Modulation of hamstrings reflexive responses during human gait

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MODULATION OF HAMSTRINGS REFLEXIVE RESPONSES DURING HUMAN GAIT

by

Bradley Wayne Floy

An Abstract

Of a thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Exercise Science in the Graduate College of The University of Iowa

May 2012

Thesis Supervisor: Professor Warren G. Darling
ABSTRACT

In humans, it is thought that both central commands and peripheral feedback from sensory receptors contribute to the control of locomotion. An important problem that exists in human locomotion research is the interactions and balance between the individual contributions of the central (CNS) and peripheral (PNS) nervous systems to the control of muscles during movement are not fully understood. Applying external perturbations such as stretches, tendon taps, and electrical stimulation to the neuromuscular system during walking can help us learn more about how the response to afferent information is modulated during locomotion. To date, most of the research looking at modulation of the response during walking has investigated the soleus and quadriceps muscles. Very little research has focused on the hamstring muscles, which are important during walking, particularly during late swing. One reason for this is that it is difficult to detect H-reflexes in hamstrings following electrical stimulation of the sciatic nerve. The purpose of this study was to demonstrate a new sciatic nerve stimulation technique and use it to study the modulation of the response to afferent feedback during walking.

This study consisted of two parts: 1) Establish the presence of an afferent mediated response (H-reflex) during prone lying in hamstrings muscles, and 2) Investigate the modulation of this afferent feedback during walking. Subjects underwent single and double pulse stimulations to the sciatic nerve during prone lying, followed by electrical stimulation at 12 different phases of the gait cycle. For each phase, stimulus response curves were created in which maximal direct (M-wave) and afferent mediated responses (H-reflex) could be determined. Maximal H-reflex (Hmax) was normalized to
maximal M-wave (Mmax) to create an H:M ratio that was used to compare modulation of the responses between phases and subjects.

Electrical stimulation of the sciatic nerve elicited detectable H-reflexes in biceps femoris during prone lying and walking. The modulation of the response to afferent feedback is not the same for all phases of the gait cycle, particularly in late swing when it has a higher amplitude than the rest of the gait cycle. This modulation was not simply related to background EMG as would be expected during isometric contractions. Thus, there must be both central and peripheral influences on the response. Understanding the control of human locomotion is important for developing rehabilitation programs for patients with lesions of the central nervous system such as stroke or spinal cord injury.

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CHAPTER I

INTRODUCTION

In humans, it is thought that both central commands and peripheral feedback from muscles contribute to the control of locomotion. During human locomotion, predictable muscle moments and powers, such as knee energy absorption in late swing, occur during each phase of the gait cycle (Inman 1966). The generation of these joint moments and powers is under the influence of both the central (CNS) and peripheral (PNS) nervous systems and may be directed by central pattern generators (Hultborn and Nielsen 2007). Central pattern generators (CPGs) found in the spinal cord are known to control locomotion patterns in many species (Sherrington 1910; Brown 1914; Duysens and Van de Crommert 1998) but have yet to be proven to be present in humans. Understanding the control of human locomotion is important for developing rehabilitation programs for patients with lesions of the central nervous system such as stroke or spinal cord injury.

An important problem that exists in human locomotion research is that the balance between the contributions of the CNS and PNS to the control of muscles during movement is not fully understood. It is believed that responses to peripheral input can contribute a substantial percentage of joint torque during movement and that these responses may be modulated differently by central influences during different tasks (Capaday and Stein 1986; Yang, Stein et al. 1991). It appears there is inconsistency between different tasks (task dependent), or even between different phases of the same task (time dependent), for a given muscle or group of muscles (Capaday and Stein 1986; Edamura, Yang et al. 1991; Faist, Blahak et al. 1999; Baken, Dietz et al. 2005; Larsen, Mrachacz-Kersting et al. 2006). The growing body of research is showing that the
contributions of the CNS and PNS are modulated differently for each muscle group throughout the different phases of the gait cycle. It is also still unclear to what extent a CPG is involved in the generation of human walking and whether sensory feedback to the spinal cord plays a significant role in human muscle activation as in other species (Sinkjaer, Andersen et al. 2000). Thus, there is a need to study the role of peripheral inputs during walking and how they are modulated throughout the gait cycle.

Afferent Feedback During Walking

The role of information from the periphery, such as that carried by group Ia, Ib, and II afferents, has been studied in the cat and human soleus muscles during walking (Yang, Stein et al. 1991; Sinkjaer, Andersen et al. 2000; Mazzaro, Grey et al. 2006). Group Ia afferents carry muscle length- and velocity-dependent information from muscle spindle fibers, while group II afferents carry length-dependent information from muscle spindle fibers. Group Ib afferents carry information from Golgi tendon organs regarding muscle tension in the musculotendinous unit. It is believed that group Ia afferents influence cat soleus electromyograph (EMG) activity during the stance phase (Nielsen and Sinkjaer 2002). However, it appears to be different in humans in that the feedback from group II afferents, and possibly from load-sensitive afferents (Ib), contribute to the amplitude modulation of the soleus muscle activity during the stance phase of the gait cycle (Sinkjaer, Andersen et al. 2000; Mazzaro, Grey et al. 2006). Despite the uncertainty of the specific roles of each type of sensory afferent, group Ia and some group II (Stauffer, Watt et al. 1976) afferent neurons have monosynaptic connections that allow sensory information to quickly influence muscle activity. Moreover, responses to
monosynaptic cutaneous afferent stimulation in cats during gait have been shown to be modulated during portions of the gait cycle (Menard, Leblond et al. 2003). These monosynaptic connections, particularly Ia afferents onto motoneurons, may be a major contributor to the control of joint torque during human locomotion. In seated subjects it was estimated that small stretches (<0.1 rad and <50ms) could produce a torque equal to as much as 20% of the maximum voluntary contraction (MVC) torque of ankle plantar flexors (Stein and Kearney 1995). During walking, it was calculated that velocity sensitive afferent information (<200ms in latency) can contribute up to 30-60% of the soleus activity during the early stance phase (Yang, Stein et al. 1991). Because of this potentially substantial contribution of reflexes to gait, it is necessary to understand their modulation in muscles important to locomotion throughout the gait cycle.

In addition to the questions about the role of afferent feedback during walking, there are also questions as to the best methodology for studying effects of this afferent input. Several techniques are commonly used to study short latency reflexes. Some studies have induced quick, short stretches to the muscle (Akazawa, Aldridge et al. 1982; Yang, Stein et al. 1991; Stein and Kearney 1995; Sinkjaer, Andersen et al. 1996; Sinkjaer, Andersen et al. 2000), while others have used a tendon tap technique to elicit responses (Van de Crommert, Faist et al. 1996; Faist, Blahak et al. 1999). Electrical stimulation of cutaneous nerves can also elicit short latency responses in muscles during walking (Duysens, Tax et al. 1996; Baken, Dietz et al. 2005). However, the most commonly used technique is the Hoffman reflex (H-reflex) (Hoffmann 1918; Akazawa, Aldridge et al. 1982; Morin, Katz et al. 1982; Capaday and Stein 1986; Capaday and Stein 1987; Crenna and Frigo 1987; Brooke, Collins et al. 1991; Edamura, Yang et al.
1991; Yang and Whelan 1993; Larsen, Mrachacz-Kersting et al. 2006; Stein and Thompson 2006; Stein, Estabrooks et al. 2007), which is the electrical analog to the stretch reflex. Each of these methods poses methodological issues. Some examples of issues with stretches and tendon taps to muscles that can arise are inconsistency in stimulus input amplitude, wearing potentially gait altering devices, variability of muscle spindle gain, and difficulty normalizing response amplitudes between subjects and studies. Eliciting and identifying H-reflexes in some muscles is extremely difficult because of either a lack of access to the peripheral nerve for stimulation or because the responses in the EMG signal are indistinguishable. The prospect of improving upon these aforementioned techniques could allow for the collection of sensory information during human gait that has not previously been studied.

There are several studies on short latency reflexes of quadriceps during human locomotion (Dietz, Discher et al. 1990; Dietz, Faist et al. 1990; Brooke, Collins et al. 1991; Mrachacz-Kersting, Lavoie et al. 2004; Larsen, Mrachacz-Kersting et al. 2006) and even more so on the soleus (Akazawa, Aldridge et al. 1982; Morin, Katz et al. 1982; Capaday and Stein 1986; Capaday and Stein 1987; Crenna and Frigo 1987; Brooke, Collins et al. 1991; Edamura, Yang et al. 1991; Yang, Stein et al. 1991; Yang and Whelan 1993; Stein and Kearney 1995; Sinkjaer, Andersen et al. 1996; Simonsen and Dyhre-Poulsen 1999; Sinkjaer, Andersen et al. 2000; Stein and Thompson 2006; Stein, Estabrooks et al. 2007). However, very little is known about the short latency reflexes in hamstrings and their role in locomotion because there has yet to be a study examining H-reflexes in the hamstring muscles. This is probably due to difficulty in stimulating the sciatic (tibial) nerve because of its location deep to the gluteus maximus. In addition, the
proximity of the nerve to the spinal cord makes the temporal occurrence of M and H responses very close and thus difficult to distinguish from each other. Therefore, the general purpose of this study was two-fold: 1) To establish a new sciatic nerve stimulation technique to elicit short latency reflexes in hamstrings, and 2) To examine the modulation of the response to afferent feedback from hamstrings during walking. The next chapter will review related research to give a background of this topic.

**Purpose of Study**

Research to date has given us insight into the modulation of the response to afferent feedback during the gait cycle in the soleus and quadriceps muscles, but is lacking for the hamstring muscles. Soleus and quadriceps are both anti-gravity muscles whose response to afferent feedback is modulated differently during active lengthening and energy absorption of the muscle in the stance phase compared to the rest of the gait cycle. The hamstring muscles undergo active lengthening and energy absorption during the late swing phase, in which it is also believed the response to afferent feedback is modulated differently than in other parts of the gait cycle (Faist, Blahak et al. 1999). One reason for the lack of studies on the hamstrings is because it is difficult to elicit and/or identify H-reflexes in the hamstrings. The purpose of this study was to identify and apply a new sciatic nerve stimulation technique to elicit afferent responses from the hamstring muscles in order to gain more information about the role afferent feedback plays during the gait cycle.
Aims and Hypotheses

The general aim of the proposed study is to investigate possible modulation of the muscle afferent contribution to hamstring muscle activity during walking. This will be accomplished through two specific aims.

**Aim 1:** Establish that the multiphasic response elicited by sacral root stimulation consists of a direct motor response followed by an afferent-mediated response.

The hypotheses tested for the first aim are:

**Hypothesis 1:** There will be a difference in the relationship between amplitudes of the first and second muscle responses with electrical stimulation intensity. The maximum direct motor (first) response amplitude (Mmax) will be greater than the maximum afferent-mediated (second) response amplitude (Hmax) following electrical stimulation. The direct motor response will increase with increasing stimulation intensity until a plateau is reached. The afferent-mediated response will increase and then decrease as stimulation intensity increases.

**Rationale:** It is important to establish that the nature of the observed responses is similar to those previously described in studies of H-reflexes in other muscles (Capaday and Stein 1986; Schieppati 1987; Stein and Capaday 1988; Edamura, Yang et al. 1991; Pierrot-Deseilligny and Burke 2005). The maximum direct motor response is reached when all available motoneuron axons have been stimulated and further increases in stimulation intensity do not result in increased response amplitude (Schieppati 1987). The maximum afferent-mediated response will increase with intensity but should never be greater than or equal to Mmax because, with increasing stimulation intensity, antidromic activity from the direct motor response prevents the afferent mediated
response from occurring in some motor neurons (Pierrot-Deseilligny and Burke 2005). Thus, after the maximum response is reached, the afferent mediated response is first decreased and then abolished at high stimulation intensities.

Hypothesis 2: There will be a difference in the afferent-mediated (2\textsuperscript{nd}) responses when using double-pulse electrical stimulation with a 50ms inter-stimulus interval. The afferent-mediated response peak to peak amplitude (PP Hwave) will be smaller due to homosynaptic depression from the first stimulus affecting the second stimulus. The direct motor response peak to peak amplitude (PP Mwave) will be the same for each of the two consecutive stimuli and will be unaffected by the double stimulus.

Rationale: When afferent neurons are electrically stimulated repeatedly with short inter-stimulus intervals (<10s), the response to the second response is depressed. This is believed to be from a delayed reuptake of neurotransmitter from the synaptic cleft (Hultborn, Illert et al. 1996). This is called homosynaptic depression, otherwise known as post-activation depression, and occurs in monosynaptic reflexes such as the H-reflex. The direct motor response is due to direct stimulation of motor axons and thus is unaffected by repeated stimuli.

Aim 2: Compare the modulation of the afferent-mediated response of hamstrings during different phases of the gait cycle.

The hypotheses tested for the second aim are:

Hypothesis 3: The maximal afferent-mediated response will be different for different phases of the gait cycle. The ratio of the maximum peak to peak amplitude of the afferent mediated response compared to the maximum peak to peak amplitude of the
direct motor response \(2^{\text{nd}}\text{max} : 1^{\text{st}}\text{max}\) will be larger during the late swing phase than in other phases of the gait cycle. Specifically, this will occur in last 15% of the gait cycle.

**Rationale:** Previous studies eliciting either H-reflexes (Morin, Katz et al. 1982; Capaday and Stein 1986; Crenna and Frigo 1987; Edamura, Yang et al. 1991) or stretch reflexes (Yang, Stein et al. 1991; Yang and Whelan 1993; Sinkjaer, Andersen et al. 1996; Sinkjaer, Andersen et al. 2000) in human soleus have shown that it is modulated differently in different parts of the gait cycle. The same has been shown for the quadriceps muscles (Dietz, Discher et al. 1990; Mrachacz-Kersting, Lavoie et al. 2004; Larsen, Mrachacz-Kersting et al. 2006). In both muscles, the time dependent effects seem to be most prominent when the muscle is actively lengthening and thus absorbing energy. In the hamstrings, energy absorption occurs in late swing and time dependent effects have been seen when eliciting stretch reflexes (Faist, Blahak et al. 1999) but have yet to be shown with H-reflexes.

