3101615

Minassian, K., Persy, I., Rattay, F., Pinter, M.M., Kern, H., Dimitrijevic, M.R., 2007. Human lumbar cord circuitries can be activated by extrinsic tonic input to generate locomotor-like activity. Hum Mov Sci 26, 275–295. https://doi.org/10.1016/j.humov.2007.01.005

Mishina, M., Senda, M., Ishii, K., Ohyama, M., Kitamura, S., Katayama, Y., 1999. Cerebellar activation during ataxic gait in olivopontocerebellar atrophy: a PET study. Acta Neurol. Scand. 100, 369–376.

Misiaszek, J.E., Cheng, J., Brooke, J.D., Staines, W.R., 1998. Movement-induced modulation of soleus H reflexes with altered length of biarticular muscles. Brain Res. 795, 25–36.

Miyai, I., Tanabe, H.C., Sase, I., Eda, H., Oda, I., Konishi, I., Tsunazawa, Y., Suzuki, T., Yanagida, T., Kubota, K., 2001. Cortical mapping of gait in humans: a near-infrared spectroscopic topography study. Neuroimage 14, 1186–1192. https://doi.org/10.1006/nimg.2001.0905

Morton, S.M., Bastian, A.J., 2007. Mechanisms of cerebellar gait ataxia. Cerebellum 6, 79–86. https://doi.org/10.1080/14734220601187741

Morton, S.M., Bastian, A.J., 2006. Cerebellar contributions to locomotor adaptations during splitbelt treadmill walking. J. Neurosci. 26, 9107–9116. https://doi.org/10.1523/JNEUROSCI.2622-06.2006

Morton, S.M., Bastian, A.J., 2004. Cerebellar control of balance and locomotion. Neuroscientist 10, 247–259. https://doi.org/10.1177/1073858404263517

Moshonkina, T.R., Shapkova, E.Y., Sukhotina, I.A., Emeljannikov, D.V., Gerasimenko, Y.P., 2016. Effect of Combination of Non-Invasive Spinal Cord Electrical Stimulation and Serotonin Receptor Activation in Patients with Chronic Spinal Cord Lesion. Bull. Exp. Biol. Med. 161, 749–754. https://doi.org/10.1007/s10517-016-3501-4

Murray, M.P., 1967. Gait as a total pattern of movement. Am J Phys Med 46, 290–333.

Mynark, R.G., Koceja, D.M., 1997. Comparison of soleus H-reflex gain from prone to standing in dancers and controls. Electroencephalogr Clin Neurophysiol 105, 135–140.

Nadeau, S., Jacquemin, G., Fournier, C., Lamarre, Y., Rossignol, S., 2010. Spontaneous Motor Rhythms of the Back and Legs in a Patient With a Complete Spinal Cord Transection. Neurorehabilitation and Neural Repair 24, 377–383. https://doi.org/10.1177/1545968309349945

Nakajima, T., Mezzarane, R.A., Hundza, S.R., Komiyama, T., Zehr, E.P., 2014. Convergence in reflex pathways from multiple cutaneous nerves innervating the foot depends upon the number of rhythmically active limbs during locomotion. PLoS One 9, e104910. https://doi.org/10.1371/journal.pone.0104910

Nakajima, T., Suzuki, S...Futatsubashi, G...Ohtsuska, H...Mezzarane, R.A...Barss, T.S...Klarner, T...Zehr, E.P...Komiyama, T., 2016. Regionally distinct cutaneous afferent populations contribute to reflex modulation evoked by stimulation of the tibial nerve during walking. J Neurophysiol jn 01011 2015. https://doi.org/10.1152/jn.01011.2015

Neagu, B., Tsang, E., Mazzella, F., Hamani, C., Moro, E., Hodaie, M., Lozano, A.M., Chen, R., 2013. Pedunculopontine nucleus evoked potentials from subthalamic nucleus stimulation in Parkinson's disease. Exp. Neurol. 250, 221–227. https://doi.org/10.1016/j.expneurol.2013.09.018

Nicolas, G., Marchand-Pauvert, V., Burke, D., Pierrot-Deseilligny, E., 2001. Corticospinal excitation of presumed cervical propriospinal neurones and its reversal to inhibition in humans. J. Physiol. (Lond.) 533, 903–919.

Nielsen, J.B., 2003. How we walk: central control of muscle activity during human walking. Neuroscientist 9, 195–204. https://doi.org/10.1177/1073858403009003012

Okamoto, T., Okamoto, K., Andrew, P.D., 2003. Electromyographic developmental changes in one individual from newborn stepping to mature walking. Gait Posture 17, 18–27.

Okamoto, T., Okamoto, K., Andrew, P.D., 2001. Electromyographic study of newborn stepping in neonates and young infants. Electromyogr Clin Neurophysiol 41, 289–296.

Palliyath, S., Hallett, M., Thomas, S.L., Lebiedowska, M.K., 1998. Gait in patients with cerebellar ataxia. Mov. Disord. 13, 958–964. https://doi.org/10.1002/mds.870130616

Pang, M.Y., Yang, J.F., 2000. The initiation of the swing phase in human infant stepping: importance of hip position and leg loading. J. Physiol. (Lond.) 528 Pt 2, 389–404.

Pang, M.Y.C., Yang, J.F., 2002. Sensory Gating for the Initiation of the Swing Phase in Different Directions of Human Infant Stepping. J. Neurosci. 22, 5734–5740. https://doi.org/10.1523/JNEUROSCI.22-13-05734.2002

Pearson, K.G., 2004. Generating the walking gait: role of sensory feedback. Prog. Brain Res. 143, 123–129. https://doi.org/10.1016/S0079-6123(03)43012-4

Pearson, K.G., Collins, D.F., 1993. Reversal of the influence of group Ib afferents from plantaris on activity in medial gastrocnemius muscle during locomotor activity. J. Neurophysiol. 70, 1009–1017. https://doi.org/10.1152/jn.1993.70.3.1009

Pearson, K.G., Misiaszek, J.E., Fouad, K., 1998. Enhancement and Resetting of Locomotor Activity by Muscle Afferentsa. Annals of the New York Academy of Sciences 860, 203–215. https://doi.org/10.1111/j.1749-6632.1998.tb09050.x

Petersen, N.T., Butler, J.E., Marchand-Pauvert, V., Fisher, R., Ledebt, A., Pyndt, H.S., Hansen, N.L., Nielsen, J.B., 2001. Suppression of EMG activity by transcranial magnetic stimulation in human subjects during walking. J. Physiol. (Lond.) 537, 651–656.

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

Pierrot-Deseilligny, E., 1996. Transmission of the cortical command for human voluntary movement through cervical propriospinal premotoneurons. Progress in Neurobiology 48, 489–517. https://doi.org/10.1016/0301-0082(96)00002-0

Plaha, P., Gill, S.S., 2005. Bilateral deep brain stimulation of the pedunculopontine nucleus for Parkinson’s disease. Neuroreport 16, 1883–1887.

Power, K.E., Lockyer, E.J., Forman, D.A., Button, D.C., 2018. Modulation of motoneurone excitability during rhythmic motor outputs. Appl Physiol Nutr Metab 43, 1176–1185. https://doi.org/10.1139/apnm-2018-0077

Prokop, T., Berger, W., Zijlstra, W., Dietz, V., 1995. Adaptational and learning processes during human split-belt locomotion: interaction between central mechanisms and afferent input. Exp Brain Res 106, 449–456.

Radhakrishna, M., Steuer, I., Prince, F., Roberts, M., Mongeon, D., Kia, M., Dyck, S., Matte, G., Vaillancourt, M., Guertin, P.A., 2017. Double-Blind, Placebo-Controlled, Randomized Phase I/IIa Study (Safety and Efficacy) with

Buspirone/Levodopa/Carbidopa (SpinalonTM) in Subjects with Complete AIS A or Motor-Complete AIS B Spinal Cord Injury. Current Pharmaceutical Design 23, 1789–1804. https://doi.org/10.2174/1381612822666161227152200

Rand, M.K., Wunderlich, D.A., Martin, P.E., Stelmach, G.E., Bloedel, J.R., 1998. Adaptive changes in responses to repeated locomotor perturbations in cerebellar patients. Exp Brain Res 122, 31–43.

Reiss, D., Marino, L., 2001. Mirror self-recognition in the bottlenose dolphin: a case of cognitive convergence. Proc. Natl. Acad. Sci. U.S.A. 98, 5937–5942. https://doi.org/10.1073/pnas.101086398

Reynolds, T.R., 1985. Stresses on the limbs of quadrupedal primates. American Journal of Physical Anthropology 67, 351–362. https://doi.org/10.1002/ajpa.1330670407

Roseberry, T.K., Lee, A.M., Lalive, A.L., Wilbrecht, L., Bonci, A., Kreitzer, A.C., 2016. Cell-Type-Specific Control of Brainstem Locomotor Circuits by Basal Ganglia. Cell 164, 526–537. https://doi.org/10.1016/j.cell.2015.12.037

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

Royal College of Physicians of London. n 80046799, 1870. Letters of Sir Charles Bell : selected from his correspondence with his brother George Joseph Bell. London : John Murray.

Ryczko, D., Dubuc, R., 2017. Dopamine and the Brainstem Locomotor Networks: From Lamprey to Human. Front. Neurosci. 11. https://doi.org/10.3389/fnins.2017.00295

Sakamoto, M., Tazoe, T., Nakajima, T., Endoh, T., Shiozawa, S., Komiyama, T., 2007. Voluntary changes in leg cadence modulate arm cadence during simultaneous arm and leg cycling. Exp Brain Res 176, 188–192. https://doi.org/10.1007/s00221-006-0742-x

Schillings, A.M., Van Wezel, B.M., Duysens, J., 1996. Mechanically induced stumbling during human treadmill walking. J Neurosci Methods 67, 11–7.

Schubert, M., Curt, A., Jensen, L., Dietz, V., 1997. Corticospinal input in human gait: modulation of magnetically evoked motor responses: Experimental Brain Research 115, 234–246. https://doi.org/10.1007/PL00005693

Schwartz, A.B., Ebner, T.J., Bloedel, J.R., 1987. Responses of interposed and dentate neurons to perturbations of the locomotor cycle. Exp Brain Res 67, 323–338.

Selionov, V.A., Ivanenko, Y.P., Solopova, I.A., Gurfinkel, V.S., 2009. Tonic central and sensory stimuli facilitate involuntary air-stepping in humans. J. Neurophysiol. 101, 2847–2858. https://doi.org/10.1152/jn.90895.2008

Shapkova, E.Y., 2004. Spinal Locomotor Capability Revealed by Electrical Stimulation of the Lumbar Enlargement in Paraplegic Patients, in: Latash, M., Levin, M. (Eds.), Progress in Motor Control: Effect of Age, Disorder, and Rehabilitation. Human Kinetics, Champaign, IL, pp. 253–289.

Shapkova, H.Y., Shapkov, Y.T., Mushkin, A.Y., 1997. Locomotor activity in paraplegic children induced by spinal cord stimulation, in: Gurfinkel, V.S., Levik, Y.S. (Eds.), Proceedings of the International Symposium on Brain and Movement. Moscow, pp. 170–171.

Sherrington, C.S., 1906. Observations on the scratch-reflex in the spinal dog. J. Physiol. (Lond.) 34, 1–50.

Shields, E.D., 2000. Concise Review: Her Name is “Lucy”, Our Three-million-year-old Ancestor. Journal of Dental Research 79, 13–20. https://doi.org/10.1177/00220345000790010101

Shik, M.L., Orlovsky, G.N., 1976. Neurophysiology of locomotor automatism. Physiol. Rev. 56, 465–501. https://doi.org/10.1152/physrev.1976.56.3.465

Shik, M.L., Severin, F.V., Orlovskiĭ, G.N., 1966. [Control of walking and running by means of electric stimulation of the midbrain]. Biofizika 11, 659–666.

Sockol, M.D., Raichlen, D.A., Pontzer, H., 2007. Chimpanzee locomotor energetics and the origin of human bipedalism. Proc. Natl. Acad. Sci. U.S.A. 104, 12265–12269. https://doi.org/10.1073/pnas.0703267104

Solopova, I.A., Selionov, V.A., Kazennikov, O.V., Ivanenko, Y.P., 2014. Effects of transcranial magnetic stimulation during voluntary and non-voluntary stepping movements in humans. Neurosci. Lett. 579, 64–69. https://doi.org/10.1016/j.neulet.2014.07.015

Solopova, I.A., Selionov, V.A., Sylos-Labini, F., Gurfinkel, V.S., Lacquaniti, F., Ivanenko, Y.P., 2015. Tapping into rhythm generation circuitry in humans during simulated weightlessness conditions. Front. Syst. Neurosci. 9. https://doi.org/10.3389/fnsys.2015.00014

Sprague, J.M., Chambers, W.W., 1953. Regulation of posture in intact and decerebrate cat. I. Cerebellum, reticular formation, vestibular nuclei. J. Neurophysiol. 16, 451–463. https://doi.org/10.1152/jn.1953.16.5.451

Stein, R.B., Capaday, C., 1988. The modulation of human reflexes during functional motor tasks. Trends Neurosci 11, 328–32.

Stringer Chris, 2016. The origin and evolution of Homo sapiens. Philosophical Transactions of the Royal Society B: Biological Sciences 371, 20150237. https://doi.org/10.1098/rstb.2015.0237

Suwa, G., Kono, R.T., Katoh, S., Asfaw, B., Beyene, Y., 2007. A new species of great ape from the late Miocene epoch in Ethiopia. Nature 448, 921–924. https://doi.org/10.1038/nature06113

Thelen, E., Fisher, D.M., Ridley-Johnson, R., Griffin, N.J., 1982. Effects of body build and arousal on newborn infant stepping. Dev Psychobiol 15, 447–453. https://doi.org/10.1002/dev.420150506

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. 112, 2006–2018. https://doi.org/10.1152/jn.00236.2014

Thibaudier, Y., Hurteau, M.-F., Telonio, A., Frigon, A., 2013. Coordination between the fore- and hindlimbs is bidirectional, asymmetrically organized, and flexible during quadrupedal locomotion in the intact adult cat. Neuroscience 240, 13–26. https://doi.org/10.1016/j.neuroscience.2013.02.028

Thorstensson, A., 1986. How is the normal locomotor program modified to produce backward walking? Exp Brain Res 61, 664–668.

Timmann, D., Horak, F.B., 1998. Perturbed step initiation in cerebellar subjects. 1. Modifications of postural responses. Exp Brain Res 119, 73–84.

Udo, M., Matsukawa, K., Kamei, H., 1979. Effects of partial cooling of cerebellar cortex at lobules V and IV of the intermediate part in the decerebrate walking cats under monitoring vertical floor reaction forces. Brain Res. 160, 559–564.

Udo, M., Oda, Y., Tanaka, K., Horikawa, J., 1976. Cerebellar control of locomotion investigated in cats: discharges from Deiters' neurones, EMG and limb movements during local cooling of the cerebellar cortex. Prog. Brain Res. 44, 445–459. https://doi.org/10.1016/S0079-6123(08)60751-7

Van Wezel, B.M., Ottenhoff, F.A., Duysens, J., 1997. Dynamic control of location-specific information in tactile cutaneous reflexes from the foot during human walking. J Neurosci 17, 3804–14.

Vasudevan, E.V., Patrick, S.K., Yang, J.F., 2016. Gait Transitions in Human Infants: Coping with Extremes of Treadmill Speed. PLoS ONE 11, e0148124. https://doi.org/10.1371/journal.pone.0148124

Vasudevan, E.V., Zehr, E.P., 2011. Multi-frequency arm cycling reveals bilateral locomotor coupling to increase movement symmetry. Experimental brain research 211, 299–312. https://doi.org/10.1007/s00221-011-2687-y

Verschueren, S.M.P., Swinnen, S.P., Desloovere, K., Duysens, J., 2002. Effects of tendon vibration on the spatiotemporal characteristics of human locomotion. Exp Brain Res 143, 231–239. https://doi.org/10.1007/s00221-001-0987-3

Villablanca, J.R., Marcus, R.J., Olmstead, C.E., 1976. Effects of caudate nuclei or frontal cortical ablations in cats. I. Neurology and gross behavior. Exp. Neurol. 52, 389–420.

Wainberg, M., Barbeau, H., Gauthier, S., 1990. The effects of cyproheptadine on locomotion and on spasticity in patients with spinal cord injuries. J. Neurol. Neurosurg. Psychiatry 53, 754–763.

White, T.D., Asfaw, B., Beyene, Y., Haile-Selassie, Y., Lovejoy, C.O., Suwa, G., WoldeGabriel, G., 2009. Ardipithecus ramidus and the Paleobiology of Early Hominids. Science 326, 64–86. https://doi.org/10.1126/science.1175802

White, T.D., WoldeGabriel, G., Asfaw, B., Ambrose, S., Beyene, Y., Bernor, R.L., Boisserie, J.-R., Currie, B., Gilbert, H., Haile-Selassie, Y., Hart, W.K., Hlusko, L.J., Howell, F.C., Kono, R.T., Lehmann, T., Louchart, A., Lovejoy, C.O., Renne, P.R., Saegusa, H., Vrba, E.S., Wesselman, H., Suwa, G., 2006. Asa Issie, Aramis and the origin of Australopithecus. Nature 440, 883. https://doi.org/10.1038/nature04629

Winter, D.A., 1987. The Biomechanics and Motor Control of Human Gait. University of Waterloo Press, Waterloo, Ontario, Canada.

Winter, D.A., Pluck, N., Yang, J.F., 1989. Backward walking: a simple reversal of forward walking? J Mot Behav 21, 291–305.

Wirz, M., van Hedel, H.J.A., 2018. Chapter 24 - Balance, gait, and falls in spinal cord injury, in: Day, B.L., Lord, S.R. (Eds.), Handbook of Clinical Neurology, Balance, Gait, and Falls. Elsevier, pp. 367–384. https://doi.org/10.1016/B978-0-444-63916-5.00024-0

Yamaguchi, T., 2004. The central pattern generator for forelimb locomotion in the cat. Prog. Brain Res. 143, 115–122. https://doi.org/10.1016/S0079-6123(03)43011-2

Yanagihara, D., Kondo, I., 1996. Nitric oxide plays a key role in adaptive control of locomotion in cat. Proc. Natl. Acad. Sci. U.S.A. 93, 13292–13297.

Yang, J.F., Gorassini, M., 2006. Spinal and brain control of human walking: implications for retraining of walking. Neuroscientist 12, 379–389. https://doi.org/10.1177/1073858406292151

Yang, J.F., Lam, T., Pang, M.Y., Lamont, E., Musselman, K., Seinen, E., 2004. Infant stepping: a window to the behaviour of the human pattern generator for walking. Can J Physiol Pharmacol 82, 662–74. https://doi.org/10.1139/y04-070

Yang, J.F., Stein, R.B., 1990. Phase-dependent reflex reversal in human leg muscles during walking. J Neurophysiol 63, 1109–17.

Yang, J.F., Stephens, M.J., Vishram, R., 1998. Infant stepping: a method to study the sensory control of human walking. J. Physiol. (Lond.) 507 (Pt 3), 927–937.

Yang, J.F., Whelan, P.J., 1993. Neural mechanisms that contribute to cyclical modulation of the soleus H-reflex in walking in humans. Exp Brain Res 95, 547–556.

Zehr, E.P., 2006. Training-induced adaptive plasticity in human somatosensory reflex pathways. Journal of applied physiology 101, 1783–94. https://doi.org/10.1152/japplphysiol.00540.2006

Zehr, E.P., 2005. Neural control of rhythmic human movement: the common core hypothesis. Exercise and sport sciences reviews 33, 54–60.

Zehr, E.P., Balter, J.E., Ferris, D.P., Hundza, S.R., Loadman, P.M., Stoloff, R.H., 2007. Neural regulation of rhythmic arm and leg movement is conserved across human locomotor tasks. The Journal of physiology 582, 209–27. https://doi.org/10.1113/jphysiol.2007.133843

Zehr, E.P., Barss, T.S., Dragert, K., Frigon, A., Vasudevan, E.V., Haridas, C., Hundza, S., Kaupp, C., Klarner, T., Klimstra, M., Komiyama, T., Loadman, P.M., Mezzarane, R.A., Nakajima, T., Pearcey, G.E., Sun, Y., 2016. Neuromechanical interactions between the limbs during human locomotion: an evolutionary perspective with translation to rehabilitation. Exp Brain Res 234, 3059–3081. https://doi.org/10.1007/s00221-016-4715-4

Zehr, E.P., Collins, D.F., Frigon, A., Hoogenboom, N., 2003. Neural control of rhythmic human arm movement: phase dependence and task modulation of hoffmann reflexes in forearm muscles. Journal of neurophysiology 89, 12–21. https://doi.org/10.1152/jn.00416.2002

Zehr, E.P., Duysens, J., 2004. Regulation of arm and leg movement during human locomotion. Neuroscientist 10, 347–61. https://doi.org/10.1177/1073858404264680

Zehr, E.P., 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. Experimental brain research 149, 260–6. https://doi.org/10.1007/s00221-003-1377-9

Zehr, E.P., Hundza, S.R., 2005. Forward and backward arm cycling are regulated by equivalent neural mechanisms. Journal of neurophysiology 93, 633–40. https://doi.org/10.1152/jn.00525.2004

Zehr, E.P., Hundza, S.R., Balter, J.E., Loadman, P.M., 2009a. Context-dependent modulation of cutaneous reflex amplitudes during forward and backward leg cycling. Motor control 13, 368–86.

Zehr, E.P., Hundza, S.R., Vasudevan, E.V., 2009b. The quadrupedal nature of human bipedal locomotion. Exercise and sport sciences reviews 37, 102–8. https://doi.org/10.1097/JES.0b013e31819c2ed6

Zehr, E.P., Komiyama, T., Stein, R.B., 1997. Cutaneous reflexes during human gait: electromyographic and kinematic responses to electrical stimulation. Journal of neurophysiology 77, 3311–25.

Zehr, E.P., Stein, R.B., 1999. What functions do reflexes serve during human locomotion? Prog Neurobiol 58, 185–205.

Zhou, R., Alvarado, L., Ogilvie, R., Chong, S.L., Shaw, O., Mushahwar, V.K., 2018. Non-gait-specific intervention for the rehabilitation of walking after SCI: role of the arms. J Neurophysiol 119, 2194–2211. https://doi.org/10.1152/jn.00569.2017

Chapter 3 - We are upright walking cats: human limbs as sensory antennae during locomotion

Abstract

Humans and cats share many characteristics pertaining to the neural control of locomotion, which has enabled the comprehensive study of cutaneous feedback during locomotion. Feedback from discrete skin regions on both surfaces of the human foot has revealed that neuromechanical responses are highly topographically organized and contribute to 'sensory steering' of our limbs during locomotion.

Pearcey GE and Zehr EP (2019). We are upright walking cats: human limbs as sensory antennae during locomotion. To be published as an invited review in Physiology (Under review as manuscript Y-00008-2019).

“It may be regarded as a physiological axiom, that all purposive movements are guided by sensations or afferent sensations of some kind.” – H. Charlton Bastian (1887).

Introduction

Locomotion includes a wide variety of motor behaviors moving the body from place to place. Humans generally walk as bipeds, but can be quadrupedal under certain conditions (Zehr et al., 2016). Despite the type, form or speed, clear evidence demonstrates the important contributions of sensory information to successful locomotion, along with virtually all habitual human movements. Kinesthesia (‘the sense of movement’) was first described by Bastian (1887) and was attributed to the skin, muscles and other deep tissues of the limbs (i.e. fascia, tendons, and joints). Sherrington (1906) later referred to and named the perception of body position as ‘proprioception’ and identified the skin as conveying significant information to the central nervous system. Yet, his experiments did not support a strong role for cutaneous feedback in the control of posture or locomotion in the cat (Sherrington, 1906), an oversight that remains one of the few times that the “father of neurophysiology” led the field in the wrong direction.

A variety of sensory organs have since been identified as contributors to proprioception: muscle spindles; Golgi tendon organs; joint receptors; and mechanoreceptors in the skin (for review, see Prochazka and Ellaway (2012)). The critical importance of sensory information from the skin arising from mechanoreceptors has emerged in recent years. Cutaneous afferent effects on motor circuits during locomotion, posture and hand function has recently been summarized nicely by Panek et al. (2014). Here, we elaborate on the integration of cutaneous sensation, from both whole nerves and discrete skin regions, in parallel with observations from habitual quadrupeds and provide evidence for considering the hands and feet as sensory antennae during locomotion.

Humans are upright walking cats

Most insights that guide our understanding of human locomotor control can be attributed to our mammalian counterpart, the feline. The neural control of locomotor behaviours of humans and cats has been reviewed more extensively elsewhere (Capaday, 2002; Dietz, 2002; Duysens and Van de Crommert, 1998; Hultborn and Nielsen, 2007;

Klarner and Zehr, 2018; Vilensky, 1987) yielding consensus that we possess locomotor characteristics that are strikingly similar to that of cats.

Mammalian locomotion is in large part controlled by neuronal networks (central pattern generators (CPGs)) within the spinal cord (Grillner, 1981; Guertin, 2009; Klarner and Zehr, 2018; Zehr, 2005). A hallmark of mammalian locomotion is the fine control resulting from interactions of a non-hierarchical three-part system comprising supraspinal centers (e.g., motor cortex, mesencephalic locomotor region, cerebellum), spinal networks (e.g., CPG, segmental interneuronal reflex pathways), and sensory feedback (e.g., skin, muscle, tendon, visual, vestibular) (Zehr, 2005). Although not 'required' to generate locomotion (Sherrington, 1910), sensory feedback plays a critical regulatory role assisting with positioning of the feet, responding to obstacles or perturbations, and tuning muscle activity across the step cycle (Pearson, 1995; Rossignol et al., 2006). While all forms of sensation are important, cutaneous feedback from the surfaces of the hands and feet provides direct information about the locomotor surface and is well suited for guiding fine adjustments to ongoing locomotion. Figure 3-1 illustrates the similarities of skin surfaces between the cat and human used while studying the role of tactile input to locomotion.

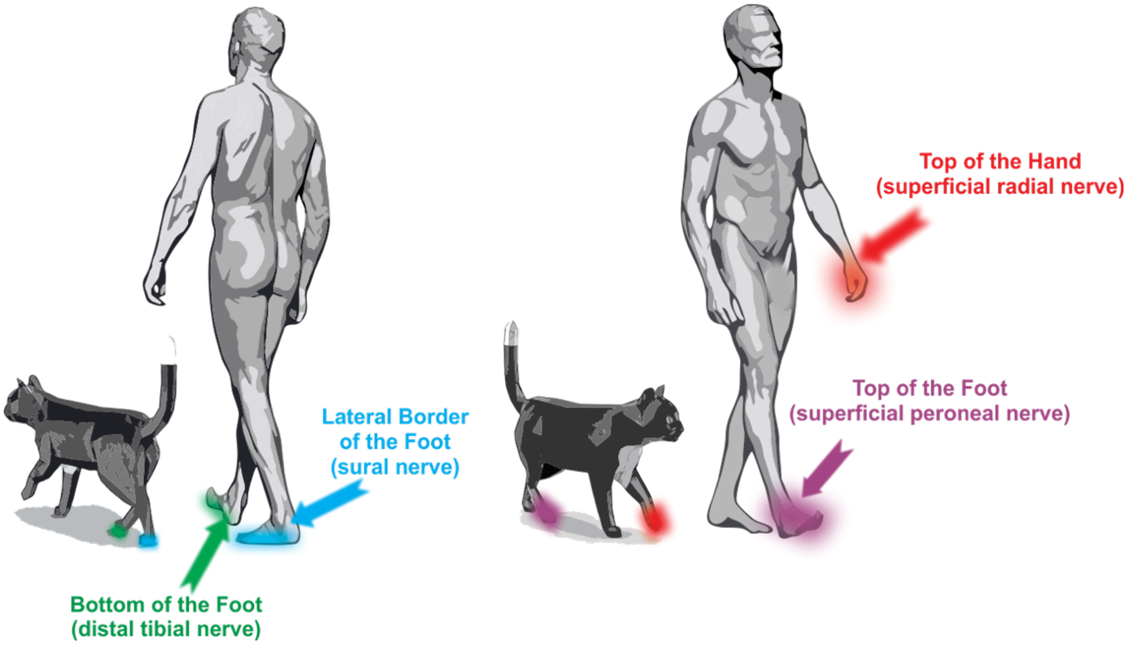

Figure 3-1: *An illustration showing the skin regions commonly studied during human locomotion. A cat is shown next to the human and similar skin regions are highlighted to*

show commonalities between species. Green represents the bottom of the foot and is innervated by the distal tibial nerve. Blue represents the lateral border of the foot and is innervated by the sural nerve. Red represents the top of the hand and is innervated by the superficial radial nerve. Purple represents the top of the foot and is innervated by the superficial peroneal nerve.

During stance, cutaneous receptors from the bottom of the feet become increasingly important if irregularities in terrain exist. For example, intact cats can walk relatively well on a treadmill but not a ladder after bilateral denervation of cutaneous nerves supplying the bottom of the hindpaws (Bouyer and Rossignol, 2003). Dorofeev et al. (2008) studied effects of nerve excision in epidural-stimulated locomotion of the decerebrate cat and revealed that cutaneous afferents ensure correct positioning of the limbs on the treadmill, modulate ipsilateral hindlimb muscle activity, and contribute to the control of stance duration. In response to lateral surface perturbations (i.e. reduced stability), an intact cat with denervation to cutaneous afferents of the hindpaw, requires more and larger steps to regain balance than pre-denervation (Bolton and Misiaszek, 2009). In humans, experiments that reduce cutaneous feedback by cooling the bottom of the foot to inactivate sensory transmission produces changes in pressure distribution (Eils et al., 2002; Nurse and Nigg, 2001; Taylor et al., 2004) and muscle activity (Nurse and Nigg, 2001) during walking that correlate with instability. Thus, cutaneous feedback plays an integral role in the maintenance of stability during stance in both cats and humans.

During swing, cutaneous feedback from the top of the foot is vital for making corrective reactions to limb perturbations and obstacles. Forssberg (1979) described the ‘stumbling corrective reaction’ in decerebrate and intact cats. Although depressed, flexion reflexes evoked by cutaneous afferents remain during fictive locomotion evoked by stimulation to the mesencephalic locomotor region (MLR) (Perreault et al., 1999). In humans, the stumbling corrective reaction is characterized by increased knee flexion and decreased dorsiflexion of the swing limb, allowing obstacle clearance and trip avoidance while preserving forward progression (Schillings et al., 1996) and is found in infants and adults (Lam et al., 2003; Pang et al., 2003; Pang and Yang, 2001; Schillings et al., 2005, 1999, 1996). Howe et al. (2015) have also highlighted the importance of cutaneous input from the top of the foot for proprioception during swing. When topical anesthetic is applied, participants exhibit altered knee and ankle joint kinematics that put them at risk of tripping. Thus, cutaneous feedback plays an integral role in obstacle avoidance during swing in both cats and humans.

"…sensation elicitable from the skin suggests that possibly different reflex motor reactions attach to the different species of end-organs undoubtedly coexisting in one and the same skin field" – Sir Charles S. Sherrington (1903).

Specificity of cutaneous feedback during locomotion

Reflex amplitudes undergo motor task- (Stein and Capaday, 1988) and phase-dependent modulation (for reviews see (Duysens et al., 2004; Rossignol et al., 2006; Zehr, 2006, 2005; Zehr and Duysens, 2004; Zehr and Stein, 1999)). Forssberg et al. (1975) originally showed that stimulation to the top of the cat paw during swing caused a stumble correction reaction yet, during stance, the same input had little effect on the same flexor motoneurons. Recently, Hurteau et al. (2017) showed that phase-dependent modulation of cutaneous reflexes is maintained with increasing speed, even after spinal transection, suggesting a spinal locus. Interestingly in humans, prior experience does not seem to affect phase modulation of cutaneous reflexes based on commonality between able-bodied participants and wheelchair users during manual wheeling (MacGillivray et al., 2013). Such nervous system tuning allows responses to be gated so that they are functionally relevant and contribute to successful execution of established and novel types of locomotion.

Both task- and phase-dependent modulation of afferent feedback during locomotion is mediated by premotoneuronal gating via spinal CPGs (Duysens et al., 2004; McCrea, 2001; Zehr, 2005). Task-dependent modulation of cutaneous reflexes has been studied between standing and walking (Komiyama et al., 2000), running (Duysens et al., 1993), and hopping (Hauglustaine et al., 2001), between level walking, incline walking, and stair climbing (Lamont and Zehr, 2006), between various frequencies of contralateral arm movements (Vasudevan and Zehr, 2011) and between static and rhythmic contractions (Brown and Kukulka, 1993; Sasada et al., 2010; Zehr et al., 2001), indicating that static and rhythmic movements are controlled by diverse neural mechanisms (Zehr, 2005). Such mechanisms also mediate phase-dependent changes in sensory feedback during rhythmic output. For example, middle latency (75-80ms) cutaneous reflexes in the ankle dorsiflexors (tibialis anterior; TA) are facilitatory during the early swing phase, but inhibitory at the swing-to-stance transition (see Duysens et al. (2004)). This ensures that regulated plantarflexion occurs with controlled establishment of foot contact of the entire bottom of the foot to increase stability (Duysens et al., 1992; Yang and Stein, 1990; Zehr and Stein, 1999). Phase-dependent reflex reversal highlights the ability of the central nervous system to alter spinal cord interneuronal excitability to flexibly and functionally modify motor output.

Modulation of sensory feedback is also location and/or nerve-specific. Nakajima et al. (2008a, 2008b) recently examined the effects of loading during passive stepping on cutaneous reflexes evoked in neurologically intact humans. In one experiment (Nakajima et al., 2008b) stimulation to the bottom of the foot induced middle latency reflexes in the ipsilateral ankle dorsiflexors that were phase-dependently modulated during loaded treadmill stepping, but not unloaded air stepping. In the second experiment (Nakajima et al., 2008a), top of the foot and bottom of the foot stimulation elicited middle latency reflexes in the ipsilateral ankle dorsiflexor that were phase-dependently modulated during any level of loading (i.e. only 33% of body weight) during passive stepping. Top of the foot stimulation evoked reflexes expressed a reversal from suppression to facilitation at early to late stance, respectively, suggesting that a combination of mechanoreceptor loading and rhythmic movement is necessary for phase-dependent modulation of cutaneous reflexes. Duysens et al. (2010) examined cutaneous reflexes evoked from the sural nerve (innervates the lateral border of the foot) in the ankle dorsiflexors during passive walking using a driven gait orthosis (DGO). They found that ankle range of motion and dorsiflexor activation was reduced at end swing, and an absence (4 out of 6) or reduction (2 out of 6) of electromyogram (EMG) suppression evoked by cutaneous reflex stimulation. Since afferent feedback is intact during passive stepping, central inputs from either spinal CPGs or descending supraspinal drive must mediate the phase-dependent reflex reversal in the ankle dorsiflexor at end swing. These experiments suggest an integrated three-part system consisting of supraspinal input, CPG activity and afferent feedback contributing to task-, phase- and nerve-dependent modulation of cutaneous reflexes.

“…stimulation of the forepaw elicits movement in the hindlimb…” Sherrington (1898)

Cutaneous feedback during locomotion is widely distributed

Activation of cutaneous afferents produce widespread modulatory effects on muscle activity throughout the body. Recent work in the cat by Hurteau et al. (2018) showed that cutaneous input to the top of the paw affects neural circuits controlling all four limbs to modify swing trajectory or stabilize the movement during stance in the stimulated limb while stabilizing the other 3 limbs. In these cats, the strength of descending pathways (i.e. from forelimb to hindlimb) seem stronger than the ascending pathways (i.e. from hindlimb to forelimb), potentially due to the risk of more serious injury with loss of forelimb support in a quadrupedal gait. Based on the earliest latencies observed, interlimb cutaneous reflexes traverse fast-conducting spinal pathways, although longer latency responses likely involve supraspinal contributions.

Zehr et al. (2001) revealed that interlimb reflexes are also widespread in humans by stimulating the top of the hand (superficial radial; SR) or foot (superficial peroneal; SP) and recording reflexes in muscles of all four limbs during isometric contractions. Similar to the cat, earliest reflexes are likely mediated by propriospinal pathways (based on latency) and may serve as a neural substrate for assisting coordination between the limbs. Using spatial facilitation, Nakajima et al. (2013) illustrated that cutaneous input from the nerves innervating the hands and feet converge on similar interneuronal pathways during “reduced” human locomotion (i.e. combined arm & leg cycling). During human walking, stimulation of the lateral border of the foot evokes reflexes in muscles throughout the body, including all limbs and the trunk (Lamont and Zehr, 2007, 2006). Furthermore, widespread effects of cutaneous feedback have a regulatory role in balance and perturbation responses. Taps to the heel or Achilles tendon and electrical stimulation of the lateral border of the foot alter back muscle erector spinae activity meaning cutaneous input from the leg may be important for maintaining balance (Clair et al., 2009).

Sensory input with mechanical consequences during human locomotion

Sensorimotor integration begins early in development. Nociceptive withdrawal-like reflexes producing muscle twitches in the sleeping neonatal rat (correlates of in utero human movements (Clancy et al., 2001)), are modulated within post-natal weeks 1-3 (Petersson et al., 2003). In humans, sophisticated sensory regulation of walking is established from a very young age. Light touch applied to the feet of infants (5.5-13 months) during weight supported treadmill stepping produces coordinated, functionally relevant responses that are modulated by phase (Lam et al., 2003). Touch to the top of the foot during swing results in a 'stumbling correction reaction' (i.e. increased ankle dorsiflexor activity, knee flexion, step cycle duration), while touch to the top of the foot during stance decreases quadriceps activation and increases stance duration. Yet touch applied to the side of the foot during forward stepping does not evoke alterations in the stepping pattern but does evoke a flexion response during swing. These reflex changes with kinematic correlates tune gait patterns and their location-, task- and phase-dependent specificity are maintained into adulthood (Rossignol et al., 2006; Zehr and Stein, 1999).

During stance, stimulation of cutaneous afferents typically produces stabilizing reactions, whereas during swing they are stumbling corrective or withdrawal reactions (Zehr and Stein, 1999). Neuromechanical responses to stimulation of nerves innervating the skin surfaces of the foot undergo nerve-specific and phase-dependent modulation, which can be completely reversed depending on the phase (i.e. reflex-reversal). For example, stimulation to the lateral border of the foot evokes eversion and dorsiflexion of the foot during stance but a withdrawal response is evoked during swing (Van Wezel et al., 1997; Zehr et al., 1998b). Withdrawal reactions evoked by stimulation to the lateral border of the foot involve dorsiflexion of the ankle whereas top of the foot evoked responses cause reduced dorsiflexion (Zehr et al., 1997). Furthermore, bottom of the foot stimulation evokes a withdrawal reaction (ankle dorsiflexion) at the stance-to-swing transition, but undergoes reflex reversal to evoke a placing reaction (ankle plantarflexion) at the swing-to-stance transition (Zehr et al., 1997). These contrasts reveal that the nervous system utilizes cutaneous information from each nerve in a context-dependent manner to move the foot away from the stimulus while minimizing mechanical disturbance and allowing locomotor progression.

Haridas and Zehr (2003) showed that stimulation of cutaneous nerves innervating the top of the foot and hand evoked opposite neuromechanical reactions at the ankle. During the stance-to-swing transition, stimulation of the ipsilateral top of the foot evoked plantarflexion, whereas stimulation of the contralateral top of the foot, as well as the ipsi- and contralateral top of the hand, evoked dorsiflexion (see figure 3-2). The observed dorsiflexion could suggest that a perturbation to other limbs results in a cautionary response by reducing the speed of forward progression (i.e. reduced toe-off propulsion), whereas stimulation to the homonymous limb results in a stumble correction reaction. In a task during which the arms are freely moving (i.e. arm swing during walking), rather than propulsive (i.e. forelimbs of a cat), cutaneous input to the hands would have functionally relevant responses that act to reduce the risk of falling, rather than contributing to stabilizing reactions (Lamont and Zehr, 2006).

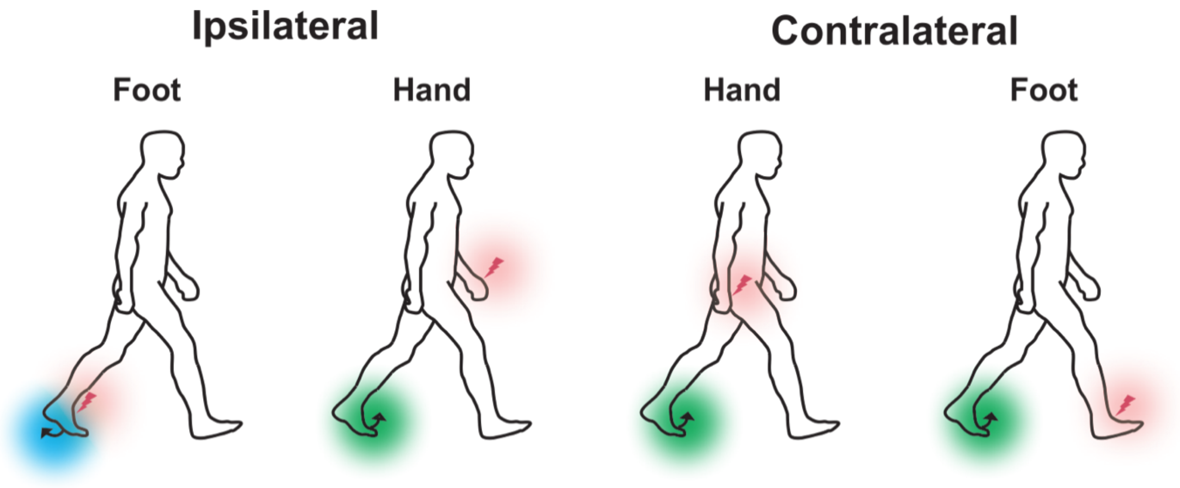

Figure 3-2: *Summary effects of stimulation to cutaneous nerves innervating the ipsi- (left) and contralateral (right) hand (superficial radial; top) and foot (superficial peroneal; bottom) dorsum on ankle dorsi- and plantarflexion during the stance-to-swing phase of gait. For clarification, the stimulated limb is highlighted in red and the affected ankle is highlighted in blue and green when plantarflexion and dorsiflexion occur, respectively. Lightning bolts indicate the location of stimulation and the arrows indicate the direction of the stimulus-induced kinematic response at the ankle. In the original experiment, EMG and kinematics were recorded bilaterally and stimulation was unilateral, however this*

diagram summarizes the effects that would occur with stimulation bilateral. [Adapted with permission from (Haridas and Zehr, 2003)].

