QUANTITATIVE ANALYSIS OF FEEDBACK DURING LOCOMOTION

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Kyla Turpin Ross

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QUANTITATIVE ANALYSIS OF FEEDBACK DURING LOCOMOTION

Approved by:

Dr. T. Richard Nichols, Advisor
Graduate School of Arts and Sciences[1]
School of Biomedical Engineering[2]
[1] *Emory University*
[2] *Georgia Institute of Technology*

Dr. Stephen P. DeWeerth
School of Biomedical Engineering
Georgia Institute of Technology

Dr. Rob Butera
School of Electrical and Computer Engineering
Georgia Institute of Technology

Dr. Ron Calabrese
Graduate School of Arts and Sciences
Emory University

Dr. Tim Cope
Boonshoft School of Medicine
Wright State University

Dr. Shawn Hochman
Graduate School of Arts and Sciences
Emory University

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LIST OF SYMBOLS AND ABBREVIATIONS

FDB	Flexor Digitorum Brevis Muscle
FDL	Flexor Digitorum Longus Muscle
FHL	Flexor Hallucis Longus Muscle
G	Gastrocnemius Muscle
LG	Lateral Gastrocnemius Muscle
MG	Medial Gastrocnemius Muscle
MLR	Mesencephalic Locomotor Region
PLAN	Plantaris Muscle
QUADS	Quadriceps Muscles
RF	Rectus Femoris Muscle
SOL	Soleus Muscle
VI	Vastus Intermedius Muscle
VL	Vastus Lateralis Muscle
VM	Vastus Medialis Muscle
XER	Crossed-Extension Reflex

SUMMARY

It is known that muscles possess both intrinsic and reflexive responses to stretch, both of which have been studied extensively. While much is known about heterogenic and autogenic reflexes during XER, these have not been well characterized during locomotion. In this study, we mapped the distribution of autogenic and heterogenic feedback in hindlimb extensor muscles using muscle stretch in the spontaneously locomoting premammillary decerebrate cat. Rather than electrical stimulation or drug administration to induce stepping, we used natural stimulation and compared stretch-evoked force responses obtained during locomotion with those obtained during XER. The goal was to ascertain whether feedback was modulated between the two states. Varying the mechanical inputs to the muscle, including introducing prior release and vibration, also enabled us to determine the underlying mechanism of changes in autogenic feedback. We found that heterogenic feedback pathways, particularly those emanating from MG, remained inhibitory during locomotion while autogenic feedback specifically in MG increased in gain. Furthermore, increases in MG gain were due to force-dependent mechanisms. This suggests that rather than an abrupt transition from inhibition to excitation with changes in motor tasks, these pathways coexist and contribute to maintaining interjoint coordination. Increases in autogenic gain provide a localized loading reflex to contribute to the completion of the movement. The results of these experiments are clinically significant, particularly for the rehabilitation of spinal cord injured patients. To effectively administer treatment and therapy for patients with compromised spinal reflexes, a complete understanding of the circuitry is required.

CHAPTER 1

INTRODUCTION

Muscles possess both intrinsic and reflexive responses to stretch, both of which have been studied extensively. Despite this, the role of proprioceptive feedback from muscle spindles and Golgi tendon organs in motor coordination, particularly in locomotion, is not well understood. While it is known that sensory feedback from muscle spindles, which provides excitatory input particularly autogenically (Eccles et al. 1957), functions to regulate the stiffness of a muscle during postural regulation (Nichols and Houk 1976), the role of force feedback from Golgi tendon organs is less clear. Force feedback inhibits extensor muscles in anesthetized (Eccles et al. 1957) and decerebrate animals (Nichols 1999). However, recent data suggest that initiation of locomotion inhibits Ib inhibition and activates an alternate, excitatory pathway that receives input at group-I strength (Pearson and Collins 1993; McCrea et al. 1995; Hultborn 2001). The role and distribution of these force-dependent and length-dependent pathways during locomotion remains controversial.

1.1 Properties of Muscle: Intrinsic

The stretch-evoked force response of a muscle contains both intrinsic and reflexive components. The extrafusal fibers of muscle, the major contributor to the intrinsic response of muscle, are known to be thixotropic, which describes the large response to small movements and small response to larger movements (Hill 1968).

Pioneering studies further characterized the mechanical response by demonstrating the short-range stiffness, the resistance to changes in length, and yielding properties of muscle (Rack 1970; Grillner 1972). More recently, experiments utilizing the method of reinnervation have been devised to remove short-latency reflexes (Cope and Clark 1993; Cope et al. 1994). These muscles, which have a permanent loss of the stretch reflex, exhibit the classical mechanical responses of muscle (Cope et al. 1994) and contribute to a loss of coordination (Abelew et al. 2000) despite maintaining normal force-generating capabilities (Cope and Clark 1993). Administering stretch to a reinnervated muscle results in a linear range of stiffness (short-range stiffness) followed by an abrupt decrease in force (yielding), presumably due to the distortion of crossbridges (Rack and Westbury 1974). However, releasing a reinnervated muscle prior to stretch exploits the history dependent properties of muscle. This prior release is physiologically relevant for a range of behaviors. For instance, muscle fibers undergo internal lengthening and shortening during locomotion (Goslow et al. 1973). This prior movement reduces the overall stretch-evoked force production of muscle (Campbell and Moss 2000) and linearizes the mechanical properties of muscle. Increasing amplitudes of prior release results in the linearization of the muscle's intrinsically nonlinear, stretch-evoked force response, whereby the range of stiffness is extended and the delay in yielding increases (Huyghues-Despointes et al. 2003). Interestingly, chemically skinned, single muscle fibers exhibit the same, history-dependent properties as the intact muscle (Huyghues-Despointes 1998), which is incidentally independent of muscle type (Kirsch et al. 1994; Huyghues-Despointes et al. 2003). Additionally, random or white noise delivered as an input to

muscle also induces a linearization of the intrinsic response of muscle (Kirsch et al. 1994).

1.2 Properties of Muscle: Reflexes

Since intrinsic and reflexive responses are both presumably important, the question is: what roles do reflexes play in movement? A large proportion of the sensory information generated and received by the cat hindlimb musculature originates from length feedback pathways. The relationship between muscle length and receptor discharge properties is complex. Despite this complexity, the functionality of length feedback is relatively well documented (Nichols et al. 1999). Projections from muscle spindles are excitatory and mainly homonymous, with some pathways distributed primarily to muscles of similar actions (Eccles et al. 1957; Nichols 1999). Length feedback functions to regulate muscular stiffness (Nichols and Houk 1976), even across varying background tension (Hoffer and Andreassen 1981), and enhance force output during stance (Stein et al. 2000; Mazzaro et al. 2006; Rossignol et al. 2006). There is even evidence of length feedback modulating during the step cycle (Sinkjaer et al. 1996) at the stance-swing transition. Additionally, length feedback of biarticular muscles enhances interjoint coupling (Nichols et al. 1999).

Like the extrafusal fibers of muscle, intrafusal fibers, specifically the muscle spindle receptors themselves, exhibit history dependence (Proske et al. 1993)**.** It is known that prior movement modulates the stretch reflex (Gregory et al. 1998), which is particularly important during motor tasks such as locomotion (Kearney et al. 1999). For

incremental stretch, the muscle spindle receptor performs a linear transformation from the change in length to force (Matthews and Stein 1969). As the magnitude of stretch increases, so does the nonlinearity of the muscle spindle response. Specifically, the receptor becomes less sensitive to stretch (Hasan and Houk 1975). Incidentally, static gamma activation is increased during movement, particularly locomotion, also contributing to the muscle spindle's sensitivity to stretch (Taylor et al. 1985; Bennett et al. 1996; Murphy et al. 2002). Intraxonal recordings of muscle spindle afferents during stretch demonstrate the initial sensitivity with an initial burst followed by a dynamic response. Introducing prior movement, by delivering triangular stretches to a muscle, gives rise to the initial burst on the first stretch, yet eliminates the burst and significantly reduces the dynamic response for subsequent stretches (Haftel et al. 2004).

Both the force-generating components and muscle spindle afferents exhibit this history dependent phenomenon, yet the interaction of the two during movement requires reconciling. When compared to intact muscles, force-responses obtained in reinnervated muscles exhibit increased linearity with increasing amplitudes of prior release. Despite this, the overall stiffness of the muscle is maintained, suggesting that the stretch reflex makes a smaller contribution to maintain stiffness during ongoing movement (Huyghues-Despointes et al. 2003).

While the properties and the roles of length feedback from muscle spindles during movement have been extensively studied, the functional role of force feedback from Golgi tendon organs, and its central processing are less understood. Traditionally, Golgi

tendon organs have been classified as providing inhibitory feedback to extensor (antigravity) muscles, particularly across joints and axes of rotation, with very few connections to the muscle of origin (Eccles et al. 1957; Nichols 1989). Unlike length feedback, force feedback does not regulate individual muscular stiffness, but it probably contributes to the regulation of whole limb stiffness and interjoint coordination due to the distributed nature of the feedback (Bonasera and Nichols 1994; Nichols et al. 1999). Additionally, the mechanics of the Golgi tendon are not particularly affected by prior movement, as are the muscle spindle receptors (Haftel et al. 2004). This is further evidence that the two rapid feedback pathways, different in distribution, sign, and mechanical properties, play important, yet distinct, roles during motor tasks.

1.3 Hindlimb Extensors: Mechanical Actions

The ankle extensors used in this study, including the gastrocnemius muscle (G), flexor hallucis longus muscle (FHL), plantaris muscle (PLAN), and soleus muscle (SOL), possess a unique architecture and mechanical action, and therefore a distinct function. The biarticular muscle G contains two separate heads, namely the medial gastrocnemius muscle (MG) and the lateral gastrocnemius muscle (LG), each of which contributes a distinct torque about the ankle joint (Lawrence and Nichols 1999). MG and LG originate on the medial and lateral sesamoid bones, respectively, and insert via the Achilles tendon onto the ventral portion of the calcaneus (Crouch 1969). As shown in Figure 1.1 (Nichols 1994), MG contributes significantly greater abduction and plantarflexion of the ankle joint than does its close synergist, LG. SOL, a uniarticular muscle, comprised entirely of

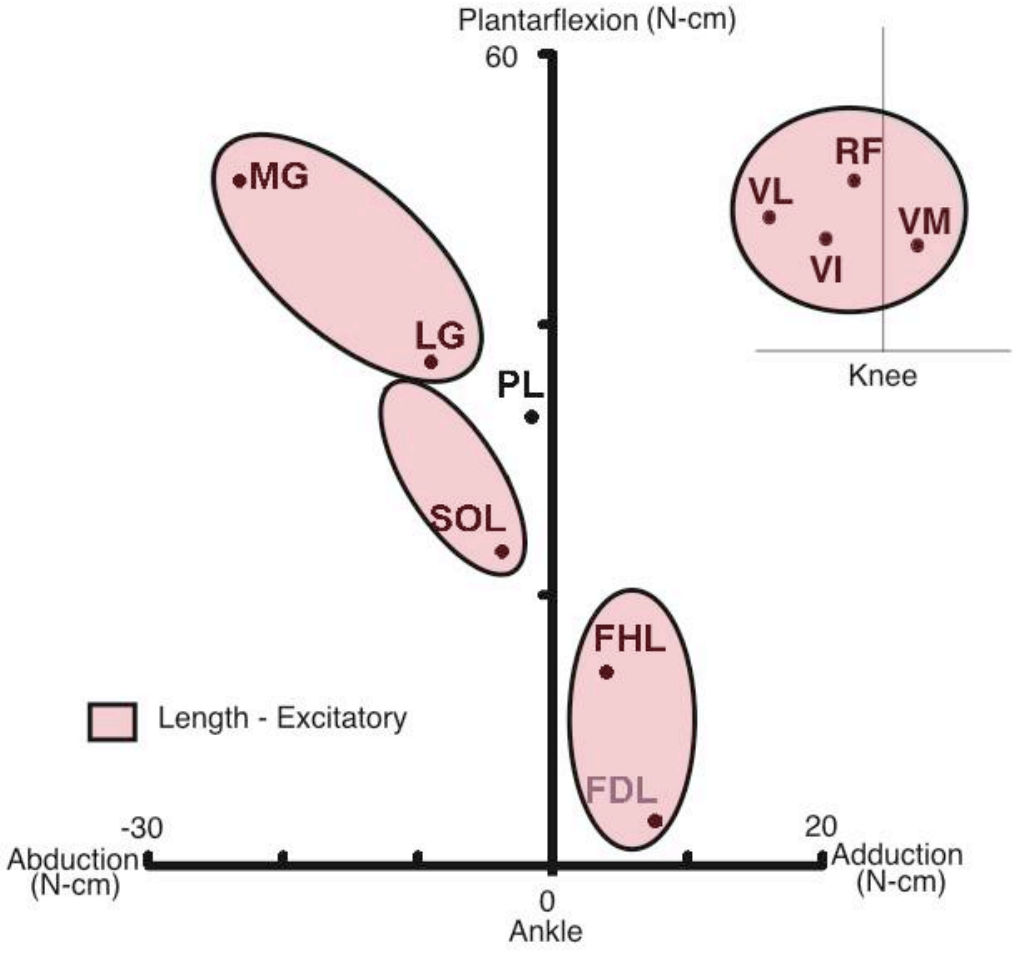


Figure 1.1 Mechanical actions about the ankle joint of cat hindlimb ankle extensors (Nichols, 1994).

slow twitch fibers, originates on the lateral side of the fibula and forms a long, thin tendon that merges with that of G to form the Achilles tendon. This muscle contributes very little to off-sagittal movement, yet still plantarflexes the ankle. Collectively, MG, LG, and SOL are known as the triceps surae, and traditionally have been classified simply as ankle extensors with similar mechanical actions.

In addition to plantarflexing the ankle, PLAN and FHL also have mechanical actions on the toes. PLAN originates on the lateral side of the patella and is closely linked to LG. The very large, broad tendon wraps around the tendons of G and SOL, lays atop the calcaneus and inserts onto the tendon of flexor digitorum brevis (Crouch 1969). The mechanical action of PLAN, traditionally also an ankle extensor, is to significantly plantarflex, yet contribute very little to the off-sagittal movement of the ankle. Making a small contribution to ankle plantarflexion, FHL is the only ankle extensor used in this study that produces an adduction rather than an abduction torque about the ankle joint. The origin of FHL is on the upper portion of the fibula and inserts into the flexor digitorum longus (FDL). Both PLAN and FHL, due to their unique insertions, work to flex the toes; additionally, FHL contributes to claw protrusion (Goslow et al. 1972; Lawrence et al. 1993; Lawrence and Nichols 1999).

The quadriceps muscles (QUADS) are comprised of 4 muscles: vastus lateralis muscle (VL), vastus medialis muscle (VM), vastus intermedius muscle (VI), and rectus femoris muscle (RF). Of these, RF is a biarticular muscle whereas the remaining are uniarticular; furthermore, VI is a homogeneous muscle comprised entirely of slow type

muscle fibers, whereas the remaining muscles are heterogeneous (Ariano et al. 1973). The entire group covers the major portions of the femur, inserts onto the patella, and serves as a powerful leg protractor. While each of the individual muscles making the QUADS possess a unique pulling direction about the patella when activated electrically, this results in pure knee extension (Abelew et al. 1996).

1.4 Hindlimb Extensors: Receptor Distribution in Cat

The distribution of muscle spindles has been studied for most of the hindlimb extensors used in these experiments. Historical studies have classified the numbers of muscle spindles in an array of hindlimb extensors, including MG, LG, and SOL (Chin et al. 1962; Eldred et al. 1974). It is known from these studies that MG contains roughly twice as many muscle spindles (62) than does LG (35). Anatomically, this suggests that length dependent excitation is twice as strong from MG onto LG than for LG onto MG. This has been confirmed in the decerebrate cat using muscle stretch (Eccles et al. 1957; Nichols 1989). The muscle spindle content in SOL is slightly lower than MG, with approximately 56. It is known that both heads of G combined also contain approximately 30% more muscle spindles than does FHL (75). The only muscle among the QUADS for which the number of muscle spindles is known is RF, which contains approximately 104 muscle spindles (Chin et al. 1962). Unlike muscle spindles, the distribution of Golgi tendon organs in each of these muscles is not entirely known. What is known is that SOL contains anywhere from 30 to 45 Golgi tendon organs (Barker 1962), and MG contains 44 Golgi tendon organs (Eldred et al. 1962). Furthermore, RF contains approximately 78 Golgi tendon organs (Barker 1962). Considering a mean value for the number of Golgi

tendon organs in SOL, these data suggest that the ratio of muscle spindles to Golgi tendon organs is preserved across muscles.

1.5 Organization of Feedback: Posture

Much research has been devoted to understanding the distribution of sensory feedback (Jankowska et al. 1981; Jankowska and McCrea 1983) and the stretch reflex, particularly in postural regulation (Houk and Rymer 1981; Nichols 1989). The organization of length and force feedback pathways in the hindlimb of the cat, as described by Eccles (1957) has been verified using the crossed-extension reflex (XER) and the mechanographic technique (Nichols 1987) to mimic perturbations during quiet stance (Nichols 1989; Bonasera and Nichols 1994; Bonasera and Nichols 1996; Nichols 1999; Wilmink and Nichols 2003). Force responses from muscles stretched were used to determine the distribution of heterogenic feedback (Nichols 1987). Under these conditions, there is asymmetric excitation between MG and LG, as previously described, and there are no known force-dependent inhibitory pathways between MG and LG (Eccles et al. 1957; Eccles et al. 1957). When activated, inhibitory pathways exist from both MG and LG onto SOL, which under quiescent conditions, are predominately excitatory (Nichols 1989). PLAN exchanges predominately force-dependent inhibition with the triceps surae (Nichols 1989) and FHL (Nichols 1994), yet a very weak, excitatory connection exists between LG and PLAN (Nichols 1989). Strong, force-dependent inhibition exists between FHL and G, SOL, and QUADS (Bonasera and Nichols 1994), and between QUADS and the triceps surae (Wilmink and Nichols 2003).

A graphical representation of these pathways under postural conditions is depicted in Figure 1.2 (Nichols 1994).

1.6 Organization of Feedback: Locomotion

The data obtained in the non-locomoting cat preparation, as described above, provide an essential mapping of the sensory feedback pathways among hindlimb extensors, yet the distribution of sensory feedback among these muscles during locomotion is not completely known. While length-dependent feedback from muscle spindles is excitatory and force-dependent feedback from Golgi tendon organs is inhibitory in postural regulation, electrophysiological studies have demonstrated that inhibitory force feedback is suppressed during locomotion and replaced by excitatory (positive) force feedback (Pearson and Collins 1993; Gossard et al. 1994; Guertin et al. 1995; Hultborn 2001). Reduced preparations, such as spontaneously locomoting decerebrate or fictive locomoting cats, have been used to study the expression of positive force feedback. In these experiments, MLR stimulation, or the administration of drugs, such as L-DOPA, or clonidine, an $\alpha 2$ adrenergic agonist, induce rhythmic, bursting or stepping behavior (McCrea, Shefchyk et al. 1995). In spontaneously locomoting preparations, the hindlimbs are often extensively denervated (Pearson, Ramirez et al. 1992; Pearson and Collins 1993). Furthermore, the electrical stimulation of extensor muscle sensory afferents elicits excitatory postsynaptic potentials (EPSPs) in extensor motoneurons during fictive locomotion, which is believed to be evidence of positive

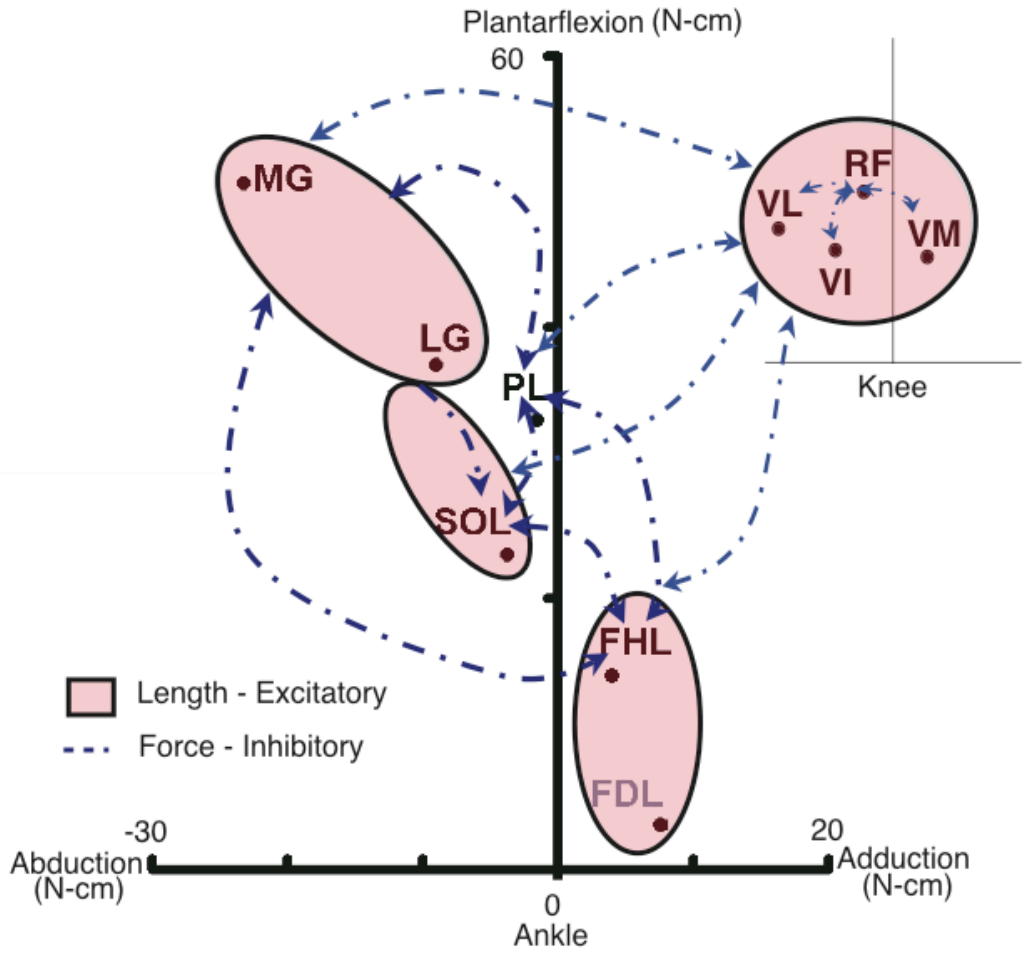


Figure 1.2 Distribution of heterogenic inhibition under postural conditions among cat hindlimb extensors (Nichols 1994).

force feedback (Gossard et al. 1994; McCrea et al. 1995).Using this technique, positive force feedback has been found between PLAN and MG (Pearson and Collins 1993), while feedback from QUADS onto the triceps surae remains inhibitory. Neither of these pathways have been shown to be recruited using natural afferent stimulation (Guertin et al. 1995).