**Hypothesis 4:** The relationship between the maximal afferent-mediated response normalized to the maximal direct motor response \(2^{\text{nd}}\text{max} : 1^{\text{st}}\text{max}\) and background muscle activity (EMG) will have a higher slope during the swing phase than in the rest of the gait cycle (stance).

**Rationale:** In the studies showing time-dependent effects of short latency reflexes during gait, the modulation of the response is not thought to be due simply to changes in motoneuron excitability as in voluntary isometric contractions (Morin, Katz et al. 1982; Capaday and Stein 1986; Brooke, Collins et al. 1991; Faist, Blahak et al. 1999; Faist, Hoefer et al. 2006). It is believed that in most parts of the gait cycle motoneuron excitability of hamstrings is directly related to muscle activation level. However, during
late swing the tendon jerk response of hamstrings is not simply related to background activity but can be modulated in a time dependent fashion by other mechanisms (Faist, Blahak et al. 1999). Specifically, the hamstrings tendon reflex is larger, in relation to background EMG, during late swing than during other phases of the gait cycle. It would be of interest to test whether results are similar for H-reflexes because the electrical stimulation technique could lead to more ways of collecting and comparing responses to afferent information due to its versatility for use during movement. This will be discussed in more detail in later sections.
CHAPTER II

REVIEW OF LITERATURE

The purpose of this section is to review related work and use that information to determine the best method to answer the question at hand, which is: During human gait, are hamstring H-reflexes modulated differently at various phases of the gait cycle? However, I must first discuss the advantages and disadvantages of different study techniques (i.e. stretch, tendon tap, electrical stimulation) and suggest why one of them is best used for the purpose of this study. I will then review the task and time dependent nature of reflexes, where the bulk of the literature has focused on the modulation of afferent feedback from the soleus and quadriceps muscles. I will lastly discuss why it is important to also study the hamstring muscles, and what related research has already been done on the hamstrings. The basis for the methodologies used in the present study will be drawn from previous related studies.

External Perturbation Techniques

As mentioned in the Introduction, because of the significant contribution of short latency reflexes to locomotion, it is beneficial to induce controlled perturbations to the neuromuscular system to gain information about control during locomotion. There are three common ways to induce short-latency reflexes in muscle: small amplitude stretches of the muscle, tendon taps using a reflex hammer or similar device, or electrically stimulating nerves to the muscle. There are advantages and disadvantages of each that need to be considered before making a decision as to which is the most appropriate technique to use in this study.
The Hoffman Reflex and Other Responses to Electrical Stimuli

The Hoffmann reflex, or H-reflex, is considered to be the electrical analog of the stretch reflex. Electrical stimulation can be applied on a mixed nerve to evoke short latency responses of the muscle. This was first described by Hoffmann (Hoffmann 1918) who demonstrated two short latency responses. The cause of the two responses is from nerve impulses traveling in both directions when an axon is stimulated. The first response (M-wave) is a direct motor response from stimulation of the motoneuron axons, and the second response (H-wave) (Magladery and McDougal 1950) is a monosynaptic response arising from afferent neuron excitation of the motoneuron in the spinal cord. The characteristics of both waves have been described in detail previously (Schieppati 1987; Pierrot-Deseilligny and Burke 2005). As the stimulus intensity is increased through repeated stimulations, the M-wave amplitude increases until a maximum is reached (Mmax), while the H-wave amplitude increases to a maximum (Hmax) and then decreases. When the stimulus intensity is low, only a few motoneurons and Ia afferents are stimulated. At intermediate intensities, most Ia afferents are stimulated without many motoneurons being in refractory, and thus the H-reflex response peaks. When the stimulus intensity is high, many motoneurons are in a refractory state or have antidromic activity and thus afferent mediated responses are not transmitted over efferent axons to the muscle. Thus, the stimulus-response (S-R) curve of the H-reflex is parabolic in nature, while the S-R curve of the M-wave is more S-shaped, plateauing at high stimulation intensities.
There are numerous considerations that must be taken into account when studying the H-reflex, as described in previous articles (Paillard 1955; Schieppati 1987). It has become widely accepted that the H-reflex is monosynaptic in nature from stimulation of Ia afferents, however it is possible that oligosynaptic inputs as well as other afferent input could influence the H-reflex. Group Ia afferents can excite motoneurons directly, but can also be inhibitory in nature via interneurons (Hultborn, Wigstrom et al. 1975). Because of relatively short distances and close conduction velocities, it is possible that non-Ia fibers as well as oligosynaptic pathways can influence the H-reflex after the initial input from monosynaptic Ia fibers (Schieppati 1987). However, with the H-reflex excitatory post-synaptic potential (EPSP) rise time being probably only a few milliseconds in duration, the effects of postsynaptic temporal summation from these other inputs is probably limited. Moreover, with electrical stimulation the Ia afferent volleys elicited are close to simultaneous whereas with a muscle stretch there is more dispersion in the Ia afferent inputs onto the spinal cord (Burke, Gandevia et al. 1983). Thus it seems likely that the H-reflex is due to monosynaptic connections from Ia afferents and is minimally influenced by other synaptic inputs.

The H-reflex amplitude can be quite variable and is dependent on many different factors. H-reflex amplitudes can vary greatly depending on level of background activity. Both sub and suprathreshold background activity can affect the size of the response. Thus H-reflex responses studied at rest have little functional value (Stein and Thompson 2006) and should be studied during the activity of interest. The H-reflex amplitude is a measure of the excitability of the motoneurons, and consequently may change in various conditions (task or time dependent) as a function of segmental and supraspinal influences.
playing upon it (Schieppati 1987). In addition, it is not merely a reflection of the excitability of motoneurons, but that it is a measure of synaptic efficacy dependent on presynaptic inhibition and neurotransmitter levels (Capaday and Stein 1987). These influences will be discussed in greater detail later in this section.

It is also important to differentiate the H-reflex from other waves that occur with electrical stimulation. One wave with a similar latency to that of the H-reflex is the F-wave. The F-wave results from backfiring of motoneurons due to antidromic conductions of action potentials (Kimura 2001). Unlike the H-reflex, the F-wave is non-synaptic and thus not affected by pre-synaptic influences. The F-wave latency can be quite variable, while the H-reflex is of constant latency under similar stimulation and recording conditions (Paillard 1955). The F-wave does not occur in many motoneurons, and when H-reflexes are present, F-waves are sparse due to a lack of recurrent activation from collisions with the F-wave (Kimura 2001). As described later in the Methodology, the response observed in this study was most likely not the F-wave but indeed an afferent mediated response because we were able to show this response propagated through a synapse whereas the F-wave is non-synaptic in nature.

Another short latency response that can occur, although not normally, is the A-wave. If a submaximal response elicits one branch of an axon but not another, the antidromic wave turns at the point of branching and travels back down the axon to the muscle and is known as the A-wave. The latency is intermediate to the M-wave and F-wave (Kimura 2001). The A-wave does not occur with supramaximal stimulations and rarely occurs in healthy individuals (Kimura 2001). In this study, stimulation does not
occur within the muscle but instead in the proximal motoneuron. Thus it was not considered to be a factor in this study.

In addition to electrical stimulation of a mixed nerve to elicit an H-reflex, electrical stimulation of cutaneous nerves can also elicit short latency reflexes. Some studies have investigated the modulation of cutaneous reflexes during walking (Duysens, Tax et al. 1996; Baken, Dietz et al. 2005). In adult humans, short latency cutaneous reflexes (<15ms) are rare to find in the legs but medium latency (15-25ms) are more common (Rowlandson and Stephens 1985). One study found that only 14% of subjects demonstrated the short latency response, while 100% demonstrated the medium latency response in biceps femoris during walking (Baken, Dietz et al. 2005). This study found much inconsistency in the responses in that they did not always follow background EMG activity, and were sometimes excitatory or suppressive depending on which response and when in the gait cycle it was elicited. Thus, afferent information from cutaneous stimulation can result in short latency responses in human hamstrings during walking, however it is highly unreliable and unpredictable.

Homosynaptic Depression

Homosynaptic depression, or post activation depression, is the phenomenon that occurs when two electrical stimuli are applied to a nerve within the range of a few milliseconds up to 10 seconds of each other. The H-reflex response of the second stimulus is reduced compared to the H-reflex of the first stimulus. The depression is only seen in the synapses that are stimulated, thus the origin of the name (Hultborn, Illert et al. 1996). Homosynaptic depression only appears in muscles at rest and disappears or is
greatly inhibited during a contraction or during activity (Stein, Estabrooks et al. 2007). It is believed that the depression is caused by modulation of presynaptic transmitter release in the monosynaptic reflex of Ia afferents (Hultborn, Illert et al. 1996). Because it is a phenomenon that occurs synaptically, the non-synaptic waves mentioned previously (M, F, A) remain unaffected. And because the depression occurs from the earliest onset of an H-wave, it is most likely associated with the Ia afferent neurons.

Comparison of Other Techniques to Electrical Stimulation

Although similar in many ways, there are also many differences between the H-reflex and stretch and tendon tap techniques. When choosing one type of external perturbation versus the other, there are benefits and drawbacks that must be taken into consideration. One problem facing both techniques is that of normalization of the responses. Normalizing the afferent mediated response to a controlled value is important when wanting to compare the response between subjects or between testing sessions. The problem with electrical stimulation of the peripheral nerves during walking is that electrode position relative to the nerve changes at different extremity positions. Thus, the effective stimulation intensity changes at different joint angles. Also, the size of the EMG response to constant nerve stimulation may vary at different muscle lengths. To overcome this, several studies have measured maximal M-wave responses at different joint angles and the respective H- and M-waves were expressed as a percentage of the maximal M-wave obtained at the same joint angle (Morin, Katz et al. 1982; Dietz, Discher et al. 1990). Similar issues occur with stretch and tendon tap techniques. In addition to changes in muscle lengths and tensions, it is difficult to maintain precise
stimulus amplitudes of the reflex hammer. Moreover, the sensitivity of the spindles is difficult to maintain throughout movement. As compared to electrical stimulation, there is no direct motor response in stretch reflexes to compare the afferent mediated responses when doing tendon tap or muscle stretches studies. Thus for comparison purposes between subjects or testing sessions it seems better to use electrical stimulation.

The testing conditions or type of activity being performed should also be considered when selecting one method over the other. Some authors have argued that tendon taps and small joint rotations are more suitable than electrical stimulation for studying reflexes because they are more natural in that they involve the fusimotor system and better resemble normal physiology that occurs during movement. Stretches activate the spindles, and thus mimic the same complete path used in real world activities. However for research purposes the fusimotor system can confound interpretation of results. With tendon taps and stretches, the muscle spindles are activated due to a change in length of the muscle. The frequency of action potentials in response to muscle stretch is dependent on the spindles’ sensitivity, which can change due to other inputs, such as gamma and beta efferents (Pierrot-Deseilligny and Burke 2005). Electrical stimulation directly excites the Ia afferents and motoneurons and thus bypasses the muscle spindle fibers. The H-reflexes and stretch reflexes are similar in that their synaptic connections are the same; however the stretch reflex and joint perturbations could be subject to inconsistency of the stimulus input to the system. The stretch of the tendon during tendon taps is often not consistent during the gait cycle; and apparatuses that induce joint rotations may change normal gait kinematics even without striking the tendon (Sinkjaer, Andersen et al. 1996). With electrical stimulation, the electrodes are much less
bothersome and gait should be closer to normal walking, at least up until time of stimulation. Thus the H-reflex may be better for studying synaptic efficacies (Capaday and Stein 1986) such as premotoneuronal or presynaptic inputs during walking.

Because of the difficulties mentioned above with tendon tap studies and a lack of understanding regarding control of muscles during movement, there is a need to study H-reflexes in addition to tendon taps in hamstring muscles. Conclusions about one type of reflex based on results from others have to be drawn with caution because of changes in stimulation and recording conditions such as electrode movement relative to underlying muscle and changes in muscle length (Faist, Hoefer et al. 2006). When functional conclusions are made, it should be kept in mind that both the application of electrical stimuli to nerves and artificial external perturbations to muscles are experimental techniques, which only to a certain extent mimic the normal physiological Ia afferent feedback (Morita, Petersen et al. 1998). Nonetheless, important information can be gathered with external perturbation techniques. With all techniques, the responses are dependent on the task being performed and the phase within the task that the response is being measured. Different muscles also respond differently based on the demands of a given task or within the same task. As coined previously (Edamura, Yang et al. 1991), this paper will use the terms “task dependent” and “time dependent” to differentiate changes between tasks, or at different points in time within the same task. I will now discuss the task and time dependent nature of reflexes.
Modulation of Responses to External Perturbations

Task Dependency

The modulation of afferent mediated responses due to external perturbations is dependent on the task being performed and varies by muscle. Soleus and quadriceps have been the two lower extremity muscles studied most extensively, mainly because of the difficulty in eliciting and identifying an H-reflex in many other leg muscles. Most of the studies to date investigating reflexes during gait have examined the response of the soleus muscle (Akazawa, Aldridge et al. 1982; Morin, Katz et al. 1982; Capaday and Stein 1986; Capaday and Stein 1987; Crenna and Frigo 1987; Brooke, Collins et al. 1991; Edamura, Yang et al. 1991; Yang, Stein et al. 1991; Yang and Whelan 1993; Stein and Kearney 1995; Sinkjaer, Andersen et al. 1996; Simonsen and Dyhre-Poulsen 1999; Sinkjaer, Andersen et al. 2000; Stein and Thompson 2006; Stein, Estabrooks et al. 2007). In addition to the soleus, quadriceps’ reflexes have also been studied during human gait (Dietz, Discher et al. 1990; Dietz, Faist et al. 1990; Brooke, Collins et al. 1991; Mrachacz-Kersting, Lavoie et al. 2004; Larsen, Mrachacz-Kersting et al. 2006). The soleus is convenient for electrical stimulation studies because of the ease of stimulating the tibial nerve in the popliteal fossa; and results in a distinct M-wave and H-reflex (Morin, Katz et al. 1982; Capaday and Stein 1986). The same holds true for stimulating the quadriceps via the femoral nerve, with it being readily accessible in the femoral triangle. Examining how studies were done on these muscles and the results obtained from them may help with the design of studies on other muscles and in other tasks.