Mechanoreceptors and their topographic organization in the foot

Mechanoreceptors in the skin are responsible for the sense of touch and contribute to proprioception and sensorimotor control (Macefield, 2005). Aβ afferents innervating four classes of cutaneous mechanoreceptors strongly contribute to cutaneous reflexes observed in human subjects (van Wezel et al., 2000). Afferents innervating cutaneous mechanoreceptors either adapt their firing patterns to sustained input slow (i.e. encode sustained input; SA) or fast (i.e. encode onset/offset; FA) and have either small (type I) or large (type II) receptive fields (Johnson, 2001; Zimmerman et al., 2014). Each class of cutaneous afferent terminates in a specific receptor and is particularly well suited for a specific form of cutaneous feedback. SAI afferents innervate Merkel cells and provide information about static touch, SAII afferents terminate in Ruffini corpuscles and provide information about skin stretch, RAI afferents innervate Meissner's corpuscles and provide information about movement across the skin, and RAII afferents terminate in Pacinian corpuscles and provide information about high frequency vibration (Johnson, 2001; Zimmerman et al., 2014). The topographic organization of mechanoreceptors in the human hand has received more attention than the foot (Macefield, 2005) likely due to the importance of the hand in manual manipulation. This led to error in thinking that the distribution of mechanoreceptors in hands and the feet are the same. A recent investigation from Strzalkowski et al. (2017) showed single afferents in the foot have higher firing thresholds, entrain less easily, and discharge fewer spikes at a given vibration amplitude compared with the hand. Cutaneous information from the bottom of the foot is essential for maintenance of balance and corrective responses to perturbations during locomotion, which means the mechanoreceptors are subjected to much higher loading stresses and differential force profiles than those found in the hand. However, the mechanical properties of the skin may contribute to differences in skin sensitivity thresholds between the hands and feet (Strzalkowski et al., 2015). Kennedy and Inglis (2002) tested assumptions of equivalency for skin receptors in the hand and foot. Microneurographic recordings identified a random distribution of all types of mechanoreceptors across the entire surface of the foot, whereas in the hand there are highly concentrated areas of type I receptors in the fingers (Johansson and Vallbo, 1980). Receptive fields of the foot were also larger than the hand and had higher activation thresholds. This can be attributed to the fact that the bottom of the foot is typically weight bearing and does not require the acuity of fine touch of the digits that has evolved as a consequence of bipedal gait. Nevertheless, all types of individual cutaneous afferents are important for the control of gait and posture. Fallon et al. (2005) observed that activation of all four classes of cutaneous receptors could evoke reflexes in motoneurons innervating muscles at the ankle. Mildren et al. (2016) have also demonstrated that fast and slowly adapting cutaneous afferents innervating the foot have roles in ankle proprioception. When cutaneous feedback is removed with anaesthetic, participants struggle to perceive the ankle angle (Mildren et al., 2017). The rich yet even distribution of mechanoreceptors around the foot make it an ideal candidate to be used as a sensory antenna to guide locomotion.

Digging down from whole afferents to discrete skin regions

Microneurographic studies of cutaneous afferents in the hand have identified that discharge from single afferents can drive motoneurons of the hand, via interneuronal connections (McNulty et al., 1999; McNulty and Macefield, 2001). Stimulation of individual digits during isometric and pincer grip contractions evoke cutaneous reflexes that are highly location and task-specific (Nakajima et al., 2006a), revealing that discrete regions of the skin produce location-specific responses that are functionally relevant within the regions innervated by an entire nerve branch. Nakajima et al. (2006b) stimulated discrete skin sites on the bottom of the foot and found differential effects of evoked responses from the heel, medial forefoot and lateral forefoot in leg muscles during sitting and standing with high spatial resolution. Single motor unit recordings of the peroneus longus (i.e. plantarflexor and everter) showed that coactivation of eversion with either the dorsi- or plantarflexors at the ankle affects the location-specificity of cutaneous reflex responses (Nakajima et al., 2009). Activation of single cutaneous afferents from the top of the foot can also produce interlimb cutaneous reflexes in the arms. Bent and Lowrey (2013) showed that all types of mechanoreceptors can evoke these interlimb responses, although the most consistent responses are from the medial region of the foot and from Type I afferents.

Stimulation of discrete skin areas during walking – evidence for 'sensory steering'

Effects of stimulating discrete skin regions of the foot on muscle activity in the legs and arms led to related exploration during walking. Reflex reversal of evoked cutaneous reflexes from whole distal tibial nerve, which innervates the skin on the bottom of the foot, in the ankle dorsiflexors are likely due to a combination of regionally distinct afferents that are phase-dependently modulated (Nakajima, 2016). During swing, stimulation of skin sites on the medial and lateral forefoot evoked facilitation, whereas at the heel there was suppression of the ankle dorsiflexors. Whole nerve stimulation evoked facilitation reminiscent of the medial forefoot during early swing, but resembled heel stimulation suppression during late swing. Were the responses from the whole nerve a simple summation of responses from discrete regions innervated by afferents found in the distal tibial nerve? Temporal summation by simultaneously stimulating the heel & whole nerve and medial forefoot & whole nerve locations was used to compare responses to those from individual discrete skin stimulation (see figure 3-3). At the stance-to-swing transition, responses from heel stimulation alone evoked inhibitory responses in ankle dorsiflexor EMG, whereas all other locations resulted in facilitation. At the swing-to-stance transition, the opposite occurred. Responses evoked from medial forefoot stimulation alone caused facilitation, whereas all others caused inhibition. These indicate there are no cancellation effects when combining heel and medial forefoot stimulation with whole nerve stimulation during gait transitions. Whole nerve stimulation is not simply a summation of combined reflexes, but rather, the regionally distinct afferents are phase-modulated and contribute to functionally relevant whole nerve responses. Stimulating regionally distinct sites reveals the precise and functionally organized skin surface serving to guide walking.

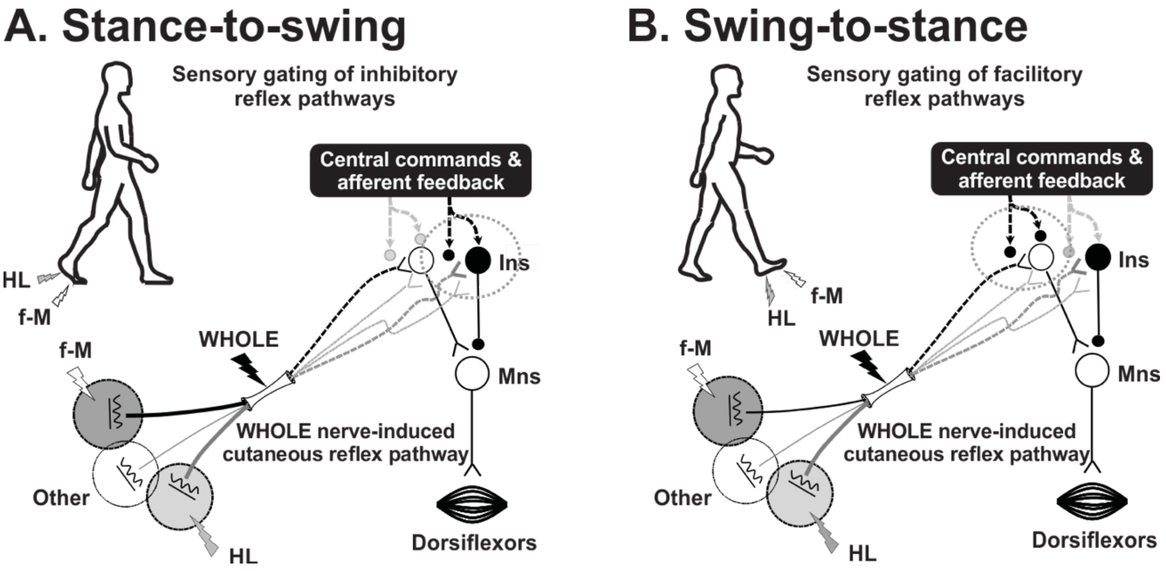

Figure 3-3: *A simplified summary of pathways involved with the gating of cutaneous reflex pathways during A) stance-to-swing and B) swing-to-stance phase transitions. The putative reflex pathways as a result of stimulation to the whole nerve (WHOLE) and discrete surfaces it innervates are shown in both A) and B). Cutaneous inputs project to interneuronal reflex pathways that can be either excitatory (white) or inhibitory (black). A) The effect of middle latency response (MLR) following forefoot medial (f-M) & WHOLE produced temporal summation of the WHOLE-reflex circuits at the stance-to-swing phase transition. Afferent inputs evoked from f-M stimulation either partly contribute to the facilitation of WHOLE-induced reflexes and/or inputs from heel (HL) were partly gated on WHOLE-reflex circuitry by locomotor-related central commands (downward black arrows; potential sites of inhibition is circled). B) In contrast, during the swing-to-stance transition, HL stimulation either partly contributes to the inhibition of WHOLE-induced reflexes and/or inputs from f-M were partly gated on WHOLE-reflex circuitry by locomotor-related central commands (downward black arrows; potential sites of inhibition are circled). WHOLE (black) HL (grey), and f-M (white) stimulation are represented by lightning bolts [recreated with permission from (Nakajima et al., 2016)].*

A pair of experiments conducted in our laboratory recently examined the neuromechanical responses evoked by discrete stimulation to the bottom (Zehr et al., 2014) and top (Klarner et al., 2017) of the foot. In both experiments, reflexes from 5 skin sites (bottom and top; total of 10 sites) were correlated to kinematic and kinetic alterations in the step cycle. Responses were highly location-specific and phase-dependent, suggesting that the foot acts to gather tactile information—as does an antenna—and provides feedback to steer the limb perturbations and maintain forward progression during walking.

Walking the topography: contributions from discrete skin regions on the bottom of the foot

Responses from stimulation to the bottom of the foot during the stance phase emphasize the role of cutaneous input in the maintenance of stability, as found during static postures (Nakajima et al., 2009, 2006b). Generally, lateral stimulation increases medial pressure, whereas medial stimulation increases lateral pressure. During late stance, effects are larger for mid-lateral than distolateral stimulation, since weight transfer increases loading of the distal portion of the foot. These responses can be attributed to a transfer of weight support in response to uneven terrain, but when stability of the forefoot is increased (i.e. late stance), stimulation to this region results in a reduced response. Heel stimulation causes facilitation of the plantarflexors (i.e. medial gastrocnemius and peroneus longus) and, during early stance, increased pressure to the distal lateral region of the foot. Essentially all bottom of the foot (except those most medial) stimulated sites evoked ankle inversion of the foot. This indicates increased reliance on the lateral portion of the foot for stability during stance on uneven surfaces, except when an object under the medial foot has increased potential for inversion ankle injury. During propulsion (i.e. terminal stance and stance to swing transition), stimulation to the bottom of the foot causes a general decrease in forefoot pressure. This decreased pressure is accompanied by facilitation of peroneus longus activity with stimulation of the lateral forefoot and facilitation of ankle dorsiflexor activity with stimulation to the medial forefoot. These location-specific responses suggest preparation of ankle eversion/inversion when propulsion occurs on uneven terrain.

Due to the relative instability of swing, expected responses would resemble subtle withdrawal or stumbling corrective responses. Indeed, location-specific withdrawal is displayed when forefoot stimulation causes dorsiflexion whereas heel stimulation causes plantarflexion (see third panel on top of figure 3-4). Stimulation to the medial side of the foot causes eversion, whereas lateral stimulation causes inversion (see third panel on bottom of figure 3-4). Kinematic responses are produced by functionally relevant changes in dorsiflexor and plantarflexor muscle activity. During the swing to stance transition, there is an overarching need for the foot to establish safe contact with the ground to initiate a solid base of support. Stimulation to the heel causes facilitation of ankle dorsiflexor activity, which acts to reduce inversion and initiate loading of the foot. Stimulation to the distal-lateral portion of the bottom of the foot causes a facilitation of peroneus longus muscle activity, as possible preparation of footfall on an uneven surface, again emphasizing the role of cutaneous feedback from the bottom of the foot in the establishment of stable ground contact.

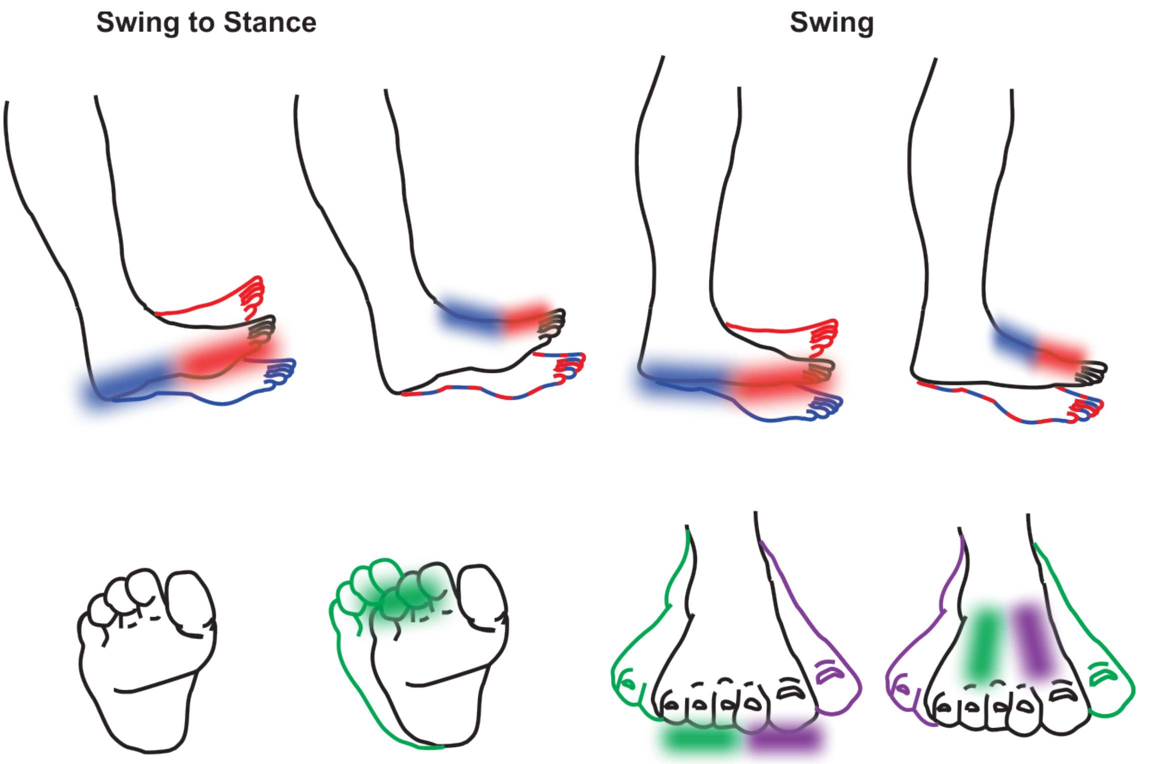

Figure 3-4: *A simplified summary of the neuromechanical effects of discrete stimulation to the top and bottom of the foot during swing to stance and swing phases from lateral (top) and frontal (bottom) view. Blurred colorations represent stimulation to the proximal (blue) or distal (red) regions in the lateral view (top), and medial (purple) or lateral (green) regions in the anterior view (bottom). The black outline of the foot represents the non-stimulated orientation of the foot, whereas colored outlines indicate the direction of the neuromechanical response to discrete tactile input. It should be noted that the amplitude*

of the neuromechanical responses are not to scale, but rather are emphasized for clarity. (summarized from (Zehr et al., 2014) and (Klarner et al., 2017)].

Topography to avoid tripping on top: discrete skin regions on the top of the foot contribute to walking

Cutaneous feedback from the top of the foot exemplifies the ability of the nervous system to avoid perturbations and continue with forward progression of the limb. Observations support the essential role of the receptors on the top of the foot during swing because responses from the top of the foot were more pronounced compared to the bottom. Stimulation of the top of the foot during swing causes a stumbling corrective response, which consists of ankle plantarflexion and knee flexion (Haridas and Zehr, 2003; Van Wezel et al., 1997; Yang and Stein, 1990; Zehr et al., 1998a, 1997). Similar to stimulation of the entire skin surface on the top of the foot, stimulation to all discrete skin regions of the bottom of the foot cause facilitation of the plantarflexors and suppression of ankle dorsiflexor activity, resulting in reduced dorsiflexion (far right on top of figure 3-4). Stimulation to the lateral regions cause inversion and facilitation of the hip adductors, whereas stimulation to the medial region cause eversion and suppression of the hip adductors (far right on bottom of figure 3-4). These responses indicate the ability of the foot to steer itself via segmental pathways, which is important for rapid obstacle avoidance prevention of tripping. Further evidence of this steering comes during the stance to swing transition. Similar to the entire top of the foot (Haridas and Zehr, 2003), stimulation of all discrete skin regions of the top of the foot cause facilitation of plantarflexor muscle activity. Yet, medial stimulation causes eversion, whereas lateral stimulation causes inversion. These responses are interpreted as obstacle avoidance, ensuring that the limb can maintain forward momentum during the propulsion into swing.

During stance, pressure changes from medio-lateral stimulation to the top of the foot were essentially opposite to those of the bottom. Generally, stimulation site and pressure changes are directly associated, such that lateral stimulation causes inversion, which is reduced with medial stimulation. During early stance, stimulation from all sites generally evoked facilitation of ankle plantarflexors associated with decreased dorsiflexion. As with the entire top of the foot (Drew and Rossignol, 1987; Zehr et al., 1998a, 1997), stimulation to any discrete skin region of the top of the foot causes plantarflexion as the foot must accept the weight of the body to avoid disturbances. When transitioning from swing to stance, stimulation to all regions of the top of the foot causes increased pressure to all sensors under the foot, indicating that perturbations to the top of the foot require more forceful ground contact in an attempt to establish stability.

Summary of cutaneous stimulation to discrete regions of the foot during walking

There is a topographic organization of neuromechanical responses evoked from the entire skin surface over the foot during walking. As with whole nerve branches innervating skin surfaces of the foot, responses are modulated by phase to induce functionally relevant alterations in muscle activity, pressure under the foot and gait kinematics. The site of tactile stimulation establishes the functional relevance of the response at a given phase of the step cycle, which can be reciprocal depending on the location and phase. Similar to stimulation to the nerve that innervates the entire lateral border of the foot (i.e. sural nerve) at the stance transition, stimulation to distinct regions of the lateral portion of the bottom of the foot evokes avoidance responses, which are not present with medial stimulation. This lateralization of responses, specifically, supports the notion of ‘sensory steering’ that was suggested by Zehr et al. (2014). The effects from cutaneous nerves innervating the skin surfaces of the hands during locomotion have been reported (for examples see (Haridas and Zehr, 2003; Klimstra et al., 2011; Zehr and Chua, 2000; Zehr and Haridas, 2003)), but the topographic organization and the resulting contributions of these discrete skin regions is an open and fruitful area requiring further investigation.

Conclusion

As in habitual quadrupeds like the cat, human walking is a relatively automated process arising from complex interactions of supraspinal input, spinal CPG activity and sensory feedback. Sensation from multiple sources is essential for precise coordination walking, but cutaneous feedback is particularly important for the maintenance of stability when the locomotor environment changes or includes obstacles and hazards. Reflexes from cutaneous innervation of the foot is highly topographically organized, phase-modulated, and functionally relevant. Ultimately, our feet act as “sensory antennae” to integrate sensorimotor interactions during walking.

References

Bastian, H.C., 1887. THE “MUSCULAR SENSE”; ITS NATURE AND CORTICAL LOCALISATION. Brain 10, 1–89. https://doi.org/10.1093/brain/10.1.1

Bent, L.R., Lowrey, C.R., 2013. Single low-threshold afferents innervating the skin of the human foot modulate ongoing muscle activity in the upper limbs. J Neurophysiol 109, 1614–25. https://doi.org/10.1152/jn.00608.2012

Bolton, D.A., Misiaszek, J.E., 2009. Contribution of hindpaw cutaneous inputs to the control of lateral stability during walking in the cat. J Neurophysiol 102, 1711–24. https://doi.org/10.1152/jn.00445.2009

Bouyer, L.J., Rossignol, S., 2003. Contribution of cutaneous inputs from the hindpaw to the control of locomotion. I. Intact cats. J Neurophysiol 90, 3625–39. https://doi.org/10.1152/jn.00496.2003

Brown, D.A., Kukulka, C.G., 1993. Human flexor reflex modulation during cycling. J Neurophysiol 69, 1212–24.

Capaday, C., 2002. The special nature of human walking and its neural control. Trends Neurosci. 25, 370–376.

Clair, J.M., Okuma, Y., Misiaszek, J.E., Collins, D.F., 2009. Reflex pathways connect receptors in the human lower leg to the erector spinae muscles of the lower back. Exp Brain Res 196, 217–27. https://doi.org/10.1007/s00221-009-1849-7

Clancy, B., Darlington, R.B., Finlay, B.L., 2001. Translating developmental time across mammalian species. Neuroscience 105, 7–17.

Dietz, V., 2002. Do human bipeds use quadrupedal coordination? Trends Neurosci 25, 462–7.

Dorofeev, I.Y., Avelev, V.D., Shcherbakova, N.A., Gerasimenko, Y.P., 2008. The role of cutaneous afferents in controlling locomotion evoked by epidural stimulation of the spinal cord in decerebrate cats. Neurosci Behav Physiol 38, 695–701. https://doi.org/10.1007/s11055-008-9034-1

Drew, T., Rossignol, S., 1987. A kinematic and electromyographic study of cutaneous reflexes evoked from the forelimb of unrestrained walking cats. J Neurophysiol 57, 1160–84.

Duysens, J., Bastiaanse, C.M., Dietz, V., Smits-Engelsman, B.M., Jansen, K., Jonkers, I., 2010. Are cutaneous reflexes from the foot preserved in passive walking in a DGO? Conf Proc IEEE Eng Med Biol Soc 2010, 3418–21. https://doi.org/10.1109/IEMBS.2010.5627887

Duysens, J., Bastiaanse, C.M., Smits-Engelsman, B.C., Dietz, V., 2004. Gait acts as a gate for reflexes from the foot. Can J Physiol Pharmacol 82, 715–22. https://doi.org/10.1139/y04-071

Duysens, J., Tax, A.A., Trippel, M., Dietz, V., 1993. Increased amplitude of cutaneous reflexes during human running as compared to standing. Brain Res 613, 230–8.

Duysens, J., Tax, A.A., Trippel, M., Dietz, V., 1992. Phase-dependent reversal of reflexly induced movements during human gait. Exp Brain Res 90, 404–14.

Duysens, J., Van de Crommert, H.W., 1998. Neural control of locomotion; The central pattern generator from cats to humans. Gait Posture 7, 131–141.

Eils, E., Nolte, S., Tewes, M., Thorwesten, L., Volker, K., Rosenbaum, D., 2002. Modified pressure distribution patterns in walking following reduction of plantar sensation. J Biomech 35, 1307–13.

Fallon, J.B., Bent, L.R., McNulty, P.A., Macefield, V.G., 2005. Evidence for strong synaptic coupling between single tactile afferents from the sole of the foot and motoneurons supplying leg muscles. J Neurophysiol 94, 3795–804. https://doi.org/10.1152/jn.00359.2005

Forssberg, H., 1979. Stumbling corrective reaction: a phase-dependent compensatory reaction during locomotion. J Neurophysiol 42, 936–53.

Forssberg, H., Grillner, S., Rossignol, S., 1975. Phase dependent reflex reversal during walking in chronic spinal cats. Brain Res 85, 103–7.

Grillner, S. (Ed.), 1981. Control of Locomotion in bipeds, tetrapods and fish., Handbook of Physiology.

Guertin, P.A., 2009. The mammalian central pattern generator for locomotion. Brain Res Rev 62, 45–56. https://doi.org/10.1016/j.brainresrev.2009.08.002

Haridas, C., Zehr, E.P., 2003. Coordinated interlimb compensatory responses to electrical stimulation of cutaneous nerves in the hand and foot during walking. Journal of neurophysiology 90, 2850–61. https://doi.org/10.1152/jn.00531.2003

Hauglustaine, S., Prokop, T., van Zwieten, K.J., Duysens, J., 2001. Phase-dependent modulation of cutaneous reflexes of tibialis anterior muscle during hopping. Brain Res 897, 180–3.

Howe, E.E., Toth, A.J., Vallis, L.A., Bent, L.R., 2015. Baseline skin information from the foot dorsum is used to control lower limb kinematics during level walking. Exp Brain Res 233, 2477–87. https://doi.org/10.1007/s00221-015-4318-5

Hultborn, H., Nielsen, J.B., 2007. Spinal control of locomotion--from cat to man. Acta Physiol (Oxf) 189, 111–121. https://doi.org/10.1111/j.1748-1716.2006.01651.x

Hurteau, M.F., Thibaudier, Y., Dambreville, C., Chraibi, A., Desrochers, E., Telonio, A., Frigon, A., 2017. Nonlinear Modulation of Cutaneous Reflexes with Increasing Speed of Locomotion in Spinal Cats. J Neurosci 37, 3896–3912. https://doi.org/10.1523/JNEUROSCI.3042-16.2017

Hurteau, M.-F., Thibaudier, Y., Dambreville, C., Danner, S.M., Rybak, I.A., Frigon, A., 2018. Intralimb and Interlimb Cutaneous Reflexes during Locomotion in the Intact

Cat. J. Neurosci. 38, 4104–4122. https://doi.org/10.1523/JNEUROSCI.3288-17.2018

Johansson, R.S., Vallbo, A.B., 1980. Spatial properties of the population of mechanoreceptive units in the glabrous skin of the human hand. Brain Res 184, 353–66.

Johnson, K., 2001. The roles and functions of cutaneous mechanoreceptors. Current Opinion in Neurobiology 11, 455–461. https://doi.org/10.1016/S0959-4388(00)00234-8

Kennedy, P.M., Inglis, J.T., 2002. Distribution and behaviour of glabrous cutaneous receptors in the human foot sole. J Physiol 538, 995–1002.

Klarner, T., Pearcey, G.E.P., Sun, Y., Barss, T.S., Kaupp, C., Munro, B., Frank, N., Zehr, E.P., 2017. Beyond the Bottom of the Foot: Topographic Organization of the Foot Dorsum in Walking. Med Sci Sports Exerc 49, 2439–2450. https://doi.org/10.1249/MSS.0000000000001389

Klarner, T., Zehr, E.P., 2018. Sherlock Holmes and the curious case of the human locomotor central pattern generator. J. Neurophysiol. 120, 53–77. https://doi.org/10.1152/jn.00554.2017

Klimstra, M.D., Thomas, E., Zehr, E.P., 2011. Biomechanical outcomes and neural correlates of cutaneous reflexes evoked during rhythmic arm cycling. Journal of biomechanics 44, 802–9. https://doi.org/10.1016/j.jbiomech.2010.12.017

Komiyama, T., Zehr, E.P., Stein, R.B., 2000. Absence of nerve specificity in human cutaneous reflexes during standing. Experimental brain research 133, 267–72.

Lam, T., Wolstenholme, C., van der Linden, M., Pang, M.Y., Yang, J.F., 2003. Stumbling corrective responses during treadmill-elicited stepping in human infants. J Physiol 553, 319–31. https://doi.org/10.1113/jphysiol.2003.043984

Lamont, E.V., Zehr, E.P., 2007. Earth-referenced handrail contact facilitates interlimb cutaneous reflexes during locomotion. Journal of neurophysiology 98, 433–42. https://doi.org/10.1152/jn.00002.2007

Lamont, E.V., 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. Experimental brain research 173, 185–92. https://doi.org/10.1007/s00221-006-0586-4

Macefield, V.G., 2005. Physiological characteristics of low-threshold mechanoreceptors in joints, muscle and skin in human subjects. Clin Exp Pharmacol Physiol 32, 135–44. https://doi.org/10.1111/j.1440-1681.2005.04143.x

MacGillivray, M.K., Klimstra, M., Sawatzky, B., Zehr, E.P., Lam, T., 2013. Prior experience does not alter modulation of cutaneous reflexes during manual wheeling and symmetrical arm cycling. Journal of neurophysiology 109, 2345–53. https://doi.org/10.1152/jn.00512.2012

McCrea, D.A., 2001. Spinal circuitry of sensorimotor control of locomotion. J Physiol 533, 41–50.

McNulty, P.A., Macefield, V.G., 2001. Modulation of ongoing EMG by different classes of low-threshold mechanoreceptors in the human hand. J. Physiol. (Lond.) 537, 1021–1032.

McNulty, P.A., Turker, K.S., Macefield, V.G., 1999. Evidence for strong synaptic coupling between single tactile afferents and motoneurones supplying the human hand. J Physiol 518 (Pt 3), 883–93.

Mildren, R.L., Bent, L.R., 2016. Vibrotactile stimulation of fast-adapting cutaneous afferents from the foot modulates proprioception at the ankle joint. J Appl Physiol (1985) 120, 855–64. https://doi.org/10.1152/japplphysiol.00810.2015

Mildren, R.L., Hare, C.M., Bent, L.R., 2017. Cutaneous afferent feedback from the posterior ankle contributes to proprioception. Neurosci Lett 636, 145–150. https://doi.org/10.1016/j.neulet.2016.10.058

Nakajima, T., Barss, T., Klarner, T., Komiyama, T., Zehr, E.P., 2013. Amplification of interlimb reflexes evoked by stimulating the hand simultaneously with conditioning from the foot during locomotion. BMC neuroscience 14, 28. https://doi.org/10.1186/1471-2202-14-28

Nakajima, T., Kamibayashi, K., Takahashi, M., Komiyama, T., Akai, M., Nakazawa, K., 2008a. Load-related modulation of cutaneous reflexes in the tibialis anterior muscle during passive walking in humans. Eur J Neurosci 27, 1566–76. https://doi.org/10.1111/j.1460-9568.2008.06120.x

Nakajima, T., Kamibayashi, K., Takahashi, M., Komiyama, T., Nakazawa, K., 2008b. Phase-dependent modulation of cutaneous reflexes in tibialis anterior muscle during passive stepping. Neurol Res 30, 46–51. https://doi.org/10.1179/016164108X268269

Nakajima, T., Sakamoto, M., Endoh, T., Komiyama, T., 2006a. Location-specific and task-dependent modulation of cutaneous reflexes in intrinsic human hand muscles. Clin Neurophysiol 117, 420–9. https://doi.org/10.1016/j.clinph.2005.09.017

Nakajima, T., Sakamoto, M., Tazoe, T., Endoh, T., Komiyama, T., 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. https://doi.org/10.1007/s00221-009-1802-9

Nakajima, T., Sakamoto, M., Tazoe, T., Endoh, T., Komiyama, T., 2006b. Location specificity of plantar cutaneous reflexes involving lower limb muscles in humans. Exp Brain Res 175, 514–25. https://doi.org/10.1007/s00221-006-0568-6

Nakajima, T., Suzuki, S...Futatsubashi, G...Ohtsuska, H...Mezzarane, R.A...Barss, T.S...Klarner, T...Zehr, E.P...Komiyama, T., 2016. Regionally distinct cutaneous afferent populations contribute to reflex modulation evoked by stimulation of the tibial nerve during walking. J Neurophysiol jn 01011 2015. https://doi.org/10.1152/jn.01011.2015

Nurse, M.A., Nigg, B.M., 2001. The effect of changes in foot sensation on plantar pressure and muscle activity. Clin Biomech (Bristol, Avon) 16, 719–27.

Panek, I., Bui, T., Wright, A.T., Brownstone, R.M., 2014. Cutaneous afferent regulation of motor function. Acta Neurobiol Exp (Wars) 74, 158–71.

Pang, M.Y., Lam, T., Yang, J.F., 2003. Infants adapt their stepping to repeated trip-inducing stimuli. J Neurophysiol 90, 2731–40. https://doi.org/10.1152/jn.00407.2003

Pang, M.Y., Yang, J.F., 2001. Interlimb co-ordination in human infant stepping. J Physiol 533, 617–25.

Pearson, K.G., 1995. Proprioceptive regulation of locomotion. Curr Opin Neurobiol 5, 786–91.

Perreault, M.C., Shefchyk, S.J., Jimenez, I., McCrea, D.A., 1999. Depression of muscle and cutaneous afferent-evoked monosynaptic field potentials during fictive locomotion in the cat. J Physiol 521 Pt 3, 691–703.

Petersson, P., Waldenstrom, A., Fahraeus, C., Schouenborg, J., 2003. Spontaneous muscle twitches during sleep guide spinal self-organization. Nature 424, 72–5. https://doi.org/10.1038/nature01719

Prochazka, A., Ellaway, P., 2012. Sensory systems in the control of movement. Compr Physiol 2, 2615–27. https://doi.org/10.1002/cphy.c100086

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

Sasada, S., Tazoe, T., Nakajima, T., Zehr, E.P., Komiyama, T., 2010. Effects of leg pedaling on early latency cutaneous reflexes in upper limb muscles. Journal of neurophysiology 104, 210–7. https://doi.org/10.1152/jn.00774.2009

Schillings, A.M., Mulder, T., Duysens, J., 2005. Stumbling over obstacles in older adults compared to young adults. J Neurophysiol 94, 1158–68. https://doi.org/10.1152/jn.00396.2004

Schillings, A.M., Van Wezel, B.M., Duysens, J., 1996. Mechanically induced stumbling during human treadmill walking. J Neurosci Methods 67, 11–7.

Schillings, A.M., Van Wezel, B.M., Mulder, T., Duysens, J., 1999. Widespread short-latency stretch reflexes and their modulation during stumbling over obstacles. Brain Res 816, 480–6.

Sherrington, C.S., 1910. Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. J Physiol 40, 28–121.

Sherrington, C.S., 1906. On the proprio-ceptive system, especially in its reflex aspects. Brain 29, 467–482.

Sherrington, C.S., 1903. Qualitative difference of spinal reflex corresponding with qualitative difference of cutaneous stimulus. J Physiol 30, 39–46.

Sherrington, C.S., 1898. Decerebrate Rigidity, and Reflex Coordination of Movements. The Journal of Physiology 22, 319–332. https://doi.org/10.1113/jphysiol.1898.sp000697

Stein, R.B., Capaday, C., 1988. The modulation of human reflexes during functional motor tasks. Trends Neurosci 11, 328–32.

Strzalkowski, N.D., Triano, J.J., Lam, C.K., Templeton, C.A., Bent, L.R., 2015. Thresholds of skin sensitivity are partially influenced by mechanical properties of the skin on the foot sole. Physiol Rep 3. https://doi.org/10.14814/phy2.12425

Strzalkowski, N.D.J., Ali, R.A., Bent, L.R., 2017. The firing characteristics of foot sole cutaneous mechanoreceptor afferents in response to vibration stimuli. J Neurophysiol 118, 1931–1942. https://doi.org/10.1152/jn.00647.2016

Taylor, A.J., Menz, H.B., Keenan, A.M., 2004. Effects of experimentally induced plantar insensitivity on forces and pressures under the foot during normal walking. Gait Posture 20, 232–7. https://doi.org/10.1016/j.gaitpost.2003.02.001

Van Wezel, B.M., Ottenhoff, F.A., Duysens, J., 1997. Dynamic control of location-specific information in tactile cutaneous reflexes from the foot during human walking. J Neurosci 17, 3804–14.

van Wezel, B.M., van Engelen, B.G., Gabreels, F.J., Gabreels-Festen, A.A., Duysens, J., 2000. Abeta fibers mediate cutaneous reflexes during human walking. J Neurophysiol 83, 2980–6.

Vasudevan, E.V., Zehr, E.P., 2011. Multi-frequency arm cycling reveals bilateral locomotor coupling to increase movement symmetry. Experimental brain research 211, 299–312. https://doi.org/10.1007/s00221-011-2687-y

Vilensky, J.A., 1987. Locomotor behavior and control in human and non-human primates: comparisons with cats and dogs. Neurosci Biobehav Rev 11, 263–274.

Yang, J.F., Stein, R.B., 1990. Phase-dependent reflex reversal in human leg muscles during walking. J Neurophysiol 63, 1109–17.

Zehr, E.P., 2006. Training-induced adaptive plasticity in human somatosensory reflex pathways. Journal of applied physiology 101, 1783–94. https://doi.org/10.1152/japplphysiol.00540.2006

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

Zehr, E.P., Barss, T.S., Dragert, K., Frigon, A., Vasudevan, E.V., Haridas, C., Hundza, S., Kaupp, C., Klarner, T., Klimstra, M., Komiyama, T., Loadman, P.M., Mezzarane, R.A., Nakajima, T., Pearcey, G.E., Sun, Y., 2016. Neuromechanical interactions between the limbs during human locomotion: an evolutionary perspective with translation to rehabilitation. Exp Brain Res 234, 3059–3081. https://doi.org/10.1007/s00221-016-4715-4

Zehr, E.P., Chua, R., 2000. Modulation of human cutaneous reflexes during rhythmic cyclical arm movement. Experimental brain research 135, 241–50.

Zehr, E.P., Collins, D.F., Chua, R., 2001. Human interlimb reflexes evoked by electrical stimulation of cutaneous nerves innervating the hand and foot. Experimental brain research 140, 495–504. https://doi.org/10.1007/s002210100857

Zehr, E.P., Duysens, J., 2004. Regulation of arm and leg movement during human locomotion. Neuroscientist 10, 347–61. https://doi.org/10.1177/1073858404264680

Zehr, E.P., Fujita, K., Stein, R.B., 1998a. Reflexes from the superficial peroneal nerve during walking in stroke subjects. Journal of neurophysiology 79, 848–58.

Zehr, E.P., 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. Experimental brain research 149, 260–6. https://doi.org/10.1007/s00221-003-1377-9

Zehr, E.P., Komiyama, T., Stein, R.B., 1997. Cutaneous reflexes during human gait: electromyographic and kinematic responses to electrical stimulation. Journal of neurophysiology 77, 3311–25.

Zehr, E.P., Nakajima, T., Barss, T., Klarner, T., Miklosovic, S., Mezzarane, R.A., Nurse, M., Komiyama, T., 2014. Cutaneous stimulation of discrete regions of the sole during locomotion produces “sensory steering” of the foot. BMC sports science, medicine and rehabilitation 6, 33. https://doi.org/10.1186/2052-1847-6-33

Zehr, E.P., Stein, R.B., 1999. What functions do reflexes serve during human locomotion? Progress in neurobiology 58, 185–205.

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

Zimmerman, A., Bai, L., Ginty, D.D., 2014. The gentle touch receptors of mammalian skin. Science 346, 950–954. https://doi.org/10.1126/science.1254229

Chapter 4 - Priming via patterned stimulation of cutaneous reflex pathways amplifies spinal cord excitability

Abstract

Priming with patterned stimulation of muscle afferents from antagonist muscles has been used to induce plasticity of spinal cord excitability as evidenced by changes in group Ia reciprocal inhibition. When assessed transiently with a condition-test pulse paradigm, stimulating cutaneous afferents innervating the foot reduces Ia presynaptic inhibition in the soleus and therefore facilitates Hoffmann (H-) reflex amplitudes. Plastic effects (i.e. priming) of longer lasting sensory stimulation (that mimics repeated ground contact loading) of cutaneous afferents innervating the foot have yet to be examined. As a first step, we examined how priming with 20 minutes of patterned and alternating stimulation between the left and right foot affects spinal cord excitability. During priming, stimulus trains (550ms; consisting of 28x1ms pulses at 50Hz, 1.2 x radiating threshold) were applied simultaneously to the sural and plantar nerves of the ankle. Stimulation to the left and right ankle was out of phase by 500ms. We evoked soleus H-reflexes and muscle compound action potentials (M-waves) prior to and following priming stimulation to provide a proxy measure of spinal cord excitability. H-reflex and M-wave recruitment curves were recorded at rest, during brief (<2 minutes) arm cycling and with sural conditioning (train of 5x1ms pulses at 2xRT with a C-T = 80ms). Data indicate a general increase in H-reflex excitability following priming via patterned sensory stimulation. Transient sural conditioning was less effective following priming, indicating that the increased excitability of the H-reflex is partially attributable to a reduction in presynaptic inhibition of Ia afferents. Priming, which enhances spinal cord excitability, may prove useful in both rehabilitation and performance settings.

New & Noteworthy

Priming via patterned stimulation of the nervous system induces neuroplasticity. Yet, accessing previously known cutaneous reflex pathways to alter muscle reflex excitability has not yet been examined. Here, we show that priming via patterned stimulation of the cutaneous afferents that innervate the foot sole can amplify spinal cord excitability, which, in this case, is attributed to reductions in presynaptic inhibition.