In both fictive and spontaneously locomoting decerebrate cat preparations, positive force feedback is known to regulate phase transitions and contribute to rhythm entrainment. Studies in spontaneously locomoting cats have shown that muscle stretch enhances extensor activity and contributes to the transition from the stance to swing phase of the step cycle (Duysens and Pearson 1980). It is believed that this positive force feedback contributes to the increase in force of the load bearing extensor muscles during the stance phase of locomotion to ensure the completion of a step. It has also been demonstrated in both the spinalized cat and fictive preparations that feedback from group Ib afferents play an important role in the entrainment of the locomotor rhythm (Pearson et al. 1992), and that extensors directly influences the extensor half-center of the central pattern generator (Pearson 1995).

Cutaneous feedback is also known to influence stepping and the control of locomotion. Historically, Sherrington demonstrated that removing cutaneous input barely impaired walking, suggesting that cutaneous input was certainly not required for locomotion (Sherrington 1910). However, it has been shown that stimulation of the dorsum of the foot during the swing phase of locomotion enhances flexion, whereas

stimulation during stance enhances the magnitude but not duration of stance, in a stumbling corrective reflex (Forssberg 1979). Similar results have been obtained by directly stimulating the purely cutaneous sural nerve in thalamic cats (Duysens and Pearson 1976). Implanted nerve cuff electrodes stimulating cutaneous nerves during unrestrained walking cats reveal that stimulating the tibial nerve, a mixed nerve, prolongs the flexion phase (Duysens and Stein 1978), confirming results obtained in premammillary decerebrate cats (Duysens 1977). This evidence concerning cutaneous feedback, combined with the proprioceptive feedback data described above, suggests that both are influential and possess unique, yet powerful effects during locomotion.

1.7 Feedback: Selective Activation

The reflexive components of stretch-evoked autogenic force responses are influenced by both length and force dependent feedback. Teasing apart the effect either pathway has on the overall force response can be difficult due to the convergence of Ia and Ib afferents onto Ib interneurons (Jankowska and McCrea 1983) and similar latencies for both rapid pathways. One method is to selectively activate either length-dependent or force-dependent pathways. For instance, vibration of the muscle-tendon unit selectively activates Ia feedback from muscle spindles due to the rapid change in length, however Ib feedback from Golgi tendon organs remains unaffected (Brown et al. 1967). Specifically, vibration at a frequency of 150 Hz activates muscle spindles in a 1:1 ratio (Clark et al. 1981; Matthews and Watson 1981).

Exploiting the mechanical properties of muscle, particularly of intrafusal fibers, can also help determine the contribution of length versus force feedback to autogenic force responses. As previously discussed, prior movement causes the intrinsic response of muscle to become more linear, while preserving the overall stiffness of the muscle. This suggests that the stretch reflex contributes less to linearization with increasing amplitudes of prior release (Huyghues-Despointes et al. 2003). Muscle also exhibits differential mechanical and reflexive responses to stretch versus release. Intrinsically, muscle displays an asymmetrical response to stretch and release, whereby stretch results in short-range stiffness and yielding, and release results in a larger, continuous decrease in force (Joyce et al. 1969; Nichols and Houk 1973; Nichols and Houk 1976). However, a reflexive muscle exhibits force symmetry with stretch and release, again supporting the premise that Ia feedback compensates for muscle nonlinearities (Nichols and Houk 1976). Muscle spindles possess this asymmetrical response to stretch and release, whereas Golgi tendon organs simply perform a linear transformation for either manipulation (Crago et al. 1976; Jami 1992; Mileusnic and Loeb 2006) .

1.8 Feedback: Task Specificity

There is evidence that the importance of length and force dependent feedback may depend on the particular motor task. For instance, muscle activation patterns change during uphill and downhill walking when compared to level walking (Smith et al. 1998; Gottschall et al. 2005). The activity in hindlimb extensors increases with increasing slope (Carlson-Kuhta et al. 1998). Conversely, forelimb activity increases while hindlimb extensor activity decreases with decreasing slope as more energy is devoted to braking

(Smith et al. 1998). Ground reaction forces also significantly increase with increasing grade (Gregor et al. 2006). Despite the increase in hindlimb extensor activity with increasing slope in these experiments, the magnitude of overall length change for these muscles, particularly for MG, actually decreases as the slope increases. Similarly, the activity in MG decreases with decreasing slope, yet the length changes increase (Gregor et al. 2006). Therefore, it has been suggested that the modulation in extensor activity is mediated by force-dependent mechanisms rather than length-dependent mechanisms. This also might suggest that the expression or distribution of positive force feedback could be similarly modulated with various motor tasks. For instance, it is conceivable that positive force feedback is more widespread during walking up a slope when larger forces are required of antigravity muscles (Gregor et al. 2006). Interestingly, the muscle activation patterns exhibited during uphill and downhill walking have been replicated simply by tilting the head to simulate slope walking. Tilting the head down, which simulates uphill walking, produces EMG activation similar to walking uphill. Likewise, tilting the head up, simulating downhill walking produces EMG activation similar to walking downhill (Gottschall et al. 2005). While transient, this head tilt method provides a unique method to possibly modulate the expression of positive force feedback.

1.9 Summary

While much is known about heterogenic and autogenic reflexes during XER, these have not been well characterized during locomotion. In this study, we utilized the spontaneously locomoting premammillary decerebrate cat to map the distribution of autogenic and heterogenic feedback in hindlimb extensor muscles, including MG, LG,

PLAN, FHL, SOL, and QUADS. We used natural stimulation, rather than electrical stimulation or drug administration, to induce stepping, and we compared stretch-evoked force responses obtained during locomotion with those obtained during XER. Additionally, previous work in this laboratory provided extensive data for postural regulation, which served as a comparison for the data obtained in this study. Varying the mechanical inputs to the muscle also enabled us to determine the underlying mechanism of changes in autogenic feedback. This data provides insight into the role of length and force feedback during the stepping cycle. We hypothesize that heterogenic force feedback pathways remain inhibitory during locomotion while positive force feedback is expressed autogenically. This suggests that there is not an abrupt transition of inhibition to excitation with changes in motor behavior. We believe that inhibitory force feedback is important for interjoint coordination, and therefore remains active for a variety of behavioral conditions. Autogenic excitation provides a localized loading reflex to contribute to the completion of the movement.

The results of these experiments are clinically significant, particularly for the rehabilitation of spinal cord injured patients. To effectively administer treatment and therapy for patients with compromised spinal reflexes, a complete understanding of the circuitry is required.

CHAPTER 2

HETEROGENIC FEEDBACK

2.1 Introduction

Sensory feedback from muscles to spinal segments plays an integral role in locomotion (Pearson 1995; Prochazka 1996; Duysens et al. 2000; Sinkjaer et al. 2000; Stein et al. 2000). Length feedback from muscle spindles projects mainly to parent motoneurons and close synergists and is thought to regulate muscular stiffness (Nichols and Houk 1976) and enhance force output during stance (Stein et al. 2000; Mazzaro et al. 2006). Additionally, length feedback is subject to modulation during the step cycle (Sinkjaer et al. 1996) at the stance-swing transition, but otherwise depends on background force independently of task.

The function of force feedback from Golgi tendon organs is less well understood. It is known that force feedback has more divergent projections than length feedback (Eccles et al. 1957; Eccles et al. 1957), particularly to muscles that cross different joints and axes of rotation (Bonasera and Nichols 1994; Bonasera and Nichols 1996; Wilmink and Nichols 2003). There are several different proposed functions ascribed to force feedback. One proposal is that force feedback regulates the stiffness of a muscle by projecting inhibition to the parent muscle (Houk 1979). A second hypothesis is that it promotes interjoint coordination via inhibitory heterogenic connections (Nichols 1994). Lastly, force feedback may serve as a "loading reflex" to support greater forces required during locomotion (Duysens and Pearson 1980; Pearson and Collins 1993; Dietz and Duysens 2000). The first two proposed functions require inhibitory force feedback and

are general purpose, while the third requires excitatory force feedback and is task-dependent. Available information has allowed the rejection of the first hypothesis, since autogenic force feedback is weak (Rymer and Hasan 1980; Nichols 1999). The second hypothesis is consistent with data obtained using intracellular recordings in anesthetized cats (Eccles et al. 1957) and was supported using natural stimulation (Nichols 1999).

Recent studies have supported the third hypothesis by electrophysiologically demonstrating that inhibitory force feedback is suppressed during locomotion and replaced by excitatory force feedback (Pearson and Collins 1993; Guertin et al. 1995; Hultborn 2001). Studies employing either clonidine (a selective $\alpha 2$ adrenergic agonist) or stimulation of the MLR to induce stepping (Pearson et al. 1992; Pearson and Collins 1993; McCrea et al. 1995) suggest that force feedback is modulated during locomotion. Specifically, force dependent feedback from Ib afferents exhibits a reflex reversal during the stance phase of locomotion, thus acting as a loading reflex, while also regulating the transition from stance to swing phase in both the fictive and spontaneously locomoting decerebrate cat preparations (Duysens and Pearson 1980; Conway et al. 1987). That is, positive force feedback contributes to the increase in force of the load bearing extensor muscles during the stance phase of locomotion to ensure the completion of this phase.

Further studies examining positive force feedback have been completed in reduced preparations. During fictive locomotion, electrical stimulation of sensory afferents originating from extensor muscles elicits excitatory postsynaptic potentials (EPSPs) in extensor motoneurons following administration of LDOPA or stimulation of

the MLR (Gossard et al. 1994; McCrea et al. 1995). Furthermore, experiments in the spinalized cat and fictive preparations have demonstrated that feedback from group Ib afferents plays an important role in the entrainment of the locomotor rhythm (Pearson et al. 1992). Based on this evidence, it has been proposed that upon initiation of locomotion, an alternate excitatory pathway receiving input from both Ia and Ib afferents emerges, while the disynaptic inhibitory pathway is suppressed (Pearson and Collins 1993). Consequently, group Ib feedback from extensors directly influences the extensor half-center of the central pattern generator (Pearson 1995).

The above evidence supports the hypothesis of an excitatory loading reflex, but also implies that the presumed coordinating influence of heterogenic inhibition is defeated during locomotion. We sought to re-investigate the issue in an actively stepping preparation using the natural stimulus of muscle stretch. We indeed found evidence for positive force feedback autogenically (Ross et al. 2002; Ross et al. 2005), however we wanted to investigate further the distribution of heterogenic feedback during locomotion. Muscles exchanging predominately force-dependent inhibition were chosen for these experiments, namely G, PLAN, FHL, and QUADS (Eccles et al. 1957; Nichols 1989). Our experiments addressed the following central question: does heterogenic force feedback among G, PLAN, FHL, and QUADS exhibit a reflex reversal with the initiation of locomotion in this preparation? We found that, under these conditions, there was not a global change from inhibition to excitation with locomotion. We propose that these heterogenic inhibitory connections could help maintain the stability and coordination of the limb. Specifically, the inhibitory force feedback could enhance interjoint coordination

and help regulate limb stiffness. Preliminary accounts of these results have been published (Ross et al. 2002; Ross et al. 2003; Ross and Nichols 2004; Ross et al. 2005).

2.2 Methods

2.2.1 Preparation

The method used to evaluate the distribution and contribution of feedback from muscle receptors is the mechanographic technique, which has been previously described (Nichols 1987). All protocols are in complete accordance with the guidelines of both the National Institutes of Health and the Emory Institutional Animal Care and Use Committee. Briefly, twenty-eight cats ranging from 3 to 6 kilograms were deeply anesthetized using isoflurane gas. A tracheotomy was performed, loosened sutures were placed around the carotid arteries, and a cannula was inserted into the external jugular vein to administer intravenous fluids during the experimental procedure. Withdrawal responses were monitored, and the level of anesthetic was adjusted accordingly.

The right hindlimb was immobilized and muscles, and the limb was prepared for careful dissection. Bone pins were inserted into the femur and tibia and then clamped to maintain the knee at a 110^{o} angle. The animal was placed in the stereotaxic frame, supported above a variable-speed treadmill. The ankle and bone pins were clamped to the treadmill frame. A temperature probe was inserted rectally and a heating pad placed under the animal to maintain a core temperature of 37^{o} C. While the ankle was maintained at 90 ^{o}, a reference suture was inserted around the tendons of the peroneus

muscles. The distance between these sutures and sutures inserted in the appropriate tendons were used to measure initial lengths of the muscles.

The appropriate muscles were dissected, carefully removing associated connective tissue to minimize mechanical coupling, yet preserving the blood supply and nerve innervation. The muscles, namely G, PLAN, and FHL of the right hindlimb were each dissected. Both PLAN and FHL were cut near their insertion onto FDB and FDL respectively. In 9 experiments, G was separated into its respective heads, MG and LG. A small bone fragment from the calcaneus was preserved during the G dissection. Each muscle was attached via the tendon to individual clamps. These tendon clamps were placed in series with myographs using strain gauges in a half bridge configuration, and four linear motors. In 5 experiments, the QUADS were dissected yet still remained attached to the patellar tendon. A small hole was drilled into the patellar tendon, through which a cable was threaded and attached to a myograph and linear motor via a pulley system. Mineral oil was used to ensure that the muscles stayed moist. Figure 2.1 depicts this experimental setup, whereby the dissected muscles of the immobilized right hindlimb are attached in series with myographs and linear motors.

A premammillary decerebration was performed, whereby the brainstem was transected rostral to the superior colliculus while preserving the mammillary bodies and subthalamic nucleus. All brain matter rostral to the transection was removed. Gelfoam and cotton were placed on the base of the cranium to minimize bleeding. Anesthesia was then titrated down and withdrawn.

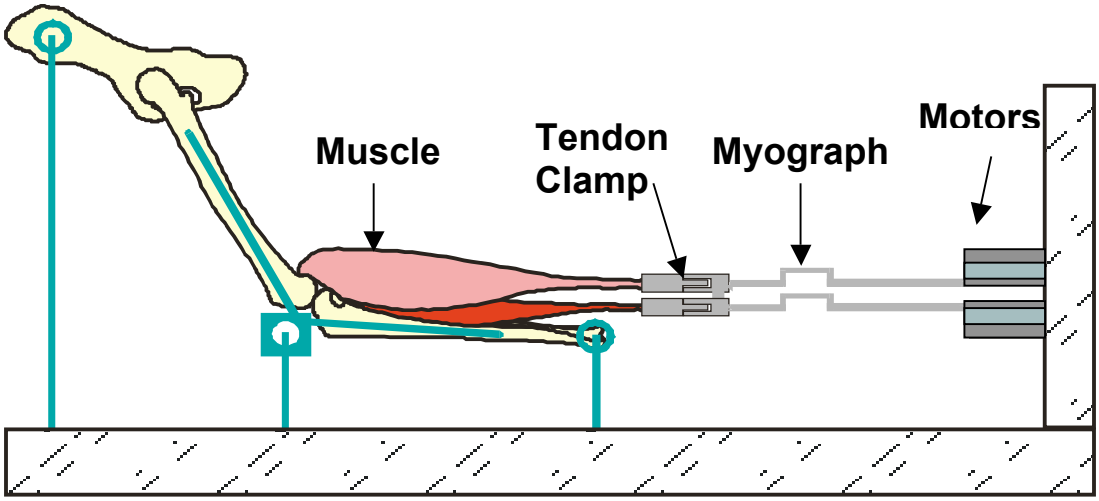


Figure 2.1 Experimental setup. The right hindlimb is immobilized, and dissected muscles are attached via tendons to myographs and linear motors.

The three remaining limbs were free to step on the treadmill while the right hindlimb remained immobilized. Stimulation of the skin beneath the tail was used to initiate stepping when spontaneous locomotion did not occur. Once locomotion data were obtained, the XER was elicited with electrical stimulation of the left posterior tibial nerve at 2 times threshold (2T). Threshold corresponds to the minimum stimulation required to elicit a force response in the dissected muscles of the contralateral limb. At the end of each experiment, the animal was euthanized with an overdose of Nembutal followed by a pneumothorax.

2.2.2 Data Acquisition

The motors used in these experiments were Parker 406LXR linear motors with an encoder resolution of 0.1 microns, maximum acceleration of approximately 50 m/s^2, and maximum load capacity of 180 Kgf. Each of the 4 linear motors were mounted on a custom-built aluminum frame and could be adjusted in the horizontal, vertical, and diagonal directions to achieve proper alignment with the appropriate muscle. The four individual frames were mounted on a rigid, outer frame.

The motors were controlled using a 6000 series *Gemini* servo drive and dSPACE board, and Simulink program. Data was acquired digitally through the dSPACE board at a sampling rate of 1000 Hz. The change in length, velocity, and hold time was specified using the data acquisition software built in Simulink with a graphical interface in ControlDesk. The typical paradigm was a 2 mm stretch at a velocity of 0.04 m/s, 100 ms hold period, and 2 mm release. All muscles were maintained at their referenced length

(see above) when the knee was fixed at 110^{o} and the ankle was at 90^{o}. The eccentric length change that an intact animal experiences during walking and trotting is approximately 4 mm (Goslow et al. 1973). The magnitude of stretch used in these experiments was 2 mm and within this physiological range. This conservative stretch was performed to preserve the tendon and to increase the longevity of the preparation while producing repeatable and robust results.

Once initiation of stepping from the three unfixed limbs commenced, the recorded force in the immobilized right hindlimb oscillated, as shown in Figure 2.2a. Random stretches were manually triggered so as to capture a significant number of trials in all phases of the step cycle. Figure 2.2b depicts the ongoing oscillations in the force with stretches administered during stepping.

The stretching paradigm in the right hindlimb was then repeated during the XER so that we could determine how feedback was reorganized with the initiation of stepping. In both behavioral conditions, the muscles were stretched in a two-state configuration. In state one, the recipient muscle was administered a ramp and hold stretch alone. In state two, the recipient muscle was then stretched with a donor muscle. Figures 2.3a and 2.3b depict the force output and length input of the recipient muscle respectively. Figures 2.3c and 2.3d depict the force output and length input of the donor muscle respectively. Additionally, Figure 2.3a contains symbols indicating the force responses obtained when the recipient muscle is stretched alone (filled circles) and stretched along with the donor muscle (open triangles). As explained below, stretch-evoked force responses obtained in

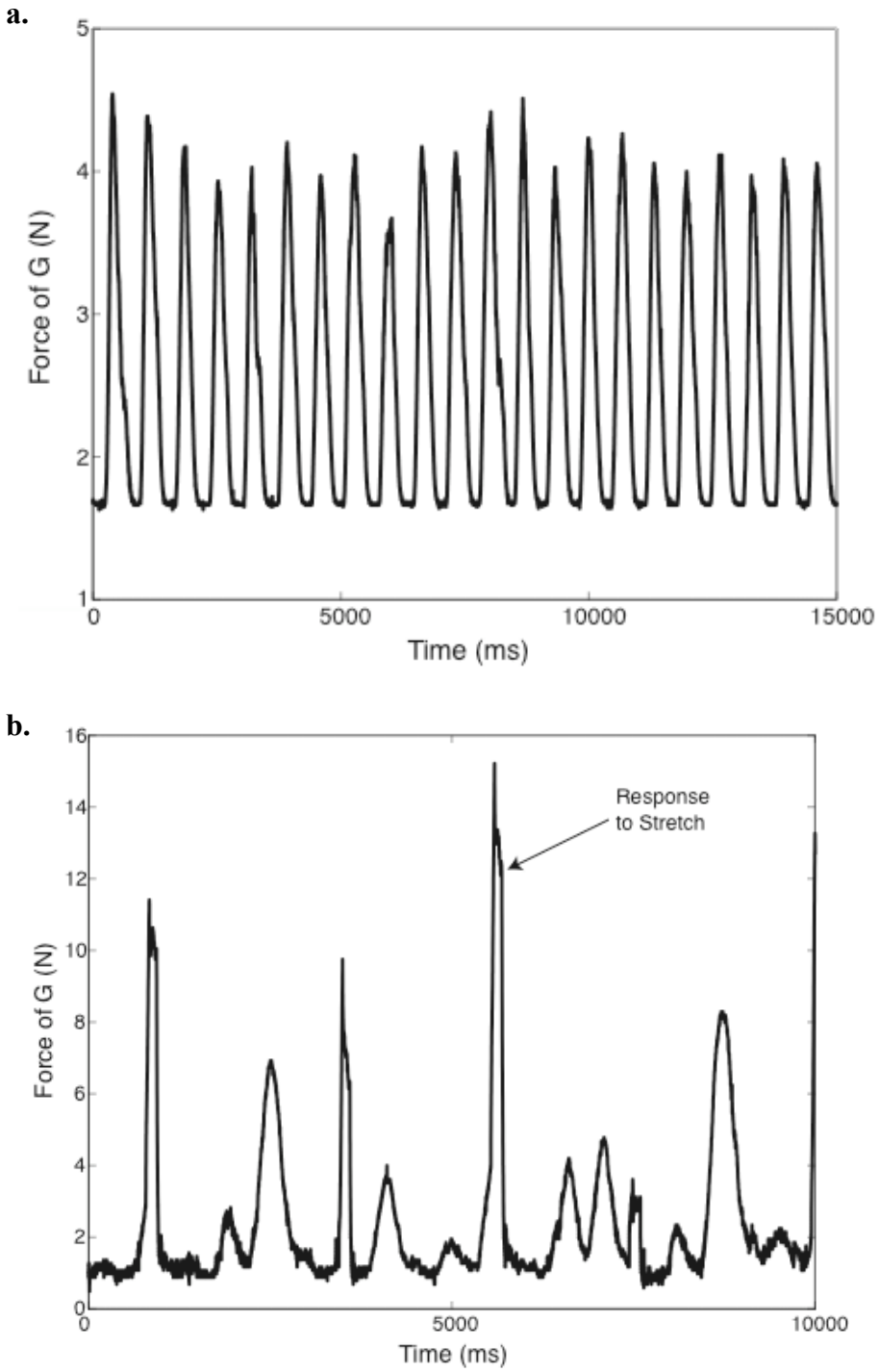


Figure 2.2. (a) Oscillations in the background force in G of the immobilized right hindlimb during stepping. (b) Ramp and hold stretches delivered on top of the oscillating background force in G. An arrow indicates the force response to a single stretch during stepping.

