

Wilfrid Laurier University

Scholars Commons @ Laurier

Theses and Dissertations (Comprehensive)

2015

Estimates of persistent inward current in human motor neurons during postural sway

Ryan Foley

Wilfrid Laurier University, fole4370@mylaurier.ca

Jayne M. Kalmar Dr.

Wilfrid Laurier University, jkalmar@wlu.ca

Follow this and additional works at: <https://scholars.wlu.ca/etd>

 Part of the Cellular and Molecular Physiology Commons, Exercise Physiology Commons, Laboratory and Basic Science Research Commons, and the Motor Control Commons

Recommended Citation

Foley, Ryan and Kalmar, Jayne M. Dr., "Estimates of persistent inward current in human motor neurons during postural sway" (2015). *Theses and Dissertations (Comprehensive)*. 1707.

<https://scholars.wlu.ca/etd/1707>

This Thesis is brought to you for free and open access by Scholars Commons @ Laurier. It has been accepted for inclusion in Theses and Dissertations (Comprehensive) by an authorized administrator of Scholars Commons @ Laurier. For more information, please contact scholarscommons@wlu.ca.

Estimates of persistent inward current in human motor neurons during postural sway

Ryan C. A. Foley

Hons. Bachelor of Science, Wilfrid Laurier University, 2012

Thesis submitted to the Faculty of Graduate Studies in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

Graduate Program in Kinesiology and Physical Education

Wilfrid Laurier University

Waterloo, Ontario

© Ryan Foley, 2014

Abstract

Persistent inward current (PIC) is a membrane property critical for increasing gain of motor neuron output. In humans, most estimates of PIC are made from plantarflexor or dorsiflexor motor units with the participant in a seated position with the knee flexed. This seated and static posture neglects the task-dependent nature of the monoaminergic drive that modulates PIC activation. Seated estimates may drastically underestimate the amount of PIC that occurs in human motor neurons during functional movement. The current study estimated PIC using the conventional paired motor unit technique which uses the difference between reference unit firing frequency at test unit recruitment and reference unit firing frequency at test unit de-recruitment (ΔF) during triangular-shaped, isometric ramps in plantarflexion force as an estimate of PIC. Estimates of PIC were also made during standing anterior postural sway, a postural task that elicits a ramped increase and decrease in soleus motor unit activation similar to the conventional seated ramp contractions. For each motor unit pair, ΔF estimates of PIC made during conventional isometric ramps in the seated posture were compared to those made during standing postural sway. Baseline reciprocal inhibition (RI) was also measured in each posture using the post-stimulus time histogram (PSTH) technique. Hyperpolarizing input has been shown to have a reciprocal relationship with PIC in seated posture and RI was measured to examine if the same reciprocal relationship holds true during functional PIC estimation. It was hypothesized that an increase in ΔF would be seen during standing compared to sitting due to greater neuromodulatory input. We found that ΔF estimates during standing postural sway were equal (2.44 ± 1.17 , $p=0.44$) to those in seated PIC estimates (2.73 ± 1.20) using the same motor unit pair. Reciprocal inhibition was significantly lower when measured in a standing posture (0.0031 ± 0.0251 , $p<0.001$) than seated (-0.0378 ± 0.0415). These results may indicate a flaw in the translation of the paired motor unit technique from isolated plantarflexion ramp contractions to a functional postural sway task even though standing recordings satisfied all validation criteria required for PIC estimation using ΔF . There is continued belief that a functional human estimate of PIC is a valuable tool for postural control research and efforts to validate a standing paradigm have been advanced by this investigation.

Keywords: Persistent inward current, paired motor unit, postural sway

TABLE OF CONTENTS

List of Figures and Tables	IV
List of Terms and Abbreviations.....	VI
Chapter 1: Introduction	1
Overview.....	2
Purpose and Objectives.....	4
Chapter 2: Review of Literature	5
Persistent Inward Current	6
Purpose of PIC in Humans.....	6
Modulation of PIC.....	8
Measuring PIC.....	9
Estimating PIC in human motor neurons	12
Caveats of the paired motor unit technique.....	14
Physiological Assumptions Made to Legitimize PIC Estimation using PMU Recordings	14
Validation Criteria Employed to Ensure the Physiological Assumptions are Met	15
Other Considerations for Valid PMU Technique PIC Estimation	16
Other Motor Neuron Properties Contributing to Changes in Excitability	17
What Traditional Human PIC Estimates are Missing	20
Hypotheses.....	21
Chapter 3: Estimates of persistent inward current in human motor neurons during postural sway.....	23
Introduction	24
Methods.....	27
Participant Recruitment	27
Experimental Protocol & Design.....	27
Apparati	28
Paired Motor Unit Technique for PIC Estimation.....	29
PSTH Technique for Reciprocal Inhibition Collection	29
Analyses.....	30
Data Acquisition	34
Statistical Analyses	36
Results	37
Effect of Posture on motor unit recruitment and muscle activation.....	38
Discussion	38
References.....	48
Chapter 4: Summary and Future Directions	71
Summary.....	72
Conclusion.....	72
Recommendations for Further Study.....	72

Reference List.....	74
Chapter 5: Appendices	83
Appendix A: Informed Consent.....	84
Appendix B: Participant Screening Questionnaire.....	87
Appendix C: Permission to Use Figures.....	88
Appendix D: Curriculum Vitae.....	91

LIST OF FIGURES AND TABLES

Chapter 2: Review of Literature

Figure 2.1: Neuromodulatory effect on muscle force output.....	7
Figure 2.2: Neuromodulatory and inhibitory inputs to spinal motor neuron pools.	9
Figure 2.3: Voltage clamp method of PIC measurement.....	10
Figure 2.4: F-I functions with varying neuromodulation.	11
Figure 2.5: Paired motor unit technique.	13
Figure 2.6: Spike threshold accommodation	18
Figure 2.7: Spike frequency adaptation	19

Chapter 3: Figures and Tables

Table 3.1: Criteria used to determine whether control motor unit firing rates provide valid estimates of PIC in the paired motor unit technique.....	54
Table 3.2: Muscle activation and ramp characteristics for seated and standing measures	55
Figure 3.1: McComas boot to measure isometric plantarflexion torque during sitting phase of experiment	56
Figure 3.2: Custom force platform to measure force during anterior posterior sway	57
Figure 3.3: Experimental protocol	58
Figure 3.4: Intramuscular and force recordings during postural sway ramp task.....	59
Figure 3.5: Assessing reciprocal inhibition	60
Figure 3.6: Spike sorting protocol	61
Figure 3.7: Filtering of the Intramuscular Recording	62
Figure 3.8: Rate-rate correlation was conducted to satisfy the physiological assumption that the reference and test unit share equal synaptic input during the contraction.....	63
Figure 3.9: Rate modulation is calculated as difference between the range of reference unit firing range.....	64
Figure 3.10: The cumulative sum PSTH technique was used to quantify reciprocal inhibition.	65

Figure 3.11: Electrode setup for stimulation to assess reciprocal inhibition	66
Figure 3.12: ePIC and RI data.	67
Figure 3.13: Correlation between baseline ePIC and reciprocal inhibition	68
Figure 3.14: Correlation between $\Delta\Delta F$ and ΔRI	69
Figure 3.15: Paired motor unit recordings during seated and standing tasks	70

LIST OF TERMS AND ABBREVIATIONS

MU: Motor unit. A single α -motor neuron and the muscle fibers it innervates.

EMG: Electromyography. A technique used for quantifying the electrical activity of a muscle; two variations: surface and intramuscular.

Spike: A term used to describe a motor unit action potential extracted from raw intramuscular EMG recording using a sorting algorithm in Spike2 software. Spikes are identified based on shape and amplitude parameters.

ISI: Inter-spike interval. The period of time between motor unit action potentials or spikes.

PIC: Persistent inward current. A motor neuron property responsible for a change in gain of the motor neuron. Largely attributed to steady, inward calcium current and facilitated by voltage gated channels located on the somatodendritic membrane regions.

PMU: Paired motor unit. Two nearby motor units activated during a voluntary contraction, ideally with slightly different recruitment thresholds.

ePIC: Estimated persistent inward current. PIC estimated in a human using the difference in firing frequencies as opposed to the direct method of intracellular recording used in animals.

ΔF : Delta F. The outcome measure of the paired motor unit technique. The difference in reference unit firing frequency between test unit recruitment and derecruitment.

SFA: Spike frequency adaptation. A motor neuron property that results in a decrease in firing rate the longer the motor neuron remains active.

STA: Spike threshold accommodation. A motor neuron property that results in an increase in excitatory input needed to generate an action potential, the slower that excitatory input is applied to the cell.

RI: Reciprocal inhibition. A spinal reflex pathway acting through 1a afferent activation causing inhibition of the α -motor neuron to the antagonist through a 1a inhibitory interneuron.

CHAPTER 1: INTRODUCTION

OVERVIEW

Over a century ago, Sir Charles Sherrington referred to the motor neuron as the ‘final common pathway’ in the neuromuscular system because the motor neuron dendrites and soma are the last site of signal integration in the neuromuscular pathway(Burke, 2007). Historically, the motor neuron was viewed as a passive summator that responds to the signals it receives. However this notion has recently been rejected in favor of a model where the motor neuron is an active integrator; not only receiving input, but amplifying or attenuating descending drive in a state-dependent and task-dependent fashion(Hamm, Turkin, Bandekar, O’Neill, & Jung, 2010; Heckman, Mottram, Quinlan, Theiss, & Schuster, 2009). Understanding the mechanisms that modulate spinal motor neuron excitability under various states and tasks is crucial in understanding neuromuscular function and control.

There are three factors that can influence motor neuron firing rate: ionotropic descending and afferent inputs, descending metabotropic neuromodulation, and intrinsic spinal motor neuron properties (Heckman et al., 2009). With ionotropic input, neurotransmitters are released from presynaptic neurons of descending pyramidal tracts and ascending sensory pathways. These neurotransmitters bind to ionotropic receptors resulting in brief depolarization or hyperpolarization of the post-synaptic membrane. The result of ionotropic input is a fast change in membrane potential ideal for action initiation or reflex loops. It is unlikely that summed ionotropic inputs are capable of accounting for the large range (almost 10 fold) in motor output the body is capable of (Heckman, Binder, & Binder, 1993; Heckman et al., 2009). The accepted explanation is that neuromodulatory input is responsible for allowing membrane excitability to vary so greatly (R. H. Lee &

Heckman, 1998a). More specifically, monoamines produced in the brain stem drive changes in intrinsic excitability in a highly state-dependent fashion(Hamm et al., 2010; Jacobs, Martín-Cora, & Fornal, 2002; Perrier, 2013). In contrast, metabotropic input acts through protein coupled membrane receptors. The end effect is carried out through a cascade of reactions originating with G-protein activation upon binding to the protein-linked membrane channel(Perrier, 2013). Metabotropic input can have a similar excitatory or inhibitory effect on the membrane as ionotropic input; however because of the metabolic cascade by which it functions the effects are longer lasting but slower. An example of metabotropic input to spinal motor neurons is neuromodulatory drive from descending neurons of extrapyramidal tracts(Hounsgaard & Hultborn, 1988). Neuromodulators can adjust the gain of the cell making it more or less responsive to direct inputs(Heckmann, Gorassini, & Bennett, 2005). Finally, the effect any ionotropic or metabotropic input has on motor neuron membrane potential can be altered by the intrinsic excitability of the membrane. This intrinsic excitability is defined by the state of multiple types of membrane ion channels(Powers, Elbasiouny, Rymer, & Heckman, 2012) that account for many of the firing behaviors outside simple summation of inputs that is still under investigation in humans.

Several different intrinsic motor neuron properties that help to determine the excitability of the cell have been observed in reduced animal preparations. These properties include, but are not limited to: afterhyperpolarization potential (AHP), persistent inward current (PIC), spike frequency adaptation (SFA) and spike threshold accommodation (STA). While STA and SFA are fairly predictable processes (they have been well modeled based on animal data using computer simulations), PIC, which is

controlled by the highly state and task-dependent neuromodulatory system, is a multi-factor, compound process with less material knowledge on how it alters firing characteristics.

PURPOSE AND OBJECTIVES

PIC, which a property responsible for altering the gain of a motor neuron, is extremely important in the generation of functionally relevant muscle force(Heckman et al., 2009; Johnson, Hyngstrom, Manuel, & Heckman, 2012). Currently, many of the human estimates if PIC take place in a seated posture even though it is a state and task-dependent phenomenon. This project aims to take the next step in studying motor neuron signal integration by capturing human data while standing. Previous work in our lab has validated the paradigm used to estimate persistent inward current, a property responsible for adjusting motor neuron gain in humans. Now that the validity of our measurements is confirmed, the focus becomes the influence of descending input. Active postural control during measurement will provide functionally relevant data that is currently unavailable in the literature.

CHAPTER 2: REVIEW OF LITERATURE

PERSISTENT INWARD CURRENT

Persistent inward current (PIC) is a lasting, inward flow of ions increasing a neurons excitability(Schwindt & Crill, 1980). PIC is an intrinsic property of the neuronal membrane capable of eliciting large magnitude changes in output. An important feature of PIC is the ability to adjust the gain of a cell, increasing or decreasing the effect of a constant ionotropic input. PIC is the major contributing property responsible for increasing a motor neuron's intrinsic excitability, increasing motor output up to ten-fold(Heckman et al., 2009). This is achieved through activation of voltage activated calcium (CAV1.3)(Heckman, Johnson, Mottram, & Schuster, 2008) and potassium (not presently known) channels in the membrane that open near firing threshold. With each receptor having slightly different properties, PIC has two distinct processes, NaPIC and CaPIC. These channels are modulated through metabotropic receptors so their activity is long-lasting; CaPIC has a slightly slower onset and is longer lasting than NaPIC(David J Bennett, Hultborn, Fedirchuk, & Gorassini, 1998). NaPIC is predominantly active in the primary phase and gives way to CaPIC after about two seconds post activation (with moderate to low neuromodulation)(Svirskis & Hounsgaard, 1997). PICs have many different characteristics depending on the state of the pathway, arousal level or task; of particular interest to this study is the regulation of PIC by monoamines and their high task-dependency.

PURPOSE OF PIC IN HUMANS

Monoamines are small-molecule neuromodulators active in many complex physiological processes; they serve an often overlooked but essential role in producing functional movement by facilitating PIC in motor neurons(Harvey, Li, Li, & Bennett, 2006; Hounsgaard & Hultborn, 1988). Studies using anesthetics have demonstrated that

decreasing or eliminating monoaminergic input greatly reduces motor output(Heckman et al., 1993). Further study showed, contractions forces over 50% MVC are predominately driven by the neuromodulatory increase in excitability and not an exponential increase in descending ionotropic drive (Figure 2.1) (R. H. Lee & Heckman, 1998a, 1998b).

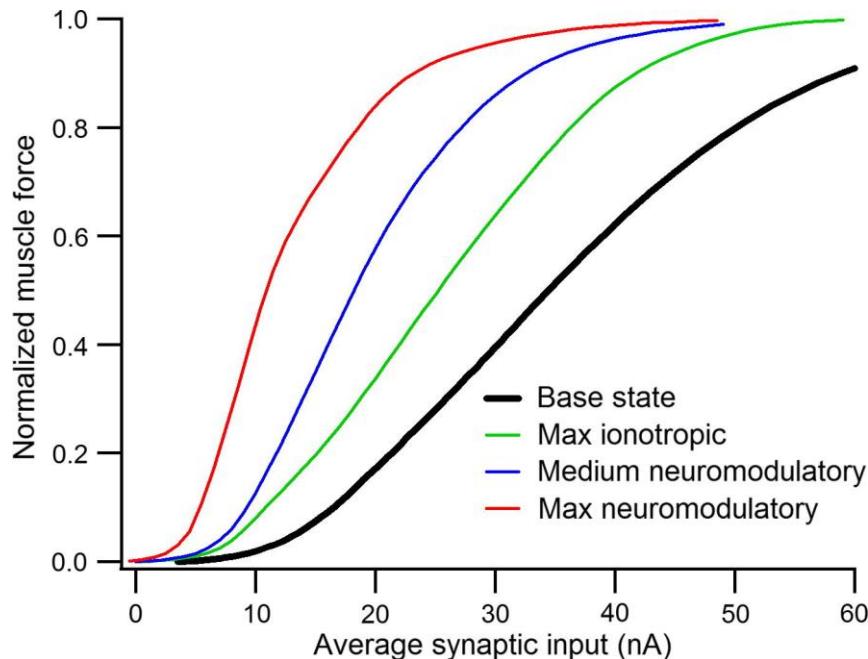


FIGURE 2.1: NEUROMODULATORY EFFECT ON MUSCLE FORCE OUTPUT. Neuromodulatory input is responsible for allowing the high levels of muscle contraction force above 50%MVC. Even high synaptic input, alone with little to no neuromodulation (thick black line), is not likely capable of producing functionally relevant movement. At a given level of synaptic input (say 20nA for example), addition of max neuromodulatory (thin red line) input can increase muscle force from 35% to over 85%. With even medium neuromodulatory drive (thin blue line), and the same synaptic input (20nA), muscle force is almost doubled from 35% to 65%. Figure taken from Heckman et al., 2009.

Functionally, this is advantageous in standing posture or locomotion; as muscles require greater input to maintain equal activation over prolonged time, descending input stays relatively similar and PIC is up regulated to compensate(ElBasiouny, Schuster, & Heckman, 2010; Heckman et al., 2009; Johnson & Heckman, 2010).

MODULATION OF PIC

Monoamines reach motor neurons via descending neuromodulatory projections from the caudal raphe nucleus, responsible for serotonin (5-HT), and the locus coeruleus, where norepinephrine (NE) is produced (Heckman et al., 2008, 2009). Although neuromodulation occurs via descending projections there is not preferential activation similar to other descending tracts (Johnson & Heckman, 2010). A general level of motor neuron gain is set by state and arousal and each task sculpts the pattern differently (Johnson et al., 2012).

As mentioned previously, high neuromodulation can greatly increase the gain of a motoneuron. While widespread increase in gain is necessary for functional levels of muscle excitation, the diffuse increase in excitability occurring via the neuromodulatory system is coupled with afferent input as a primary means of generating selective inhibition (Figure 2.2) (Nielsen, Crone, & Hultborn, 2007).

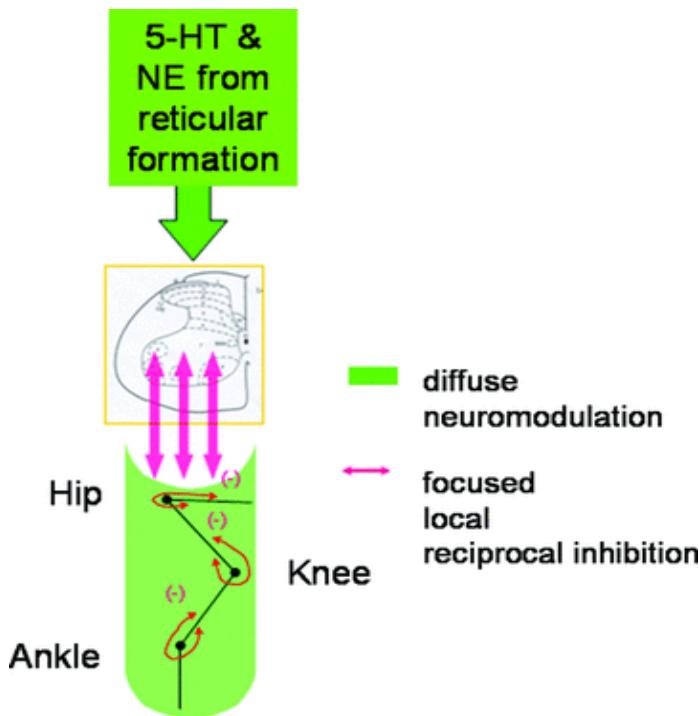


FIGURE 2.2: NEUROMODULATORY AND INHIBITORY INPUTS TO SPINAL MOTOR NEURON POOLS. Neuromodulatory input to the spinal motor neurons is diffuse and used to control excitability in a widespread manner. This generalized excitability is then shaped by selective inhibition to deactivate PIC in certain pools to create functional movement patterns. Taken from (Johnson & Heckman, 2010).

Reciprocal inhibition is one of the pathways preventing excitation from the neuromodulatory system from generating constant co-contraction of antagonist muscles at multiple limb segments(Johnson & Heckman, 2010; Johnson et al., 2012). A unique characteristic of PIC is that even brief hyperpolarizing stimulus can eliminate the depolarizing Ca^{2+} current, such as ascending 1a afferent reciprocal inhibition(Heckman et al., 2009; Johnson & Heckman, 2010). In this pathway, 1a afferent sensory neuron carries the information of muscle stretch spindles to the CNS (Crone & Hultborn, 1987). This pathway results in a direct excitation of the homologus muscle and inhibition of the antagonist muscle via the 1a inhibitory interneuron (Kernell, 2006). The reciprocal relationship between inhibition and PIC is important to functional control of movement(Johnson & Heckman, 2010; Johnson et al., 2012).

MEASURING PIC

Several techniques can be used to measure PIC directly in reduced animal preparations. Early research focused predominantly on the self-sustained firing ability of motor neurons(Schwindt & Crill, 1980). Demonstrated by continued neuronal firing following termination of depolarizing ionotropic input, this ability to maintain two steady firing states (with and without extrinsic activation) is also referred to as bistability (Svirskis & Hounsgaard, 1997). However, this technique cannot yield direct information on the strength of PIC, only presence or absence. The activation of PIC is also dependent on the

membrane voltage at the time of ionotropic input. Demonstrating the voltage-gated nature of channels responsible for PIC (Binder, 2002; Elias, Chaud, & Kohn, 2012), a cell voltage clamped in a hyperpolarized state does not initiate a PIC when a depolarizing current is introduced, compared to one clamped at a normal resting potential (Figure 2.3).