Introduction

Neuroplasticity is “the capacity for continuous alteration of the neural pathways and synapses of the living brain and nervous system in response to experience or injury” (Merriam-Webster 2019). These experiences come from training of a motor skill and sensations that we feel. In rehabilitation, maladaptive neuroplasticity due to an injury is reversed through practice or training of motor skills, however, external stimuli to induce sensations have also been used to target specific neural circuits (Field-Fote 2015). This ‘priming’, is a change in a behaviour induced by previous stimuli, and has most prominently been studied in learning experiments (Stoykov and Madhavan 2015). In rehabilitation settings, however, whether the goal is to attenuate (Estes et al. 2017) or augment (Leech et al. 2018) spinal cord excitability, priming has received increasing attention.

Priming techniques that specifically target tactile afferents to induce neuroplasticity include transcutaneous electrical nerve stimulation (TENS), vibration, trans spinal direct current stimulation (tsDCS), and others. The specific targeted neural pathway of these sensory stimulation protocols can vary substantially. For example, TENS applied over a muscle belly likely excites both the underlying tactile mechanoreceptors and the group Ia afferents from the underlying muscle (Hamilton et al. 2018; Radhakrishnan and Sluka 2005). Furthermore, the skin overlying the leg provides very little functional relevance for motor outputs compared to that of the surface of the feet. For example, the moving limbs have end points that interact with our environment, and these end points (i.e. the hands and feet) provide functionally relevant feedback about interactions with the environment (Klarner et al. 2017; Zehr et al. 2014; Zehr and Stein 1999).

For over a century, cutaneous reflex pathways have been studied in mammals (including humans) to provide information about the integration of cutaneous sensations to motor output. They are evoked via non-noxious stimulation of either the afferents that innervate tactile mechanoreceptors (i.e. Pacinian, Meissner’s, and Ruffini corpuscles and Merkel discs) or the mechanoreceptors themselves within the skin of the hands and feet (Hurteau et al. 2018; Zehr 2006). Activation causes sensations of tactile vibration, buzzing, fluttering and/or tingling. These polysynaptic pathways contain an unknown number of interneurons that produce inhibition or facilitation of motoneurons (Hurteau et al. 2018).

In general, preceding stimulation (i.e. with a condition-test interval <150ms) to cutaneous afferents innervating the feet, used to evoke cutaneous reflexes, facilitates the soleus SOL Hoffmann (H-) reflex (Iles 1996). Furthermore, repetitive stimulation to cutaneous afferents innervating skin over the hand causes alterations in the recruitment thresholds of motor units, such that smaller units are recruited with higher thresholds and larger units are recruited at lower thresholds (Garnett and Stephens 1981). This is somewhat contradictory to the orderly recruitment of motor units (Henneman 1985).

During natural movement, it is quite rare for sensations to occur continuously, rather they occur with a phasic pattern. For example, reaching to grasp an object will provide sensory information only when appropriate (i.e. when contact is made with the object to be grasped), whereas locomotion provides rhythmic sensory feedback that differs from phase to phase (Duysens and Pearson 1998). Therefore, if the intent is to alter the excitability of spinal circuits involved in movement, optimal priming should provide sensory information that is similar to real-life experiences rather than continuous stimulation which may be interpreted as noise. Indeed, Perez and colleagues (Perez et al. 2003) showed that the pattern of sensory stimulation to the common peroneal nerve was crucial to induce plasticity of the Ia reciprocal inhibition pathway. The purpose of this experiment was to examine the effects of repeated and patterned stimulation to afferents innervating the plantar surfaces of the feet by using the SOL H-reflex as a proxy of spinal cord excitability. We hypothesized that repeated activation of the cutaneous reflex pathways, that reduce presynaptic inhibition (PSI) acutely, would cause increased excitability of the SOL H-reflex that can be, at least partially, attributed to reductions in Ia PSI.

Methods

Participants

Eight neurologically intact participants (5 female) were recruited from the university student population. Participants were apparently healthy and provided written and signed informed consent prior to partaking in any experimental protocols. The protocol was approved by the Human Research Ethics Board at the University of Victoria and was conducted in accordance with the Declaration of Helsinki.

Experimental protocol

Participants were prepared for electromyographic (EMG) recordings upon arrival to the Rehabilitation Neuroscience Laboratory. Once familiarized with protocols, participants sat comfortably in the seat of an arm and leg cycle ergometer (Sci-Fit Pro 2, Tulsa, OK) with the foot pedals removed and feet secured into custom restraints (see figure 4-1 for experimental set-up) in a position identical to Chapter 5. Bilateral recruitment curves of the SOL H-reflex were sampled, followed by bilateral SOL H-reflexes with a fixed M-wave size both while the participant sat quietly. Next, a bilateral recruitment curve and fixed M-wave H-reflex recordings were sampled while the participant performed a brief (<2 minutes) bout of arm cycling. Next, a cutaneous (sural) conditioned recruitment curve of the SOL H-reflex was sampled, followed by a set of cutaneous conditioned SOL H-reflexes with a fixed M-wave size both while the participant sat quietly. Priming via patterned sensory stimulation was then provided for 20 minutes to as the participant sat quietly in the seat. Immediately after the patterned sensory stimulation the recruitment curves and H-reflexes with a fixed M-wave size were repeated in the same order as prior to the stimulation.

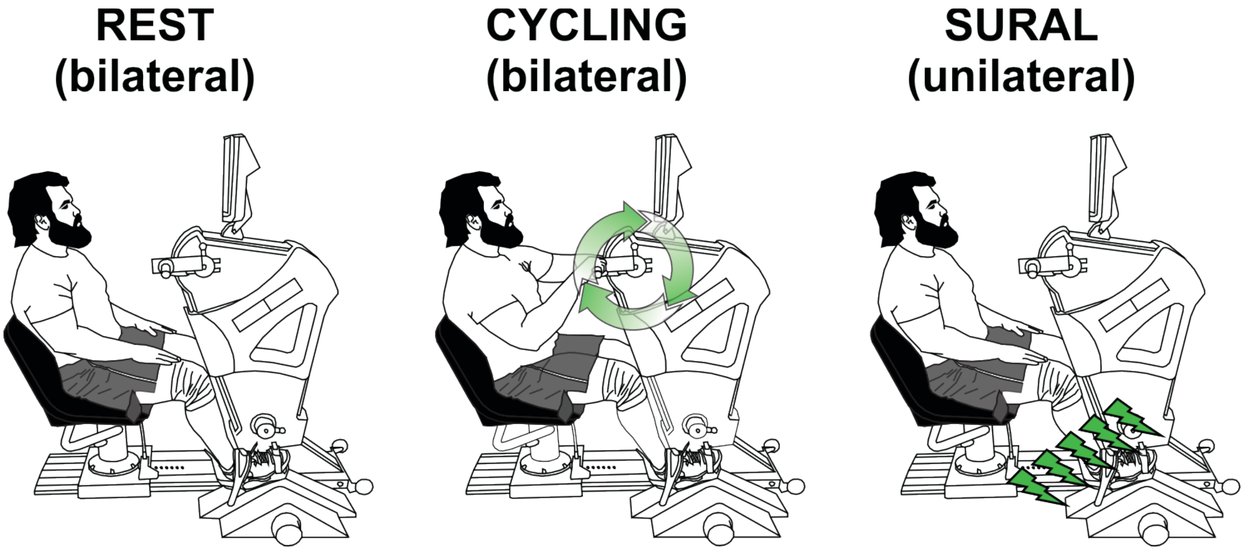

Figure 4-1: *Experimental set-up for each condition used to measure H-reflexes. Quite sitting (REST) is depicted in the left panel, arm cycling (CYCLING; indicated by the circular arrows) in the middle panel, and sural conditioning (SURAL; indicated by the five lightning bolts over the ankle) in the right panel. Words in parentheses indicate whether H-reflexes were evoked in the bilateral or unilateral SOL for recording.*

Priming via patterned sensory stimulation

Patterned sensory stimulation was delivered to the bilateral ankles in an attempt to simulate ground contact during walking. Prior to the experimental protocol, perceptual threshold (PT) and radiating threshold (RT) was determined for each stimulation site. PT was defined as the lowest current required to evoke the smallest detectable tactile sensation, whereas RT was defined as the minimum current required to cause clear radiating paresthesia of the innervation area (Nakajima et al. 2014; Pearcey et al. 2017). Stimulation parameters were chosen to create radiating paresthesia along the plantar and lateral border of the foot, and was characterized as “buzzing”, “vibrating”, “fluttering” and/or “tingling”. Careful attention was given to ensure there were no visible muscle contractions in muscles of the foot or any noxious sensations. Therefore, trains of stimulation were applied simultaneously to the plantar (posterior/inferior to the medial malleolus) and sural (posterior/inferior to the lateral malleolus) nerves of the right and left foot in an alternating fashion (see figure 4-2A for electrode placement) for 20 minutes while participants sat comfortably. Stimulation parameters were chosen to simulate average contact times during typical gait, which is based on the assumption that the typical young adult has a mean preferred walking speed of ~1.4 m/s (Bohannon 1997), and has a stride frequency of ~60 strides per minute while walking at that speed (Hansen et al. 2017). To create simulated contact during walking, pulse trains consisted of 28x1 ms pulses applied for 550ms (i.e. 50Hz pulse frequency within the train) at 1.2 times the radiating threshold. The trains were applied to each foot with a 1Hz frequency and duty cycle of 11:9. Stimulation overlapped on both feet for a period of 50ms twice in each cycle, at the beginning and end of each train. See figure 4-2B for a visual representation of the stimulation.

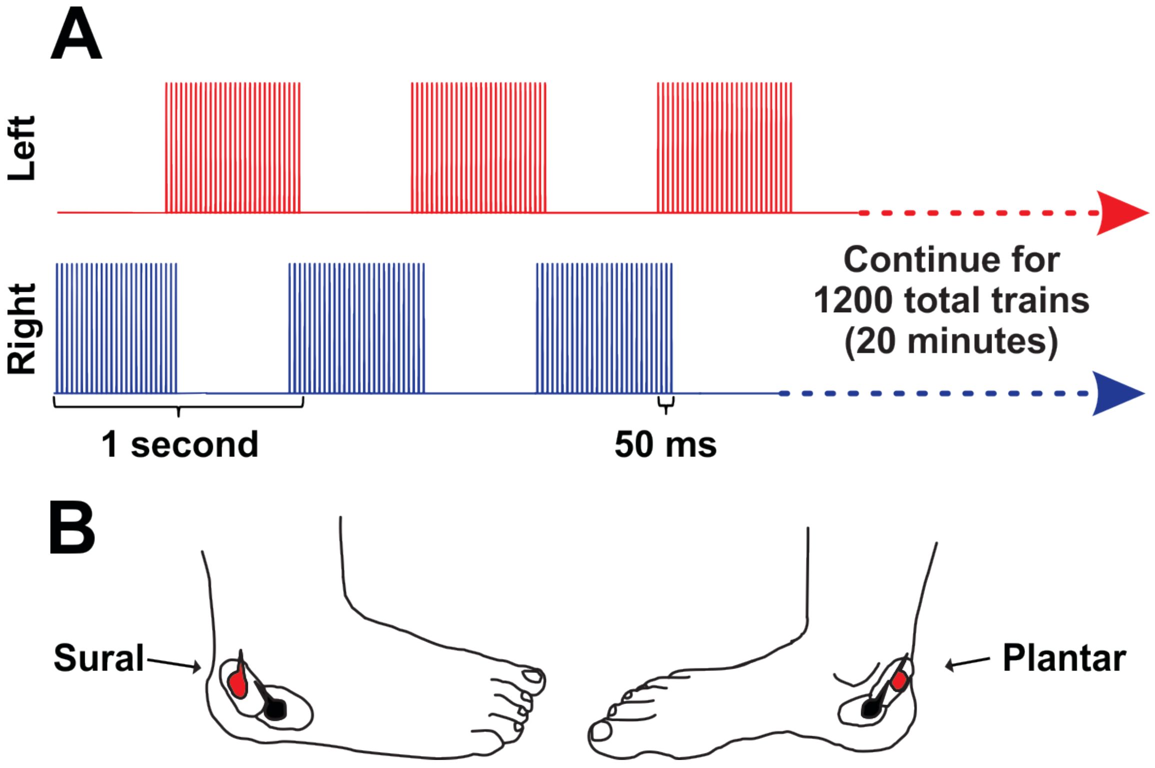

Figure 4-2: *Stimulation characteristics are shown in A. Simultaneous 1ms pulses were delivered to the sural and plantar nerves in trains that lasted 550ms at a rate of 50Hz, had an 11:9 duty cycle, and alternated between the feet. The red trace represents pulses to the left foot whereas the blue trace represents pulses to the right foot. Stimulation electrodes were placed inferior and posterior to the lateral (sural nerve; left panel/lateral view) and medial (plantar nerve; right panel/medial view) malleoli, as depicted in B.*

Hoffmann (H-) reflex stimulation

To evoke H-reflexes in the bilateral SOL muscles, single 1 ms square wave electrical pulses were applied to the popliteal fossa with bipolar surface electrodes (Thought Technology Ltd., Montreal, QC, Canada) using a Digitimer (model DS7A, Medtel, NSW, Australia) constant current stimulator. A non-contact milliammeter (mA-2000, Bell Technologies, Orlando, FL, USA) was used to measure current delivered for each stimulus. Simultaneous recruitment curves (40 sweeps) were recorded at rest pre- and post-priming. Bilateral H-reflexes with an amplitude corresponding to ~75% H_{max} on the ascending limb of the recruitment curve with a small (i.e. to minimize antidromic effects) but measurable M-wave amplitude were recorded following each recruitment curve. This amplitude was chosen to ensure we were within the range of H-reflex amplitudes that are sensitive to both facilitation and inhibition from presynaptic inhibitory input (Crone et al. 1990). Furthermore, it was essential that there was a measurable M-wave amplitude to provide a control for the level of stimulation throughout the experiment (Zehr 2002).

Recruitment (stimulus-response) curves

Bilateral stimulus-response curve stimuli were delivered pseudo randomly between 1 and 3 s for a total of 40 sweeps. Stimulus intensity was increased and decreased incrementally (ranged from 0.1 to 1mA per increment) based on the excitability of the reflex pathway in different individuals, while ensuring that supramaximal M-wave amplitudes were achieved by increasing larger increments once the H-reflex amplitude started to decrease in size (i.e. after the ascending limb, for examples see (Klimstra and Zehr 2008)).

Constant M-wave, H-reflex recordings

Following each recruitment curve, the current required to evoke a constant M-wave that corresponded to ~5% of M_{max} was delivered randomly between 1 and 3 s for a total of 10 sweeps to both legs simultaneously. Pre-priming H-reflex amplitudes at this stimulation intensity evoked a response that was ~75% of H_{max}. The recorded 10 sweeps were averaged and compared.

Arm cycling conditioning of H-reflexes

To provide a conditioning paradigm that increases Ia PSI, we had participants perform low intensity arm cycling (1 Hz/60 rpm, 50 watts) while we sampled bilateral H-reflexes from their resting SOL. Arm cycling activates cervicolumbar propriospinal networks that increase Ia PSI through presynaptic inhibitory interneurons, causing a general suppression of H-reflexes in the SOL (Frigon *et al.*, 2004; Zehr *et al.*, 2004; Brooke & Zehr, 2006; Loadman & Zehr, 2007). During this conditioning, stimulation to evoke SOL H-reflexes was given at the same position in the cycling phase (i.e. when the right hand was top dead center and left hand was bottom dead center of the arm cycling phase) to reduce the variability associated with phase-dependent modulation of H-reflexes during arm cycling (de Ruiter et al. 2010).

Cutaneous conditioning of H-reflexes

To provide a conditioning paradigm that reduces Ia PSI, the right sural nerve was stimulated with a train of five 1 ms pulses delivered at 300 Hz at two times the radiating threshold. The interval between the sural nerve conditioning and the test H-reflex (C-T interval) was 80 ms (Frigon *et al.*, 2004; Zehr *et al.*, 2004; Brooke & Zehr, 2006; Loadman & Zehr, 2007).

Electromyography

Following cleansing the skin surfaces with isopropyl alcohol, bipolar surface electrodes were placed over the mid-muscle bellies of the soleus (SOL), tibialis anterior (TA) and vastus lateralis (VL) of the participant's dominant leg. A common ground electrode was placed over each patella. EMG signals were preamplified (x500-100 for SOL and x5000 for TA and VL) and band pass filtered (10-1000 Hz for SOL and 100-300 Hz for TA and VL) (GRASS P511, AstroMed). These parameters are consistent with previous experiments in our laboratory (Balter and Zehr 2007; Pearcey et al. 2017; Vasudevan and Zehr 2011; Zehr et al. 2012). After conversion to a digital signal, data were sampled at 5000 Hz using custom built sweep based acquisition software (LABVIEW, National Instruments, TX, USA).

Control data

To illustrate the stability of recruitment curves recorded with these, we obtained data from a separate experiment (Chapter 5 of this dissertation). Data from the eight participants who also participated in the current experiment were extracted for analysis and comparison. The CONTROL condition from Chapter 5 required participants to sit in the same position as the current experiment for one hour and recruitment curves of the SOL H-reflexes and M-waves were recorded with the same methodologies as the current experiment. Data from the 8 participants who also completed this experiment in figure 4-3.

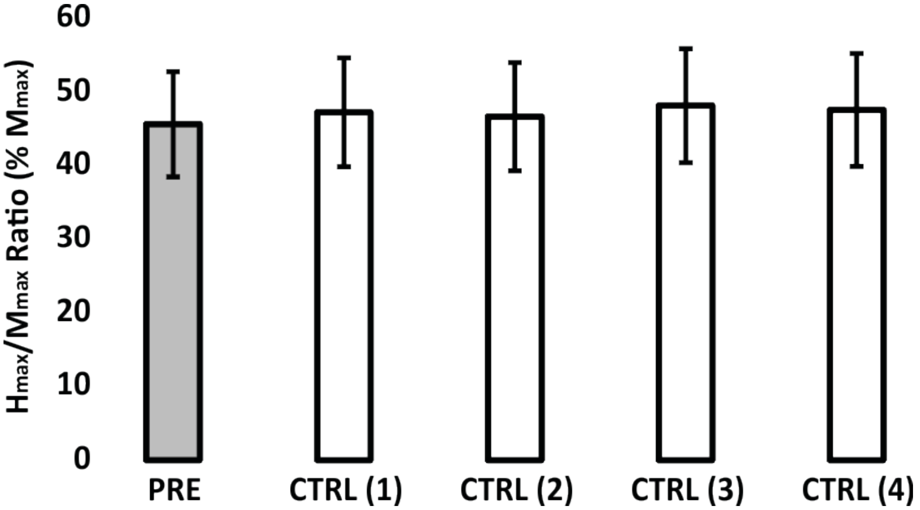

Figure 4-3: *A comparison of H_{max}/M_{max} ratios obtained at rest prior to priming stimulation and from four time points in Chapter 5. The group average (±SE) H_{max}/M_{max} ratio recorded in the right SOL of participants who performed both experiments (n = 8) is shown prior to priming stimulation (PRE; grey) and at four control timepoints separated by 10 minutes (CTRL; unfilled).*

Statistics

Statistical procedures were performed using SPSS 18.0 (Chicago, Illinois). A three factor (PRIMING x CONDITIONING x LEG) repeated measures (RM) ANOVA was performed on dependent variables (i.e. H-reflex amplitude and recruitment curve parameters) for the REST and CYCLING. When we examined the effects of sural conditioning, a two factor (CONDITIONING x TIME) RM ANOVA was performed on data from the right leg only. If significant interaction effects were identified and direction of change from PRE was predicted because of a priori hypotheses, one-tailed paired samples t-tests were performed. In all cases, statistical significance was set at $p \leq 0.05$. Results are reported as means ± SD in text (SE in figures). To provide an indication of the magnitude of the effect of transient conditioning and priming, Cohen's d effect sizes were calculated (Cohen 1977).

Results

Priming via patterned sensory stimulation increases spinal cord excitability

H-reflex excitability is generally increased following 20 minutes of priming via patterned sensory stimulation to the cutaneous nerves innervating the foot soles (see figure 4-4A). This is shown in the single subject traces of H-reflexes recorded on the ascending limb of the recruitment curve with matching M-wave amplitudes (figure 4-4B) and in the single subject recruitment curves (figure 4-4C). A 3 way (PRIMING x CONDITIONING x LEG) RM ANOVA revealed that there was a significant main effect of PRIMING ($F_{(1,7)} = 10.974$, $p = 0.016$), but no significant effect for LEG ($F_{(1,7)} = 0.036$, $p = 0.856$). H_{max}/M_{max} ratios recorded at rest were increased by 11.5 ± 18.29% ($p = 0.018$, d = 0.24) and 12.9 ± 18.04% ($p = 0.033$, d = 0.30) for the right and left SOL, respectively. H_{max}/M_{max} ratios recorded during cycling were increased by 21.6 ± 15.76% ($p = 0.015$, d = 0.29) and 18.1 ± 19.97% ($p = 0.043$, d = 0.30) for the right and left SOL, respectively. Unlike H-reflex excitability measured at rest and during cycling, H_{max}/M_{max} ratios recorded following a brief train of stimuli to the sural nerve were similar ($p = 0.669$, d = 0.058) PRE (52 ± 22.95% M_{max}) and POST (53 ± 22.67% M_{max}) patterned nerve stimulation.

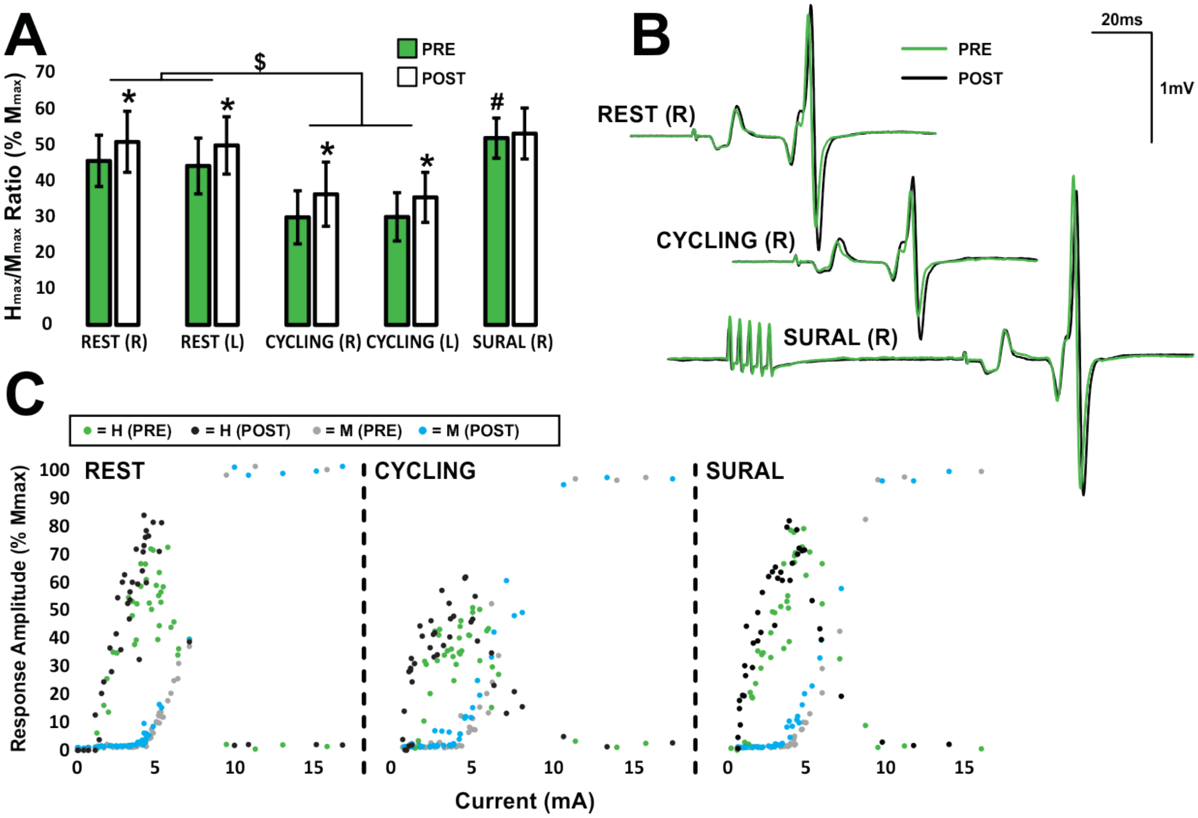

Figure 4-4: *The effects of priming stimulation on H-reflex excitability across various conditions. The group average (±SE) is shown for* H_{max}/M_{max} *ratios recorded prior to (PRE; green) and following (POST; unfilled) priming stimulation during REST, CYCLING and following sural conditioning (SURAL) in A. Letters in brackets indicate the leg in which the H-reflexes were recorded. The asterisks indicates a significant (*$p < 0.05$*) difference from PRE to POST, cash sign indicates a significant (*$p < 0.05$*) difference between REST and CYCLING, whereas pound indicates a significant (*$p < 0.05$*) difference between REST and SURAL at PRE. An individual's mean of 10 traces prior to (PRE; green) and following (POST; black) priming stimulation is shown in B for H-reflexes recorded from the right leg at rest (REST; top), during arm cycling (CYCLING; middle) and following brief conditioning stimuli to the sural nerve (SURAL; bottom). An individual's recruitment curve recorded from the right leg at rest (REST; left), during arm cycling (CYCLING; middle) and following brief conditioning stimuli to the sural nerve (SURAL; right) is shown in C. The green and black dots are H-reflex amplitudes during PRE and POST, respectively, whereas the grey and cyan dots are M-wave amplitudes during PRE and POST, respectively.*

Sural conditioning reduces presynaptic inhibition prior to priming but is less effective following priming

H-reflex excitability is generally increased following a brief train of stimuli to the sural nerve. This is shown in the single subject traces of H-reflexes recorded on the ascending limb of the recruitment curve with matching M-wave amplitudes (figure 4-4B in the bottom traces) and in the single subject recruitment curves (figure 4-4C to the far right). A 2 way (PRIMING x CONDITIONING) RM ANOVA revealed that there was a significant main effect of CONDITIONING ($F_{(1,7)} = 9.117$, $p = 0.004$) and TIME ($F_{(1,7)} = 7.111$, $p = 0.037$). Prior to priming, the H_{max}/M_{max} ratio was facilitated by 19.9 ± 11.56% ($p = 0.02$, d = 0.3).

In order to compare the effectiveness of sural conditioning PRE and POST priming, we created a sural conditioning ratio. This ratio was calculated as the difference between the SURAL and REST H_{max}/M_{max} ratio as a percentage of REST. The effect of conditioning was reduced ($p = 0.043$, d = 0.97) from 19.9 ± 11.56% (PRE) by nearly half to 10.23 ± 8.05% (POST). Group means for the sural conditioning ratio (figure 4-5A), an individual's individual traces with match M-waves (figure 4-5B) and an individual's recruitment curve with sural conditioning (figure 4-5C) can been seen in figure 4-5.

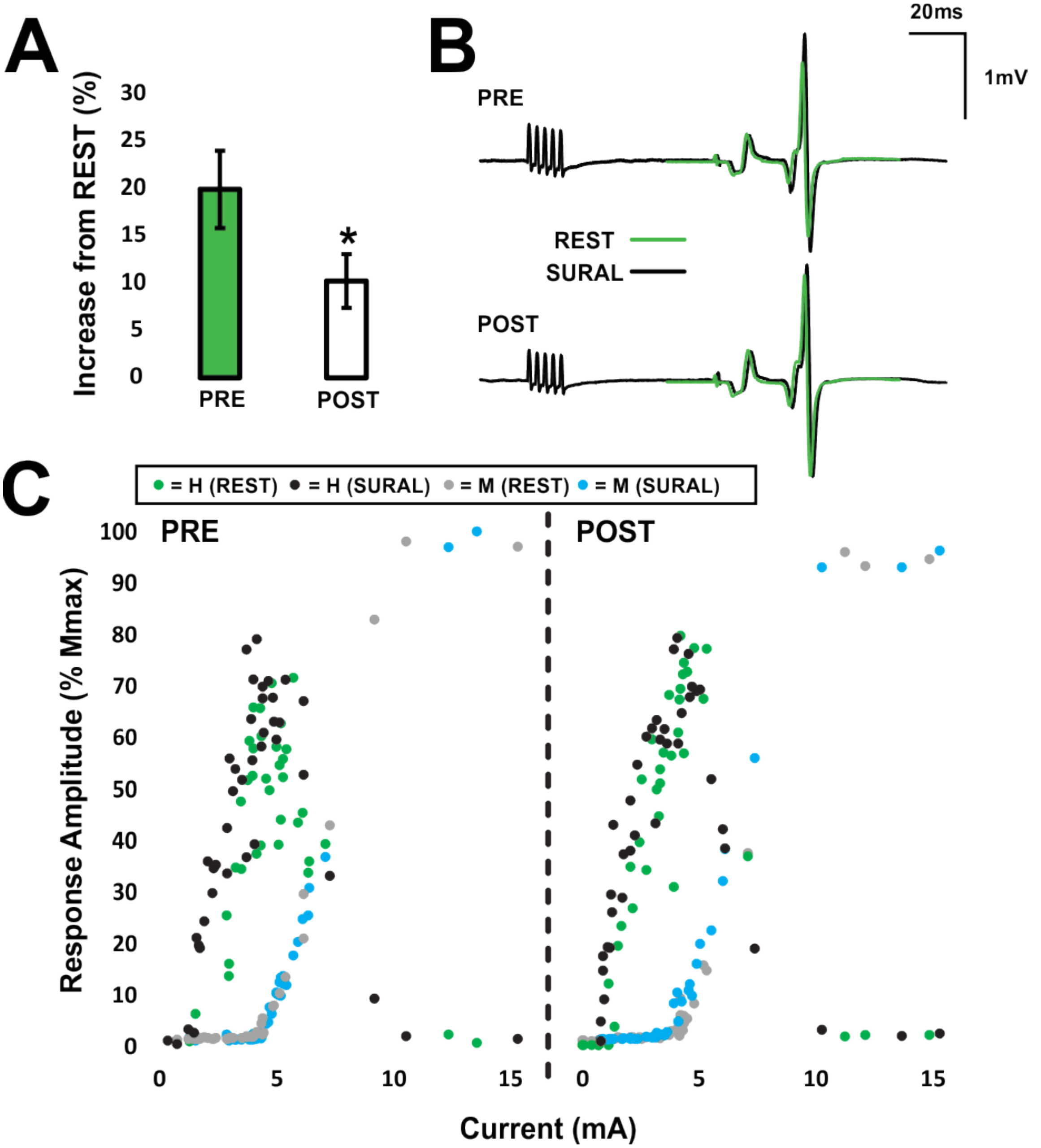

Figure 4-5: *The effects of conditioning from a brief train of stimuli applied to the sural nerve on H-reflex excitability. The group average (±SE) is shown for* H_{max}/M_{max} *ratios recorded prior to (PRE; green) and following (POST; unfilled) priming stimulation in A. The asterisks indicates a significant (*$p < 0.05$*) difference from PRE to POST. An individual's mean of 10 traces without conditioning stimulation (REST; green) and with sural conditioning (SURAL; black) prior to (PRE; top) and following (POST; bottom) priming stimulation is shown in B. An individual's recruitment curve prior to (PRE; left)*

and following (POST; right) priming stimulation is shown in C. The green and black dots are H-reflex amplitudes during REST and SURAL, respectively, whereas the grey and cyan dots are M-wave amplitudes during REST and SURAL, respectively.

H-reflex excitability is reduced during brief arm cycling pre and post priming

H-reflex excitability is generally reduced during a brief bout of arm cycling (shown in figure 4-4). This is shown in the single subject traces of H-reflexes recorded on the ascending limb of the recruitment curve with matching M-wave amplitudes (figure 4-4B when you compare the top and middle traces) and in the single subject recruitment curves (figure 4-4C when you compare the left and middle panels). A 3 way (PRIMING x CONDITIONING x LEG) RM ANOVA revealed that there was a significant main effect of CONDITIONING ($F_{(1,7)} = 8.047$, $p = 0.03$), but no significant effect for LEG ($F_{(1,7)} = 0.036$, $p = 0.856$). For the right SOL H_{max}/M_{max} ratio, there was a similar ~30% suppression from REST to CYCLING both PRE (31.4 ± 28.64%, $p = 0.012$, d = 0.77) and POST (29.2 ± 26.48%, $p = 0.014$, d = 0.59) priming. Similarly, for the left SOL H_{max}/M_{max} ratio, there was a similar ~30% suppression from REST to CYCLING both PRE (28.4 ± 31.3%, $p = 0.027$, d = 0.81) and POST (29 ± 29.62%, $p = 0.021$, d = 0.73) priming.

Discussion

The most important finding from this study is that 20 minutes of priming via patterned stimulation to cutaneous afferents innervating the plantar surfaces of the feet causes facilitation of SOL H-reflex excitability. Since sensory enhancement via sural nerve conditioning is less effective following patterned stimulation (i.e. priming) compared to before, we suggest that this SOL H-reflex facilitation is, at least partially, due to reduction in Group Ia presynaptic inhibition.

The importance of stimulation characteristics

Repetitive transcutaneous electrical nerve stimulation (i.e. TENS) has been used clinically for nearly half a century to treat pain (Claydon et al. 2011) and improve rehabilitation strategies for a variety of neurological dysfunctions (Francini et al. 1981; Levin and Hui-Chan 1992; Ng Shamay S and Hui-Chan Christina W.Y. 2007). There has been a reported absence of consistent effects of TENS on SOL H-reflex amplitudes (Goulet et al. 1994), however, methodological considerations including the stimulation parameters (i.e. frequency, pulse duration, intensity, etc.) and location likely account for these inconsistencies. For example, Serrano-Munoz et al. (2017) showed that two different therapists were inconsistent at providing a current density of TENS that elicits a "strong but comfortable sensation, just below motor threshold." In doing so, they revealed that lower current densities tended to increase the SOL H-reflex, whereas higher current densities tended to decrease the SOL H-reflex. This intensity-dependent effect on the excitability of the SOL H-reflex has also been reported previously during both transient conditioning (Delwaide et al. 1981) and sustained stimulation (Hardy et al. 2002). Transient stimulation (seven 1ms pulses at 300Hz) of the sural nerve causes a general biphasic facilitation of SOL H-reflexes in both the ispsi- and contralateral limb when stimulus intensity causes tactile sensation (2.5x radiating threshold), whereas noxious sensation (4.5x radiating threshold) causes inhibition (Delwaide et al. 1981). In the work of Hardy and colleagues (2002), 15 minutes of TENS at sensory threshold to the skin over the dorsi- or plantarflexors caused the SOL H-reflex to increase, however stimulation at 1.5x the motor threshold either reduced or had no effect on the H-reflex amplitude. Adding to the discrepancies between effects of TENS on H-reflex excitability, reports by the same group have suggested that TENS either causes reductions in SOL H-reflex amplitudes

(Walsh et al. 2000), or has no effect (Cramp et al. 2000), when applied over the sural nerve. Sensations experienced by participants were described as feeling the "strong but comfortable" sensation beneath the electrodes. The diagrams show that the electrodes were placed proximal to the ankle near the achilles tendon and, since no indication of stimulation intensity is given, it could have been too high to cause facilitation of H-reflexes. Hence, these studies used stimulation parameters not typically associated with evoking cutaneous reflexes, whereas simulation with parameters more similar to those used to evoke cutaneous reflexes (i.e. 1ms pulse duration, with radiating paresthesia into the innervation area) enhances H-reflex excitability during fatiguing cycling sprints or during non-fatiguing cycling (Pearcey et al. 2017).

It is important to note that the stimulation provided in the current experiment was provided in a temporal pattern that resembles ground contact during human locomotion (i.e. 1Hz step frequency, alternating between the feet). Cutaneous input has powerful effects on spinal central pattern generating circuits, such that stimulation to the plantar surface of the foot in the cat causes entrainment of the locomotor cycle (Duysens and Pearson 1998). Using patterns of stimulation that resemble sensations experienced during locomotion have been proven vital when inducing plasticity of human spinal circuits. For example, Perez and colleagues (2003) used rhythmic bursts of stimulation (ten 1ms pulses at 100Hz every 1.5 seconds for a total of 1000 trains) to the common peroneal nerve to increase the strength of reciprocal inhibition from the TA to the SOL. When they provided constant stimulation (one 1ms pulse every 150ms for a total of 10,000 pulses) at the same intensity, there was no enhancement of reciprocal inhibition. Similarly, stimulation to the tibial nerve just above the threshold intensity required to evoke a SOL H-reflex causes suppression of the H-reflex amplitude when stimulation was delivered in a pattern (three 1ms pulses at 50Hz every 200 ms for a total of 600 pulses), but not after constant stimulation (1ms pulses applied at 15Hz for a total of 600 pulses) (Jimenez et al. 2018).

Possible mechanisms involved in the plasticity of H-reflex excitability

Although reports of plasticity of H-reflex excitability induced by TENS has been extensively examined, the mechanisms responsible for that plasticity have not been elucidated. Similar to some experiments with TENS (Arsenault et al. 1993; Hardy et al.

2002), we have provided a method of supplying patterned and continuous stimulation to cutaneous afferents that causes a general facilitation of H-reflex excitability. Given the fact that the sural conditioning of the SOL H-reflex was less effective after priming, we suggest that H-reflex plasticity due to patterned stimulation in the current experiment arises from reductions in Ia presynaptic inhibition. Locomotor networks converge onto Ia terminals via presynaptic inhibitory interneurons (Rudomin 1990). As reported previously (Frigon et al. 2004), and as shown in the arm cycling condition both PRE and POST priming, locomotor activity of the arms is able to access locomotor networks to amplify Ia PSI, and therefore reduce the SOL H-reflex amplitude. On the contrary, trains of stimuli that are applied to the cutaneous afferents preceding the tibial nerve stimulation required to evoke an H-reflex, reduces Ia PSI, and therefore facilitates the amplitude of the SOL H-reflex (Delwaide et al. 1981; Demaire et al. 1989; Demaire and Ciancia 2000; Iles 1996). Here there was a ~20% facilitation in H-reflex amplitudes as a result of sural conditioning prior to priming. Reductions in the sural conditioning effect following priming suggests that there may be a ceiling effect for reductions in Ia PSI that can be reached. Therefore, the plasticity in the Ia presynaptic inhibitory interneuronal pathway, which is the likely source of the H-reflex facilitation, was already

Functional implications of amplifying spinal cord excitability

Targeted neuroplasticity that increases the excitability of spinal circuits has certainly been postulated to improve function after spinal cord injury (Thompson and Wolpaw 2015). Indeed, rodents with incomplete spinal cord injuries who can increase their H-reflex through operant conditioning (Chen et al. 2014a, 2014b) show improved walking function (i.e. longer steps and improved stepping symmetry). Although hyperexcitability of reflexes is observed in those with spinal cord injury, it has been suggested that amplifying the excitability of these pathways may facilitate their locomotor capacity (Leech et al. 2018). This amplification of spinal cord excitability may also prepare neurologically impaired individuals to perform physical bouts of effort. Fatigue is a common and debilitating consequence of neurological impairments including multiple sclerosis (MS) (Chalah et al. 2015; Hameau et al. 2017; Tur 2016), stroke (Knorr et al. 2012), spinal cord injury (SCI) (Papaiordanidou et al. 2014), and cerebral palsy (CP) (Neyroud et al. 2017). Stroke survivors characterize fatigue as a tremendous sense of tiredness, feeling of exhaustion, and lack of physical and mental energy that impede activities of daily living (De Groot et al. 2003). In fact, 46% of stroke patients rate fatigue as their most debilitating symptom (Michael et al. 2006). Neuromuscular fatigue of central origin almost certainly contributes to self-reported fatigue post-stroke (Knorr et al. 2012), in patients with MS (Hameau et al. 2017) and SCI (Papaiordanidou et al. 2014), which often interferes with the rehabilitation process. Therefore, mitigating neuromuscular fatigue represents a potential therapeutic target for reducing self-reported fatigue in the abovementioned neurologically impaired patients. Currently, though mitigating neuromuscular fatigue in a neurologically intact, let alone neurologically impaired, population is currently not well understood. A previous report from our lab (Pearcey et al. 2017) showed that stimulation to cutaneous afferents can enhance spinal cord excitability and reduce fatigue, therefore priming the central nervous system through known cutaneous reflex pathways may be able mitigate fatigue of future movements.

Conclusion

Priming via patterned sensory stimulation of cutaneous afferents innervating the plantar surfaces of the feet induces short-term spinal cord plasticity. After applying stimulation with parameters that elicit non-painful sensations to the feet soles that mimic ground contact phases of walking, H-reflexes amplitudes were significantly facilitated. This facilitation persisted during quiescent sitting and when there was rhythmic activity of the arms. The facilitation was absent when H-reflexes were conditioned by a brief train of stimuli to the sural nerve. Taken together, these results suggest that priming via patterned sensory stimulation causes a reduction in Ia presynaptic inhibition. Potential applications of priming via sensory stimulation includes rehabilitation and sport performance settings, where mitigating reductions in spinal cord excitability can enhance or prolong motor output.

References

Arsenault AB, Belanger AY, Durand MJ, De Serres SJ, Fortin L, Kemp F. Effects of TENS and topical skin anesthesia on soleus H-reflex and the concomitant influence of skin/muscle temperature. *Arch Phys Med Rehabil* 74: 48–53, 1993.