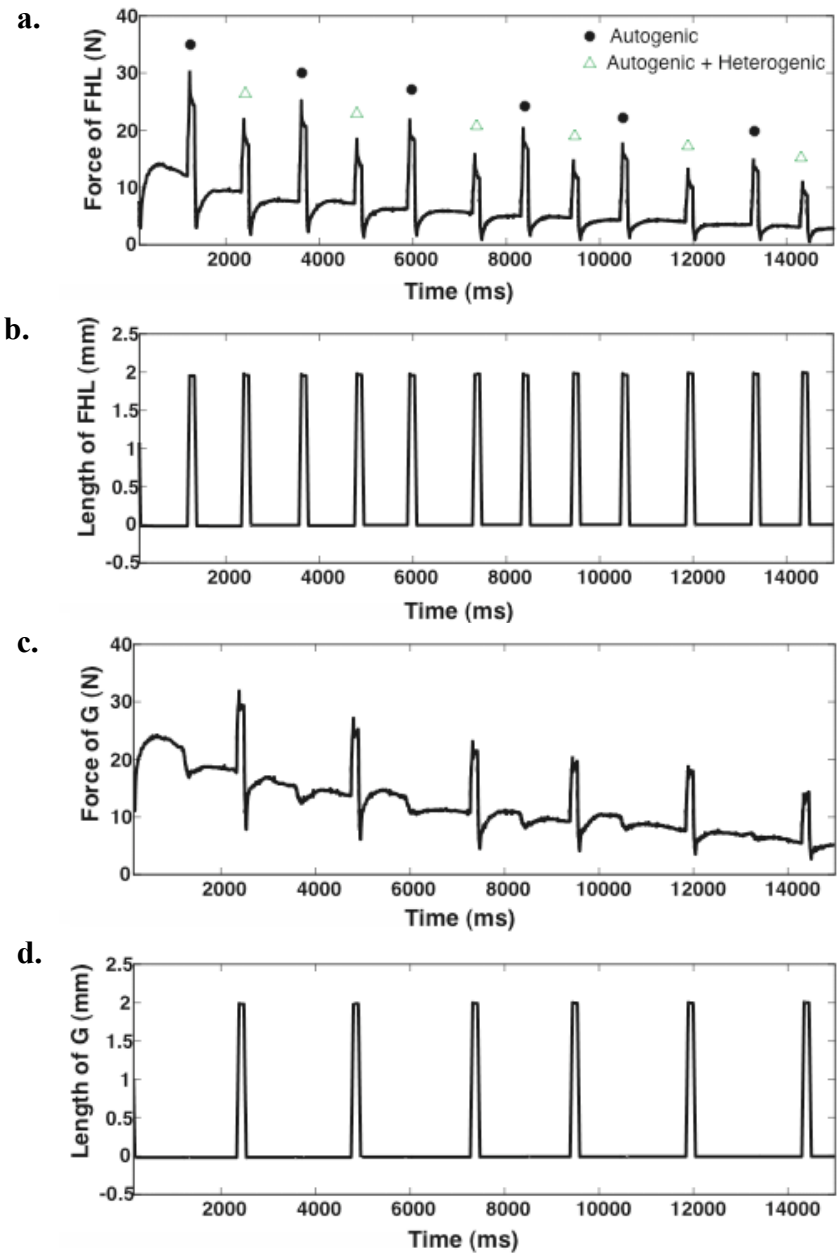


Figure 2.3 (a) Recipient muscle stretch-evoked force response during XER. Symbols above recipient stretches indicate responses obtained when the muscle is stretched alone (filled circles) and together with the donor muscle (open triangles). (b) Recipient muscle length input to two-state stretch. (c) Donor muscle stretch-evoked force response during XER. (d) Donor muscle length input for two-state stretch. A two-state stretch is performed to ascertain strength and sign of heterogenic feedback between a recipient and donor muscle. The stimulation of the tibial nerve in the left hindlimb at 2 T evokes an increase in the background force of the recipient and donor muscles, FHL and G respectively (a, c). As the background force declines, ramp and hold stretches (2 mm, 0.04 m/s stretch, 100 ms hold period), are delivered to the recipient and donor muscles (b, d).

the recipient muscle using this two-state stretch paradigm allowed us to measure the effects of feedback from donor to recipient. Briefly, filled circles represent the autogenic response, while the open triangles represent the autogenic response modified by the heterogenic input. This symbol convention remains consistent throughout this chapter for evaluating the strength and sign of heterogenic feedback. Finally, calculating the voltage outputs with no load and with a 1 kg load completed a two-point calibration of the strain gauges. A linear interpolation between the two points was completed in Matlab version 7.01 to calculate fits for each of the four myographs.

2.2.3 Data Analysis:

Force measurements were used to discern heterogenic feedback pathways when muscles were alternatively stretched. Measured forces of the recipient muscle consisted of intrinsic properties of muscles as well as contributions from reflex action (autogenic and heterogenic). Data acquired during the experiment was organized by state, whereby state one corresponded to data obtained when the recipient muscle was stretched alone (filled circles), while state two represented those from stretching the donor and recipient muscles together (open triangles). Comparing recipient muscle responses during state one with those during state two reveals the heterogenic contribution. Each file contained data for a particular muscle combination (i.e. G to FHL), where half of the stretches occurred in state one and half in state two. Force output and length input of FHL, G, PLAN, and QUADS, was recorded for each stretch.

Software in Matlab version 7.01 was then used to analyze the data. Briefly, the background force of the muscle was calculated as an average of the force during the interval 10 ms prior to the beginning of the stretch, during the isometric hold period. This brief period was used to account for any shift in the background force during locomotion. A baseline was then fit to the 10 ms prior to stretch and the 10 ms following the return to the initial position to account for a shifting baseline. Force responses were eliminated if the deviation in the force during either of these 10 ms periods prevented the calculation of an accurate baseline. The baseline was constructed by performing a linear interpolation from the mean force response just prior to the stretch to the mean force after the end of the release. The entire baseline was then subtracted from the overall force response. Figure 2.4a depicts a sample force trace, the baseline calculation described above, and the resulting baseline subtracted force data. Figure 2.4b depicts a single stretch administered during stepping; despite the oscillations in the background force, a baseline calculation can be performed.

To evaluate the strength and sign of heterogenic feedback during either locomotion or XER, individual force responses at specific time points were obtained from the baseline subtracted force data, and background force was obtained from the original force trace. Figure 2.5 depicts the typical analysis, whereby force responses for a specific timepoint are plotted as a function of background force. Each data point represents a response of the recipient muscle obtained when the muscle was either stretched alone (filled circles) or response of the recipient muscle when it was stretched with another muscle (open triangles), and individual responses represent an individual

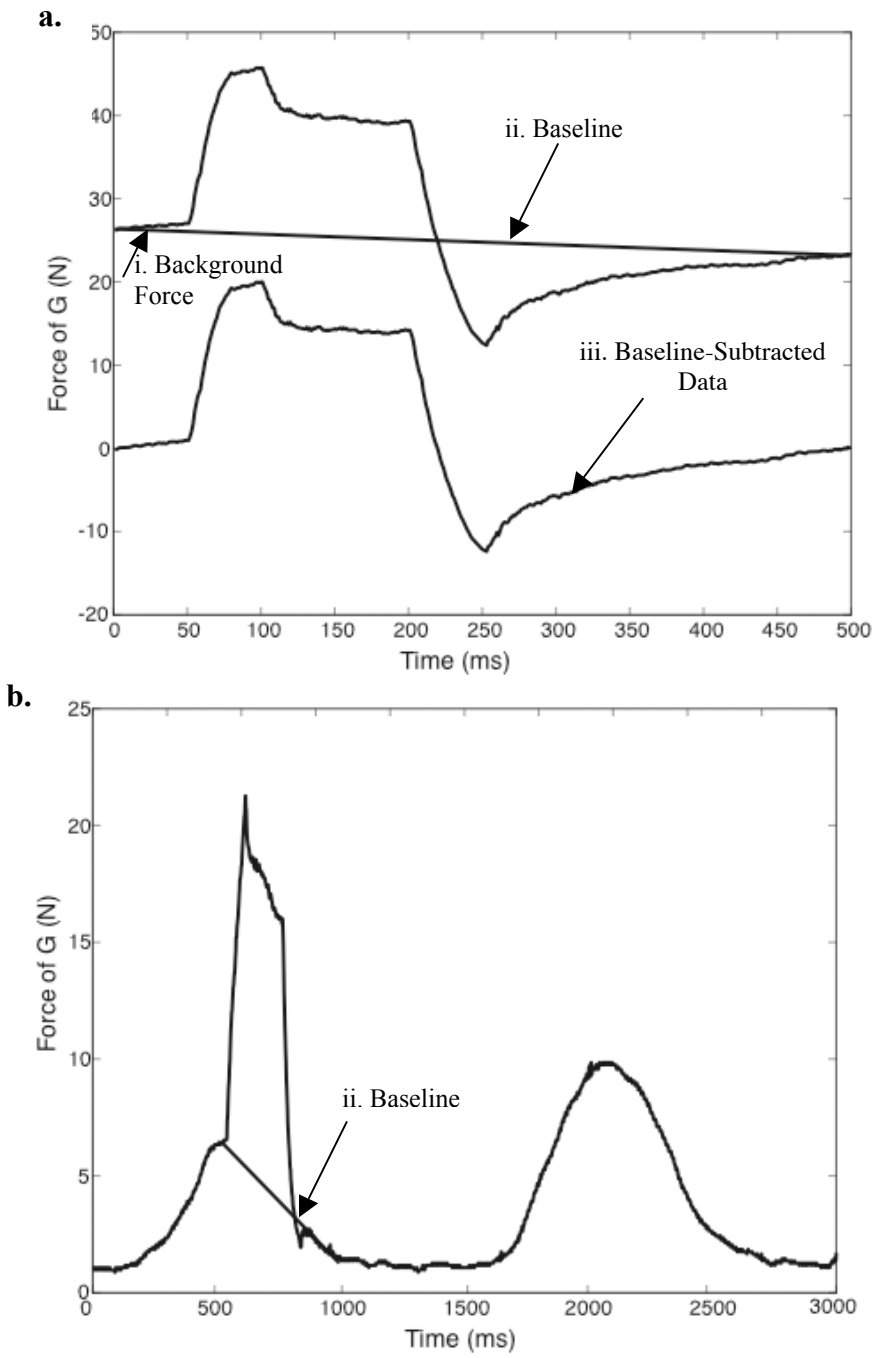


Figure 2.4 (a) Individual force response: background force (i.) of an individual stretch is calculated as the average of the force over 10 ms just prior to the ramp and hold stretch; baseline (ii.) is calculated for each stretch by performing a linear interpolation between the first 10 ms and last 10 ms of data; baseline is subtracted from each individual trace to yield the baseline-subtracted data (iii.). (b) Baseline calculated for an individual stretch despite a shifting background force during stepping.

stretch. Polynomial fits and 95% confidence intervals were fit to each population of data for a given time point.

Force responses were obtained at 3 separate time points to assess the strength and sign of heterogenic feedback at various phases of the ramp and hold stretch. Responses obtained 10 ms following the beginning of the stretch represent the mechanical phase, in that this timepoint occurs prior to any influence of reflex activity (Figure 2.5a*)*. Separation of the two populations of data in the mechanical phase suggests mechanical coupling between the two muscles. Force responses that occur 50 ms following the beginning of the stretch represent the dynamic phase of heterogenic feedback (Figure 2.5b). A similar analysis was done for the end of the hold period, corresponding to 100 ms following the beginning of the stretch, the static phase, as shown in Figure 2.5c. The filled circles represent responses obtained when the muscle was stretched alone, while open triangles represent responses obtained when the muscle was stretched with another muscle. To monitor the donor's neural input onto the recipient muscle, force responses were obtained in the donor muscle rather than the recipient muscle when both muscles were stretched (open triangles). The polynomial fits for force responses obtained at the dynamic and static phase timepoints were plotted as a function of donor muscle background force, as shown in Figure 2.5d. Additionally, differences for both the dynamic and static phases in the recipient muscle were calculated by subtracting the polynomial fits, as shown in Figure 2.5e.

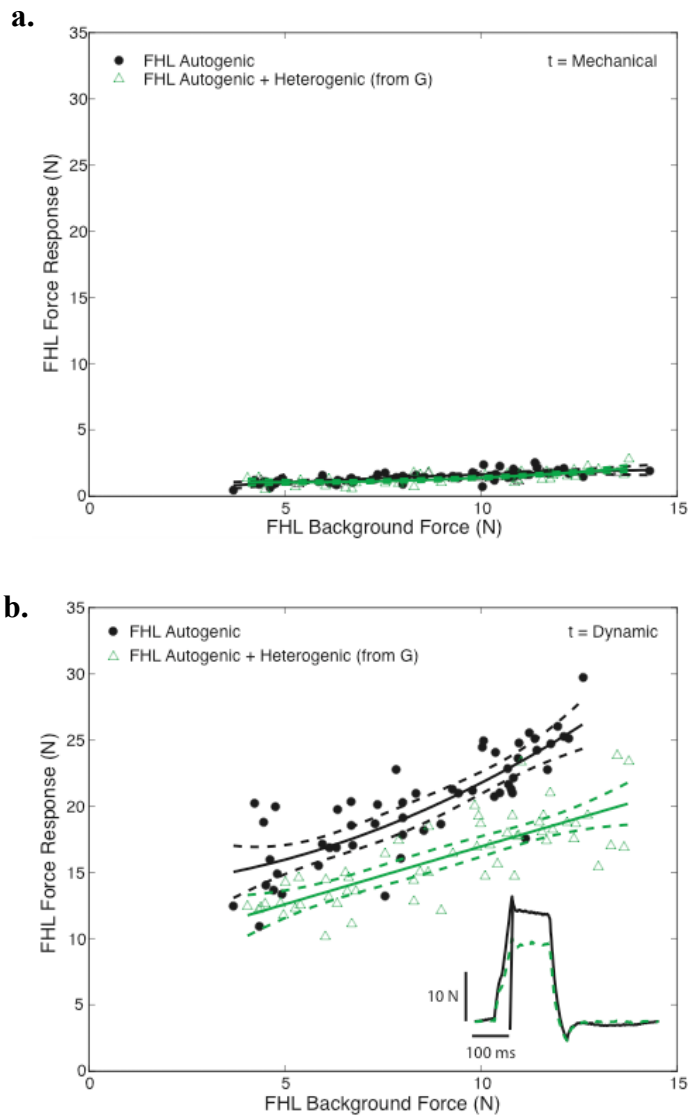
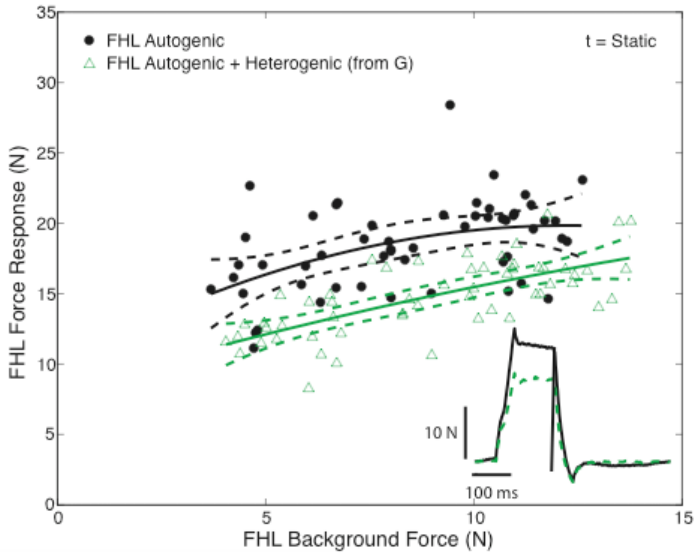


Figure 2.5 (a) Heterogenic inhibition from G onto FHL, where G is the donor muscle and FHL is the recipient muscle during locomotion for the mechanical phase, (b) dynamic phase, and (c) static phase. Filled circles and open triangles represent FHL force responses from stretches occurring in state one and state two, respectively. Polynomials and 95% confidence intervals are fit to each population of data, and statistical tests reveal that the populations for the dynamic and static phases are distinctly separated ($p<0.01$). Two traces matched at 10 N background force in FHL from state one (solid line) and state two (dashed line) have been superimposed to illustrate the magnitude of inhibition from G onto FHL during locomotion, and the vertical line indicates the sample time. Heterogenic inhibition from G onto FHL is more force-dependent during the dynamic phase than during the static phase. The magnitude of force responses in G during the static versus the dynamic phase (d) indicates the input signal for the recipient muscles, and differs slightly from the magnitude of inhibition from G onto FHL (e).

c.



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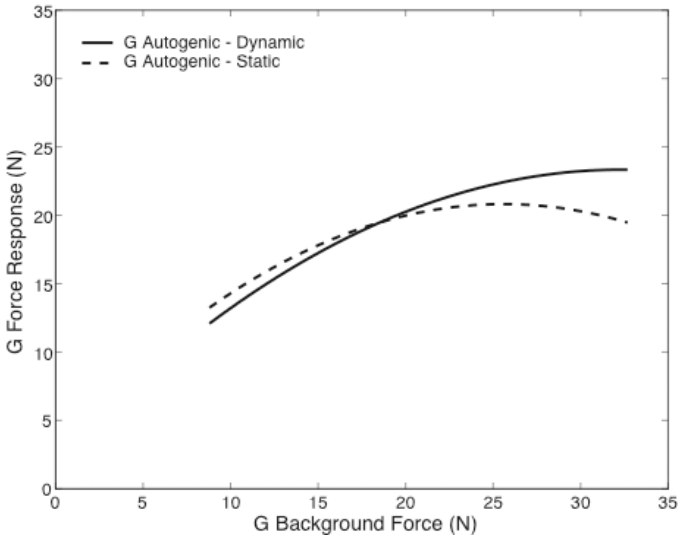


Figure 2.5 (continued)

e.

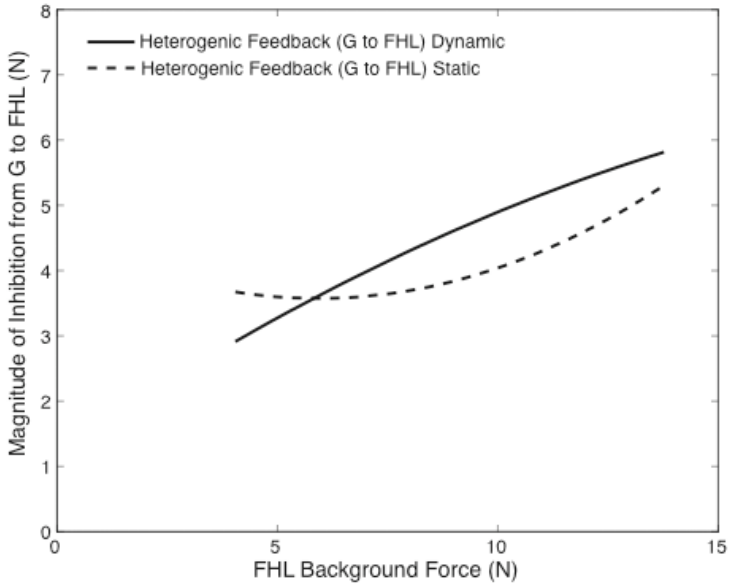


Figure 2.5 (continued)

Statistics were performed using Statistica 6.0 and Excel to test the separation of the data populations. Two regression models were fit to the data. In the full model, force response at the specified time point was the dependent variable (Y). The predictors in the full model included the grouping variable (X_2), representing state one or state two, background force (x_1), background force squared (x_1^2), the grouping and background force crossed term (x_1X_2), and the grouping variable multiplied by the squared background force term ($x_1^2X_2$). The reduced model lacked all terms containing the grouping variable, thus pooling the data into one population. The following equation (Kutner et al. 1996) represents the full model:

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x^2 + \beta_3 X_2 + \beta_4 x_1 X_2 + \beta_5 x_1^2 X_2$$

The reduced model eliminates all grouping variables, and thus reduces to the following equation:

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x^2$$

The sum of squared errors (SSE) and degrees of freedom (DF) were obtained from both the full and reduced models, as well as the mean squared errors (MSE) from the full model. An F statistic was calculated using the following equation:

$$F = ((SSE(Reduced) - SSE(Full))/(DF(Full)-DF(Reduced))/MSE(Full)$$

This F test was performed to test the following null hypothesis:

$H_o = \beta_3 = \beta_4 = \beta_5 = 0$

While the 95% confidence intervals represent the validity of the polynomial fit to the data points, a p-value < 0.01 from the statistical test stated above rejects the null hypothesis, and statistically proves that the two populations are distinctly different.

While Figure 2.5 represents data from specific time points, the magnitude of the inhibition was explored for the entire time-course of the ramp and hold profile. To achieve this, polynomial fits of the two populations of data were calculated for every 5 ms of the entire response. For each 5 ms time-point, the polynomial representing the data obtained when the muscle was stretched alone was subtracted from the polynomial representing the data obtained when two muscles were stretched together. A surface plot was then created from each of the difference calculations. A common range in background force of the muscle for both populations was also computed and represented on the z-axis. Figure 2.6 depicts the time-course of the heterogenic inhibition exhibited in Figure 2.5.