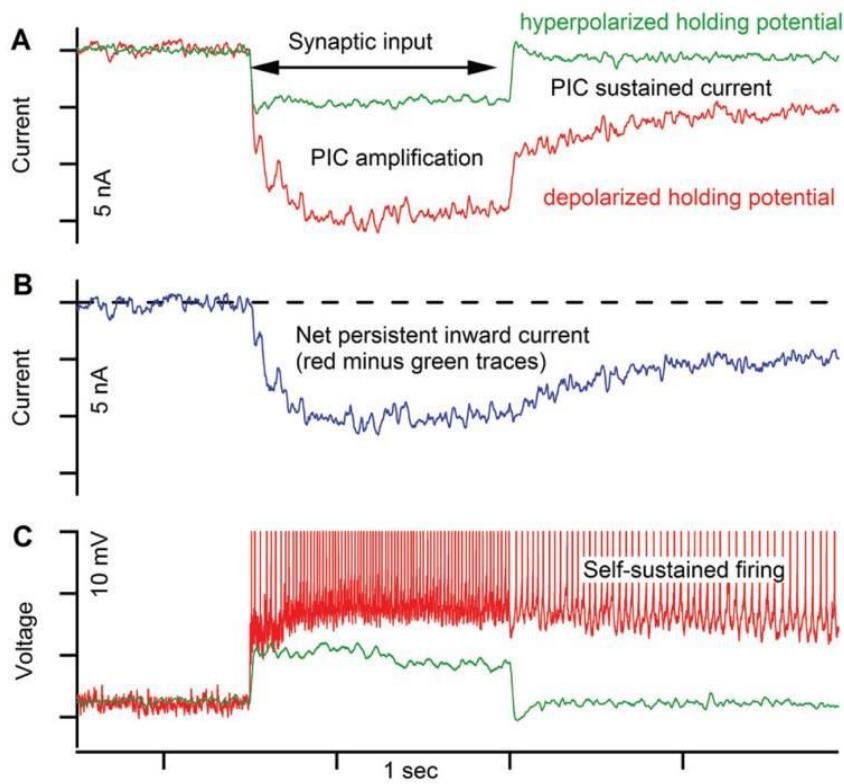


FIGURE 2.3: VOLTAGE CLAMP METHOD OF PIC MEASUREMENT. Under voltage clamp, PIC can be seen as a downward deflection in current that is greater than the current from synaptic input. This can be seen by the difference between red and green tracings in panel A. The green tracing is synaptic input under voltage clamp with neuromodulatory drive removed. The red tracing is the inward current in response to the same input with neuromodulatory drive intact. Panel B demonstrates the net amplitude of the PIC generated current. This technique can demonstrate the amount of PIC amplification in nA; a measurement not possible in human subjects. Although PIC cannot generate any cell excitation without synaptic input (panel C, green line), PIC creates around threshold voltage necessary to have functional cell firing (panel C, red line). A second characteristic of a motor neuron with PIC is self-sustained firing; this firing persists long after removal

of current and is terminated via inhibitory input to the cell. Figure taken from Heckman et al. 2009.

Another reduced prep technique used to investigate PIC is the use of frequency current relationships (Figure 2.4) that compare firing frequency to the input current into a cell. Distinct phases are seen by sharp changes in slope of the line at a given level of monoaminergic input. The steepest-sloped (secondary phase) segments of the function are the result of PIC, allowing the neuron to fire more often at a given ionotropic input(Heckman et al., 2009).

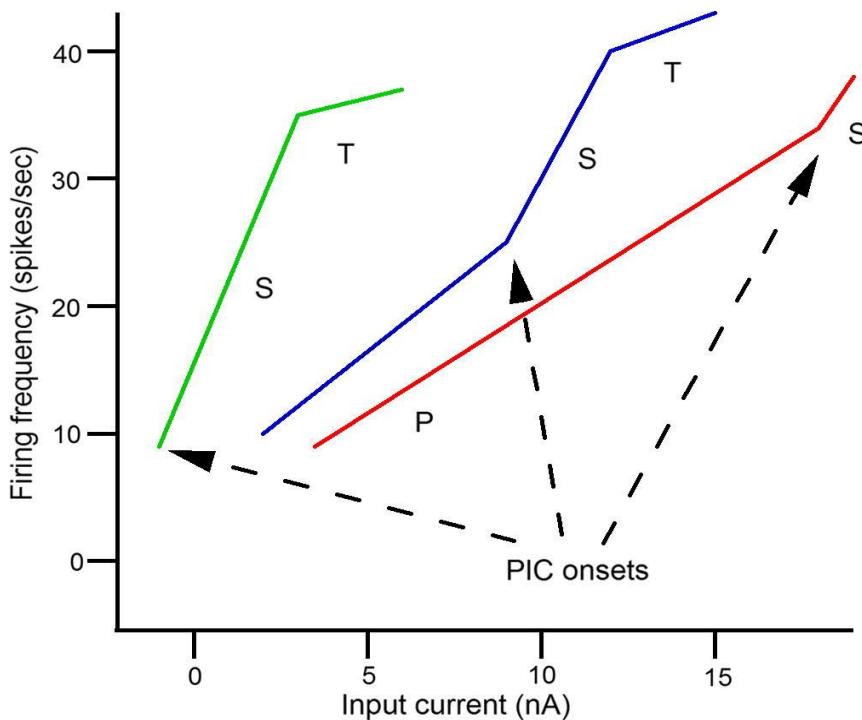


FIGURE 2.4: F-I FUNCTIONS WITH VARYING NEUROMODULATION. F-I functions are created by injecting current into neurons. These plots can tell us how the neuron translates input into firing frequency under strict conditions in animal preparations. A sharper slope indicates that the neuron has increased gain. Each line denotes a different neuromodulatory input; increasing in drive from low (red line) to moderate (blue line) to high (green line). **P** denotes the primary phase or “base state of the F-I function. **S** (secondary) is the range in which PIC is most active and **T** (tertiary) is beyond PIC activation. PIC activation occurs via voltage dependent membrane channels, therefore the more PIC the less current needed to initiate firing. Also, presence of PIC at firing onset means the neuron is in an immediate

state of high gain (note the high slope of the S range of the green tracing immediately from the onset of firing). During the primary phase Figure taken from Heckman et al. 2009.

ESTIMATING PIC IN HUMAN MOTOR NEURONS

It is clear that making intracellular recordings from human motor neurons to investigate PIC is not possible, so other methods such as self-sustained firing(Gorassini, Bennett, & Yang, 1998; Heckman et al., 2008; Walton, Kalmar, & Cafarelli, 2002) or paired-motor unit recordings are used. A technique commonly implemented to measure PIC in humans is known as the paired-motor unit (PMU) technique. It garnered attention as an isolate measure for PIC in humans and has been used to study various pathologies since inception by Gorassini and colleagues(D J Bennett, Li, Harvey, & Gorassini, 2001; Gorassini, Yang, Siu, & Bennett, 2002) . This paradigm compares firing rates at recruitment to firing rates at derecruitment to provide an estimate of intrinsic excitability. Firing rates are measured during a voluntary isometric torque ramp contraction. Thus, if the motor neuron is a linear summator, or passive integrator, as was once thought, then the difference between firing rates at these two time points would be minimal. However, this is not the case; motor unit firing persists below the level of synaptic drive at which it was recruited (Heckman et al., 2009). This difference in firing rate reflects changes in motor neuron excitability independent of synaptic input. Quantifiable difference in firing rate, an estimate of the intrinsic excitability of the neuron, is the currently the best measure available for PIC study in humans. The lowest threshold unit to be recruited in the ramp contraction is commonly referred to as the ‘reference’ or ‘reporter’ unit. ΔF , mentioned above, is calculated as the difference in control unit firing rate between the time of a second, or ‘test’ unit recruitment and derecruitment. An example of the paired-motor unit technique can be seen in Figure 2.5.

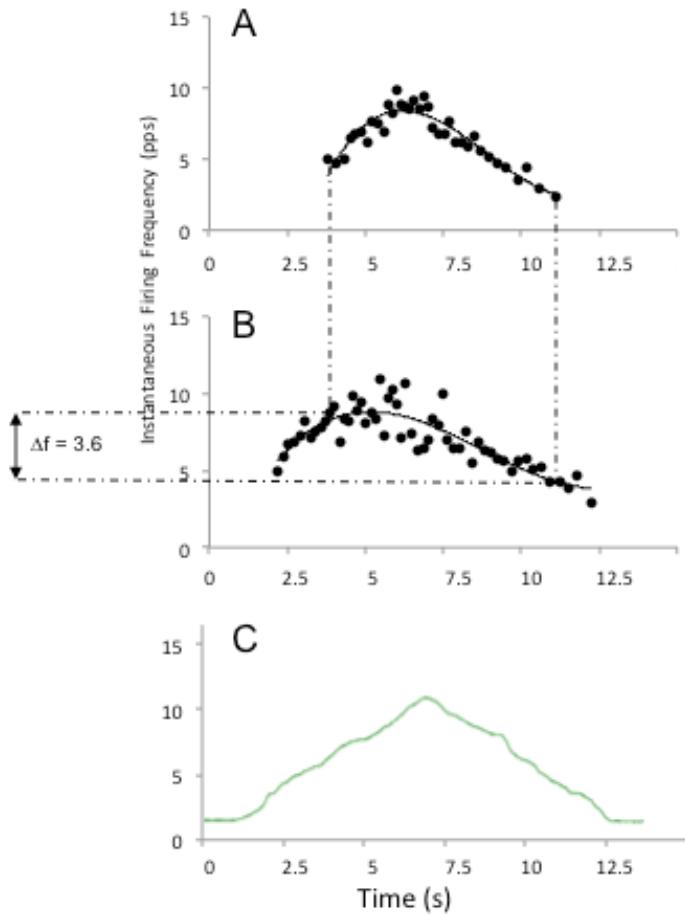


FIGURE 2.5: PAIRED MOTOR UNIT TECHNIQUE. A ramp contraction generated the recruitment of multiple units (C). These units' firing frequencies are plotted and compared to a designated reference unit which is generally the first unit recruited (B). With only synaptic input, hypothetically test unit (A) recruitment and derecruitment would occur at the same reference unit firing frequency (an estimate of descending ionotropic input). However, observations show a scenario such as the data shown above, where test unit firing persists beyond the firing rate at recruitment. The difference between the 2 firing rates is ΔF , an estimate of PIC in humans.

To estimate PIC using PMU data the instantaneous firing frequencies of two motor units are determined and plotted over time. The reference unit is lowest threshold unit that can be isolated with high consistency and the test unit is a unit of higher threshold and with a recruitment onset of two seconds post-recruitment of the reference unit. The resulting

plot of control unit instantaneous firing frequencies over the ramp usually resembles a skewed quadratic curve. While force output in this technique is essential to recruit the appropriate motor units, the plot of reference unit firing frequency is used as the estimate of synaptic input from descending drive.

CAVEATS OF THE PAIRED MOTOR UNIT TECHNIQUE

PHYSIOLOGICAL ASSUMPTIONS MADE TO LEGITIMIZE PIC ESTIMATION USING PMU RECORDINGS

Several assumptions exist in order to interpret paired motor units ΔF values as a valid measure of PIC. In animal models synaptic input can be measured directly(Powers, Nardelli, & Cope, 2008), but in human research this is not possible. Thus, with several assumptions to assert the human model is acting in a predictable manner, PIC can be estimated using firing patterns from two similar motor units, a pair. The first assumption is that the firing rate of the lowest-threshold ('reference') unit recorded in the ramp contraction is an accurate estimate of net excitatory synaptic drive (Gorassini et al., 2002; Heckmann et al., 2005). Crucial to demonstrating amplification of synaptic input it to have a quantifiable synaptic input to begin with. The next assumption is that there is PIC saturation of the reference unit; meaning the frequency current of that neuron is in a steady state where it can be a linear index of synaptic input. For the second assumption, the reference unit must be a sensitive indicator to the adjustments in synaptic input, with firing rate fluctuating proportionally to the increase in drive to the motor neuron (Gorassini et al., 2002). This is important because only times of test unit recruitment and derecruitment are used to determine ΔF . Finally there is the assumption that the reference unit and the test unit share common synaptic drive. Logically if there is not a common synaptic drive,

reference unit firing rates at test recruitment would have nothing to do with the level of synaptic input to recruit the test unit. It is assumed that PIC saturation of the reference unit is reached before test unit onset. Time to PIC saturation is long (up to 2 seconds), and is reflective of the non-linear portion of motor unit firing rate when there is not a fixed relationship between firing frequency and net input current(Gorassini et al., 2002; Udina, D'Amico, Bergquist, & Gorassini, 2010). Again, before PIC saturation it cannot be assumed that reference unit firing rates are a linear indicator of input to the cell.

VALIDATION CRITERIA EMPLOYED TO ENSURE THE PHYSIOLOGICAL ASSUMPTIONS ARE MET

The work of Stephenson and Maluf to outline criteria for reducing within subject variability of ΔF (Stephenson & Maluf, 2011) but also the validity of ΔF , provides this investigation with a number of validation tests for standing estimates to meet. This study examined an extremely large amount of single motor unit recording data and analyzed it using the paired-motor unit technique to outline specific criteria to reduce variability and increase validity. To satisfy assumptions and obtain the most valid data this study employs each of the 5 recommendations for choosing motor unit pairings. Each recommendation is made in order to ensure the physiological assumptions made by the technique explained earlier remain intact and ΔF is a valid measure of PIC in humans. These ‘validation criteria’, as they are commonly referred to, are laboratory calculations that exist to verify the physiological assumptions based on motor neuron firing rate behaviors under various circumstances. In the same order the physiological assumptions were listed, the criteria to validate the technique are as follows 1) A minimum of 1 second be left between recruitment of the reference unit and the recruitment of the test unit. This ensures PIC saturation of the reference unit and ΔF is not being calculated during the primary phase of firing. 2) There

is a rate-rate correlation coefficient greater than $r=0.7$. This ensures the 2 units have similar levels of descending drive and satisfies one of the assumptions of the paired-motor unit technique(Gorassini et al., 2002). 3) The rate modulation of the reference unit must not be within 0.5pps of ΔF . This is indicative in either a saturation of discharge rate in the reference unit or recruitment of the test too close to peak force. 4) Duration of test motor unit activity should be kept in a similar range whenever possible. This reduces the contaminating effect spike frequency adaptation can have on ΔF . 5) The rate of firing rate modulation should not be above 1pps. This ensures that firing rate is slow enough to ensure PIC saturation before additional units are recruited. Firing rates increase over 1pps show inflated PIC.

OTHER CONSIDERATIONS FOR VALID PMU TECHNIQUE PIC ESTIMATION

Even beyond validity criteria for the paired motor unit technique, there are several experimental considerations for reliable data collection. These include accounting for the warm-up time of PIC and choosing motor unit pairs with recruitment at similar activation levels. Findings by Bennett & Hultborn (1998) indicate PIC has a prolonged warm-up time, beyond the scope of PIC activation. As described previously, PIC takes several seconds to activate once a motor unit has been recruited(David J Bennett et al., 1998; Gorassini et al., 2002). However, there is also a long duration warm-up time that can occur as an experiment progresses. Continued activation of PIC-dependent, slow motor units leads to an increase in neuromodulatory drive to the motor neuron pool. This up regulation of PIC serves to alleviate demand from descending cortical drive, allowing the system to perpetuate signals, using ascending input to regulate firing rates(Heckman et al., 2009). Implications for the current study are likely larger for standing collection as more generalized activation is required to maintain balance. Seated measures will likely have

less non-linearity introduced via- warm-up but it is equally important to recognize and mitigate the possibility of insufficient PIC warm-up. Similarly to warm-up considerations, investigations have shown that ΔF is positively correlated to the percentage of muscle activation at test unit onset (Stephenson & Maluf, 2011). While this is not part of the validity criteria needed to prove satisfaction of PMU technique assumptions, it is a necessary consideration in order to provide reliable PIC estimates. Investigations using this technique should only compare motor unit pairs with test unit activation at similar activation levels at the point of test unit recruitment.

OTHER MOTOR NEURON PROPERTIES CONTRIBUTING TO CHANGES IN EXCITABILITY

Paramount to the discussion of estimated PIC measurement (ePIC) using the PMU technique is that ΔF is a function of PIC with very few other contributing factors. In order for this to be true, ΔF should be a good reflector of changes in PIC; and minimally affected by changes to other intrinsic motor neuron properties. However, the validity of the paired-motor unit analysis has recently been questioned in a simulation study conducted by Revil & Fuglevand (2012). This suggests that a single ramp contraction does not isolate for PIC. The longer the rate of rise in the ramp the more susceptible ΔF becomes to contamination by spike threshold accommodation (Figure 2.6).

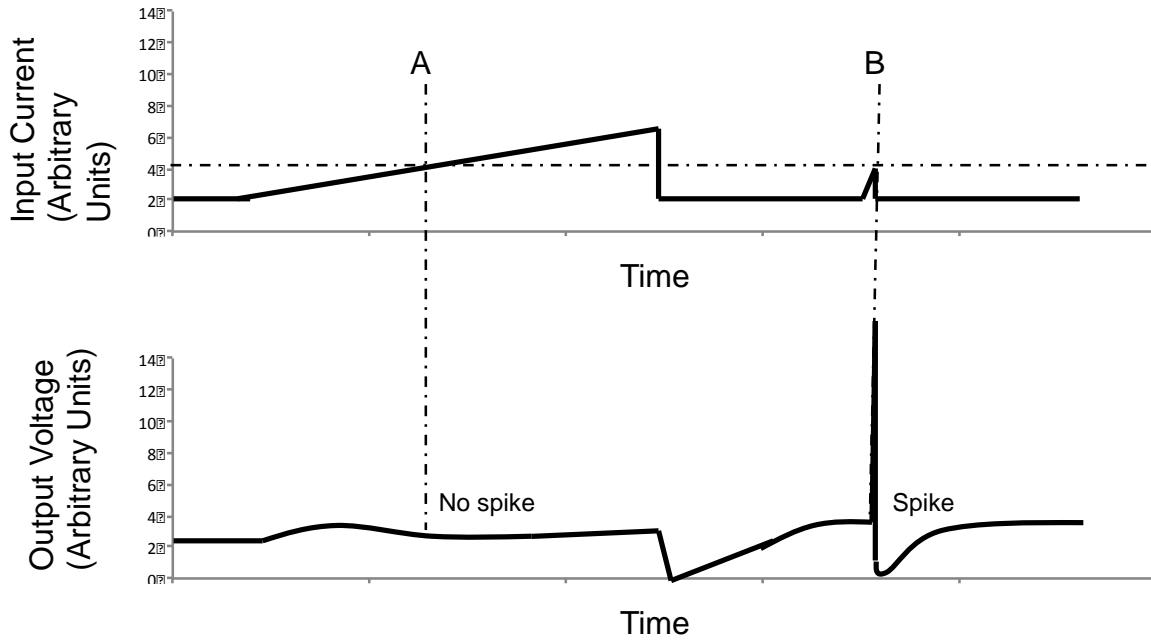


FIGURE 2.6: SPIKE THRESHOLD ACCOMMODATION is the increase in membrane firing threshold in response to slowly rising depolarizing currents. In this example, a current input of 2 arbitrary units (top panel, A) does not generate a spike (bottom panel, A) when current rises slowly. When current rises quickly (top panel, B) then the same current (2 arbitrary units) will generate a spike (bottom panel, B). Adapted from a lecture by Yaeger, L. *Neural Networks: Spike Neuron Models*, Indiana University

Additionally the longer the duration of the total ramp time the more susceptible ΔF becomes to contamination by spike frequency adaptation (Figure 2.7).

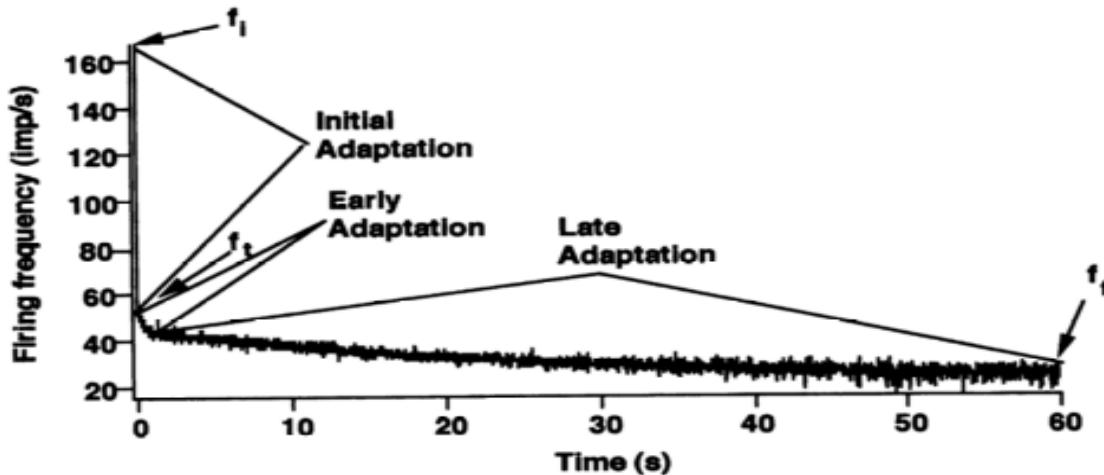


FIGURE 2.7: SPIKE FREQUENCY ADAPTATION is the decline in firing rate of a neuron over time in response to a stable current input. Initial adaptation takes place in the first 1-6 ISIs. Early adaptation takes place up to 2 seconds post-recruitment and late adaptation is a slow exponential decrease beyond this point for as long as activation occurs. (Sawczuk, A., Powers R. K., 1995)

The authors suggested the use of a series of ramps using differing rates of rise and differing plateaus to clarify what proportion of the ΔF value obtained is truly PIC. For example, if a ramp is too short it does not allow for PIC saturation before onset of the test unit. However, if the rate of rise is too slow SFA will inflate ΔF value as a function of time. Moreover, if the total ramp time is too long SFA will increase ΔF also as a function of time (Revil & Fuglevand, 2012). A recent study from this laboratory using the paired motor unit technique has found results very similar to the simulation findings of Revil & Fuglevand using human PMU recordings(Vandenberk & Kalmar, 2014). SFA and STA both introduce nonlinearities into current estimates of PIC. However, measuring reciprocal inhibition at several joint angles allowed the investigator to conclude that although there are confounding variables, shorter ramps, approximately 10s in duration with no plateau

still serve as a valid estimate of PIC. This study will employ the paired-motor unit technique as well as reciprocal inhibition while following these new guidelines.

WHAT TRADITIONAL HUMAN PIC ESTIMATES ARE MISSING

Currently paired motor unit assessments of PIC occur during isometric contractions with the participant seated. This increases the repeatability of recordings in addition to reducing the influence of joint synergists in force production. This increases signal clarity but removes the ability to generalize the findings to human postural control and dynamic movement. Moreover, the highly state dependent nature of intrinsic excitability makes generalization between postures difficult. Locomotor behavior can affect the voltage threshold of a neuron (Krawitz et al. 1996) and neuromodulatory input changes with the speed of locomotion(Jacobs & Fornal, 1999a; Jacobs et al., 2002).