Balter JE, Zehr EP. Neural coupling between the arms and legs during rhythmic locomotor-like cycling movement. *J Neurophysiol* 97: 1809–18, 2007.

Bohannon RW. Comfortable and maximum walking speed of adults aged 20-79 years: reference values and determinants. *Age Ageing* 26: 15–19, 1997.

Chalah MA, Riachi N, Ahdab R, Creange A, Lefaucheur JP, Ayache SS. Fatigue in Multiple Sclerosis: Neural Correlates and the Role of Non-Invasive Brain Stimulation. *Front Cell Neurosci* 9: 460, 2015.

Chen Y, Chen L, Liu R, Wang Y, Chen XY, Wolpaw JR. Locomotor impact of beneficial or nonbeneficial H-reflex conditioning after spinal cord injury. *J Neurophysiol* 111: 1249–1258, 2014a.

Chen Y, Chen L, Wang Y, Wolpaw JR, Chen XY. Persistent beneficial impact of H-reflex conditioning in spinal cord-injured rats. *J Neurophysiol* 112: 2374–2381, 2014b.

Claydon LS, Chesterton LS, Barlas P, Sim J. Dose-specific Effects of Transcutaneous Electrical Nerve Stimulation (tens) on Experimental Pain: A Systematic Review. *The Clinical Journal of Pain* 27: 635–647, 2011.

Cohen J. Statistical power analysis for the behavioral sciences (Rev. ed.). Hillsdale, NJ, US. 1977.

Cramp FL, Noble G, Lowe AS, Walsh DM, Willer JC. A controlled study on the effects of transcutaneous electrical nerve stimulation and interferential therapy upon the

RIII nociceptive and H-reflexes in humans. *Archives of Physical Medicine and Rehabilitation* 81: 324–333, 2000.

Crone C, Hultborn H, Mazieres L, Morin C, Nielsen J, Pierrot-Deseilligny E. Sensitivity of monosynaptic test reflexes to facilitation and inhibition as a function of the test reflex size: a study in man and the cat. *Exp Brain Res* 81: 35–45, 1990.

De Groot MH, Phillips SJ, Eskes GA. Fatigue associated with stroke and other neurologic conditions: Implications for stroke rehabilitation. *Arch Phys Med Rehabil* 84: 1714–20, 2003.

Delwaide PJ, Crenna P, Fleron MH. 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, 1981.

Demaire C, Ciancia F. Sural nerve facilitation of the soleus muscle H-reflex in high level gymnasts. *Eur J Appl Physiol* 82: 446–50, 2000.

Demaire C, Honore J, Le Bizec J, Coquery JM. Specific modulation of the Hoffmann reflex cutaneous facilitation during a reaction-time task. *Exp Brain Res* 74: 592–602, 1989.

Duysens J, Pearson KG. From cat to man: basic aspects of locomotion relevant to motor rehabilitation of SCI. *NeuroRehabilitation* 10: 107–118, 1998.

Estes SP, Iddings JA, Field-Fote EC. Priming Neural Circuits to Modulate Spinal Reflex Excitability. *Front Neurol* 8: 17, 2017.

Field-Fote EC. Exciting recovery: augmenting practice with stimulation to optimize outcomes after spinal cord injury. *Prog Brain Res* 218: 103–26, 2015.

Francini F, Maresca M, Procacci P, Zoppi M. The effects of non-painful transcutaneous electrical nerve stimulation on cutaneous pain threshold and muscular reflexes in normal men and in subjects with chronic pain. *Pain* 11: 49–63, 1981.

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

Garnett R, Stephens JA. Changes in the recruitment threshold of motor units produced by cutaneous stimulation in man. *J Physiol (Lond)* 311: 463–473, 1981.

Goulet C, Arsenault AB, Levin MF, Bourbonnais D, Lepage Y. Absence of consistent effects of repetitive transcutaneous electrical stimulation on soleus H-reflex in normal subjects. *Arch Phys Med Rehabil* 75: 1132–1136, 1994.

Hameau S, Zory R, Latrille C, Roche N, Bensmail D. Relationship between neuromuscular and perceived fatigue and locomotor performance in patients with multiple sclerosis. *Eur J Phys Rehabil Med* 53: 833–840, 2017.

Hamilton LD, Mani D, Almuklass AM, Davis L, Vieira TM, Botter A, Enoka RM. Electrical nerve stimulation modulates motor unit activity in contralateral biceps brachii during steady isometric contractions. *J Neurophysiol* , 2018. doi:10.1152/jn.00235.2018.

Hansen EA, Kristensen LAR, Nielsen AM, Voigt M, Madeleine P. The role of stride frequency for walk-to-run transition in humans. *Sci Rep* 7, 2017.

Hardy SGP, Spalding TB, Liu H, Nick TG, Pearson RH, Hayes AV, Stokic DS. The effect of transcutaneous electrical stimulation on spinal motor neuron excitability in people without known neuromuscular diseases: the roles of stimulus intensity and location. *Phys Ther* 82: 354–363, 2002.

Henneman E. The size-principle: a deterministic output emerges from a set of probabilistic connections. *J Exp Biol* 115: 105–112, 1985.

Hurteau M-F, Thibaudier Y, Dambreville C, Danner SM, Rybak IA, Frigon A. Intralimb and Interlimb Cutaneous Reflexes during Locomotion in the Intact Cat. *J Neurosci* 38: 4104–4122, 2018.

Iles JF. Evidence for cutaneous and corticospinal modulation of presynaptic inhibition of Ia afferents from the human lower limb. *J Physiol* 491 (Pt 1): 197–207, 1996.

Jimenez S, Mordillo-Mateos L, Dileone M, Campolo M, Carrasco-Lopez C, Moitinho-Ferreira F, Gallego-Izquierdo T, Siebner HR, Valls-Solé J, Aguilar J, Oliviero A. Effects of patterned peripheral nerve stimulation on soleus spinal motor neuron excitability. *PLoS ONE* 13: e0192471, 2018.

Klarner T, Pearcey GEP, Sun Y, Barss TS, Kaupp C, Munro B, Frank N, Zehr EP. Beyond the Bottom of the Foot: Topographic Organization of the Foot Dorsum in Walking. *Med Sci Sports Exerc* 49: 2439–2450, 2017.

Klimstra M, Zehr EP. A sigmoid function is the best fit for the ascending limb of the Hoffmann reflex recruitment curve. *Exp Brain Res* 186: 93–105, 2008.

Knorr S, Rice CL, Garland SJ. Perspective on neuromuscular factors in poststroke fatigue. *Disabil Rehabil* 34: 2291–9, 2012.

Leech KA, Kim HE, Hornby TG. Strategies to augment volitional and reflex function may improve locomotor capacity following incomplete spinal cord injury. *J Neurophysiol* 119: 894–903, 2018.

Levin MF, Hui-Chan CW. Relief of hemiparetic spasticity by TENS is associated with improvement in reflex and voluntary motor functions. *Electroencephalogr Clin Neurophysiol* 85: 131–142, 1992.

Merriam-Webster. Definition of PLASTICITY [Online]. 2019.https://www.merriam-webster.com/dictionary/plasticity [17 Jan. 2019].

Michael KM, Allen JK, Macko RF. Fatigue after stroke: relationship to mobility, fitness, ambulatory activity, social support, and falls efficacy. *Rehabil Nurs* 31: 210–7, 2006.

Nakajima T, Mezzarane RA, Hundza SR, Komiyama T, Zehr EP. Convergence in reflex pathways from multiple cutaneous nerves innervating the foot depends upon the number of rhythmically active limbs during locomotion. *PLoS One* 9: e104910, 2014.

Neyroud D, Armand S, De Coulon G, Sarah RDDS, Maffiuletti NA, Kayser B, Place N. Plantar flexor muscle weakness and fatigue in spastic cerebral palsy patients. *Res Dev Disabil* 61: 66–76, 2017.

Ng Shamay S, Hui-Chan Christina W.Y. Transcutaneous Electrical Nerve Stimulation Combined With Task-Related Training Improves Lower Limb Functions in Subjects With Chronic Stroke. *Stroke* 38: 2953–2959, 2007.

Papaiordanidou M, Varray A, Fattal C, Guiraud D. Neural and muscular mechanisms of electrically induced fatigue in patients with spinal cord injury. *Spinal Cord* 52: 246–50, 2014.

Pearcey GEP, Noble SA, Munro B, Zehr EP. Spinal Cord Excitability and Sprint Performance Are Enhanced by Sensory Stimulation During Cycling. *Front Hum Neurosci* 11: 612, 2017.

Perez MA, Field-Fote EC, Floeter MK. Patterned sensory stimulation induces plasticity in reciprocal ia inhibition in humans. *J Neurosci* 23: 2014–8, 2003.

Radhakrishnan R, Sluka KA. Deep tissue afferents, but not cutaneous afferents, mediate transcutaneous electrical nerve stimulation-Induced antihyperalgesia. *J Pain* 6: 673–680, 2005.

Rudomin P. Presynaptic inhibition of muscle spindle and tendon organ afferents in the mammalian spinal cord. *Trends Neurosci* 13: 499–505, 1990.

de Ruiter GC, Hundza SR, Zehr EP. Phase-dependent modulation of soleus H-reflex amplitude induced by rhythmic arm cycling. *Neurosci Lett* 475: 7–11, 2010.

Serrano-Muñoz D, Gómez-Soriano J, Bravo-Esteban E, Vázquez-Fariñas M, Taylor J, Avendaño-Coy J. Intensity matters: Therapist-dependent dose of spinal transcutaneous electrical nerve stimulation. *PLOS ONE* 12: e0189734, 2017.

Stoykov ME, Madhavan S. Motor priming in neurorehabilitation. *J Neurol Phys Ther* 39: 33–42, 2015.

Thompson AK, Wolpaw JR. Targeted neuroplasticity for rehabilitation. *Prog Brain Res* 218: 157–72, 2015.

Tur C. Fatigue Management in Multiple Sclerosis. *Curr Treat Options Neurol* 18: 26, 2016.

Vasudevan EV, Zehr EP. Multi-frequency arm cycling reveals bilateral locomotor coupling to increase movement symmetry. *Experimental brain research* 211: 299–312, 2011.

Walsh DM, Noble G, Baxter GD, Allen JM. Study of the effects of various transcutaneous electrical nerve stimulation (TENS) parameters upon the RIII nociceptive and H-reflexes in humans. *Clin Physiol* 20: 191–199, 2000.

Zehr EP. Considerations for use of the Hoffmann reflex in exercise studies. *Eur J Appl Physiol* 86: 455–68, 2002.

Zehr EP. Training-induced adaptive plasticity in human somatosensory reflex pathways. *J Appl Physiol (1985)* 101: 1783–94, 2006.

Zehr EP, Loadman PM, Hundza SR. Neural control of rhythmic arm cycling after stroke. *Journal of neurophysiology* 108: 891–905, 2012.

Zehr EP, Nakajima T, Barss T, Klarner T, Miklosovic S, Mezzarane RA, Nurse M, Komiyama T. Cutaneous stimulation of discrete regions of the sole during locomotion produces “sensory steering” of the foot. *BMC sports science, medicine and rehabilitation* 6: 33, 2014.

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

Chapter 5 – Sensory enhancement to amplify voluntary force output during ramping submaximal isometric contractions

Abstract

Stimulation to cutaneous nerves alters the perceived heaviness of an object, such that stimulation that causes tactile sensations reduces the perceived heaviness of an object. This suggests that either descending commands are facilitated or the perception of effort is facilitated when tactile sensation is enhanced. Sensory stimulation can also mitigate decrements in motor output and spinal cord excitability that occur with fatigue. The effects of sensory stimulation applied with the appropriate timing of voluntary force output have not yet been examined. Therefore, the purpose of this study was to examine effects of sensory enhancement to nerves innervating opposed skin areas of the foot (top, superficial peroneal; SP, or bottom, distal tibial; TIB) on voluntary plantarflexion (PF) or dorsiflexion (DF) ramp contractions. Stimulation trains were applied for 2 seconds at either a uniforn 150Hz frequency or a modulated frequency from 50-150Hz, and were delivered at the initiation of the contraction. Participants traced a line with their contraction force, while receiving real-time visual feedback. Participants were instructed to perform a ramp contraction (slope ~10% maximal voluntary contraction (MVC)/s) to ~20% MVC and then to hold ~20% MVC for 2 seconds. Cutaneous reflexes were evoked 75ms after the hold was initiated, which corresponded to 75ms after sensory enhancement. The integrated force output was greater for all sensory enhancement conditions compared to control trials during plantarflexion, however, only uniform sensory enhancement (of both nerves) amplified force output during dorsiflexion. Cutaneous reflexes evoked after sensory enhancement were unaltered, however, neuromechanical twitch amplitudes following cutaneous reflex stimulation were generally facilitated after sensory enhancement. Taken together, these results indicate that sensory enhancement can amplify force output, that is, at least partially, due to alterations in the excitability of interneuronal networks within the spinal cord.

New & Noteworthy

The efficacy of appropriately timed sensory stimulation to enhance sensations and amplify force output has not been examined. Here we show sensory stimulation that elicits tactile sensations can amplify force output during both plantarflexion and dorsiflexion. This amplification in force occurs irrespective of whether the cutaneous field that is stimulated resides on the surface which is producing the force, or the opposing surface. This information may provide insights for the development of technologies to improve performance and/or rehabilitation.

Introduction

To generate voluntary force, supraspinal projections either directly (i.e. cortico-motoneuronal pathways) or indirectly (i.e. via propriospinal interneurons) provide motor commands to activate spinal motoneurons (Lemon, 2008), which in turn cause muscle fibres to contract. During increasing efforts, there is orderly recruitment of motor units from slow twitch, low force and fatigue resistant units to progressively faster, stronger and more fatigable units, according to Henneman's size principle (Henneman, 1985). Active perception of force output relies on a combination of central motor commands, sensory feedback and cerebellar function (Bhanpuri et al., 2012), suggesting that humans can utilize a combination of feedforward and feedback strategies to predict and react to changes in force output based on the environmental constraints of a task. These descending commands can be influenced by sensory feedback both at supraspinal centers and within the spinal cord, through interneuronal pathways and directly onto spinal motoneurons (Pierrot-Deseilligny, 1996). Descending pathways from the brainstem release monoamines onto motoneurons which facilitate persistent inward currents (PICs) and exert gain control of synaptic inputs onto motoneurons (Heckman and Enoka, 2012). PICs provide the gain control of descending motor commands at the level of the spinal motoneuron (Johnson et al., 2017) by changing the intrinsic electrical properties of the cell. Therefore, sensory inputs onto motoneurons, either excitatory and inhibitory, are amplified depending on the state of the neuromodulatory drive.

The sense of movement, otherwise known as kinesthesia, was first described by Bastian (1887), who attributed the sense of bodily movement to the skin, muscles and other deep textures of the limbs. Although not specifically mentioned at the time, these deep textures have since been identified as the fascia, tendons, and joints. Force control can be maintained in the absence of peripheral feedback by incorporating other sources of sensory feedback, such as vision (Rothwell et al., 1982). Likewise, stability of force output is not compromised when vision is completely absent (Limonta et al., 2015), suggesting that peripheral feedback is capable of supporting force control independently. Transient disruptions in cutaneous feedback can impair the ability to match force from one limb to another (Choi et al., 2013). In terms of measuring the contributions of sensations to the generation of force, there has been a plethora of work done to examine deficits resulting from the absence of sensory feedback. For example, a ring block digital anaesthesia reduces maximal force production of the finger flexors by about 25% (Shim et al., 2012). Furthermore, when deprived of afferent feedback from the active muscle, motor units cannot maintain firing rates (Macefield et al., 1993). During fatiguing isometric contractions, reductions in group Ia presynaptic inhibition (PSI) have been correlated with the ability to sustain contraction force (Baudry et al., 2011) and brief enhancement of cutaneous afferents of the foot reduces group Ia PSI, as evidenced in conditioned H-reflex experiments (Delwaide et al., 1981; Iles, 1996). Therefore, stimulation of cutaneous afferents seems like a likely candidate to amplify motor output via, at least partially, reductions in PSI.

Many previous experiments (summarized in Zehr et al (2006)) have incorporated brief (i.e. ≤20 ms trains of stimuli) input to cutaneous afferents to examine polysynaptic reflex pathways during a variety of tasks. Stimulation of cutaneous reflex pathways typically causes facilitation of ongoing muscle activity both at the spinal and supraspinal levels (Jenner and Stephens, 1982). We have recently become interested in testing the efficacy of sustained sensory stimulation (i.e. >300ms trains of stimuli) to previously identified reflex pathways to amplify motor output, rather than using the stimulation as a probe to examine reflex pathways. The difference between what we will further refer to as ‘sensory enhancement’ and cutaneous reflex stimulation is the duration in which it is applied. We believe that this extended duration causes lasting activation of a sensory pathway (i.e. sensory enhancement) to amplify motor output, rather than a brief train, as is common for cutaneous reflexes, which causes a perturbation. It is also important to note that this type of stimulation is different from applying transcutaneous electrical nerve stimulation (TENS) as sensory enhancement causes activation of cutaneous innervation areas of the distal limb segment (i.e. the foot) rather than the tactile and/or muscle afferents of the muscle beneath the electrodes. In a recent experiment (Pearcey et al., 2017), we applied trains of stimulation to afferents innervating skin surfaces of the foot during the power phase of cycling. This stimulation was applied during both non-fatiguing cycling and fatiguing cycling sprints. Interestingly, sensory stimulation enhanced spinal cord excitability during non-fatiguing cycling and mitigated reductions in power output during the fatiguing cycling, suggesting that sensory enhancement can amplify motor output.

Although the loci of enhancement are yet to be entirely elucidated, it could be that the perceptions of effort are altered when cutaneous afferents are activated. This is supported by Aniss et el. (1988) who showed that non-noxious cutaneous reflex stimulation reduced the perceived heaviness of a weight lifted by the index finger. Alternatively, it is possible that input-output integration of motor commands is altered due to changes in spinal cord excitability, which may result in the amplification of descending commands such that similar commands give rise to earlier recruitment of motor units.

Regardless of the mechanism, the purpose of the current experiment was to examine whether sensory enhancement can amplify force output during a simple ramp and hold isometric contraction of the ankle plantar- and dorsiflexors. To tease out whether there is a skin surface-dependent effect on amplifying force output during contractions in opposite directions, we stimulated opposing surfaces of the foot; bottom (distal tibial nerve) and top (superficial peroneal nerve). We hypothesized that 1) sensory enhancement to the bottom of the foot (BOT) would amplify force output during plantarflexion and sensory enhancement to the top of the foot (TOP) would amplify force output during dorsiflexion, and 2) sensory enhancement to skin surfaces irrelevant to the task (i.e. opposite to the surface producing the force) would not amplify force output. As a proxy of interneuronal excitability, we also measured cutaneous reflexes during the initial hold portion of the contraction, 75 ms after the sensory enhancement terminated.

Methods

Participants

Twelve neurologically intact participants (5 female) were recruited from the university student population. Participants were apparently healthy (i.e. no history of cardiovascular, metabolic or neuromuscular impairment) and provided written and signed informed consent prior to partaking in any experimental protocols. The protocol was approved by the Human Research Ethics Board at the University of Victoria and was conducted in accordance with the Declaration of Helsinki.

Experimental timeline

Upon arrival, participants performed two maximal voluntary contractions (MVC) of the plantarflexors and dorsiflexors. We determined their perceptual and radiating thresholds of stimulation to the superficial peroneal and distal tibial nerves. Stimulation intensities to be used for sensory enhancement and cutaneous reflexes were then applied to ensure the participants could tolerate it (i.e. non-noxious). Participants then practiced performing the ramp and hold plantarflexion contractions. When participants were able to keep their force within ± 2% MVC of the target in 9/10 trials, their practice ended. Participants were then required to "rate the level of effort required to perform the task" on a visual analog scale from 0-10 where 0 meant no effort and 10 meant absolute maximal effort (see appendix 1). Participants then performed all plantarflexion trials outlined in table 5-1. After each set of trials involving sensory enhancement (without cutaneous reflexes), participants were asked to 1) rate the effort required to produce force compared to control (i.e. no sensory enhancement), 2) rate the difficulty to trace the line compared to control (i.e. no sensory enhancement), and 3) rate the sensation of stimulation (see appendix 2). They then performed a plantarflexion MVC, took a break if requested, and moved on to the dorsiflexion trials. To do this, they practiced performing the ramp and hold dorsiflexion contractions until they were able to keep their force within ± 2% MVC of the target in 9/10 trials. They then performed all dorsiflexion trials outlined in table 5-1. Questions asked during the plantarflexion contractions were also asked during dorsiflexion, and finally, they performed a dorsiflexion MVC. Conditions with no cutaneous reflex stimulation consisted of 10 trials, whereas conditions with cutaneous reflex stimulation consisted of 15 trials to ensure enough sweeps to create an appropriate mean trace to create a subtracted trace. The entire protocol had a typical duration of 75 minutes.

Table 5-1: A summary of all experimental conditions performed by participants in order. *Plantarflexion and dorsiflexion are abbreviated as PF and DF, top of foot and bottom of foot are abbreviated as TOP and BOT and superficial peroneal and distal tibial are abbreviated as SP and TIB, respectively.*

Condition	*Contraction Type*	*Sensory Enhancement*	*Cutaneous Reflex Stimulation*
NCP	PF	none	none
NSP	PF	none	SP
NTP	PF	none	TIB
USCP	PF	Uniform 150Hz to TOP	none
USTP	PF	Uniform 150Hz to TOP	TIB
UTCP	PF	Uniform 150Hz to BOT	none
UTSP	PF	Uniform 150Hz to BOT	SP
RSCP	PF	Modulated 50-150Hz to TOP	none
RSTP	PF	Modulated 50-150Hz to TOP	TIB
RTCP	PF	Modulated 50-150Hz to BOT	none
RTSP	PF	Modulated 50-150Hz to BOT	SP
NCD	DF	none	none
NSD	DF	none	SP
NTD	DF	none	TIB
USCD	DF	Uniform 150Hz to TOP	none
USTD	DF	Uniform 150Hz to TOP	TIB
UTCD	DF	Uniform 150Hz to BOT	none
UTSD	DF	Uniform 150Hz to BOT	SP
RSCD	DF	Modulated 50-150Hz to TOP	none
RSTD	DF	Modulated 50-150Hz to TOP	TIB
RTCD	DF	Modulated 50-150Hz to BOT	none

RTSD	DF	Modulated 50-150Hz to BOT	SP

Isometric force recordings

Participants sat in a custom-fitted chair designed to minimize extraneous movements, with both feet securely fastened to plates on the floor, bracing over the knees and ankles and knees fixed at 90 degrees of flexion (see figure 5-1A). Forces were measured with a strain gauge (Omegadyne Ltd. Model 101-500). After being allowed a “test run” to ensure that the right movements were being produced, participants completed four total trials of 5 s maximal voluntary isometric contractions that alternated between plantarflexion and dorsiflexion for a total of 2 contractions of each movement. Maximum values were determined offline by taking the mean value of a 500ms duration around the largest reading generated over the course of the two trials.

For ramp and hold contractions, participants were provided with real-time feedback of their force output and asked to trace a line which corresponded to a rise in force of 10% MVC/s and hold at ~20% MVC for 2s. There was a 10s break between contractions within each trial. An example contraction is shown in figure 5-1B.

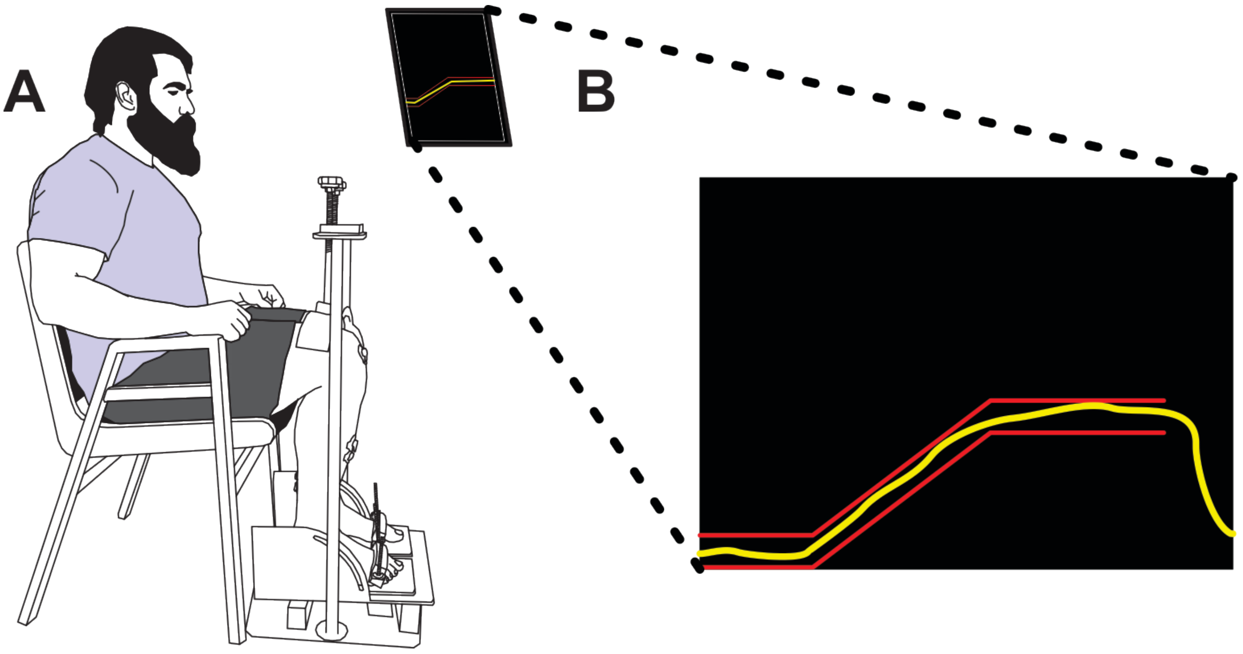

Figure 5-1*: A graphical sketch of the set-up that was used throughout the experimental protocol is shown in A. In B, a representative trace of one control trace (i.e. no stimulation) is shown from the perspective of what a participant could see.*

Electromyography

Bipolar surface electrodes were placed over the mid-muscle bellies of the soleus (SOL) and tibialis anterior (TA) of the participant's dominant leg. EMG signals were preamplified (x5000) and band pass filtered (100-300 Hz) (GRASS P511, AstroMed). These parameters are consistent with previous experiments in our laboratory (Balter and Zehr, 2007; Pearcey et al., 2017; Vasudevan and Zehr, 2011). Data were sampled at 5000 Hz using a custom-built continuous acquisition software (LABVIEW, National Instruments, TX, USA).

Cutaneous reflex stimulation

Cutaneous reflexes evoked during the hold portion of the contraction were used to provide insight into the excitability of the interneuronal networks within the spinal cord. Reflexes were evoked via surface stimulation of the superficial peroneal nerve (SP; innervates the dorsal surface of the foot) or the distal tibial nerve (TIB; innervates the plantar surface of the foot). Electrodes were placed on the midline of the anterior crease of the ankle (SP) and posterior/inferior to the medial malleolus of the ankle (TIB), both in a bipolar configuration. Prior to beginning the experiment, perceptual (PT) and radiating (RT) thresholds were found for each participant. Intensities were then set to 2 x RT for reflex trials, providing it was tolerated by the participant. Reflex stimulation was delivered as trains of 5 x 1.0 ms pulses at 300Hz (P511 Astro-Med Grass Instrument) by a Grass S88 stimulator with SIU5 stimulus isolation and a CCUI constant current unit (Astro-Med Grass Instrument, West Warwick, RI). Trains were triggered at a latency of 2075ms after the threshold of force output (>1%MVC) was exceeded.

Sensory enhancement stimulation

Sensory enhancement was delivered during the ascending portion of the contraction to examine whether it would alter force output or cutaneous reflex amplitudes evoked during the hold portion of the contraction. The SP and TIB nerves were stimulated in separate trials to examine specific effects of sensory enhancement from skin surfaces on the top and bottom of the foot during both plantarflexion and dorsiflexion. Nerves were stimulated using the same electrodes and stimulator outlined in the previous paragraph, however, intensities were set to 1.2 x RT. Stimulus trains were 2000ms in duration. During modulated stimulation (MOD) trials, 1ms square wave stimuli started at 50Hz and increased to 150Hz at a rate of 50Hz/s. During uniforn stimulation (UNI) trials, 1ms square wave stimuli were applied at a rate of 150Hz. Trains were triggered when the threshold of force output (>1%MVC) was exceeded.

Force output data analysis

Offline data were analyzed using custom written MATLAB programs (Version R2011b, The Mathworks, Natick, MA, USA). To determine the difference in force output during the isometric contraction, integration was used to determine the area under the curve of the mean force trace for each trial. Force traces were integrated over the first 2 or final 2 seconds of the contraction, which corresponds to the ascending and constant force portions of the contraction.

Cutaneous reflex data analysis

Offline data were analyzed using custom written MATLAB programs (Version R2011b, The Mathworks, Natick, MA, USA). For both dorsiflexion and plantarflexion trials, data from the control trials (NCP, NCD) were averaged across 10 sweeps and subtracted from the trials with only reflex stimulation applied (NSP, NTP, NSD, NTD). Subtracted data were rectified and low-pass filtered using 4th order dual pass Butterworth filters at 100Hz and then averaged across 15 sweeps. Cutaneous reflexes were determined as the difference between the control EMG and the maximal inhibition or facilitation amplitude during the early latency window (50-75ms post-stimulation), middle latency (75-120ms post-stimulation). Net cutaneous reflexes were determined from the average cumulative reflex EMG over 150ms post-stimulation (ACRE150). Post-stimulation force was determined as the difference between the pre-stimulation force and the peak in a window from 140ms to 220ms post stimulation. This was chosen because correlated changes in kinematics of the gait cycle corresponding to cutaneous input have been previously established within this time window (Klimstra et al., 2011; Zehr et al., 1997). Cutaneous reflexes were measured in the SOL and TA for the plantarflexion and dorsiflexion trials, respectively.

To determine the effects of enhancement (uniform or modulated stimulation during the ascending portion of the contraction) on cutaneous reflex amplitudes, trials with sensory enhancement but no reflexes were averaged across all 10 sweeps and subtracted from the trials with both sensory enhancement and reflex stimulations. Early latency, middle latency, net cutaneous reflexes and cutaneous stimulation evoked changes in force between 150-220ms were calculated as described in the previous paragraph.

Early latency, middle latency, and net cutaneous reflexes were normalized to the maximum EMG obtained during pre-test MVCs. Post-stimulation force was normalized to the maximal plantarflexion or dorsiflexion force from pre-test MVCs.

Statistics

Statistical procedures were performed using SPSS 18.0 (Chicago, Illinois). For between condition comparisons, a one factor (CONDITION) repeated measure ANOVA was run separately on plantarflexion and dorsiflexion variables to determine main effects for force output and reflex modulation. If significant main effects were identified and direction of change from control was predicted because of a priori hypotheses, one-tailed paired samples t-tests were performed. In all cases, statistical significance was set at $p \leq 0.05$. Results are reported as means ± SD in text (SE in figures).

Results

Sensory enhancement amplifies force output

Sensory enhancement caused a general facilitation of force output in all conditions during the ramp of force production, but this facilitation completely disappeared after stimulation terminated. The force enhancement was robust and consistent throughout trials (see figure 5-2 for an example of a typical participant's individual traces).

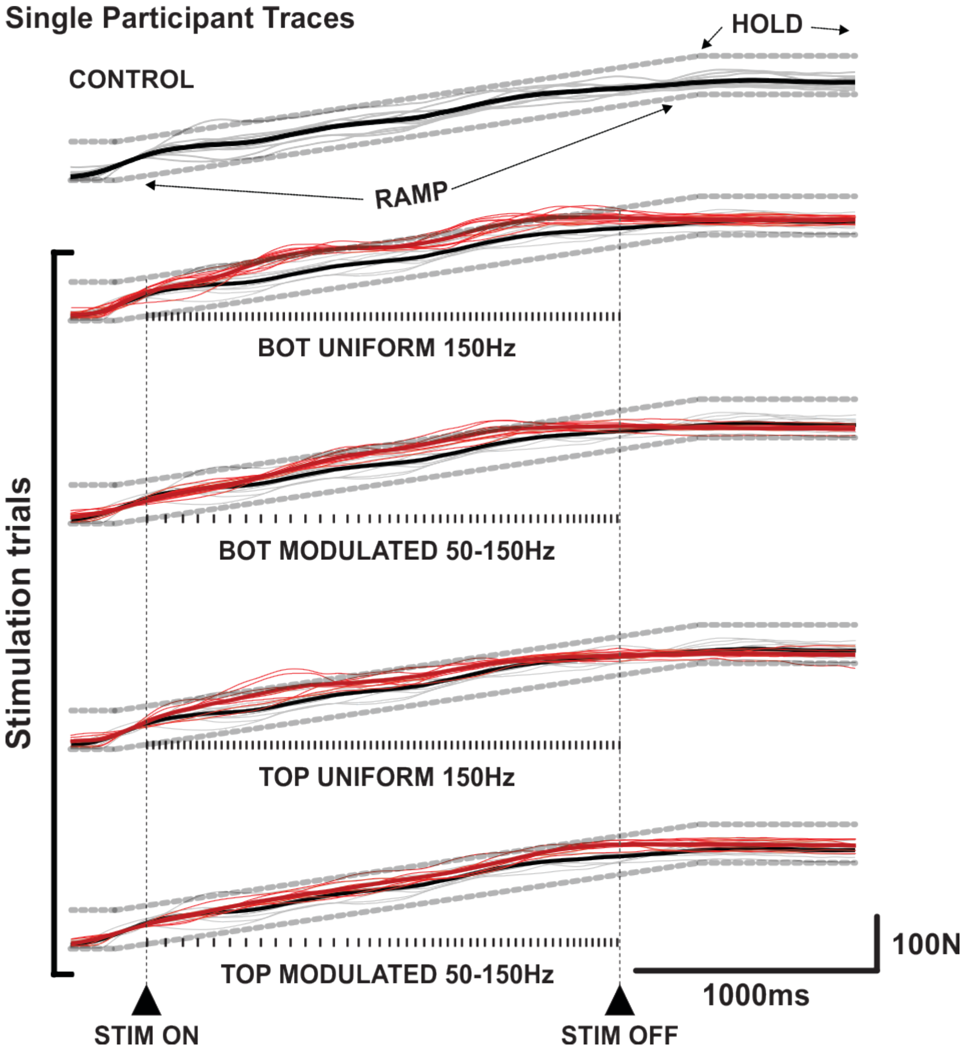

Figure 5-2*: A typical participant's force traces are shown for the control, BOT UNI, BOT MOD, TOP UNI and TOP MOD during plantarflexion. In the sensory enhancement conditions (red lines), the trials are overlaid onto the control trials (grey lines). Means are*

depicted as thick lines (thick red = mean of sensory enhanced trials, thick black = mean of control trials), whereas thin lines are the individual trials. Dotted lines represent the target boundaries that participants were instructed to stay within.

Sensory enhancement to amplify force is especially clear when examining figures 3 and 5, which show sensory enhancement trials plotted against non-stimulated control trials, for plantarflexion and dorsiflexion contraction trials. In general, the effect was clearer in the plantarflexion compared to dorsiflexion contractions, and in the uniform frequency conditions compared to the modulated frequency conditions. In fact, the facilitation of force output appears to be graded with the increased frequency of stimulation during the plantarflexion trials (see right panels of figure 5-3).

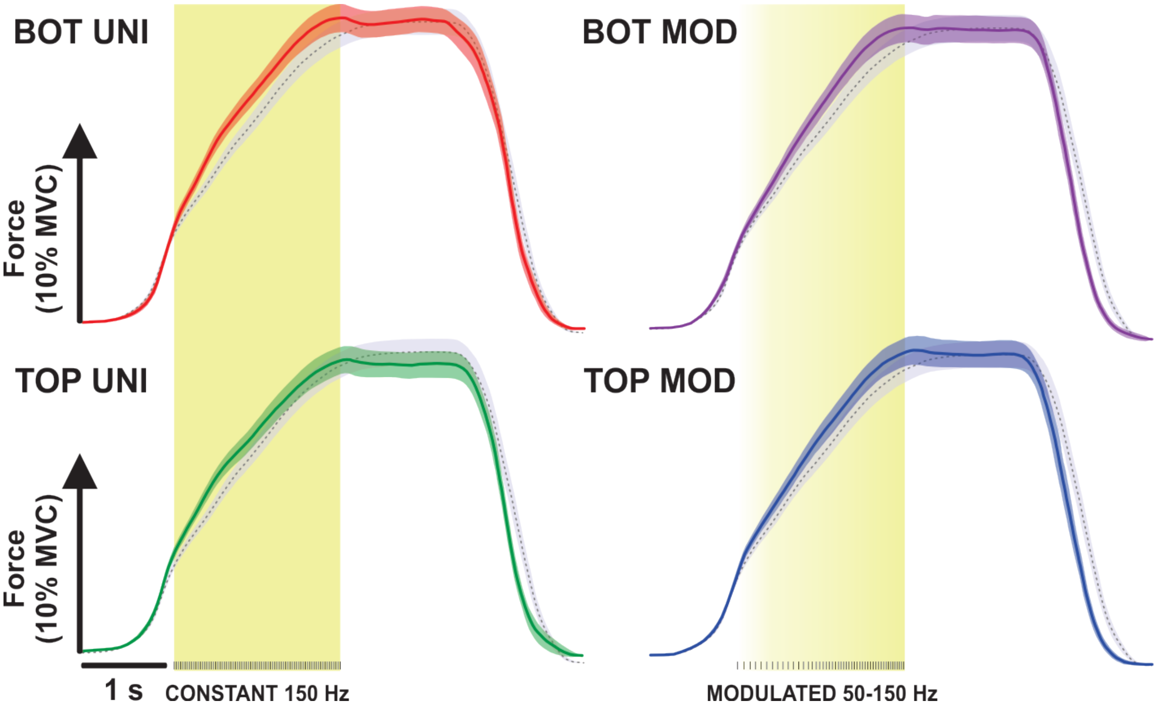

***Figure 5-3**: Group mean ± 95% confidence intervals are plotted for each sensory enhancement condition (colored; red = BOT UNI, green = TOP UNI, magenta = BOT MOD, and blue = TOP MOD) against the control condition (grey) during plantarflexion. All data are normalized to MVC prior to averaging across participants. Shaded yellow boxes indicate the time that the stimulation was applied, and the hue of the yellow indicates the frequency.*

During the increasing portion of the plantarflexion contractions (i.e. the ramp), the RM ANOVA revealed a significant effect of condition ($F_{(4,44)} = 9.436$, $p < 0.0001$) on the integrated area under the curve of force output. The integrated force was 6.6 and 8.5% greater ($p = 0.007$ and 0.006), compared to control, when sensory enhancement was applied to the TOP during UNI and MOD, respectively. The integrated force was 12.8 and 10.7% greater ($p = 0.00008$ and 0.002), compared to control, when sensory enhancement was applied to the BOT during UNI and MOD, respectively. There were no differences between conditions during the hold portion of the plantarflexion contractions ($F_{(4,44)} = 1.468$, $p = 0.228$). Mean force traces (± 95% confidence intervals) are shown for all sensory enhancement conditions compared to control in figure 5-3. Group data for the mean area under the curve of the RAMP and HOLD portions of the contraction are displayed in figure 5-4 A and C, respectively, for each condition.

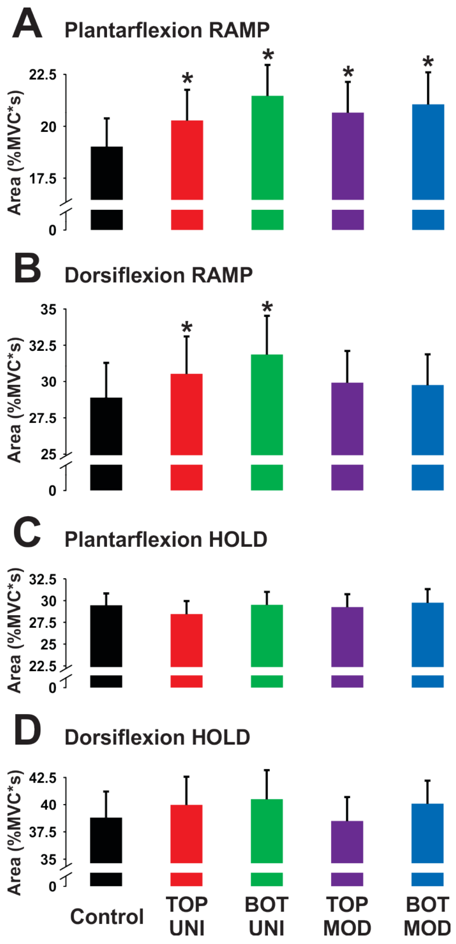

Figure 5-4*: The mean area under the curve of the ramp (A and B) and hold (C and D) contractions for the control (black), TOP UNI (red), BOT UNI (green), TOP MOD (magenta), and BOT MOD (blue). Error bars represent standard error and asterisks indicate significant differences from control.*

During the increasing portion of the dorsiflexion contractions, the RM ANOVA revealed a significant effect of condition ($F_{(4,44)} = 2.619$, $p = 0.049$) on the integrated area under the curve of force output. The integrated force was 5.6% greater ($p = 0.02$), compared to control, when sensory enhancement was applied to the TOP during UNI, but was not facilitated when the sensory enhancement was applied during MOD. Similarly, the integrated force was 10.2% greater ($p = 0.009$), compared to control, when sensory enhancement was applied to the BOT during UNI, but was not facilitated when the sensory enhancement was applied during MOD. There were no differences between conditions during the hold portion of the dorsiflexion contractions ($F_{(4,44)} = 1.251$, $p = 0.305$). Mean force traces (± 95% confidence intervals) are shown for all sensory enhancement conditions compared to control in figure 5-5. Group data for the mean area under the curve of the RAMP and HOLD portions of the contraction are displayed in figure 5-4 B and D, respectively, for each condition.