2.3 Results

The purpose of these studies was to determine the distribution of the feedback among ankle extensor muscles in the hindlimb of the cat. Heterogenic feedback pathways among G, including MG and LG, PLAN, FHL, and QUADS are predominately force-dependent and inhibitory under conditions of quiet stance (Bonasera and Nichols 1994; Nichols 1999; Wilmink and Nichols 2003). This paper details results from twenty-eight

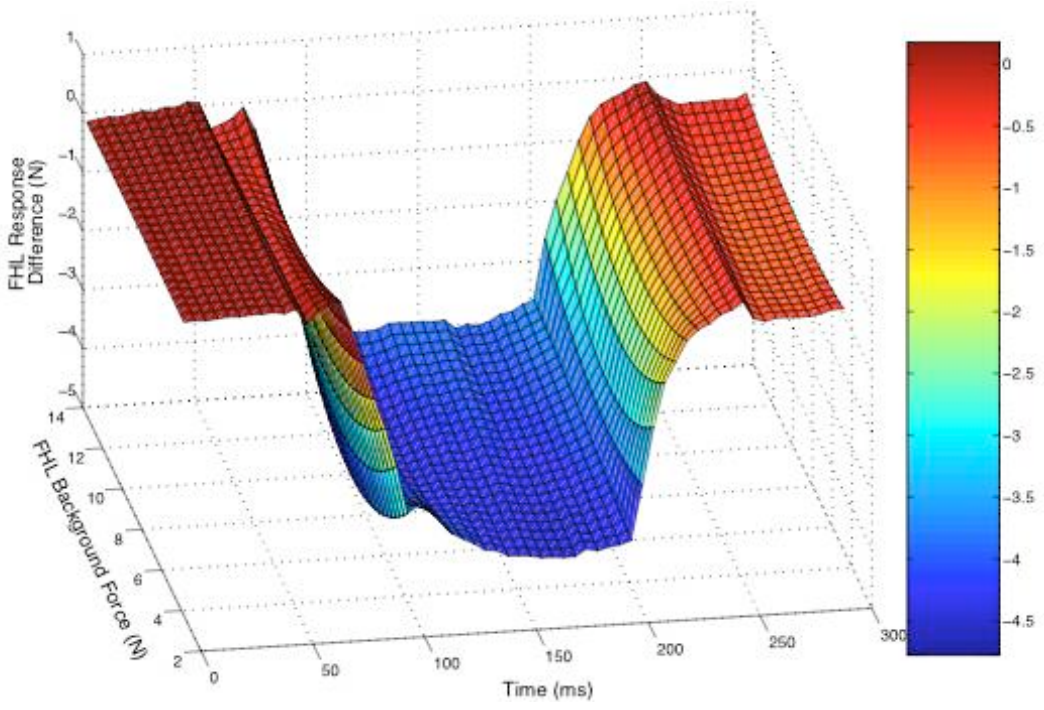


Figure 2.6 A three-dimensional surface that quantifies the magnitude of heterogenic inhibition from G onto FHL during locomotion as a function of force and time. To quantify the magnitude of heterogenic inhibition from G onto FHL during locomotion, response differences are calculated for every 5 ms over the ramp and hold stretch by subtracting the polynomial fits for state two from the polynomial fits from state one. A three-dimensional surface is created from the series of response difference calculations. The heterogenic inhibition from G onto FHL during locomotion remains relatively constant over time and FHL background force.

experiments, and examines the feedback pathways among G, MG, LG, PLAN, FHL, and QUADS. There was a range in the characteristics of locomotion among the twenty-eight preparations. Three (11%) animals did not exhibit any stepping despite skin stimulation, 7 (25%) and 11 (39%) animals produced stepping with the aid of skin stimulation with and without oscillations in the background force of the muscles in the immobilized limb respectively, and 7 (25%) animals exhibited stepping and oscillations without stimulation. The main observation from these studies was that heterogenic inhibition was maintained between G, PLAN, FHL, and QUADS with the initiation of locomotion. Specifically, the heterogenic inhibition from G onto either FHL or PLAN was the most robust and consistent result. Bidirectional force feedback between FHL and PLAN is weak under conditions of XER (Nichols 1994). While inhibition from FHL onto PLAN was found during locomotion, little to no inhibition was found from PLAN onto FHL during either locomotion or XER in the premammillary decerebrate preparation. During locomotion, the feedback from QUADS onto G was stronger than that from G onto QUADS. This is in contrast to the symmetric responses found between these muscles in a non-locomoting preparation (Wilmink and Nichols 2003)

2.3.1 G contributes inhibition to FHL during locomotion

The heterogenic feedback from G onto FHL was examined in seventeen total experiments, sixteen of which exhibited stepping behavior. Of these experiments evaluating the interaction between G and FHL during locomotion, twelve demonstrated inhibition from G onto FHL, as previously shown in the intercollicular decerebrate cat (Bonasera 1994). The remaining 4 preparations did not exhibit heterogenic inhibition in

either the locomotion or XER states. Figure 2.5 depicts a representative example of the heterogenic inhibition from G onto FHL during stepping. Heterogenic inhibition is not due to a purely mechanical event (Figure 2.5a*)* and increases with increasing background force for the dynamic response, as indicated by the divergence in the polynomial fits in Figure 2.5b. Of the twelve experiments exhibiting heterogenic inhibition from G onto FHL, 50% (6) exhibited this force-dependent trend in the inhibition for the dynamic response, 25% (3) exhibited inhibition that remained constant with increasing background force, 17% (2) displayed greater inhibition at lower and higher background forces (parabolic), and 8% (1) decreased inhibition with increasing background force. Force-dependency decreases and variability increases for the static response (Figure 2.5c). For the static response, 25% (3) demonstrated an increasing inhibition from G onto FHL, 50% (6) remained constant with increasing background force, and 25% (3) exhibited greater inhibition at lower and higher background forces (parabolic).

Additionally, the magnitude of the net reflex, as indicated by the separation between force responses obtained in state one and state two is greater in the dynamic phase than in the static phase (Figure 2.5). While there is some variability and scatter among the data points, the relatively tight 95% confidence intervals in both conditions indicate that the polynomial fits effectively represent the data. Our criterion for significant inhibition is that the p-value<0.01. In most, but not all cases, this also corresponds to non-overlapping confidence intervals. In this example, the confidence intervals are clearly separate for the two populations of data, confirming that there is

heterogenic inhibition from G onto FHL. Additionally, multiple regression yielded $p<0.01$, thus statistically proving that these populations are distinctly different.

Traces inset in Figure 2.5b and Figure 2.5c are force responses for state one (solid line) and state two (dashed line). The background force for both conditions was matched at the mean background force of approximately 10 N. Baselines were subtracted from both traces to better illustrate the magnitude and time course of the inhibition from G onto FHL. As shown by these traces, the magnitude of inhibition remains relatively constant during the hold period.

To better understand why the inhibition might increase with force, we reasoned that if the inhibition is force related, it should increase if the force response of the donor muscle increases. Therefore, for the interaction from G onto FHL, we obtained responses in the donor muscle, G during the dynamic phase. Figure 2.5d represents the donor responses, and therefore the input signal from G onto FHL during locomotion for both the static and dynamic time points. The polynomial fits for the static and dynamic time points were subtracted (Figure 2.5e) to visualize the response difference for comparison with the donor plot. Note that both the donor responses (Figure 2.5d) and the heterogenic responses from G onto FHL (Figure 2.5e) increase with increasing background force, thus suggesting a force-dependent trend in the data.

Figures 2.5b and 2.5c show heterogenic inhibition from G to FHL data during locomotion for one time-point, dynamic and static respectively. To examine the trend of

the feedback throughout the time course of the ramp and hold stretch, a three-dimensional plot was created as previously described. Figure 2.6 represents the time-course of heterogenic feedback from G onto FHL during locomotion. The surface represents the difference between the two populations of data as a function of time and FHL background force. The maximum response difference is approximately 5 N, and is sustained throughout the 100 ms hold period. There is some slight force-dependency at the earlier time points that is reduced at the end of the hold period. While heterogenic inhibition from G onto FHL during locomotion was robust, the opposing interaction was explored. Weak, yet statistically significant, inhibition from FHL onto G occurred in 40% (6) of the fifteen experiments devoted to exploring the interaction (not illustrated).

2.3.2 G contributes inhibition to PLAN during locomotion

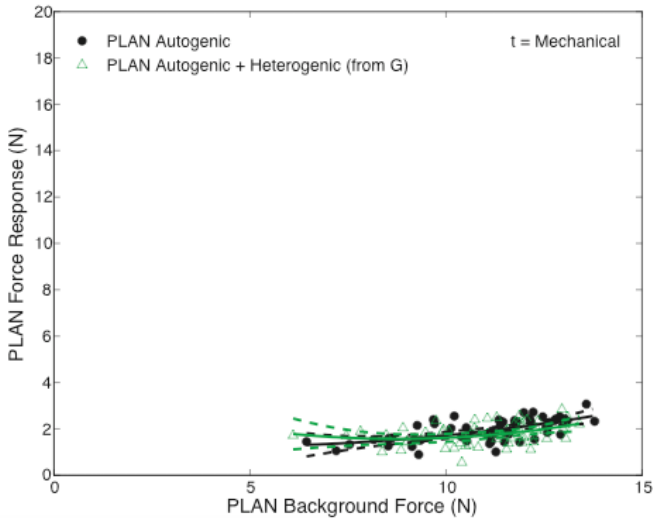
Feedback from G onto PLAN was examined in twelve experiments, eleven of which exhibited stepping. Of these experiments, 9 exhibited heterogenic inhibition from G onto PLAN. The remaining preparations did not exhibit heterogenic inhibition in either the locomotor or XER. Figure 2.7a demonstrates that there is no statistical difference between the two populations of data during the mechanical phase, thus ruling out the possibility of mechanical coupling between these two muscles. Figure 2.7b depicts responses acquired 50 ms following the beginning of the ramp, representing the dynamic phase of the feedback from G onto PLAN during locomotion. While there is slight overlap in the confidence intervals at the lowest background force, approximately 6 N, the two populations are clearly separated at higher background forces. Multiple regression yielded $p<0.01$, thus statistically proving that these populations are distinctly

different. Of the 9 experiments exhibiting heterogenic inhibition from G onto PLAN, 56% (5) exhibited this force-dependent trend in the inhibition for the dynamic response, 22% (2) displayed greater inhibition at lower and higher background forces (parabolic), 11% (1) exhibited inhibition that remained constant with increasing background force, and 11% (1) decreased inhibition with increasing background force.

Force responses in the recipient muscle, PLAN, for state one (solid line) and state two (dashed line) are inset in Figure 2.7b and Figure 2.7c to illustrate the magnitude and timing of heterogenic inhibition from G onto PLAN. Each trace was chosen at the mean PLAN background force of 10 N, and the baselines were subtracted to better visualize the inhibition. The inhibition from G onto PLAN remained constant over the 100 ms hold period. Additionally, during locomotion, the magnitude of inhibition from G onto PLAN (Figure 2.7b) was less than the inhibition from G onto FHL (Figure 2.5b). Figure 2.7b depicts force responses in the static phase of feedback and illustrates the increase in variability when compared to the dynamic phase (Figure 2.7c). For the static response, 33% (3) exhibited the force-dependent trend in inhibition from G onto PLAN, 33% (3) remained constant with increasing background force, 22% (2) exhibited a parabolic distribution, and 11% (1) decreased with increasing force.

To monitor the signal coming from G, the donor muscle, onto the recipient muscle, PLAN, force responses from G were plotted for both the static and dynamic time points, as shown in Figure 2.7d. The polynomial fits for responses obtained in state two were subtracted from the polynomial fits for responses obtained in state one for both the

a.



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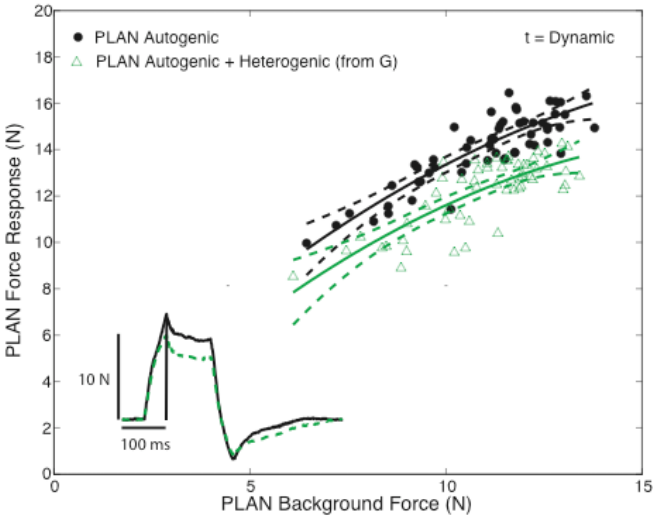
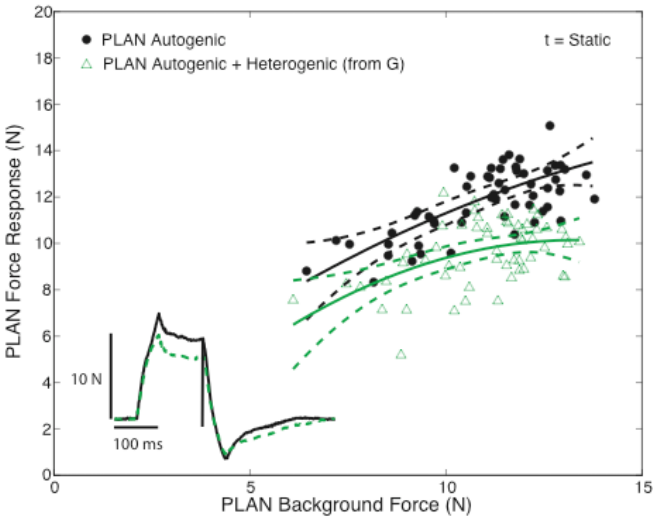


Figure 2.7 (a) Heterogenic inhibition from G onto PLAN, where G is the donor muscle and PLAN is the recipient muscle during locomotion for the mechanical phase. (b) Heterogenic inhibition from G onto PLAN during locomotion for the dynamic phase. (c) Heterogenic inhibition from G onto PLAN during locomotion for the static phase. The same conventions as Figure 2.5 apply. Two traces matched at 10 N background force in PLAN from state one (solid line) and state two (dashed line) have been superimposed to illustrate the magnitude of inhibition from G onto PLAN during locomotion, and the vertical line indicates the sample time. Heterogenic inhibition from G onto PLAN during locomotion remains independent of force during the dynamic phase, yet increases with increasing force during the static phase. Variability also increases with increasing time. The magnitude of force responses in G for the static versus the dynamic phase (d) indicates the input signal for the recipient muscle and differs slightly from the magnitude of inhibition from G onto PLAN (e).

c.



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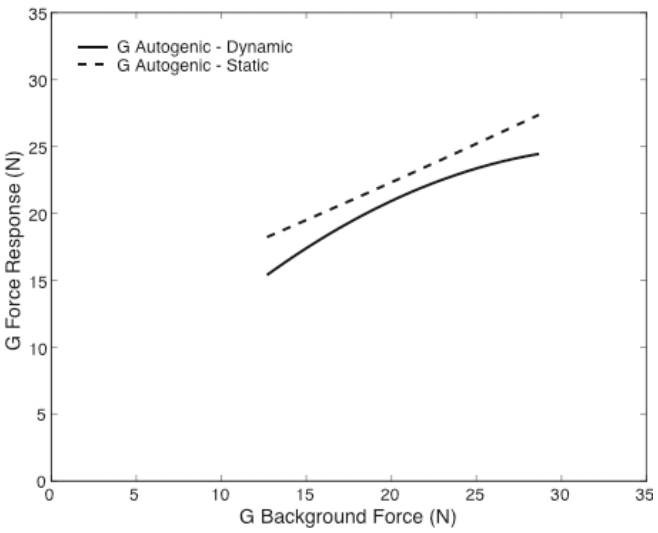


Figure 2.7 (continued)

e.

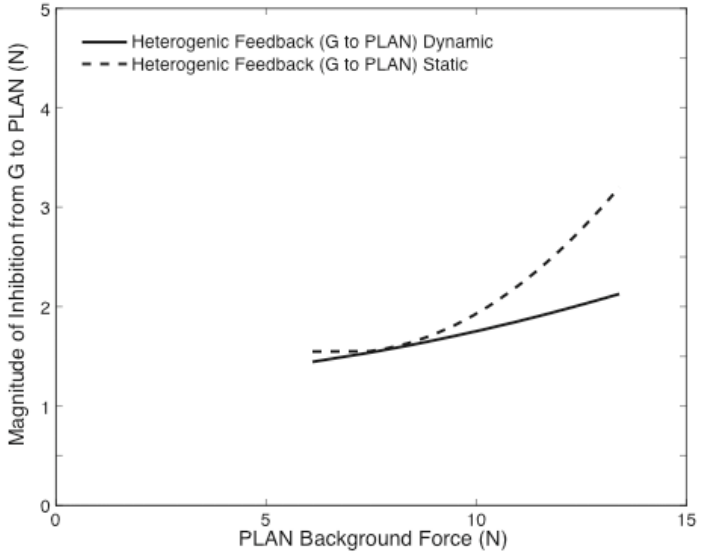


Figure 2.7 (continued)

static and dynamic time points (Figure 2.7e) to visualize the response difference for comparison with the donor plot. The slope of the polynomial fit to the donor responses in Figure 2.7d at the dynamic time point is similar to the increase in the magnitude of separation in the populations of data shown in Figure 2.7e.

To assess the magnitude and time-course of the heterogenic inhibition from G onto PLAN during locomotion, a three-dimensional difference plot was created as previously described, as shown in Figure 2.8. The inhibition reaches a peak of 2 N and increases in force-dependency at the end of the hold period. The feedback from PLAN onto G was also examined during locomotion, yet yielded less robust results than the opposing interaction. Weak, yet statistically significant inhibition from PLAN onto G occurred in 23% (3) of the thirteen experiments devoted to exploring the interaction.

2.3.3 Heterogenic inhibition emanates from MG not LG during locomotion

We evaluated the strength and sign of heterogenic feedback from the two heads of G, namely MG and LG, to determine the relative contribution of heterogenic inhibition onto FHL and PLAN. Feedback from MG onto FHL was examined in 8 experiments, 6 of which exhibited stepping. Of these experiments, 4 exhibited heterogenic inhibition from MG onto FHL. The remaining preparations did not exhibit heterogenic inhibition in either the locomotor or XER. Figure 2.9a depicts responses acquired 50 ms following the beginning of the ramp, representing the dynamic phase of the feedback from MG onto FHL during locomotion. While there is slight overlap in the confidence intervals over the

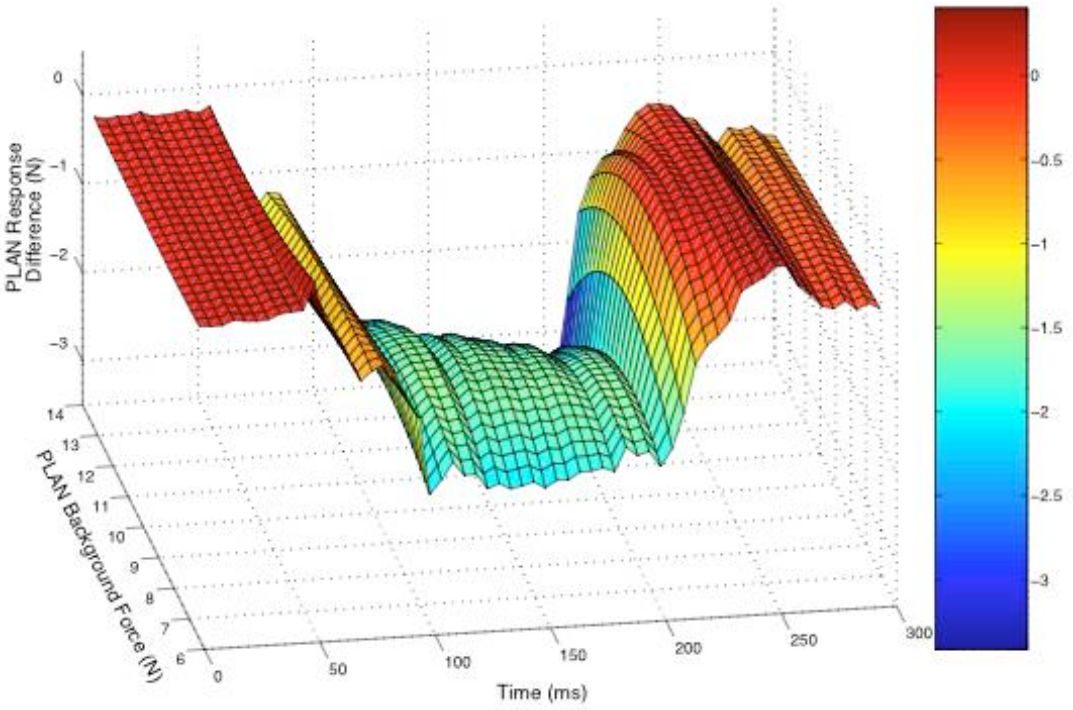


Figure 2.8 A three-dimensional surface that quantifies the magnitude of heterogenic inhibition from G onto PLAN during locomotion as a function of force and time. The magnitude of heterogenic inhibition from G onto PLAN during locomotion for the entire time-course of the ramp and hold stretch was calculated in the same manner as Figure 2.6. The heterogenic inhibition from G onto PLAN during locomotion increases slightly over time, and remains slightly dependent on PLAN background force at longer latencies.

range of background force, multiple regression yielded $p<0.01$, thus statistically proving that these populations are distinctly different.

Force responses in the recipient muscle, FHL for state one (solid line) and state two (dashed line) are inset in Figure 2.9a and Figure 2.9b to illustrate the magnitude and timing of heterogenic inhibition from MG onto FHL. Each trace was chosen at the mean FHL background force of 6 N, and the baselines were subtracted to better visualize the inhibition. The inhibition from MG onto FHL was greatest in the dynamic phase and decreased slightly in the static phase. To assess the magnitude and time-course of the heterogenic inhibition from MG onto FHL during locomotion, a three-dimensional difference plot was created as previously described, as shown in Figure 2.10. The inhibition reaches a peak of approximately 5 N and remains constant throughout the hold period.

A similar trend was demonstrated for feedback from MG onto PLAN. Of the 7 stepping preparations evaluating this interaction, 5 exhibited heterogenic inhibition from MG onto PLAN. Figure 2.11a depicts responses acquired 50 ms following the beginning of the ramp, representing the dynamic phase of the feedback from MG onto PLAN during locomotion. While there is slight overlap in the confidence intervals over the range of background force, multiple regression yielded $p<0.01$, thus statistically proving that these populations are distinctly different.

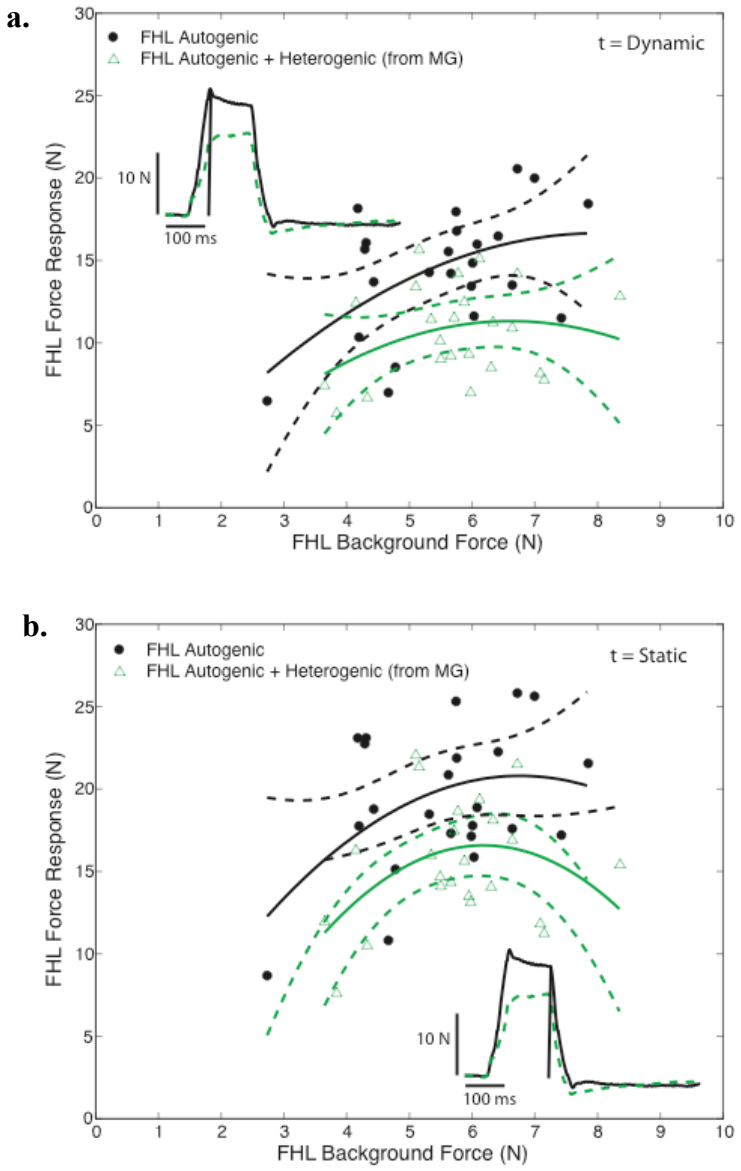


Figure 2.9 (a) Heterogenic inhibition from MG onto FHL, where MG is the donor muscle and FHL is the recipient muscle during locomotion for the dynamic phase. (b) Heterogenic inhibition from MG onto FHL during locomotion for the static phase. The same conventions as Figure 2.5 apply. Two traces matched at 6 N background force in FHL from state one (solid line) and state two (dashed line) have been superimposed to illustrate the magnitude of inhibition from MG onto FHL during locomotion, and the vertical line indicates the sample time. Heterogenic inhibition from MG onto FHL during locomotion increases with increasing force during the dynamic phase, yet remains independent of force during the static phase. Variability also increases with increasing time.