Standing posture changes the activity of several pathways that would possibly alter PIC in motor neurons. One that has yet to be discussed in this review is the input of the vestibulospinal tract onto the motor neuron. Movement of the head results in the displacement of fluid in the sensory organs (semicircular canals and otoliths); fluid pushing on a structure known as the cupula transduces rotation (semicircular canals) and acceleration (otoliths) into neural signals though sensory neurons attached to hair cell receptors(Day & Fitzpatrick, 2005). Sensory signals from the hair cells are relayed to bilateral cerebellar nuclei(Liang, Bácskai, Watson, & Paxinos, 2014). Animal studies have shown that the direct pathway from vestibulospinal nuclei onto the spinal motor neurons work via reflex modulation and serve as the system's gyroscope, determining the head's relative orientation in space(Ijspeert, 2002). Descending vestibulospinal input onto interneurons that participate in central pattern generators (Sasaki, Asawa, Katsuno, Usami,

& Taguchi, 2001) of the spinal cord also becomes more active during standing than sitting(Highstein & Holstein, 2012). The majority of descending vestibular tracts that originate in the Dieter's nucleus project to upper limb motor neurons. The remaining projects to lower limb motor neurons are diffuse and deliver small depolarizing stimuli, EPSPs (Westcott, Powers, Robinson, & Binder, 1995). However, although synaptic potentials appear to remain the same across the motor pool, the effective synaptic current has been shown to be larger in F type motor units compared to S type units(Westcott et al., 1995).

With the introduction of postural sway to a paired motor unit protocol, vestibulospinal input previously absent in the traditional seated paradigm increases the synaptic input to the motor neuron. This is not ideal as the PMU technique assumes synaptic input is increasing in a linear fashion due to increased descending drive. However, because the units being measured are predominately low threshold, slow motor units, less affected by vestibulospinal depolarization, we can cautiously move forward with investigation of PIC using a postural sway.

This project aims to use adapt the conventional seated paradigm to estimate PIC in humans to a paradigm that employs a standing posture, something functionally relevant to postural control, which has not yet been attempted.

HYPOTHESES

- 1) PMU recordings made during anterior postural sway will meet previously published validation criteria used to ensure that the physiological assumptions underlying the PMU technique are met.

- 2) ΔF will be larger in the standing condition compared to the seated condition, while all validation criteria are satisfied, indicating a valid measure of PIC

**CHAPTER 3: ESTIMATES OF PERSISTENT INWARD CURRENT IN
HUMAN MOTOR NEURONS DURING
POSTURAL SWAY**

Ryan C. A. Foley, Jayne M. Kalmar

Department of Kinesiology and Physical Education
Wilfrid Laurier University
Waterloo, Ontario, Canada

INTRODUCTION

Persistent inward current (PIC) is a depolarizing influx of Ca^{2+} into the somatodendritic region of a motor neuron(Heckman et al., 2008). Although facilitated by voltage-gated Ca^{2+} channels(David J Bennett et al., 1998), the magnitude of this depolarizing current is controlled by monoamines via G-protein linked, metabotropic membrane receptors(R. Lee & Heckman, 1999). PIC acts to increase the excitability of a motor neuron resulting in a larger output for a given input; analogous to how a gain dial on an amplifier increases the outgoing signal(Heckman et al., 2009). Monoaminergic input to motor neurons exists through direct projections originating in brain stem nuclei that produce these neuromodulatory monoamines (serotonin (5HT) and norepinephrine (NE)). Thus, these neuromodulatory tracts have the ability to adjust motor neuron gain through the differential release of 5HT or NE(Hounsgaard & Hultborn, 1988).

Many recent investigations have set out with the goal of determining validity for paired motor unit estimates of persistent inward current (PIC). These estimated PIC (ePIC) experiments have used interventions and technique variations to outline criteria needed to validate assumptions of the paired motor unit technique(Stephenson & Maluf, 2011; Wienecke, Zhang, & Hultborn, 2009). Other investigations have made recommendations on optimizing the parameters of the ramp contractions performed during collection(Revill & Fuglevand, 2011; Vandenberk & Kalmar, 2014). This investigation aimed to further progress persistent inward current research using a new variation of the paired motor unit technique.

Several reviews and articles have cited the likelihood of PIC as a mechanism to decrease central drive while maintaining postural muscle activation(Brownstone, 2006;

Heckman et al., 2009; Johnson & Heckman, 2010). This is a logical assertion as slow motor units that make up the majority of anti-gravity musculature have the largest recorded contribution of PIC to their overall excitability. Along the same path of reasoning, it has been hypothesized that PIC varies in a state and task dependent fashion (Heckman et al., 2009; Hyngstrom, Johnson, Miller, & Heckman, 2007) . Expectedly, this change in motor neuron excitability would be driven by differential release of monoamine through neuromodulatory tracts during different arousal states. Previous work has indirectly shown this increase in neuromodulation to the motor neuron during differential arousal through firing of brain stem neurons where descending monoaminergic drive originates (Jacobs et al., 2002). This indirect measure of PIC activation has been shown to vary during sleep states as well as high arousal compared to resting levels(Trulson, Jacobs, & Morrison, 1981). Particularly interesting to the current study was the finding indicating variation in neuromodulatory drive with a change in task(Veasey & Fornal, 1995), such as sitting to walking and further to running(Jacobs & Fornal, 1999b). These findings provided sufficient evidence to warrant an investigation into the task dependency of PIC in human motor neurons.

Currently, most persistent inward current estimates in human motor neurons come via the paired motor unit (PMU) technique. Over the last decade, the technique has evolved to have specific criterions to validate assumptions made when estimating excitability from repetitive firing of motor unit action potentials. Beyond validation, several studies have used the PMU technique with interventions to explore the range of human motor neuron gain. Some of these findings reflect increased PIC with chronic spinal cord injury (Norton, Bennett, Knash, Murray, & Gorassini, 2008; Venugopal,

Hamm, Crook, & Jung, 2011) or with drug administration such as amphetamine (Udina et al., 2010) or caffeine (Walton et al., 2002)

Traditionally PMU ePIC measurements of the lower leg postural muscles occur with the shank clamped in a plantarflexion dynamometer, or McComas Boot (Figure 3.1). This method is quite successful at isolating the soleus from the gastrocnemius by mechanical advantage and the best way to obtain controlled voluntary contractions. However, in order to best serve the experimental protocol the body is taken out of any scenario resembling postural control. While there remains a multitude of questions to be answered by way of seated ePIC measurements, this study set out to provide insight on a more functionally relevant estimate of human persistent inward current.

This investigation aimed to create a novel standing protocol to mirror the way in which seated ePIC measures take place. This involved having a participant standing on a custom platform (Figure 3.2) that measured pressure change only in the anterior/posterior direction. Thus, a voluntary forward postural sway was now equivalent to the plantarflexion ramp contractions commonly used in PMU studies.

It was hypothesized that PMU recordings made during anterior postural sway would meet previously published validation criteria used to ensure that the physiological assumptions underlying the PMU technique are met. In PMU recordings that satisfied all validation criteria, indicating a valid measure of PIC, ΔF would be larger in the standing condition compared to the seated condition.

METHODS

PARTICIPANT RECRUITMENT

A total of 10 participants (6 female) aged 19-25 (mean=22.4 ± 1.64) were recruited for this investigation resulting in 15 useable motor unit pairs (n=15; 6 participants had one useable motor unit pairs, 3 participants had two useable motor unit pairs, 1 participant had three useable motor unit pairs). Prior to participation, participants were screened to ensure no prior history of neurological disease, recent leg injury (past 6 months), recent concussion (past 6 months) and no chronic use of substances that may alter neural excitability such as nicotine, amphetamines or selective serotonin re-uptake inhibitors (SSRIs). This project was approved by the university ethics board and conforms to the Declaration of Helsinki. All participants provided written informed consent.

Prior to study enrollment, prospective participants were completed an orientation to familiarize them with the standing postural sway task, seated isometric torque plantarflexion and to ensure that they were comfortable with the intramuscular EMG electrodes. The 30-minute orientation served to answer any participant questions and to allow them to practice the ramp contractions such that they could perform these ramps with accuracy.

EXPERIMENTAL PROTOCOL & DESIGN

To investigate state-dependent changes in PIC a novel standing postural sway technique was developed and tested along with traditional paired motor unit estimates of persistent inward current. Testing began after recording and stimulation electrode set-up with the person in an upright, seated posture (3.3). The participant slowly rose to a standing posture with their right foot on the custom force platform. Their left foot was

positioned on a fixed platform of equal height. Six inches between the feet allowed for a comfortable and stable stance. The participant performed a series of standing ramp contractions followed by standing reciprocal inhibition measurement. With the help of the experimenter, the participant then slowly transitioned (a distance of 1m between apparatus) to a seated posture and placed their right foot into a McComas boot(Figure 3.3). The participant performed seated ramp contractions followed by seated reciprocal inhibition collection. To ensure repeatability of measures and minimal intramuscular electrode shift, the participant rose again to perform a single standing ramp contraction.

APPARATI

To estimate persistent inward current during isometric plantarflexion contractions in a seated position, the right leg was positioned in a McComas boot lower leg dynamometer (Marsh, Sale, McComas, & Quinlan, 1981) custom built by York University Technical Department (York University, Toronto, Ontario, Canada). A built-in transducer measured isometric plantarflexion and dorsiflexion torque. Isometric plantarflexion amplitude was displayed on a computer monitor and participants were asked to trace a triangular ramp contraction to 10% MVC. Ramp contractions were a constant 5s to peak and 10s in total duration. Data for the standing protocol was collected with the participant positioned with feet five inches apart, hand at their sides and looking straight ahead. A custom-built force platform measured anterior posterior sway. This was achieved using a load cell (S-type load cell, Durham Instruments Inc., Pickering, Ontario, Canada) anterior to the toes to reflect an anterior shift in the area of the foot bearing weight. This provided clear biofeedback for anterior postural sway. As the participant leaned forward, and an increase in pressure was placed on the forefoot, an increase in force could be seen on the screen (Figure 3.4). The output of the load cell

was zeroed to a resting stance when the participant was asked to maintain a comfortable stance without any forward sway. As a participant leaned forward there was a positive deflection in the force, indicating forward sway, which returned to baseline as they returned to a resting stance. Peak force was designated as the most forward leaning position the participant could consistently achieve and maintain for three seconds while their heels remained in contact with the platform. This maximum forward lean was used as the benchmark for ramp height and as a plantarflexion MVC is not possible during standing posture one was not recorded.

PAIRED MOTOR UNIT TECHNIQUE FOR PIC ESTIMATION

The paired motor unit technique used to estimate pic in this investigation requires a comparison of the firing rates of two motor units from the ramp contraction. The first unit to be recruited, and lowest threshold in the ramp, is referred to as the ‘reference unit’. A second, higher threshold unit, recruited later in the ramp is used as the ‘test unit’. Instantaneous firing rate was plotted for both units over the duration of the best ramp performed. Plots were fitted with a fourth order polynomial curve to obtain smoothed firing frequency for any given time in the ramp. Estimation of persistent inward current was obtained by calculating the difference in reference unit firing frequency between points of test unit onset and offset. This difference in firing frequency, known as ΔF , reflects prolonged test unit firing that persists beyond the removal of synaptic drive needed to originally activate the unit.

PSTH TECHNIQUE FOR RECIPROCAL INHIBITION COLLECTION

Reciprocal inhibition of each reference motor unit was estimated using the post-stimulus time histogram (PSTH) technique (Aymard, Chia, Katz, Lafitte, & Pénicaud, 1995). Electrical stimulation of the common peroneal nerve (CPN) activated the

reciprocal inhibitory pathway at the axons of 1a afferent sensory neurons during a sustained low-level contraction in which the participant had only one or two active units visible on the intramuscular EMG recordings. Once a constant firing rate of the reference soleus motor unit was established a sequencing script was used to elicit soleus spike-triggered stimulation of the common peroneal nerve approximately. The sequence was set up to deliver pulses to the common peroneal nerve approximately 80ms prior to a soleus motor unit firing to optimize reciprocal inhibition of the soleus motor unit (Figure 3.5). 80 sub-motor threshold stimuli were delivered over four minutes of the constant low-level plantarflexion contraction.

ANALYSES

PAIRED MOTOR UNIT RECORDINGS: Single motor unit recordings were sorted online by a spike-sorting algorithm using Spike2 software (version 7.02, CED Limited, Cambridge, England). Spike recordings were recognized based on amplitude and shape and fit into templates for each active motor unit (Figure 3.6). However, several units in each ramp had their shape skewed when multiple units fired simultaneously. In order to rectify that the unit of interest had indeed fired manual inspection and sorting of the spike data was needed. Prior to manual sorting, recordings were subjected to an offline hum-remove filter prior to analysis. This filter (Figure 3.7) decomposed repetitive sequences of oscillating baseline noise to aid manual sorting of motor units missed by online sorting. Once all spikes fired for the motor unit of interest had been identified during a ramp instantaneous firing frequencies for both reference and test unit were exported and plotted as previously described as per the paired motor unit technique.

To assert that ΔF is a dependable estimate of persistent inward current, there are several physiological assumptions made by the paired motor unit technique that must be accurate. This is done through the use of validation criteria, which PMU recordings must meet in order to be considered as useable data before statistical analyses. While several independent investigations have contributed to the exact criteria needed for validation, it was critical for the current investigation to examine all possible points of error in the paired motor unit technique before conclusively determining a standing variation to be a valid estimation of PIC. This examination began with an affirming review of the assumptions the paired-motor unit technique makes and why.

The first major assumption of the technique is that there is a shared, common synaptic drive to both the reference and the test unit. A difference in drive between the two units of a pair would be undeniable evidence that any PIC estimation from that data is void. Several studies recommend inspection of motor unit pair rate-rate correlations to (Powers et al., 2008) validate the shared drive assumption (Gorassini et al. 2002, Stephenson & Maluf, 2011, Udina et al. 2010). The rate-rate correlation coefficient is then a measure of common synaptic modulation between two concurrently active motor units. This coefficient was calculated by plotting averaged instantaneous firing frequency (200ms bins(Powers et al., 2008)) for both reference and test unit for the duration of the ramp. Mean firing frequency values were correlated to obtain a Pearson's r, correlation coefficient (Figure 3.8). A minimum value of $r = 0.7$ or $r^2 = 0.5$ is need to pass the paired motor unit technique assumption that there is equally shared synaptic drive to both reference and control unit. For this experiment all motor unit pairs with a $r^2 < 0.5$ were excluded from statistical analysis.

Paired motor unit analysis also assumes full activation of the reference unit at the time of test unit recruitment. A corollary to this is that the reference unit is a linear indicator of the next excitability of the motor neuron. PIC is a long lasting depolarization, however it is also has a relatively slow activation (Bennett 2001). Previous research has shown that it may take up to 2s for full PIC onset, or saturation (Udina 2010). If the test unit is recruited before PIC saturation of the reference unit, while the reference unit is in an unstable state of excitability, the assumption is broken. However, experimental data has shown that only recruitment intervals below 1s have poor validity, likely with shorter durations leading to smaller, and sometimes negative ΔF values. This investigation insured that only motor unit pairs with recruitment intervals $>1s$ were analyzed.

In addition, the paired-motor unit technique assumes that the reference motor unit firing rate is sensitive enough to detect changes in the next excitatory input. Meaning, if continually increasing excitation was supplied to the motor neuron, the firing rate would increase proportionally. The index of excitability the reference unit firing rate provides is a strong factor in the validity of ΔF . To eliminate cases where the reference unit firing rate was saturated after test unit recruitment, and did not increase sufficiently to satisfy the aforementioned assumption, a validation criterion for rate modulation was introduced. Rate modulation is calculated as difference between the range of reference unit firing range ($ff_{max} - ff_{min}$ on Figure 3.9) and ΔF for that motor unit pair. Motor unit pairs with a reference unit rate modulation value within 0.5pps of ΔF do not satisfy the assumption of equal and continually increasing excitation to all motor unit in the pool.

Similarly to the previously mentioned assumption that reference unit firing frequency can only be a linear indicator of excitability once PIC saturation has occurred, ΔF is only truly estimated when done so under test unit PIC saturation. Several studies have investigated PIC saturation times yielding somewhat unanimous results. Two seconds of test unit activity is required to assert with reasonable certainty that ΔF is an accurate estimate of persistent inward current. As such, only test units with a minimum of 2s of consistent firing were included in analysis.

The idea that human ΔF measures are reflective only of persistent inward current is not a traditional assumption like the previous three discussed, but if this were to be proven untrue, PMU would prove far less useful for understanding motor neuron excitability. First shown in a simulation study (Revil & Fuglevand 2012) and later in humans (Vandenberk & Kalmar 2014) other motor neuron properties such as spike frequency adaptation and spike threshold accommodation can heavily influence persistent inward current if specific ramp parameters are not met. More specifically, the longer the total duration of the ramp, there is an increase in spike frequency adaptation. Where SFA is a time-dependent phenomenon, spike threshold accommodation increases with slower rates of rise. Although the direct contributions of each property (PIC, SFA, & STA) to ΔF were quantified in the isolated computer simulations (Revil & Fuglevand 2012), *in vivo* study cannot make the same distinction. Rather, a novel use of the relationship between PIC and reciprocal inhibition at different joint angles was exploited to verify results found through the simulations (Vandenberk & Kalmar 2014). As such, both studies recommend limiting rate of rise to ~2% MVC/s and total ramp duration of 10s.

Beyond the ramp criteria needed to satisfy assumptions of the paired motor unit technique are some other aspects of muscle activation and motor unit firing characteristics that could have varied between postures. It has been shown that ΔF is positively correlated with muscle activation at test unit onset (Stephenson and Maluf 2011). Soleus muscle activation at test unit recruitment was calculated by normalizing 0.5s (0.25s before and 0.25s after the point of recruitment) of soleus surface EMG during the ramp contraction to 0.5s of soleus surface EMG during an MVC. This data was then compared to provide a normalized estimate of muscle activation at both test and reference unit recruitments between standing and seated.

RECIPROCAL INHIBITION: A post-stimulus time histogram was used to quantify this difference in firing times for a span of up to 0.4s using 50ms bins. A difference histogram was created from the control PSTH and stimulation PSTH. A cumulative sum of this difference histogram was then plotted with a larger deviation below the x axis denoting larger inhibition (Figure 3.10). Where past investigations have used the peak negative value, in the 180ms-305ms range to identify inhibition during the 2nd ISI, this investigation used trapezoidal integration (MS Excel 2007) over the same window. Area under the curve was found to be more reflective of total occurring inhibition and resistant to large, but brief deflections.

DATA ACQUISITION

Data acquisition and analysis were completed using Spike2 software (version 7.02, CED Limited, Cambridge, England). Analog-to-digital conversion and sequencing of electrical stimuli were carried out through a 64-bit Micro1401-3 unit (CED Limited, Cambridge, England).

INTRAMUSCULAR EMG: Single motor unit recordings were obtained using 50.8- μm Formvar-insulated stainless steel wires (California Fine Wire Company, Grover Beach, CA, USA). Three wires were inserted into the lateral aspect of the soleus (2 cm distal to the inferior border of the gastrocnemius lateral head as determined by muscle palpation) on the right leg using a 27-gauge BD PrecisionGlide™ Needle (Becton, Dickinson Company, Franklin Lakes, NJ, USA)(Figure 3.11). The electrode was secured using a hooked end on the wires, which provided stability through basic movements but was easily removed upon experiment completion. To complete the electrode set-up, the wires were input into a 10x preamplifier (EQ electrodes, Chalfont, PA, USA) secured to the leg with an adhesive pad.

SURFACE EMG: Ag-AgCl electrodes epoxy-embedded with a x10 preamplifier (EQ electrodes, Chalfont, PA, USA) were positioned over the tibialis anterior and lateral soleus (Figure 3.11). Electrodes had recording surfaces of 0.5cm^2 and an interelectrode distance of 1.2 cm. Preamplifiers input to a custom-built, variable gain 2nd stage amplifier (York University Machine Shop, Toronto, Canada). A ground was placed on the medial tibia. All skin contacts were cleaned using scrubbing alcohol pads and electroconductive gel was applied to contacts to enhance the electrode-skin interface. Intramuscular EMG signals were sampled at 20,000 Hz with all other surface EMG inputs sampled at 2,000Hz. Force was sampled at 150Hz from both the force platform and the McComas boot. Online filtering of intramuscular signals was performed using a Neurolog System (Amplifier insert: NL106, Filter insert: NL126, Digitimer Inc., Hertfordshire, England). An online band-pass filter was applied to intramuscular recordings, attenuating signal outside a 200-3,000 Hz range. Low-end cut off was altered

slightly to optimize signals during each experimental session. Surface EMG was highpass filtered with a corner edge cutoff frequency of 20Hz, as recommended (De Luca 2010, Winters 1980). Force data was low-pass filtered online at 50Hz and all data was subjected to an online 60Hz notch filter.

NERVE STIMULATION: A 2.5cm² carbonized rubber stimulation electrode was positioned over the common peroneal nerve, just lateral to the head of the fibula (Figure 3.11) to activate the nerve to the antagonist when measuring reciprocal inhibition of the soleus. A Digitimer constant current stimulator (model DS7AH, Digitimer Inc., Hertfordshire, England) was used to deliver stimuli for reciprocal inhibition quantification. Stimulation to elicit reciprocal inhibition was set at 80% of soleus motor threshold (defined as the stimulus intensity needed to elicit a >50µV response tibialis anterior Mwave for at least 50% of stimulations). Threshold was assessed during a comfortable standing position. All pulses were 1ms in duration.

STATISTICAL ANALYSES

Prior to statistical analyses data were inspected for outliers using an acceptance range of ± 2 standard deviations. Identified outliers were replaced using mean substitution. Two-tailed dependent samples t-tests with an alpha level set at 0.05 were used to compare means of standing and seated conditions in all variables measured. All statistical tests were carried out using STATISTICA software built in t-test and correlation functions (Statsoft Inc., Tulsa, OK, USA). Finally a correlation between ePIC (ΔF) and RI was conducted only for those participants, which demonstrated reciprocal inhibition.