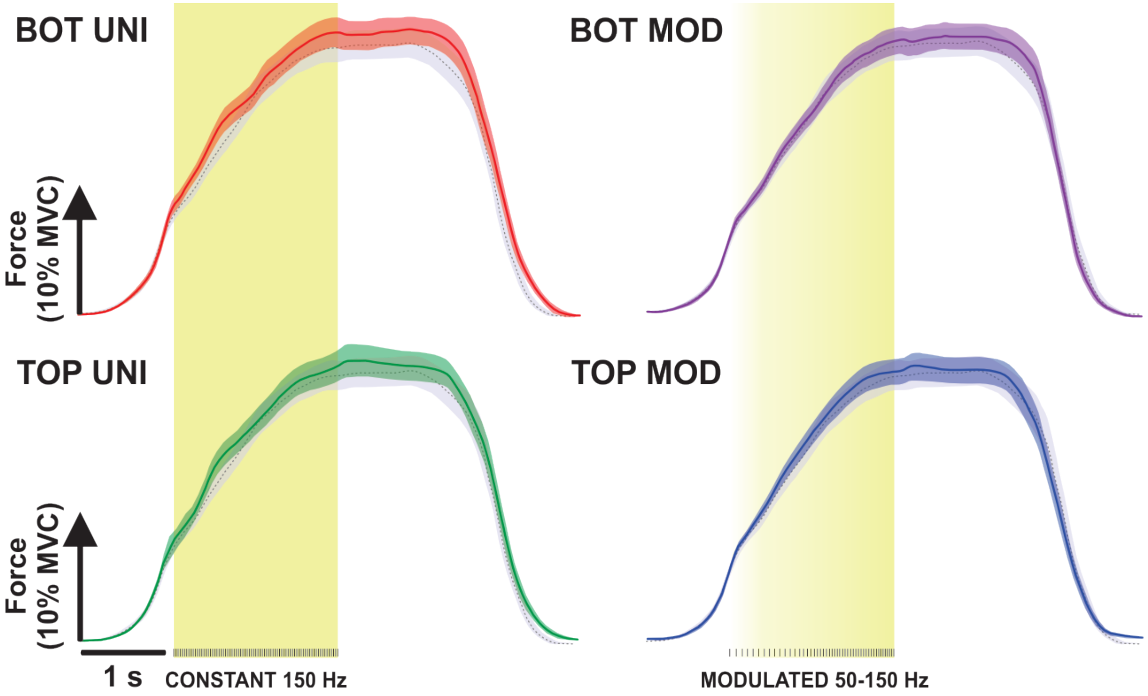

Figure 5-5*: Group mean ± 95% confidence intervals are plotted for each sensory enhancement condition (colored; red = BOT UNI, green = TOP UNI, magenta = BOT MOD, and blue = TOP MOD) against the control condition (grey) during dorsiflexion. All data are normalized to MVC prior to averaging across participants. Shaded yellow boxes indicate the time that the stimulation was applied, and the hue of the yellow indicates the frequency.*

Sensory enhancement alters evoked force after cutaneous reflex stimulation

No significant differences were observed for early latency, middle latency and net cutaneous reflex amplitudes between conditions. Net reflexes measured in the SOL evoked from TIB (control = -2.8 ± 3.17%, UNI enhanced = -3.8 ± 5.99%, MOD enhanced = -5.6 ± 5.61%) and SP (control = -0.4 ± 2.82%, UNI enhanced = 0.5 ± 3.24%, MOD enhanced = -0.5 ± 3.64%) during plantarflexion were generally inhibitory and did not differ between conditions (TIB $p > 0.16$, SP $p > 0.32$). Net reflexes measured in the TA evoked from TIB (control = 1.3 ± 2.21%, UNI enhanced = 0.96 ± 2.93%, MOD enhanced = 2 ± 1.64%) and SP (control = -0.56 ± 1.94%, UNI enhanced = 0.93 ± 3.38%, MOD enhanced = 0.98 ± 4.65%) during dorsiflexion were generally facilitory and did not differ between conditions (TIB $p > 0.27$, SP $p > 0.24$).

Inhibitory effects of cutaneous reflex stimulation on post-stimulation force between 140-220ms were weakened after sensory enhancement. During plantarflexion, significant facilitation was observed in the post-stimulation force for all the sensory enhanced conditions, regardless of stimulation type or nerve. The RM ANOVA revealed significant effect of condition for force evoked by stimulation to both SP ($F_{(2,22)} = 7.521$, $p = 0.003$) and TIB ($F_{(2,22)} = 3.575$, $p = 0.045$). Without sensory enhancement, SP cutaneous reflex stimulation caused a slight reduction in plantarflexion force (-0.39 ± 0.68%MVC), whereas SP cutaneous reflex stimulation caused slight increases in plantarflexion force after sensory enhancement during both UNI (0.17 ± 0.38%MVC, $p = 0.016$) and MOD (0.17 ± 0.2%MVC, $p = 0.015$) stimulation from the BOT. Without sensory enhancement, TIB cutaneous reflex stimulation caused a slight reduction in plantarflexion force (-0.11 ± 0.87%MVC), whereas TIB cutaneous reflex stimulation caused slight increases in plantarflexion force after sensory enhancement during trials with UNI (0.41 ± 0.58%MVC, $p = 0.044$) and MOD (0.36 ± 0.66%MVC, $p = 0.038$) stimulation to the TOP (see Figure 5-6A).

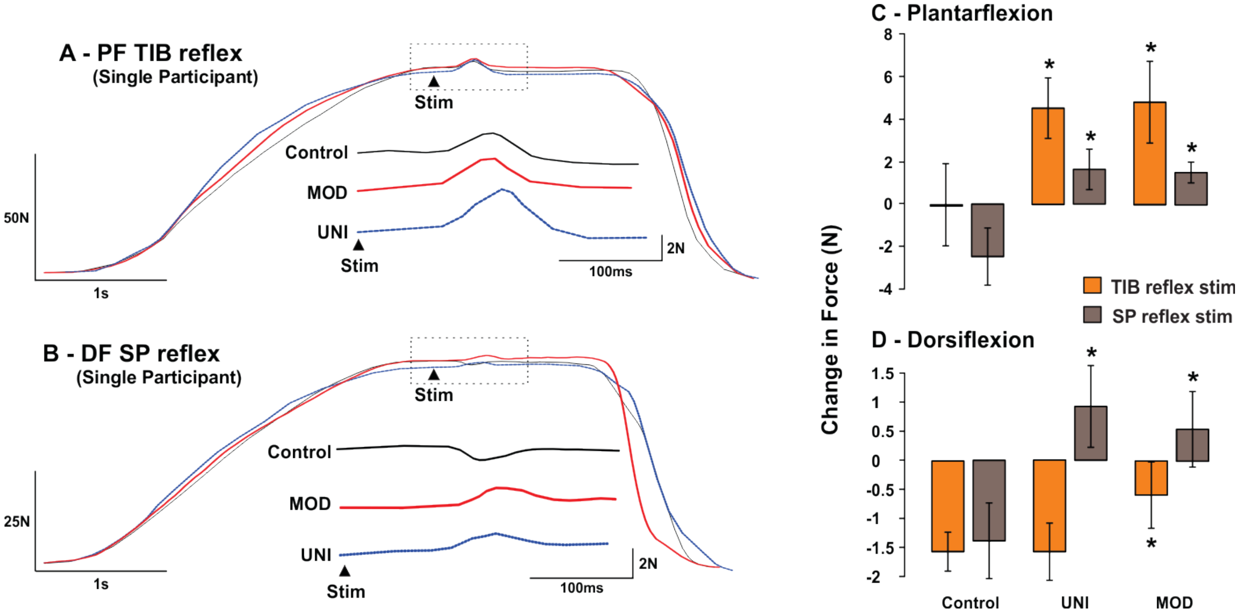

Figure 5-6: *The group mean of evoked changes in force output following cutaneous reflex stimulation for plantarflexion (A) and dorsiflexion (B) contractions. Error bars indicate standard error and asterisks indicate significant difference from the no sensory conditioning conditions ($p < 0.05$).*

During dorsiflexion, reductions in the inhibitory effect due to sensory enhancement were also observed in the post-stimulation force in all conditions except UNI stimulation applied to the TOP. The RM ANOVA revealed significant effect of condition for force evoked by stimulation to both SP ($F_{(2,22)} = 8.992$, $p = 0.002$) and TIB ($F_{(2,22)} = 3.730$, $p = 0.042$). Without sensory enhancement, SP cutaneous reflex stimulation caused a slight reduction in dorsiflexion force (-0.63 ± 0.88%MVC), whereas SP cutaneous reflex stimulation caused slight increases in dorsiflexion force after sensory enhancement during both UNI (0.4 ± 0.12%MVC, $p = 0.005$) and MOD (0.16 ± 0.09%MVC, $p = 0.011$) stimulation from the BOT. Without sensory enhancement, TIB cutaneous reflex stimulation caused a slight reduction in dorsiflexion force (-0.73 ± 0.59%MVC), whereas TIB cutaneous reflex stimulation caused a slightly smaller reduction in dorsiflexion force after sensory enhancement of MOD stimulation of the TOP (-0.1 ± 0.84%MVC, $p = 0.035$). After sensory enhancement of UNI (0.74 ± 0.82%MVC, $p = 0.942$) to the TOP, there was no change in the reduction of dorsiflexion force caused by cutaneous reflex stimulation to TIB (see Figure 5-6B).

Sensory enhancement alters the perception of effort

Participants rated the level of effort to perform the task as being 3.6 ± 1.1 and 3.9 ± 1.6 (scored out of 10) for the plantarflexion and dorsiflexion control tasks, respectively. These ratings of level of effort did not differ from one another ($p = 0.379$). In general, participants did not perceive the effort required to produce force during sensory enhancement to be different from control. Group mean ratings of effort compared to control ranged from -0.2 to 0.5. During plantarflexion, the difficulty of tracing the line compared to control was significantly (p = 0.0005) harder during UNI stimulation (BOT = -1.3 ± 1.6, TOP = -1.5 ± 1.6) compared to MOD stimulation (BOT = -0.4 ± 1.5, TOP = 0.3 ± 1.3). In general, the sensation of stimulation was neither pleasant or unpleasant (i.e. ratings close to 0), with the exception of UNI stimulation to the BOT, which was rated as slightly unpleasant (-1.2 ± 1.3). During dorsiflexion trials, similar trends in data were present, however there were no significant differences between conditions.

Discussion

The most important finding from this experiment was that sensory enhancement caused facilitation of force output. This facilitation was present during stimulation of both the top and bottom of the foot, during both plantar- and dorsiflexion, and during both UNI and MOD stimulation. The participants did not perceive the stimulation as altering their ability to produce force, however they did perceive the task of tracing the line to be more difficult. The participants also perceived the stimulation as a neutral sensation (i.e. not pleasant nor unpleasant). Cutaneous reflex amplitudes were not altered following sensory enhancement, however there was a general facilitation of force 140-220ms following cutaneous reflex stimulation, suggesting a general facilitation of interneuronal networks involved with the integration of cutaneous input that contributes to ongoing force output.

Work by Aniss and colleagues (1988) showed that non-noxious stimulation to the digital nerve that evokes radiating paresthesia can reduce the perceived heaviness of a 500g but not 100g object. On the contrary, repetitive stimulation to the cutaneous nerves studied here (i.e. sensory enhancement of the TOP or BOT) disrupts cutaneous input during a force matching task, however, does not affect position matching (Choi et al., 2013). The authors also examined the effect of cutaneous stimulation on the mean force when participants were instructed to hold a low level (i.e. ~10% MVC) isometric dorsiflexion contraction. They found that there was no change in the mean force from either SP or TIB stimulation and suggested that cutaneous input does not cause under/over shoot of force control. The contractions in the current experiment required participants to gradually increase force up to ~20% MVC. Thus, they differed from the contractions from the work of Choi and colleagues in three ways; 1) the stimulation was provided to enhance sensations throughout the entire ramp of the contraction rather than to disrupt cutaneous feedback, 2) there was increasing descending drive throughout the ramp of the contraction, and 2) the effort was greater (almost double).

During increasing efforts of isometric voluntary contractions, it is well accepted that motor units are recruited in an orderly fashion, such that smaller low threshold units are recruited first, followed by larger higher threshold units (Henneman, 1985). Recruitment order is altered during 50Hz stimulation of the index finger, which resembles “firm gripping of the finger”, prompting the recruitment threshold of small units to increase and the recruitment thresholds of larger units to decrease (Garnett and Stephens, 1981). This alteration of recruitment order in the presence of cutaneous input has also been shown in the decerebrate cat (Kanda et al., 1977), suggesting that the mechanisms responsible, at least partially, lie within the spinal cord (see mechanisms in other section). Given this alteration in recruitment thresholds, and as suggested by Garnett and Stephens (1981), less descending drive is required to produce a given force. Presumably because of this, in the current experiment, the same descending drive resulted in amplified force output. It is possible that the enhancements of force only become apparent at slightly higher force outputs, since perceived heaviness is not altered via cutaneous stimulation with light (i.e. 100g) but is with heavier (i.e. 500g) weight (Aniss et al., 1988).

Spinal mechanisms other than alterations in recruitment thresholds of motor units may also play a role in the amplification of force. Constitutive activity of Ia afferent terminals onto the homonymous motoneuron pool provides a net facilitation of motoneuron firing rates and if Ia feedback is removed, firing rates plummet by about 30% (Macefield et al., 1993). During fatiguing efforts, reductions in presynaptic inhibition are associated with increased time to task failure (Baudry et al., 2011). Recent work (Chapter 3) has shown that patterned stimulation to cutaneous afferents of the feet can reduce Ia presynaptic inhibition. Therefore, this reduction in presynaptic inhibition may play a role in the amplification of force that occurs with sensory enhancement stimulation to the TOP and BOT.

Supraspinal contributions to the force amplification resulting from sensory enhancement should also be considered. Indeed, there are many examples that have shown increased cortical excitability in response to sensory stimulation. Repetitive stimulation the radial and ulnar nerves facilitates motor evoked potentials of hand muscles and causes an expansion of the cortical representation that can elicit a motor evoked potential in those muscles (Ridding et al., 2001). Repetitive stimulation to the SP nerve also facilitates the maximal MEP that can be evoked in the ipsilateral tibialis anterior muscle (Knash et al., 2003). By using blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI), Golaszewski and colleagues (2004) were able to show that sensory stimulation of the hand using a specialized mesh glove increases neuronal activity that is localized within the sensorimotor cortex. Using the same mesh glove, Golaszewski and colleagues (2010) found that sensory stimulation causes cortical excitability to increase. They concluded that the strength of corticospinal projections and inhibitory and faciliatory intracortical mechanisms all played a role in the increased excitability. Since the task in the current experiment required participants to voluntarily produce force, any increase in motor cortical excitability would cause an increased force output, compared to the desired command. Indeed, plasticity in corticomotor excitability have been previously suggested to increase muscle activity and grip force following 15 minutes of sensory stimulation over the forearm flexors (Dickstein and Kafri, 2008).

Absence of nerve specificity

There was ~10% facilitation of plantarflexion force when sensory enhancement was delivered to the TOP and BOT. Similarly, uniform stimulation to both nerves amplified force output during dorsiflexion. This suggests a general facilitation effect of cutaneous input on voluntary force output no matter the skin surface that is stimulated. A lack of nerve-specificity has previously been observed during tonic contractions, when compared to reflexes evoked during walking (Komiyama et al., 2000). Similar effects have also been observed when examining the conditioning effects of cutaneous input on muscle reflexes. For example, brief stimulation to both the ipsi- and contralateral calf or anterior thigh increases excitability of the patellar stretch reflex (Burke et al., 1989) and cutaneous stimulation applied to cutaneous afferents dispersed throughout the body, including the lower and upper limbs as well as the head, all facilitate the soleus H-reflex (Delwaide and Crenna, 1984). TENS to the Achilles or TA tendon facilitates the SOL H-reflex (Arsenault et al., 1993), as does TENS applied over the muscle bellies of the tibialis anterior or triceps surae (Hardy et al., 2002). Therefore, it is not surprising that sensory enhancement applied to either the TOP or BOT in the current experiment caused similar amplifications in force during both plantar- and dorsiflexion.

The role of stimulation frequency

Force amplification is graded with the frequency of sensory enhancement during plantarflexion. As seen in figure 5-3, force amplification begins immediately at the onset of UNI stimulation and remains relatively constant, whereas during the MOD stimulation, the amplification appears to gradually increase with stimulation frequency to a maximal amplification just before the offset of stimulation. This likely arises from increased temporal summation of cutaneous input at higher frequencies. In the dorsiflexion trials, there was only significant facilitation of force output with stimulation at a uniform frequency. This can probably be explained by the increases variability of dorsiflexion contractions compared to plantarflexion. On the contrary, the MOD stimulation was less unpleasant than the UNI stimulation, suggesting that in practice, lower frequency onset that increases with levels of effort may provide a more suitable option in some instances.

Practical implications

Previous work from our lab has shown that patterned sensory stimulation applied to nerves innervating the skin of the foot can increase H-reflex excitability during cycling and mitigate reductions in power output when cycling sprints are performed (Pearcey et al., 2017). This ergogenic effect of sensory stimulation became most evident later in the sprinting protocol, when central commands are inadequate to drive motoneurons efficiently (Collins et al., 2018; Pearcey et al., 2015). Therefore, sensory stimulation represents the potential to become a therapeutic tool to amplify force output when fatigue and/or neurological impairment have impaired the ability to activate muscles. Caution must be advised when extrapolating these findings to maximal activation of muscles, however, since sensory stimulation has only yet been shown to be effective during sub-maximal activation of muscles.

Conclusion

The results from this experiment suggest that sensory enhancement stimulation facilitates force output during an incremental isometric contraction. It appears that this facilitation is a general effect evoked from multiple nerves that occurs during both plantar- and dorsiflexion at the ankle that is graded with stimulation frequency, where higher frequency sensory stimulation induces greater facilitation of force output. The mechanisms underlying the observed amplified force output remain unclear, however one likely candidate remains the reduction in group Ia PSI that has previously been observed in response to cutaneous conditioning. A second candidate is the increased excitability of the corticospinal pathway that has been observed with TENS and cutaneous stimulation of the hand with a mesh glove. Future experiments should try to elucidate whether other mechanisms, such as changes in spinal motoneuron properties or other supraspinal mechanisms play a role.

References

Aniss, A.M., Gandevia, S.C., Milne, R.J., 1988. Changes in perceived heaviness and motor commands produced by cutaneous reflexes in man. J. Physiol. (Lond.) 397, 113–126.

Arsenault, A.B., Belanger, A.Y., Durand, M.J., De Serres, S.J., Fortin, L., Kemp, F., 1993. Effects of TENS and topical skin anesthesia on soleus H-reflex and the concomitant influence of skin/muscle temperature. Arch Phys Med Rehabil 74, 48–53.

Balter, J.E., Zehr, E.P., 2007. Neural coupling between the arms and legs during rhythmic locomotor-like cycling movement. J Neurophysiol 97, 1809–18. https://doi.org/10.1152/jn.01038.2006

Bastian, H.C., 1887. The “muscular sense”; its nature and cortical localisation. Brain April, 89.

Baudry, S., Maerz, A.H., Gould, J.R., Enoka, R.M., 2011. Task- and time-dependent modulation of Ia presynaptic inhibition during fatiguing contractions performed by humans. J Neurophysiol 106, 265–73. https://doi.org/10.1152/jn.00954.2010

Bhanpuri, N.H., Okamura, A.M., Bastian, A.J., 2012. Active force perception depends on cerebellar function. J. Neurophysiol. 107, 1612–1620. https://doi.org/10.1152/jn.00983.2011

Burke, J.R., Kamen, G., Koceja, D.M., 1989. Long-latency enhancement of quadriceps excitability from stimulation of skin afferents in young and old adults. J Gerontol 44, M158-163.

Choi, J.T., Lundbye-Jensen, J., Leukel, C., Nielsen, J.B., 2013. Cutaneous mechanisms of isometric ankle force control. Exp Brain Res 228, 377–384. https://doi.org/10.1007/s00221-013-3570-9

Collins, B.W., Pearcey, G.E.P., Buckle, N.C.M., Power, K.E., Button, D.C., 2018. Neuromuscular fatigue during repeated sprint exercise: underlying physiology and methodological considerations. Appl Physiol Nutr Metab 43, 1166–1175. https://doi.org/10.1139/apnm-2018-0080

Delwaide, P.J., Crenna, P., 1984. Cutaneous nerve stimulation and motoneuronal excitability. II: Evidence for non-segmental influences. J Neurol Neurosurg Psychiatry 47, 190–196.

Delwaide, P.J., Crenna, P., Fleron, M.H., 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.

Dickstein, R., Kafri, M., 2008. Effects of antecedent TENS on EMG activity of the finger flexor muscles and on grip force. Somatosens Mot Res 25, 139–146. https://doi.org/10.1080/08990220802131416

Garnett, R., Stephens, J.A., 1981. Changes in the recruitment threshold of motor units produced by cutaneous stimulation in man. J. Physiol. (Lond.) 311, 463–473.

Golaszewski, S.M., Bergmann, J., Christova, M., Nardone, R., Kronbichler, M., Rafolt, D., Gallasch, E., Staffen, W., Ladurner, G., Beisteiner, R., 2010. Increased motor cortical excitability after whole-hand electrical stimulation: a TMS study. Clin Neurophysiol 121, 248–254. https://doi.org/10.1016/j.clinph.2009.09.024

Golaszewski, S.M., Siedentopf, C.M., Koppelstaetter, F., Rhomberg, P., Guendisch, G.M., Schlager, A., Gallasch, E., Eisner, W., Felber, S.R., Mottaghy, F.M., 2004. Modulatory effects on human sensorimotor cortex by whole-hand afferent electrical stimulation. Neurology 62, 2262–2269.

Hardy, S.G.P., Spalding, T.B., Liu, H., Nick, T.G., Pearson, R.H., Hayes, A.V., Stokic, D.S., 2002. The effect of transcutaneous electrical stimulation on spinal motor neuron excitability in people without known neuromuscular diseases: the roles of stimulus intensity and location. Phys Ther 82, 354–363.

Heckman, C.J., Enoka, R.M., 2012. Motor unit. Compr Physiol 2, 2629–2682. https://doi.org/10.1002/cphy.c100087

Henneman, E., 1985. The size-principle: a deterministic output emerges from a set of probabilistic connections. J. Exp. Biol. 115, 105–112.

Iles, J.F., 1996. Evidence for cutaneous and corticospinal modulation of presynaptic inhibition of Ia afferents from the human lower limb. J Physiol 491 (Pt 1), 197–207.

Jenner, J.R., Stephens, J.A., 1982. Cutaneous reflex responses and their central nervous pathways studied in man. J Physiol 333, 405–19.

Johnson, M.D., Thompson, C.K., Tysseling, V.M., Powers, R.K., Heckman, C.J., 2017. The potential for understanding the synaptic organization of human motor commands via the firing patterns of motoneurons. J Neurophysiol 118, 520–531. https://doi.org/10.1152/jn.00018.2017

Kanda, K., Burke, R.E., Walmsley, B., 1977. Differential control of fast and slow twitch motor units in the decerebrate cat. Exp Brain Res 29, 57–74.

Klimstra, M.D., Thomas, E., Zehr, E.P., 2011. Biomechanical outcomes and neural correlates of cutaneous reflexes evoked during rhythmic arm cycling. Journal of biomechanics 44, 802–9. https://doi.org/10.1016/j.jbiomech.2010.12.017

Knash, M.E., Kido, A., Gorassini, M., Chan, K.M., Stein, R.B., 2003. Electrical stimulation of the human common peroneal nerve elicits lasting facilitation of cortical motor-evoked potentials. Exp Brain Res 153, 366–377. https://doi.org/10.1007/s00221-003-1628-9

Komiyama, T., Zehr, E.P., Stein, R.B., 2000. Absence of nerve specificity in human cutaneous reflexes during standing. Experimental brain research 133, 267–72.

Lemon, R.N., 2008. Descending pathways in motor control. Annu. Rev. Neurosci. 31, 195–218. https://doi.org/10.1146/annurev.neuro.31.060407.125547

Limonta, E., Rampichini, S., Cè, E., Esposito, F., 2015. Effects of visual feedback absence on force control during isometric contraction. Eur. J. Appl. Physiol. 115, 507–519. https://doi.org/10.1007/s00421-014-3036-1

Macefield, V.G., Gandevia, S.C., Bigland-Ritchie, B., Gorman, R.B., Burke, D., 1993. The firing rates of human motoneurones voluntarily activated in the absence of muscle afferent feedback. J Physiol 471, 429–43.

Pearcey, G.E., Murphy, J.R., Behm, D.G., Hay, D.C., Power, K.E., Button, D.C., 2015. Neuromuscular fatigue of the knee extensors during repeated maximal intensity intermittent-sprints on a cycle ergometer. Muscle Nerve 51, 569–79. https://doi.org/10.1002/mus.24342

Pearcey, G.E.P., Noble, S.A., Munro, B., Zehr, E.P., 2017. Spinal Cord Excitability and Sprint Performance Are Enhanced by Sensory Stimulation During Cycling. Front Hum Neurosci 11, 612. https://doi.org/10.3389/fnhum.2017.00612

Pierrot-Deseilligny, E., 1996. Transmission of the cortical command for human voluntary movement through cervical propriospinal premotoneurons. Progress in Neurobiology 48, 489–517. https://doi.org/10.1016/0301-0082(96)00002-0

Ridding, M.C., McKay, D.R., Thompson, P.D., Miles, T.S., 2001. Changes in corticomotor representations induced by prolonged peripheral nerve stimulation in humans. Clin Neurophysiol 112, 1461–1469.

Rothwell, J.C., Traub, M.M., Day, B.L., Obeso, J.A., Thomas, P.K., Marsden, C.D., 1982. Manual motor performance in a deafferented man. Brain 105 (Pt 3), 515–542.

Shim, J.K., Karol, S., Kim, Y.-S., Seo, N.J., Kim, Y.H., Kim, Y., Yoon, B.C., 2012. Tactile feedback plays a critical role in maximum finger force production. J Biomech 45, 415–420. https://doi.org/10.1016/j.jbiomech.2011.12.001

Vasudevan, E.V., Zehr, E.P., 2011. Multi-frequency arm cycling reveals bilateral locomotor coupling to increase movement symmetry. Experimental brain research 211, 299–312. https://doi.org/10.1007/s00221-011-2687-y

Zehr, E.P., 2006. Training-induced adaptive plasticity in human somatosensory reflex pathways. J Appl Physiol (1985) 101, 1783–94. https://doi.org/10.1152/japplphysiol.00540.2006

Zehr, E.P., Komiyama, T., Stein, R.B., 1997. Cutaneous reflexes during human gait: electromyographic and kinematic responses to electrical stimulation. Journal of neurophysiology 77, 3311–25.

Chapter 6 – Exploiting cervicolumbar connections enhances short-term spinal cord plasticity induced by rhythmic movement

Abstract

Arm cycling causes suppression of soleus (SOL) Hoffmann (H-) reflex that outlasts the activity period. Arm cycling presumably activates propriospinal networks that modulate Ia presynaptic inhibition. Interlimb pathways are thought to relate to the control of quadrupedal locomotion, allowing for smooth, coordinated movement of the arms and legs. We examined whether the number of active limb pairs affects the amount and duration of activity-dependent plasticity of the SOL H-reflex. On separate days, fourteen participants completed 4 randomly ordered 30 minute experimental sessions: 1) quiet sitting (CTRL); 2) arm cycling (ARM); 3) leg cycling (LEG); and, 4) arm and leg cycling (A&L) on an ergometer. SOL H-reflex and M-wave amplitudes were evoked via electrical stimulation of the tibial nerve. M-wave and H-reflex recruitment curves were recorded while the participants sat quietly prior to, 10 and 20 minutes into, immediately after, and at 2.5, 5, 7.5, 10, 15, 20, 25, and 30 minutes after each experimental session. Normalized maximal H-reflexes were unchanged in CTRL but were suppressed by >30% during the ARM, LEG and A&L. H-reflex suppression outlasted activity duration for ARM (≤2.5 mins), LEG (≤5 mins), and A&L (≤30 mins). The duration of reflex suppression after A&L was greater than the algebraic summation of ARM and LEG. This non-linear summation suggests that using the arms and legs simultaneously—as in typical locomotor synergies—amplifies networks responsible for the short-term plasticity of lumbar spinal cord excitability. Enhanced activity of spinal networks may have important implications for the implementation of locomotor training for targeted rehabilitation.

New and Noteworthy

Modulation of reflexes outlasts an activity duration, however the effects of using the arms and legs together compared to separately is unknown. Here we show that the coupling of the arms and legs together during locomotor activity has little effect on the magnitude of reflex modulation ***during*** activity, yet enhances the duration of modulation ***after*** the

---

Pearcey GE and Zehr EP (2019). Exploiting cervicolumbar connections enhances short-term spinal cord plasticity induced by rhythmic movement. Under revision in Experimental Brain Research (EXBR-D-19-00201) activity has been ceased. These findings may have major implications for the implementation of locomotor retraining following a neurological insult.

Introduction

Physical training can produce short- and long-term plasticity of spinal cord excitability. For example, H-reflex amplitudes are reduced in highly trained ballet dancers (Koceja *et al.*, 1991; Nielsen *et al.*, 1993), with balance training (Trimble & Koceja, 2001), and with training to walk backwards (Schneider & Capaday, 2003). Amplitude modulation of H-reflexes also occurs during acute performance of a motor task. During rhythmic movement, H-reflexes are modulated in a phase-dependent manner by central pattern generators (CPGs) and afferent feedback arising from the rhythmic movement (Brooke *et al.*, 1997; Zehr & Stein, 1999; Zehr & Duysens, 2004; Zehr *et al.*, 2016). Interestingly, short duration (<2 minutes) passive cyclical movement of the leg is sufficient to cause amplitude modulation of the SOL H-reflex that outlasts movement cessation by ~4s (Misiaszek *et al.*, 1995). When leg-cycling is performed for 30 minutes, SOL H-reflex amplitudes are reduced by ~40% for at least 10 minutes after cycling has ceased (Motl & Dishman, 2003), suggesting that activity-dependent plasticity of H-reflexes is not constrained to the timeframe that the activity is performed. Further work (Mazzocchio *et al.*, 2006; Meunier *et al.*, 2007) has shown that a single bout of skilful cycling, which requires cognitive effort to alter the frequency of movement to various target cadences, can cause short-term spinal cord plasticity (Mazzocchio *et al.*, 2006) that persists ~24 hours after the cycling session (Meunier *et al.*, 2007). The short-term spinal cord plasticity resulting from skilful cycling, is more pronounced than that of non-skilful cycling, suggesting that spinal cord plasticity can be further increased when descending drive to those spinal circuits is emphasized.

Plasticity of spinal cord circuits during and after movement cessation is not isolated to the moving limb (Brooke *et al.*, 1997). Indirect evidence from human reflex experiments suggests that, similar to other animals, we possess locomotor networks in the spinal cord (i.e. CPGs) capable of modifying rhythmic output. In addition to CPG's, humans have retained cervico- and thoraco-lumbar connections (Dietz, 2002; Zehr & Duysens, 2004; Zehr *et al.*, 2016), which can affect the excitability of reflexes in remote limbs (i.e. limbs that are not involved in movement). These interlimb effects on spinal cord excitability occur from legs to arm and from arms to leg. Amplitude modulation of H-reflexes evoked in the forearm (flexor carpi radialis; FCR) during walking (Domingo *et al.*, 2014), leg cycling (Zehr *et al.*, 2007; Nakajima *et al.*, 2013), and passive leg stepping (Nakajima *et al.*, 2011; Nakajima *et al.*, 2016) provide evidence for remote effects of leg movement on the spinal cord excitability in the arms. In support of effects of the arms on the legs, SOL H-reflexes are reduced in amplitude during rhythmic arm swing (Hiraoka, 2001; Hiraoka & Iwata, 2006; Massaad *et al.*, 2014) and rhythmic arm cycling (Frigon *et al.*, 2004; Zehr *et al.*, 2004; Loadman & Zehr, 2007; Barzi & Zehr, 2008; Javan & Zehr, 2008; Dragert & Zehr, 2009; Hundza & Zehr, 2009; de Ruiter *et al.*, 2010; Hundza *et al.*, 2012; Kaupp *et al.*, 2018). In the work of Javan and Zehr (2008), rhythmic activity of the arms caused modulation of reflexes in the legs that outlasted the activity duration, evident of short-term plasticity of spinal cord excitability.

Combined arm and leg movement may enhance movement-induced plasticity of spinal circuits by exploiting the interactions of afferent feedback and pattern generating networks of the arms and legs (i.e. cervicolumbar connections). In a study by de Ruiter *et al.* (2010), participants cycled with just the ipsilateral arm, just the contralateral arm, or with both arms and noted that the greatest SOL H-reflex amplitude modulation occurred when participants cycled with both arms. Subsequently, Mezzarane *et al.* (2011) showed that exploiting cervicolumbar connections during rhythmic stepping increases modulation of reflexes in the leg. Nakajima *et al.* (2014) expanded upon this idea when they had participants perform arm only, contralateral leg only or arm and contralateral leg recumbent stepping while evoking cutaneous reflexes from two nerves separately and from both nerves simultaneously in the resting leg. Combined cutaneous reflexes were facilitated with increased magnitude based on the number of rhythmically active limbs. This facilitation was greater than the algebraic sum of the two reflexes alone, suggesting a facilitation effect from descending drive and/or CPG related activity that is dependent upon the number of rhythmically active limbs. In a more recent experiment, Sasada *et al.* (2016) provided further evidence for a common neural element that is only active during coordinated arm and leg movement by showing a facilitation of cutaneous reflexes in the upper limbs during combined arm and leg cycling. This facilitation during combined arm and leg (A&L) cycling was greater than the sum of facilitation during arm only and leg only cycling.

Recent suggestions (Dietz, 2002; Ferris *et al.*, 2006; Zehr *et al.*, 2009; Zehr, 2016) to exploit cervicolumbar connections in human locomotor rehabilitation training by including rhythmic arm movement with rhythmic leg movements has shown great success. Having participants perform rhythmic arm cycling while also performing functional electrical stimulated leg cycling improved corticospinal drive to the lower limbs and supports the notion that active engagement of the upper limbs facilitates the restoration of function following spinal cord injury (Zhou *et al.*, 2017). Furthermore, combined A&L cycling training has been shown to cause vast improvements in walking function, strength and neurophysiological integrity in chronic stroke participants (Klarner *et al.*, 2016a, b). Indeed, arm cycling training alone was also able to improve walking function and neurophysiological integrity in chronic stroke participants (Kaupp *et al.*, 2018). During both arm and A&L cycling, a notable outcome was the regulation of hyperactive stretch reflexes in the plantar flexors during arm cycling. Unfortunately, it is unclear whether there is a clear advantage of A&L cycling compared to arm only or leg only cycling when it comes to modulation of reflexes in the legs following activity. Therefore, the purpose of this experiment was to determine whether the active engagement of the arms and legs together, rather than separately, enhances the activity-dependent modulation of the soleus H-reflex pathway in neurologically intact participants.

Methods

Participants

Fourteen neurologically intact participants (170.9 ± 10.16 cm, 69.4 ± 13.51 kg, 24.9 ± 3.34 years, 7 females) were recruited from the university student population. Participants were apparently healthy (i.e. no history of cardiovascular, metabolic or neuromuscular impairment) and provided written and signed informed consent prior to partaking in any experimental protocols. The protocol was approved by the Human Research Ethics Board at the University of Victoria and was conducted in accordance with the Declaration of Helsinki.

Experimental Protocol

Participants began each experimental session sitting as depicted in the far left panel of figure 6-1 (i.e. control). On 4 separate days, they then either sat in a resting position or performed arm, leg, or A&L cycling on a Sci-fit arm and leg cycle ergometer (Sci-Fit Pro 2, Tulsa, OK) in a seated position with the center of the crank set to the same height as their acromion. For leg and A&L cycling, the feet were strapped onto pedals with the knee just slightly bent at the furthest portion of the pedal stroke. For arm cycling, the feet were strapped into footplates with hip, knee and ankle angles kept constant throughout all experimental procedures. For A&L cycling, arm and leg cranks were mechanically coupled with a 90^o out of phase offset to maintain a constant rigid relation between arm and leg movement. The intensity and duration of cycling was kept constant at 60 revolutions per minute (RPM), at a workload of 20W (minimum workload setting of the ergometer) for 30 minutes in all conditions. H-reflex and M-wave recruitment curves were recorded 10 and 20 minutes into each session. After the first 30 minutes in each session, H-reflex and M-wave recruitment curves were recorded immediately and 2.5-, 5-, 7.5-, 10-, 15-, 20-, 25- and 30-minutes post-cycling.

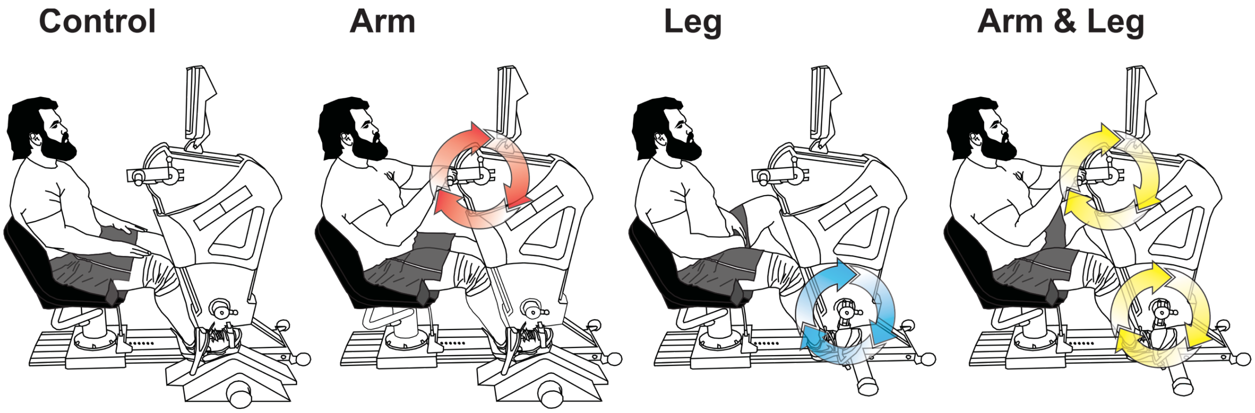

Figure 6-1: *A representation of the activity performed in each of the conditions. All PRE and POST H-reflex measures were taken in the control position as shown in the far left panel (i.e. Control).*

Electromyography

Bipolar surface electrodes were placed bilaterally over the mid-muscle bellies of the soleus (SOL), tibialis anterior (TA) and vastus lateralis (VL). Electrode positions were marked and recorded in relation to anatomical landmarks and placed by the same experimenter each day for consistency. To reduce variation in placement, anatomical landmarks and measurements taken from the first session were used on subsequent sessions. EMG signals from SOL were preamplified (x500) and band pass filtered (10-1000 Hz) and, similarly, EMG signals from TA and VL preamplified (x5000) and band pass filtered (100-300 Hz) (GRASS P511, AstroMed). These parameters are consistent with previous experiments in our laboratory (Balter & Zehr, 2007; Vasudevan & Zehr, 2011; Zehr *et al.*, 2012; Pearcey *et al.*, 2017). After conversion to a digital signal, data were sampled at 2000 Hz using a custom-built continuous acquisition software (LABVIEW, National Instruments, TX, USA).

Transcutaneous nerve stimulation

To evoke H-reflexes in the SOL muscle, single 1 ms square wave electrical pulses were applied to the right tibial nerve at the popliteal fossa with bipolar surface electrodes (Thought Technology Ltd., Montreal, QC, Canada) using a Digitimer (Medtel, NSW, Australia) constant current stimulator (model DS7A). A non-contact milliammeter (mA-2000, Bell Technologies, Orlando, FL, USA) was used to measure current delivered for each stimulus. Recruitment curves were recorded at pre-, during cycling, and post-cycling. For pre-activity recruitment curves, 40 sweeps were recorded, however to allow for higher resolution in time, only 20 sweeps were recorded for during and post-cycling time points. During pre- and post-cycling collection participants had their feet secured on foot plates however during activity for the leg and A&L conditions, reflexes were evoked when the right foot passed the position that corresponded to the same joint angles of the right leg. Ankle and knee angles (ankle ~90°, knee ~110°) were monitored throughout the experimental sessions to ensure there were no changes. Stimulus-response curve stimuli were delivered pseudo randomly between 1 s and 3 s. Stimulus intensity was increased and decreased incrementally (ranged from 0.1 mA to 1 mA per increment) based on the excitability of the reflex pathway in different individuals, while ensuring that supramaximal M-wave amplitudes were achieved by increasing larger increments once the H-reflex amplitude started to decrease in size (i.e., after the peak H-reflex amplitude was reached, for examples see (Klimstra & Zehr, 2008)).