Modulation of the soleus reflexes has been shown to be dependent on the task performed. In static conditions, reflex modulation is directly associated with EMG
activity. However, Akazawa et al. (1982) (Akazawa, Aldridge et al. 1982) showed in the cat that soleus H-reflex modulation during walking was not caused solely by changes in background EMG, thus concluding there must be some task dependent effects in the cat. When comparing EMG matched standing isometric contractions versus walking, stretch reflexes were lower during standing than walking in cats (Akazawa, Aldridge et al. 1982). Differences also occur in humans, but different than the cat in that stretch reflexes as well as H-reflexes are lower during walking than in static contractions (Capaday and Stein 1986; Brooke, Collins et al. 1991; Edamura, Yang et al. 1991). When comparing walking versus running in humans, H-reflexes during running were lower despite higher peak EMG levels during the stance phase (Capaday and Stein 1987; Edamura, Yang et al. 1991).

While modulation of soleus reflexes is task dependent, the speed of movement does not appear to affect the response. H-reflex amplitude has been shown to be no different within the same task (e.g. walking or running) performed at different speeds; but also different when performed at the same speed during different tasks (e.g. running versus walking) (Edamura, Yang et al. 1991), although, these findings conflicted with those of a similar study (Simonsen and Dyhre-Poulsen 1999).

Quadriceps muscles’ afferent mediated responses also appear to be task dependent in nature. Quadriceps stretch reflex amplitudes obtained during isometric contractions were found to be lower than during the gait cycle despite matched joint angles and background EMG activity (Mrachacz-Kersting, Lavoie et al. 2004). When specifically investigating the vastus medialis in a different study, the H-reflex was depressed during walking compared to matched EMG levels during standing (Brooke, Collins et al. 1991).
However, slightly different results were shown in the other muscles during a different task when it was found that the rectus femoris and vastus medialis H-reflexes were increased during early stance and decreased during the remainder of the gait cycle compared to EMG matched prone-lying (Dietz, Faist et al. 1990). Similar findings were obtained by the same group (Dietz, Discher et al. 1990) when studying tendon tap reflexes during standing versus walking. The response was nearly the same in early stance as controlled standing, and decreased through the remainder of the gait cycle.

In summary, it appears that reflex amplitude varies depending on the muscle and task. Human soleus reflex amplitudes are lowest during running, slightly greater during walking, and even higher during standing contractions despite similar background EMG levels. Human quadriceps reflex amplitudes also varied for prone lying, standing, or walking. Thus it appears that short latency reflexes are influenced by the task at hand and not just by the background activity of the muscle.

*Time Dependency*

As mentioned above, afferent mediated responses can differ based on the task being performed; however they can also change at different phases or times within the same task. The relative amplitude of the response is dependent partially on the time of the gait cycle the perturbation occurs. During human gait, reflexes play some important roles such as contributing to muscle activity during normal walking and responding to unexpected external perturbations (Nielsen and Sinkjaer 2002). Again, soleus and quadriceps have received the most attention in studies looking at time dependency. It is worthwhile to review the functions of soleus and quadriceps muscles at this time because
the functions appear to be very much associated with their modulation seen during activity.

Studies eliciting either H-reflexes (Morin, Katz et al. 1982; Capaday and Stein 1986; Crenna and Frigo 1987; Edamura, Yang et al. 1991) or stretch reflexes (Yang, Stein et al. 1991; Yang and Whelan 1993; Sinkjaer, Andersen et al. 1996; Sinkjaer, Andersen et al. 2000) in human soleus at different times during walking found time dependent effects in addition to the task dependent effects previously mentioned. During gait, the soleus is predominantly inactive at heel strike while the pretibial muscles absorb energy as they slow the foot into plantarflexion during footfall. The soleus is most active during mid to late stance (Winter and Yack 1987). The soleus and other ankle plantar flexors absorb mechanical energy by active lengthening during this time to resist ankle dorsiflexion as the body moves over the foot. During this time of resisted stretch, muscle activity is increased presumably from increased muscle spindle firing or possibly CPG activation, which is why this time period is of most interest in soleus reflex studies. The soleus is not active during swing because the ankle dorsiflexors (e.g., tibialis anterior), generate energy during midswing to elevate the toes for ground clearance.

Just as in multi-task studies, within task studies also found that soleus H-reflex and stretch reflex amplitude did not follow background EMG amplitude. Typically, motoneuron excitability is higher when there is peripheral input from muscle spindles arriving during a movement. However, modulation of reflexes during walking is not simply related to background EMG as in standing, thus showing that some central mechanisms must be involved in the modulation of reflexes during walking. During standing isometric contractions, much of the EMG is probably from central origins
because the spindles are not undergoing much length change, although they are active due to gamma/beta drive and other peripheral inputs do occur from group Ib afferents due to increases in muscle tension activating Golgi Tendon Organs. During walking, some of the EMG results from the peripheral muscle spindles firing because lengthening of the muscles occurs at various points in the cycle. Thus both central and peripheral inputs to motor neurons may contribute to muscle activity. Stretch reflexes have been induced during walking by a custom fit device that changes ankle joint angles throughout the entire gait cycle (Sinkjaer, Andersen et al. 1996). With this technique it was found that soleus stretch reflexes were higher throughout the stance phase than in the swing phase. Increases in soleus H-reflex amplitude have also been observed throughout the stance phase, while being decreased during swing (Capaday and Stein 1987; Crenna and Frigo 1987). Thus, it appears that modulation is phase-specific, in the case of the soleus being highest when resisting lengthening of the muscle.

Just as in soleus, quadriceps’ stretch and H-reflex modulation is not simply related to activation, but is also time dependent (Dietz, Discher et al. 1990; Mrachacz-Kersting, Lavoie et al. 2004; Larsen, Mrachacz-Kersting et al. 2006). The quadriceps are important during early stance as they prevent collapsing of the knee in order to support body weight. The knee extensors absorb energy around heel strike to prevent excessive knee flexion (about first 10% of stride), and then generate energy in knee extension to support body weight during mid-stance (10-30% of stride). From mid to late stance the quadriceps are predominantly inactive. The hip flexors and rectus femoris are then active again during early swing to generate hip flexion. The hip flexor function of rectus femoris assists in advancing the lower extremity forward and elevating the limb for the
toes to clear the ground during early swing (Eng, Winter et al. 1997). The quadriceps are relatively quiet during the remainder of the swing phase until just prior to heel strike.

Stretch, tendon-tap, and H-reflexes have all shown time-dependent effects in quadriceps. Stretch reflexes have been elicited using a portable device that induced somewhat unexpected knee flexion throughout the gait cycle (Mrachacz-Kersting, Lavoie et al. 2004). It was found that reflex size is larger during the early stance phase as compared to the late swing phase. It is significantly modulated during the progression from the late swing to the early stance phase, increasing as the stance phase progresses.

When performing tendon taps using a reflex hammer to induce a stretch reflex in quadriceps, it was found that in early stance the response was similar to EMG controlled standing, but was reduced for the remainder of the gait cycle (Dietz, Discher et al. 1990). When looking at quadriceps H-reflexes, it was similarly shown for there to be a slight increase in H-reflex at knee yielding, while there was a decreased response for the rest of the cycle compared to isolated contractions (Dietz, Faist et al. 1990). Another study showed higher H-reflexes of the quadriceps in early stance (4% of stride) than in any other part of the gait cycle, including prior to and right at heel strike (0% of stride) (Larsen, Mrachacz-Kersting et al. 2006). These results are opposite to that seen in soleus, in that soleus H-reflex amplitude is highest when absorbing energy. It appears that in soleus and quadriceps, there is a fair amount of evidence to show task and time dependent effects on the modulation of afferent mediated responses, although findings vary with measurement techniques (electrical stimulation vs. stretch vs. tendon tap). The evidence for such variations in afferent-mediated responses in other lower extremity muscles is much less abundant as the only other muscles that have been studied with these
techniques is tibialis anterior (Brooke, Collins et al. 1991) and gastrocnemius (Makihara, Segal et al. 2012).

Studies Involving the Hamstring Muscles

We have learned from studies on the soleus and quadriceps that modulation of short latency reflexes is dependent on the function of a muscle for a given task, or portion of a task, such as walking. Similar studies need to also be done for the hamstring muscles because they are also very important during gait and currently there is very little research on short latency reflexes in hamstrings during walking. First, however, we must understand the function of the hamstrings throughout the gait cycle. In quadrupeds, the hamstrings are mostly knee flexors, but in humans their primary function is to produce hip extension and resist knee extension (Duysens, van Wezel et al. 1998). At heel strike and continuing through mid-stance, the hamstrings and other hip extensors are generating energy to extend the hip and support the torso against gravity. In some subjects small hamstrings activity is also seen in late stance for propulsion, but it is less than during late swing and was not observed in all subjects in this study. Inter subject variability in biceps femoris EMG is not uncommon during walking. In early swing, the hamstrings are quiet as the hip is flexing to advance the swing limb. The foot is elevated through passive knee flexion occurring as a result of hip flexion. The hamstrings are rapidly stretched at the end of the swing phase and there is a large burst of EMG activity in this period (Winter and Yack 1987) called the “extensor burst” (Duysens and Van de Crommert 1998). This burst is presumably from stretch reflexes (Prochazka, Westerman et al. 1976) or CPG activation due to rapid lengthening of the muscle at this time.
burst of activity contributes to muscle energy absorption because the muscle is actively
lengthening to slow down hip flexion and limit acceleration of knee extension in late
swing (Duysens, van Wezel et al. 1998). Thus, hamstrings are absorbing energy in late
swing in order to decrease the velocity of the leg at heel strike. It is thought that the
extensor burst is afferent-mediated because it is reduced during fictive locomotion of cats
(Duysens, van Wezel et al. 1998). It remains unclear as to which peripheral receptors
(i.e. group Ia, Ib, II, cutaneous) are responsible for the afferent control of hamstring
activity.

Only a few studies have attempted to test in humans if the extensor burst is
afferent mediated. Two studies to date have investigated tendon tap reflexes of biceps
femoris during human gait using a reflex hammer to elicit an afferent mediated response
(Van de Crommert, Faist et al. 1996; Faist, Blahak et al. 1999). One study used a
‘reduced gait’ to reduce changes in stimulus intensity due to knee movement while
subjects walked on a split treadmill (Van de Crommert, Faist et al. 1996). They found
that reflex amplitude was greatest in terminal swing, and not always related to level of
muscle activation. This study was complemented by a similar study that used the same
tendon tap technique to assess the modulation pattern of the biceps femoris reflex during
the normal step cycle against the modulation found during reduced gait (Faist, Blahak et
al. 1999). They also investigated whether the general reflex depression found during
locomotion versus standing for soleus and quadriceps is also present in biceps femoris. It
was shown that reflexes elicited during reduced gait, as used in the previous study, did
not differ from normal treadmill walking. The highest reflex responses occurred in late
swing, and that short-latency reflexes of the biceps femoris evoked by a tendon tap are
generally smaller during locomotion than during a standing condition with equivalent voluntary EMG activity. Thus, reductions in tendon tap reflexes are present in muscles during the swing phase when the function is not anti-gravity in nature, just as in muscles such as soleus and quadriceps that occur during stance when supporting the body against gravity.

There has been one attempt to study afferent modulation of hamstrings activity during gait using electrical stimulation. A new technique was recently used that allowed for studying all the lower extremity muscles at the same time (Courtine, Harkema et al. 2007; Dy, Gerasimenko et al. 2010). They applied percutaneous stimulation between T11 and T12 spinous processes to elicit multisegmental monosynaptic responses (MMRs) in multiple leg muscles bilaterally. They found not only task dependent modulation, but also time dependent modulation throughout the gait cycle of all muscles that changed in accordance with EMG. One major downside of this study is that the technique does not allow for comparing between subjects or testing sessions because of changes in recording conditions such as muscle length and electrode position. Because they were stimulating dorsal spinal roots, they did not elicit a direct motor response (M-wave). With peripheral stimulation studies, the H-reflex amplitude is compared with the maximum direct motor response (Mmax) amplitude for each condition allowing for comparisons between subjects and testing sessions. Because multiple muscles were excited with each stimulus, they were unable to elicit maximal responses for each muscle. A second limitation of this study is that only 12 stimulations occurred in each of 16 different bins, with a range of 70ms within each bin. That is a wide range of time during gait and there could be very different biomechanical or physiological events occurring from the beginning to the end
of each bin. A more precise latency of stimulation would be preferable. Lastly, because of the location of the stimulus, it was not selective of a particular muscle group thus there was heteronymous facilitation from a number of Ia afferents being stimulated. Thus, the MMRs, although monosynaptic, were a sum of a number of different muscle groups afferent neurons (Courtine, Harkema et al. 2007).
CHAPTER III

METHODOLOGY

Subjects and Testing Procedure

The data collection for this project took place in the Biodynamics Laboratory (520 FH) within the Department of Integrative Physiology at the University of Iowa, Iowa City, IA. A total of 21 subjects (7 Male) ages 19-37 (mean 22.8) enrolled in the study (See Table III-1). Subjects had no known history of neurological disorders or lower extremity impairments. The sample size was based on related work with similar outcomes of interest in which the range was between 8-13 subjects (Dietz, Discher et al. 1990; Van de Crommert, Faist et al. 1996; Faist, Blahak et al. 1999; Larsen, Mrachacz-Kersting et al. 2006; Courtine, Harkema et al. 2007). Anthropometric data, menstrual cycle period, and electrode placement positions were measured from each subject along with EMG and footswitch data. This study was approved by the University of Iowa Institutional Review Board.