RESULTS

ASSUMPTIONS OF THE PAIRED MOTOR UNIT TECHNIQUE

All motor unit pairs had a test unit activation ≥ 1 s after reference unit recruitment (2.28 ± 0.91 s), ensuring complete PIC activation of the reference unit. Pearson correlations for each motor unit pair met the $r^2 \geq 0.5$ requirement ($r^2 = 0.85 \pm 0.13$) to ensure that reference unit and test unit share a common level of synaptic drive (Table 3.1). The rate modulation (difference between the range of reference unit firing range [$ff_{max} - ff_{min}$]) and ΔF for a motor unit pair) for each ramp was ≥ 0.5 (2.61 ± 1.38). Finally, duration of ramp rise (5.57 ± 0.64 s) and decline (5.83 ± 0.50 s) corresponded with previously recommended duration guidelines to minimize the contributing effects of other intrinsic motor neuron properties that could contribute to nonlinear firing (Revill & Fuglevand, 2011; Vandenberk & Kalmar, 2014).

PERSISTENT INWARD CURRENT AND RECIPROCAL INHIBITION

A two-tailed t-test revealed there was no main effect of posture on estimates of spinal excitability. No difference was found between postures ($p=0.442$) with mean standing ePIC measurements of 2.44 ± 1.17 pps) and seated estimates of 2.73 ± 1.20 pps. Mean and individual data can be seen in Figure 3.12 (left). Figure 3.12 (right) also depicts the mean and individual reciprocal inhibition data between postures. A standing posture resulted in significantly less ($p>0.001$) reciprocal inhibition (0.003 ± 0.025) than in a seated position (-0.038 ± 0.042). There was no relationship between PIC and RI for standing or seated posture when ΔF and CumSum area were correlated (standing: $r=-.224$, $p=0.421$; seated: $r=.232$, $p=0.405$). Pearson analysis of the difference in ePIC between in standing compared to seated posture ($\Delta\Delta F$) and the difference in

inhibition between postures (Δ RI) was moderately correlated ($r=0.934$, $p<0.001$, *RI data with a positive value was excluded for this correlation) (Figure 3.14).

EFFECT OF POSTURE ON MOTOR UNIT RECRUITMENT AND MUSCLE ACTIVATION

There was no significant ($p=0.233$) in reference unit firing frequency at recruitment between standing and seated as measured with only the first inter-spike interval (standing, 5.06 ± 1.16 pps; seated, 5.49 ± 1.12 pps) or using an average from the first 3 inter-spike intervals (standing, 6.18 ± 1.49 pps; seated, 6.14 ± 0.85 pps; $p=0.094$) (Table 3.2). Similarly, test unit firing frequency at recruitment did not differ between postures using the first inter-spike interval (standing: 5.25 ± 1.51 pps, seated: 4.63 ± 1.69 pps; $p=0.373$) or when estimated using the first three inter-spike interval average (standing: 6.06 ± 1.35 pps, seated: 6.59 ± 1.32 pps; $p=0.300$) (Table 3.2).

Soleus muscle activation at motor unit recruitment was significantly different between postures (Table 3.2) for both the reference (standing: 12.70 ± 5.82 %EMG_{max}, seated: 9.38 ± 4.66 %EMG_{max}, $p>.001$) and test units (standing: 22.66 ± 7.17 %EMG_{max}, seated: 14.38 ± 6.00 %EMG_{max}, $p>.001$). Finally, there was significantly longer time period between reference unit recruitment and test unit recruitment (Table 3.1) in a seated posture (2.55 ± 0.87 s) than a standing posture (2.00 ± 0.90 s) ($p=.020$).

DISCUSSION

Two possible outcomes were hypothesized when this novel protocol was developed to estimate PIC during a standing postural task. The first hypothesis was simply that PMU recordings could be made during standing anterior postural sway that

had recruitment patterns and firing rate modulation similar to those made during seated isometric plantarflexion contractions . The second hypothesis was that if valid PMU recordings could be made during standing anterior postural sway, then , ΔF estimates of PIC made from standing PMU recordings will be greater than seated measures due to increased raphe spinal drive to motor neurons during a standing postural task (Heckman et al., 2009; Jacobs & Fornal, 1999a; Jacobs et al., 2002; Johnson & Heckman, 2010). Alternatively, we hypothesized that standing ΔF estimates of PIC would not satisfy previously published validation criteria ((Gorassini et al., 2002; Powers et al., 2008; Stephenson & Maluf, 2011; Vandenberk & Kalmar, 2014)) due to confounding physiological processes (Wienecke et al., 2009) in which case standing PMU recordings would not be a viable approach to assessing PIC during a functional postural task.

It was expected that ePIC during a standing posture would be significantly greater than that in the seated measurement. Furthermore, it was expected that reciprocal inhibition would be greater in standing than seated. As hypothesized, baseline reciprocal inhibition was significantly lower in standing compared to seated posture as indicated by more negative cumulative sums of the difference PSTH (standing: 0.003 ± 0.025 , seated: -0.038 ± 0.042 , $p<0.001$). Unexpectedly however, standing estimates of PIC were no different than estimates made during seated isometric contractions (standing ΔF : 2.438 pps ± 1.169 , seated ΔF 2.727 pps ± 1.197). The investigation was able to capture postural sway PMU recordings that closely resemble equivalent seated PIC estimates (Figure 3.15).

The current literature provides ample evidence warranting the hypothesis that persistent inward current would increase in the anti-gravity muscles to facilitate standing

posture. PIC plays an important role in motor functionally relevant movement, particularly in the anti-gravity musculature (ElBasiouny et al., 2010; Heckman et al., 2008, 2009; Jacobs et al., 2002; Johnson & Heckman, 2010). In the present study, mean ΔF were nearly identical in standing and seated posture. It should be noted that there is a large range in $\Delta\Delta F$ (the difference between seated and standing ΔF) within participants (individual standing ePIC ranged from 1.5% to 515% of seated ePIC), and it is possible that we did not have adequate statistical power to detect a difference. The motor unit pairs, which exhibited the largest $\Delta\Delta F$, had much greater ePIC standing than seated, exhibiting the hypothesized result of increased PIC with standing posture. Two motor unit pairs obtained from the same participant demonstrated the opposite trend, a drastic increase in ePIC when seated (standing $\Delta\Delta F$ 1.5% and 16% respectively). However, the majority motor unit pairs demonstrated little deviation in ePIC between postures. Even with a sample of fifteen motor unit pairs, which provides adequate statistical power in other studies, (Udina et al., 2010; Vandenberk & Kalmar, 2014), the possibility of type II statistical error cannot be omitted.

Baseline reciprocal inhibition did change as expected with posture; decreasing significantly ($p<0.001$) in the standing measurement (0.003 ± 0.025) when compared to seated measurement (-0.038 ± 0.042). This was expected as there is a slight dorsiflexion about the ankle during standing measurements due to the participant initiated, voluntary lean required to activate the firing of the measured motor unit. Whereas, when using the PSTH technique for seated measurement of RI the plantarflexion torque produced to activate the low threshold unit does not result in movement about the ankle when clamped in a McComas boot (Marsh et al., 1981). Furthermore, functional postural

stability requires co-contraction not present during seated RI measurement. Previous research supports the notion that with standing posture there is a disinhibition of the α -motor neuron via presynaptic inhibition (Cattagni, Martin, & Scaglioni, 2014; Nielsen & Kagamiharat, 1992) possibly explaining the decrease in reciprocal inhibition measured through this investigation. Disinhibition of the alpha motor neuron promotes co-contraction advantageous to maintaining tonic activity of antigravity muscle with only slight changes in activation for postural sway compensatory movements (Katz & Meunier, 1988; Nielsen & Kagamiharat, 1992). However, because reciprocal inhibition and PIC interact in order to create functional movement, the reflex loop is differentially modulated when standing vs. during postural sway. An earlier investigation which estimated reciprocal inhibition at rest using conditioned H reflexes instead of using the PSTH technique (which can only be conducted during voluntary contraction) demonstrated increased reciprocal in individuals in quiet stance (Kasai, Kawanishi, & Yahagi, 1998). However, when dorsiflexion for a postural sway was initiated, a large decrease in RI was measured and hypothesized to promote co-contraction for a more stable support structure for the body. This decrease in RI upon initiation of postural sway is in line with the current investigation and may be a limitation to the investigation of standing PIC-RI interaction using the PSTH technique.

There has been past debate over using ΔF as a true estimate of PIC as several possible confounding factors have been identified (Revill & Fuglevand, 2011; Vandenberk & Kalmar, 2014; Wienecke et al., 2009). However, over several years, the use of the paired motor unit technique has been refined steps taken improve the validity of the method (Stephenson & Maluf, 2011; Udina et al., 2010; Vandenberk & Kalmar,

2014). Several recommendations and criteria have been outlined to ensure that ΔF values derived from paired motor unit recordings provide valid estimates of PIC in the conventional seated posture. The importance of PIC to functionally relevant movement like standing has been previously discussed, but functional PIC estimation has not been examined in humans. It is not known if the criteria outlined to ensure the validity of seated paired motor unit estimates of PIC in the conventional seated posture would be met if the paired motor unit technique was used to assess PIC during a functional and dynamic postural sway. Thus, all validation criteria available to the authors at the time of investigation (Gorassini et al., 2002; Stephenson & Maluf, 2011) were employed to ensure physiological assumptions of the paired motor unit technique were met for standing data. In brief, we found that 1) control motor units were likely to have saturated PICs prior to recruitment of the test motor unit (there was a minimum of 1s between control of test unit recruitment), 2) control and test motor units appear to share a common motor drive (all but three pairs analyzed had rate-rate correlations >0.7), 3) the control motor unit remained a sensitive indicator of changes in synaptic drive (rate modulation of the reference unit was always within 0.5pps of ΔF). Thus, all previously published validation criteria were satisfied in both seated and standing postural sway paired motor unit estimates of PIC.

This investigation conducted a rigorous examination of motor unit firing patterns for possible differences between standing and seated postures to determine whether a standing postural-sway protocol to estimate PIC during functional movement is feasible. Although all motor unit pairs met previously published criteria to provide valid estimates of PIC, we sought to determine whether the measures of validity differed between

postures. Although rate-rate correlations for both postures exceeded the $r^2=0.5$ threshold set by Gorassini et al. (2002) to ensure that control and test motor unit shared a common level of synaptic drive, standing had significantly lower mean rate-rate correlation coefficient than seated. Although minimum accepted criteria were met, lower rate-rate correlations indicate that standing estimates may have scenarios where synaptic drive differs. Studies have shown that presynaptic inhibition, which is a motor neuron specific input (not diffuse throughout the pool such as neuromodulatory input), change with posture (Nielsen & Kagamiharat, 1992). Changes in descending inputs (like presynaptic inhibition) which are more active during functional tasks may disrupt the relationship between motor unit pairs necessary for valid PIC estimation. Another validation criterion that differed between postures was the time between reference and test unit recruitment. Standing recordings showed a significantly shorter recruitment interval (standing: 2.00 s \pm 0.90, seated: 2.55 s \pm 0.87, $p=0.0204$) between reference and test motor units of a pair. Although paired motor units met published criteria for ensuring that the PIC of the reference unit was fully saturated prior to test unit recruitment (minimum of 1s of reference unit firing prior to a test unit onset), it is still possible that the reference motor unit PIC was not fully saturated. This investigation followed recommendations that a minimum of 1s should separate recruitment of reference and test units (D J Bennett et al., 2001; Powers et al., 2008), however other recommendations have called for a separation of 2s or more to allow for PIC saturation of the reference unit (Gorassini et al., 2002; Stephenson & Maluf, 2011). One possibility for the lack of PIC difference measured between postures could be that standing the (assumed) larger PIC during postural sway has a comparably longer time to reach saturation, resulting in an underestimation of

standing PIC. This may also be a consequence of the difference between muscle activation across the conditions. While ramp parameters were similar enough to have the same motor unit pairs activated in sequence in both standing and seated postures, a higher overall level of activation may have recruited additional MUs to the standing ramp contractions. Standing posture, with known increases in co-contraction to stabilize postural sway(Katz & Meunier, 1988; Nielsen & Kagamiharat, 1992), likely requires higher recruitment to overcome antagonist resistance and produce functional movement.

The methodology used in this study employed a number of constraints to ensure differences between postures could not be attributed to nonlinear rhythmic firing properties other than PIC which would contribute to ΔF (such as SFA and STA). These constraints include limiting the duration of the contraction and the rate of force production to minimize the contribution of SFA and STA respectively. These constraints differ from validation criteria in that failing to meet them does not result in data that explicitly violate the physiological assumptions of the paired motor unit technique, but rather increase the likelihood that intrinsic properties other than PIC contribute to ΔF . It has been suggested that ramp duration should be no longer than 10s to provide ΔF values that reflect predominantly PIC (Revill & Fuglevand, 2011; Vandenberk & Kalmar, 2014). Even though participants were instructed to trace ramps with a 5-s rise and 5-s fall, seated ramps had a significantly longer duration of ramp rise (standing: $5.24 \text{ s} \pm 0.37$, seated: $5.90 \text{ s} \pm 0.70$, $p=0.025$). This is a limitation as increased ramp duration has been shown to inflate ΔF most likely via spike frequency adaptation (Vandenberk & Kalmar, 2014). The ramp contraction is a difficult fine motor skill that takes a large amount of practice to perform masterfully and the difference seen is most likely due to the task complexity and

participant focus. However, a difference in duration was not detected for ramp decline, and the difference seen in rise duration (0.65s) can be considered negligible when observing the magnitude or ramp duration increase needed to significantly confound ΔF (Revill & Fuglevand, 2011; Vandenberk & Kalmar, 2014).

One apparent imbalance in the study design is the performance of MVCs in seated posture prior to data collections where no such max efforts predicated standing PIC estimation. This non-counterbalanced design was put in place in an attempt to normalize seated ramp contraction forces across participants. However, it is acknowledged that these maximal effort contractions may have introduced additional non-linearities into the paired motor unit technique. Specifically, a muscle potentiated by previous high force contractions will more demonstrate greater force output with equivalent activation(Hodgson, Docherty, & Robbins, 2005). Postactivation potentiation (PAP) is the result of phosphorylation of myosin regulatory light chain proteins, rendering actin-myosin more sensitive to calcium ions in subsequent activation(Hamada, Sale, MacDougall, & Tarnopolsky, 2000). This potentiation does indeed affect the soleus (Miyamoto, Fukunaga, & Kawakami, 2009) but is a much more prevalent phenomenon in type II and IIA muscle fibers(Hamada et al., 2000). Increased muscle potentiation may have resulted in the overestimation of activation in seated posture based on force output since standing posture did not have a maximal warm up compared to seated measurements. Although this is not ideal for paired motor unit recordings to have confounding variables, standing data collection did closely mimic a functional task, the goal of the experiment, and the effect of potentiation of soleus ΔF should be minimal.

With co-contraction known to aid in executing controlled movement, it is possible that participants used different strategies to achieve the force ramps. Some participants may have used tibialis anterior activation to moderate fluctuating soleus activation during both standing and seated protocols. The investigators realize that differing levels of co-contraction between participants may account for differing levels of observed reciprocal inhibition and possibly PIC. If a participant co-contracted to achieve the necessary ramp contraction it would decrease the reciprocal inhibition to the alpha motor neuron, inflating the estimated PIC value obtained. However, participants showed a significant ($p=0.0147$) decrease in TA activation in seated posture (ramp rise: 0.0067 ± 0.0023 ; ramp decline: 0.0067 ± 0.0023) compared to standing (ramp rise: 0.0268 ± 0.0106 ; ramp decline: 0.0218 ± 0.0078). This indicates that a strategy of increased co-contraction did not account for the increase in seated PIC observed in this investigation. Admittedly, this was not a primary analysis planned during experimental design. As such, there is no tibialis anterior MVC available to normalize RMS amplitude of raw TA surface EMG with. This may create disparities in measured TA activation and percentage of maximal activation between participants based on electrode placement. However, the standard deviations of each condition range similar to the mean with no outliers. Once again this finding indicates that a strategy of increased co-contraction was not a major contributor to the increased PIC observed in the seated posture.

The current findings indicate PIC estimates via the paired motor unit technique have limitations when transferred to a functional postural sway task. Nonetheless, estimates of PIC during a standing posture are still warranted given that PIC is expressed to a greater extent in antigravity muscles (ElBasiouny et al., 2010; Heckmann et al.,

2005; Johnson & Heckman, 2010) and the majority of scenarios where PIC is relevant to human movement are during high force output tasks, scenarios when constant input is not necessary (i.e standing) and locomotion. Future directions might be to make paired motor unit recordings to estimate PIC during isometric contractions in a standing posture. One such apparatus would have the participant performing a standing plantarflexion calf raise against an immovable shoulder restraint. This set-up would mimic the static muscle lengths and joint angles seen in the conventional seated posture but require activation of all antigravity muscle groups and reflect differences in motor neuron gain expected in a standing posture. Further improvements to a standing protocol may result in a functionally relevant variation of the paradigm that would be applicable to many populations experiencing postural control deficits (i.e. older adults, mild traumatic brain injury, stroke, Parkinson 's disease).

REFERENCES

Aymard, C., Chia, L., Katz, R., Lafitte, C., & Pénicaud, a. (1995). Reciprocal inhibition between wrist flexors and extensors in man: a new set of interneurones. *The Journal of Physiology*, 487 Pt 1, 221–35

Bennett, D. J., Hultborn, H., Fedirchuk, B., & Gorassini, M. (1998). Synaptic Activation of Plateaus in Hindlimb Motoneurons of Decerebrate Cats Synaptic Activation of Plateaus in Hindlimb Motoneurons of Decerebrate Cats. *Journal of Neurophysiology*, 80:2023-2037,2023–2037.

Bennett, D. J., Li, Y., Harvey, P. J., & Gorassini, M. (2001). Evidence for plateau potentials in tail motoneurons of awake chronic spinal rats with spasticity. *Journal of Neurophysiology*, 86(4), 1972–82.

Brownstone, R. M. (2006). Beginning at the end: repetitive firing properties in the final common pathway. *Progress in Neurobiology*, 78(3-5), 156–72.

Cattagni, T., Martin, a, & Scaglioni, G. (2014). Is spinal excitability of the triceps surae mainly affected by muscle activity or body position? *Journal of Neurophysiology*, 111(12), 2525–32.

ElBasiouny, S. M., Schuster, J. E., & Heckman, C. J. (2010). Persistent inward currents in spinal motoneurons: important for normal function but potentially harmful after spinal cord injury and in amyotrophic lateral sclerosis. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, 121(10), 1669–79.

Gorassini, M., Yang, J. F., Siu, M., & Bennett, D. J. (2002). Intrinsic activation of human motoneurons: possible contribution to motor unit excitation. *Journal of Neurophysiology*, 87(4), 1850–8.

Hamada, T., Sale, D. G., MacDougall, J. D., & Tarnopolsky, M. a. (2000). Postactivation potentiation, fiber type, and twitch contraction time in human knee extensor muscles. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, 88, 2131–2137.

Heckman, C. J., 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(3), 264–75.

Heckman, C. J., Mottram, C., Quinlan, K., Theiss, R., & Schuster, J. (2009). Motoneuron excitability: the importance of neuromodulatory inputs. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, 120(12), 2040–54.

Heckmann, C. J., Gorassini, M. a, & Bennett, D. J. (2005). Persistent inward currents in motoneuron dendrites: implications for motor output. *Muscle & Nerve*, 31(2), 135–56.

Hodgson, M., Docherty, D., & Robbins, D. (2005). Post-activation potentiation: Underlying physiology and implications for motor performance. *Sports Medicine*, 35(7), 585–595.

Hounsgaard, J., & Hultborn, H. (1988). Bistability of alpha-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *The Journal of Physiology*, 345–367.

Hyngstrom, A. S., Johnson, M. D., Miller, J. F., & Heckman, C. J. (2007). Intrinsic electrical properties of spinal motoneurons vary with joint angle. *Nature Neuroscience*, 10(3), 363–9.

Jacobs, B. L., & Fornal, C. a. (1999). Activity of serotonergic neurons in behaving animals. *Neuropsychopharmacology*, 21(2 Suppl), 9S–15S.

Jacobs, B. L., Martín-Cora, F. J., & Fornal, C. a. (2002). Activity of medullary serotonergic neurons in freely moving animals. *Brain Research. Brain Research Reviews*, 40(1-3), 45–52.

Johnson, M. D., & Heckman, C. J. (2010). Interactions between focused synaptic inputs and diffuse neuromodulation in the spinal cord. *Annals of the New York Academy of Sciences*, 1198, 35–41.

Kasai, T., Kawanishi, M., & Yahagi, S. (1998). Posture-dependent modulation of reciprocal inhibition upon initiation of ankle dorsiflexion in man. *Brain Research*, 792(1), 159–163.

Katz, R., & Meunier, S. (1988). Changes in presynaptic inhibition of 1a fibres in man while standing, 417–437.

Lee, R., & Heckman, C. (1999). Enhancement of bistability in spinal motoneurons in vivo by the noradrenergic α 1 agonist methoxamine. *Journal of Neurophysiology*, 2164–2174.

Marsh, E., Sale, D., McComas, J., & Quinlan, J. (1981). Influence of joint position on ankle dorsiflexion in humans. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, 51(1), 160–7.

Miyamoto, N., Fukunaga, T., & Kawakami, Y. (2009). Evidence for intermuscle difference in postactivation potentiation in the human triceps surae: A mechanomyographic study. *Muscle and Nerve*, 39(May), 703–706.

Nielsen, B. Y. J., & Kagamiharat, Y. (1992). The regulation of disynaptic reciptoca Ia inhibition during co-contraction. *Journal of Physiology, Paris*, 373–391.

Norton, J. a, Bennett, D. J., Knash, M. E., Murray, K. C., & Gorassini, M. a. (2008). Changes in sensory-evoked synaptic activation of motoneurons after spinal cord injury in man. *Brain: A Journal of Neurology*, 131(Pt 6), 1478–91.

Powers, R. K., Nardelli, P., & Cope, T. C. (2008). Estimation of the contribution of intrinsic currents to motoneuron firing based on paired motoneuron discharge records in the decerebrate cat. *Journal of Neurophysiology*, 100(1), 292–303.

Revill, A. L., & Fuglevand, A. J. (2011). Effects of persistent inward currents, accommodation, and adaptation on motor unit behavior: a simulation study. *Journal of Neurophysiology*, 106(3), 1467–79.