Statistics

Statistical procedures were performed using SPSS 18.0 (Chicago, Illinois). For between condition group comparisons, a two factor (CONDITION x TIME) repeated measure ANOVA was run to determine main and interaction effects for reflex modulation across the four conditions. If significant interactions were found and direction of change was predicted because of priori hypotheses, paired samples t-tests were performed. In all cases, statistical significance was set at $p \leq 0.05$. Results are reported as means ± SD in text (SE in figures).

Results

M-wave excitability

Maximal M-wave recordings were consistent at PRE between conditions, however there were changes in M_{max} amplitudes as a result of cycling with the legs. At PRE, group averaged M_{max} amplitudes were 10957 ± 831.8μV, 10802.2 ± 854.8μV, 10700 ± 680.1μV, 11041 ± 937.5μV for CTRL, ARM, LEG and A&L, respectively. The RM ANOVA (4 (condition)x12(time)) revealed a significant interaction effect of time and condition ($F_{(33,396)} = 1.651$, $p = 0.015$). For all time points after the cessation of cycling, M_{max} values were increased by approximately 10% (range of 9.5-10.9%, p ranged from 0.000127 to 0.01) during LEG, and approximately 7% (range of 6.3-7.8%, p ranged from 0.035 to 0.05) during A&L. There were no changes in M_{max} during CTRL or ARM. This suggests that after rhythmic leg movement, the peripheral excitability of the soleus muscle is increased.

H-reflex excitability

H-reflex excitability was drastically reduced during ARM, LEG and A&L activity, and remained suppressed following all forms of rhythmic activity. Separate RM ANOVAs (4 (condition) x 12 (time)) were run for the H_{max}/M_{max} ratio and each variable of the H-reflex recruitment curves (**slope**, **H@th**, **H@50**, **H@100**, **c@th**, **c@50**, **c@max**). The RM ANOVA for the H_{max}/M_{max} ratio revealed a significant effect for time ($F_{(11,132)} = 25.722$, $p < 0.0001$), condition ($F_{(3,36)} = 9.446$, $p < 0.0001$) and an interaction effect of time and condition ($F_{(33,396)} = 4.419$, $p < 0.0001$), however, the H_{max}/M_{max} ratio at pre was similar between conditions as shown in table 6-1. PRE recruitment curve values are shown in table 6-2 and did not differ between conditions. Effects of the RM ANOVA for each recruitment curve variable are displayed in table 6-3.

Table 6-1: *Pre group average and standard deviation values for maximal M-wave (Mmax), H-reflex (Hmax) and the ratio between the Mmax and Hmax for each condition.*

Condition	***M_{max} (μV)***	***H_{max} (μV)***	***H_{max}/M_{max} ratio (%)***
CTRL	10957 ± 831.8	4870 ± 1963.3	47.5 ± 22.23

ARM	10802 ± 854.7	5036 ± 1958.2	46.8 ± 18.58
LEG	10669 ± 680.1	5607 ± 2553.5	52.9 ± 22.43
A&L	11041 ± 937.5	5506 ± 1850.4	50.0 ± 15.26

Table 6-2: *Pre group average and standard deviation values for recruitment curves in each condition. c@th = stimulation current required to evoke the smallest H-reflex, c@50% = stimulation current required to evoke an H-reflex 50% of maximum amplitude, c@max = stimulation current required to evoke the maximum H-reflex, H@th = H-reflex size at the current required to evoke the smallest H-reflex from PRE, H@50% = H-reflex size at the current required to evoke 50% of the maximal H-reflex from PRE, and H@100% = H-reflex size at the current required to evoke the maximal H-reflex from PRE.*

Condition	***Slope***	***c@th (mA)***	***c@50 (mA)***	***c@max (mA)***	***H@th*** (%M_{max})	***H@50*** (%M_{max})	***H@100*** (%M_{max})
CTRL	**3.2 ± 2.24**	**56.7 ± 14.95**	**66.3 ± 11.44**	**75.8 ± 8.82**	**5.7 ± 2.32**	**23.6 ± 9.46**	**41.6 ± 16.77**
ARM	**3.3 ± 3.20**	**56.8 ± 14.21**	**69.1 ± 6.41**	**81.3 ± 6.27**	**5.3 ± 2.61**	**22.0 ± 11.23**	**38.7 ± 19.65**
LEG	**3.1 ± 2.82**	**60.5 ± 8.46**	**71.1 ± 6.06**	**81.6 ± 8.01**	**5.6 ± 2.81**	**23.5 ± 11.68**	**41.2 ± 20.43**
A&L	**3.4 ± 2.92**	**55.2 ± 17.43**	**65.2 ± 11.94**	**75.2 ± 8.98**	**5.8 ± 3.06**	**24.9 ± 13.05**	**43.8 ± 23.11**

Table 6-3: *Main effects from the repeated measures ANOVA performed on each recruitment curve variable. F-ratios are reported with p-values in parenthesis. Asterisks indicate a significant effect. c@th = stimulation current required to evoke the smallest H-reflex, c@50% = stimulation current required to evoke an H-reflex 50% of maximum amplitude, c@max = stimulation current required to evoke the maximum H-reflex, H@th = H-reflex size at the current required to evoke the smallest H-reflex from PRE, H@50% = H-reflex size at the current required to evoke 50% of the maximal H-reflex from PRE, and H@100% = H-reflex size at the current required to evoke the maximal H-reflex from PRE.*

Variable	*Condition*		*Time*		*Interaction*	
Slope	*$F_{(3,36)} = 3.253$*	*(0.033)**	*$F_{(11,132)} = 3.262$*	*(0.001)**	$F_{(33,396)} = 1.287$	(0.138)
c@th	$F_{(3,36)} = 0.276$	(0.843)	*$F_{(11,132)} = 3.950$*	*(< 0.001)**	$F_{(33,396)} = 1.34$	(0.104)
c@50%	$F_{(3,36)} = 1.821$	(0.161)	*$F_{(11,132)} = 16.288$*	*(< 0.001)**	*$F_{(33,396)} = 2.554$*	*(< 0.001)**
c@max	*$F_{(3,36)} = 3.123$*	*(0.038)**	*$F_{(11,132)} = 16.332$*	*(< 0.001)**	*$F_{(33,396)} = 3.762$*	*(< 0.001)**
H@th	$F_{(3,36)} = 1.204$	(0.322)	*$F_{(11,132)} = 11.771$*	*(< 0.001)**	*$F_{(33,396)} = 1.457$*	*(0.05)**
H@50%	*$F_{(3,36)} = 4.41$*	*(0.01)**	*$F_{(11,132)} = 26.419$*	*(< 0.001)**	*$F_{(33,396)} = 2.804$*	*(< 0.001)**
H@100%	*$F_{(3,36)} = 7.702$*	*(< 0.001)**	*$F_{(11,132)} = 34.738$*	*(< 0.001)**	*$F_{(33,396)} = 4.709$*	*(< 0.001)**

Modulation of H-reflexes during Activity

In general, H-reflex excitability was greatly reduced during all forms of rhythmic activity. There was no change in H_{max}/M_{max} ratio during CTRL at time points 10 (47.2 ± 21.86%) and 20 (48.6 ± 22.84%), compared to PRE (47.5 ± 22.23%). There was, however, a reduction of 40.4 ± 28.80% and 37.9 ± 28.57% during ARM, 42.8 ± 29.36% and 40.3 ± 26.91% during LEG and 48.2 ± 22.00% and 50.0 ± 21.86% during A&L at 10 and 20, respectively. The reductions in H_{max}/M_{max} ratio were similar between conditions. In all conditions except CTRL, the slope of the recruitment curve was reduced by ~40% at both 10 and 20, however these reductions in slope did not differ between conditions nor between time points. There was a general increase in current required to evoke 50% H_{max} and H_{max}, but not the threshold H-reflex, at 10 and 20 for ARM, LEG, and A&L. The increased current required to evoked H_{max} at 20 was greater for the LEG ($p = 0.013$) and A&L ($p = 0.011$) conditions compared to ARM, suggesting that activity of the legs was driving this effect. The H-reflex amplitude was reduced during ARM (~35%), LEG (~70%) and A&L (~70%) at the current required to get 50% of H_{max} (all $p < 0.01$) and H_{max} (all $p < 0.001$) from PRE but were not significantly different from each other. See figure 6-2 for a single subject's sigmoidal fits for recruitment curves that were recorded at all time-points in comparison to PRE in figure 6-2.

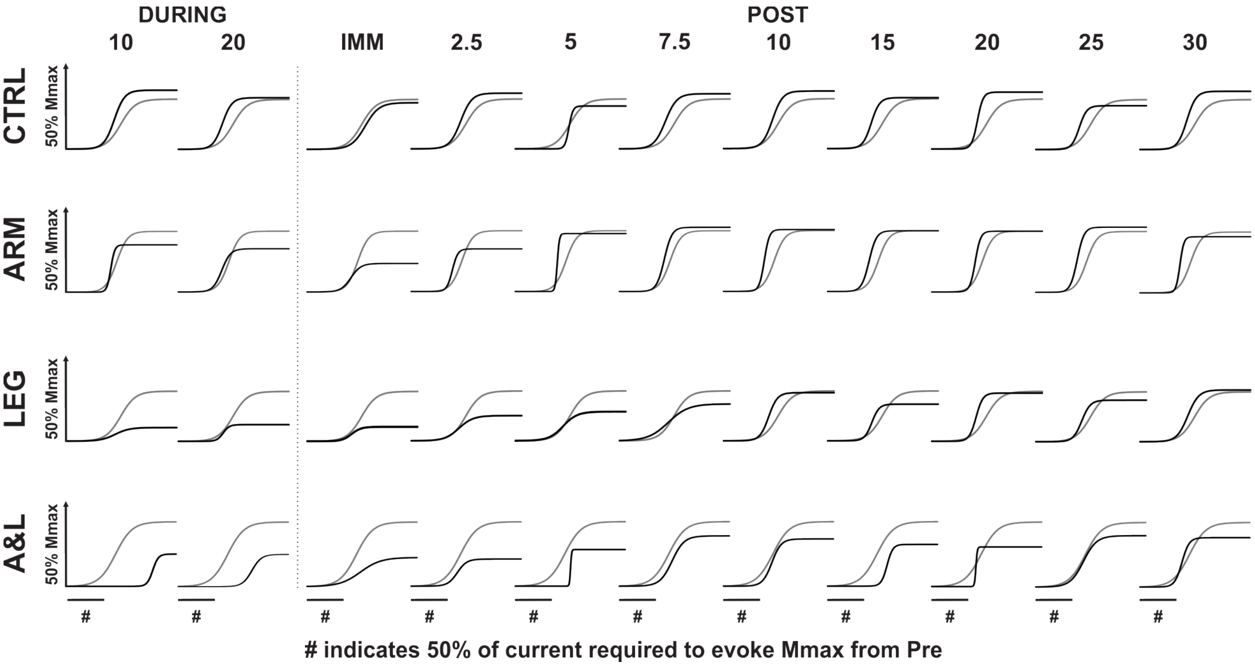

Figure 6-2: *Lines represent the sigmoidal fit of each H-reflex recruitment curve that was recorded for a single subject. The grey lines are the PRE curves, whereas the black lines are the curves from the corresponding time point and condition. CTRL is on top, ARM is second from the top, LEG is third from the top and A&L is at the bottom.*

Modulation of H-reflexes after activity has ceased

After activity, there was persistent modulation of the H-reflex pathway in all conditions, other than CTRL. During ARM, the H_{max}/M_{max} ratio was significantly (p = 0.0019) reduced by 19.7 ± 20.54% immediately after cycling, however returned to PRE values by 2.5 minutes post-cycling. The H-reflex amplitude evoked at the current required to evoke H_{max} during PRE (i.e. **H@100%**) remained reduced for up to 10 minutes, indicating a reduced excitability at the same relative input between time points. During LEG, the H_{max}/M_{max} ratio was significantly reduced by 28.5 ± 21.54% and 10 ± 14.67% immediately and 2.5 minutes post-cycling, respectively, however returned to PRE values by 5 minutes post-cycling. The H-reflex amplitudes evoked at the current required to evoke 50% of H_{max} (i.e. **H@50%**) and current required to evoke H_{max} (i.e. **H@100%**) during PRE both remained reduced for up to 10 minutes. During A&L, the H_{max}/M_{max} ratio was significantly reduced by >20% up to 5, >10% up to 25, and by ~8% at 25 and 30 minutes post-cycling, compared to PRE (see figure 6-3). The reduction in H_{max}/M_{max} ratio during A&L was greater than that of ARM immediately post, and was greater than that of ARM and LEG at all other time points up to, but not including, 30 minutes post-cycling (see figure 6-3). Furthermore, the current required to evoke the maximal H-reflex (i.e. **c@max**) was increased for up to 25 minutes and the H-reflex amplitudes evoked at the current required to evoke 50% of H_{max} (i.e. **H@50%**) and current required to evoke H_{max} (i.e. **H@100%**) during PRE remained reduced for up to 15 minutes each.

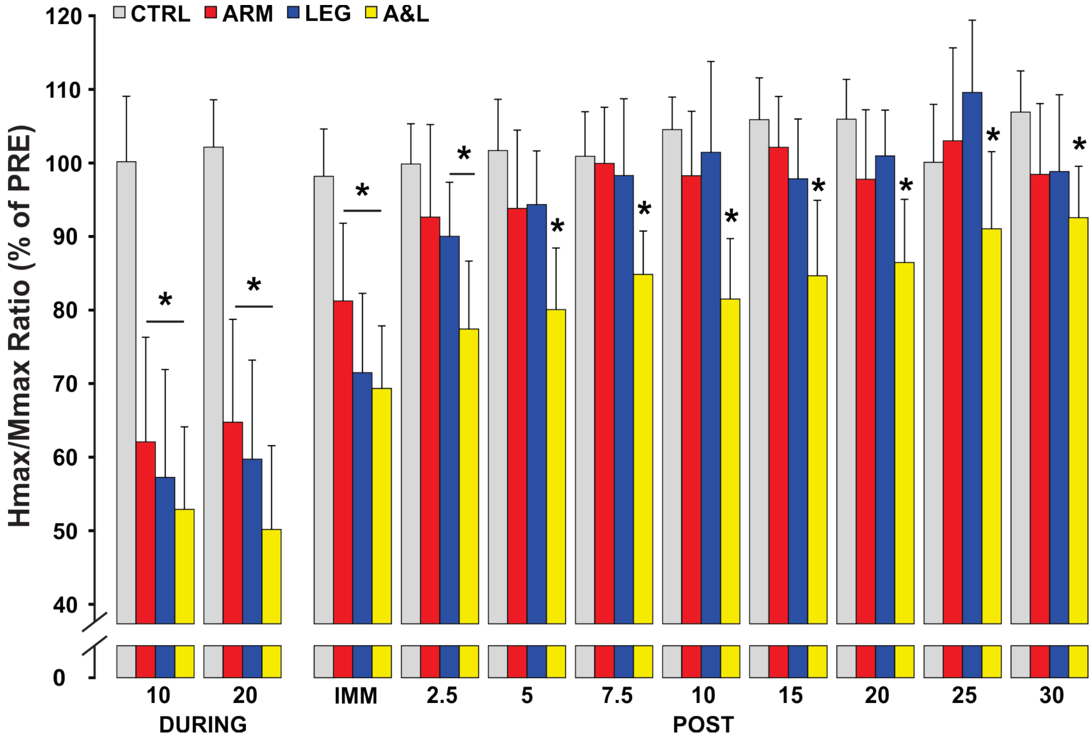

Figure 6-3: *Group averaged H_{max}/M_{max} ratios for the control (CTRL; grey fill), arm (ARM; red fill), leg (LEG; blue fill), and combined arm and leg (A&L; yellow fill) conditions. All values are made relative to the recruitment curve measured on that day at PRE. Asterisks indicate a significant reduction from PRE and error bars indicate standard error of the group.*

Discussion

The main finding of this experiment is that exploiting cervicolumbar connections during rhythmic movement enhances the suppressive effects of cycling on soleus H-reflexes after the activity has ceased. The prolonged H-reflex suppression is greatest when combined arm and leg rhythmic activity is performed, followed by rhythmic activity of the limbs where reflexes are measured (i.e. the legs in this case), followed by rhythmic activity of remote limbs (i.e. arms in this case). These findings build on previous findings from our lab (Javan & Zehr, 2008) that show a persistent short-term plasticity of H-reflexes in the legs induced by arm cycling. Furthermore, these findings support the working hypothesis that humans have retained cervicolumbar connections throughout evolution that can be exploited to enhance targeted rehabilitation strategies and augment neuroplasticity (Zehr, 2016).

Rhythmic leg movement causes prolonged suppression of H-reflex excitability in the legs, which is accompanied by a facilitation of peripheral excitability

Little attention has been given to the lasting effects of rhythmic leg movement on H-reflex excitability after a task has ceased. Initially, Misiaszek *et al.* (1995) showed that short duration (<2 minutes) of passive cycling reduced SOL H-reflexes for ~4 seconds. More recently, Motl and colleagues (Motl & Dishman, 2003; Motl *et al.*, 2003) have shown that 30 minutes of low intensity leg cycling reduces SOL H-reflex excitability for ~10 minutes. In line with those findings, our current results show that leg cycling reduced H_{max}/M_{max} ratios for up to 5 minutes, however, H-reflex amplitudes at stimulus intensities required to evoke 50% and H_{max} from PRE were reduced for up to 10 minutes. Interestingly, we also found that M_{max} amplitudes were facilitated by ~10% following the leg cycling. Unfortunately, there are few reports in the literature that have examined the effects of non-fatiguing leg cycling on peripheral excitability. In an experiment aimed to examine the effects of various warm-up routines on knee extensor function, Girard *et al.* (2009) found that M_{max} amplitude of the vastus lateralis muscle tended to increase following a running warm-up. We believe that this is the first report of non-fatiguing cycling causing an increase in M_{max}, however, the extent to which various parameters of cycling can increase peripheral excitability (i.e. M_{max}) are not known.

Rhythmic arm movement causes prolonged suppression of H-reflex excitability in the legs

H-reflex suppression due to rhythmic activity of a remote limb pair (arms or legs), opposite to the resting limbs (legs or arms) has been well-documented (Hiraoka, 2001; Frigon *et al.*, 2004; Zehr *et al.*, 2004; Hiraoka & Iwata, 2006; Loadman & Zehr, 2007; Zehr *et al.*, 2007; Dragert & Zehr, 2009; Hundza & Zehr, 2009; de Ruiter *et al.*, 2010; Nakajima *et al.*, 2011; Hundza *et al.*, 2012; Nakajima *et al.*, 2013; Domingo *et al.*, 2014; Massaad *et al.*, 2014; Nakajima *et al.*, 2016). Our current findings coincide with the previous reports of Javan and Zehr (2008) and Nakajima *et al.* (2016) as we saw a ~40% suppression of SOL H-reflex amplitudes (H_{max}/M_{max}) during arm cycling activity. Following the cessation of arm cycling, however, H_{max}/M_{max} values in the current experiment returned to pre-cycling values within the first 2.5 minutes. The H-reflex amplitude at the current required to evoke H_{max} pre-cycling was reduced for up to 10 minutes, indicating a prolonged suppression of H-reflex excitability. The duration of prolonged suppression in the current experiment is less than that of Javan and Zehr (2008) but closer to the duration reported in Nakajima *et al.* (2016). This is probably due to the method used to sample H-reflexes, since H-reflexes at ~50-70% of H_{max} are more susceptible to influences from Ia PSI from presynaptic inhibitory interneurons (Crone *et al.*, 1990), than are the responses at H_{max}. Nevertheless, a similar trend has been shown in the current experiment, illustrating the potential of rhythmic activity of a remote limb pair to cause prolonged suppression of H-reflex excitability.

Rhythmic combined arm and leg activity amplifies prolonged suppression of H-reflex excitability in the legs

Interactions of arm and leg rhythmic movements during activity have been well documented. Recent work by Nakajima *et al.* (2014) used spatial facilitation to examine the convergence of common reflex pathways during various rhythmic and locomotor-like movements of the arms and legs. A greater facilitation of reflex amplitudes was found when both the arms and legs were used together, compared to the summation of using the limbs separately, suggesting a weighting of facilitation according to the number of rhythmically active limbs. This highlights common interneuronal reflex pathways that contribute to excitability differences when the arms and legs work together during a locomotor task (Zehr, 2016). Subsequently, Sasada *et al.* (2016) provided evidence for a common neural element of the early latency cutaneous reflex pathway, which receives convergent input from the arms and legs, that is only active when the arms and legs are used together. Indeed, Zehr et al. (2007) showed that H-reflex amplitudes evoked at the current required for H_{max} PRE were reduced to a similar extent between LEG and A&L, whereas during ARM they were reduced to a lesser extent. Although somewhat surprising, these findings support of the notion that there are arm-to-leg neural interactions during locomotor-like movements that are dominated by the legs. During A&L movement in the current experiment, the suppression of SOL H-reflex excitability was greater than during ARM but similar to LEG. This leg dominated suppression in SOL H-reflex excitability is thought to reflect an active physiological filtering of excessive afferent feedback (Brooke *et al.*, 1997) that occurs during rhythmic movement of the limb in which the reflex is measured (see Brooke and Zehr (2006)). However, the extent to which the interactions of arm and leg movements interact following the cessation of movement have not been examined until now. After the cessation of A&L movement, we found prolonged suppression of SOL H-reflexes lasted for at least 25 minutes, compared to less than 10 for LEG and ARM added together. This finding suggests that using the arms and legs together in a locomotor context activates a common neural element that contributes to augmenting the prolonged suppression of the SOL H-reflex following the cessation of rhythmic activity.

Mechanisms responsible for prolonged suppression of H-reflexes

Recent decades of experiments have provided indisputable evidence that reflex pathways are malleable, and the excitability depends on the context in which they are evoked, the task being performed and the phase within the task (Stein & Capaday, 1988; Zehr & Stein, 1999). The central nervous system, and in particular descending and afferent input onto interneurons and motoneurons, modulates the expression of a reflex based upon the task performed (Zehr, 2006). Multiple reviews have summarized the effects of rhythmic leg activity on H-reflexes evoked in the legs (see the following reviews: (Brooke *et al.*, 1997; Brooke, 2004; Brooke & Zehr, 2006)). In short, the gain of SOL H-reflexes is higher during phases of stepping, walking, running, and cycling when the SOL is active, whereas the gain is reduced when the SOL is inactive and returning to that phase (Brooke *et al.*, 1997). However, compared to lying, sitting or standing, SOL H-reflex gain is lower during rhythmic movement of the legs, and as movement frequency increases SOL H-reflex gain decreases. This adaptive gain control is attributable to Ia presynaptic inhibition (PSI) (summarized in (Zehr, 2006)).

Conditioning of H-reflexes with movement and sensory inputs known to modulate Ia PSI provided evidence that interlimb effects on H-reflex excitability are mediated by Ia PSI (Frigon *et al.*, 2004; Nakajima *et al.*, 2013) likely stemming from CPG related activity and/or afferent feedback related to movement (Brooke *et al.*, 1997). Although the mechanism causing the prolonged suppression of SOL H-reflex excitability following rhythmic movement is not entirely clear, it has been suggested it may result from activation of persistent inward currents within the spinal interneurons mediating Ia PSI (Javan & Zehr, 2008). Persistent inward currents have been identified within the central nervous system of several species (Brown & Griffith, 1983; Murase *et al.*, 1986; Oyama *et al.*, 1986; Hounsgaard & Kiehn, 1989; Fraser & MacVicar, 1996; Lee & Heckman, 1998a, b; Smith & Perrier, 2006; Abbinanti *et al.*, 2012; Hultborn *et al.*, 2013; Wang *et al.*, 2015), including humans (Collins *et al.*, 2002; Heckman *et al.*, 2008; Wilson *et al.*, 2015; Johnson *et al.*, 2017), and have been shown to play a role in the activity of interneurons within the ventral horn of the turtle spinal cord (Smith & Perrier, 2006), commissural interneurons within the mouse spinal cord (Abbinanti *et al.*, 2012) and inhibitory interneurons within the human and primate cortex (Wang *et al.*, 2015). Plateau-like behaviour of the Ia PSI interneuron (as studied in the current experiment) can be immediately terminated when a brief sensory input is provided (Javan & Zehr, 2008) similar to the termination of plateau-like behaviour of motoneurons by inhibitory post-synaptic inputs in vivo (Lee & Heckman, 1998a, b). Persistent activity of the Ia PSI interneurons has been proposed as the simplest explanation for short-term plasticity of SOL H-reflexes resulting from rhythmic movement (Javan & Zehr, 2008). Therefore, activation of the common interneuronal networks that receive convergent information from both the arms and legs when they are used together (Nakajima *et al.*, 2014; Sasada *et al.*, 2016; Zehr, 2016) could facilitate the plateau-like activity of inhibitory spinal interneurons, thus resulting in more prolonged suppression of the SOL H-reflex pathway when the arms and legs are used together, rather than separately.

Clinical Translation

Priming neural circuits has predominantly been thought of as a means of facilitating voluntary motor output in context of rehabilitation (Stoykov & Madhavan, 2015). Recently, however, Estes *et al.* (2017) provided evidence for the use of physical therapeutic/electroceutic interventions to reduce spinal reflex excitability and therefore reduce spasticity. Since spasticity is associated with hyperexcitable Ia reflex excitability (Levin & Hui-Chan, 1993), and A&L activity causes the longest lasting suppression of H-reflex excitability following activity of the modalities tested in the current experiment, it seems logical that A&L activity could be used as a primer of spinal reflex circuits to reduce spasticity. Indeed, arm (Sosnoff & Motl, 2010) and leg (Motl *et al.*, 2006; Motl *et al.*, 2007; Sosnoff *et al.*, 2009; Sosnoff & Motl, 2010) cycling has been shown to reduce H-reflex excitability and improve spasticity for individuals with multiple sclerosis. The extent to which cervicolumbar connections can be exploited to improve spasticity in various clinical populations remains to be seen.

Conclusion

The results from the current experiment provide support for the hypothesis that exploiting cervicolumbar connections can enhance spinal cord plasticity. These observations add to the continually expanding body of literature illustrating that rhythmic arm and leg movements are functionally integrated during locomotion with characteristics reminiscent of our quadrupedal ancestors (Zehr, 2016). Targeted rehabilitation strategies should incorporate rhythmic activity of both the arms and legs together in order to augment neuroplasticity in spinal networks.

References

Abbinanti MD, Zhong G & Harris-Warrick RM. (2012). Postnatal emergence of serotonin-induced plateau potentials in commissural interneurons of the mouse spinal cord. *Journal of neurophysiology* **108,** 2191-2202.

Balter JE & Zehr EP. (2007). Neural coupling between the arms and legs during rhythmic locomotor-like cycling movement. *J Neurophysiol* **97,** 1809-1818.

Barzi Y & Zehr EP. (2008). Rhythmic arm cycling suppresses hyperactive soleus H-reflex amplitude after stroke. *Clin Neurophysiol* **119,** 1443-1452.

Brooke JD. (2004). Somatosensory paths proceeding to spinal cord and brain--centripetal and centrifugal control for human movement. *Canadian journal of physiology and pharmacology* **82,** 723-731.

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

Brooke JD & Zehr EP. (2006). Limits to fast-conducting somatosensory feedback in movement control. *Exercise and sport sciences reviews* **34,** 22-28.

Brown DA & Griffith WH. (1983). Persistent slow inward calcium current in voltage-clamped hippocampal neurones of the guinea-pig. *The Journal of physiology* **337,** 303-320.

Collins DF, Gorassini M, Bennett D, Burke D & Gandevia SC. (2002). Recent evidence for plateau potentials in human motoneurones. *Adv Exp Med Biol* **508,** 227-235.

Crone C, Hultborn H, Mazieres L, Morin C, Nielsen J & Pierrot-Deseilligny E. (1990). Sensitivity of monosynaptic test reflexes to facilitation and inhibition as a function of the test reflex size: a study in man and the cat. *Experimental brain research* **81,** 35-45.

de Ruiter GC, Hundza SR & Zehr EP. (2010). Phase-dependent modulation of soleus H-reflex amplitude induced by rhythmic arm cycling. *Neuroscience letters* **475,** 7-11.

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

Domingo A, Klimstra M, Nakajima T, Lam T & Hundza SR. (2014). Walking phase modulates H-reflex amplitude in flexor carpi radialis. *Journal of motor behavior* **46,** 49-57.

Dragert K & Zehr EP. (2009). Rhythmic arm cycling modulates Hoffmann reflex excitability differentially in the ankle flexor and extensor muscles. *Neuroscience letters* **450,** 235-238.

Estes SP, Iddings JA & Field-Fote EC. (2017). Priming Neural Circuits to Modulate Spinal Reflex Excitability. *Front Neurol* **8,** 17.

Ferris DP, Huang HJ & Kao PC. (2006). Moving the arms to activate the legs. *Exercise and sport sciences reviews* **34,** 113-120.

Fraser DD & MacVicar BA. (1996). Cholinergic-dependent plateau potential in hippocampal CA1 pyramidal neurons. *J Neurosci* **16,** 4113-4128.

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. *Journal of neurophysiology* **91,** 1516-1523.

Girard O, Carbonnel Y, Candau R & Millet G. (2009). Running versus strength-based warm-up: acute effects on isometric knee extension function. *European journal of applied physiology* **106,** 573-581.

Heckman CJ, Johnson M, Mottram C & Schuster J. (2008). Persistent inward currents in spinal motoneurons and their influence on human motoneuron firing patterns. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* **14,** 264-275.

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 & Iwata A. (2006). Cyclic modulation of H-reflex depression in ipsilateral and contralateral soleus muscles during rhythmic arm swing. *Somatosens Mot Res* **23,** 127-133.

Hounsgaard J & Kiehn O. (1989). Serotonin-induced bistability of turtle motoneurones caused by a nifedipine-sensitive calcium plateau potential. *The Journal of physiology* **414,** 265-282.

Hultborn H, Zhang M & Meehan CF. (2013). Control and role of plateau potential properties in the spinal cord. *Curr Pharm Des* **19,** 4357-4370.

Hundza SR, de Ruiter GC, Klimstra M & Zehr EP. (2012). Effect of afferent feedback and central motor commands on soleus H-reflex suppression during arm cycling. *J Neurophysiol* **108,** 3049-3058.

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.

Javan B & Zehr EP. (2008). Short-term plasticity of spinal reflex excitability induced by rhythmic arm movement. *Journal of neurophysiology* **99,** 2000-2005.

Johnson MD, Thompson CK, Tysseling VM, Powers RK & Heckman CJ. (2017). The potential for understanding the synaptic organization of human motor commands via the firing patterns of motoneurons. *Journal of neurophysiology* **118,** 520-531.

Kaupp C, Pearcey GEP, Klarner T, Sun Y, Cullen H, Barss TS & Zehr EP. (2018). Rhythmic arm cycling training improves walking and neurophysiological integrity in chronic stroke: the arms can give legs a helping hand in rehabilitation. *Journal of neurophysiology* **119,** 1095-1112.

Klarner T, Barss TS, Sun Y, Kaupp C, Loadman PM & Zehr EP. (2016a). Exploiting Interlimb Arm and Leg Connections for Walking Rehabilitation: A Training Intervention in Stroke. *Neural Plast* **2016,** 1517968.

Klarner T, Barss TS, Sun Y, Kaupp C, Loadman PM & Zehr EP. (2016b). Long-Term Plasticity in Reflex Excitability Induced by Five Weeks of Arm and Leg Cycling Training after Stroke. *Brain Sci* **6**.

Klimstra M & Zehr EP. (2008). A sigmoid function is the best fit for the ascending limb of the Hoffmann reflex recruitment curve. *Exp Brain Res* **186,** 93-105.

Koceja DM, Burke JR & Kamen G. (1991). Organization of segmental reflexes in trained dancers. *International journal of sports medicine* **12,** 285-289.

Lee RH & Heckman CJ. (1998a). Bistability in spinal motoneurons in vivo: systematic variations in persistent inward currents. *Journal of neurophysiology* **80,** 583-593.

Lee RH & Heckman CJ. (1998b). Bistability in spinal motoneurons in vivo: systematic variations in rhythmic firing patterns. *Journal of neurophysiology* **80,** 572-582.

Levin MF & Hui-Chan C. (1993). Are H and stretch reflexes in hemiparesis reproducible and correlated with spasticity? *J Neurol* **240,** 63-71.

Loadman PM & Zehr EP. (2007). Rhythmic arm cycling produces a non-specific signal that suppresses Soleus H-reflex amplitude in stationary legs. *Experimental brain research* **179,** 199-208.

Massaad F, Levin O, Meyns P, Drijkoningen D, Swinnen SP & Duysens J. (2014). Arm sway holds sway: locomotor-like modulation of leg reflexes when arms swing in alternation. *Neuroscience* **258,** 34-46.

Mazzocchio R, Kitago T, Liuzzi G, Wolpaw JR & Cohen LG. (2006). Plastic changes in the human H-reflex pathway at rest following skillful cycling training. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* **117,** 1682-1691.

Meunier S, Kwon J, Russmann H, Ravindran S, Mazzocchio R & Cohen L. (2007). Spinal use-dependent plasticity of synaptic transmission in humans after a single cycling session. *The Journal of physiology* **579,** 375-388.

Mezzarane RA, Klimstra M, Lewis A, Hundza SR & Zehr EP. (2011). Interlimb coupling from the arms to legs is differentially specified for populations of motor units comprising the compound H-reflex during "reduced" human locomotion. *Experimental brain research* **208,** 157-168.

Misiaszek JE, Brooke JD, Lafferty KB, Cheng J & Staines WR. (1995). Long-lasting inhibition of the human soleus H reflex pathway after passive movement. *Brain research* **677,** 69-81.

Motl RW & Dishman RK. (2003). Acute leg-cycling exercise attenuates the H-reflex recorded in soleus but not flexor carpi radialis. *Muscle & nerve* **28,** 609-614.

Motl RW, Knowles BD & Dishman RK. (2003). Acute bouts of active and passive leg cycling attenuate the amplitude of the soleus H-reflex in humans. *Neuroscience letters* **347,** 69-72.

Motl RW, Snook EM & Hinkle ML. (2007). Effect of acute unloaded leg cycling on spasticity in individuals with multiple sclerosis using anti-spastic medications. *Int J Neurosci* **117,** 895-901.

Motl RW, Snook EM, Hinkle ML & McAuley E. (2006). Effect of acute leg cycling on the soleus H-reflex and modified Ashworth scale scores in individuals with multiple sclerosis. *Neuroscience letters* **406,** 289-292.

Murase K, Ryu PD & Randic M. (1986). Substance P augments a persistent slow inward calcium-sensitive current in voltage-clamped spinal dorsal horn neurons of the rat. *Brain research* **365,** 369-376.

Nakajima T, Kamibayashi K, Kitamura T, Komiyama T, Zehr EP & Nakazawa K. (2016). Short-Term Plasticity in a Monosynaptic Reflex Pathway to Forearm Muscles after Continuous Robot-Assisted Passive Stepping. *Frontiers in human neuroscience* **10,** 368.

Nakajima T, Kitamura T, Kamibayashi K, Komiyama T, Zehr EP, Hundza SR & Nakazawa K. (2011). Robotic-assisted stepping modulates monosynaptic reflexes in forearm muscles in the human. *Journal of neurophysiology* **106,** 1679-1687.

Nakajima T, Mezzarane RA, Hundza SR, Komiyama T & Zehr EP. (2014). Convergence in reflex pathways from multiple cutaneous nerves innervating the foot depends upon the number of rhythmically active limbs during locomotion. *PloS one* **9,** e104910.

Nakajima T, Mezzarane RA, Klarner T, Barss TS, Hundza SR, Komiyama T & Zehr EP. (2013). Neural mechanisms influencing interlimb coordination during locomotion in humans: presynaptic modulation of forearm H-reflexes during leg cycling. *PloS one* **8,** e76313.

Nielsen J, Crone C & Hultborn H. (1993). H-reflexes are smaller in dancers from The Royal Danish Ballet than in well-trained athletes. *Eur J Appl Physiol Occup Physiol* **66,** 116-121.

Oyama Y, Akaike N & Nishi K. (1986). Persistent calcium inward current in internally perfused snail neuron. *Cell Mol Neurobiol* **6,** 71-85.

Pearcey GEP, Noble SA, Munro B & Zehr EP. (2017). Spinal Cord Excitability and Sprint Performance Are Enhanced by Sensory Stimulation During Cycling. *Frontiers in human neuroscience* **11,** 612.

Sasada S, Tazoe T, Nakajima T, Futatsubashi G, Ohtsuka H, Suzuki S, Zehr EP & Komiyama T. (2016). A common neural element receiving rhythmic arm and leg activity as assessed by reflex modulation in arm muscles. *Journal of neurophysiology* **115,** 2065-2075.

Schneider C & Capaday C. (2003). Progressive adaptation of the soleus H-reflex with daily training at walking backward. *Journal of neurophysiology* **89,** 648-656.

Smith M & Perrier JF. (2006). Intrinsic properties shape the firing pattern of ventral horn interneurons from the spinal cord of the adult turtle. *Journal of neurophysiology* **96,** 2670-2677.

Sosnoff J, Motl RW, Snook EM & Wynn D. (2009). Effect of a 4-week period of unloaded leg cycling exercise on spasticity in multiple sclerosis. *NeuroRehabilitation* **24,** 327-331.

Sosnoff JJ & Motl RW. (2010). Effect of acute unloaded arm versus leg cycling exercise on the soleus H-reflex in adults with multiple sclerosis. *Neuroscience letters* **479,** 307-311.

Stein RB & Capaday C. (1988). The modulation of human reflexes during functional motor tasks. *Trends Neurosci* **11,** 328-332.

Stoykov ME & Madhavan S. (2015). Motor priming in neurorehabilitation. *J Neurol Phys Ther* **39,** 33-42.

Trimble MH & Koceja DM. (2001). Effect of a reduced base of support in standing and balance training on the soleus H-reflex. *Int J Neurosci* **106,** 1-20.

Vasudevan EV & Zehr EP. (2011). Multi-frequency arm cycling reveals bilateral locomotor coupling to increase movement symmetry. *Experimental brain research* **211,** 299-312.

Wang B, Yin L, Zou X, Ye M, Liu Y, He T, Deng S, Jiang Y, Zheng R, Wang Y, Yang M, Lu H, Wu S & Shu Y. (2015). A Subtype of Inhibitory Interneuron with Intrinsic Persistent Activity in Human and Monkey Neocortex. *Cell Rep.*

Wilson JM, Thompson CK, Miller LC & Heckman CJ. (2015). Intrinsic excitability of human motoneurons in biceps brachii versus triceps brachii. *Journal of neurophysiology* **113,** 3692-3699.

Zehr EP. (2006). Training-induced adaptive plasticity in human somatosensory reflex pathways. *Journal of applied physiology* **101,** 1783-1794.

Zehr EP, Barss TS, Dragert K, Frigon A, Vasudevan EV, Haridas C, Hundza S, Kaupp C, Klarner T, Klimstra M, Komiyama T, Loadman PM, Mezzarane RA, Nakajima T, Pearcey GE & Sun Y. (2016). Neuromechanical interactions between the limbs during human locomotion: an evolutionary perspective with translation to rehabilitation. *Exp Brain Res* **234,** 3059-3081.

Zehr EP, Barss, T. S., Dragert, K., Frigon, A., Vasudevan, E. V., Haridas, C., Hundza, S. R., Kaupp, C., Klarner, T., Klimstra, M., Komiyama, T., Loadman, P. M., Mezzarane, R. A., Nakajima, T., Pearcey, G. E., Sun, Y. (2016). Neuromechanical interactions between the limbs during human locomotion—an evolutionary perspective and translation to rehabilitation. *Experimental brain research*.

Zehr EP & Duysens J. (2004). Regulation of arm and leg movement during human locomotion. *Neuroscientist* **10,** 347-361.

Zehr EP, Frigon A, Hoogenboom N & Collins DF. (2004). Facilitation of soleus H-reflex amplitude evoked by cutaneous nerve stimulation at the wrist is not suppressed by rhythmic arm movement. *Experimental brain research* **159,** 382-388.

Zehr EP, Hundza SR & Vasudevan EV. (2009). The quadrupedal nature of human bipedal locomotion. *Exercise and sport sciences reviews* **37,** 102-108.

Zehr EP, Klimstra M, Johnson EA & Carroll TJ. (2007). Rhythmic leg cycling modulates forearm muscle H-reflex amplitude and corticospinal tract excitability. *Neurosci Lett* **419,** 10-14.

Zehr EP, Loadman PM & Hundza SR. (2012). Neural control of rhythmic arm cycling after stroke. *Journal of neurophysiology* **108,** 891-905.

Zehr EP & Stein RB. (1999). What functions do reflexes serve during human locomotion? *Progress in neurobiology* **58,** 185-205.

Zhou R, Alvarado L, Kim S, Chong SL & Mushahwar VK. (2017). Modulation of Corticospinal Input to the Legs by Arm and Leg Cycling in People with Incomplete Spinal Cord Injury. *Journal of neurophysiology***,** jn 00663 02016.