Surface EMG data were collected during all trials from six muscles of the right lower extremity: biceps femoris, rectus femoris, vastus lateralis, tibialis anterior, soleus, and semitendinosus. Bipolar Ag-AgCl electrodes (Delsys; 0.1x1 cm, inter-electrode distance 1cm) were placed on the bellies of the muscles according to SENIAM guidelines. The reference electrode was placed on the head of the right fibula. The signal was amplified (1000x) and band-pass filtered (20-450hz). Pressure sensitive switches were taped under the heel of the participants to act as a footswitch. The EMG
TABLE III-1. Subject information.

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An ‘x’ represents data collected and used in a condition. Latency of the 2nd response is reported in milliseconds. Height is measured in centimeters. For females, the number of days since start of last menstrual cycle is listed, if known.

#Subject menstruates once every 3 months
^Subject could not accurately recall day
FIGURE III-1. Order of participation of events for study.
and footswitch signals as well as stimulation current was digitized (5K Hz) using custom-made data acquisition software. Stimulation current was measured by recording an output directly from the stimulator using the data acquisition software. Because the stimulation waveform was square in nature, the amplitude of the top plateau of the wave was measured and thus represented stimulus amplitude strength.

The study required two visits to the laboratory, one as an introduction to the study and another for data collection. Data collection took place in two experimental parts (See Figure III-1): 1. A screening test to determine if a biceps femoris H-reflex could be elicited in each subject; and 2. Characterization of the presumed H-reflex modulation during walking.

**Experiment 1: Screening Test and Neural Structures Responsible for the Different Responses to Sacral Root Stimulation.**

The screening test was used to determine if distinguishable H-reflexes could be elicited from potential subjects for treadmill walking. The attempt to elicit H-reflexes in subjects at rest was done with the subject lying face-down on a dynamometer bench. Sacral spinal nerves or their roots were stimulated using a high-voltage stimulator (Digitimer Type D185) at intensities ranging from 0 to 2 Amperes (1ms duration). Stimulating electrodes (circular, 2cm diameter) were placed over the sciatic nerve in the right lower extremity (the diffusing electrode over the L5 spinous process and the stimulation electrode placed 15 cm distally at a 45 degree angle) (Figure III-2). Stimulus amplitude was measured by recording the output of the stimulator with the data acquisition software. The first experimental manipulation was done to determine the optimal level of stimulation intensity that elicits the highest 2nd response amplitude. This
was accomplished by electrically stimulating the muscle at pseudorandomly selected intensities and recording the muscle responses. The direct motor response (M-wave: first response) increases in amplitude as a function of stimulation intensity whereas the afferent-mediated response (second response: presumed to be the H-reflex wave) increases, reaches a maximum and decreases as a function of stimulation intensity (Schieppati 1987) (Figure III-3).

Furthermore, short interval (50ms) double-pulse stimulations at the optimal stimulation intensity for eliciting the 2nd response were done in the same position to test if the 2nd response was afferent-mediated. This inter-stimulus interval has been used previously to demonstrate homosynaptic depression in previous studies (Courtine, Harkema et al. 2007; Minassian, Persy et al. 2007) and falls within the recommended time interval of 10-50ms or greater than 400ms (Schieppati 1987). The double-pulse stimulations were used to determine whether the first pulse caused an inhibition of the 2nd response to the second pulse (Stein and Thompson 2006). If inhibition is present, it lends support for the presence of the H-reflex (Figure III-4). This is known as post-activation depression, or homosynaptic depression. If there is a difference (first response larger), then it can be presumed that an H-reflex is present. Because not all subjects demonstrate or have an identifiable H-reflex in the hamstring muscles, those subjects who did not show one were excluded from the remainder of the study. If the subjects demonstrated an H-reflex in this condition, they then took part in the walking portion (Experiment 2) of the study.
FIGURE III-2. Location of electrode placement in Experiment 1. Red: diffusing electrode, black: stimulating electrode, blue: recording electrode for biceps femoris.
FIGURE III-3. Example of hamstring EMG responses to electrical stimulation over the sacral spinal roots. A) Example of the raw EMG response following stimulation for subject SS20080514. Dashed vertical lines represent the start of the M (green) and H (blue) waves. B) Example of stimulus-response curve of biceps femoris from subject BF20080416 (PI of study). Closed squares represent peak to peak values (PP) of the direct motor response (Mraw). Open circles represent peak to peak values of the afferent mediated response (2nd response).
FIGURE III-4. Examples of hamstring EMG responses following double-pulse stimulation. A) Paired stimuli overlaid in time from subject ML20071003 following first stimulus (solid line) and following a second stimulus 50ms after the first stimulus (dashed line). Note the lack of a second response following the second stimulus. B) Same subject and responses to two stimuli 50ms apart plotted continuously over time. C) Example of EMG response in which the H-reflex was not inhibited following second stimulation in female subject AP20080604. Two stimuli are overlaid in time just as in graph (A). D) Example of a subject (KM20080527) in which there is believed to be an H-reflex (arrow) that was not inhibited. In all graphs, red vertical dashed lines represent time of second stimulation, green lines represent start of M-wave, and blue lines represent start of H-wave.
Figure III-4 – continued

C)

D)
**Experiment 2: Stimulation During Walking**

The second experiment was performed only on those subjects in whom a 2\textsuperscript{nd} response was detected in single pulse stimulation and inhibited with double pulse stimulation. This experiment involved subjects walking at their normal, comfortable walking speed on a treadmill. Subjects were given breaks whenever they felt they needed one or at least between every two walking conditions. Electrical stimulation was applied with the same electrode configuration as in the screening test. Stimulations occurred randomly at 12 different latencies throughout the gait cycle. The latencies were calculated at 0, 6.25, 12.5, 25, 37.5, 50, 62.5, 68.75, 75, 81.25, 87.5, and 93.75\% of the gait cycle (Figure III-5).

**FIGURE III-5.** Example of hamstrings EMG pattern during a gait cycle without any electrical stimulation from one trial of subject BF20080416. Vertical lines represent times when stimulations were given on other walking cycles.
These latencies were chosen in order to include a higher density of stimulations during the late swing phase when hamstrings are most active. Stimulations at one of the latencies were given once every 4-6 seconds. For each latency, a minimum of 40 stimulations were given with varying intensities in order to create a S-R curve for biceps femoris. Within a given latency, the 40+ stimulations were given in a pseudorandom order of intensity. The order among the 12 latencies was varied between subjects so that S-R curves were not always done in the same order among all subjects.

An online analysis of the motor response as a function of electrical stimulation intensity was used to ensure that the entire motor recruitment curve was recorded. This was only able to be done for one muscle, biceps femoris, due to limitations of recording software. It also helped to keep the number of stimulations per subject within a tolerable amount. It is necessary to produce a S-R curve at each time period because a maximum motor response (Mmax) must be determined at each point in the gait cycle. Mmax can change with either stimulation electrode or recording electrode movement on the skin relative to the nerve; and also due to changes in muscle length. Footswitch, EMG, and electrical stimulation data were collected 100ms before and 3 seconds after heel strike for each gait cycle in which a stimulus was applied.

**Data Analysis**

*Calculation of First and Second Muscle Responses*

Due to overlap between the 1st (M-wave) and 2nd response (presumed H-wave) waves to electrical stimuli, a post-hoc signal processing procedure was applied. The procedure is similar to that of Larsen et al. (2006) (Larsen, Mrachacz-Kersting et al.
For each condition, the trial which elicited the maximum direct motor response (Mmax) was identified. The M-wave in the trial containing Mmax was localized and the signal isolated and used for comparison purposes as described below. 2. On the trials in which the EMG showed a 2nd response, the corresponding signal part was also isolated. 3. The signal magnitudes at the starting points of the two isolated signal segments were determined. The Mmax signal segment was multiplied with the ratio of the starting signal value for the 2nd response:Mmax to give us a corrected M-wave (Mcorr) 4. The Mcorr signal segment was subtracted from the raw 2nd response wave, and the remaining signal was the 2nd response amplitude, which was measured as the peak to peak (PP) amplitude. By normalizing to Mmax in each individual excitability curve, it ensured that changes in EMG amplitude due to changes in muscle geometry during a gait cycle would not affect the true result (Larsen, Mrachacz-Kersting et al. 2006). Normalizing to Mmax has been done in many previous studies and is the preferred method for normalization of EMG (Morin, Katz et al. 1982; Capaday and Stein 1986; Dietz, Faist et al. 1990). This normalization procedure also served a second important purpose: identification of stimulation artifact. Stimulation artifact has been seen previously in studies in this lab (Peterson 2009) and elsewhere (Myklebust 1984), and can affect M-wave measurement if not identified. In the present study, a clear distinction between the end of the stimulus artifact and the start of the M-wave could not be seen, but it is not believed that stimulus artifact affected PP M-wave amplitude. Previous studies have estimated human motoneuron axon conduction velocity to be around 40 m/s (Borg and Borg 1987; Frijns, Laman et al. 1997; Brooke and Zehr 2006). Based on electrode distances measured on
the subjects in this study and using a conduction velocity of 40 m/s, the predicted latencies to M-wave onset are between 5-8ms. Assessment of M-wave amplitude during data analysis showed that the PP M-wave amplitude calculation was no different when using a starting latency of 5ms vs 10ms for the M-wave. The calculated M-wave PP amplitude was the exact same in all walking subjects when both start times were used because neither the maximum nor minimum peaks fell between 5 and 10ms. It is highly doubtful that stimulation artifact would last long enough to affect the 2nd response, but if it had, the cancellation technique described above should have eliminated it (See Figure III-6). A raw M-wave with stimulation artifact is shown in Figure III-7. This is the trial in which the start time was closest to influencing the PP Mwave because the peak minimum EMG amplitude was at exactly 10ms latency.

**Calculation of Background EMG During Walking**

EMG data during walking was collected during each trial. For each point in the gait cycle studied, mean rectified EMG was calculated for a 20ms time period before the time when stimulation occurred. In previous studies, background EMG sampling windows of 50ms in rectus femoris H-reflexes (Dietz, Faist et al. 1990; Larsen, Mrachacz-Kersting et al. 2006), and ~45ms (4% of gait cycle) (Yang and Whelan 1993) and 10ms (Morin, Katz et al. 1982) have been used in soleus H-reflex studies. The shorter time period in this study was decided upon because the stimulation latencies were sometimes within 65ms of each other and it was thought that a shorter window would give a better representation of the background activity immediately before each designated stimulation latency. All trials in which the background EMG was ± two
standard deviations away from the mean for that condition were discarded from analysis. Also, all trials in which the latency of stimulation recorded by EMG did not match the latency on the footswitch were discarded from analysis. The mean of all trials at each latency was used to compare background EMG with the $2^{nd}:1^{st}$ response ratio.

**Statistical Analysis**

*Experiment 1*

Latencies of the H-reflex were measured and correlated with subject height using linear regression. This was done to gain information about the relationship between latency and subject height for comparison purposes with other studies. In this regression, subject height was the independent variable while latency of the H-reflex was the dependent variable. H-reflex latency was identified by raw EMG data from the double-pulse stimulations. The start of the deviation of the response following the second stimulation compared to the response following the first stimulation was identified as the onset of the H-reflex. There were 17 (7 male) subjects included in this analysis.

To test if the measured first and second stimulus-responses respond differently to increasing electrical stimulation intensity (See Hypothesis 1), the stimulus-response curves were fitted with Boltzmann or Boltzmann-like non-linear regression curves as used in related studies (Capaday 1997; Stein, Estabrooks et al. 2007). The peak to peak values for the corrected $1^{st}$ and $2^{nd}$ response waves were plotted against stimulation intensity for each of the points in the gait cycle stimulated, with stimulation intensity being the independent variable. Typically, about 40 stimulations for each condition were used to create a curve. The shapes of the curves were distinct for both responses.
FIGURE III-6. Procedure for isolating the second response from overlapping M-wave. Top trace shows a raw Mmax signal. The second trace shows a raw Mmax (black line) and a raw signal with a 2nd response present (gray line). The third trace shows a corrected Mmax signal (dashed black line) compared to a raw 2nd response signal (gray line). The bottom trace shows the result when the raw 2nd response signal is subtracted from corrected M-wave. The result is a corrected 2nd response. A) Example is from a normal walking trial of subject ML20071003. B) Example is from same subject during quiet, prone lying. Note the stimulus artifact (<5ms), followed by the M-wave (5-20ms) and 2nd response (>20ms).
Figure III-6 – continued

B)
FIGURE III-7. Example of an Mmax trial from subject CD20080626 in late swing at 87.5% of the gait cycle. Green vertical dashed line is placed at a latency of 5ms and blue vertical dashed line is placed at a latency of 10ms. The small red horizontal lines are the minimum and maximum M-wave peaks. In this example, the minimum falls at a latency of 10ms. Thus the PP amplitude for the M-wave does not differ when choosing 5ms vs 10ms for the start of the M-wave. In all other trials and subjects, the peaks never fell between those times.

The corrected M-wave S-R curves were fitted with a Boltzmann sigmoidal equation:

\[ Y = \text{Min} + (\text{Max}-\text{Min})/(1 + \exp((V50-X)/\text{Slope})) \]

In this equation, \( Y \) is response, \( \text{Max} \) and \( \text{Min} \) refer to minimum and maximum values of the curve, \( V50 \) is the stimulus level where half the maximum response is reached, \( x \) is stimulus amplitude (milliamps), and \( \text{Slope} \) is steepness (width) of the curve (Stein, Estabrooks et al. 2007). The corrected 2nd response S-R curves were found to be best fitted with a modified Boltzmann equation that did not include the descending portion of the curve. The equation was the same as for the Boltzmann, except the constraint was added so the range did not include the descending portion of the curve. This modified
Boltzmann has been presumed to be appropriate for the ascending portion of the H-reflex S-R curve (Klimstra and Zehr 2008). The constraint was easily added by ending the data range to be fitted at the stimulation intensity where the response intensity began to decrease. Examples of the curve fits to the S-R curve are shown in Figure III-8.