- Stephenson, J. L., & Maluf, K. S. (2011). Dependence of the paired motor unit analysis on motor unit discharge characteristics in the human tibialis anterior muscle. *Journal of Neuroscience Methods*, 198(1), 84–92.
- Trulson, M., Jacobs, B., & Morrison, A. (1981). Raphe unit activity during REM sleep in normal cats and in pontine lesioned cats displaying REM sleep without atonia. *Brain Research*, 226, 75–91.
- Udina, E., D'Amico, J., Bergquist, A. J., & Gorassini, M. a. (2010). Amphetamine increases persistent inward currents in human motoneurons estimated from paired motor-unit activity. *Journal of Neurophysiology*, 103(3), 1295–303.
- Vandenberk, M. S., & Kalmar, J. M. (2014). An evaluation of paired motor unit estimates of persistent inward current in human motoneurons. *Journal of Neurophysiology*, 111(9), 1877–84.
- Veasey, S., & Fornal, C. (1995). Response of serotonergic caudal raphe neurons in relation to specific motor activities in freely moving cats. *The Journal of Neuroscience*, 15(July), 5346–5359.
- Venugopal, S., Hamm, T. M., Crook, S. M., & Jung, R. (2011). Modulation of inhibitory strength and kinetics facilitates regulation of persistent inward currents and motoneuron excitability following spinal cord injury. *Journal of Neurophysiology*, 106(5), 2167–79.
- Walton, C., Kalmar, J. M., & Cafarelli, E. (2002). Effect of caffeine on self-sustained firing in human motor units. *The Journal of Physiology*, 545(2), 671–679.

Wienecke, J., Zhang, M., & Hultborn, H. (2009). A prolongation of the postspike afterhyperpolarization following spike trains can partly explain the lower firing rates at derecruitment than those at recruitment. *Journal of Neurophysiology*, 102(6), 3698–710.

TABLE 3.1: CRITERIA USED TO DETERMINE WHETHER CONTROL MOTOR UNIT FIRING RATES PROVIDE VALID ESTIMATES OF PIC IN THE PAIRED MOTOR UNIT TECHNIQUE. All motor unit pairs analyzed in this experiment exceeded the minimum accepted value for published validation criteria (Gorassini et al., 2002; Powers et al., 2008; Stephenson & Maluf, 2011). However, there were differences between postures. When seated, there was a significantly longer time between reference unit and test unit onset. An asterisk denotes significant differences between seated and standing postures.

Posture	Time Between Reference and Test unit Recruitment	Rate Modulation	Rate-Rate Correlation Coefficient
Minimum accepted value ^{a,b,c}	>1s ^b >2s ^{a,c}	Minimum difference of 0.5pps ^{a,c}	$r^2 \geq 0.5$ or $r \geq 0.7^{a,c}$
Standing	$2.00 \pm 0.90^*$	2.51 ± 1.34	$0.79 \pm 0.16^*$
Seated	2.55 ± 0.87	2.70 ± 1.46	0.90 ± 0.053

a. Gorassini et al., 2002

b. Powers et al., 2008

c. Stephenson and Maluf, 2011

TABLE 3.2: MUSCLE ACTIVATION AND RAMP CHARACTERISTICS FOR SEATED AND STANDING MEASURES. An asterisk denotes significant differences between seated and standing posture. Particularly interesting is that standing posture consistently showed a higher percentage of soleus activation. However, this was not accompanied by a notable increase in the number of active motor units in intramuscular EMG recordings as would be expected with such a large disparity in activation. Instantaneous firing frequencies at recruitment of test or reference units were quite low when only the first ISI is isolated.

	Posture	
	Standing	Seated
sEMG at peak of ramp (RMS)	$28.92 \pm 14.24^*$	16.55 ± 6.46
Instantaneous firing frequency of reference unit at recruitment (pps)		
Only first ISI	5.06 ± 1.16	5.49 ± 1.12
Average of first 3 ISIs	6.18 ± 1.49	6.14 ± 0.85
Instantaneous firing frequency of test unit at recruitment (pps)		
Only first ISI	5.24 ± 1.51	4.63 ± 1.69
Average of first 3 ISIs	6.06 ± 1.35	6.59 ± 1.32
Duration of ramp rise (s)	$5.24 \pm 0.37^*$	5.90 ± 0.70
Duration of ramp decline (s)	5.83 ± 0.65	5.96 ± 0.62
muscle activation at reference unit recruitment (%) maximal sEMG RMS)	$12.70 \pm 5.82^*$	9.38 ± 4.66
muscle activation at test unit recruitment (%) maximal sEMG RMS)*	$22.66 \pm 7.17^*$	14.38 ± 6.00

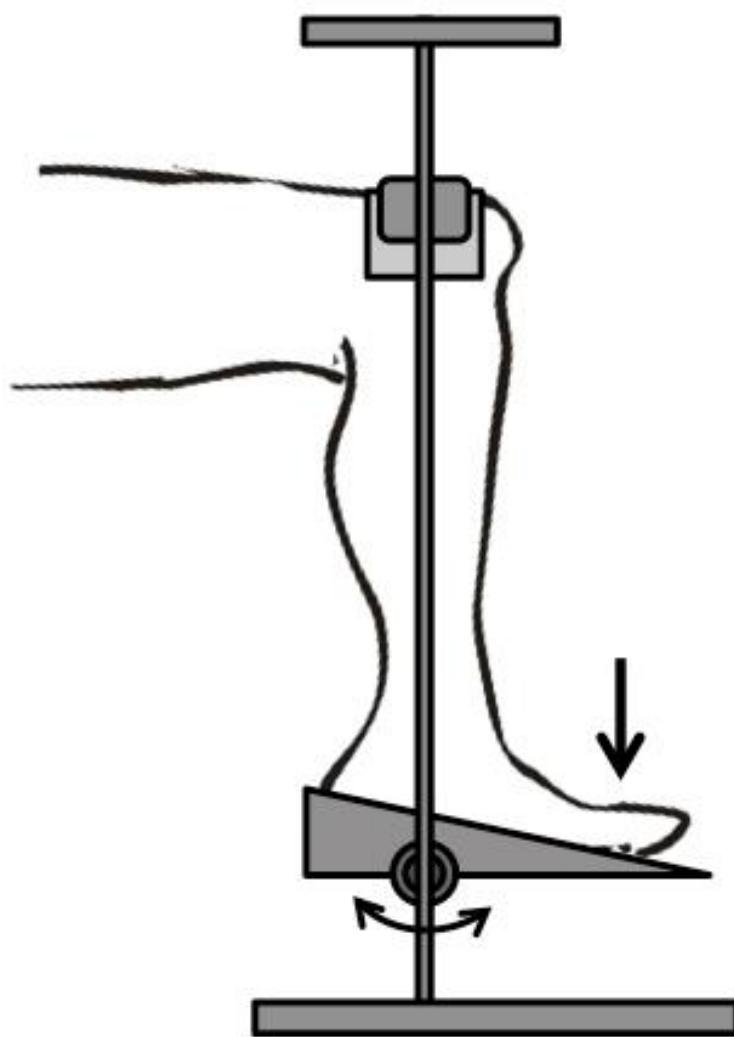


FIGURE 3.1: MCCOMAS BOOT TO MEASURE ISOMETRIC PLANTARFLEXION TORQUE DURING SITTING PHASE OF EXPERIMENT. The leg is clamped from the top of the knee (flexed at 90°) to the foot platform. A resistive transducer is used to capture isometric ankle plantarflexion torque. The ankle joint angle can be adjusted however it was held at 0° to agree with the standing joint angle.

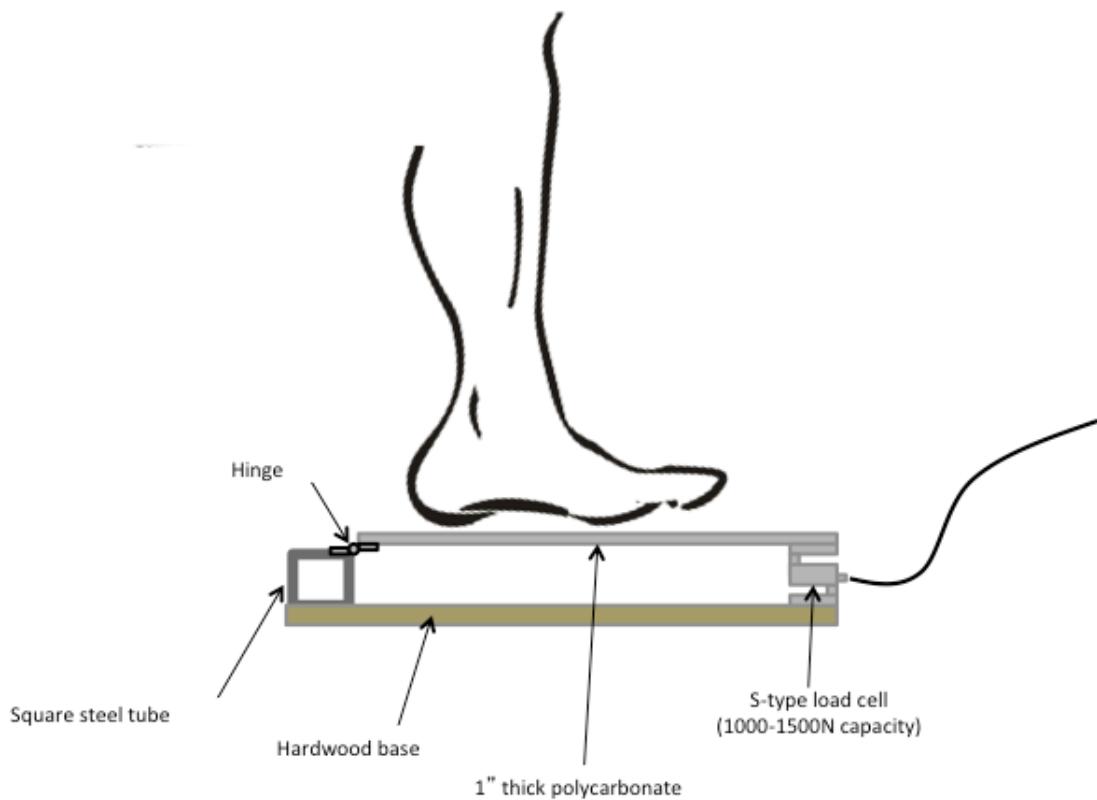


FIGURE 3.2: CUSTOM FORCE PLATFORM TO MEASURE FORCE DURING ANTERIOR POSTERIOR SWAY. An S-type load cell under front of platform was zeroed to have feedback read zero during neutral stance. A hinge in the rear ensured system sensitivity for low force, small adjustments in posture. Participants had their foot traced onto paper to ensure it was in the same spot on the platform in the event of repositioning.

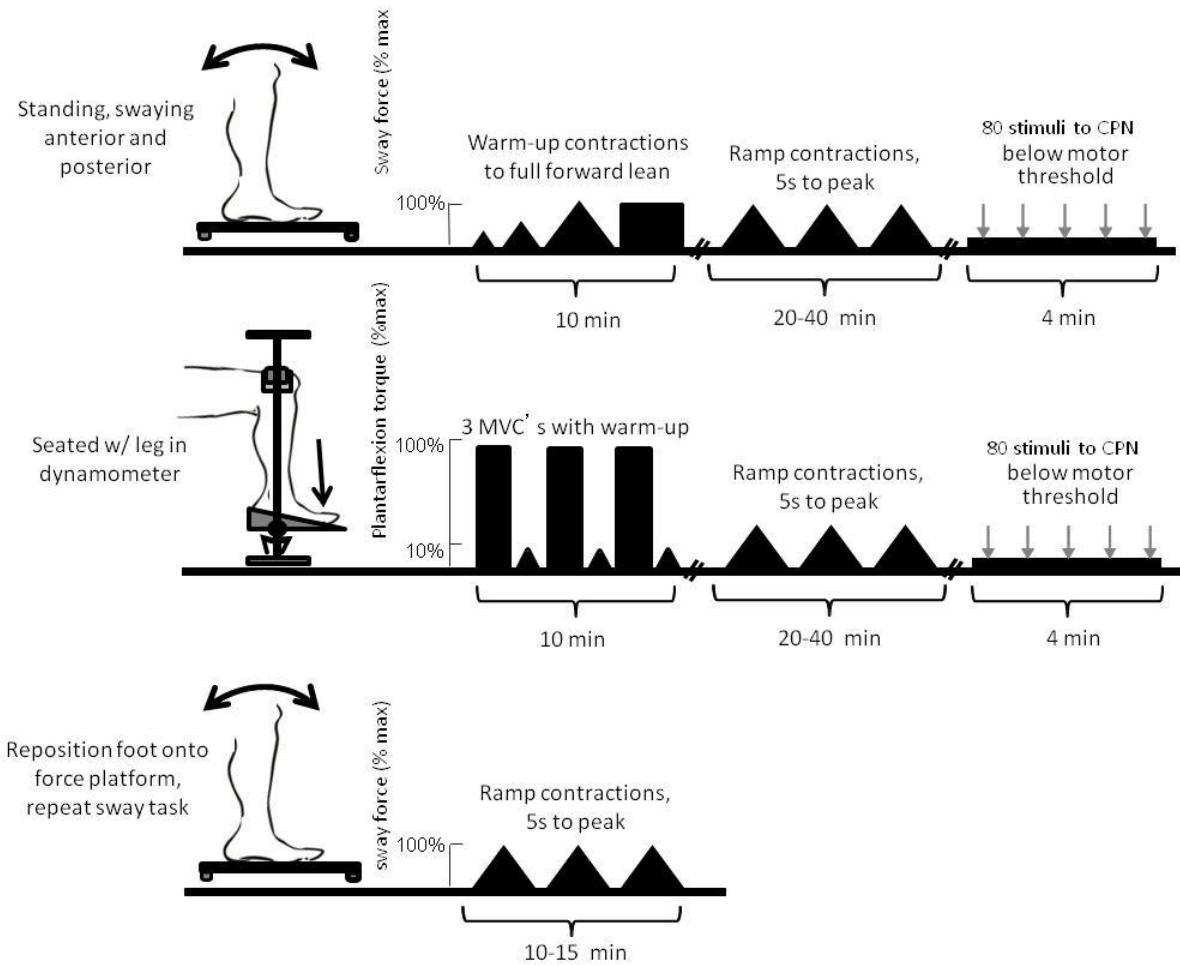


FIGURE 3.3: EXPERIMENTAL PROTOCOL. Participants began in a standing posture. Electrodes and intramuscular needle were positioned. Set-up was a large portion of the experiment and always conducted methodically to ensure recording quality. Participants performed warm-up calf raises and slowly transitioned to the postural sway task. Participants were given a ramp tracing on a transparency to trace on the screen by leaning forward and back. This was repeated until 3 smooth ramps were collected. To assess reciprocal inhibition, 80 stimuli were delivered to the CPN during a very slight forward lean. The participant was then seated with their leg in the McComas boot. 3 Maximal plantarflexion contractions were used for MVC. Participants again performed ramp contractions, this time to 10%MVC. Reciprocal inhibition data was then collected during a low level contraction using 80 stimuli to the nerve to the antagonist. Participants were then transitioned back to standing to collect one more ramp to ensure the same motor units could be followed through posture transition in both directions.

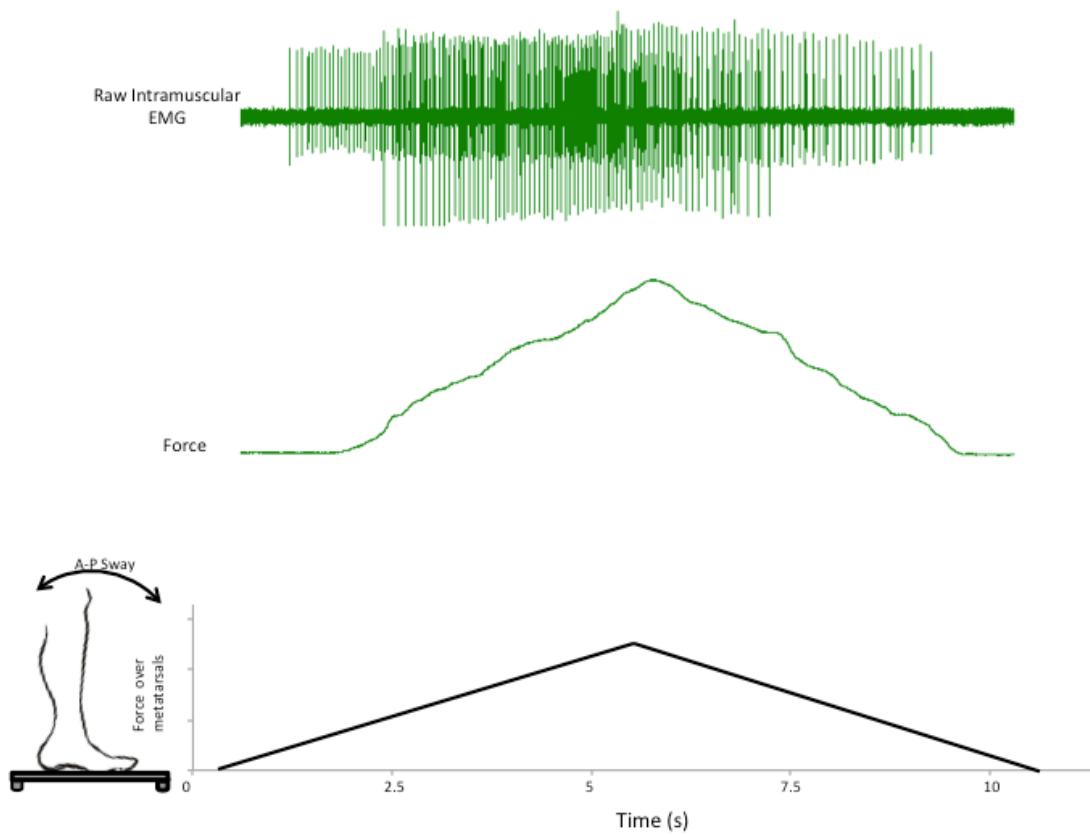


FIGURE 3.4: INTRAMUSCULAR AND FORCE RECORDINGS DURING POSTURAL SWAY

RAMP TASK: Similarly to the isometric plantarflexion ramps in the seated position, standing ramp templates were provided over the screen on a transparency for participants. Peak ramp force was normalized to the most comfortable anterior lean position that the participant could maintain consistent force output. Participants were instructed to position their weight over the heels during the non-sway phase and this value of force output was zeroed. Participants had multiple practice attempts to ensure reliable ramps could be produced during the experimental protocol.

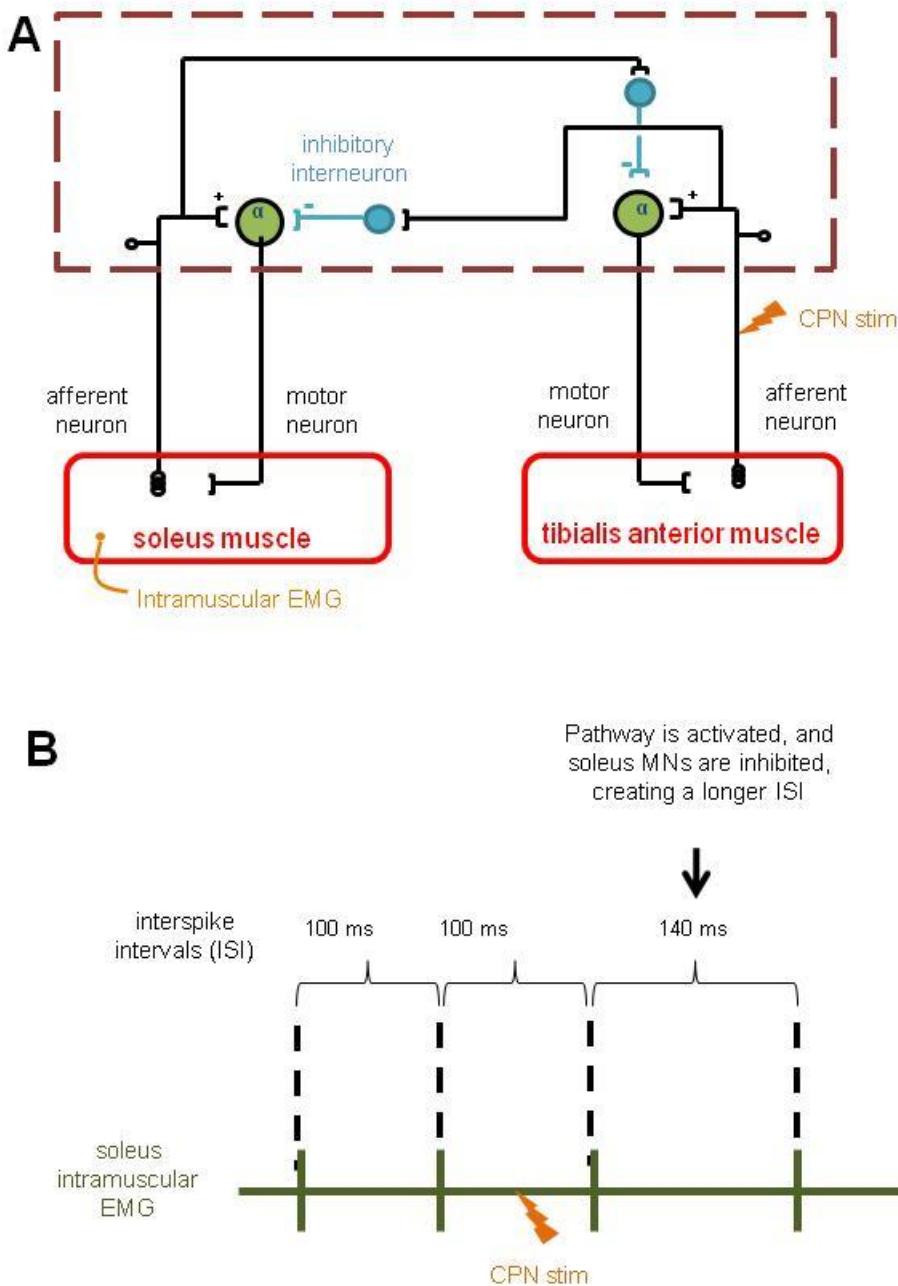


FIGURE 3.5: ASSESSING RECIPROCAL INHIBITION. The common peroneal nerve (CPN) was stimulated at motor threshold during light plantarflexion to activate the reciprocal inhibitory pathway (A), and inhibit soleus motor neurons (MNs). This results in a delay in soleus motor unit discharge and an increased interspike interval (B). This is an example of the effect of one stimuli of the 80 stimuli used generate the PSTH shown in Figure 15.