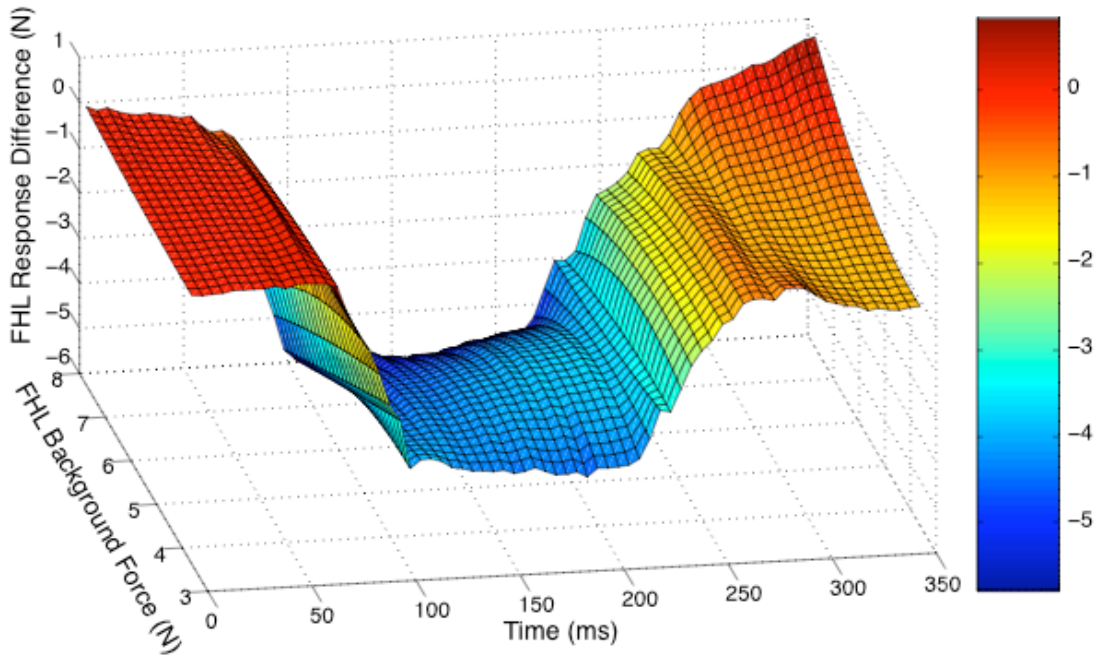


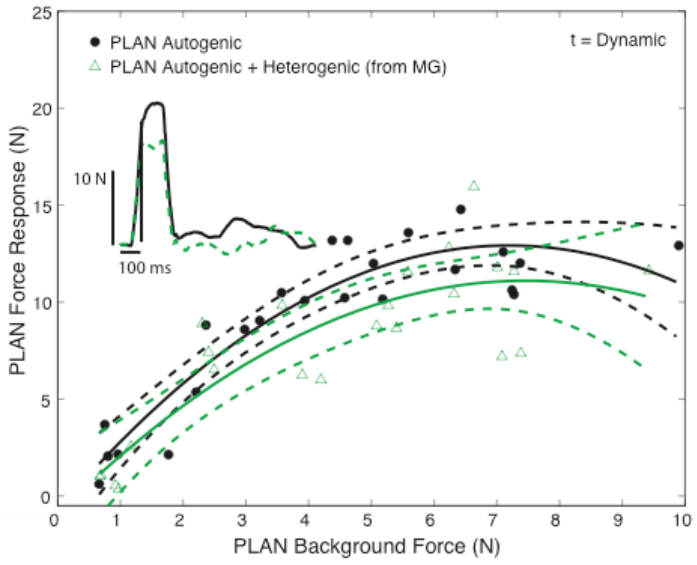
Figure 2.10 A three-dimensional surface that quantifies the magnitude of heterogenic inhibition from MG onto FHL during locomotion as a function of force and time. The magnitude of heterogenic inhibition from MG onto FHL during locomotion for the entire time-course of the ramp and hold stretch was calculated in the same manner as Figure 2.6. The heterogenic inhibition from MG onto FHL during locomotion remains constant over time, and is slightly dependent on FHL background force.

Force responses in the recipient muscle, PLAN for state one (solid line) and state two (dashed line) are inset in Figure 2.11a and Figure 2.11b to illustrate the magnitude and timing of heterogenic inhibition from MG onto PLAN. Each trace was chosen at the mean PLAN background force of 5 N, and the baselines were subtracted to better visualize the inhibition. The inhibition from MG onto PLAN was greatest in the dynamic phase and decreased slightly in the static phase. To assess the magnitude and time-course of the heterogenic inhibition from MG onto PLAN during locomotion, a three-dimensional difference plot was created as previously described, as shown in Figure 2.12. The inhibition reaches a peak of approximately 4 N and exhibits slight force dependency throughout the hold period. Feedback from LG onto FHL and LG onto PLAN was examined in 4 and 3 stepping preparations respectively. Of these experiments, 2 exhibited statistical excitation for LG onto PLAN during locomotion; this is most likely due to the weak, length-dependent excitatory pathway exchanged between the two muscles. There was no evidence for force-dependent inhibition from LG onto either FHL or PLAN.

2.3.4 Weak inhibition between QUADS and G exists during locomotion

Due to the distribution of force feedback pathways that cross joints and influence interjoint coordination, the interactions between QUADS and G during locomotion were explored in 5 experiments, all of which exhibited stepping. Figure 2.13a illustrates the heterogenic inhibition that exists during locomotion from QUADS onto G. Force traces from state one (solid line) and state two (dashed line) are matched at a G background force of 6 N. While there is some overlap in the confidence intervals of the two polynomial fits, these two populations are statistically distinct ($p<0.01$). The magnitude

a.



b.

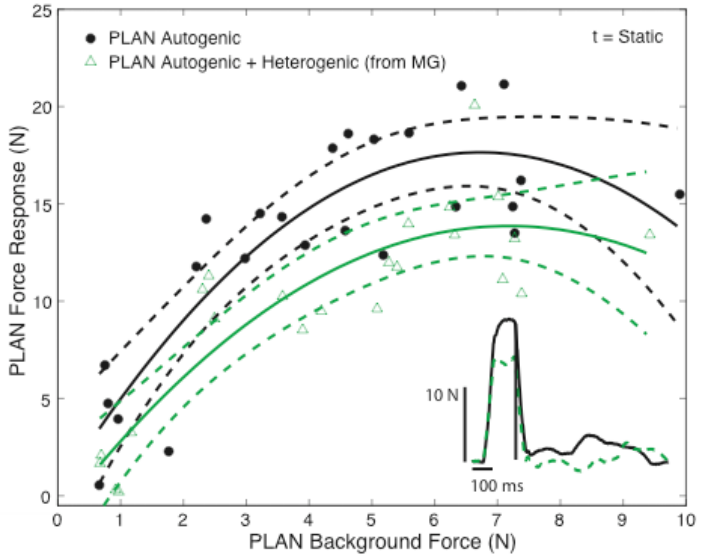


Figure 2.11 (a) Heterogenic inhibition from MG onto PLAN, where MG is the donor muscle and PLAN is the recipient muscle during locomotion for the dynamic phase. (b) Heterogenic inhibition from MG onto PLAN during locomotion for the static phase. The same conventions as Figure 2.5 apply. Two traces matched at 5 N background force in PLAN from state one (solid line) and state two (dashed line) have been superimposed to illustrate the magnitude of inhibition from MG onto PLAN during locomotion, and the vertical line indicates the sample time. Heterogenic inhibition from MG onto PLAN during locomotion increases with increasing force during the dynamic phase, yet remains independent of force during the static phase. Variability also increases with increasing time.

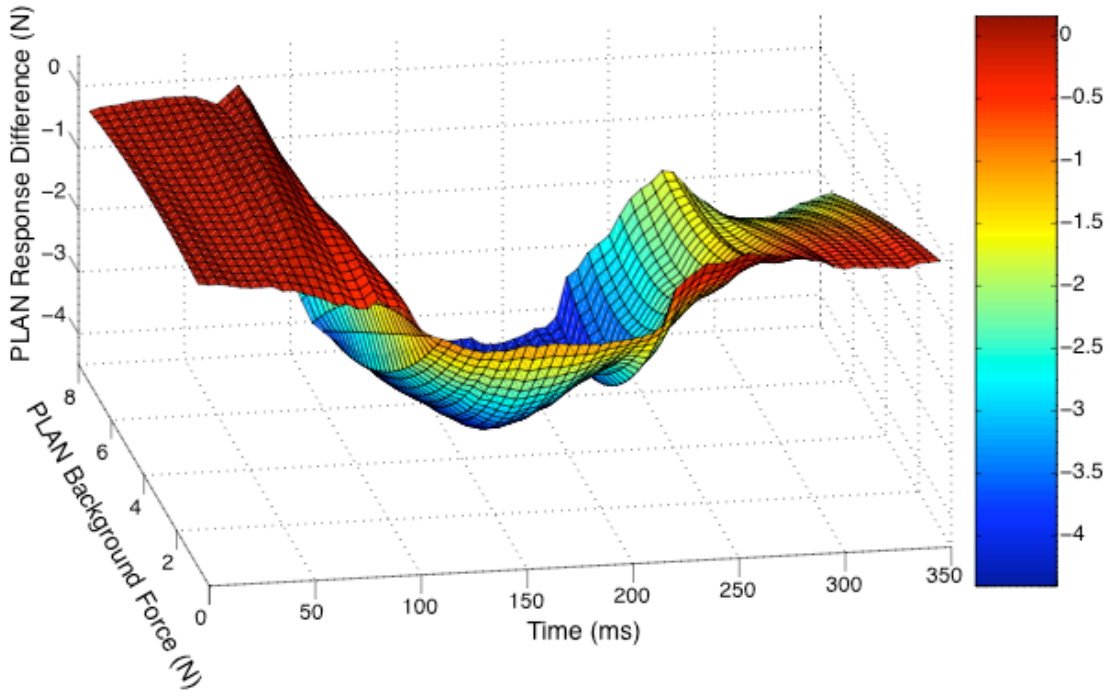


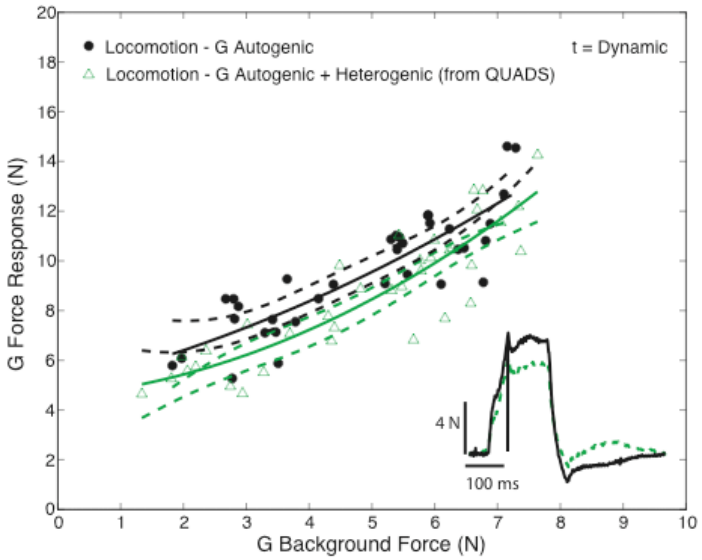
Figure 2.12 A three-dimensional surface that quantifies the magnitude of heterogenic inhibition from MG onto PLAN during locomotion as a function of force and time. The magnitude of heterogenic inhibition from MG onto PLAN during locomotion for the entire time-course of the ramp and hold stretch was calculated in the same manner as Figure 2.6. The heterogenic inhibition from MG onto PLAN during locomotion is slightly dependent on PLAN background force.

of heterogenic inhibition from QUADS onto G was greater during XER than during locomotion, as indicated in Figure 2.13b. Force traces from state one (solid line) and state two (dashed line) are matched at a G background force of 3.5 N. Statistical tests reveal that these two populations are statistically separate ($p<0.01$). The opposing interaction of the feedback from G onto QUADS was also inhibitory during locomotion. However, the magnitude of inhibition from G onto QUADS was significantly smaller, yet still statistically significant ($p<0.01$), than that from QUADS onto G, and it diminished at longer latencies (not shown). Statistically significant heterogenic inhibition from G onto QUADS and QUADS onto G during locomotion was found in 60% (3) of the experiments.

2.3.5 Weak Inhibition is exchanged between FHL and PLAN

Feedback from FHL onto PLAN was examined in twelve experiments, eleven of which produced stepping. Of these experiments, 4 exhibited heterogenic inhibition from FHL onto PLAN. The remaining preparations did not exhibit heterogenic inhibition in either the locomotion or XER state. Figure 2.14a depicts responses acquired at the 200 ms time point, representing the static phase of the feedback from FHL onto PLAN during locomotion. While there is slight overlap in the confidence intervals at the lowest background force, a p-value<0.01 indicates that these populations are distinctly different. While this example presented depicts force-dependence and significant inhibition, this was not a consistent result across locomoting preparations. Figure 2.14b depicts the inhibition from FHL onto PLAN during the XER, in the same preparation. It is evident that the magnitude of inhibition at the static timepoint is similar in both behavioral

a.



b.

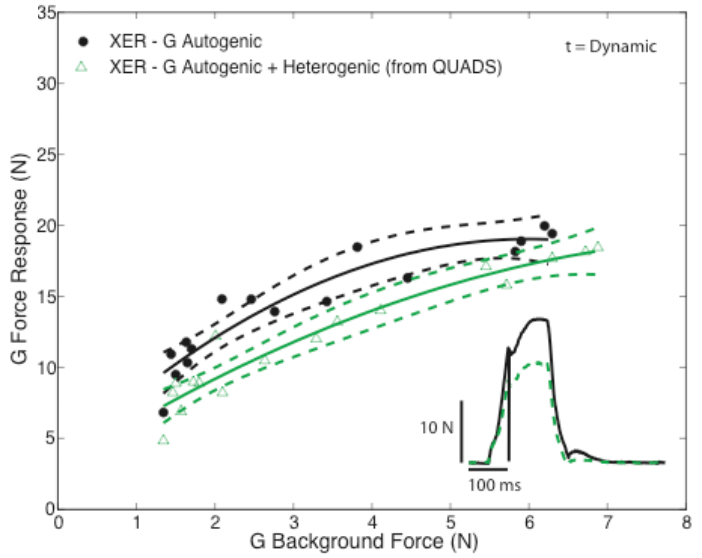


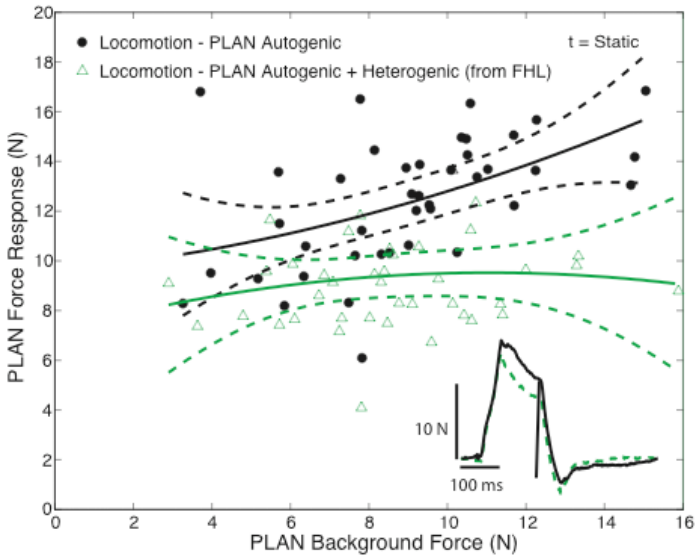
Figure 2.13 (a) Heterogenic inhibition from QUADS onto G, where QUADS is the donor muscle and G is the recipient muscle during locomotion for the dynamic phase. (b) Heterogenic inhibition from QUADS onto G during XER for the dynamic phase. The same conventions as Figure 2.5 apply. Two traces matched at 6 N (a) and 3.5 N (b) background force from state one (solid line) and state two (dashed line) have been superimposed to illustrate the magnitude of inhibition from QUADS onto G during locomotion and XER respectively, and the vertical line indicates the sample time. Heterogenic inhibition from QUADS onto G is greater during XER than during locomotion, and remains independent of force during the dynamic phase in both behavioral conditions.

conditions. Force responses in the recipient muscle, PLAN, for state one (solid line) and state two (dashed line) are inset in Figure 2.14a and Figure 2.14b to illustrate the magnitude and timing of heterogenic inhibition from FHL onto PLAN. Each trace was chosen at the mean PLAN background force of 7 N, and the baselines were subtracted to better visualize the inhibition. The separation of these data is statistically significant ($p<0.01$). Feedback from PLAN onto FHL was examined in thirteen experiments, twelve of which exhibited stepping. Of these experiments, only 1 experiment exhibited weak inhibition from PLAN onto FHL during locomotion.

2.3.6 Heterogenic inhibition is similar during locomotion and XER

Previous studies have mapped the organization of heterogenic feedback in the intercollicular decerebrate cat under conditions of XER (Bonasera and Nichols 1994; Nichols 1999). Figures 2.14 and 2.15 both compare force responses during locomotion and XER. Figure 2.15 compares the heterogenic inhibition exhibited from G onto FHL during locomotion (Figure 2.15a) with that during XER (Figure2. 15b) in the same premammillary decerebrate animal. While FHL achieved higher force responses during XER than during locomotion for comparable background forces in FHL, the relative separation between the polynomial fits remains similar in both conditions. Inset traces are responses for state one (solid line) and state two (dashed line) matched at an FHL background force of 5 N. Under both locomotion and XER, the separation of the populations of data is statistically significant ($p<0.01$). These traces further support the similar magnitude in heterogenic inhibition from G onto FHL during both locomotion and XER.

a.



b.

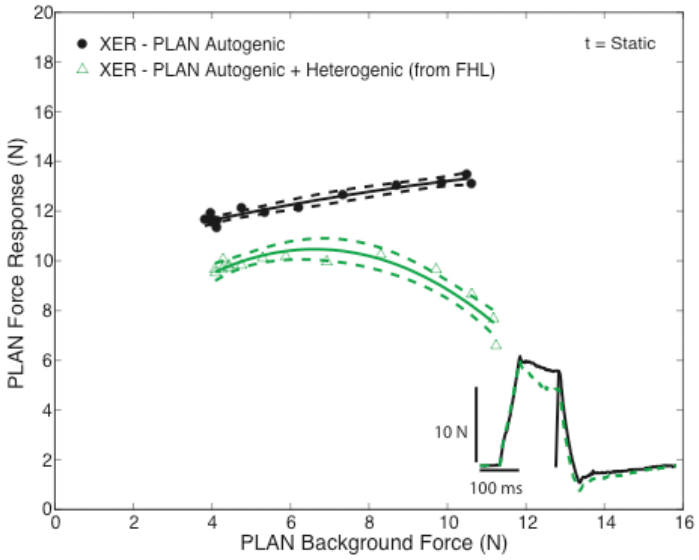
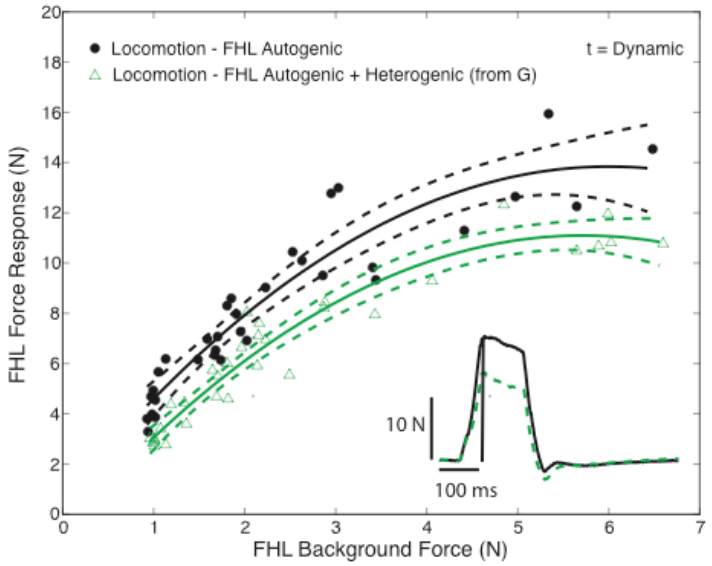


Figure 2.14 (a) Heterogenic inhibition from FHL onto PLAN, where FHL is the donor muscle and PLAN is the recipient muscle during locomotion for the static phase. (b) Heterogenic inhibition from FHL onto PLAN during XER for the static phase. Heterogenic inhibition from FHL onto PLAN is similar in strength and sign during locomotion (a) and XER (b). The same conventions as Figure 2.5 apply. Matched traces at a background force of 7 N for state one (solid line) and state two (dashed line) have been superimposed and inset to demonstrate the trend of heterogenic inhibition in both behavioral states, and the vertical line indicates the sample time.

a.



b.