The maximum values (Hmax & Mmax) of each fitted curve were used for amplitude comparison purposes between the two responses. This was done to test whether the Mmax is larger than the maximum 2nd response in all testing conditions. To test for the difference in the maximum 2nd response and Mmax, a two-way repeated measures analysis of variance (ANOVA) was applied for all maximum 2nd response and Mmax values calculated during the experiment. Wave type (M and 2nd) and stimulation latency (0, 6.25, 12.5, 25, 37.5, 50, 62.5, 68.75, 75, 81.25, 87.5, and 93.75% of gait cycle) were the independent variables with latency being the repeating factor. Tukey’s Multiple Comparison post-hoc test was used when appropriate. After the maximum 1st response was shown to be larger than the maximum 2nd response, the maximum values of each curve were then used to create a 2nd max:1st max response ratio for each subject at each latency of stimulation. The 2nd max:1st max ratios are used in later analysis.

In order to determine if an H-reflex is present (see Hypothesis 2) during the double-pulse stimulation, the peak-to-peak amplitudes of the second response following the first versus the second stimuli were compared using a paired t-test (Stein, Estabrooks et al. 2007). The second response following the first stimulus was close to its maximum obtainable value for these recording conditions for each subject. If the second response is decreased after the second stimulus due to homosynaptic depression, it lends support
FIGURE III-8. Examples from subject SS20080514 during walking showing the range over which the S-R curve was fitted with a modified Boltzmann equation. The point at which the line ends represents the range of stimulation amplitudes used to produce the regression fit. Graphs are from A) 12.5% B) 37.5% and C) 68.75% of the gait cycle.
C)

for the second response being afferent mediated. The peak-to-peak amplitudes of the first response following both stimuli were also compared using a paired t-test. This response should not change because it is a direct motor response and not afferent-mediated and thus not affected by homosynaptic depression.

Experiment 2

One of the aims of this paper was to determine if the afferent mediated response is phase dependent, i.e. it is different during different phases of the gait cycle. The 2nd response to 1st response ratio is the best measure of this response. In order to test for a difference in 2nd response to 1st response ratio between different phases of the gait cycle (See Hypothesis 3), one-way repeated measures ANOVA was applied with latency as the repeated factor (Faist, Blahak et al. 1999; Mrachacz-Kersting, Lavoie et al. 2004; Larsen,
Mrachacz-Kersting et al. 2006). The maximum values of each response (2\textsuperscript{nd} max and Mmax) at each latency for each subject were used in the comparison. Tukey’s Multiple Comparison post-hoc test was used to find differences in comparisons for the ratio between each phase of the gait cycle.

Linear regression analysis was used to calculate the slope of the background EMG activity and 2\textsuperscript{nd}:1\textsuperscript{st} ratio (See Hypothesis 4). The 2\textsuperscript{nd}:1\textsuperscript{st} ratio versus normalized background EMG plot was fitted with linear regression twice: once for only the stance phase and once for only the swing phase. Linear regression fits have been used previously for comparing 2\textsuperscript{nd}:1\textsuperscript{st} ratio versus background EMG (Edamura, Yang et al. 1991). This was done for each subject separately, with the slopes averaged for all subjects. The purpose of separating the swing phase was to test whether 2\textsuperscript{nd} responses differed during this time period because the muscle is actively lengthening for part of it. It was expected that the slope would be higher during the swing phase than in stance, suggesting facilitation of the reflex response during this phase.

Level of significance was set at p<0.05 for all tests. GraphPad Prism and SPSS Statistical Analysis Software were used for data and statistical analysis.
CHAPTER IV
RESULTS

A total of 21 subjects (7 Male) enrolled in the study (See Table III-1) and took part in Experiment 1. Of the 14 females, four did not qualify for Experiment 2 because they did not have an identifiable 2nd response and thus ended the study after Experiment 1. Five subjects (1 male) did not participate in Experiment 2 because they took part in Experiment 1 before Experiment 2 was added to the study and were not available to return to laboratory. This left 12 subjects (6 male) who participated in the study in its entirety. During data analysis, one female subject was found to have unusable data in Experiment 2 due to technical problems and thus was not included in the analysis. For analysis purposes the usable data included 17 subjects (7 male) in Experiment 1, and 11 subjects (6 male) in Experiment 2.

Experiment 1: Screening Test

Of the six muscles recorded, only biceps femoris EMG muscle responses were analyzed because, along with semitendinosus, they were of primary interest for the purposes of this study. Semitendinosus’ responses appeared to be similar to biceps femoris’ responses during data collection, however they were not analyzed because the stimulation technique used in this study only allowed for only one EMG recording channel to have an appropriate stimulus-response curve. During data collection, the peak to peak value of the first response was measured and plotted versus stimulation intensity to create a stimulus-response curve. The experimenter could only see a single (biceps femoris) stimulus-response curve develop during the experiment and thus could only
ensure that a full plot which included Mmax was collected for one muscle (biceps femoris). It is possible that complete stimulus response curves were created for other muscles (i.e. semitendinosus), however it could not be verified until after data collection was complete. When data from other muscles was reviewed post-hoc, it was found that complete stimulus response curves were not obtained for semitendinosus in most conditions, thus Mmax and 2nd:1st response ratios could not be created for analysis.

Relationship of Subject Height to H-reflex Latency

H-reflex latency was positively correlated with subject height (Figure IV-1) \( (R^2 = 0.61, F_{1,15} = 24.32, p < 0.001) \) as expected, because of the greater distance an impulse must travel in taller subjects. The average latency in this study was 21.9 ms \( (SD = 2.08) \), which falls within a comparable range to those previously found in hamstring stretch and tendon tap studies (20.3–21.9ms) (Fiemert, Bumann-Melney et al. 2005) (See Appendix). It makes intuitive sense that H-reflex latencies should be several milliseconds less than stretch reflexes because: 1) The electrical stimulation site was closer to the spinal cord than the site of the tendon tap and 2) A bypass of the fusimotor system with electrical stimulation. The slightly longer latency in this study is probably due to the difficulty of the investigator precisely separating the end of the M-wave with the start of the H-reflex as mentioned previously. Normally the H-reflex is identified as the first deflection from baseline (Pierrot-Deseilligny and Burke 2005), but with the present technique the M- and H-waves were close together making it difficult to determine a deflection point. To aid in the identification of the H-reflex onset, the responses to the two stimulations given in the double-pulse technique were superimposed
(e.g., Fig. III-4). This showed a change in the second response to the second stimulus versus the second response following the first stimulus. Because inhibition was occurring after the second stimulus, the response was not identical to that from the first stimulus and a change in the response after the second stimulus would closely represent the start of the afferent mediated response. The EMG peak to peak values of both responses were the main outcome variables used in this study. Importantly, the peaks and minima of the EMG of the responses did not occur near beginning or the end of the responses. Thus it is highly doubtful that a few milliseconds of error in the chosen start of the responses affected any of the results in the study. Examples of the chosen start of H-reflex for four of the subjects are shown in Figure IV-2.

I also attempted to measure 2nd response latency using another method, specifically time to the first peak or minimum in the response because this point can be identified more accurately than onset. In one study using hamstring tendon taps (Friemert, Bumann-Melney et al. 2005), the mean onset latency was 21.9ms with a mean time to first peak being 26.7ms. In the present study, the time to the first peak was 25.2ms. These latencies to the first peak of the 2nd response seem to be more appropriate because they are shorter than during stretch reflexes. That is, stretch reflexes elicit spindle activation leading to a longer conduction pathway than stimulation of spindle afferents at the sacral spinal roots. Thus the chosen onsets for the 2nd responses in this study were probably after the actual onset by a few milliseconds.
FIGURE IV-1. Demonstration of H-reflex latency as it relates to subject height. The plotted line is the best fit linear regression. Each point represents one subject for either males (squares) or females (triangles). A) Plot of height to H-reflex onset latency and B) plot of time to first peak or minima of H-reflex.

A)

B)
FIGURE IV-2. Examples of raw EMG responses. A) Raw EMG traces from four subjects following double-pulse stimulation. Arrow is directed at the point in time where it is believed the H-reflex began. This point in time was used in further analysis as the H-reflex latency. Solid lines are responses to first stimulus, dashed lines are responses to second stimulus. B) Raw EMG traces from subject ML20071003 following single pulse stimulation at four different intensities. Blue vertical dashed lines represent point in time where H-reflex probably began for that trial. Arrow is pointed at first peak of presumed H-reflex. Time to first peak was consistent in these examples falling between 23-24ms, whereas start of the response showed a range of a few milliseconds between trials.
Figure IV-2 – continued

B)
Responses to Single Pulse Stimulations While Prone

The M-wave S-R curves during prone lying were fit with a Boltzmann sigmoidal equation (n=17, mean $R^2 = 0.95$, range 0.85-0.99). The corrected H-reflex S-R curves during prone lying were found to be best fit with a modified Boltzmann equation that did not include the descending portion of the curve (n=17, mean $R^2 = 0.85$, range 0.62-0.97). For both types of curve fits, there were some curves in which the chosen fit did not converge. In some cases, either the maximum stimulator output was reached or the maximum tolerable stimulus amplitude was given for a particular subject without seeing a plateau in the M-wave S-R curve. This could have been due to contamination from nearby muscles in the EMG recordings of biceps femoris (Pierrot-Deseilligny and Burke 2005). When there was a data set that could not be fitted with a regression, the maximum response value for all stimulations in that condition for either the M-wave or H-reflex was used as $H_{max}$ or $M_{max}$ respectively. For the prone lying subjects, only 1/17 M-waves and none of the H-reflex S-R curves analyzed were unable to be fitted with the chosen regression equations, thus the maximum value obtained was used for analysis in that subject. Examples of fitted curves during prone lying are shown in Figure IV-3.

During single pulse stimulation at rest, the maxima of each subject’s peak to peak values of the direct motor response (i.e. M-wave) were higher than the maxima of each subjects’ peak to peak values of the afferent-mediated response (i.e., H-wave). During prone lying for all subjects, $M_{max}$ was significantly higher than the $2^{nd}$ response max ($t_{16} = 6.40, p <0.0001$). This held true for all 17 subjects and is demonstrated in Figure IV-4. This is not a novel finding and was easily predicted, but is important to establish when identifying responses to stimulation. The range of $2^{nd}$max/$M_{max}$ ratios was 0.12-0.69
(mean 0.34) which is similar to the range of Hmax/Mmax ratios (i.e., 0.04-0.40) observed in quadriceps femoris during standing isometric contractions (Larsen, Mrachacz-Kersting et al. 2006).

Responses to Double-pulse Stimulations

The group mean of all subjects’ maximum 2nd response peak to peak value during double-pulse stimulation was significantly reduced following the second stimulus ($t_{11} = 2.999, p = 0.0121$) as predicted in Hypothesis 2. Twelve subjects took part in the double pulse stimulations, with each subject seeing a reduction. From the first to second stimulus, the mean peak to peak value of the 2nd response dropped from 0.3776 mV ($\pm 0.1037$) to 0.1276 mV ($\pm 0.0334$) implying that homosynaptic depression was occurring. The direct motor response was not affected by the second stimulus ($t_{11} = 0.057, p = 0.96$). These values remained consistent between the two stimuli: 0.7763 mV ($\pm 0.1534$) to 0.7783 mV ($\pm 0.1544$) (Figure IV-5). See Figure IV-6 for examples of the responses following double pulse stimulation.

Experiment 2: Stimulation during walking

Comparison of Direct Motor and Afferent Mediated Responses During Walking

The M-wave S-R curves were fit with a Boltzmann sigmoidal equation during walking for all 12 times of stimulation (n=11, mean $R^2 = 0.95$, range 0.85-0.99 for regressions that converged). The corrected H-reflex S-R curves were found to be best fit with a modified Boltzmann equation that did not include the descending portion of the curve during walking (n=11, mean $R^2 = 0.67$, range 0.15-0.97). For the 12 time periods
FIGURE IV-3. Examples of S-R curve regressions for individual subjects during prone lying. A) Example of subject CD20080626 showing good regression fits. B) Example of subject EF20080701 showing poor regression fits (this was the one case in which the chosen regression did not produce a maximum value for the fit for the M-wave).
FIGURE IV-4. Demonstration of individual maximum peak to peak values for the M-wave and H-reflex during prone lying. The lines show individuals’ paired Mmax and Hmax responses for all 17 subjects, Mmax (closed squares) Hmax (open circles).

FIGURE IV-5. Peak to peak M-wave values following the first and second stimuli during the double-pulse technique. Each pair of points represents one subject.
FIGURE IV-6. Demonstration and examples of EMG traces and values following double pulse stimulation. A) Example of a typical raw EMG response for subject SS20080514 following the first stimulus (solid line) and following a second stimulus 50ms after the first stimulus (dashed line). Note the lack of a second response following the second stimulus. B) Responses of the same trial as (A) but continuous over time rather than overlaid. The data is the same for both plots. C) Plots of the 12 subjects’ individual values for both responses following both stimuli. Horizontal lines represent means for each group. D) Peak to peak 2nd response values for the two stimuli for all 12 subjects. Values for each subject are connected by a line.
Figure IV-6 – continued

C)

![Graph C]

D)
in the gait cycle of the 11 subjects who participated in the walking portion of the study there was a total of 2 out of 132 M-wave and 14 out of 132 H-reflex S-R curves that did not converge. Examples of fitted curves are shown in Figure IV-7.

During walking, the maximum direct motor response (Mmax) was higher than the maximum of the afferent mediated response (Hmax) for each subject. There was a main effect of wave (M vs H) \( (F_{1,110} = 34.53, p < 0.0001) \) but not latency \( (F_{11,110} = 1.14, p = 0.333) \). There was a significant interaction of wave x latency \( (F_{11,110} = 2.54, p = 0.0048) \) (See Figure IV-8). As mentioned during discussion of prone stimulation, this is not an original finding and was easily predicted, but important to demonstrate to help validate that the 2\textsuperscript{nd} wave is an H-reflex.