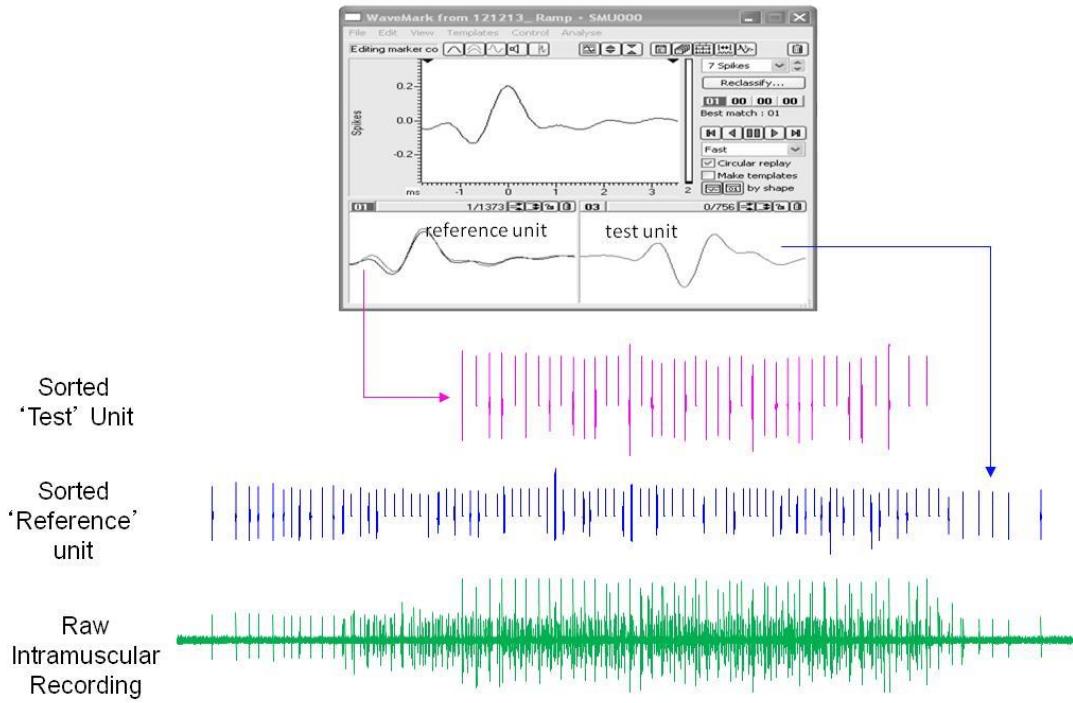


FIGURE 3.6: SPIKE SORTING PROTOCOL used to identify control and test motor units. Intramuscular recordings (bottom trace) were made via fine wire electrodes inserted into the soleus muscle. Spike sorting software (Spike2, Cambridge Electronic Design) was used to identify reference (middle trace) and test (top trace) motor units coded according to amplitude and shape (top figure). Templates were made using an algorithm and then manually sorted by the investigator to account for sorting errors due to superimposed spikes or amplitude changes.

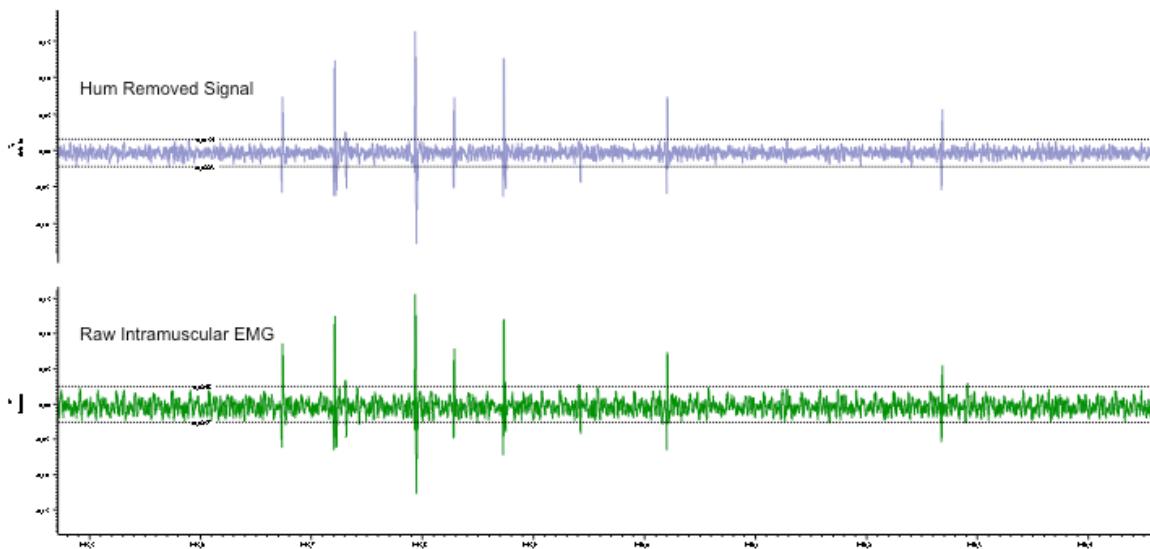


FIGURE 3.7: FILTERING OF THE INTRAMUSCULAR RECORDING. In addition to a bandpass hardware filter, a “Hum Remove” online digital filter (Spike 2 software (version 7.1.2), Cambridge Electronic Design, Cambridge Inc., United Kingdom) was used to remove noise during data collection. Data was broken into 400 bins and an algorithm detected the most predominant resonating frequency in each. A filter was then applied for each epoch, resulting in a marked reduction in oscillating baseline noise. This improved accuracy of manual sorting, especially among low threshold motor units.

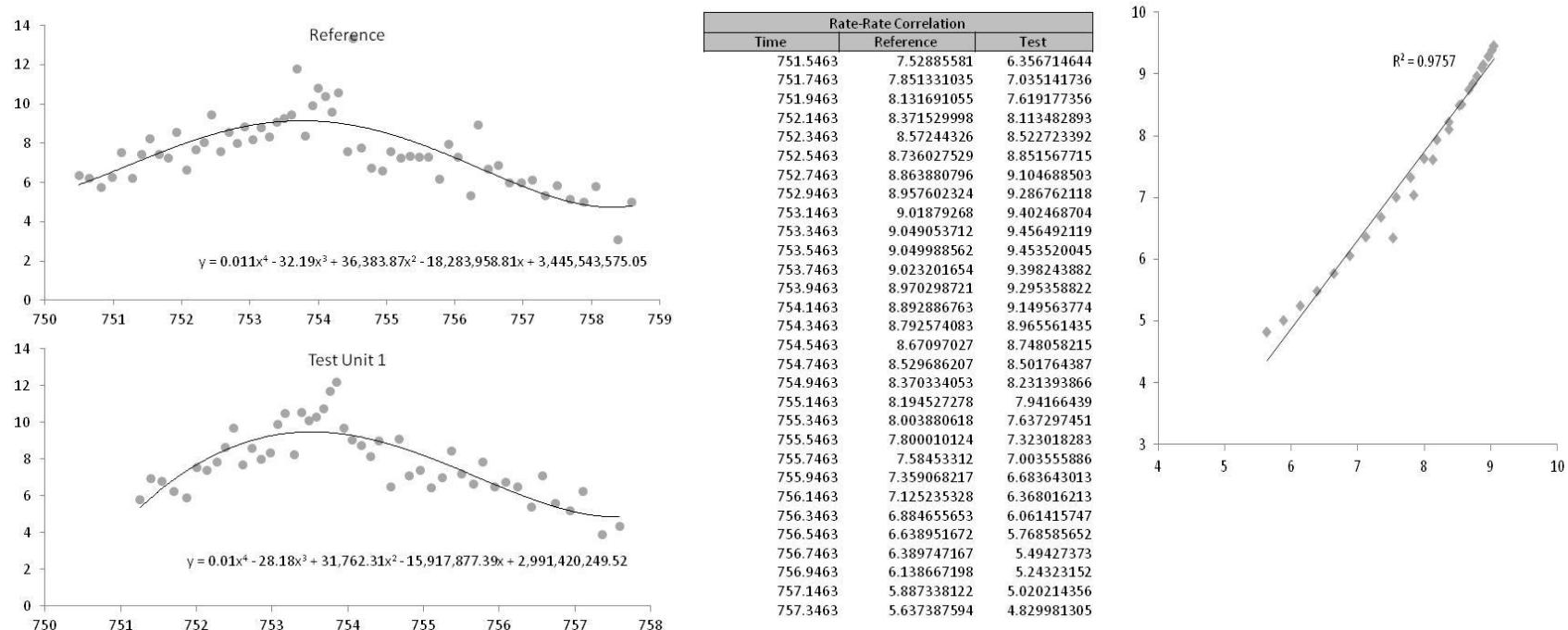


FIGURE 3.8: RATE-RATE CORRELATION WAS CONDUCTED TO SATISFY THE PHYSIOLOGICAL ASSUMPTION THAT THE REFERENCE AND TEST UNIT SHARE EQUAL SYNAPTIC INPUT DURING THE CONTRACTION. For this to be true, and the paired motor unit technique valid, the firing rates of both motor units must vary in accordance with the other. This is confirmed by correlating the mean firing rate values (200ms bins, mean values of firing rate (pps)) for each motor unit over the duration of the test unit activity. Pearson's correlation coefficient was then calculated from the plotted data (test firing rate vs reference firing rate)

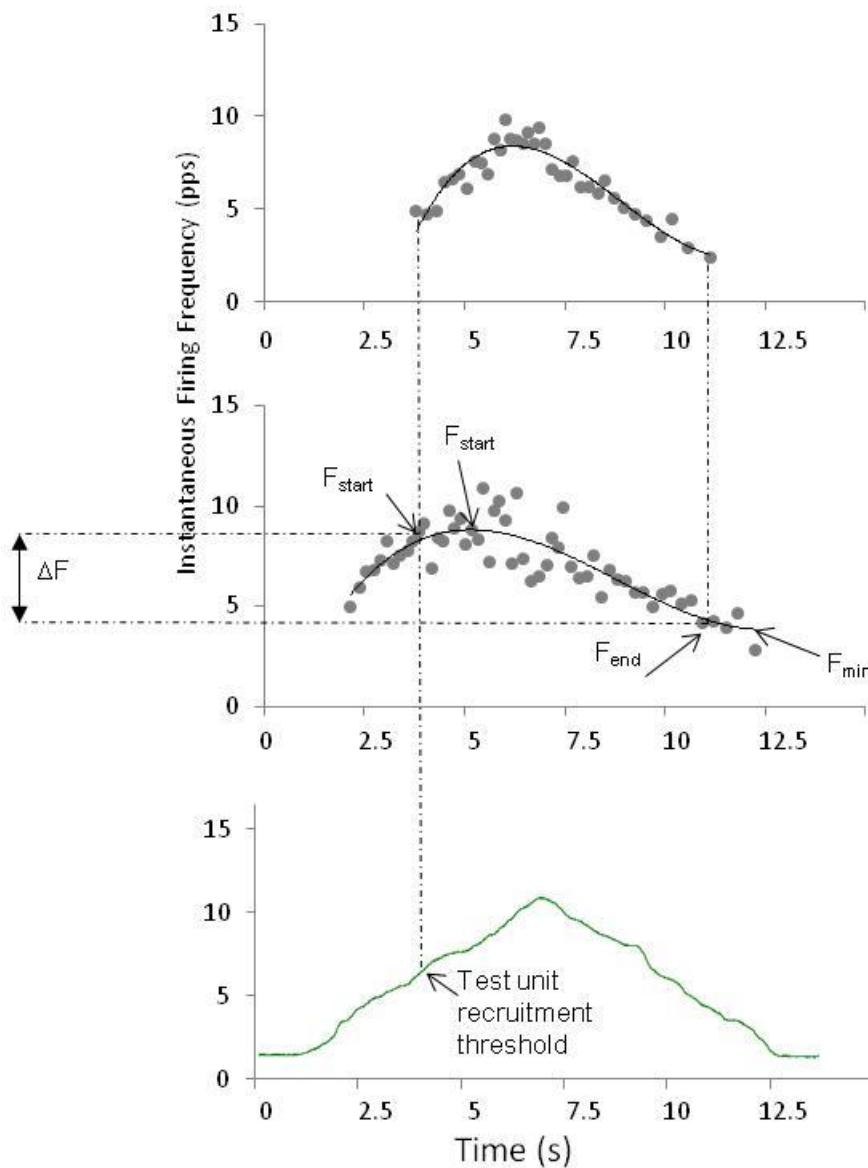


FIGURE 3.9: RATE MODULATION IS CALCULATED AS DIFFERENCE BETWEEN THE RANGE OF REFERENCE UNIT FIRING RANGE. Peak reference unit firing frequency (F_{max}) to the last calculated firing frequency of the reference unit (F_{min}) is quantified and compared to the calculated ΔF value ($F_{start} - F_{end}$) for that motor unit pair. If ΔF is within 0.5pps of the rate modulation the pair does not meet the assumption that the reference unit is a sensitive indicator of net excitatory input to the motor neuron for the duration of the ramp contraction.

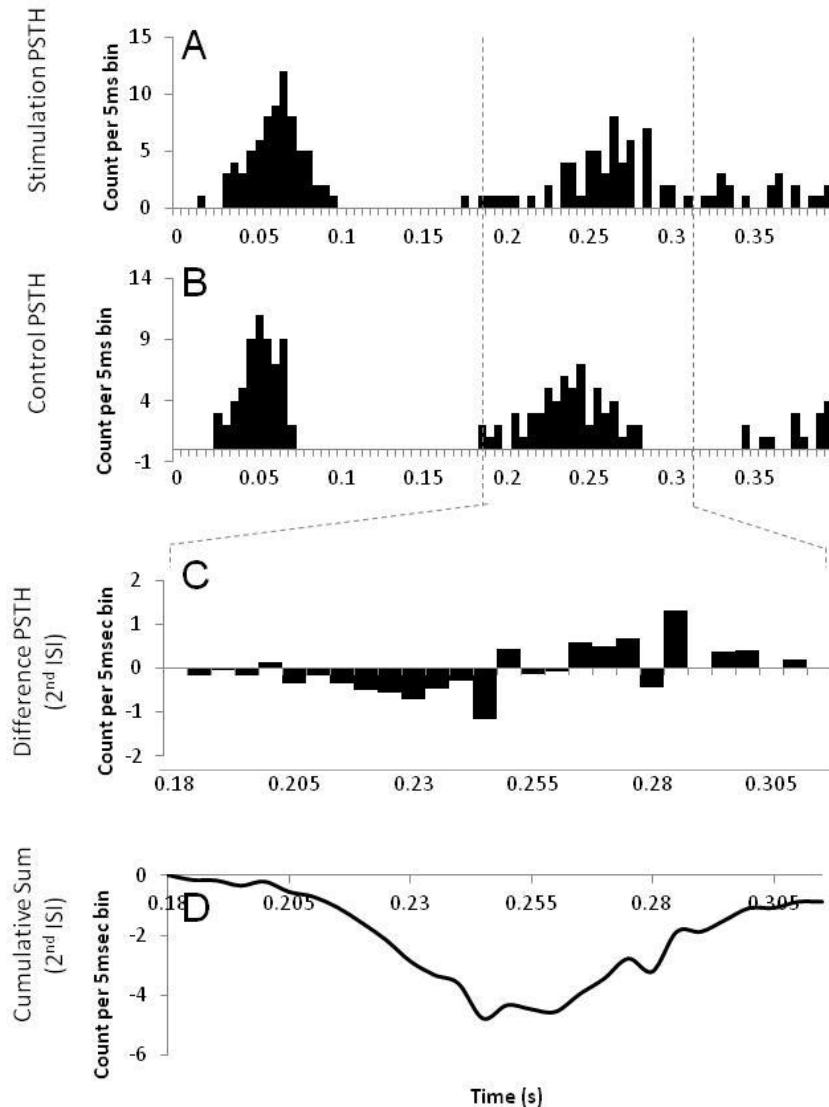


FIGURE 3.10: THE CUMULATIVE SUM PSTH TECHNIQUE WAS USED TO QUANTIFY RECIPROCAL INHIBITION.

Stimulation PSTH (A): The CPN was stimulated to activate the reciprocal inhibitory pathway. The number of spikes (count) were plotted in 5-ms bins for 400 ms after each stimulus.

Control PSTH (B): This PSTH reflects the interspike intervals during soleus motor unit activity without stimulation of the nerve to the antagonist (i.e. CPN).

Difference PSTH for 2nd ISI (C): Spike counts from the control PSTH were subtracted from the stimulation PSTH to quantify reciprocal inhibition. A negative value signifies inhibition.

Cumulative Sum of 2nd ISI (D): Difference PSTH counts were cumulatively added. This cumulative sum is used to detect changes from the mean and the timing of these changes. A negative deflection indicates inhibition and the amount of inhibition was quantified by area under the curve (trapezoidal integration)

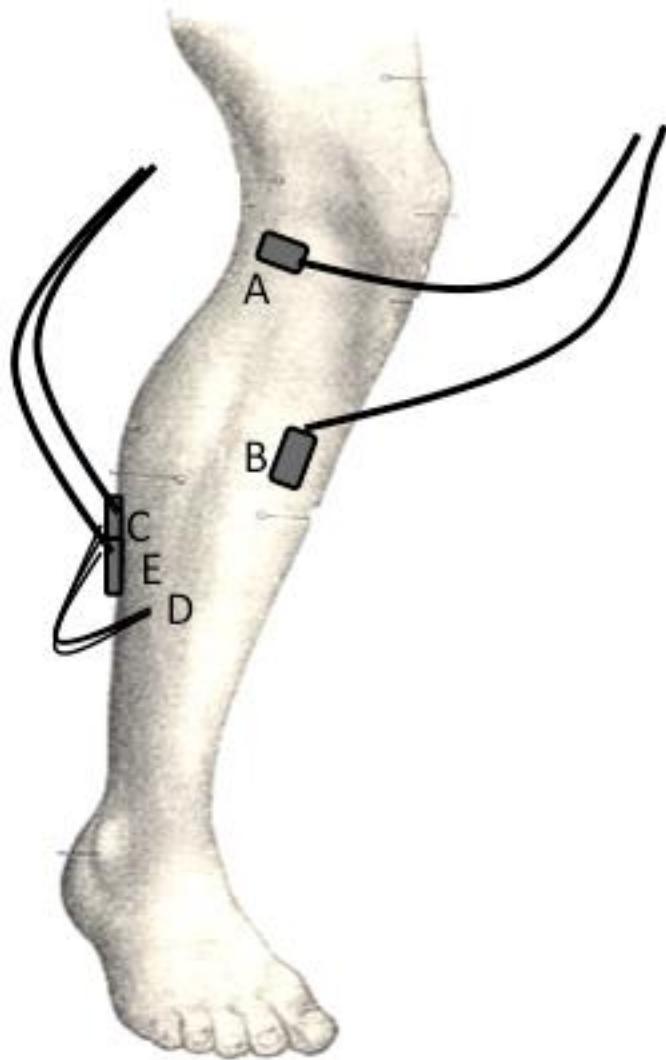


FIGURE 3.11: ELECTRODE SETUP FOR STIMULATION TO ASSESS RECIPROCAL INHIBITION. **A** stimulation electrode for RI placed over the CPN. **B** surface EMG on the TA to measure stimulation threshold for CPN stimulation. **C** Surface EMG placed on electrode to measure overall activity of soleus during contraction. Intramuscular electrode wires (**D**) fed into preamplifier (**E**) secured to leg.

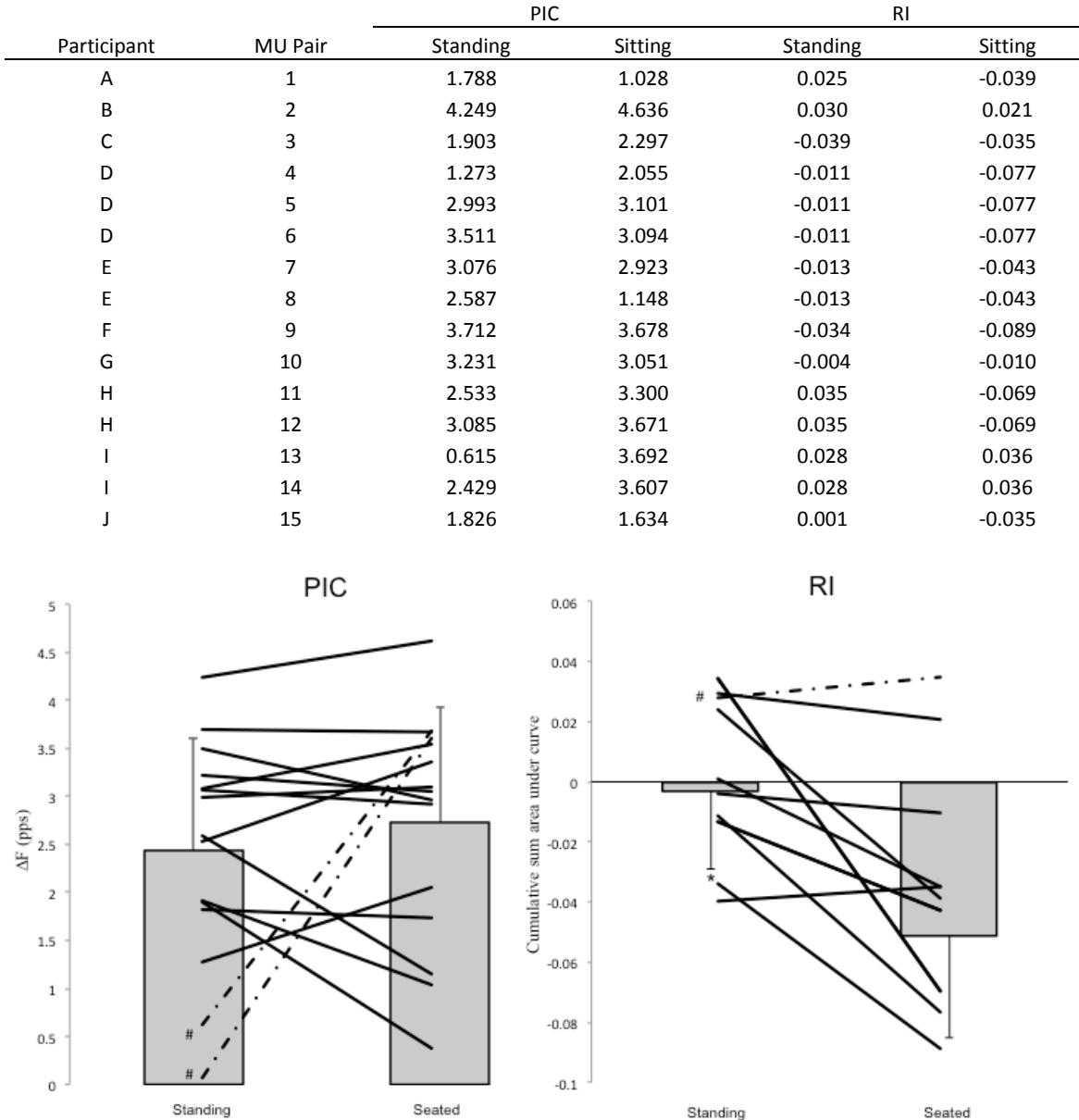


FIGURE 3.12: ePIC AND RI DATA. Top: Pic and RI values by participant. Bottom: PIC values (left) showed no significant difference between standing and seated posture. This trend of comparable ePIC values between postures is reflected by group means (grey bars) and by the majority of individual data (solid black lines). However, two participants (dotted lines, 1&2) showed marked increases in ePIC from standing to seated. Reciprocal inhibition did show a significant ($p<0.001$) difference between postures. Note that a larger negative value denotes increased reciprocal inhibition. The two individuals who registered a large increase in PIC did not have that correlate to a decrease in reciprocal inhibition in the seated posture. The # denotes the participant with the single largest changes in ePIC between postures; comparing this to their Reciprocal inhibition data indicates this large discrepancy was the result of a change in RI in that individual.