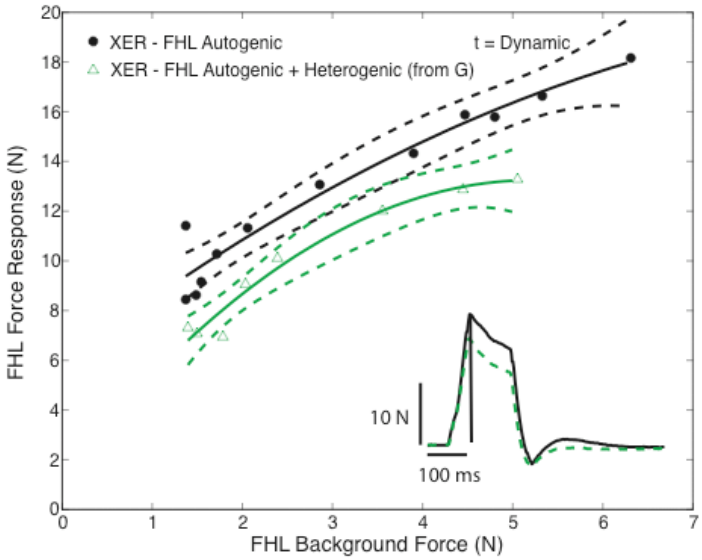


Figure 2.15 (a) Heterogenic inhibition from G onto FHL, where G is the donor muscle and FHL is the recipient muscle during locomotion for the dynamic phase. (b) Heterogenic inhibition from G onto FHL during XER for the dynamic phase. Heterogenic inhibition from G onto FHL is similar in strength and sign during locomotion (a) **and XER** (b)**. The same conventions as Figure 2.5 apply. Matched traces at a background force of 5 N for state one (solid line) and state two (dashed line) have been superimposed and inset to demonstrate the trend of heterogenic inhibition in both behavioral states, and the vertical line indicates the sample time.**

2.4 Discussion

In this study, we have attempted to address the organization and functional implications of heterogenic force feedback among ankle extensors, namely G, PLAN, FHL, and QUADS during spontaneous locomotion in premammillary decerebrate cats. Although recent data suggests that positive force feedback is autogenic (Ross et al. 2002; Ross et al. 2005), we found that heterogenic feedback linking ankle extensor muscles remained inhibitory with the initiation of, and during locomotion. Specifically, the largest magnitude of force-dependent inhibition emanated from G onto either PLAN or FHL. Furthermore, this inhibition emanated exclusively from MG, rather than both heads of G. Weak inhibitory interactions were found in the opposing direction, and between FHL and PLAN and between G and QUADS. Furthermore, the magnitude and of heterogenic inhibition from G onto FHL remained relatively constant between stepping and XER. While the differences in force response between locomotion and XER were similar, the autogenic force responses were actually higher in the XER case for a similar background force. Despite the variability in locomotion, from spontaneous stepping with oscillations in the background force to aided stepping with stimulation, the results remain consistent across preparations. The following discussion addresses these results from both a neural and biomechanical perspective, and evaluates the possible functions of heterogenic inhibition during locomotion.

Mechanical effects can explain the decreased autogenic force responses in hindlimb extensors during locomotion when compared to XER. This phenomenon is most likely due to the internal movement of muscle fibers during the changing activation levels

of locomotion, despite the fact that these muscle-tendon units are held isometric. The internal shortening and lengthening of the muscle fibers during stepping are likely to increase cross-bridge detachment and therefore turnover rate, which decreases force and presumably the effective muscle stiffness (Joyce et al. 1969; Rack and Westbury 1969; Kirsch et al. 1994). Therefore, the response to stretch during locomotion is lower than that during XER, where the internal length and stiffness of the muscle fiber, and the activation remain constant. Despite this decrease in force response, there are examples of specific muscles overcoming this mechanical effect to exhibit increases in autogenic gain during locomotion (Ross et al. 2002; Ross et al. 2005).

Previous studies suggest that positive force feedback is more widespread during locomotion than in our studies. Possible factors in the expression of positive force feedback during locomotion include the manner of inducing locomotion or activation of Ib or force-related afferents. Studies have utilized electrical nerve stimulation at group I strength to evaluate force feedback for pathways lacking Ia connections (Pearson and Collins 1993; Guertin et al. 1995), or electrical stimulation of the MLR to evoke stepping (Guertin et al. 1995; McCrea et al. 1995; Donelan and Pearson 2004). Previous work has revealed important differences between synchronous, electrical stimulation and natural stimulation (Enriquez-Denton et al. 2002). Our use of natural stimulation is one factor that might explain the different patterns of force feedback from previous studies. In addition, it is not clear from the literature whether this might be due to quantitative differences in the distribution of positive force feedback between experiments utilizing spontaneous locomotion versus MLR stimulation. The use of external manipulations to

induce locomotion, such as MLR or skin stimulation, could underlie different experimental results, should the effect not only be due to changes in behavioral state.

Information regarding the changes in the distribution of heterogenic inhibition can also be gleaned from previous studies in both locomoting and non-locomoting cat preparations. Intracellular recordings from motoneurons in anesthetized cats revealed the organization of force feedback from Golgi tendon organs in the hindlimb musculature of the cat (Eccles et al. 1957). This organization, whereby Ib afferent feedback is weak autogenically and distributed widely to muscles crossing joints, was verified using muscle stretch in an intercollicular decerebrate cat (Nichols 1989; Nichols 1999). Specifically, feedback coming from MG onto other ankle extensors, namely PLAN and FHL, was force dependent (Bonasera and Nichols 1994; Nichols 1994). Additionally, in the intercollicular decerebrate cat, there was greater heterogenic inhibition from the QUADS onto the triceps surae than in the reverse direction (Wilmink and Nichols 2003). Guertin and colleagues described a similar, asymmetric relationship between the triceps surae and the QUADS using nerve and MLR stimulation in the fictive locomoting cat preparation (Guertin et al. 1995). In the current study, rather than a global change from inhibition to excitation with locomotion, the inhibitory heterogenic relationships among these three ankle extensors and the QUADS were preserved in similar ratios to the previous studies. Specifically, Bonasera (Bonasera and Nichols 1994) documented the number of experiments demonstrating heterogenic inhibition between G and FHL in intercollicular decerebrate cats. However, while we found similar relationships in our

premammillary, decerebrate cats during stepping, the strength of inhibition was greater in the intercollicular preparations than in the premammillary preparations.

Comparison of data from intercollicular to premammillary preparations suggests an alternative explanation for the varying distribution of force feedback in the different studies. For instance, the lack of evidence for heterogenic, positive force feedback in this study might be explained by the experimental preparation, as it is possible that the distribution of excitatory force feedback is dependent on the motor task. For example, while muscle activation patterns during treadmill locomotion closely resemble those observed during level walking (Smith et al. 1998; Gottschall et al. 2005) it is possible that positive force feedback is more widespread during walking up a slope when larger forces are required of antigravity muscles (Gregor et al. 2006). In contrast, heterogenic inhibition might be more important during downslope walking when active lengthening of extensors predominates during stance (Nichols et al. 1999). This has important implications for previous studies evaluating the reflex reversal of force-dependent inhibition to excitation in fictively locomoting cats (Gossard et al. 1994; McCrea et al. 1995). In these preparations, there is a lack of sensory feedback modulation, and the specific motor task is unknown. We propose that this array of experimental paradigms, from intercollicular, to spontaneously stepping preparations, to paralyzed fictive preparations, represents a behavioral spectrum that ranges from predominately inhibitory force feedback to more widespread excitatory force feedback.

There are several physiological explanations for MG serving as the major source of inhibition during locomotion. First, the distribution of Golgi tendon organs in G, FHL, and PLAN could influence the amount of inhibition present during locomotion. That is, if MG possessed the greatest number of Golgi tendon organs, this could possibly explain the large magnitude of force-dependent inhibition coming from this muscle during locomotion. Unfortunately, this information is unknown for all of these muscles. What is known is that the combined medial and lateral heads of G possess approximately 60% more muscle spindles than FHL (Chin et al. 1962). It is also known that the ratio of muscle spindles to Golgi tendon organs may be preserved across muscles (Eldred et al. 1974).

The strength of the inhibition emanating from MG during locomotion could also be due to biomechanical factors. First, there could be a presumed dependence of inhibition on the background force and response of the donor muscle, as seen in previous studies (Bonasera and Nichols 1994). However, the magnitudes of inhibition did not always correlate with the donor responses or forces in our study. Muscle architecture, including the physiological cross-sectional area and force generation capabilities of each muscle, could be a contributing factor. It is known that G, including both the medial and lateral heads, has the greatest physiological cross-sectional area and largest pinnation angle (Sacks and Roy 1982), and is therefore poised to generate the greatest force. While G, FHL, and PLAN all act to extend the ankle, G is the only muscle that inserts into the calcaneus. FHL and PLAN both have mechanical linkages to the metatarsal phalanges

(MTP) joint with insertions into the flexor digitorum longus and flexor digitorum brevis, respectively.

The last proposed explanation implicates a central mechanism for weighting the relative strengths of these force-dependent feedback pathways. Experiments performed in intercollicular decerebrate cats using intramuscular stimulation revealed the mechanical contribution of individual muscles to the endpoint force, and therefore the action about an individual joint (Lawrence and Nichols 1999). While G, FHL, and PLAN all have mechanical actions at the ankle, G, specifically the MG, generates the greatest non-sagittal moment as a large abduction torque about the joint. This non-sagittal component could play an important role in stability during locomotion, and possibly implicates a central organization of neural inhibitory connections among these muscles to create a stabilizing effect. That is, negative force feedback could favor those muscles with the greatest non-sagittal moment, and induce stability by increasing the base of support during locomotion.

There are functional consequences of heterogenic inhibition, namely interjoint coordination. Mechanically, MG has action at both the ankle and knee joints. PLAN also exerts a high ankle torque and has mechanical action on the MTP joint. While FHL has a smaller ankle torque, it also produces an adduction torque and acts on the MTP and distal interphalangeal (IP) joints (Goslow et al. 1972; Lawrence and Nichols 1999). Like the biomechanical actions of these muscles, the distribution of force feedback crosses multiple joints and axes of rotation (Wilmink and Nichols 2003). Therefore, heterogenic

inhibition provides cross-joint and cross-axis coupling that, in some cases, parallels the mechanical coupling of biarticular muscles (Nichols 1994). Additionally, heterogenic inhibition, in combination with excitatory length feedback, contributes to the regulation of whole-limb stiffness (Nichols and Houk 1976; Nichols et al. 1999). It is important to also note that while the greatest amount of heterogenic inhibition emanates from MG in our study, we have also provided evidence for positive force feedback that is autogenic and mainly associated with MG (Ross et al. 2002; Ross et al. 2005).

CHAPTER 3

AUTOGENIC FEEDBACK

3.1 Introduction

Proprioceptive feedback from muscle spindles and Golgi tendon organs plays an important, yet unique, role during locomotion (Pearson 1995; Prochazka 1996; McCrea 1998; Duysens et al. 2000; Hultborn 2001). Muscle spindles possess a complex relationship between muscle length and receptor discharge properties (Matthews 1964). Matched to intrinsic properties to regulate muscle stiffness, it is well documented that muscle spindles provide feedback, particularly to the muscle of origin (Eccles et al. 1957; Nichols 1999). This reflex component is most important during active lengthening (Mazzaro et al. 2006). Conversely, the structure of Golgi tendon organs results in a relatively simple transduction of force, however the functional role of force feedback, and its central processing are poorly understood.

Traditionally, Golgi tendon organs have been classified as providing inhibitory feedback to extensor (antigravity) muscles, particularly to muscles other than the muscle of origin (Eccles et al. 1957). This distributed network of inhibition connects mainly muscles that cross joints and axes of rotation (Nichols 1989; Bonasera and Nichols 1994; Wilmink and Nichols 2003). Functionally, this may promote interjoint coordination and stability (Nichols 1999). Recent studies suggest that an excitatory pathway from Golgi tendon organs is opened with the initiation of locomotion, and Ib inhibition is suppressed (Pearson and Collins 1993). It is hypothesized that this positive force feedback provides a loading reflex that reinforces the force in a muscle, particularly during the stance phase of

locomotion (Duysens and Pearson 1980; Pearson and Collins 1993), and promotes the transition from stance to swing phase (Lam and Pearson 2002). Additionally, Ib feedback from Golgi tendon organs has the ability to entrain the locomotor rhythm (Gossard et al. 1994).

Positive force feedback has been observed in reduced experimental preparations with varying methods to evoke locomotion. There is evidence for the reflex reversal in the electrophysiological recordings in extensor motoneurons of fictively locomoting cats (Guertin et al. 1995; McCrea et al. 1995). These preparations lack sensory feedback, which plays an important role in the modulation of locomotion. While the alternating bursting in the motoneurons mimic locomotion, whether or not the animal is in a state of locomotion is somewhat unknown. Additionally, some of these studies employ clonidine (a selective $\alpha 2$ adrenergic agonist), L-DOPA, or stimulation of the MLR to induce stepping, and electrical stimulation of afferents for extensor activation (Pearson et al. 1992; Pearson and Collins 1993; McCrea et al. 1995). While these studies generally suggest a global change from inhibition to excitation with the initiation of locomotion, it has been noted that some group I inhibitory pathways exchanged between the triceps surae and QUADS remain during locomotion (Guertin et al. 1995).

Previous studies in this laboratory suggest that inhibitory pathways are not replaced with excitatory ones. Instead, heterogenic feedback between hindlimb extensors in spontaneously locomoting premammillary decerebrate cats remains inhibitory (Ross and Nichols Submitted). Preliminary findings suggest that positive force feedback

manifests itself as an increase in autogenic gain during locomotion (Ross et al. 2002; Ross et al. 2005). We utilized a spontaneously locomoting premammillary cat and muscle stretch as a natural input to the muscle to investigate the strength and sign of autogenic feedback. Hindlimb extensors were chosen for these experiments, namely G, including both heads of G, MG and LG, PLAN, FHL, SOL, and QUADS (Eccles et al. 1957; Nichols 1989). Our experiments addressed the following central question: does autogenic force feedback in G, MG, LG, PLAN, FHL, SOL and QUADS exhibit a reflex reversal with the initiation of locomotion in this preparation? We found that, under these conditions, there was not a global change from inhibition to excitation with the initiation of locomotion. Instead, there was an increase in excitation in G, specifically in MG, during locomotion. We propose that the coexistence of these excitatory and inhibitory pathways could promote a stable system. Specifically, the selective autogenic gain in G could serve as the loading reflex, while the heterogenic inhibitory connections could help maintain the stability and coordination of the limb.

3.2 Methods

3.2.1 Preparation

The methods used to determine the strength and sign of feedback have been described previously (see Chapter 2). The mechanographic technique was used to evaluate the distribution and contribution of feedback from muscle receptors (Nichols 1987). Briefly, nineteen cats ranging from 3 to 6 kilograms were deeply anesthetized using isoflurane gas. The animal was surgically prepared by performing a tracheotomy, preparing carotid arteries for ligation, and cannulating the external jugular vein for fluid

delivery. Withdrawal responses were monitored, and the level of anesthetic was adjusted accordingly.

The right hindlimb was immobilized, bone pins were inserted into the femur and tibia, and the leg was clamped to maintain the knee at a 110^{o} angle. The animal was placed in the stereotaxic frame, supported above a variable-speed treadmill. The core temperature was maintained at 37^{o} C. Reference sutures inserted around the tendons of the peroneus muscles and sutures inserted into the muscle tendons were used to determine the initial lengths of the muscles.

The G, PLAN, SOL, and FHL of the right, immobilized hindlimb were dissected. Both PLAN and FHL were cut near their insertion onto FDB and FDL respectively. In 5 experiments, the G was separated into its respective heads, MG and LG. Each muscle was attached via their tendon to myographs and linear motors. In 5 experiments, the QUADS were dissected and attached to a myograph and motor via a cable and pulley system. Mineral oil was used to ensure that the muscles stayed moist.

A premammillary decerebration was performed, whereby the brainstem was transected rostral to the superior colliculus while preserving the mammillary bodies and subthalamic nucleus. All brain matter rostral to the transection was removed. Gelfoam and cotton were placed on the base of the cranium to minimize bleeding. Anesthesia was then titrated down and withdrawn.

Three limbs were free to step on the treadmill while the right hindlimb remained immobilized. Stimulation of the skin beneath the tail was used to initiate stepping when spontaneous locomotion did not occur. Once locomotion data were obtained, XER was elicited with electrical stimulation of the left posterior tibial nerve at 2 times threshold. At the end of each experiment, the animal was euthanized with an overdose of Nembutal followed by a pneumothorax.

3.2.2 Data Acquisition

The Parker 406LXR linear motors used in this study were mounted on a custom-built aluminum frame and could be adjusted in the horizontal, vertical, and diagonal directions so that the motor could be properly aligned with the appropriate muscle. Data was acquired digitally through the *dSPACE* board at a sampling rate of 1000 Hz. Data acquisition software in *Simulink* and a graphical interface in *Controldesk* were used to specify the change in length, velocity, and hold time. The typical paradigm was a 2 mm stretch at a velocity of 0.04 m/s, 100 ms hold period, and 2 mm release. All muscles were maintained at their referenced length (see above) when the knee was fixed at 110^{o} and the ankle was at 90^{o}.

Once initiation of stepping from the three unfixed limbs commenced, random stretches were manually triggered so as to capture a significant number of trials in all phases of the step cycle. Oscillations in the background force of G in the right hindlimb, force responses due to stretch during stepping are shown in Figure 3.1a. Symbols above stretches illustrate the force responses obtained when the muscle is stretched alone during

locomotion (filled circles). Figure 3.1b depicts the length input to the muscle during locomotion, which corresponds in time to the force responses depicted in Figure 3.1a.

The stretching paradigm in the right hindlimb was then repeated during XER, as shown in Figures 3.2a and 3.2b, so that we could determine how autogenic feedback was reorganized with the initiation of stepping. Symbols above stretches in Figure 3.2a illustrate the force responses obtained when the muscle is stretched alone during XER (open circles). As described below, force responses obtained during locomotion (filled circles) were compared to those obtained in the XER (open circles) to evaluate the change in autogenic gain with the initiation of locomotion. Finally, individual myographs were calibrated using a two-point calibration.

3.2.3 Data Analysis:

Force measurements were used to discern autogenic feedback pathways when muscles were stretched. Comparing recipient muscle responses during locomotion (filled circles) with those during XER (open circles) reveals the autogenic gain between the behavioral states. Software in Matlab version 7.01 was used to analyze the data. Briefly, the background force of the muscle was calculated as an average of the force 10 ms prior to the beginning of the stretch, during the isometric hold period. A baseline was constructed by performing a linear interpolation from the mean force response just prior to the stretch to the mean force after the end of the release. The entire baseline was then subtracted from the overall force response.

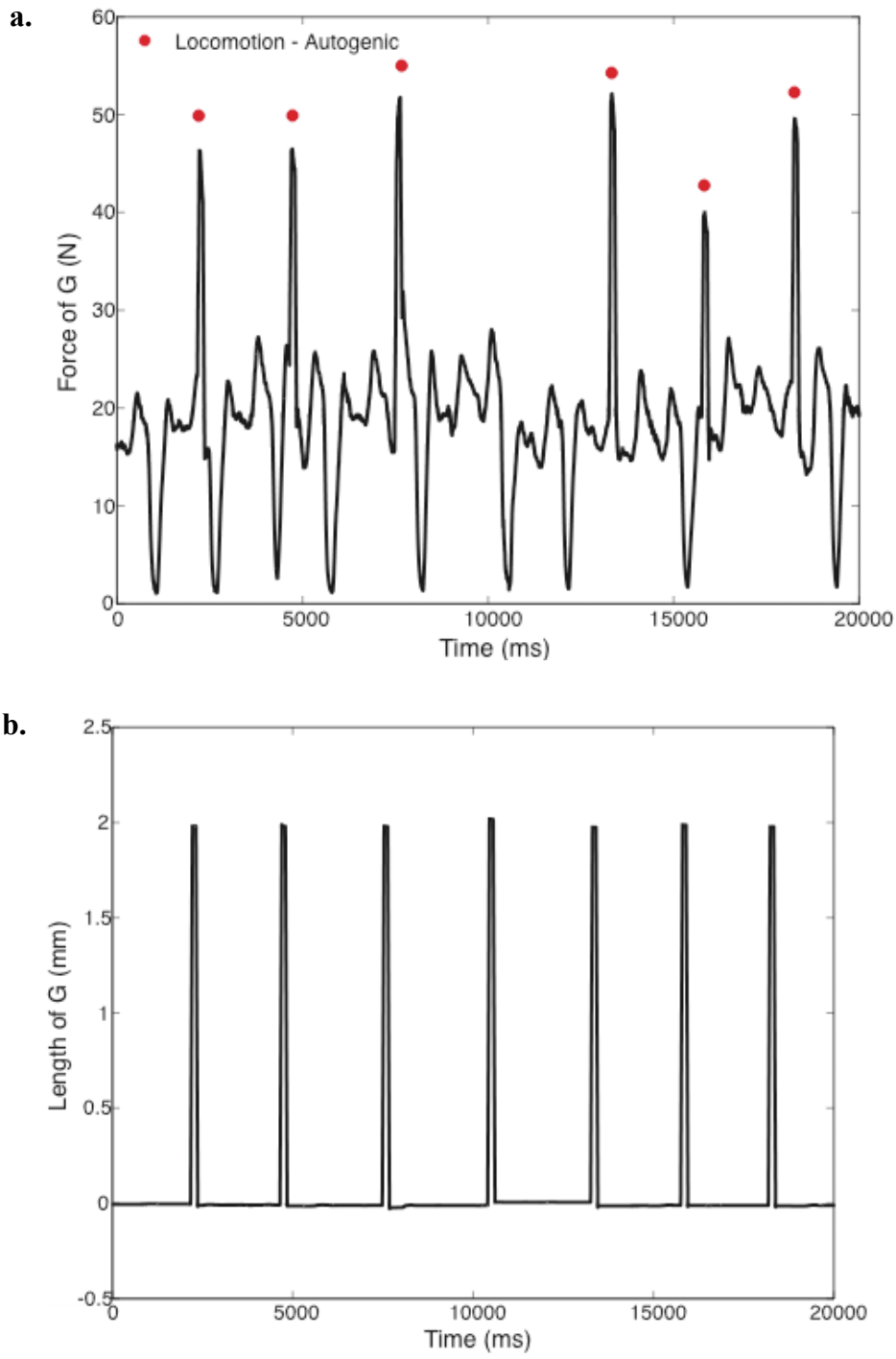


Figure 3.1. (a) Ramp and hold stretches delivered on top of the oscillating background force in G. Symbols above stretches indicate responses obtained when the muscle is stretched alone (filled circles) during locomotion. (b) Ramp and hold length input to G during locomotion.