**Time Dependency of Responses to Stimulation During Walking**

When normalized to the maximum M-wave, the maximum H-reflex was increased during late swing compared to the rest of the gait cycle. There was a main effect of latency of stimulation \( (F_{11,110} = 6.97, p < 0.0001) \) because the mean peak to peak Hmax:Mmax response ratio was significantly higher during late swing than during the rest of the gait cycle. The Hmax:Mmax ratio at 87.5%, 93.75%, and 100% of the gait cycle were significantly higher than the rest of gait cycle, but not different from each other. This appeared to be due to Hmax being greatest just before and at heel strike (Figure IV-8a). There was also a trend for Mmax to be lowest during late swing and early stance (Figure IV-8a), although it was not statistically significant \( (F_{11,110} = 1.733, p = 0.0752) \). The group mean Hmax:Mmax ratios were thus higher at the end of the swing phase and at heel strike (Figures IV-9 and IV-10).
FIGURE IV-7. Demonstration of regression curve fits during walking. A) An example of well-fit M-wave and 2\textsuperscript{nd} response S-R curves for female subject LB20080603 during walking at 81.25\% of the gait cycle. B) Example of a poorly-fitted M-wave S-R curve for male subject TP20080513 at 0\% of the gait cycle. C) Example of a poorly fitted 2\textsuperscript{nd} response S-R curve for female subject LB20080603 at 62.5\% of the gait cycle. Closed squares represent peak-to-peak values of the M wave, and open circles represent peak-to-peak values of the H-reflex. The data were fitted with either Boltzmann or modified Boltzmann regression curves, respectively. D) Example of raw EMG responses at each time in the gait cycle following stimulation at a stimulus amplitude of 0.50 amps for subject OD20080520. Center graph is raw background EMG for this subject during one complete gait cycle without stimulation. At this stimulation intensity, both M and 2\textsuperscript{nd} responses are seen in the raw EMG trace.

A)
Figure IV-7 – continued

B)  

C)
Figure IV-7 - continued

D)
FIGURE IV-8. Mean (±SD) values for all subjects of the maximum M and H waves following stimulation during each of the 12 latencies during the gait cycle. A) Group means of 11 subjects’ response values throughout the gait cycle. B) Group means for 11 subjects’ M and H waves at each of the 12 latencies of stimulation.

A)

![Graph A]

B)

![Graph B]
FIGURE IV-9. Top graph depicts the Hmax:Mmax EMG response ratio throughout the course of the gait cycle. Data points are the mean of 11 subjects (±SE). Lower graph shows background EMG for 20ms prior to stimulation at designated times throughout the gait cycle. Data points are also mean for 11 subjects.
FIGURE IV-10. Example of H:M ratio throughout the gait cycle for one male (ML20071001) and one female (CD20080626). Top trace for each represents H:M ratios throughout the gait cycle. The bottom traces represent mean rectified EMG normalized to the corresponding Mmax value for that period in the gait cycle.
Figure IV-10 – continued
FIGURE IV-11. M-wave stimulus-response curves for two subjects during the late swing phase. Mmax was typically lower in late swing compared to the rest of the gait cycle, and showed normal S-R curves.

Influence of Background Activity on Hmax/Mmax Response Ratios

It was hypothesized that normalized H-reflex amplitude (Hmax/Mmax) would have a higher slope in swing than in the remainder of the gait cycle (stance). The slope of the relationship between Hmax/Mmax and background EMG for each phase was calculated for each subject. The slopes from the individual subjects was used to create group means for both stance and swing. All non-significant fits were treated as having zero slope. For the stance phase, 5 of 11 subjects showed significant fits, while for the swing phase 7 of 11 subjects showed significant fits. For the stance phase the mean slope was 0.054 (±0.086, range 0-0.262) while the swing phase was 0.341 (±0.679, range 0-2.33). A paired t-test was used to test for a difference between the two groups. There was no difference between the slopes of the stance and swing phase (Fig IV-12a, \( p = 0.20, t_{10} = 1.371 \)). The lack of a significant effect is probably due to the high inter-subject variability, large number of non-significant fits giving zero slopes, and lack of consistent changes in slope for the different phases among subjects.
FIGURE IV-12. Hmax/Mmax response ratios during walking versus background EMG during the 20ms prior to stimulation. A) Slopes of all 11 subjects for stance and swing phases. Horizontal bar represents mean for all subjects. B) Example of subject OD20080520 who demonstrated a significant fit during the swing phase (right graph) and a non-significant fit during the stance phase (left graph).

A)
CHAPTER V
DISCUSSION

This study showed that it is possible to elicit both a direct motor response (M-wave) and an afferent-mediated response (H-reflex) in human hamstrings muscles using sciatic nerve stimulation both while laying prone and walking on a treadmill. This is the first time to my knowledge that complete S-R curves for both the M and H-waves have been created in human hamstring muscles. This study also showed that the human hamstring H-reflex is time-dependent, as the relative amplitude is larger in the late swing phase than during any other phase of the gait cycle. Lastly, this study showed that the H-reflex amplitude is not simply directly related to background EMG levels during walking. This is consistent with the results of previous studies using other techniques on the hamstrings muscle and with studies of soleus and quadriceps H-reflexes during gait. Thus, it seems likely there are other spinal or supraspinal task and time dependent influences on the H-reflex.

Experimental Technique and Characterization of H-reflex

The aim of the first part of this study was to establish a new technique for eliciting hamstring H-reflexes through sciatic nerve stimulation. We are fairly certain that the short latency responses seen in this study are indeed M-waves and H-reflexes as claimed because: 1) The latency of the first peak of the evoked H-reflex falls within a comparable range to other studies on hamstring muscle tendon taps and stretch reflexes; 2) The M-wave increased in amplitude with increasing stimulation intensity until a plateau was reached; while the H-reflex increased and then decreased in amplitude with increasing
stimulation intensity; 3) The H-reflex was never higher in amplitude than the M-wave; and 4) The H-reflex was inhibited by homosynaptic and presynaptic depression following a double-pulse stimulation while the M-wave was unaffected.

Latency of H-reflex

The latencies of the H-reflexes in this study were directly and strongly correlated with subject height, i.e. the H-reflex latency increased with taller subjects due to a longer distance the impulses had to travel. This is not a novel finding but does lend some small support for the responses being afferent mediated. If the presumed H-reflex was some other type of afferent mediated response, stimulation artifact, or did not travel to the spinal cord and back, the latency would not be well correlated to subject height. We measured distances between the spinal cord and stimulating and recording electrodes and showed the taller subjects had longer distances for the impulse to travel and thus had longer latencies of the response to stimulation.

The latencies of the H-reflex (mean 21.9ms ± 2.1, range 18.4 - 25.6ms) observed in this study are within a reasonable range for a monosynaptic reflex response in hamstrings. The hamstring tendon-tap latency (Van de Crommert, Faist et al. 1996) 21.9ms ± 3.1 and stretch reflex latency (Friemert, Bumann-Melnyk et al. 2005) 20.3ms ± 3.5 are both close in range to the H-reflex latency seen in this study. MMR responses (Courtine, Harkema et al. 2007) are slightly faster at 17.8ms ± 1.5 as expected because the stimulation occurred much closer to the spinal cord. Thus, the time frame for the responses seen in this study fall close to, although slightly longer, than expected values. It would be reasonable to expect the H-reflex latencies to be slightly less than those found
in stretch reflex and tendon tap studies due to the site of electrical stimulation being several centimeters proximal to the location of the muscle spindles. There are a few possible reasons why H-reflexes latencies in this study were longer than stretch reflexes in other studies. First, even after the isolation procedure used in this study to separate the M-wave and H-reflex, the start of the H-reflex was not always obvious and could have been erroneously measured. However, the major outcome variable used in this study is peak to peak amplitude of the responses. As mentioned earlier, differences of up to 5ms in the start time of the responses would have had no effect on the calculated peak to peak amplitude values used in the results. Neither the positive nor negative peaks in the EMG occurred near the chosen start of the wave. Secondly, it was unclear in previous studies the distances from the spinal cord to the site of the perturbation or recording electrodes. It is possible that the stimuli traveled similar distances in both studies, however the delay expected from the time of the tendon tap or stretch to activation of the muscle spindles is unaccounted for. Lastly, it has been shown that latency of H-reflexes is dependent on the duration of the stimulation (Mogyoros, Kiernan et al. 1997). The latency increases shown were not greater than 1ms between stimulus durations of 0.05-1.0ms in that study. In the present study the stimulation duration was 1ms, but even with a 1ms increase in latency it still does not fully account for the longer latency we observed.

Even though the chosen onset of the H-reflex latency may have been unclear, the time to first peak of the response was easily identified. The latency of this peak was found to be 1.5ms shorter than the time to first peak of hamstring stretch reflexes (Friemert, Bumann-Melnyk et al. 2005) and 2ms shorter than for tendon taps (Faist, Blahak et al. 1999). This finding is in line with the expectations mentioned previously of
H-reflexes having a slightly shorter latency than tendon taps or stretch reflexes because the site of electrical stimulation is closer to the spinal cord than the site of tendon taps and stretch reflexes. Thus, it appears that the latency of the observed H-reflexes is close to expected values despite the ambiguity in identifying the actual start of the response.

**Stimulus-Response Curves**

The stimulus-response curves elicited in this study for both the M-wave and H-reflex are representative of typical curves shown in other studies. In the prone condition, the Boltzmann and modified Boltzmann equations used for the M-wave and H-reflex S-R curves resulted in very high correlations in most cases. A minimum of 40 stimulations were given to each subject allowing full S-R curves to be created in most cases. As expected, the M-wave S-R curves increased and remained at a plateau with increasing stimulation intensities. In contrast, H-reflex S-R curves increased, reached a maximum, and then decreased with increasing stimulation intensity. The decrease in H-reflex PP amplitude is because antidromic motor volleys set up in the motoneuron axons collide with and prevent transmission of the H-reflex response. The characteristics of these curves are similar to those described previously and shown in other studies (Schieppati 1987; Pierrot-Deseilligny and Burke 2005). However, this is the first study to my knowledge that has produced complete S-R curves for hamstring M-waves and H-reflexes during prone lying and walking.

On occasion it was observed that the M-wave S-R curve did not plateau at high stimulation intensities. I propose a few possible reasons for this. In some conditions, as the S-R curve was being created, it became apparent that further increases in stimulation
intensity would not result in a plateau at high intensities. As long as the subject could tolerate it, repeated stimulations were made at the high intensities in attempt to create a plateau; but often the subjects reported extreme discomfort and did not wish to continue at high intensities. A second possible explanation for the lack of plateau is that the subtraction procedure to eliminate stimulus artifact and differentiate between M and H-waves did not completely eliminate the stimulus artifact in the M-wave. If there was not complete elimination of the artifact in the Mmax trials, this would affect the corrected H-waves because Mmax was used in the subtraction procedure. Complete elimination of stimulus artifact most likely did not occur, as has been seen in previous studies using this technique (Peterson 2009). Although the technique may not be perfect, to my knowledge there is not a better method for handling this issue. One last potential explanation for the lack of plateau in the M-wave S-R curve is that the EMG recording was probably not isolated to biceps femoris but contaminated by responses in other muscles. If this occurred, it would explain both why stimulator maximum output or maximum subject tolerance was reached prior to achieving plateau in M-wave response. EMG crosstalk from neighboring muscles could lead to Mmax sometimes being an overestimation of true biceps femoris Mmax at high stimulation intensities (Pierrot-Deseilligny and Burke 2005) and thus lead to a plateau not being seen in the M-wave S-R curve in some subjects. It is also worth noting that in long muscles, such as biceps femoris, there is great variation in Mmax due to large changes in muscle geometry (Simonsen and Dyhre-Poulsen 1999). The relative or absolute muscle fiber lengths and diameters as well as pennation angle can change based on musculotendinous tension and/or joint angles (Gerilovsky, Tsvetinov et al. 1989). In addition, changes in distance between the
stimulating electrode and the sciatic nerve may change with changes in hip flexion (Kamiya, Tanabe et al. 2006) Although this might not make it more difficult to obtain maximum M-wave values, it does stress the importance of doing so for comparison purposes during different functional tasks such as walking.

It was also noticed in this study that not all H-reflex S-R curves returned to zero, and/or they only had a few points in the descending portion of the curve. One likely explanation is that the subtraction technique described previously incorrectly resulted in non-zero values of the H-wave at high stimulation intensities. When the M-wave is large, the tail end of the wave returns from a peak (usually negative) value back towards background EMG values. In the absence of an H-reflex, the post-processing tail end of the M-wave should theoretically be a flat line. However, this was often not the case. This resulted in many non-zero values calculated for the H-wave at high stimulation intensities despite not appearing to be an H-reflex present. Regardless, this issue had little, if any, effect on the results of this study. The descending portion of the H-reflex S-R curve was not included in fitting of the curves. Thus, the outcome variable of Hmax was not affected.

Relative Amplitudes of Mmax and Hmax

The maximum values of the S-R curves for the M- and H-waves were determined and used as the outcome variables Mmax and Hmax, respectively. We believe these values are appropriate representations of the maximum peak to peak amplitudes for each condition. It could be argued that the true maximum value in each condition is the highest single PP value measured. However we believe the calculated maximum of the S-
R curve fit is better because it is not subject to a random outlier that may have occurred during stimulations.