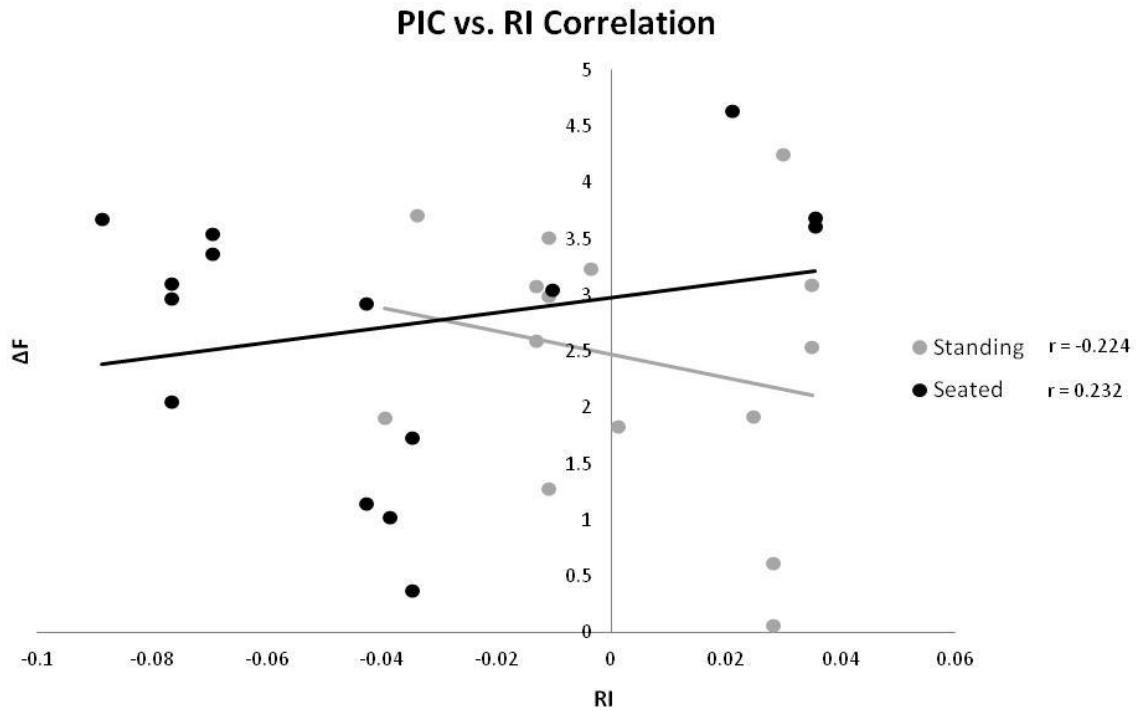


FIGURE 3.13: CORRELATION BETWEEN BASELINE EPIC AND RECIPROCAL INHIBITION. A decrease in PIC is expected with increased reciprocal inhibition (more negative values). This investigation found no relationship exists at baseline between reciprocal inhibition and estimates of PIC in either standing or seated posture (standing: $r=-.224$, $p=0.421$; seated: $r=.232$, $p=0.405$).

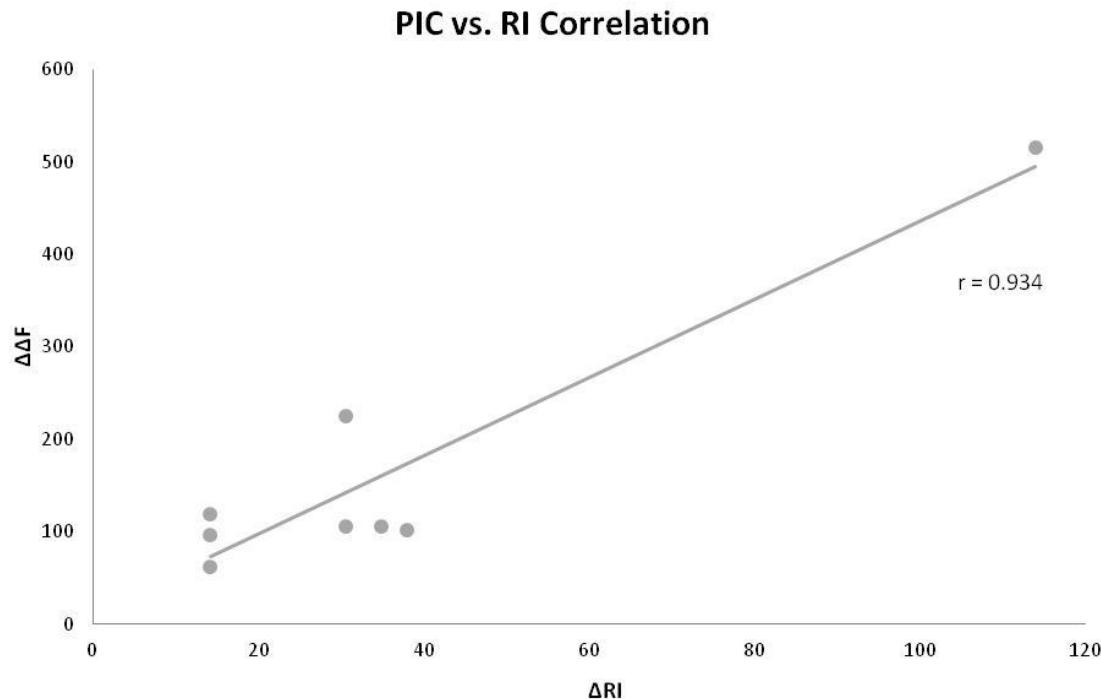


FIGURE 3.14: CORRELATION BETWEEN $\Delta\Delta F$ AND ΔRI . Acknowledging that there is a slight dorsiflexion of the ankle $\Delta\Delta F$ and ΔRI were correlated to demonstrate which units increase in ΔF from seated to standing was accounted for by ΔRI , the reduction in reciprocal inhibition. When the outlying point is removed, no relationship exists, and the change in PIC is not accounted for by the change in RI between postures ($p < 0.001$ including all points).

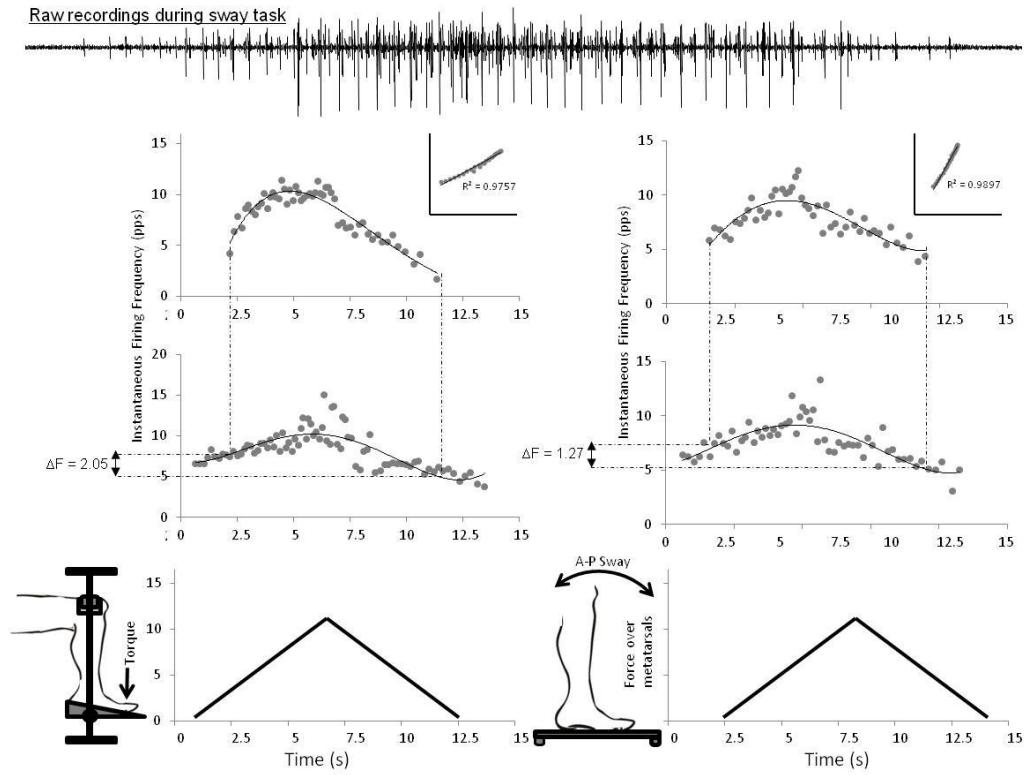


FIGURE 3.15: PAIRED MOTOR UNIT RECORDINGS DURING SEATED AND STANDING TASKS. Conventional paired motor unit recordings are made in a seated posture (traces and figure on left). This has always provided investigators with reliable, repeatable measures, but is divergent from what likely happens during upright standing posture. In order to obtain PMU estimates of PIC during standing posture a standing forward sway protocol was designed. In the current study, isometric plantarflexion torque (seated technique) was replaced with an anterior postural way. This force was quantified using a load cell placed under the anterior portion of the custom platform where the participants stood. PMU plots for both seated and standing resemble recordings obtained by past investigations. Furthermore, standing estimates also meet all PMU technique criteria, further validating standing ePIC collection.

CHAPTER 4: SUMMARY AND FUTURE DIRECTIONS

SUMMARY

1. Standing paired motor unit recordings met all published validation criteria, satisfying the physiological assumptions of the technique and indicating ΔF was a valid measure of PIC.
2. Standing ΔF did not differ from seated ΔF indicating no difference in persistent inward current between postures.
3. Certain validation criteria (rate-rate correlation) and technique constraints (% muscle activation at test unit recruitment) significantly differed between seated and standing.
4. Reciprocal inhibition was significantly greater in a seated posture than when measured with a standing postural sway.

CONCLUSION

This study provided the first functional estimates of persistent inward current in humans, demonstrating that a postural sway ramp contraction could provide paired motor unit recordings that meet all published validation criteria for the technique. While operating within other constraints to limit confounding factors influence on ΔF , differences between standing and conventional seated PIC estimates indicate further investigation is needed before concluding that standing ΔF is a valid index of persistent inward current.

RECOMMENDATIONS FOR FURTHER STUDY

The current experiment demonstrated it is possible to obtain ΔF estimates of PIC during a functional postural sway, however elimination of the dorsiflexion associated with an anterior postural sway may be a better method for standing PIC estimation moving forward. Creating an apparatus whereby the participants shoulders could be restrained from superior

movement, a standing isometric plantarflexion task could be performed. This would not only allow for a more controlled pace of contraction but regulate the contraction strength to anywhere from zero to one hundred percent MVC. Furthermore, such an apparatus could be accommodating of ankle joint angle changes so that the relationship between RI and PIC could be investigated while standing, similar to the seated Δ RI- $\Delta\Delta$ F correlations of Vandenberk & Kalmar 2014.

REFERENCE LIST

- Aymard, C., Chia, L., Katz, R., Lafitte, C., & Pénicaud, (1995). Reciprocal inhibition between wrist flexors and extensors in man: a new set of interneurones. *The Journal of Physiology*, 487, 221–35.
- Bennett, D. J., Hultborn, H., Fedirchuk, B., & Gorassini, M. (1998). Synaptic Activation of Plateaus in Hindlimb Motoneurons of Decerebrate Cats Synaptic Activation of Plateaus in Hindlimb Motoneurons of Decerebrate Cats. *J Neurophysiol* 80:2023-2037,, 2023–2037.
- Bennett, D. J., Li, Y., Harvey, P. J., & Gorassini, M. (2001). Evidence for plateau potentials in tail motoneurons of awake chronic spinal rats with spasticity. *Journal of Neurophysiology*, 86(4), 1972–82.
- Binder, M. D. (2002). Integration of synaptic and intrinsic dendritic currents in cat spinal motoneurons. *Brain Research. Brain Research Reviews*, 40(1-3), 1–8.
- Brownstone, R. M. (2006). Beginning at the end: repetitive firing properties in the final common pathway. *Progress in Neurobiology*, 78(3-5), 156–72.
- Burke, R. E. (2007). Sir Charles Sherrington's the integrative action of the nervous system: a centenary appreciation. *Brain: A Journal of Neurology*, 130(Pt 4), 887–94.
- Cattagni, T., Martin, a, & Scaglioni, G. (2014). Is spinal excitability of the triceps surae mainly affected by muscle activity or body position? *Journal of Neurophysiology*, 111(12), 2525–32.

- Crone, C., & Hultborn, H. (1987). Reciprocal Ia inhibition between ankle flexors and extensors in man. *Journal of Physiology*, 163–185.
- Day, B. L., & Fitzpatrick, R. C. (2005). The vestibular system. *Current Biology : CB*, 15(15), R583–6.
- ElBasiouny, S. M., Schuster, J. E., & Heckman, C. J. (2010). Persistent inward currents in spinal motoneurons: important for normal function but potentially harmful after spinal cord injury and in amyotrophic lateral sclerosis. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, 121(10), 1669–79.
- Elias, L. A., Chaud, V. M., & Kohn, A. F. (2012). Models of passive and active dendrite motoneuron pools and their differences in muscle force control. *Journal of Computational Neuroscience*, 33(3), 515–31.
- Gorassini, M. a, Bennett, D. J., & Yang, J. F. (1998). Self-sustained firing of human motor units. *Neuroscience Letters*, 247(1), 13–16.
- Gorassini, M., Yang, J. F., Siu, M., & Bennett, D. J. (2002). Intrinsic activation of human motoneurons: possible contribution to motor unit excitation. *Journal of Neurophysiology*, 87(4), 1850–8.
- Hamada, T., Sale, D. G., MacDougall, J. D., & Tarnopolsky, M. a. (2000). Postactivation potentiation, fiber type, and twitch contraction time in human knee extensor muscles. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, 88, 2131–2137.

Hamm, T. M., Turkin, V. V., Bandekar, N. K., O'Neill, D., & Jung, R. (2010). Persistent currents and discharge patterns in rat hindlimb motoneurons. *Journal of Neurophysiology*, 104(3), 1566–77.

Harvey, P. J., Li, X., Li, Y., & Bennett, D. J. (2006). 5-HT₂ receptor activation facilitates a persistent sodium current and repetitive firing in spinal motoneurons of rats with and without chronic spinal cord injury. *Journal of Neurophysiology*, 96(3), 1158–70.

Heckman, C. J., Binder, M. D., & Binder, D. (1993). Computer simulations of motoneuron firing rate modulation Computer Simulations of Motoneuron. *Journal of Neurophysiology*, 1005–1008.

Heckman, C. J., 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(3), 264–75.

Heckman, C. J., Mottram, C., Quinlan, K., Theiss, R., & Schuster, J. (2009). Motoneuron excitability: the importance of neuromodulatory inputs. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, 120(12), 2040–54.

Heckmann, C. J., Gorassini, M. a, & Bennett, D. J. (2005). Persistent inward currents in motoneuron dendrites: implications for motor output. *Muscle & Nerve*, 31(2), 135–56.

Highstein, S. M., & Holstein, G. R. (2012). The anatomical and physiological framework for vestibular prostheses. *Anatomical Record (Hoboken, N.J.: 2007)*, 295(11), 2000–9.

Hodgson, M., Docherty, D., & Robbins, D. (2005). Post-activation potentiation: Underlying physiology and implications for motor performance. *Sports Medicine*, 35(7), 585–595.

Hounsgaard, J., & Hultborn, H. (1988). Bistability of alpha-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *Journal of Physiology*, 345–367.

Hyngstrom, A. S., Johnson, M. D., Miller, J. F., & Heckman, C. J. (2007). Intrinsic electrical properties of spinal motoneurons vary with joint angle. *Nature Neuroscience*, 10(3), 363–9.

Ijspeert, A. (2002). Locomotion, vertebrate. *The Handbook of Brain Theory and Neural Networks*, (3).

Jacobs, B. L., & Fornal, C. a. (1999a). Activity of serotonergic neurons in behaving animals. *Neuropsychopharmacology*, 21, 9S–15S.

Jacobs, B. L., Martín-Cora, F. J., & Fornal, C. a. (2002). Activity of medullary serotonergic neurons in freely moving animals. *Brain Research. Brain Research Reviews*, 40(1-3), 45–52.

- Johnson, M. D., & Heckman, C. J. (2010). Interactions between focused synaptic inputs and diffuse neuromodulation in the spinal cord. *Annals of the New York Academy of Sciences*, 1198, 35–41.
- Johnson, M. D., Hyngstrom, A. S., Manuel, M., & Heckman, C. J. (2012). Push-pull control of motor output. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 32(13), 4592–9.
- Kasai, T., Kawanishi, M., & Yahagi, S. (1998). Posture-dependent modulation of reciprocal inhibition upon initiation of ankle dorsiflexion in man. *Brain Research*, 792(1), 159–163.
- Katz, R., & Meunier, S. (1988). Changes in presynaptic inhibition of 1a fibers in man while standing. *Brain*, 417–437.
- Lee, R. H., & Heckman, C. J. (1998a). Bistability in Spinal Motoneurons In Vivo: Systematic Variations in Persistent Inward Currents Bistability in Spinal Motoneurons In Vivo: Systematic Variations in Persistent Inward Currents. *Journal of Neurophysiology*, 583–593.
- Lee, R. H., & Heckman, C. J. (1998b). Bistability in Spinal Motoneurons In Vivo: Systematic Variations in Rhythmic Firing Patterns Bistability in Spinal Motoneurons In Vivo: Systematic Variations in Rhythmic Firing Patterns. *Journal of Neurophysiology*, 572–582.

Lee, R., & Heckman, C. (1999). Enhancement of bistability in spinal motoneurons in vivo by the noradrenergic α 1 agonist methoxamine. *Journal of Neurophysiology*, 2164–2174.

Liang, H., Bácskai, T., Watson, C., & Paxinos, G. (2014). Projections from the lateral vestibular nucleus to the spinal cord in the mouse. *Brain Structure & Function*, 219(3), 805–15.

Marsh, E., Sale, D., McComas, a J., & Quinlan, J. (1981). Influence of joint position on ankle dorsiflexion in humans. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, 51(1), 160–7.

Miyamoto, N., Fukunaga, T., & Kawakami, Y. (2009). Evidence for intermuscle difference in postactivation potentiation in the human triceps surae: A mechanomyographic study. *Muscle and Nerve*, 39(May), 703–706.

Nielsen, B. Y. J., & Kagamiharat, Y. (1992). The regulation of disynaptic reciprocal Ia inhibition during co-contraction. *Journal of Physiology, Paris*, 373–391.

Nielsen, J. B., Crone, C., & Hultborn, H. (2007). The spinal pathophysiology of spasticity--from a basic science point of view. *Acta Physiologica (Oxford, England)*, 189(2), 171–80.

Norton, J. a, Bennett, D. J., Knash, M. E., Murray, K. C., & Gorassini, M. a. (2008). Changes in sensory-evoked synaptic activation of motoneurons after spinal cord injury in man. *Brain: A Journal of Neurology*, 131(Pt 6), 1478–91.

- Perrier, J. (2013). Modulation of the intrinsic properties of motoneurons by serotonin. *Current Pharmaceutical Design*, (28), 4371–4384.
- Powers, R. K., Elbasiouny, S. M., Rymer, W. Z., & Heckman, C. J. (2012). Contribution of intrinsic properties and synaptic inputs to motoneuron discharge patterns: a simulation study. *Journal of Neurophysiology*, 107(3), 808–23.
- Powers, R. K., Nardelli, P., & Cope, T. C. (2008). Estimation of the contribution of intrinsic currents to motoneuron firing based on paired motoneuron discharge records in the decerebrate cat. *Journal of Neurophysiology*, 100(1), 292–303.
- Revill, A. L., & Fuglevand, A. J. (2011). Effects of persistent inward currents, accommodation, and adaptation on motor unit behavior: a simulation study. *Journal of Neurophysiology*, 106(3), 1467–79.
- Sasaki, O., Asawa, S., Katsuno, S., Usami, S., & Taguchi, K. (2001). Gait initiation in bilateral vestibular loss. *Auris, Nasus, Larynx*, 28(4), 295–9.
- Sawczuk, A., Powers R. K., and B. (1995). Spike frequency adaptation studied in hypoglossal motoneurons of the rat. *Journal of Neurophysiology*, 1799–1810.
- Schwindt, P. C., & Crill, W. E. (1980). Properties of a persistent inward current in normal and TEA-injected motoneurons Properties of a Persistent Inward Current in Normal and TEA-Injected Motoneurons. *J Neurophysiol* 43, 1700–1724.

Stephenson, J. L., & Maluf, K. S. (2011). Dependence of the paired motor unit analysis on motor unit discharge characteristics in the human tibialis anterior muscle. *Journal of Neuroscience Methods*, 198(1), 84–92.