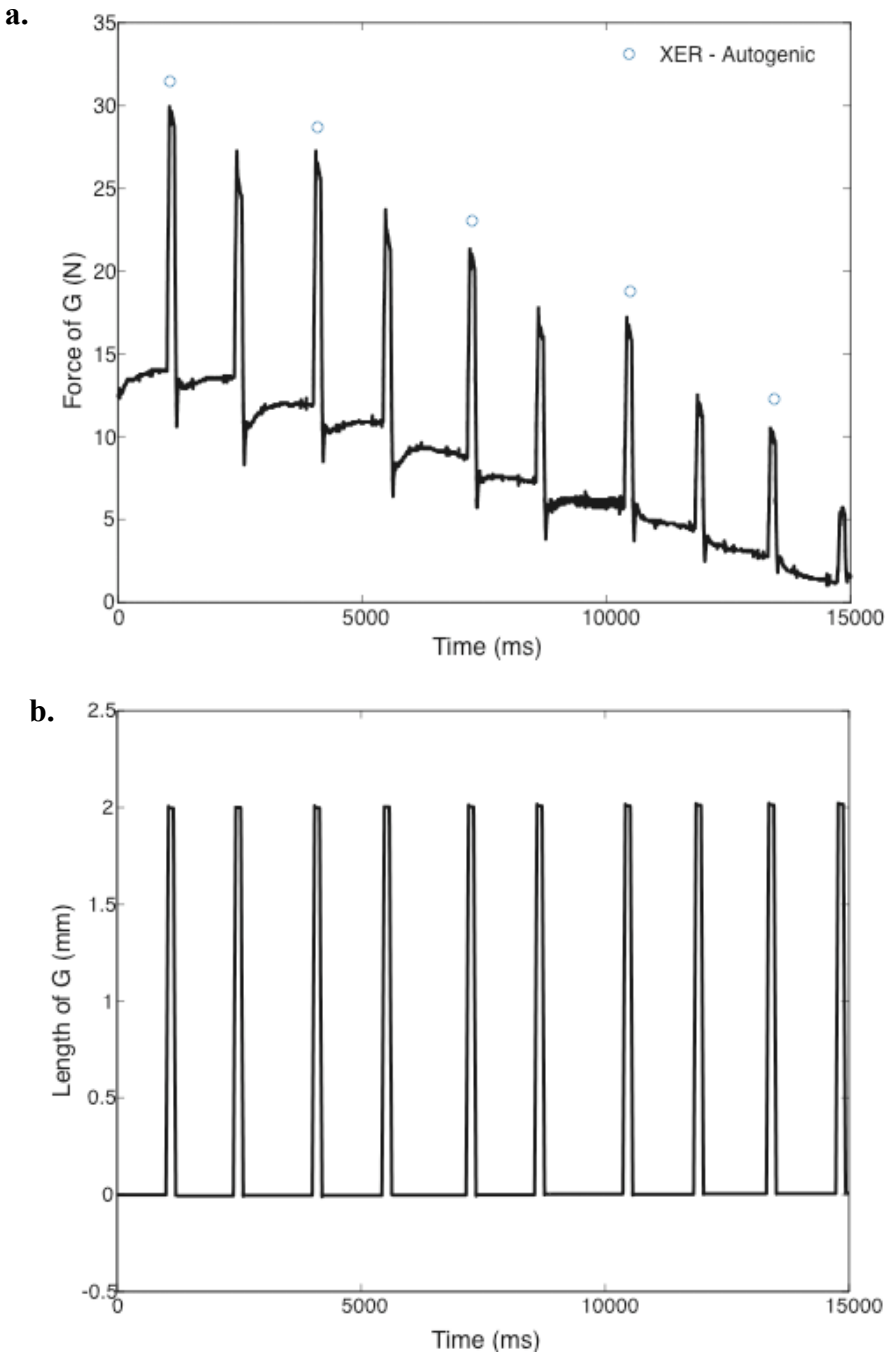


Figure 3.2 (a) Recipient muscle stretch-evoked force response during XER. Symbols above stretches indicate responses obtained when the muscle is stretched alone (open circles) during XER. (b) Recipient muscle length input to two-state stretch. The stimulation of the tibial nerve in the left hindlimb at 2 T evokes an increase in the background force of the recipient muscle, G (a). As the background force declines, ramp and hold stretches (2 mm, 0.04 m/s stretch, 100 ms hold period), are delivered to the recipient muscle (b).

To evaluate autogenic feedback between the behavioral states, individual force responses at specific time points were obtained from the baseline subtracted force data, and background force was obtained from the original force trace. As shown in Figure 3.3, force responses for a specific timepoint are plotted as a function of background force. Each data point represents a response obtained when the muscle stretched alone during locomotion (filled circles) or XER (open circles), and individual responses represent an individual stretch. Polynomial fits and 95% confidence intervals were fit to each population of data for a given time point.

Three time points were used to assess the strength and sign of autogenic feedback. Responses obtained 10 ms following the beginning of the stretch represent the mechanical phase, and separation of the two populations of data in this phase suggests a mechanical artifact. Force responses that occur 50 ms and 100 ms following the beginning of the stretch represent the dynamic and static phase of autogenic feedback Figures 3.3c and 3.3d respectively). The filled circles represent responses obtained when the muscle was stretched alone during locomotion, while open circles represent responses obtained when the muscle was stretched alone during XER. Additionally, individual traces from both behavioral conditions were matched at similar background forces and expanded in time to visualize the timecourse of both mechanical and reflexive effects as shown in Figures 3.3e and 3.3f.

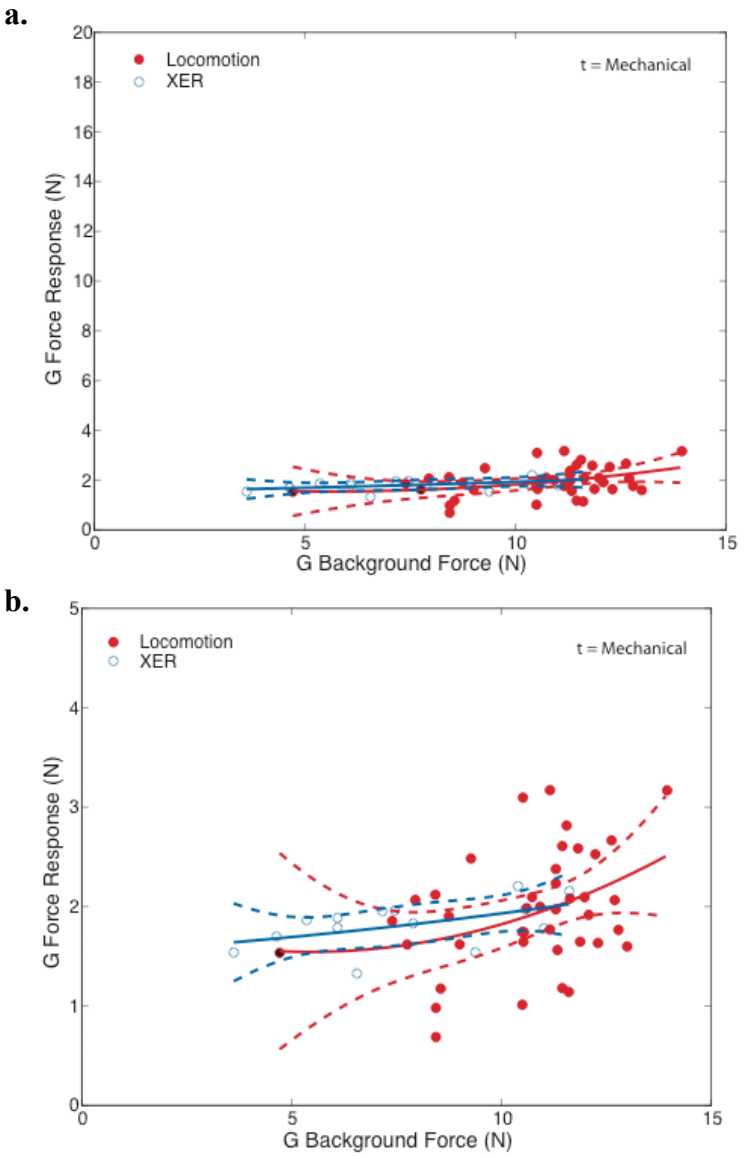
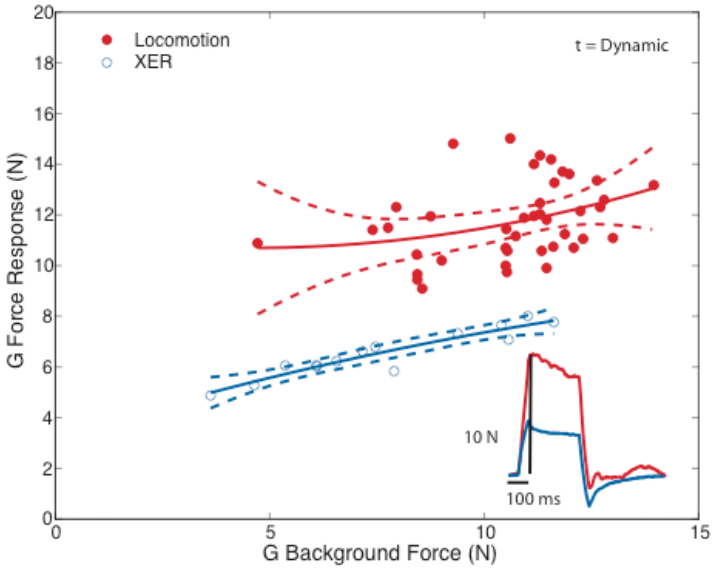


Figure 3.3 (a) Autogenic responses for G during locomotion and XER for the mechanical phase. (b) Expanded view of the autogenic responses of G for the mechanical phase. (c) Autogenic excitation of G for the dynamic phase, and (d) the static phase. Filled circles and open circles represent G force responses from stretches occurring during locomotion and XER, respectively. Polynomials and 95% confidence intervals are fit to each population of data, and statistical tests reveal that the populations are distinctly separated ($p<0.01$). Two traces matched at 10 N background force in G from locomotion (solid line) and XER (dashed line) have been superimposed to illustrate the magnitude of excitation from G, and the vertical line indicates the sample time. Two traces matched at a background force of 5 N and 12 N have been expanded in time to illustrate that the autogenic excitation occurs approximately 17 ms following the beginning of stretch (e, f).

c.



d.

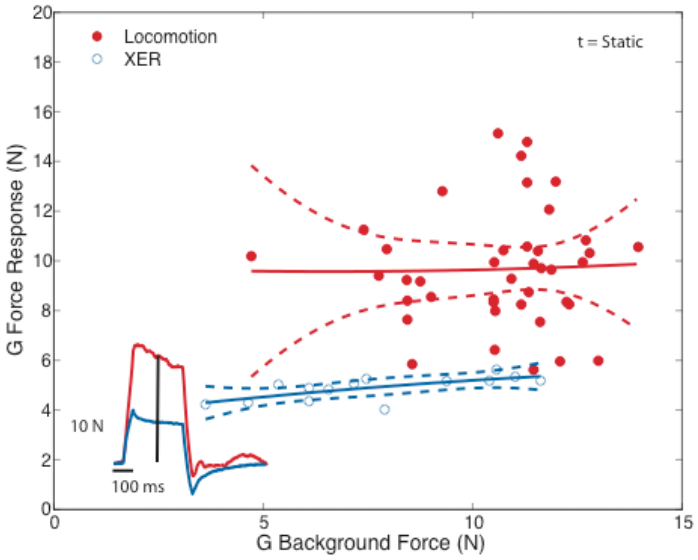
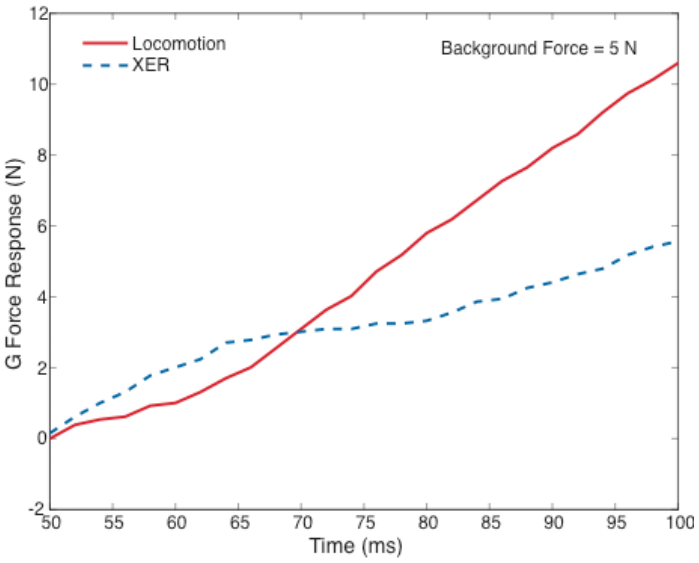


Figure 3.3 (continued)

e.



f.

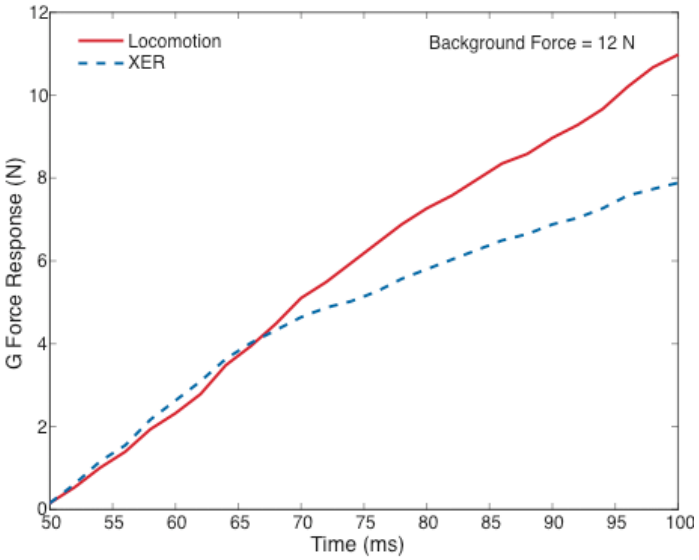


Figure 3.3 (continued)

Statistics were performed using Statistica 6.0 and Excel to test the separation of the data populations. Similar to the statistics previously described, two regression models were fit to the data. In the full model, force response at the specified time point was the dependent variable (Y). The predictors in the full model included the grouping variable (X_2), representing force responses during locomotion or XER, background force (x_1), background force squared (x_1^2), the grouping and background force crossed term (x_1X_2), and the grouping variable multiplied by the squared background force term ($x_1^2X_2$). The reduced model lacked all terms containing the grouping variable, thus pooling the data into one population. An F statistic was calculated by obtaining the sum of squared errors (SSE) and degrees of freedom (DF) from both the full and reduced models, as well as the mean squared errors (MSE) from the full model. A p-value < 0.01 from the statistical test stated above rejects the null hypothesis, and statistically proves that the two populations are distinctly different.

While Figure 3.3 represents data from one time point, the difference in the autogenic feedback between behavioral states was explored for the entire time-course of the ramp and hold profile. Polynomial fits of the two populations of data were calculated for every 5 ms of the entire response. For each time-point, the polynomial representing the force responses obtained during XER was subtracted from the polynomial representing the data obtained during locomotion. A surface plot was then created from each of the difference calculations as shown in Figure 3.4. A common range in background force of the muscle for both populations was also computed and represented on the z-axis.

3.3 Results

3.3.1 Autogenic feedback increases in G during locomotion

The strength of the increase in autogenic gain in G during locomotion was examined in sixteen total experiments, all of which exhibited stepping behavior. Four experiments were rejected due to poor XER activation. Of the twelve remaining experiments evaluating the force responses in G both during locomotion and XER, 9 demonstrated autogenic excitation in G. The remaining 3 preparations did not exhibit increases in autogenic gain during locomotion, but rather demonstrated no significant difference in the force responses between the two behavioral states. Figure 3.3 depicts a representative example of the increase in autogenic gain in G during stepping. This autogenic excitation is not due to a purely mechanical event, as demonstrated by the overlapping force responses shown in Figures 3.3a and 3.3b. The expanded view of these mechanical responses, as shown in Figure 3.3b, demonstrates that while the confidence intervals are overlapping for the entire range of background force, there is a slight depression at lower background forces. There is a clear increase in autogenic gain between locomotion and XER in the dynamic phase, as depicted in Figure 3.3c, however there is not a clear force-dependent trend in the separation. Of the 9 experiments exhibiting autogenic excitation in G, 44% (4) exhibited a force-dependent trend in the excitation for the dynamic response, 22% (2) decreased excitation with increasing background force, and 33% (3) possessed little overlapping background force for the two populations, therefore a clear relationship could not be determined. The same relative relationships exist for the static responses. However, in 1 experiment, the responses,

which were not statistically different for the dynamic phase, became statistically inhibitory at the later time point, in part due to the wide scatter in data points.

Additionally, the variability and scatter among the data points increases slightly for the static phase (Figure 3.3d), as demonstrated by the widening of 95% confidence intervals. Our criterion for significant depression is that the p-value<0.01. In most, but not all cases, this also corresponds to non-overlapping confidence intervals. In this example, the confidence intervals are clearly separate for the two populations of data, confirming that there is autogenic excitation from G. Additionally, multiple regression yielded $p<0.01$ for both the dynamic and static phases, thus statistically proving that these populations are distinctly different.

Traces inset in Figures 3.3c and 3.3d are force responses during locomotion (solid line) and during XER (dashed line). The background force for both conditions was matched at the mean background force of approximately 10 N. Baselines were subtracted from both traces to better illustrate the magnitude and time course of the autogenic excitation from G. As shown by these traces, the magnitude of excitation remains relatively constant during the hold period.

To evaluate the time course of the autogenic excitation, individual traces from locomotion and XER were matched at 5 N and 12 N, in Figures 3.3e and 3.3f respectively, and have been plotted at the beginning of the stretch. Traces matched at 5N correspond to stretches occurring in the swing phase of locomotion, and illustrate that

there is a significant mechanical effect that results in decreased force responses during this phase of locomotion (Figure 3.3e). However, the excitatory reflex response overcomes this mechanical effect with a latency of approximately 17 ms following the beginning of the stretch. Traces matched at 12N represent the stance phase of locomotion, as shown in Figure 3.3f. While the initial inhibitory response is decreased in magnitude compared to the swing phase, the mechanical response is still inhibitory, although smaller, in the stance phase. Additionally, the autogenic excitatory response overcomes the mechanical response on a similar time course to the swing phase, approximately 18 ms.

Figures 3.3c and 3.3d depict autogenic excitation from G during locomotion for one time-point, dynamic and static respectively. To examine the trend of the feedback throughout the time course of the ramp and hold stretch, a three-dimensional plot was created as previously described. Figure 3.4 represents the time course of autogenic feedback from G during locomotion. The surface represents the difference between the force responses obtained during locomotion and XER as a function of time and G background force. The maximum response difference is approximately 6 N, and declines slightly throughout the hold period.

The influence of locomotor phase on the autogenic gain of G was explored further. Force responses for the dynamic (Figure 3.5a) and static time points (Figure 3.5b) were plotted for an experiment clearly displaying stepping in the three, unrestrained limbs, and oscillations in the background force of the immobilized limb. Polynomial fits

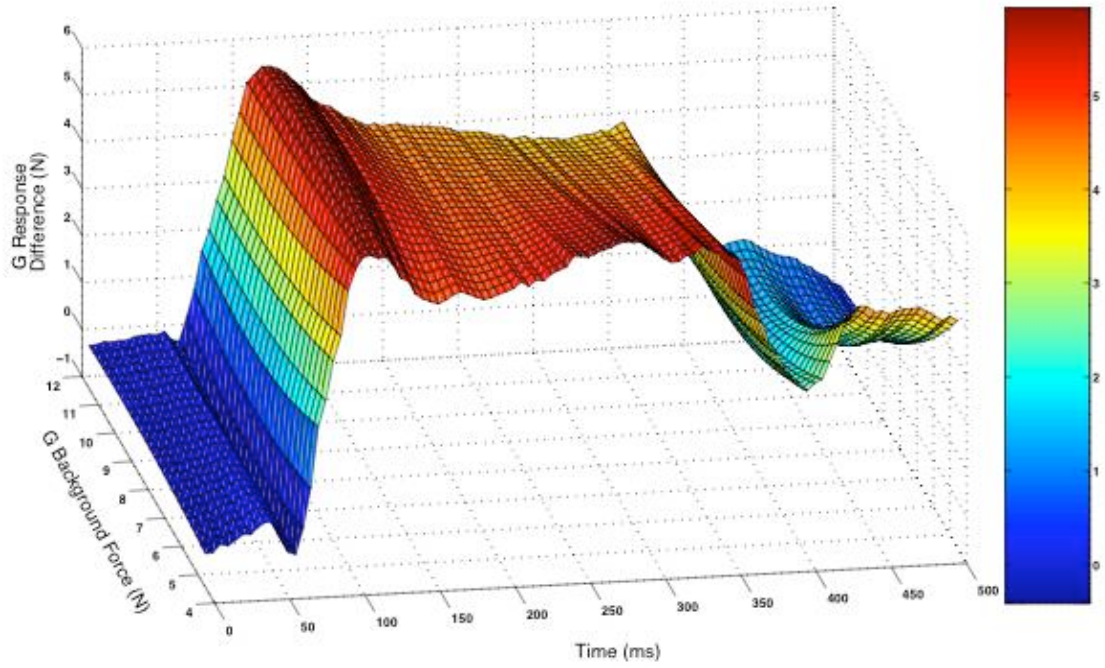


Figure 3.4 A three-dimensional surface that quantifies the magnitude of autogenic excitation from G during locomotion as a function of force and time. To quantify the magnitude of autogenic excitation from G during locomotion, response differences are calculated for every 5 ms over the ramp and hold stretch by subtracting the polynomial fits for XER from the polynomial fits from locomotion. A three-dimensional surface is created from the series of response difference calculations. The autogenic excitation from G during locomotion remains relatively constant over time and G background force.

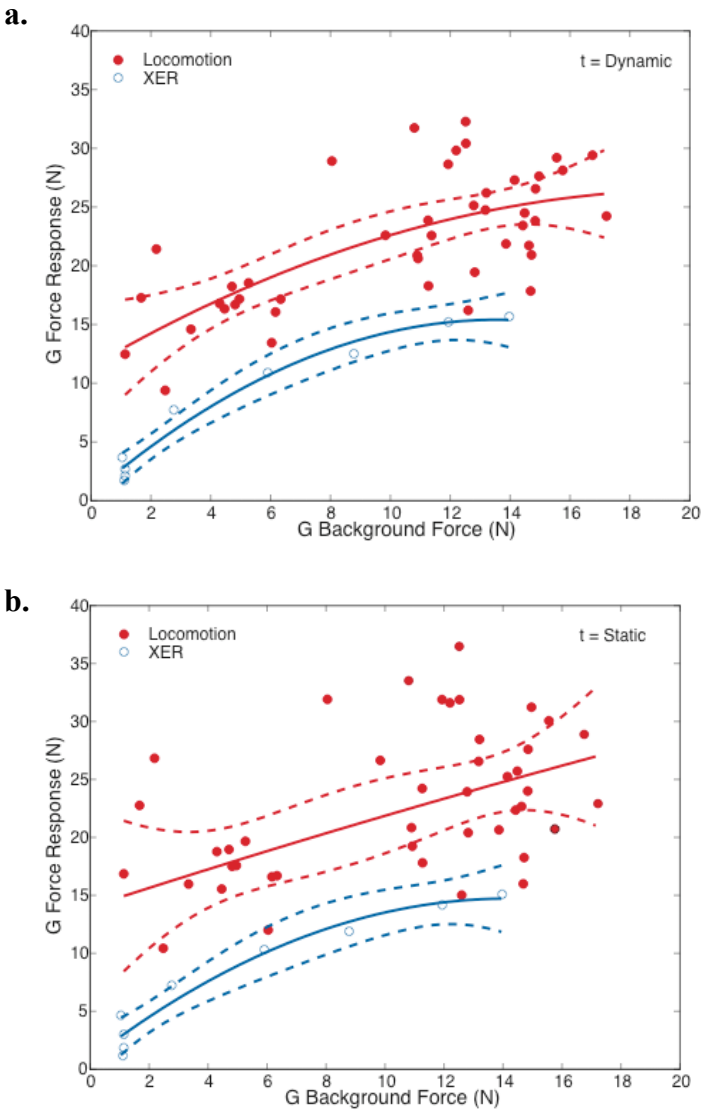
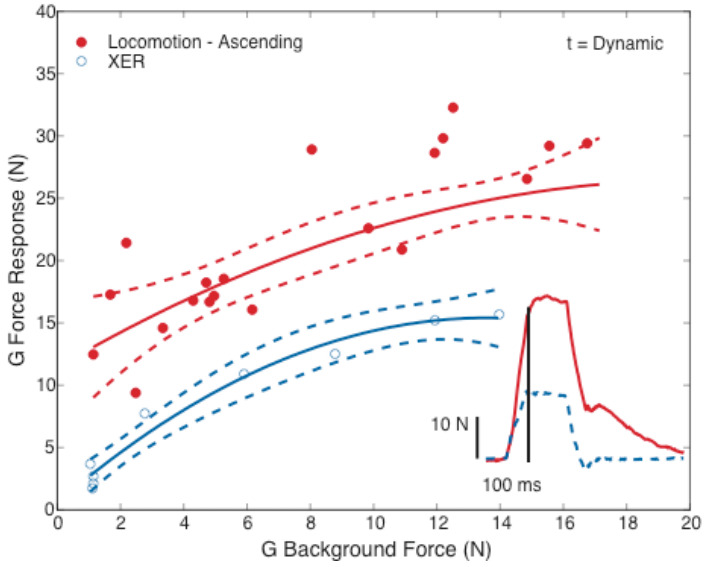


Figure 3.5. (a) Autogenic excitation of G during locomotion and XER for the dynamic and the (b) static timepoints for all phases of locomotion. The same conventions as Figure 3.3 apply. Individual stretches are categorized by phase, and force responses in G for the dynamic and static timepoints are displayed for the ascending phase (c,d), descending phase (e,f), and peak of stance (g,h). Original polynomial fits and 95% confidence intervals appear on each figure to demonstrate the quality of the fits to each phase of locomotion. Two traces matched at 14 N background force in G from locomotion (solid line) and XER (dashed line) have been superimposed to illustrate the magnitude of autogenic excitation from G during the various phases, and the vertical line indicates the sample time.

c.



d.