When lying prone, the Mmax in all subjects was always higher than the Hmax within a given S-R curve. Mmax also always occurred at higher stimulation intensities than Hmax. Although this makes intuitive sense and was not a novel finding, it is important to demonstrate because, if for some reason the H-reflex is higher in amplitude than the M-wave, it would suggest there are some methodological issues with the study. Methodological issues could include incorrectly identifying the H-reflex, improperly subtracting out the stimulus artifact, or improper placement of the recording and stimulating electrodes. The importance of correctly calculating Mmax and Hmax individually for each condition should not be understated. Calculating Mmax for each condition provides a means of normalizing the H-reflex. Comparing the H-reflex response with the M-wave provides an estimate of the percentage of the motoneuron pool firing during the reflex and allows for comparisons when changes in muscle geometry occur due to changes in muscle length or contraction (Pierrot-Deseilligny and Burke 2005). In this study, the ratio of Hmax:Mmax ranged from 0.04-0.83 (mean 0.24 +/- 0.055). During quiescent, prone lying the range was 0.12-0.66 (mean 0.34 +/- 0.17). In other walking studies, the range of quadriceps Hmax:Mmax was 0.10-0.36 (mean 0.21 +/- 0.06) (Larsen, Mrachacz-Kersting et al. 2006), and for soleus 0.33-0.43 (Morin, Katz et al. 1982). The lowest ratios occurred during midstance, which is also when background EMG was relatively low. The ratios that were on the higher end of our range occurred during late swing when background EMG was high. Also contributing to the high ratios during late swing and lower ratios during midstance is that Mmax was typically higher
during mid to late stance and lower during late swing. We are not sure why the range of ratios in this study is higher than in other studies using different muscles. However, because in all the studies the same task was used (walking), the larger range in our study might simply be a characteristic of CNS control over afferent-mediated responses of the hamstrings muscle during different phases of walking.

*Homosynaptic Depression*

Inhibition of the H-reflex in the prone position was achieved in this study using a paired-pulse technique with an inter-stimulus interval of 50ms. The M-wave PP amplitude did not change following the second stimulus due to it being non-synaptic in nature. These results confirmed the hypothesis and were consistent with findings in previous studies (Courtine, Harkema et al. 2007; Minassian, Persy et al. 2007; Stein, Estabrooks et al. 2007). We showed the PP amplitude of the second response following the second stimulus to be 12-81% (mean 35%) of the size of the response to the first stimulus. In other studies, the PP amplitudes of the second response following the second stimulus was found to be 30-50% in quadriceps, 10-30% in soleus (Courtine, Harkema et al. 2007; Stein, Estabrooks et al. 2007), and about 20% of the size of the second response following the first stimulus in hamstrings (Courtine, Harkema et al. 2007). The double pulse stimulations were elicited using a stimulation intensity close to that which elicits Hmax. In hindsight, this was probably not the most appropriate stimulation amplitude because if the actual amplitude used is slightly higher than the amplitude at which Hmax occurs, it would fall on the descending portion of the SR curve. This could confound interpretation of the results in this study if there was a drift in stimulation intensity.
because the first stimulus could have occurred at the peak, while the second stimulus could have been on the descending edge of the SR curve. Thus, what was thought to be homosynaptic depression could have actually been collision of antidromic action potentials (Grospretre and Martin 2011). It would have been ideal to perform the double pulse stimulations at or near the stimulation intensity which elicits an H-wave of 50% Hmax. The mean intensity used in the double pulse stimulations was 0.07 milliamps (11.7%, range -0.18-0.0008) less than mean intensity that elicited Hmax during prone lying. Only one subject had a stimulation intensity during double pulse that was greater than that used for Hmax, with that difference being being so small (0.0008 milliamps) that on the S-R curve its predicted value is no different than Hmax. Because intensities used were less than or right at those which elicit Hmax, I am confident that it did not affect the results or change the conclusions that can be drawn from them.

The inhibition of the second response is believed to be caused by homosynaptic depression, otherwise known as postactivation depression (PAD) and presynaptic inhibition. Homosynaptic depression occurs in monosynaptic connections at inter-stimulus intervals (ISI) up to 10 seconds. It is most prominent for ISIs below 1-2s, and virtually non-existent for ISIs of 5-10s (Pierrot-Deseilligny and Burke 2005). It occurs as a result of repeated attempts at exciting the motoneuron by Ia afferents. At short intervals there is not enough time for neurotransmitter reuptake in the synaptic cleft and thus the second stimulus fails to cause an action potential (Stein, Estabrooks et al. 2007). Homosynaptic depression is seen only in subjects at rest and disappears at about 15-20% activation (Stein and Thompson 2006). In addition to electrical stimulation of a mixed nerve to elicit an H-reflex, electrical stimulation of cutaneous nerves can also elicit short
latency reflexes. In adult humans, short latency cutaneous reflexes (<15ms) are rare to find in the legs but medium latency (15-25ms) are more common (Rowlandson and Stephens 1985). There is much inconsistency in the cutaneous reflex responses in that they do not always follow background EMG activity, and are sometimes excitatory or suppressive depending on which response and when in the gait cycle it is elicited (Baken, Dietz et al. 2005). Thus, afferent information from cutaneous stimulation can result in short latency responses in human hamstrings during walking, but these responses are highly unreliable and unpredictable. Thus, the presence of homosynaptic depression in the second response seen in the present study lends support to the response being monosynaptic in nature and thus likely an H-reflex.

This study used an ISI of 50ms, however using a longer ISI (>400ms) may have provided additional insight. At shorter ISIs (<400ms), such as used in this study, the depression could be attributed at least partially to presynaptic inhibition from primary afferent depolarization. This can occur via heteronymous or homonymous stimulation. Presynaptic inhibition normally does not last longer than 300-400ms. Stimulation of the homonymous afferent with longer ISIs can cause inhibition by homosynaptic depression (Pierrot-Deseilligny and Burke 2005). Homosynaptic depression can be elicited at longer ISIs up to 10s, with the depression being attributed to a delay in neurotransmitter reuptake in the synaptic cleft (Stein, Estabrooks et al. 2007). Thus, it appears that using a longer ISI greater than 400ms may have been useful to ensure homosynaptic depression was the cause of the inhibition. Regardless, a 50ms ISI was used in the present study as recommended previously (Schieppati 1987).
Advantages of New Technique

The technique introduced in this study is a new approach to studying hamstring afferent feedback during walking in humans. Previous work has been done with tendon taps (Van de Crommert, Faist et al. 1996; Faist, Blahak et al. 1999), stretch reflexes (Friemert, Bumann-Melnyk et al. 2005), and most recently multisegmental monosynaptic responses (MMR) have been introduced (Courtine, Harkema et al. 2007; Minassian, Persy et al. 2007; Dy, Gerasimenko et al. 2010). To my knowledge this is the first time H-reflexes have been elicited in the hamstrings.

The H-reflex, short latency stretch reflex, and tendon tap are all dependent on monosynaptic excitation from homonymous afferents. Each of the techniques are different in several ways and have physiological caveats that should be considered: 1) While walking causes less synchronized, low frequency, and less burst-like afferent activity, external perturbations evoke more synchronized, high frequency, burst-like activity in many different afferents (Nielsen and Sinkjaer 2002). Single pulse electrical stimulation as used to elicit the H-reflex results in a synchronous volley with a single action potential in large cutaneous afferents as well as Ia and Ib afferents. 2) During walking, afferent feedback is ‘expected’ by the CNS- it is built into the motor program for the movement, whereas afferent activity from external perturbations of electrical stimulation deviates from the expected afferent activity. The effects of these different afferents in central networks cannot be assumed to be the same. 3) Tendon tap produces a dynamic movement which results in a prolonged activation of muscle spindles and afferent firing. The afferent volley is dependent on fusimotor effects, and since spindle sensitivity is influenced by gamma drive, the afferent volley from tendon taps and
stretches can be affected indirectly by the gamma system (Paillard 1955). Moreover, the short latency stretch reflex is overlapped by a medium latency response due to group II volley from muscle spindle secondary endings (Pierrot-Deseilligny and Burke 2005) making it difficult to distinguish. Because afferent volleys from electrical stimulation, tendon taps, and normal walking are all not the same, information gained from one technique may not necessarily be applicable to other techniques.

The various stimulation techniques also have issues in measurement techniques that should be considered. 1) When using the H-reflex in proximal muscles, e.g. biceps femoris, the tail end of the M-wave can overlap with the H-reflex. Tendon taps and stretches do not have this issue because of the lack of a direct motor response. This overlap of M-wave and H-reflex can make it difficult to identify the onset of the H-wave and amplitude measurements can be problematic when the reflex is superimposed on background muscle activity during walking. 2) The M-wave allows for normalization of the H-reflex due to changes in muscle length and geometry during movement. This cannot be done with tendon taps or MMR thus making it a more versatile technique and allows for more comparisons across conditions and subjects.

In summary, there are caveats and measurement issues that should be considered with all techniques, but we feel electrical stimulation is very useful because of the consistency in afferent volleys elicited and the ability to normalize it for comparisons across subjects and testing sessions. Thus we think the advantages of single pulse electrical stimulation to induce H-reflexes in the hamstrings outweigh the stated caveats.
Modulation of H-reflex During Gait

Stimulus Response Curves

The stimulus-response curves elicited for both the M-wave and H-reflex were similar to those seen in prone lying. Just as in the prone condition, the Boltzmann and modified Boltzmann equations used for the M-wave and H-reflex S-R curves resulted in very high correlations. The same equations as in prone lying were used for both tasks with the same outcome variables (Mmax & Hmax) calculated from the elicited S-R curves. It was noticed that Mmax and Hmax had large changes throughout the gait cycle, with Mmax being greatest during late stance and Hmax being greatest during late swing. These large changes were not surprising considering the long length of biceps femoris (Simonsen and Dyhre-Poulsen 1999) and the change in recording conditions during movement. This observation adds further evidence for the need to normalize the H-reflex for each phase of the gait cycle.

Task Dependency

Task dependency of the biceps femoris H-reflex was not tested in this study, even though similar data collection methods were used with prone lying and walking. Comparison of the two tasks would not have been appropriate due to differing background EMG activity between quiescent prone lying and walking. The aim of this study was not to compare hamstring H-reflexes during different tasks, thus we did not match background EMG levels to those found during different phases of the gait cycle. However, it is thought that the reflex responses differ in various tasks (Morin, Katz et al. 1982). The relative amplitude of the short latency response and the slope of the stimulus-
response curve could be steeper in different tasks even at similar background EMG and stimulus intensity (Capaday 1997). This has been investigated numerous times in other studies (Morin, Katz et al. 1982; Capaday and Stein 1986; Capaday and Stein 1987; Yang and Whelan 1993). Some studies have addressed this issue by matching soleus EMG during standing to those during either the swing phase (Yang and Whelan 1993) or stance phase (Morin, Katz et al. 1982) of gait. In those studies, soleus H-reflex remained depressed during both phases of the gait cycle even when the standing static contraction had a similar level of background EMG. Thus, the reflex response is apparently task dependent based on results from other studies.

**Time Dependency**

One of the primary findings of this study was a phase dependent modulation of biceps femoris H-reflex during human locomotion. The amplitude of the maximum H-reflex relative to the maximum M-wave was largest in late swing phase of the gait cycle, specifically in the last 15% of the gait cycle. This was consistent with results found in related studies on stretch reflexes (Friemert, Bumann-Melnyk et al. 2005) and tendon taps (Van de Crommert, Faist et al. 1996; Faist, Blahak et al. 1999) in hamstrings. We found that when the H-reflex was not normalized, there appeared to be small increases in the H-reflex in late swing and early stance. It was also observed that the Mmax values were clearly largest in late stance (~60% of the gait cycle), and lowest around heel strike. A possible cause for lower Mmax values during late swing is that true Mmax was not being reached. However in this study we are confident that true Mmax was reached because plateaus are easily seen with good curve fits for the subjects in that phase. Some
examples are shown in Figure IV-11. Thus, when H-reflexes were normalized to Mmax, the H-reflexes were clearly largest in late swing. We believe this normalization to Mmax is important due to the changes in muscle geometry and relative position of recording and stimulating electrodes during gait.

During this study, it was found that H-reflex response amplitude was not related to background EMG amplitude during specific phases of the gait cycle. The correlation between the average Hmax:Mmax ratio and average background EMG activity for all subjects throughout the entire gait cycle was high ($R^2 = 0.896$). However, when removing the late swing phase from the linear fit, and thus significantly shortening the range of values, the $R^2$ value drops to 0.520. Typically during static contractions, increases in reflex amplitude will parallel background EMG and represent changes in the excitability of the motoneuron pool (Akazawa, Aldridge et al. 1982; Stein, Estabrooks et al. 2007). However, during different tasks and different phases of the same task, changes in response amplitude only partially parallel with the increases in background EMG activity in hamstrings (Van de Crommert, Faist et al. 1996), soleus (Yang and Whelan 1993) and quadriceps (Larsen, Mrachacz-Kersting et al. 2006). These studies came to this conclusion by finding Hmax:Mmax ratios during various phases of the gait cycle and comparing them to matched EMG levels during standing isometric contractions. In some phases of the gait cycle, the matched Hmax:Mmax ratio showed large differences between the two tasks. These phases were primarily those in which the muscles were actively lengthening. During human gait, the hamstring muscles are actively lengthened during late swing. We hypothesized that the slope of the normalized H-reflex amplitude and background EMG would be higher during swing than during stance based on
findings from related studies showing larger reflex responses during muscle lengthening (Yang and Whelan 1993; Van de Crommert, Faist et al. 1996; Larsen, Mrachacz-Kersting et al. 2006). This was not shown to be the case, probably because of the high variability in the slopes among subjects during both stance and swing and the large number of non-significant relationships between background EMG and H-reflex amplitude.

It was thought that modulation of biceps femoris H-reflexes would be different during active lengthening, such as that seen in soleus in late stance and quadriceps just after heel strike. Because hamstring H-reflexes relative to background EMG were not different during late swing compared to the rest of the gait cycle, we cannot conclude that biceps femoris H-reflexes are modulated differently during the late swing phase. Thus, it appears there is no evidence for phase dependent modulation of the response independent of excitation of the motoneuron pool (background EMG).