Chapter 7 - Spinal cord excitability and sprint performance are enhanced by sensory stimulation during cycling

Abstract

Spinal cord excitability, as assessed by modulation of Hoffmann (H-) reflexes, is reduced with fatiguing isometric contractions. Furthermore, spinal cord excitability is reduced during non-fatiguing arm and leg cycling. Presynaptic inhibition of Ia terminals is believed to contribute to this suppression of spinal cord excitability. Electrical stimulation to cutaneous nerves reduces Ia presynaptic inhibition, which facilitates spinal cord excitability, and this facilitation is present during arm cycling. Although it has been suggested that reducing presynaptic inhibition may prolong fatiguing contractions, it is unknown whether sensory stimulation can alter the effects of fatiguing exercise on performance or spinal cord excitability. Thus, the aim of this experiment was to determine if sensory stimulation can interfere with fatigue-related suppression of spinal cord excitability, and alter fatigue rates during cycling sprints. 13 participants randomly performed three experimental sessions that included: unloaded cycling with sensory stimulation (*CONTROL + STIM*), sprints with sensory stimulation (*SPRINT + STIM*), and sprints without stimulation (*SPRINT*). Seven participants also performed a fourth session (*CONTROL*), which consisted of unloaded cycling. During SPRINT and SPRINT + STIM, participants performed 7, 10s cycling sprints interleaved with 3min rest. For *CONTROL* and *CONTROL + STIM*, participants performed unloaded cycling for ~30min. During *SPRINT + STIM* and *CONTROL + STIM*, participants received patterned sensory stimulation to nerves of the right foot. H-reflexes and M-waves of the right soleus were evoked by stimulation of the tibial nerve at multiple time points throughout exercise. Sensory stimulation facilitated soleus H-reflexes during unloaded cycling, whereas sprints suppressed soleus H-reflexes. While receiving sensory stimulation, there was less suppression of soleus H-reflexes and slowed reduction in average power output, compared to sprints without stimulation. These results demonstrate that sensory stimulation can substantially mitigate the fatiguing effects of sprints.

Pearcey GE, Noble SA, Munro B and Zehr EP (2017). Spinal Cord Excitability and Sprint Performance Are Enhanced by Sensory Stimulation During Cycling. Frontiers in Human Neuroscience. 2017 Dec 18;11:612. doi: 10.3389/fnhum.2017.00612. eCollection 2017.

New and Noteworthy

Spinal cord excitability is reduced with fatigue and acute stimulation to cutaneous afferents can increase spinal cord excitability. Here we showed that sensory stimulation to cutaneous afferents of the foot could mitigate reductions in spinal cord excitability and power output that occurred during fatiguing cycling sprints.

Introduction

It is well established that intermittent, maximal effort bouts of exercise result in decrements in performance. In many cases, these performance decrements are due to neuromuscular fatigue that is a combination of peripheral and central fatigue mechanisms (Billaut, Basset et al. 2006, Billaut and Basset 2007, Racinais, Bishop et al. 2007, Mendez-Villanueva, Hamer et al. 2008, Girard, Bishop et al. 2013, Girard, Bishop et al. 2013, Pearcey, Murphy et al. 2014, Pearcey, Bradbury-Squires et al. 2016, Monks, Compton et al. 2017). Although not the only mechanism, central fatigue resulting from high intensity cycling (Amann 2012, Amann, Venturelli et al. 2013, Sidhu, Weavil et al. 2014, Sidhu, Weavil et al. 2017) is believed to result from group III/IV muscle afferent feedback, which subsequently alters corticospinal excitability and the level of muscle activation (Pearcey, Bradbury-Squires et al. 2016). In felines, fatiguing stimulation of the gastrocnemius-soleus muscles results in suppression of monosynaptic Ia activation of synergistic motoneurones, which is primarily due to increased pre-synaptic inhibition (Kalezic, Bugaychenko et al. 2004). Furthermore, fatiguing voluntary (Kukulka, Moore et al. 1986, Iguchi and Shields 2012), non-voluntary electrically-evoked (Garland and McComas 1990) and sub-maximal voluntary (Kuchinad, Ivanova et al. 2004) contractions of the plantarflexors, and voluntary contractions of the intrinsic hand muscles (Duchateau and Hainaut 1993, Duchateau, Balestra et al. 2002) cause suppression of Hoffmann (H)-reflex amplitudes. Prolonged running also causes drastic suppression of the Soleus H-reflex for at least 30 minutes (Racinais, Girard et al. 2007). Although it seems likely that H-reflex amplitudes would be reduced, it is currently unknown whether cycling sprints affect spinal reflex excitability in humans.

Although the effects of sprint cycling on H-reflex amplitudes are not known, non-fatiguing arm (Frigon, Collins et al. 2004, Loadman and Zehr 2007, Barzi and Zehr 2008, Hundza and Zehr 2009, de Ruiter, Hundza et al. 2010, Palomino, Hundza et al. 2011) and leg (Motl and Dishman 2003, Motl, Knowles et al. 2003, Motl, O'Connor P et al. 2004, Motl, Snook et al. 2006) cycling effects on H-reflexes have been studied extensively. With both paradigms, there is suppression of soleus (SOL) H-reflex amplitudes. Cycling-induced suppression has been attributed to increased presynaptic inhibition from Ia presynaptic inhibitory interneurons (Brooke, Cheng et al. 1997, Frigon, Collins et al. 2004).

Since the available evidence suggests that fatiguing isometric contractions and non-fatiguing cycling both cause suppression of the SOL H-reflex amplitude, it seems likely that fatiguing cycling would do the same.

The importance of afferent input to motoneurons during voluntary contractions was demonstrated by Macefield et al. (1993). They showed that firing rates of the tibialis anterior (TA) are reduced when deprived of afferent feedback from the contracting muscle. More recently, Baudry et al. (2011) have shown that reductions in presynaptic inhibition of Ia afferents are associated with an increased time that a fatiguing contraction can be sustained. One method known to reduce presynaptic inhibition of Ia afferents is electrical stimulation of cutaneous afferents (Hagbarth 1952). Electrical stimulation of nerves innervating the skin of the hand (Zehr, Frigon et al. 2004) and foot (Demaire, Honore et al. 1989, Iles 1996, Brooke, Cheng et al. 1997, Frigon, Collins et al. 2004) can decrease Ia presynaptic inhibition and, therefore, increase the amplitude of H-reflexes. Furthermore, cutaneous electrical stimulation applied during and after cycling has been shown to "cancel" the inhibitory effects of cycling on H-reflex amplitudes (Frigon, Collins et al. 2004, Javan and Zehr 2008). Therefore, since the facilitation of sensory stimulation and fatiguing effects of exercise on the H-reflex both act via Ia inhibitory mechanisms, the combination of sensory stimulation during fatiguing cycling may offset one another, resulting in less decrement of spinal cord excitability and cycling performance.

Thus, the purpose of the current study was to 1) confirm that fatigue via intermittent sprints will decrease spinal reflex excitability, 2) determine whether patterned sensory stimulation can increase spinal reflex excitability during unloaded cycling, and 3) determine if patterned sensory stimulation can interact with fatigue to mitigate suppression of reflexes and decrements in performance. Based on previous fatiguing and non-fatiguing cycling literature, we hypothesize that H-reflex amplitudes would decrease as a result of the fatigue induced from intermittent sprints. Furthermore, due to the fact that sensory stimulation reduces pre-synaptic inhibition of Ia interneurons, we hypothesize that patterned sensory stimulation would increase H-reflex amplitudes during unloaded cycling. Lastly, we hypothesize that patterned sensory stimulation would have an interaction effect during cycling that would decrease the rate of power decrement and offset the suppression of H-reflexes due to fatigue.

Methods

Participants

Thirteen volunteers (24.3 ± 3.10 years, 174.3 ± 7.43 cm, 74.5 ± 12.03 kg) of both sexes (3 female) participated in this experiment. Participants had no known history of metabolic or neuromuscular impairment. Participants provided written and signed informed consent in accordance with the Human Research Ethics Board at the University of Victoria and the project was performed in accordance with the Declaration of Helsinki.

Experimental protocols

In order to examine the effects of sensory stimulation of the right foot on fatigue and H-reflexes during fatiguing cycling, the following three experimental protocols were used: 1) *No Sprint (CONTROL +STIM)*, 2) *No Stim Sprint (SPRINT)*, and 3) *Stim Sprint (SPRINT + STIM)*. All three protocols were randomly performed at the same time of day (± 60 min) with a minimum of 48 hours (up to 7 days) between protocols. To ensure there were no effects of the unloaded cycling used in the outlined experiments on H-reflex amplitudes, seven participants performed a fourth condition (*CONTROL*). Participants were instructed to wear the same clothing and shoes for all experimental testing and asked not to perform heavy exercise, eat, drink caffeine, smoke, or drink alcohol 4 hours prior to arriving at the laboratory.

Upon arrival, participants were informed of the protocol. The duration of each experimental testing session was the same for all four protocols, but the set-up time was slightly shorter for the *SPRINT* and *CONTROL* protocols because they did not require set-up of sensory stimulation of the foot. For all reflex measures, each participant's posture was tightly monitored and controlled. That is, the seat height was adjusted to the comfort of the participant, knowing that they were to remain seated throughout the entire experiment. The selected seat height was then kept constant between all four protocols. Participants' feet were secured on the pedals with nylon straps, and not altered throughout the session. Furthermore, during times that reflexes were recorded, the participants were instructed to hold the handlebars in a predetermined location with elbows and shoulders extended and asked to stare at a predetermined target directly in front of them (see Fig 5B for position). Following set-up, participants performed a standard warm-up of 5 min cycling at 70-80 rpm with a resistance of 1 kg. The participants then rested while resting

M-H recruitment curves were performed. Finally, 10 H-reflexes with constant M-wave amplitude and 3 M_{max} were collected during unloaded cycling at 60 rpm and averaged.

The participants then either performed a 10 s sprint without any sensory stimulation (*SPRINT*), a sprint with sensory stimulation (*SPRINT + STIM*), or continued to cycle (unloaded) at 60 rpm for 10 s while receiving sensory stimulation (*CONTROL +STIM*), or continued to cycle (unloaded) at 60 rpm for 10 s without stimulation (*CONTROL*). Participants then immediately returned to 3 minutes of cycling (unloaded) at 60 rpm while M_{max} (3 sweeps) and H-reflexes (10 sweeps) were evoked. During the evoked M_{max} and H-reflex trials, participants were instructed to maintain the same posture as the pre-exercise test. When recording was complete, they were asked to cycle at 60 rpm and were free to move their upper body and talk until the next sprint (*SPRINT* and *SPRINT + STIM*), 10 s of sensory stimulation (*CONTROL + STIM*), or 10 s of unloaded cycling (*CONTROL*). The participants then repeated this procedure for a total of seven times. After the 7th repetition of this procedure, participants performed a 10 min cool-down by cycling at 70-80 rpm with 1 kp of resistance. M_{max} and H-reflexes were evoked at the 5- and 10-minute marks (participants reverted back to 60 rpm unloaded cycling for collection). After the final M_{max} and H-reflexes were recorded, M-H recruitment curves were recorded at rest in the same position as pre-exercise (see figure 7-1A for a timeline of each session).

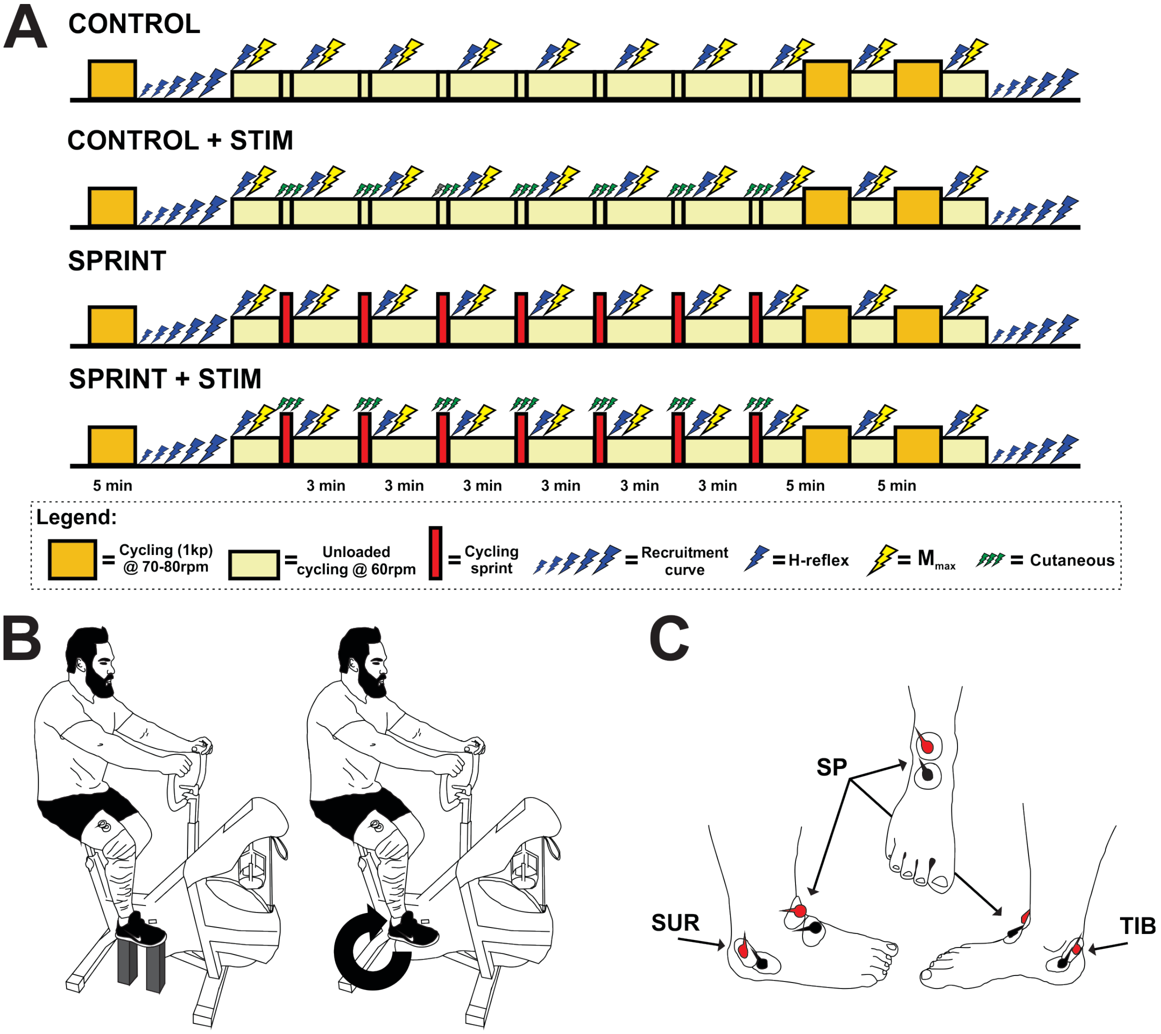

Figure 7-1. *A) Timeline of each experimental session. The legend below the timelines outlines the symbol meanings. B) An illustration of the experimental set-up. The left portion of the illustration shows the position of the pre and post session M-H recruitment curve set-up for each participant. The right portion shows the position of H-reflex and* M_{max} *stimuli during cycling. This position was also the position that the train of sensory stimulation was evoked during the SPRINT + STIM and CONTROL + STIM conditions. C) An illustration of the approximate electrode positions on the right foot for sensory stimulation. Views are of the lateral, frontal and medial from left to right, respectively.*

General experimental arrangements

Soleus H-reflex stimulation

To evoke H-reflexes in the SOL muscle, single 1 ms square wave electrical pulses were applied to the right popliteal fossa with bipolar surface electrodes (Thought Technology Ltd., Montreal, QC, Canada) using a Digitimer (Medtel, NSW, Australia) constant current stimulator (model DS7A). A non-contact milliammeter (mA-2000, Bell Technologies, Orlando, FL, USA) was used to measure current delivered for each stimulus. Recruitment curves (40 sweeps) were recorded at rest pre- and post-exercise. H-reflexes with an amplitude corresponding to ~75% H_{max} on the ascending limb of the recruitment curve with constant and measureable M-wave amplitude were recorded during unloaded cycling throughout each experimental session. This amplitude was chosen to ensure we were within the range of H-reflex amplitudes that are sensitive to both facilitation and inhibition from presynaptic inhibitory input (Crone, Hultborn et al. 1990) Furthermore, it was essential that there was a measureable M-wave amplitude to control the level of motoneuron excitability throughout the experiments (Zehr 2002).

Stimulus-response curves

At rest, participants had their right foot at the 2 o'clock position of the cycle ergometer, secured with wooden blocks under the pedals and heel of the right foot (See figure 7-1B for set-up). Knee and ankle angles (knee ~90^{o}, knee ~120^{o}) were monitored by the same investigator with a manual goniometer and kept constant from pre- to post-exercise and from session to session. Stimulus-response curve stimuli were delivered pseudo randomly between 1 and 3 s for a total of 40 sweeps. Stimulus intensity was increased and decreased incrementally (ranged from 0.1 to 1mA per increment) based on the excitability of the reflex pathway in different individuals, while ensuring that supramaximal M-wave amplitudes were achieved by increasing larger increments once the H-reflex amplitude started to decrease in size (i.e. after the ascending limb, for examples see Klimstra and Zehr (2008)).

Constant M-wave and H-reflex stimuli during cycling

To monitor the H-reflex excitability throughout the experiment, the current required to evoke a constant M-wave that corresponded to ~5% of M_{max} was delivered randomly every 1-2 pedal strokes at the 2 o'clock position during unloaded cycling (60 rpm). Pre-exercise H-reflex amplitudes at this stimulation intensity evoked a response that was ~75% of H_{max}. A total of 10 sweeps were recorded, averaged and compared at each time point.

Supramaximal M-wave stimuli during cycling

To account for any peripheral changes in excitability throughout the experimental sessions, three supramaximal M-wave (M_{max}) stimuli were delivered immediately after recording the 10 H-reflexes but at the same point in the cycle as the H-reflexes. Stimulation intensity was set to 140% of the value that produced M_{max} during the H-M recruitment curve recorded at rest, prior to cycling.

Sensory Stimulation

To induce sensory stimulation of the right foot, trains of 15 x 1 ms pulses (50 Hz) were applied simultaneously to the superficial peroneal, tibial, and sural nerves with bipolar surface electrodes (See figure 7-1C for electrode positions). Grass S88 stimulators (Grass Instruments, AstroMed) connected in series with a SIU5 isolator and CCU1 constant current units were used to deliver the stimuli. The onset of sensory stimuli was triggered when the pedal passed the 2 o'clock position in the CONTROL + STIM and SPRINT + STIM conditions. Stimulation lasted for 10s during the sprint (SPRINT + STIM) or at the same 10s time point during unloaded cycling (CONTROL + STIM). Prior to the experimental protocol, perceptual threshold (PT) and radiating threshold (RT) was determined for each site. PT was defined as lowest current required to evoke the smallest detectable tactile sensation, whereas RT was defined as the minimum current required to cause clear radiating paresthesia of the innervation area (Nakajima, Mezzarane et al. 2014). Stimulation intensity was set to 1.0 x RT in order to induce a non-noxious cutaneous sensation. Participants described the sensation as "tingling", "fluttering" and/or "vibrating".

Electromyographic recordings

Skin surfaces were shaved and then cleaned with alcohol wipes. Bipolar configurations of Ag-AgCl surface electrodes (Thought Technology Ltd., Montreal, QC, Canada) were fixed to the skin over muscles of the right SOL, tibialis anterior, vastus lateralis, and flexor carpi radialis. Ground electrodes were fixed to bony landmarks (patella and lateral olecranon) that were electrically neutral. EMG recordings were amplified (500-

1000 times for SOL and 5000 times for all other muscles) and filtered (100-1000 Hz for SOL and 100-300 Hz for all other muscles, P511 Grass Instruments, AstroMed).

Cycle ergometer sprints

All cycling occurred on a Monark cycle ergometer (Ergomedic 894 E, Monark Exercise AB, Vansbro, SWE). Unloaded cycling was maintained at 60 ± 5 rpm between sprints. For all sprints, participants were instructed to accelerate slowly to 60 rpm and then accelerate as hard as possible, at which point the weight basket (9% of body weight) would drop and they would cycle as hard as possible for 10 s. For all subsequent sprints, the participants were already cycling at 60 rpm (unloaded) and were given a 30 s warning and then a 3 s countdown of when they were to sprint again (see figure 7-1A for timeline). No verbal encouragement was provided during the sprints, to maintain consistent external motivation between sessions. All power output data was recorded using Monark Wingate software and stored on a computer for further analysis.

Data analysis

All EMG data were acquired at a sampling rate of 5000 Hz with a 12-bit A/D converter connected to a personal computer running custom LabView version 8.0 (National Instruments, Austin, TX, USA). EMG data other than SOL were full wave rectified. SOL H-reflex and M-wave data were analysed from single unrectified 100 ms sweeps. H-reflex and M-wave peak-to-peak amplitudes were analysed in all trials with custom written Matlab version R2011b (Mathworks, Nantick, MA) and then normalized to M_{max} amplitude. For recruitment curves, data was then imported into custom written LabView software where it was fit with a sigmoid function (Klimstra and Zehr 2008). Pre-stimulus EMG activity was calculated as the root mean square value 20 ms prior to stimulus onset, and these amplitudes were normalized to M_{max}.

Statistical Analysis

Mean power output was compared between conditions using a two-way repeated measures (RM) ANOVA (2 conditions (*SPRINT* vs. *SPRINT + STIM*) X 7 sprints). M_{max}, H-reflex and corresponding M-wave amplitudes, and background EMG were compared between conditions with a two-way RM ANOVA (4 conditions X 10 time points). Current required to evoke H-reflex threshold, H_{max} amplitude, H-reflex @ 50% of current required to produce H_{max} and slope of the H-reflex recruitment curve at rest were compared from pre- to post-exercise with a two-way RM ANOVA (4 conditions X 2 time points (pre and post-exercise)).

Pairwise comparisons were performed on significant main effects (condition and time) and interactions using paired t-tests. Data is expressed as means ± SD, except in figures where it is expressed as means ± SE for clarity. Significant differences were determined as $p < 0.05$ in all cases and all ANOVA and t-tests were performed using SPSS version 22 (SPSS, Chicago, IL, USA).

To provide qualitative information about effects of sensory stimulation, we included magnitude based-inferences. Effect sizes (Cohen's *d*) on the interaction effects in the mean changes between conditions (*SPRINT + STIM* and *SPRINT*) were determined. The interaction effect of time and sensory stimulation was calculated from the mean difference between pre-exercise and each time point (post-sprint 1, 2, 3, 4, 5, 6, 7, post-5 mins and post-10 mins) for the *SPRINT + STIM* and *SPRINT* conditions. The two differences were then subtracted to estimate the effect of sensory stimulation at each time point. The following criteria were used to assess qualitative descriptors of standardised effects: trivial (<0.2), small (0.2–0.5), moderate (0.5–0.8), and large (>0.8) (Cohen 1988). Effects with 95% confidence limits that overlap the threshold for small positive and negative effects were defined as unclear. Effect sizes, which were clearly small or larger, were defined as substantial (Pearcey, Bradbury-Squires et al. 2015). All magnitude-based inference calculations were performed in Excel version 2011 (Microsoft Corporation, Redmond, WA).

Results

Power output

Effects of fatigue on power output

Sprint power is reduced with repeated bouts. The average power output of the first sprint between sessions was not significantly different (*SPRINT + STIM* = 745 ± 171.3 watts; *SPRINT* = 758 ± 171.5 watts; $p = 0.84$), but to reduce inter-session variability, each sprint was made relative to the first sprint of that session. For both the *SPRINT + STIM* and *SPRINT* conditions, as the sprint number increased, the mean power decreased (see figure 7-2). The 2 (condition) x 7 (time) RM ANOVA revealed that there was a significant (main effect time, $p < 0.001$) decrease in average power output in during both sessions. There was a substantial small to very large effect (d ranged from 0.34 to 5.60) of sprint number on average power output (see table 7-1).

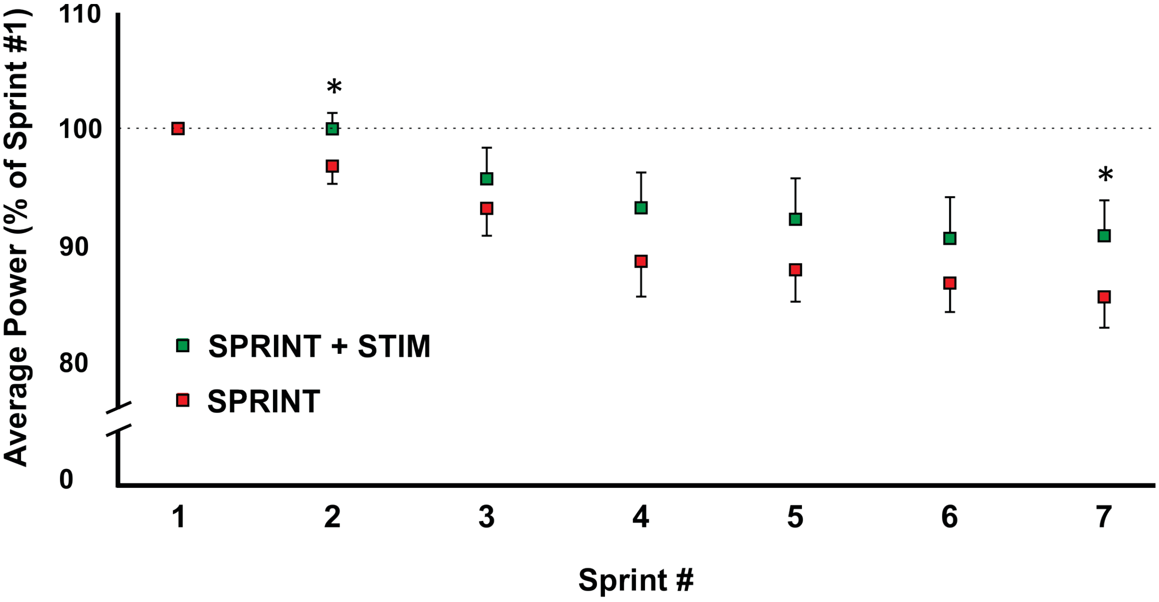

***Figure 7-2**. A graphical representation of how the average power output changed from sprints 1 through 7. All values were made relative to sprint 1 of the corresponding session. Asterisks indicate significant (p < 0.05) differences between SPRINT + STIM and SPRINT conditions. All values are means ± standard error.*

Table 7-1: *Effect sizes and 95% confidence intervals of between condition differences in percent change of average power from sprint 1 to each sprint (top) and effect sizes of decrease in average power from sprint 1 to each sprint within each condition (bottom).*

% decrease in avg power	**Sprint #**					
	2	**3**	**4**	**5**	**6**	**7**
Cohen's D (condition)	0.41	0.14	0.26	0.28	0.28	0.45
95% CI	0.24-3.90	-1.28-3.45	-0.08-5.17	-0.24-5.07	-0.35-4.84	1.23-6.29

Cohen's D (time)	**S**	1.67	2.68	3.56	4.36	5.37	5.60
	S + S	0.34	1.87	2.50	2.49	2.93	3.26

Effects of sensory stimulation on performance measures

Sensory stimulation mitigates fatigue-related decline in power output. Overall, sensory stimulation resulted in smaller decrements of average power throughout the sprint protocol. The 2 (condition) x 7 (time) RM ANOVA revealed that there was a significant interaction effect ($p = 0.034$). Pairwise t-tests revealed that the decrease in average power was significantly greater for the *SPRINT* than *SPRINT + STIM* at sprint 2 ($p = 0.048$) and 7 ($p = 0.036$). Between-condition effects of sensory stimulation on power output are provided in table 7-1.

Spinal Cord Excitability

Spinal Cord Excitability Control Measures

Pre-exercise M_{max} and H-reflexes evoked during unloaded cycling were similar between conditions. M_{max} amplitudes were not significantly ($p = 0.61$) different between or within conditions. Furthermore, H-reflexes were not significantly ($p = 0.45$) different pre-exercise between conditions, but to reduce inter-session variability, all H-reflex amplitudes were made relative to the pre-exercise value. H-reflex amplitudes evoked at pre in each condition as a percentage of both H_{max} and M_{max} are presented in table 7-2. An exemplary subject's H-reflex recordings for each condition and time point are shown in figure 7-3. Furthermore, group mean stimulation intensity used to evoke H-reflexes and M-wave amplitudes recorded with H-reflexes at all time points for all conditions are shown in figure 7-4. There were no significant effects observed for time ($p = 0.91$ [stim], $p = 0.55$ [M-wave]) or condition ($p = 0.75$ [stim], $p = 0.32$ [M-wave]), for both stimulation intensity and M-wave amplitude. To ensure that differences in muscle activity were not contributing to the differences in H-reflexes between time points and conditions, we plotted the group mean pre-stimulus EMG activity of the SOL, TA and VL in figure 7-5. No significant time (p = 0.12 [SOL], 0.46 [TA], 0.67 [VL]) or condition (p = 0.42 [SOL], 0.72 [TA], 0.29 [VL]) effects were observed.

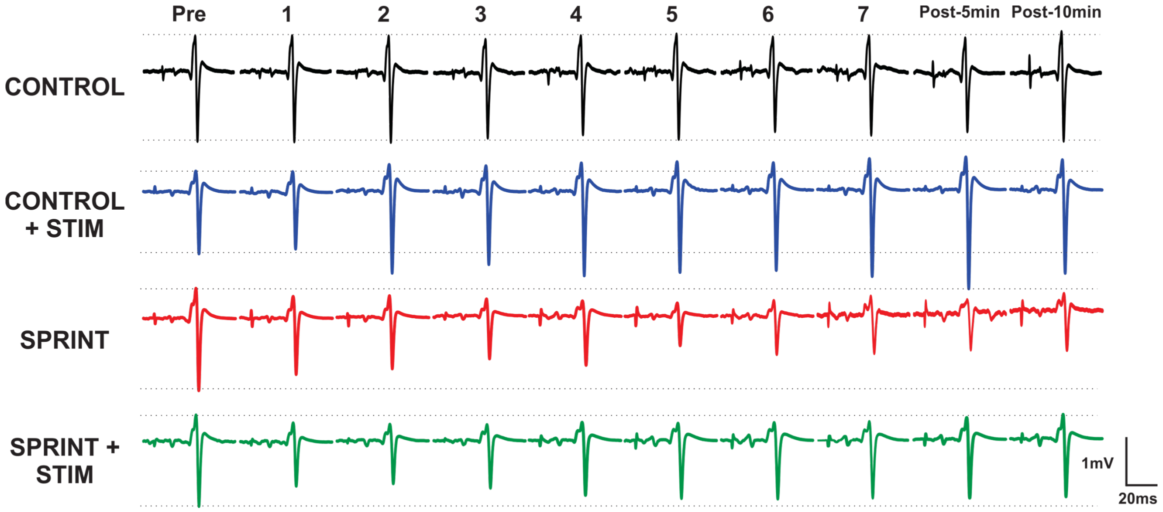

Figure 7-3. *Depicts a single subject's average of 10 H-reflex recordings at each time point, for each condition. Horizontal dotted lines indicate the pre-exercise H-reflex amplitude of each session for ease of comparison.*

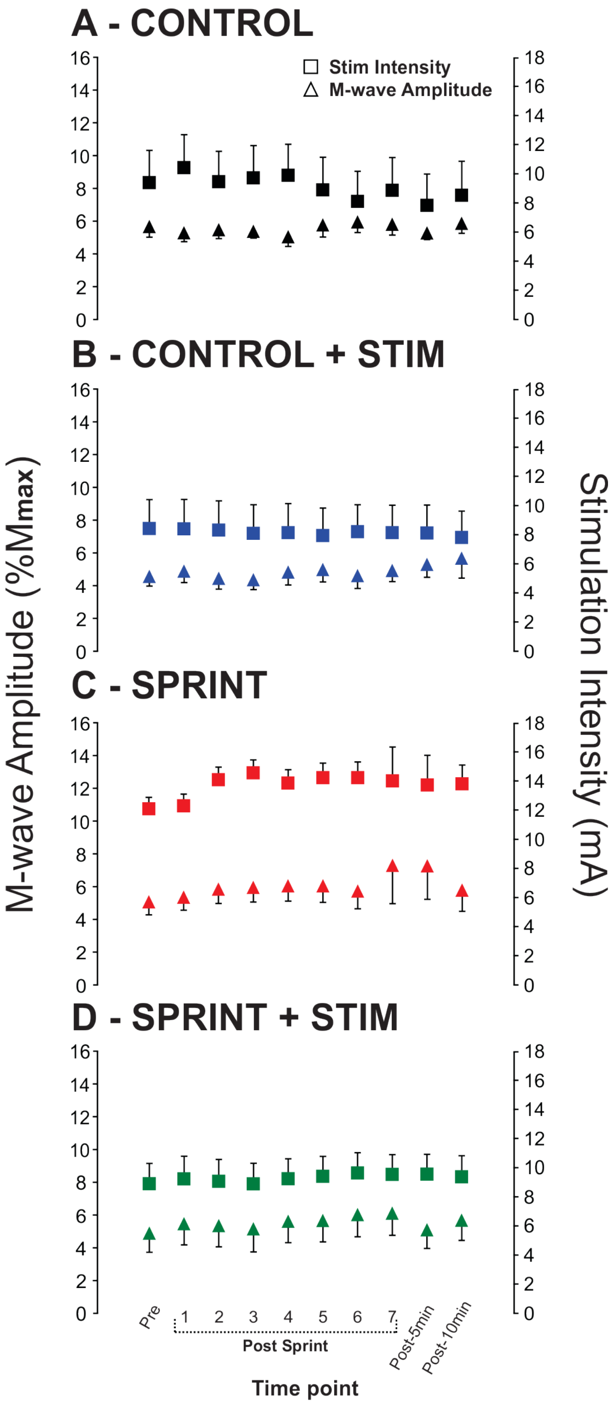

Figure 7-4. *Group means of M-wave amplitudes that accompanied H-reflexes (primary y-axis) and stimulation intensities required to evoke H-reflexes (secondary y-axis) at each time point (x-axis) are represented by triangles and squares, respectively. Each panel displays data from the following condition: A) CONTROL, B) CONTROL + STIM, C) SPRINT, and D) STIM + SPRINT.*

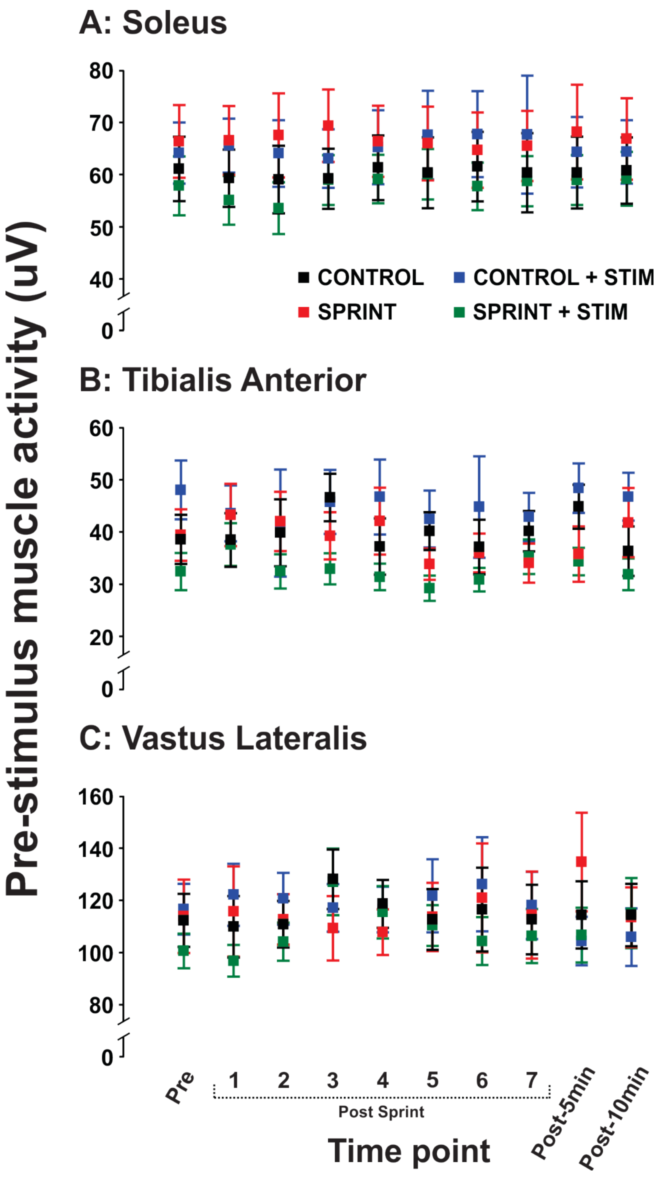

***Figure* 7-5**. *Group means of the rectified EMG amplitude averaged over 20 ms prior to stimulus onset is displayed for the soleus (A), tibialis anterior (B), and vastus lateralis (C) for all time points that H-reflexes were evoked during unloaded cycling. In all panels, CONTROL is black, CONTROL + STIM is blue, SPRINT is red, and SPRINT + STIM is green.*

Table 7-2: *Group averaged values for stimulation current, M-wave amplitude, H-reflex amplitudes as a percentage of H_{max} and H-reflex amplitudes as a percentage of M_{max} for H-reflexes evoked during unloaded cycling at PRE.*

Condition	**Stimulation Current (mA)**		**M (% of M_{max})**		**H (% of H_{max})**		**H (% of M_{max})**	
	Mean	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
CONTROL	8.4	7.16	4.6	2.13	77.4	13.65	40.8	16.73
CONTROL + STIM	9.6	8.23	5.1	2.85	75.9	22.71	38.0	15.94
SPRINT	8.9	6.05	5.4	4.52	77.1	19.68	43.5	22.24
SPRINT + STIM	9.4	8.06	5.3	2.18	75.8	21.73	41.4	20.60

Pre H-M recruitment curves were similar between conditions. All parameters of the recruitment curves recorded at rest prior to the protocol were stable (e.g. were not statistically different (p = 0.63, 0.70, 0.83 for H@th, H@50 and H@100, respectively)) between sessions. Figure 7-6A displays a single subject plot of the pre-exercise recruitment curves between the SPRINT and SPRINT +STIM conditions whereas figure 7-6B displays the post-exercise recruitment curves between conditions.

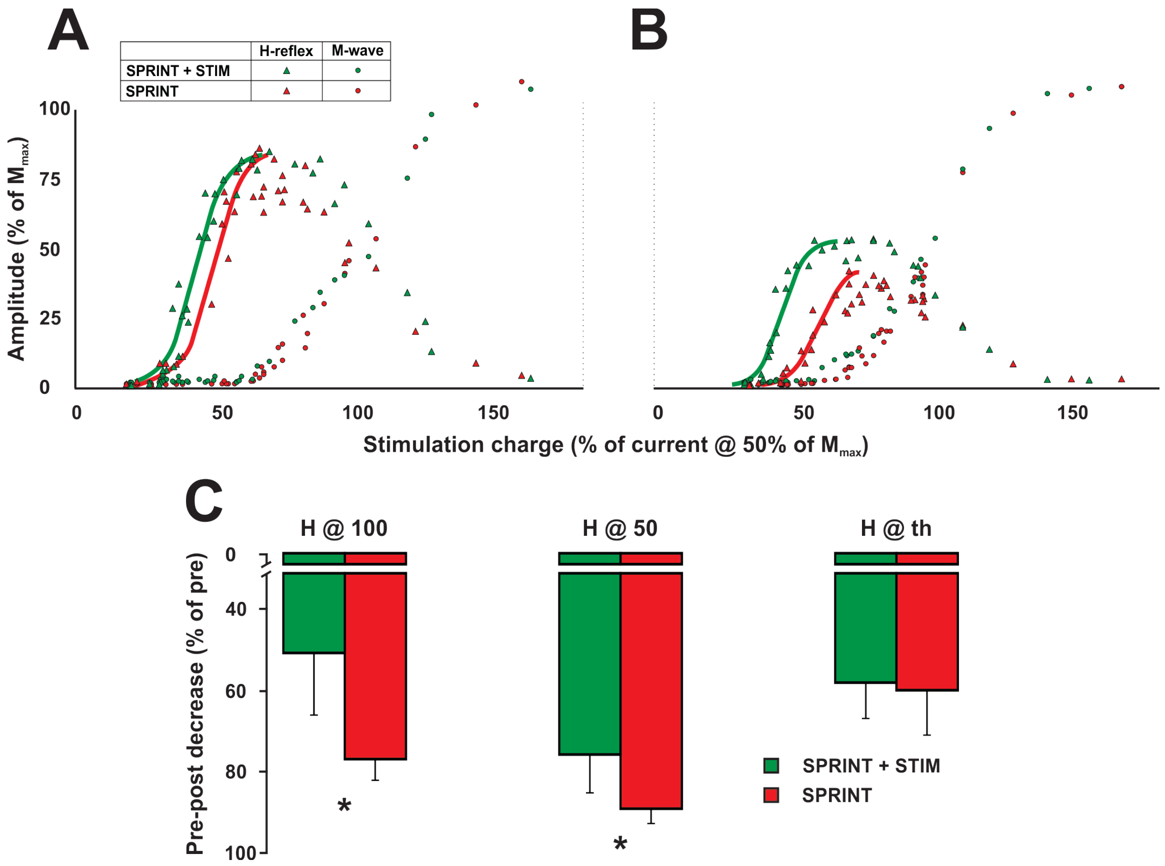

***Figure 7-6**. Single subject M-H recruitment curve raw data for the SPRINT (red) and STIM + SPRINT (green) conditions at A) pre, and B) post session. Triangles indicate H-reflex peak-to-peak amplitudes (y-axis), circles indicate M-wave amplitudes (y-axis) at the corresponding stimulation intensity (x-axis), solid red and green lines represent the sigmoid fit that was used to calculate the variables for the recruitment curve analysis similar to Klimstra et al. (2008). Group averages of pre to post session percent changes in recruitment curve variables are shown in C). These variables indicate the amplitude of the post H-reflex when measured at the same relative stimulation intensity that evoked a H-reflex at threshold (H@th), halfway up the ascending limb (H@50) and at the peak of the ascending limb (H@100). Please see Klimstra et al. (2008) for detailed descriptions of the methods used to determine these variables. Asterisks indicate significant ($p < 0.05$) differences in the decrease pre to post session between conditions. Values are group means ± standard error.*

Effects of sensory stimulation on spinal cord excitability

Sensory stimulation facilitates H-reflexes evoked during control cycling. During *CONTROL*, there was no amplitude modulation of H-reflexes over time (p = 0.23), however, sensory stimulation applied for 10 s caused a general increase of the H-reflex amplitudes throughout the *CONTROL + STIM* session. The 4 (condition) x 10 (time) RM ANOVA revealed that there was a significant (main effect condition, $p = 0.016$, main effect time, p = 0.008, interaction p = 0.035) difference in H-reflex amplitudes during the *CONTROL + STIM* condition. In fact, at all time points other than post 1 (p = 0.28), pairwise comparisons revealed that *CONTROL + STIM* H-reflex amplitudes were greater than all other conditions (p ranged from < 0.001 to 0.03). The effects of sensory stimulation were substantially small to moderate (d ranged from 0.36-0.67) which resulted in increased H-reflex amplitudes throughout the experimental protocol (see figure 7-3 and 7-7; table 7-3).