Svirskis, G., & Hounsgaard, J. (1997). Depolarization-Induced Facilitation of a Plateau-Generating Current in Ventral Horn Neurons in the Turtle Spinal Cord Depolarization-Induced Facilitation of a Plateau-Generating Current in Ventral Horn Neurons in the Turtle Spinal Cord. *Journal of Neurophysiology*, 77(6), 1740–1742.

Trulson, M., Jacobs, B., & Morrison, A. (1981). Raphe unit activity during REM sleep in normal cats and in pontine lesioned cats displaying REM sleep without atonia. *Brain Research*, 226, 75–91.

Udina, E., D'Amico, J., Bergquist, A. J., & Gorassini, M. a. (2010). Amphetamine increases persistent inward currents in human motoneurons estimated from paired motor-unit activity. *Journal of Neurophysiology*, 103(3), 1295–303.

Vandenberk, M. S., & Kalmar, J. M. (2014). An evaluation of paired motor unit estimates of persistent inward current in human motoneurons. *Journal of Neurophysiology*, 111(9), 1877–84.

Veasey, S., & Fornal, C. (1995). Response of serotonergic caudal raphe neurons in relation to specific motor activities in freely moving cats. *The Journal of Neuroscience*, 15(July), 5346–5359.

- Venugopal, S., Hamm, T. M., Crook, S. M., & Jung, R. (2011). Modulation of inhibitory strength and kinetics facilitates regulation of persistent inward currents and motoneuron excitability following spinal cord injury. *Journal of Neurophysiology*, 106(5), 2167–79.
- Walton, C., Kalmar, J. M., & Cafarelli, E. (2002). Effect of caffeine on self-sustained firing in human motor units. *The Journal of Physiology*, 545(2), 671–679.
- Westcott, S., Powers, R., Robinson, F., & Binder, M. (1995). Distribution of vestibulospinal synaptic input to cat triceps surae motoneurons. *Experimental Brain Research*, 107(1), 1–8.
- Wienecke, J., Zhang, M., & Hultborn, H. (2009). A prolongation of the postspike afterhyperpolarization following spike trains can partly explain the lower firing rates at derecruitment than those at recruitment. *Journal of Neurophysiology*, 102(6), 3698–710.

CHAPTER 5: APPENDICES

APPENDIX A: INFORMED CONSENT

WILFRID LAURIER UNIVERSITY INFORMED CONSENT STATEMENT

Estimates of persistent inward current in human motor neurons during postural sway

You are invited to participate in a research study at Wilfrid Laurier University. The purpose of our study is to provide further insight into task dependent nature of a motoneuron property known as persistent inward current.

Student Investigator:

Name: Ryan Foley
 Institution: Wilfrid Laurier University
 Phone: (519) 884-0710 ext. 3334
 Email: fole4370@mylaurier.ca

Supervisor:

Name: Dr. Jayne Kalmar (13)
 Institution: Wilfrid Laurier University
 Phone: (519) 884-0710 ext. 2033
 Email: jkalmar@wlu.ca

INFORMATION

Ten participants will take part in this research study. The aim of our study is to investigate the relationship between a spinal reflex pathway and a property of spinal motor neurons. Specifically, we will be studying an intrinsic property of spinal neurons that sets their level of excitability during different tasks and states. We are also interested in how inhibition acts to adjust the excitability of motor neurons during different postures. To date this has only been investigated in participants free of nervous system pathologies. We aim to identify task dependency of motor neuron excitability using a functionally relevant task.

The experiment will take place in room NC119 of the Northdale Campus at Wilfrid Laurier University, which is located on the corner of Hickory Street and Hazel Street (66 Hickory St. W). Upon arrival, you can dial the lab extension (x3334) from the outdoor keypad at the main entrance and a member of the laboratory will meet you there at the entrance.

We will use electrical nerve stimulation to quantify the degree of reciprocal inhibition at different time periods throughout the experiment. Electrodes will be attached to the skin over a nerve in your leg and when stimulated it will cause muscles in your lower-leg to contract. Intramuscular electrodes that are made out of very fine wires will be inserted into your leg to record the electrical activity within the muscle when you contract your leg voluntarily. This will be repeated in a sitting and a standing condition on one experimental day. This electrode will only be used to record electrical responses and never to stimulate the muscle.

These procedures are safe and have been used routinely in research settings for more than 40 years; however, some participants may find the sensation unpleasant. If you find these procedures uncomfortable, you may withdraw from the study at any time. A 30-minute orientation will take place on the same day as the experiment. The purpose of the orientation is to introduce you to the techniques employed in this study (nerve stimulation and intramuscular recordings). Following this orientation session, we will assess these preliminary recordings. If the recordings meet our criteria, we will continue with the experiment and finish that same day. The experimental protocol will take approximately 2.5 hours. You be compensated with \$30 for participating.

Initials _____

RISKS

The electrical stimulation applied to the mixed nerve through a constant current stimulator will cause an involuntary muscle “twitch” in the target muscle. You may find this stimulation unpleasant; however, constant current stimulation is a noninvasive procedure that does not cause damage to the nerve or other tissues.

The initial insertion of the intramuscular electrodes may be associated with a stinging sensation due to the alcohol used to clean your skin. There is also a remote risk of infection with the insertion of intramuscular electrodes. To reduce this risk the needles and electrodes are sterilized using an autoclave and your skin is prepared with alcohol. The researcher will also be wearing medical-grade, non-latex gloves during any manipulation of the needle or the electrode wires. Needles, electrodes and razors (used to shave skin around electrodes) are never reused.

Some participants may find the electrode uncomfortable during the initial few contractions after insertion. This typically subsides after some muscle use and is mostly unnoticed.

There occasionally may be localized bruising (<0.5cm diameter) around the site of electrode insertion similar to what you might observe following a blood test. This bruising subsides within 48 hours and is not typically associated with any discomfort.

BENEFITS

You will not benefit directly from participating in this study. However, this study will help us understand the neural control of muscles in both healthy populations and those with concussions. It will provide valuable insight into the state-dependency of commonly used motor unit recording techniques.

CONFIDENTIALITY

All data collected in this study will be stored indefinitely in NC119 and will only be accessible by the investigators. All measures will be taken to ensure your privacy and all your data will be coded and identified by a participation code. Group results will be submitted for publishing in various research journals. Individual results will remain completely confidential and not published to ensure your privacy.

COMPENSATION

You be compensated with \$30 for participating in this study.

CONTACT

If you have questions at any time about the study or the procedures you may contact the researcher or supervisor.

This project has been reviewed and approved by the University Research Ethics Board. If you feel you have not been treated according to the descriptions in this form, or your rights as a participant in research have been violated during the course of this project, you may contact Dr. Robert Basso, Chair, University Research Ethics Board, Wilfrid Laurier University, (519) 884-1970, extension 4994 or rbasso@wlu.ca.

PARTICIPATION

Your participation in this study is voluntary; you may decline to participate without penalty. If you decide to participate, you may withdraw from the study at any time without penalty and without loss of benefits to which you are otherwise entitled.

Initials _____

FEEDBACK AND PUBLICATION

The results will be presented for completion of the Graduate Thesis project and at the American College of Sports Medicine and Exercise Neuroscience Group conferences. We will also be making submissions to an appropriate scientific journal, such as the Journal of Neuroscience or Journal of Neurotrauma.

If you would/ would not like to receive a summary of the results of this study, please indicate below:

No Feedback

Email

CONSENT

I have read and understand the above information. I have received a copy of this form. I agree to participate in this study.

Participant's signature_____

Date _____

Investigator's signature_____ Date _____

APPENDIX B: PARTICIPANT SCREENING QUESTIONNAIRE

Participant Screening Questionnaire

Name: _____
 Sex (circle one): Male / Female
 Date of Birth: _____
 Phone Number: (____) _____
 Email: _____

For investigator use only
 Participant Code:

Please check the following that apply, or write 'no' beside the question:

- I suffer from an acute or chronic ankle injury.
- I have previously been diagnosed with a neurological disorder.
- I have been diagnosed with a traumatic head injury by a physician in the past year.
- I have suffered what I think to have been a concussion in the past year but did not see a physician regarding it.
- I am a smoker.
- I am currently taking any prescription medication.
If checked for 'yes', please list:
 1) _____
 2) _____
 3) _____
 4) _____
 5) _____
- I am currently taking any herbal or sport supplements.
If checked for 'yes', please list:
 1) _____
 2) _____
 3) _____
 4) _____
 5) _____

APPENDIX C: PERMISSION TO USE FIGURES

Figures 2.1, 2.3, 2.4

This is a License Agreement between Ryan CA Foley ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center (CCC). It consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

Supplier	Elsevier The Boulevard, Langford Kidlington, Oxford, OX5 1GB, UK
Registered Company Number	1982084
Customer name	Ryan CA Foley
Customer address	24 Haliburton Avenue Toronto, ON M9B4Y4
License number	3497190376636
License date	Oct 27, 2014
Licensed content publisher	Elsevier
Licensed content publication	Clinical Neurophysiology
Licensed content title	Motoneuron excitability: The importance of neuromodulatory inputs
Licensed content author	C.J. Heckman, Carol Mottram, Kathy Quinlan, Renee Theiss, Jenna S
Licensed content date	December 2009
Licensed content volume number	120
Licensed content issue number	12
Number of pages	15
Start Page	2040
End Page	2054
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	3
Format	print
Are you the author of this Elsevier article?	No
Will you be translating?	No
Title of your thesis/dissertation	Estimates of persistent inward current in human motor neurons due to
Expected completion date	Dec 2014
Estimated size (number of pages)	75
Elsevier VAT number	GB 494 6272 12

Permissions price	0.00 CAD
VAT/Local Sales Tax	0.00 CAD / 0.00 GBP
Total	0.00 CAD

Figure 2.2

This is a License Agreement between Ryan CA Foley ("You") and John Wiley and Sons ("John Wiley and Sons") provided by Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by John Wiley and Sons payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

License Number	3497190801550
License date	Oct 27, 2014
Licensed content publisher	John Wiley and Sons
Licensed content publication	Annals of the New York Academy of Sciences
Licensed content title	Interactions between focused synaptic inputs and diffuse neuromodulation in the dorsal root ganglion
Licensed copyright line	© 2010 New York Academy of Sciences
Licensed content author	M.D. Johnson,C.J. Heckman
Licensed content date	Jun 10, 2010
Start page	35
End page	41
Type of use	Dissertation/Thesis
Requestor type	University/Academic
Format	Print
Portion	Figure/table
Number of figures/tables	1
Original Wiley figure/table number(s)	Figure 7
Will you be translating?	No
Title of your thesis / dissertation	Estimates of persistent inward current in human motor neurons during postural muscle activation
Expected completion date	Dec 2014
Expected size (number of pages)	75
Total	0.00 CAD
Terms and Conditions	

APPENDIX D: CURRICULUM VITAE

Ryan C.A. Foley, B.Sc.

**Graduate Student, Department of Kinesiology & Physical Education
Faculty of Science, Wilfrid Laurier University**

Qualifications Profile:

- Neural control of human movement
- Waveform acquisition, processing and analysis
- Transcranial magnetic stimulation and intramuscular electromyography

Education:

Sept. 2012 - present **Master of Science, Kinesiology and Physical Activity**
Wilfrid Laurier University, Department of Kinesiology
Supervisor: Dr. J. M. Kalmar

Estimates of persistent inward current in human motor neurons during postural sway

Advanced Coursework Completed:

Instrumentation and Digital Signal Processing in Biophysical Research
KIN612 – University of Waterloo

Statistical Reasoning & Experimental Analysis
KP620 – Wilfrid Laurier University

Sept. 2008 - May 2012 **Bachelor of Science, Kinesiology & Physical Education**
Wilfrid Laurier University, Department of Kinesiology

Honours degree

Advanced Coursework Completed:

Advanced Biomechanics
KP451 – Wilfrid Laurier University

Neuromuscular Function in Exercise
KP425 – Wilfrid Laurier University

Research Grants:

2011 – 2012	\$700.00	FOSSA Research Grant (WLU) Training-induced adaptations in Interhemispheric Inhibition
--------------------	----------	--

Honours and Awards:

Summer 2014	\$5,500.00	Graduate Studentship (WLU) Faculty of Graduate Studies, Wilfrid Laurier University
Summer 2013	\$6,000.00	Graduate Studentship (WLU) Faculty of Graduate Studies, Wilfrid Laurier University
Sept. 2013	\$3,000.00	Graduate Scholarship
Sept. 2013	\$3,750.00	Graduate Entrance Scholarship
June 2013	\$300.00	Graduate Travel Assistantship Faculty of Graduate Studies, Wilfrid Laurier University
Apr. 2013		MHAD4 Poster Presentation Finalist 4 th Annual Muscle Health Awareness Day, York University
Apr. 2012		Dean's List Faculty of Science, Wilfrid Laurier University
Apr. 2010		Dean's List Faculty of Science, Wilfrid Laurier University
June 2008		Senior School Letter Michael Power/St. Joseph's High School
Sept. 2004 – June 2008		Honour Roll Michael Power/St. Joseph's High School

Research Experience:

Graduate Student Wilfrid Laurier University, Waterloo, Ontario, Canada	Sept. 2012-present
<ul style="list-style-type: none"> • Supervisor: Dr. Jayne Kalmar 	

- Study design, research, instrument design, data collection, analysis and manuscript preparation for master's thesis project investigating a functional technique for human persistent inward current estimation
- Mentorship and training of incoming Masters researcher

Research Assistant **May 2013-Aug 2013**

Wilfrid Laurier University, Waterloo, Ontario, Canada

- Mentored undergraduate thesis students in data collection (TMS & intramuscular EMG) and analysis techniques using Spike2 and Signal software

Undergraduate Research Assistant **May 2012-Aug 2012**

Wilfrid Laurier University, Waterloo, Ontario, Canada

- Perfected study design and recollected all participants from undergraduate thesis work
- Analyzed MEP activation data for senior graduate student and aided with design of a script for normalized mirror activation analysis

Undergraduate Thesis Student **Sept. 2011-Apr. 2012**

Wilfrid Laurier University, Waterloo, Ontario, Canada

- Supervisor: Dr. Jayne Kalmar
- Researched the effects of a novel, unimanual training intervention on interhemispheric inhibitory signals between motor cortices using transcranial magnetic stimulation

Manuscripts in preparation:

Foley, R.C.A., & Kalmar, J.M., (in preparation). Estimates of persistent inward current in human motor neurons during postural sway. *Journal of Neurophysiology*, (submission in January 2015)

Foley, R.C.A., & Kalmar, J.M., (in preparation). Training-Induced adaptation in interhemispheric inhibition. *Journal of Applied Physiology*, (submission in March 2015)

Invited Presentations:

Estimates of Persistent Inward Current During Standing Posture, Department of Kinesiology, Wilfrid Laurier University, January 2013. 1-hour seminar presentation

Reciprocal Inhibition and Persistent Inward Current: Experimental Approach to Motor Neuron Excitability, KP425 – Neuromuscular Function in Exercise, Wilfrid Laurier University, November 13th, 2013

Reciprocal Inhibition: The importance of inhibitory input on the alpha-motor neuron, Department of Kinesiology, Wilfrid Laurier University, January 2013. 1-hour seminar presentation

A Neuromuscular Approach to Muscle Cramps During Long-Duration Exercise , KP422 – Advanced Human Physiology, Wilfrid Laurier University, October 16th, 2012

Non-refereed contributions:

Foley, R.C.A., & Kalmar, J.M., (2014). Estimates of persistent inward current in human motor neurons during postural sway (poster presentation at the Society for Neuroscience Annual Meeting, Washington, D.C., USA)

Foley, R.C.A. and J.M. Kalmar (2013) Training Induced Adaptation of Interhemispheric Inhibition (oral presentation, Exercise Neuroscience Group, Oshawa, ON, June 2013)

Foley, R.C.A. and J.M. Kalmar (2013) Training Induced Adaptation of Interhemispheric Inhibition (poster presentation at the American College of Sports Medicine Meeting, Indianapolis, IN, USA)

Foley, R.C.A. and J.M. Kalmar (2013) Interhemispheric Inhibition During Bimanual Training: Acute and Chronic Adaptations (poster presentation at the Muscle health Awareness Day, Toronto, ON, April 2013)

Other Academic Contributions:

- ❖ **Neuromuscular Physiology Demonstrator** at BrainWorks Day Camp for children, Wilfrid Laurier University, August 2013
- ❖ **Guest lecturer** for Kinesiology Graduate Primer, “TMS: introduction to MEPs and Paired-Pulse Research”, August 20, 2012
- ❖ **Undergraduate Thesis Poster Presentation Evaluator**, April 2, 2013
- ❖ Volunteer tour guide at Kinesiology **Graduate Program Open House**, March 26, 2013
- ❖ Volunteer tour guide at Kinesiology **Graduate Program Open House**, November 15, 2012

Work Experience:

Kinesiology Lab Technician (part- time) **Aug. 2014-present**
University of Ontario Institute of Technology, Oshawa, Ontario, Canada

- Responsible for the daily operation and maintenance of the kinesiology teaching lab equipment including metabolic carts, ECG-treadmill stress testing systems, cycle ergometers, load cells, digital goniometers and PowerLab compact data acquisition systems
- Assist with the design and production of several apparatus for the teaching and research labs

Teaching Assistant **Sept. 2012-Apr. 2014**

Wilfrid Laurier University, Waterloo, Ontario, Canada

Human Physiology (2nd year course) Jan. 2014-Apr. 2014

- Assisted with set-up of labs, marking of weekly quizzes and exams

Exercise Physiology Labs (3rd year course) Sept. 2013-Dec. 2013

- Ran students through VO₂Max, Wingate and strength testing protocols using Medisoft metabolic carts and Humac Norm multi-joint dynamometers

Neuromuscular function in Exercise (4th year course) Sept. 2012-Dec. 2013

- Provided direction, marked final research topic papers and weekly quizzes

Bio-Dynamics of Physical Activity (1st year course) Jan. 2013-Apr. 2013

- Assisted students with the transition to university quality writing and referencing techniques as well as organizational and study skills and marked final research papers

Advanced Exercise Physiology (4th year course) Sept. 2012-Dec. 2012

- Marked weekly quizzes and met with students having trouble with course material to explain advanced physiological concepts

Sales Associate (part- time)

Nov. 2009-Sept.2014

FGL Sports Ltd., SportChek, Waterloo, Ontario, Canada

- Footwear, hardgoods and softgoods sales associate offering expertise in running mechanics, skate sharpening and snowsports apparel.

Inclinometer Monitoring Specialist

May 2010-Sept. 2011

Monir Precision Monitoring Inc., Mississauga, Ontario, Canada

- Monitored numerous large-scale excavation sites using MEMS inclinometer system and assisted in borehole and pile installation and set-up targets for total station monitoring in confined-space TTC subway tunnels

Lifesaving and Swim Instructor

June 2006-Sept.2010

City of Toronto, Etobicoke, Ontario, Canada

- Swim Instructor for ages ranging from infant (less than one year) to adults with a primary focus on swimming efficiency and survival skills

Sales Associate (part- time)

Sept. 2005-June 2008

Sporting Life Inc., Etobicoke, Ontario, Canada

- Footwear sales associate and 2-times representative at the Toronto International Bicycle Show

Volunteer service:

- **WSIB Student Ergonomist** – Occupational Health Clinic
 - Assisted with ergonomic assessments of injured workers

- Researched and catalogued new ergonomic products to be used in the workplace
- **Student-Kinesiologist** – Justine Blainey Wellness Centre
 - Observed chiropractic treatment performed by Dr. Blake Broker
 - Implemented patient stretching and home exercise plans
- **Rehabilitation Assistant** – Sun Life Movement Disorders Research and Rehabilitation Centre
 - Assisted in implementation of exercise plans aimed at reducing Parkinson's Disease symptoms

Certifications:

- ❖ **Canadian Society for Exercise Physiology Certified Personal Trainer** – August 2014 to present
- ❖ **Standard First Aid/ CPR-C** – May 2003 to present
- ❖ **Fall Restraint and Fall Arrest Safety Training** – May 2010 to Dec. 2011
- ❖ **WHIMIS Certificate** – Sept. 2004
- ❖ **Confined Space Safety Training** – May 2010 to Dec. 2011
- ❖ **Tri-Council Policy Training in Ethical Conduct for Research Involving Humans** – Oct. 2011

Professional Development/Courses Taken:

- CSEP-CEP Workshop (in progress)
- CSEP-CPT Workshop
- Statistical Reasoning & Experimental Design (graduate level)
- Instrumentation & Digital Signal Processing in Biophysical Research (graduate level)
- Neurocognition of Human Movement (graduate level)
- Advanced Biomechanics
- Neuromuscular Function in Exercise
- Advanced Fitness Assessment
- Endocrinology
- Biopsychology
- Sports Medicine
- Genetics
- Cell and Molecular Biology
- Exercise Physiology
- Human Physiology

Extra-Curricular Activities:

Intramural Handball 2013
 Wilfrid Laurier University, Waterloo, ON

Intramural Soccer 2012

Wilfrid Laurier University, Waterloo, ON

Intramural Ultimate Frisbee	2012
Wilfrid Laurier University, Waterloo, ON	
Intramural Ice Hockey	2010-2014
Wilfrid Laurier University, Waterloo, ON	
Intramural Ball Hockey	2010-2012
Wilfrid Laurier University, Waterloo, ON	
Intramural Dodgeball	2009-2012
Wilfrid Laurier University, Waterloo, ON	
Ski & Snowboard Club Member	2009-2012
Wilfrid Laurier University, Waterloo, ON	
Kin Games Participant	2011 & 2012
Wilfrid Laurier University, Waterloo, ON	
Student Council Vice President	2013
MPSJ, Etobicoke, ON	
Grade 9 Orientation Head Executive	2007
MPSJ, Etobicoke, ON	
Grade 9 Orientation Co-Executive	2006
MPSJ, Etobicoke, ON	
Senior Boys Softball Team	2008
MPSJ, Etobicoke, ON	
Cross Country Team	2004-2007
MPSJ, Etobicoke, ON	

References:

Dr. Jayne Kalmar
 Associate Professor
 Kinesiology, Wilfrid Laurier University
 (519) 884-0710 ext. 2033

Dr. Michael Cinelli
 Associate Professor
 Kinesiology, Wilfrid Laurier University
 (519) 884-0710 ext. 4127