**Possible Causes of H-reflex Modulation**

There are several possible mechanisms underlying modulation of the H-reflex during walking and other activities. Some of those include changes in background EMG, postsynaptic inhibition (Akazawa, Aldridge et al. 1982), reciprocal inhibition, and presynaptic inhibition or CPG modulation (Hultborn, Wigstrom et al. 1975; Akazawa, Aldridge et al. 1982; Morin, Katz et al. 1982; Capaday and Stein 1987; Hultborn, Meunier et al. 1987; Hultborn, Meunier et al. 1987). I will address each of these mechanisms in subsequent paragraphs.
Background EMG

In addition to descending input, background EMG activity during active lengthening contractions of individual muscles during walking could be caused by several different types of afferent input. As mentioned in the Introduction, some possible sources of afferent activity during walking that have been proposed have included group Ia afferents (Nielsen and Sinkjaer 2002), group Ib, group II afferents (Sinkjaer, Andersen et al. 2000), and cutaneous receptor afferents (Mazzaro, Grey et al. 2006). Sinkjaer et al. (2000) used ischaemic block and lidocaine injections during plantar flexor unloading to show that peripheral afferents other than Ia, such as Ib and group II, contribute to the activation during the stance phase of human walking. Pharmaceutical depression of the group II afferents reduces the soleus EMG responses to ankle dorsiflexion, while the anaesthetic block of cutaneous and proprioceptive afferents of the foot did not affect the soleus EMG amplitude modulation in response to ankle perturbations during the stance phase of walking. Thus it is unlikely that cutaneous receptors affect EMG activity during active lengthening contractions, and that it is mostly the result of Ia, Ib, or group II afferents. Regardless of the type of afferent neuron they all appear to significantly affect reflex amplitude in response to plantarflexor unloading.

Even though peripheral inputs may have a large influence on the amplitude of background EMG, they do not account for all the changes in the amplitude of the response to stimulation during walking. Yang & Whelan (1993) trained subjects to activate background soleus EMG during the swing phase when it is not normally active. Soleus H-reflexes remained depressed during the swing phase when compared to stance, despite similar levels of background EMG. Moreover, in the same subjects during
standing with the leg in the air and equal background EMG activity, H-reflex values were increased and similar to values seen in previous studies. Thus, afferent activity alone does not appear to account for changes in H-reflex amplitude. These observations lend support to the idea that there is a significant central control of H-reflexes related to task dependency.

*Postsynaptic Inhibition*

Most authors agree that the postsynaptic excitability of motoneurons is dependent on Ia firing rate, and is not affected by the type of motor activity (Morin, Katz et al. 1982). Postsynaptic inhibition can reduce the H-reflex, but will also result in reductions in EMG (Stein and Kearney 1995). Heteronymous oligosynaptic reflexes in human quadriceps have been shown to be similar during walking and standing, while H-reflexes in the same muscle were reduced during walking compared to standing (Brooke, Collins et al. 1991). Because background EMG activity was similar in both cases, it is unlikely that post-synaptic excitability was changed, thus the authors concluded that pre-synaptic mechanisms must be the cause of the H-reflex inhibition. That is, because H-reflexes are monosynaptic in nature, and postsynaptic inhibition was unchanged as indicated from background EMG, only presynaptic mechanisms are available to modulate the H-reflex.

*Reciprocal Inhibition*

It has also been suggested that activation of the antagonist muscle could alter H-reflexes by reciprocal inhibition (Edamura, Yang et al. 1991). A couple of studies have addressed this issue and found that it is not likely to cause the modulation of reflexes seen
during walking. Edamura and colleagues (Edamura, Yang et al. 1991) observed tibialis anterior and soleus activity during running and walking and found that EMG activity of these muscles was not always reciprocal in nature; and H-reflex amplitude in all subjects did not necessarily coincide with background EMG amplitude in soleus and tibialis anterior. Moreover, Yang & Whelan (1993) (Yang and Whelan 1993) trained subjects to activate soleus or to deactivate tibialis anterior at times in the gait cycle opposite to their normal firing. Despite these manipulations to create unnatural muscle activities, the H-reflexes remained as they would during normal gait, thus dissociating background soleus EMG activity and reciprocal inhibition from tibialis anterior as potential causes of H-reflex variation. Larsen et al. (2006) (Larsen, Mrachacz-Kersting et al. 2006) measured hamstring activity during H-reflexes of quadriceps and found that modulation of the quadriceps H-reflex does not follow classic reciprocal inhibition patterns either. Because reciprocal inhibition has not yet been shown to affect H-reflexes during walking in other muscles, it is unlikely that reciprocal inhibition caused the modulation in the present study on hamstrings.

**Presynaptic Inhibition**

It has generally been accepted that the changes in H-reflex excitability are primarily caused by presynaptic inhibition during walking (Hultborn, Meunier et al. 1987; Hultborn, Meunier et al. 1987; Schieppati 1987; Brooke, Cheng et al. 1995; Stein 1995; Stein and Kearney 1995; Schneider, Lavoie et al. 2000). Authors of a variety of related studies all agree that presynaptic mechanisms are most likely responsible, including studies on soleus H-reflex (Morin, Katz et al. 1982; Edamura, Yang et al. 1991;
Yang and Whelan 1993), quadriceps H-reflex (Dietz, Discher et al. 1990; Dietz, Faist et al. 1990; Larsen, Mrachacz-Kersting et al. 2006), and cat biceps femoris H-reflex (Duysens et al. 1998). Directly measuring presynaptic inhibition in humans is difficult, but studies that have been able to maintain steady background EMG and use either vibrations or short trains of stimuli at short latencies have come to the conclusion, after excluding postsynaptic inhibition as a potential mechanism, that modulation of short latency reflexes likely comes from presynaptic inhibition (Stein 1995).

Presynaptic inhibition features include 1) A depression of the monosynaptic excitatory postsynaptic potential (ESPS) recorded across the postsynaptic membrane without any change in its time course, and 2) No change in the postsynaptic membrane potential or in the excitability of the motoneuron (Willis 2006). Short latency reflexes which are not monosynaptically evoked may not show the depression of reflex magnitude because walking enhances and modulates primary afferent depolarization leading to presynaptic modulation (Brooke, Cheng et al. 1995). According to Dietz et al 1990 (Dietz, Faist et al. 1990), modulation of the H-reflex during walking occurs only in monosynaptic connections because polysynaptic pathways do not contribute significantly (Fournier et al. 1986), particularly in the early part of the H-reflex. Because of the short latency of the monosynaptic reflex, especially the onset of the response, they believe that presynaptic inhibition is probably the only cause of the modulation during walking.

It is highly likely that presynaptic inhibition was the source of modulation in the current study because electrical stimulation during walking was used instead of tendon taps or stretch reflexes. In one study, electrically evoked H-reflexes were more sensitive to presynaptic inhibition than mechanically evoked tendon tap and stretch reflexes.
(Morita, Petersen et al. 1998), although in that study the responses were elicited with subjects at rest and may not apply to conditions during activity. However, to make this assumption it is assumed that for a given level of EMG the same population of motor units is firing. The distribution of inputs to a motor neuron pool could be different during different tasks such that the distribution of membrane potentials and hence the excitability to the H-reflex volley could vary (Stein and Kearney 1995). In this experiment, the same task is being performed (walking), however the muscle does have different roles in various parts of the gait cycle, which could be associated with different inputs to the motor neurons of particular muscles.

Capaday (Capaday 1997) noted that the H-reflex is a measure of efficacy of synaptic transmission when measurements are made at matched levels of motor activity, thus neuromodulators could affect the excitability of the motoneuron differently in different tasks. For a fixed level of alpha-motoneuron pool activity and stimulus intensity, the H-reflex output depends on the level of presynaptic inhibition of Ia-afferent terminals in the spinal cord. As mentioned by Schieppati (Schieppati 1987), it would be of great interest to know the effects of descending input, different afferent input, and oligosynaptic input on the motoneuron activity either directly or indirectly via presynaptic mechanisms during different activities. However, recording intracellularly in humans is not currently possible, thus presynaptic inhibition can only be measured indirectly and is preferably done by H-reflexes rather than tendon tap or stretch (Stein and Kearney 1995).
Methodological Considerations and Limitations

A limit of note in this new technique is that only one muscle EMG recording was able to be fully analyzed at a time. With changes to the data acquisition software, it may be possible to obtain the same results with two or more muscles at a time. The downside to this would be that in order to obtain complete S-R curves for multiple muscles, many more stimulations would probably be needed per condition. After performing data collection in this study, it is thought that adding even more stimulations would create problems with subject comfort, fatigue, and willingness to complete the study.

In some subjects it was difficult to distinguish between M- and H-waves, especially during the intensities where smaller H-waves were elicited. The precautions taken (based on recommendations of Larsen et al. 2006) because of this were: 1) Full stimulus-response curves were created at each latency including Mmax and H-wave amplitudes were extracted post-hoc, 2) a post-hoc correction of H-reflex was made because of a possible time-domain overlap of the H and M-waves, 3) A stimulus duration of 1ms was used because this duration tends to give a better separation of M and H waves (Larsen, Mrachacz-Kersting et al. 2006). Despite the precautions taken, it is possible that the chosen start to the H-reflex was inaccurate and resulted in the reported latencies being longer than expected. As discussed earlier it is highly unlikely this affected our outcome variable measurements of peak-to-peak M-wave and H-wave amplitude. But by measuring the time to first peak of the H-wave, we were still able to show the time to first peak of the response was in line with expected values in other related studies. The difficulty in separating the M and H waves is probably one of the biggest drawbacks of
this new technique, but the peak to peak amplitudes are likely not affected and thus this technique can still be very useful.

Another limitation was the use of the stimulation intensity at which Hmax occurs for the double pulse stimulations. As discussed previously, this is dangerous as it has the potential for small changes in stimulus amplitude to result in responses that fall on the descending portion of the S-R curve and confound interpretation of results. Although it did not appear to have negative consequences in this study, future studies should avoid the possibility by using stimulus intensities that fall on the ascending portion of the curve by using a stimulation intensity that will elicit an H-reflex that is close to 50% of the amplitude of Hmax amplitude.

**Functional Significance and Future Considerations**

Hopefully the technique shown in this study and the information gained from it can be applied to patients with neuromuscular deficits during walking. It is believed this method is a valid technique that can be used in future studies to gain quantitative and qualitative information about posterior thigh muscle control. The short latency afferent-mediated response was reduced using the double pulse stimulation, while the direct motor response was unaffected. Thus the response was likely monosynaptic and probably an H-reflex. Because the response can be normalized to the direct motor response, it can be used for comparisons in different tasks and between subjects. With other techniques, i.e. tendon tap or MMR, it is difficult to normalize the responses and compare between subjects or between testing sessions. In patients with neurological lesions such as stroke or spinal cord injury, variability in gait patterns exist and can make it difficult to compare
between subjects. They often have varying degrees of spasticity and motor control, especially in incomplete spinal cord patients. Adding a bulky device such as a reflex hammer may make it even more difficult to ambulate. Movement disorders resulting from CNS injury may affect the ability of humans to modulate presynaptic inhibition, which might lead to some of the symptoms observed in these conditions. Despite variability amongst patient conditions, this technique could still be used on a case by case basis to assess afferent mediated responses and how they may contribute to impairment. In addition to research purposes, sciatic nerve stimulation may also be able to be applied for therapeutic purposes such as in FES (Functional Electrical Stimulation) or to assess the effects of therapy designed to normalize the gait pattern. There have been numerous studies to date on possible roles of short latency responses during walking of soleus and quadriceps, but this is the first to examine how such responses in hamstrings may vary during walking. This is also the first study to my knowledge that has elicited complete S-R curves for the M and H-waves in humans during different phases of the gait cycle.
APPENDIX

Summary of reflex latencies and conduction velocities found in literature review. Reflex types are designated at either H-reflex (H), tendon taps (TT), stretch reflexes (Stretch), or multisegmental monosynaptic responses (MMR), and conduction velocity is designated as (CV).

<table>
<thead>
<tr>
<th>Study</th>
<th>Type</th>
<th>Muscle</th>
<th>Latency or CV</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borg (1987)</td>
<td>CV</td>
<td>Motoneuron</td>
<td>39.8 m/s</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>Brooke (1991)</td>
<td>H</td>
<td>Vastus medialis</td>
<td>19.9 ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brooke &amp; Zehr (2006)</td>
<td>Ia CV</td>
<td></td>
<td>40-90 m/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bruhn et al. (2011)</td>
<td>Stretch</td>
<td>Biceps femoris</td>
<td>21.9 ms</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Courtine et al. (2007)</td>
<td>MMR</td>
<td>Biceps femoris</td>
<td>17.8 ms</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MMR</td>
<td>Rectus femoris</td>
<td>15.9 ms</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MMR</td>
<td>Soleus</td>
<td>25.0 ms</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Friemert et al (2005)</td>
<td>TT</td>
<td>Biceps femoris</td>
<td>21.9 ms</td>
<td>3.1</td>
<td>17-28</td>
</tr>
<tr>
<td></td>
<td>Stretch</td>
<td>Biceps femoris</td>
<td>20.3 ms</td>
<td>3.5</td>
<td>15.4-25.8</td>
</tr>
<tr>
<td>Frijns et al. (1997)</td>
<td>H</td>
<td>Soleus</td>
<td>30.2 ms</td>
<td>2.1</td>
<td>24.9-36.2</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>Soleus</td>
<td>35.2 ms</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>Rectus femoris</td>
<td>20.8 ms</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Larsen et al. (2006)</td>
<td>H</td>
<td>Vastus lateralis &amp; Rectus femoris</td>
<td>20.1/20.7 ms</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Mrachacz-Kersting et al. (2004)</td>
<td>Stretch</td>
<td>Quadriceps</td>
<td></td>
<td></td>
<td>18-23</td>
</tr>
<tr>
<td>Shefner 1994</td>
<td>CV</td>
<td>Motoneuron</td>
<td>52.4 m/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinkjaer (1996)</td>
<td>Stretch</td>
<td>Soleus</td>
<td>42.0 ms</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Van de Crommert (1996)</td>
<td>TT</td>
<td>Biceps femoris</td>
<td>21 ms</td>
<td>2</td>
<td>18-24</td>
</tr>
</tbody>
</table>
REFERENCES