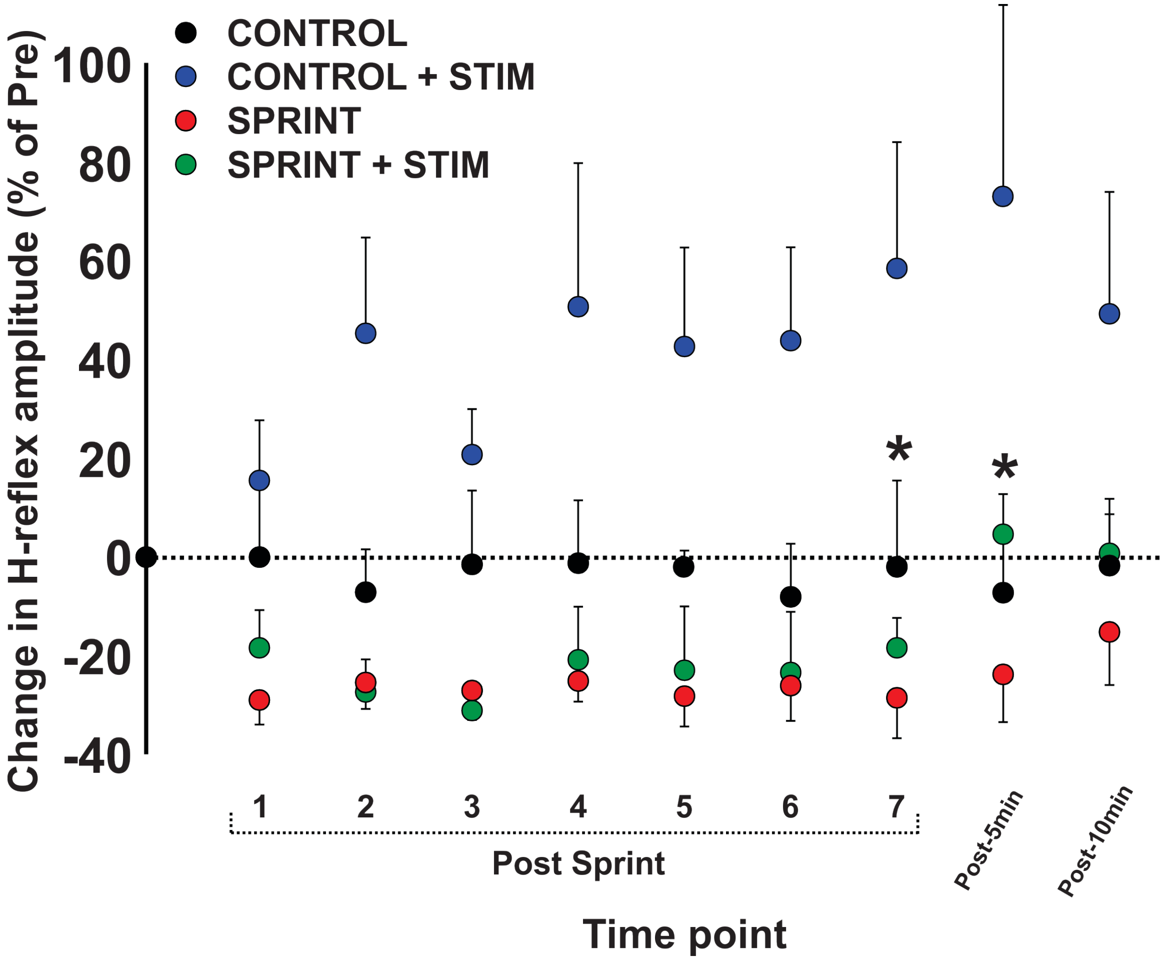

***Figure** 7-7. Depicts the group average H-reflex amplitudes relative to the M_{max} measured at the same time point. All values were made relative to the pre-exercise average of the corresponding session for comparison. Values are group means ± standard error. Asterisks indicate significant ($p < 0.05$) differences between SPRINT + STIM and SPRINT conditions. For clarity of display, all other significant differences were omitted and can be found in text.*

***Table 7-3:** Effect sizes and 95% confidence intervals of the between condition difference of the percent change of H-reflex amplitude from pre-sprint 1 to each time-point (top) and effect sizes of the change in H-reflex amplitude from pre-sprint 1 to each sprint within each condition (bottom). C = CONTROL, C+S = CONTROL + STIM, S = SPRINT, S+S = SPRINT + STIM.*

Reflex amplitude % change		**Time point**								
	Comparison	**Post-1**	**Post-2**	**Post-3**	**Post-4**	**Post-5**	**Post-6**	**Post-7**	**5 min post**	**10 min post**
Cohen's D (condition)	**C+S - S**	1.14	1.09	1.52	0.73	1.02	1.09	1.02	0.72	0.77
	C+S - S+S	0.98	1.14	1.64	0.59	0.77	0.88	0.86	0.47	0.50
	S - S+S	0.38	0.19	0.20	1.01	0.83	0.56	0.92	1.00	0.62
	C - C+S	-0.16	-1.55	-0.38	-1.26	-5.08	-1.52	-0.94	-1.77	-1.03
	C - S+S	0.39	0.61	0.53	0.41	2.02	0.37	0.27	-0.24	-0.01
	C - S	0.59	0.62	0.50	0.63	2.91	0.51	0.46	0.37	0.21
95% CI (condition)	**C+S - S**	35.00 - 43.06	55.03 - 64.77	35.99 - 43.63	54.93 - 66.22	53.17 - 63.18	53.73 - 63.72	68.23 - 78.95	72.21 - 85.83	47.81 - 59.47
	C+S - S+S	29.41 - 38.12	56.81 - 67.77	38.61 - 43.63	41.38 - 54.94	37.03 - 49.35	40.91 - 52.92	55.53 - 67.67	43.86 - 58.56	28.06 - 40.61
	S - S+S	1.77 - 8.73	1.39 - 6.25	1.76 - 6.63	-0.35 - 8.04	-0.30 - 10.17	-0.36 - 6.96	-0.81 - 8.24	0.24 - 23.04	0.14 - 14.35
	C - C+S	-11.6 - -3.14	-49.50 - -40.29	-22.62 - -15.45	-58.84 - -47.96	-52.42 - -43.90	-59.02 - -50.10	-60.34 - -49.63	-79.77 - -67.30	-55.95 - -45.65
	C - S+S	15.02 - 21.84	14.50 - 20.72	23.46 - 29.90	14.49 - 20.20	16.73 - 21.59	10.26 - 16.40	12.25 - 18.85	-13.32 - -6.41	-4.33 - 2.91
	C - S	24.67 - 30.83	15.30 - 20.77	21.96 - 28.37	24.06 - 29.90	25.16 - 30.13	14.96 - 21.31	23.74 - 29.88	12.27 - 18.72	7.03 - 14.12
Cohen's D (time)	**C+S**	0.36	0.67	0.57	0.46	0.58	0.65	0.65	0.52	0.55
	S	1.92	1.55	1.47	1.92	1.47	1.19	2.12	0.80	0.49
	S+S	0.99	1.39	1.76	0.35	0.30	0.36	0.81	0.24	0.14
	C	-0.02	0.25	0.03	0.03	0.18	0.23	0.04	0.18	0.06

Effects of fatigue on spinal cord excitability

*H-reflexes evoked during unloaded cycling are suppressed following intermittent sprints***.** There was general suppression of H-reflexes evoked after each of the seven sprints for both the *SPRINT* and *SPRINT + STIM* conditions, when compared to pre-exercise. A 4 (condition) x 10 (time) RM ANOVA revealed that there was a significant (main effect condition, $p = 0.016$, main effect time, $p = 0.008$, interaction $p = 0.035$) change in H-reflex amplitude during the *SPRINT + STIM* and *SPRINT* conditions. Pairwise comparisons revealed that *SPRINT* (all $p < 0.001$) and *SPRINT + STIM* (p ranged from < 0.001 to 0.022) H-reflex amplitudes were reduced after sprints 1 through 7. There were both trivial and substantial small to large (*d* ranged from 0.14 to 1.92) effects of intermittent sprints to reduce H-reflex amplitudes (see table 7-3). Interestingly, there was no longer significant suppression of H-reflex amplitudes measured at 5 (p = 0.38) or 10 min (p = 0.39) post sprint 7 for the *SPRINT* + *STIM* condition, whereas the suppression remained at 5 (p = 0.005) and 10 min (p = 0.036) post-sprint 7 for the *SPRINT* condition.

Interaction of sensory stimulation and fatigue on spinal cord excitability

Sensory stimulation mitigates fatigue-related suppression of H-reflexes measured during unloaded cycling following intermittent sprints. This is evidenced by a reduction of H-reflex suppression in the *SPRINT* + *STIM* compared to *SPRINT* condition. A 4 (condition) x 10 (time) RM ANOVA revealed that there were significant (main effect condition, p = 0.016, main effect time, p = 0.008, interaction effect, p = 0.035) differences between SPRINT and SPRINT + STIM. Paired t-tests revealed that the *SPRINT* + *STIM* H-reflexes were significantly less suppressed immediately (p = 0.006) and 5 mins after sprint 7 (p = 0.019) compared to the *SPRINT* condition (see figure 7-3 and 7-7; table 7-3).

Sensory stimulation mitigates fatigue-related suppression of resting H-M recruitment curves measured post-exercise. A 4 (condition) x 2 (time) RM ANOVA for each variable revealed that from pre- to post-experimental protocol, there were significant main effects for time that indicated an increase in the slope of the H recruitment curve ($p < 0.001$) and current required to evoke 50% of H_{max} (p = 0.003) and H_{max} (p = 0.018) and, furthermore, a decrease in the post H-reflex amplitude evoked at 50% of the current required to get H_{max} during the pre-exercise test ($p < 0.001$) and the post H-reflex amplitude evoked at the current required to evoke the pre-exercise H_{max} ($p < 0.001$). Main condition effects were observed for the H-reflex amplitude evoked at the current used to evoke H_{max} during the pre recruitment curve (p = 0.034) and H-reflex amplitude evoked at 50% of current required to evoke H_{max} during the pre recruitment curve (p = 0.045). Pairwise t-tests revealed that both of these variables were greater for the *SPRINT* condition compared to the *SPRINT* + *STIM* condition (see figure 7-6C).

Discussion

The main results from this experiment show that sensory stimulation can alter the effects of fatigue; furthermore, spinal cord excitability is reduced by fatigue and enhanced by sensory stimulation. These data reveal for the first time the potential effects of cutaneous sensory input interacting with fatigue-induced suppression of neural function and performance outcomes. These observations further our understandings of the effects of cutaneous input on human locomotion. Potential mechanisms and methodological considerations are discussed below.

Sensory stimulation reduces decrements in performance measures

With increasing bouts of intermittent sprints, power output decreased significantly. This is a consistent finding with previous work that has examined the fatigue of intermittent sprints during arm (Pearcey, Bradbury-Squires et al. 2016) and leg cycling (Billaut, Basset et al. 2006, Billaut and Basset 2007, Racinais, Bishop et al. 2007, Mendez-Villanueva, Hamer et al. 2008, Girard, Bishop et al. 2013, Girard, Bishop et al. 2013, Pearcey, Murphy et al. 2014). A noteworthy finding of the current experiment was that there was significantly less decrement in average power output of the second and seventh sprints in the presence of sensory stimulation compared to sprints without stimulation. Although the mechanism is not entirely clear, it is possible that information from distal afferent nerve stimulation interferes with group III/IV afferent information, which has been speculated to contribute to fatigue during intense exercise (Amann, Proctor et al. 2008, Amann, Runnels et al. 2011, Sidhu, Weavil et al. 2014, Sidhu, Weavil et al. 2017). Since stimulation of the distal tibial, sural and superficial peroneal nerves can activate multiple sensory afferents, trains of stimuli applied during each pedal stoke may act to send volleys of action potentials to the spinal cord and supraspinal centres. Although it is unclear if enhanced sensory information may interact with the perception of difficulty resulting from an intense bout of exercise, it appears that there are slight interactions of peripheral nerve stimulation with repeated sprint ability. Further work is needed to understand the exact mechanisms contributing to these effects.

Sensory stimulation facilitates spinal cord excitability during unloaded cycling

During unloaded control cycling, we stimulated the right foot each time the foot passed the 2 o’clock position for a total of 10 pedal strokes, interleaved by 3 min of unloaded cycling. This stimulation facilitated the H-reflex amplitude by ~7~27% of pre-session values throughout the experimental protocol. The amount of facilitation appeared to be least after the first bout of stimuli and greatest 5 min following the final bout of stimuli. This relationship suggests that, perhaps, there was a cumulative effect of sensory stimulation on the facilitation of the SOL H-reflex. We attribute the facilitation of the H-reflex to result from reduced Ia presynaptic inhibition. In the cat, Brink, Jankowska et al. (1984) showed that stimulation of group I afferents has premotoneuronal effects on Ia reflex excitability, such that stimulation can reduce presynaptic inhibition and, therefore, increase Ia reflex excitability. Low intensity stimulation of the sural nerve increases H-reflex amplitudes at C-T intervals of 70-90 ms (Demaire and Ciancia 2000) and when stimulation of sural and common peroneal nerves are combined with condition-test intervals of 70-250 ms, there is significant decreases in presynaptic inhibition (Iles 1996). Thus, it is apparent that group I afferents affect the excitability of the H-reflex. In the current experiment, a low intensity combination of sural, tibial, and superficial peroneal stimulation of the foot resulted in facilitation of H-reflexes that were measured ~10~30 s following the sensory stimulation. Although a persistent effect of sensory stimulation has not been observed before, we suggest that there is a cumulative effect of the stimulation that can interact with the voluntary output during cycling, which acts to reduce presynaptic inhibition and therefore facilitate the SOL H-reflex.

Fatigue reduces spinal cord excitability

Intermittent sprints induced suppression of SOL H-reflex amplitude. During non-fatiguing cycling, it has been speculated that suppression of the H-reflex occurs via Ia presynaptic inhibition (Brooke, Cheng et al. 1997, Frigon, Collins et al. 2004) that likely arise from central pattern generator and/or descending supraspinal inputs. Therefore, since intermittent sprints and the subsequent rest periods in this experiment consisted of rhythmic cycling, increased presynaptic inhibition should be noted as a contributing factor to the suppression of the H-reflexes. Furthermore, electrically-stimulated submaximal fatiguing isometric contractions (Garland and McComas 1990), sustained maximal voluntary isometric contractions (MVC) (Duchateau and Hainaut 1993), sustained 25 and 50% MVCs (Duchateau, Balestra et al. 2002), sustained low (25% MVC) and high (42-66% MVC) force plantarflexion (Kuchinad, Ivanova et al. 2004) and intermittent MVCs (Iguchi and Shields 2012) all cause approximately ~40~50% reduction in H-reflex peak-to-peak amplitudes. In the current experiment, the H-reflex amplitudes were only decreased by ~11~27%. Since the fatigability of the plantarflexors is not as substantial as some other muscle groups (i.e. knee flexors) during cycling sprints (Rampinini, Connolly et al. 2016), it is possible that the SOL in the current experiment was not fatigued to the extent of the aforementioned isometric fatigue studies (Garland and McComas 1990, Duchateau and Hainaut 1993, Duchateau, Balestra et al. 2002, Kuchinad, Ivanova et al. 2004). However, unlike the amount of amplitude modulation, the timeline of recovery of the H-reflex amplitude in the current experiment was perhaps longer than that of isometric contraction experiments. H-reflex amplitudes recorded 5-10 mins following the final sprint remained lower than values recorded prior to sprint 1 (see figure 7-7) for the *SPRINT* condition. This trend was altered in the SPRINT + STIM condition, such that H-reflex amplitudes were back to pre-sprint values <5 mins following sprint 7. This recovery is similar to the recovery of H-reflex amplitudes seen in the work of Kuchinad, Ivanova et al. (2004). In the current experiment, the fatigue-related suppression after each sprint was quite similar, and did not increase with an increased number of sprints (i.e. more fatigue). The onset of suppression of H-reflexes in isometric contractions is early, and this suppression remains somewhat consistent throughout fatigue protocols (Duchateau, Balestra et al. 2002, Kuchinad, Ivanova et al. 2004, Iguchi and Shields 2012). These data indicate that the overall suppression of H-reflexes may arise from initial accumulation of metabolites in the muscles, which then activate group III/IV muscle afferents, similar to fatigue during locomotor exercise (Blain, Mangum et al. 2016). Fatigue sensitive afferents can project to the spinal cord to inhibit extensor motoneuron output directly (Martin, Smith et al. 2006). It has also been shown that capsaicin sensitive group III/IV muscle afferents mediate inhibition of Ia input onto motoneurons (Pettorossi, Della Torre et al. 1999) through either presynaptic inhibitory interneurons or through direct synaptic connections onto group Ia afferent terminals (Della Torre, Lucchi et al. 1996). Fatigue sensitive afferents also project to supraspinal regions (Sidhu, Weavil et al. 2017) and likely act to alter supraspinal excitability of the corticospinal tract (Pearcey, Bradbury-Squires et al. 2016), which can alter descending commands on presynaptic inhibition.

Although peripheral changes in excitability (i.e. sarcolemma excitability) can contribute to a reduction in H-reflex amplitude, these changes would be evident by a decrease in the M_{max} amplitude. Since M_{max} amplitudes did not significantly decrease, but H/M_{max} did decrease throughout the SPRINT protocol, it can be concluded that these changes were not due to a decrease in peripheral excitability. The lack of a decrease in M_{max} amplitude throughout a sprint protocol is similar to that of the M_{max} amplitudes evoked in the knee flexors in previous studies (Billaut, Kerris et al. 2013, Girard, Bishop et al. 2013, Hureau, Olivier et al. 2014, Pearcey, Murphy et al. 2014). Decreased excitability of Ia afferent axonal excitability (hyperpolarization) can also contribute to the suppression of H-reflex amplitudes. If this were the case in the current experiment, one would expect to see an increase in current required to elicit H-reflexes, which would indicate an alteration in the axonal thresholds. There were no significant differences in stimulation intensity after each sprint or in pre- to post-session current required to evoke H-reflexes during the M-H recruitment curves. Thus, decreased excitability of Ia afferent axons likely is not responsible for the suppression of H-reflexes in the current experiment.

Sensory stimulation interacts with fatigue-related reductions in spinal cord excitability

H-reflex amplitudes immediately and 5 mins after sprint 7 were significantly greater in the *SPRINT + STIM* condition compared to the *SPRINT* condition. This suggests that the presynaptic inhibition of the *SPRINT + STIM* condition was slightly less than the *SPRINT* condition. Above, we have discussed how the intermittent sprints increase presynaptic inhibtion of the Ia terminals on SOL motoneurons and that sensory stimulation during unloaded cycling reduces said presynaptic inhibition. Furthermore, we pointed out that there seems to be a cumulative effect of sensory stimulation that results in a greater facilitation of reflexes later in the session, whereas the increased presynaptic inhibition resulting from fatigue appears to be similar throughout the session. Therefore, the interactions of the effects of sensory stimulation and fatigue would likely be greatest later in the session. This is supported by a similar suppression of H-reflex amplitude between the *SPRINT + STIM* and *SPRINT* conditions early in the session, whereas later in the session, there is a divergence between the relative amplitudes of reflexes between the two conditions. Since, reductions in afferent input can lower firing rates of motoneurons (Macefield, Gandevia et al. 1993) and reduced presynaptic inhibition (i.e. increased afferent input to motoneurons) has been linked to the ability to maintain sustained isometric contractions (Baudry, Maerz et al. 2011), it then seems possible that the reductions in group Ia presynaptic inhibition may have contributed to the improved performance of the cycling sprints.

Conclusion

The novel interaction of stimulating nerves innervating the skin of the foot with the fatigue-related decrements in power and suppression of H-reflexes has been highlighted in the current study. This suggests that enhancing feedback from the skin may moderate exercise-related fatigue. The interactions between enhanced sensory input and fatiguing exercise implies that other modalities that alter the sensory transmission from the skin during fatiguing exercise may also have beneficial effects. Future experiments should be conducted to determine whether accessories, apparel or footwear, which can alter sensory feedback from the skin, are able to interact with exercise-related fatigue.

References

Amann, M. (2012). "Significance of Group III and IV muscle afferents for the endurance exercising human." Clin Exp Pharmacol Physiol 39(9): 831-835.

Amann, M., L. T. Proctor, J. J. Sebranek, M. W. Eldridge, D. F. Pegelow and J. A. Dempsey (2008). "Somatosensory feedback from the limbs exerts inhibitory influences on central neural drive during whole body endurance exercise." J Appl Physiol (1985) 105(6): 1714-1724.

Amann, M., S. Runnels, D. E. Morgan, J. D. Trinity, A. S. Fjeldstad, D. W. Wray, V. R. Reese and R. S. Richardson (2011). "On the contribution of group III and IV muscle afferents to the circulatory response to rhythmic exercise in humans." J Physiol 589(Pt 15): 3855-3866.

Amann, M., M. Venturelli, S. J. Ives, J. McDaniel, G. Layec, M. J. Rossman and R. S. Richardson (2013). "Peripheral fatigue limits endurance exercise via a sensory feedback-mediated reduction in spinal motoneuronal output." J Appl Physiol (1985) 115(3): 355-364.

Barzi, Y. and E. P. Zehr (2008). "Rhythmic arm cycling suppresses hyperactive soleus H-reflex amplitude after stroke." Clin Neurophysiol 119(6): 1443-1452.

Baudry, S., A. H. Maerz, J. R. Gould and R. M. Enoka (2011). "Task- and time-dependent modulation of Ia presynaptic inhibition during fatiguing contractions performed by humans." J Neurophysiol 106(1): 265-273.

Billaut, F. and F. A. Basset (2007). "Effect of different recovery patterns on repeated-sprint ability and neuromuscular responses." J Sports Sci 25(8): 905-913.

Billaut, F., F. A. Basset, M. Giacomoni, F. Lemaitre, V. Tricot and G. Falgairette (2006). "Effect of high-intensity intermittent cycling sprints on neuromuscular activity." Int J Sports Med 27(1): 25-30.

Billaut, F., J. P. Kerris, R. F. Rodriguez, D. T. Martin, C. J. Gore and D. J. Bishop (2013). "Interaction of central and peripheral factors during repeated sprints at different levels of arterial O2 saturation." PLoS One 8(10): e77297.

Blain, G. M., T. S. Mangum, S. K. Sidhu, J. C. Weavil, T. J. Hureau, J. E. Jessop, A. D. Bledsoe, R. S. Richardson and M. Amann (2016). "Group III/IV muscle afferents limit the intramuscular metabolic perturbation during whole body exercise in humans." J Physiol 594(18): 5303-5315.

Brink, E., E. Jankowska and B. Skoog (1984). "Convergence onto interneurons subserving primary afferent depolarization of group I afferents." J Neurophysiol 51(3): 432-449.

Brooke, J. D., J. Cheng, D. F. Collins, W. E. McIlroy, J. E. Misiaszek and W. R. Staines (1997). "Sensori-sensory afferent conditioning with leg movement: gain control in spinal reflex and ascending paths." Prog Neurobiol 51(4): 393-421.

Cohen, J. (1988). Statistical power analysis for the behavioral sciences. New Jersey, Lawrence Erlbaum.

Crone, C., H. Hultborn, L. Mazieres, C. Morin, J. Nielsen and E. Pierrot-Deseilligny (1990). "Sensitivity of monosynaptic test reflexes to facilitation and inhibition as a function of the test reflex size: a study in man and the cat." Exp Brain Res 81(1): 35-45.

de Ruiter, G. C., S. R. Hundza and E. P. Zehr (2010). "Phase-dependent modulation of soleus H-reflex amplitude induced by rhythmic arm cycling." Neurosci Lett 475(1): 7-11.

Della Torre, G., M. L. Lucchi, O. Brunetti, V. E. Pettorossi, P. Clavenzani and R. Bortolami (1996). "Central projections and entries of capsaicin-sensitive muscle afferents." Brain Res 713(1-2): 223-231.

Demaire, C. and F. Ciancia (2000). "Sural nerve facilitation of the soleus muscle H-reflex in high level gymnasts." Eur J Appl Physiol 82(5-6): 446-450.

Demaire, C., J. Honore, J. Le Bizec and J. M. Coquery (1989). "Specific modulation of the Hoffmann reflex cutaneous facilitation during a reaction-time task." Exp Brain Res 74(3): 592-602.

Duchateau, J., C. Balestra, A. Carpentier and K. Hainaut (2002). "Reflex regulation during sustained and intermittent submaximal contractions in humans." J Physiol 541(Pt 3): 959-967.

Duchateau, J. and K. Hainaut (1993). "Behaviour of short and long latency reflexes in fatigued human muscles." J Physiol 471: 787-799.

Frigon, A., D. F. Collins and E. P. Zehr (2004). "Effect of rhythmic arm movement on reflexes in the legs: modulation of soleus H-reflexes and somatosensory conditioning." J Neurophysiol 91(4): 1516-1523.

Garland, S. J. and A. J. McComas (1990). "Reflex inhibition of human soleus muscle during fatigue." J Physiol 429: 17-27.

Girard, O., D. J. Bishop and S. Racinais (2013). "Hot conditions improve power output during repeated cycling sprints without modifying neuromuscular fatigue characteristics." Eur J Appl Physiol 113(2): 359-369.

Girard, O., D. J. Bishop and S. Racinais (2013). "Neuromuscular adjustments of the quadriceps muscle after repeated cycling sprints." PLoS One 8(5): e61793.

Hagbarth, K. E. (1952). "Excitatory and Inhibitory Skin Areas for Flexor and Extensor Motoneurones." Acta Physiol Scand 26(Supplementum 94).

Hundza, S. R. and E. P. Zehr (2009). "Suppression of soleus H-reflex amplitude is graded with frequency of rhythmic arm cycling." Exp Brain Res 193(2): 297-306.

Hureau, T. J., N. Olivier, G. Y. Millet, O. Meste and G. M. Blain (2014). "Exercise performance is regulated during repeated sprints to limit the development of peripheral fatigue beyond a critical threshold." Exp Physiol 99(7): 951-963.

Iguchi, M. and R. K. Shields (2012). "Cortical and segmental excitability during fatiguing contractions of the soleus muscle in humans." Clin Neurophysiol 123(2): 335-343.

Iles, J. F. (1996). "Evidence for cutaneous and corticospinal modulation of presynaptic inhibition of Ia afferents from the human lower limb." J Physiol 491 (Pt 1): 197-207.

Javan, B. and E. P. Zehr (2008). "Short-term plasticity of spinal reflex excitability induced by rhythmic arm movement." J Neurophysiol 99(4): 2000-2005.

Kalezic, I., L. A. Bugaychenko, A. I. Kostyukov, A. I. Pilyavskii, M. Ljubisavljevic, U. Windhorst and H. Johansson (2004). "Fatigue-related depression of the feline monosynaptic gastrocnemius-soleus reflex." J Physiol 556(Pt 1): 283-296.

Klimstra, M. and E. P. Zehr (2008). "A sigmoid function is the best fit for the ascending limb of the Hoffmann reflex recruitment curve." Exp Brain Res 186(1): 93-105.

Kuchinad, R. A., T. D. Ivanova and S. J. Garland (2004). "Modulation of motor unit discharge rate and H-reflex amplitude during submaximal fatigue of the human soleus muscle." Exp Brain Res 158(3): 345-355.

Kukulka, C. G., M. A. Moore and A. G. Russell (1986). "Changes in human alpha-motoneuron excitability during sustained maximum isometric contractions." Neurosci Lett 68(3): 327-333.

Loadman, P. M. and E. P. Zehr (2007). "Rhythmic arm cycling produces a non-specific signal that suppresses Soleus H-reflex amplitude in stationary legs." Exp Brain Res 179(2): 199-208.

Macefield, V. G., S. C. Gandevia, B. Bigland-Ritchie, R. B. Gorman and D. Burke (1993). "The firing rates of human motoneurones voluntarily activated in the absence of muscle afferent feedback." J Physiol 471: 429-443.

Martin, P. G., J. L. Smith, J. E. Butler, S. C. Gandevia and J. L. Taylor (2006). "Fatigue-sensitive afferents inhibit extensor but not flexor motoneurons in humans." J Neurosci 26(18): 4796-4802.

Mendez-Villanueva, A., P. Hamer and D. Bishop (2008). "Fatigue in repeated-sprint exercise is related to muscle power factors and reduced neuromuscular activity." Eur J Appl Physiol 103(4): 411-419.

Monks, M. R., C. T. Compton, J. D. Yetman, K. E. Power and D. C. Button (2017). "Repeated sprint ability but not neuromuscular fatigue is dependent on short versus long duration recovery time between sprints in healthy males." J Sci Med Sport 20(6): 600-605.

Motl, R. W. and R. K. Dishman (2003). "Acute leg-cycling exercise attenuates the H-reflex recorded in soleus but not flexor carpi radialis." Muscle Nerve 28(5): 609-614.

Motl, R. W., B. D. Knowles and R. K. Dishman (2003). "Acute bouts of active and passive leg cycling attenuate the amplitude of the soleus H-reflex in humans." Neurosci Lett 347(2): 69-72.

Motl, R. W., J. O'Connor P and R. K. Dishman (2004). "Effects of cycling exercise on the soleus H-reflex and state anxiety among men with low or high trait anxiety." Psychophysiology 41(1): 96-105.

Motl, R. W., E. M. Snook, M. L. Hinkle and E. McAuley (2006). "Effect of acute leg cycling on the soleus H-reflex and modified Ashworth scale scores in individuals with multiple sclerosis." Neurosci Lett 406(3): 289-292.

Nakajima, T., R. A. Mezzarane, S. R. Hundza, T. Komiyama and E. P. Zehr (2014). "Convergence in reflex pathways from multiple cutaneous nerves innervating the foot depends upon the number of rhythmically active limbs during locomotion." PLoS One 9(8): e104910.

Palomino, A. F., S. R. Hundza and E. P. Zehr (2011). "Rhythmic arm cycling differentially modulates stretch and H-reflex amplitudes in soleus muscle." Exp Brain Res 214(4): 529-537.

Pearcey, G. E., D. J. Bradbury-Squires, J. E. Kawamoto, E. J. Drinkwater, D. G. Behm and D. C. Button (2015). "Foam rolling for delayed-onset muscle soreness and recovery of dynamic performance measures." J Athl Train 50(1): 5-13.

Pearcey, G. E., D. J. Bradbury-Squires, M. Monks, D. Philpott, K. E. Power and D. C. Button (2016). "Arm-cycling sprints induce neuromuscular fatigue of the elbow flexors and alter corticospinal excitability of the biceps brachii." Appl Physiol Nutr Metab 41(2): 199-209.

Pearcey, G. E., J. R. Murphy, D. G. Behm, D. C. Hay, K. E. Power and D. C. Button (2014). "Neuromuscular fatigue of the knee extensors during repeated maximal intensity intermittent-sprints on a cycle ergometer." Muscle Nerve.

Pettorossi, V. E., G. Della Torre, R. Bortolami and O. Brunetti (1999). "The role of capsaicin-sensitive muscle afferents in fatigue-induced modulation of the monosynaptic reflex in the rat." J Physiol 515 (Pt 2): 599-607.

Racinais, S., D. Bishop, R. Denis, G. Lattier, A. Mendez-Villaneuva and S. Perrey (2007). "Muscle deoxygenation and neural drive to the muscle during repeated sprint cycling." Med Sci Sports Exerc 39(2): 268-274.

Racinais, S., O. Girard, J. P. Micallef and S. Perrey (2007). "Failed excitability of spinal motoneurons induced by prolonged running exercise." J Neurophysiol 97(1): 596-603.

Rampinini, E., D. R. Connolly, D. Ferioli, L. A. T. A, G. Alberti and A. Bosio (2016). "Peripheral neuromuscular fatigue induced by repeated-sprint exercise: cycling vs. running." J Sports Med Phys Fitness 56(1-2): 49-59.

Sidhu, S. K., J. C. Weavil, T. S. Mangum, J. E. Jessop, R. S. Richardson, D. E. Morgan and M. Amann (2017). "Group III/IV locomotor muscle afferents alter motor cortical and corticospinal excitability and promote central fatigue during cycling exercise." Clin Neurophysiol 128(1): 44-55.

Sidhu, S. K., J. C. Weavil, M. Venturelli, R. S. Garten, M. J. Rossman, R. S. Richardson, B. S. Gmelch, D. E. Morgan and M. Amann (2014). "Spinal mu-opioid receptor-sensitive lower limb muscle afferents determine corticospinal responsiveness and promote central fatigue in upper limb muscle." J Physiol 592(22): 5011-5024.

Zehr, E. P. (2002). "Considerations for use of the Hoffmann reflex in exercise studies." Eur J Appl Physiol 86(6): 455-468.

Zehr, E. P., A. Frigon, N. Hoogenboom and D. F. Collins (2004). "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(3): 382-388.

Chapter 8 - General Conclusions

The main objective of this dissertation was to provide a foundation and evidence for exploiting evolutionarily conserved pathways to enhance targeted plasticity in human spinal cord circuits. Within this primary focus, evolutionarily conserved pathways were identified that could preferentially either increase or decrease spinal cord excitability through activation of Ia presynaptic inhibitory interneurons. Presynaptic inhibitory interneurons can therefore be thought of as having the potential to either increase or decrease Ia reflex excitability depending on the most desirable outcome. This section will summarize the findings to support this framework encompassing the exploitation of evolutionarily conserved pathways to promote plasticity in human spinal cord circuits.

An evolutionarily conserved pathway to increase spinal cord excitability

In the cat, acute cutaneous stimulation increases excitability of the Ia reflex (Crone et al., 1990; Schieppati and Crenna, 1984), decreases the threshold of recruitment for high threshold motor units (Kanda et al., 1977) and increases extensor activity during and prolongs the stance phase of walking (Duysens and Pearson, 1976). In humans, acute stimulation of cutaneous afferents also increases Ia reflex excitability (Crone et al., 1990; Iles, 1996; Jenner and Stephens, 1982), reduces perception of effort when lifting heavy objects (Aniss et al., 1988) and also reduces the recruitment threshold of high threshold motor units (Garnett and Stephens, 1981). Thus, the findings of chapter 4 (i.e. increased spinal cord excitability following 15 minutes of cutaneous stimulation) should not come as a surprise. More interesting observations were revealed in chapter 5, which showed that appropriately timed stimulation to cutaneous afferents of the foot could amplify force output. This provides functional application of the increased spinal cord excitability that may be used in more applied situations. For example, appropriately timed stimulation of cutaneous afferents during walking in humans with spinal cord injury may be able to facilitate extensor activity to provide enough propulsion to enhance locomotor rehabilitation. However, further work is required to substantiate this idea.

An evolutionarily conserved pathway to decrease spinal cord excitability

Decades of work have shown task-dependent modulation of spinal cord excitability. In particular, rhythmic movement of either the homonymous or a heteronymous limb can reduce Ia reflex excitability (Brooke et al., 1997, 1995), and alterations in spinal cord excitability persist beyond the cessation of activity (Javan and Zehr, 2008; Motl and Dishman, 2003). Interactions of rhythmic activity between the arms (forelimbs) and legs (hindlimbs), similar to those used in habitual quadrupeds, have been shown to amplify the convergence of multiple cutaneous reflex pathways (Nakajima et al., 2014), and would suggest to amplify the effects of rhythmic activity on spinal cord excitability. Indeed, results from chapter 6 show that spinal cord plasticity induced by rhythmic movement is amplified when using the arms and legs together compared to using the arms or legs separately. If the goal is to reduce spinal cord excitability, cervicolumbar connections can be exploited to amplify the plastic effects of rhythmic movement. This may have substantial impacts when guiding and supporting rehabilitation strategies that aim to reduce the excitability Ia reflex pathways to reduce spasticity.

Interactions of conserved pathways that have opposite effects on spinal cord excitability

Rhythmic movement and fatigue reduce spinal cord excitability, and the reductions in spinal cord excitability associated with fatigue can have debilitating consequences. Currently, there are very few ergogenic aids that target spinal cord excitability to mitigate fatigue. The findings of chapter 7 suggest that plasticity of spinal cord excitability induced by both fatigue and cutaneous stimulation are, at least partially, mediated by similar pathways within the spinal cord. As such, using cutaneous stimulation during fatiguing exercise can mitigate the reductions in spinal cord excitability and subsequent decrements in performance. It remains to be seen if similar effects can be observed in a clinical population to improve activities of daily living.

Future directions

Knowledge of plasticity within the human spinal cord has been progressing (Wolpaw, 2018), albeit at a slow rate. However, precise pathways to encourage optimal plasticity in various populations, including both athletic and neurological impaired, needs further investigation. For example, does increased spinal cord excitability associated with sensory enhancement improve athletic performance? Does increased spinal cord excitability improve functional outcomes of neurologically impaired individuals who cannot optimally activate their muscles? Does amplified plasticity of spinal cord circuits in neurologically intact individuals translate into improved clinical outcomes for those with neurological impairment? Finally, to what extent does the plasticity in one direction negatively impact the functional outcomes of individuals with neurological impairment? The answers to these questions will provide further knowledge required to successfully implement the ideas presented in this dissertation.

References

Aniss, A.M., Gandevia, S.C., Milne, R.J., 1988. Changes in perceived heaviness and motor commands produced by cutaneous reflexes in man. J. Physiol. (Lond.) 397, 113–126.

Brooke, J.D., Cheng, J., Collins, D.F., McIlroy, W.E., Misiaszek, J.E., Staines, W.R., 1997. Sensori-sensory afferent conditioning with leg movement: gain control in spinal reflex and ascending paths. Prog Neurobiol 51, 393–421.

Brooke, J.D., Cheng, J., Misiaszek, J.E., Lafferty, K., 1995. Amplitude modulation of the soleus H reflex in the human during active and passive stepping movements. J Neurophysiol 73, 102–11.

Crone, C., Hultborn, H., Mazieres, L., Morin, C., Nielsen, J., Pierrot-Deseilligny, E., 1990. Sensitivity of monosynaptic test reflexes to facilitation and inhibition as a function of the test reflex size: a study in man and the cat. Exp Brain Res 81, 35–45.

Duysens, J., Pearson, K.G., 1976. The role of cutaneous afferents from the distal hindlimb in the regulation of the step cycle of thalamic cats. Exp Brain Res 24, 245–55.

Garnett, R., Stephens, J.A., 1981. Changes in the recruitment threshold of motor units produced by cutaneous stimulation in man. J. Physiol. (Lond.) 311, 463–473.

Iles, J.F., 1996. Evidence for cutaneous and corticospinal modulation of presynaptic inhibition of Ia afferents from the human lower limb. J Physiol 491 (Pt 1), 197–207.

Javan, B., Zehr, E.P., 2008. Short-term plasticity of spinal reflex excitability induced by rhythmic arm movement. J Neurophysiol 99, 2000–5. https://doi.org/10.1152/jn.01315.2007

Jenner, J.R., Stephens, J.A., 1982. Cutaneous reflex responses and their central nervous pathways studied in man. J Physiol 333, 405–19.

Kanda, K., Burke, R.E., Walmsley, B., 1977. Differential control of fast and slow twitch motor units in the decerebrate cat. Exp Brain Res 29, 57–74.

Motl, R.W., Dishman, R.K., 2003. Acute leg-cycling exercise attenuates the H-reflex recorded in soleus but not flexor carpi radialis. Muscle Nerve 28, 609–14. https://doi.org/10.1002/mus.10479

Nakajima, T., Mezzarane, R.A., Hundza, S.R., Komiyama, T., Zehr, E.P., 2014. Convergence in reflex pathways from multiple cutaneous nerves innervating the foot depends upon the number of rhythmically active limbs during locomotion. PLoS One 9, e104910. https://doi.org/10.1371/journal.pone.0104910

Schieppati, M., Crenna, P., 1984. Natural cutaneous stimulation induces late and long-lasting facilitation of extensor motoneurons in the cat. Brain Res. 293, 259–267.

Wolpaw, J.R., 2018. The negotiated equilibrium model of spinal cord function: The spinal cord: a negotiated equilibrium. The Journal of Physiology 596, 3469–3491. https://doi.org/10.1113/JP275532