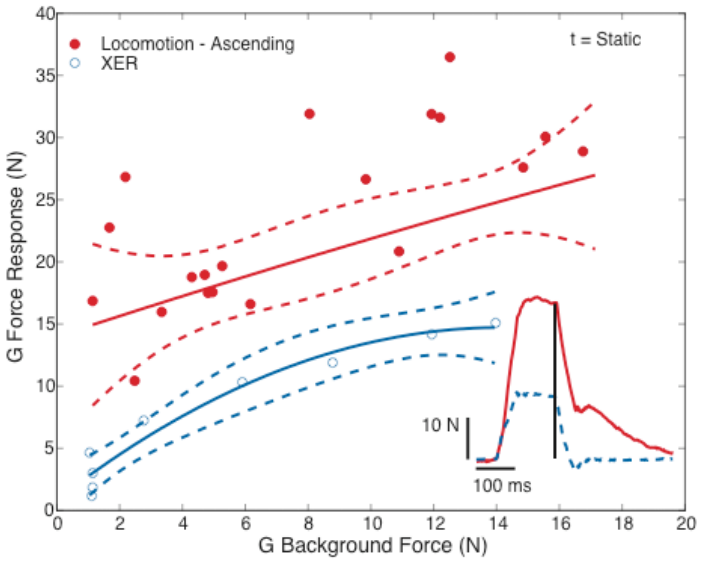
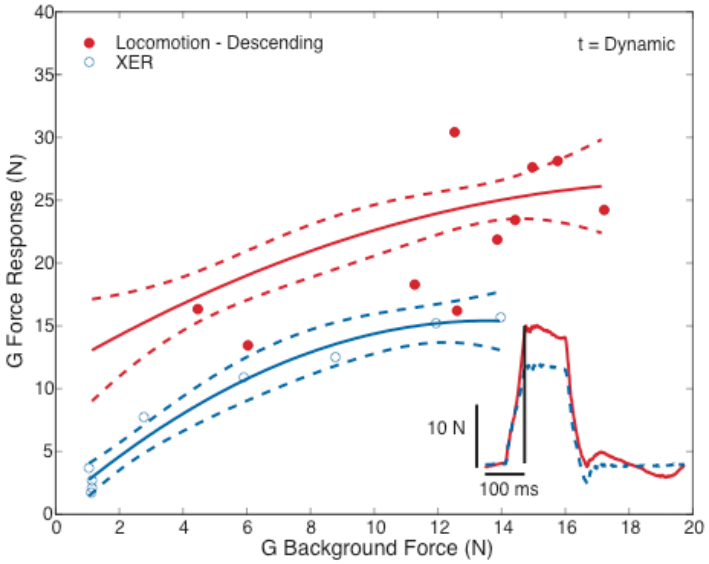


Figure 3.5 (continued)

e.



f.

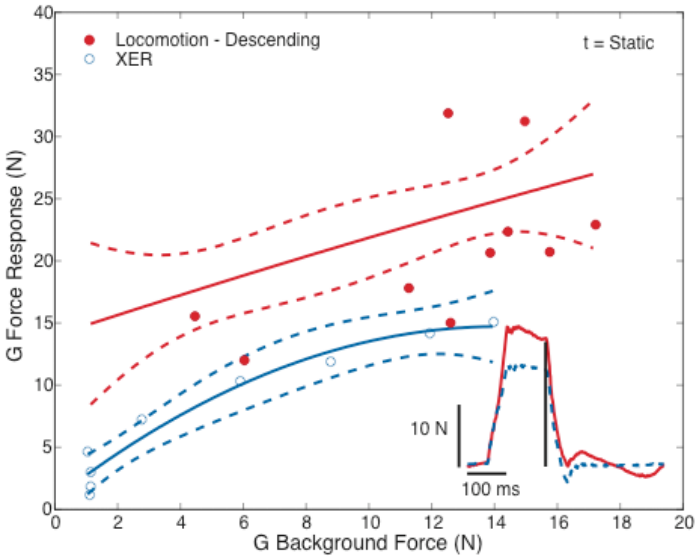
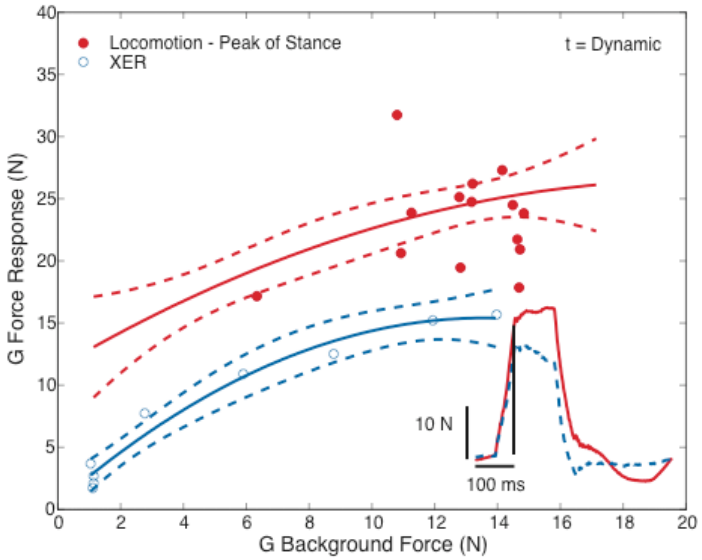


Figure 3.5 (continued)

f.



g.

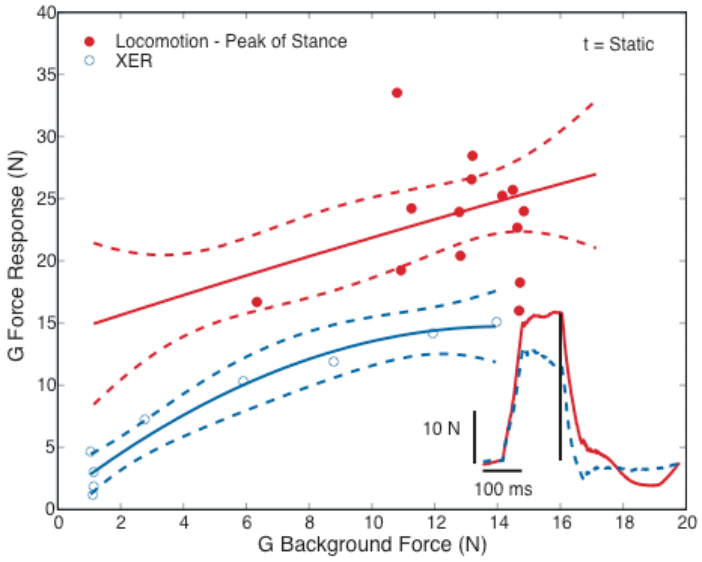


Figure 3.5 (continued)

and 95% confidence intervals were plotted for data obtained during locomotion and XER. Each stretch was then categorized by phase: swing or stance. Traces were separated based on the oscillations in the raw force trace. Stance was further delineated into the upswing, downswing, or plateau of stance phase. Only responses occurring during stance were included in the analysis. Individual force responses for the dynamic and static phases occurring during the upswing (Figures 3.5c and 3.5d), downswing (Figures 3.5e and 3.5f), and plateau (Figures 3.5g and 3.5h) of stance were compared with force responses obtained during XER. In each case, the original polynomial fits and 95% confidence intervals were plotted to illustrate the relative distribution of force responses for each phase and accuracy of the analysis method. Individual traces from the corresponding phase matched at a background force of 14 N for locomotion (solid line) and XER (dashed line) are inset in each figure, and a vertical line illustrates the sample time.

This phase analysis was further explored by evaluating the magnitude of force responses at different points of the step cycle. As shown in Figure 3.6a, the magnitude of recipient muscle force responses is plotted and synchronized in time to the force oscillations occurring in muscle (Figure 3.6b). These locomotion force responses are then compared to responses obtained during XER, as shown in Figure 3.6c, to illustrate that the increase in autogenic gain is exhibited across all phases of locomotion. In addition to this analysis, individual G force traces matched at a background force of 10 N for the upswing (Figure 3.7a) and downswing (Figure 3.7b) of locomotion (solid line) and XER (dashed line) illustrate the increase in autogenic gain in for both phases. In Figure 3.7c, traces from the upswing (solid), downswing (dotted), and plateau (dash-dot)

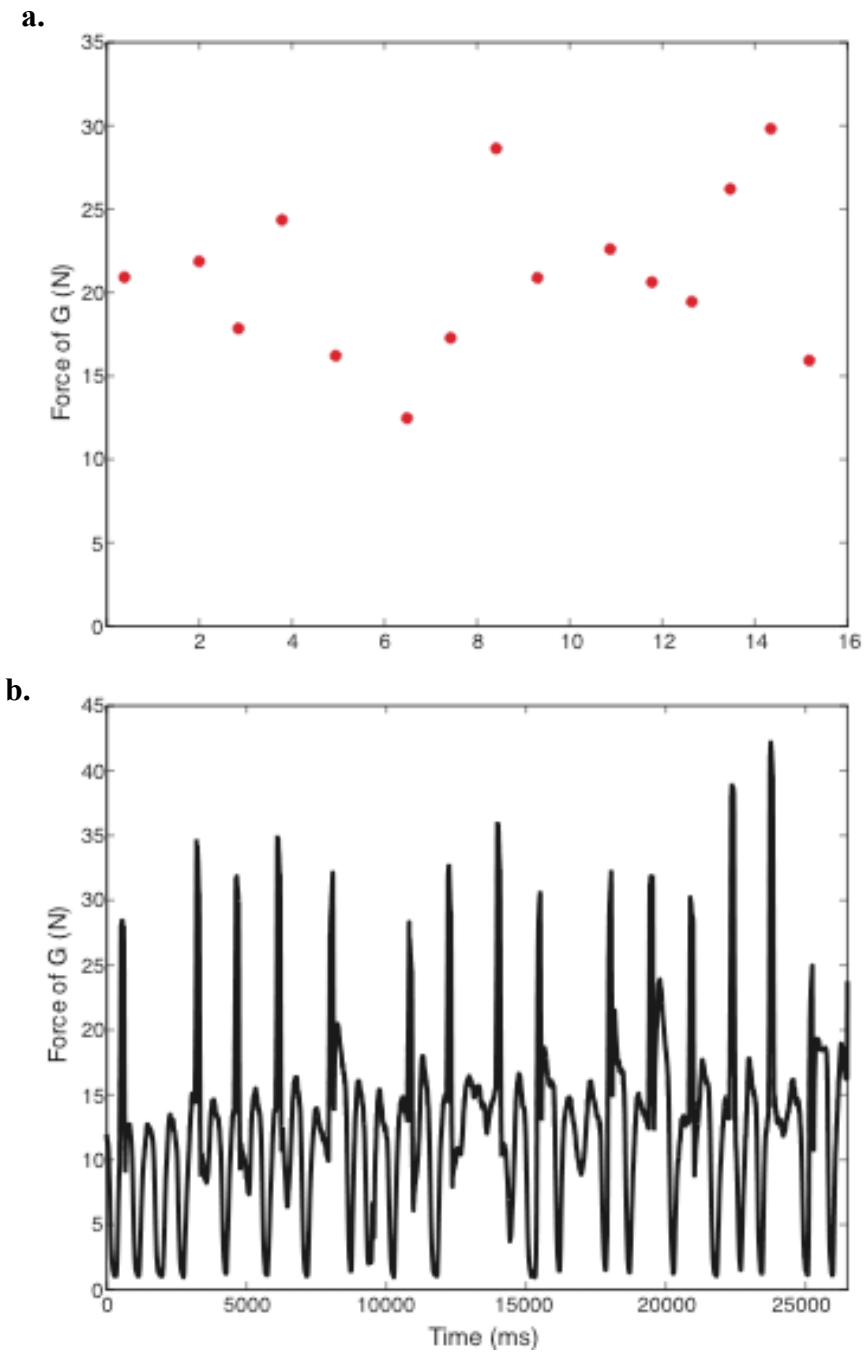


Figure 3.6 (a) Force responses of G during locomotion synchronized in time with force oscillations during stepping (b). (c) Autogenic excitation of G for the dynamic phase during locomotion. The same conventions as Figure 3.3 apply.

c.

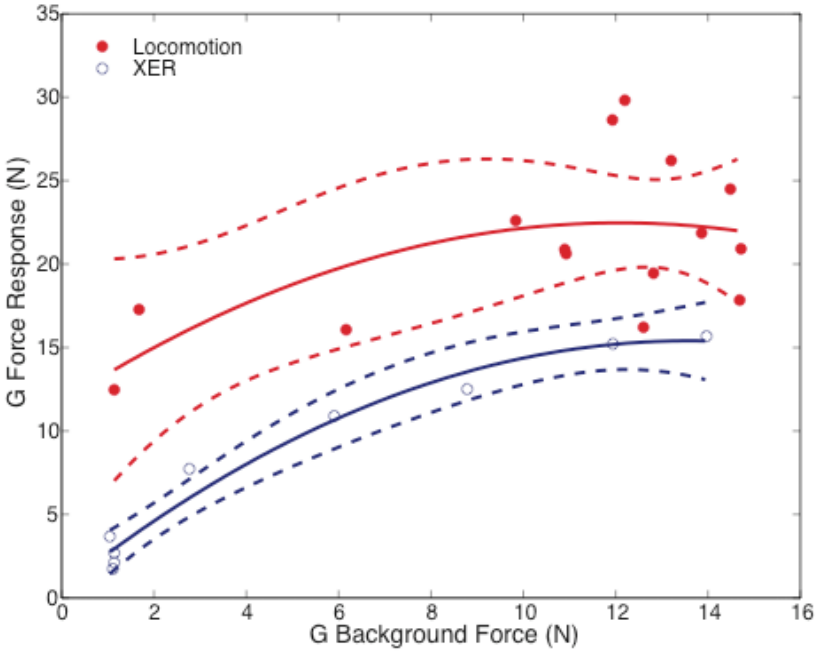


Figure 3.6 (continued)

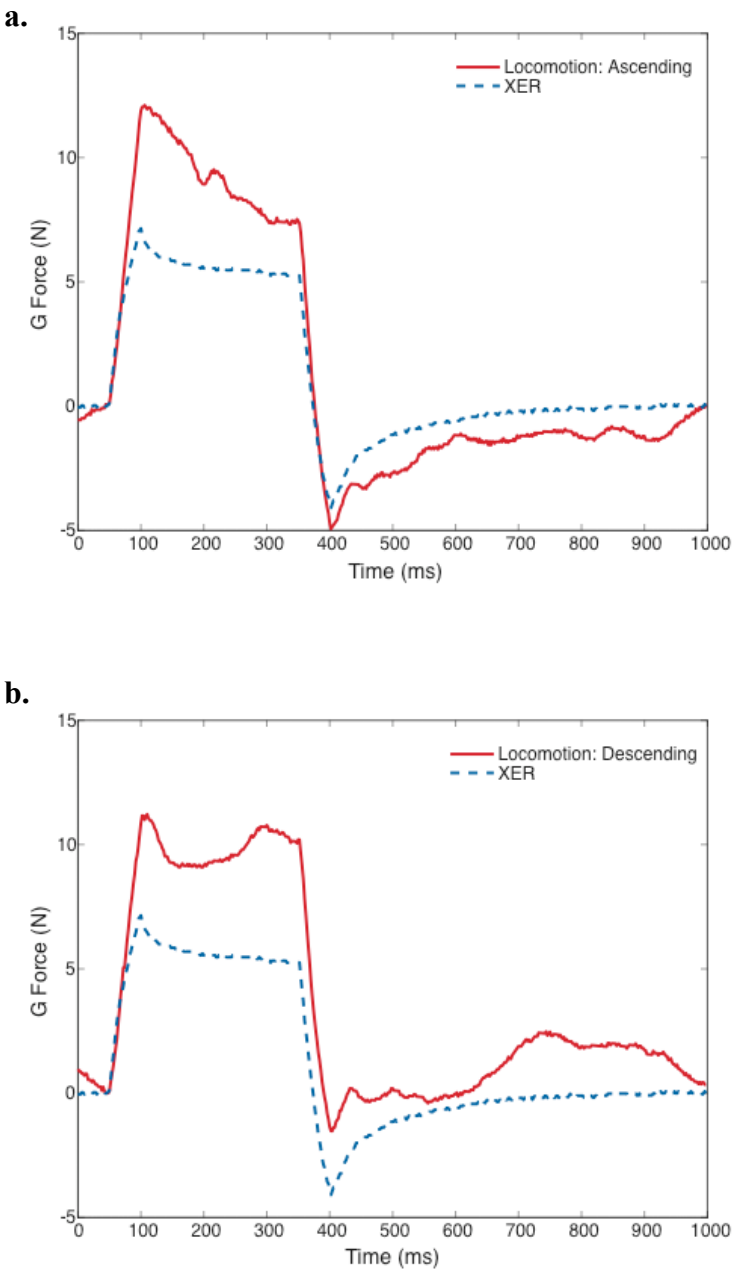


Figure 3.7. (a) Stretch-evoked autogenic excitation in G during locomotion during the ascending phase and (b) the descending phase. Two traces matched at 10 N background force in G during locomotion (solid line) and during XER (dashed line) have been superimposed to illustrate the magnitude of autogenic excitation of G during locomotion in the ascending phase (a) versus the descending phase (b). (c) Traces from ascending, descending, and peak of stance, and XER superimposed to emphasize the relative strengths of G autogenic excitation during locomotion.

c.

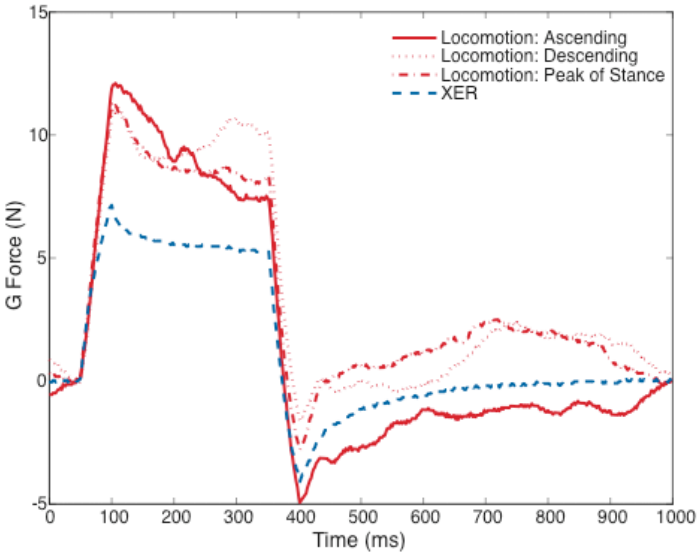


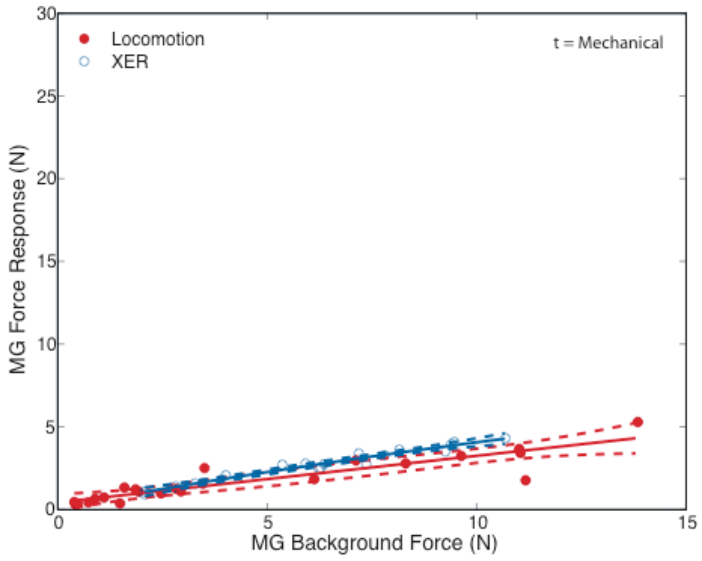
Figure 3.7 (continued)

of stance during locomotion and XER (dashed) have been superimposed to illustrate that G responses obtained during all periods of stance exhibit increases in autogenic gain when compared to responses obtained during XER (Figure 3.7c). Furthermore, traces occurring on the upswing exhibited greater stiffness, therefore achieved higher forces than did those traces occurring on the downswing or plateau.

3.3.2 Autogenic feedback increases selectively in MG

Autogenic feedback from MG was examined in 8 experiments, all of which exhibited stepping behavior. Of these experiments, 1 experiment was eliminated due to MG nerve damage, and another due to poor XER activation. Of the 6 remaining experiments, all exhibited increased autogenic excitation from MG during locomotion. Figure 3.8a demonstrates that there is a statistical difference between the two populations of data during the mechanical phase, thus suggesting that the depression is due to a mechanical effect. Statistical tests yield a p-value<0.01, thus supporting that these data populations are separate. Figure 3.8c depicts responses acquired 50 ms following the beginning of the ramp, representing the dynamic phase of the autogenic feedback from MG during locomotion. While there is slight overlap in the confidence intervals at the lowest background force, the two populations are clearly separated at higher background forces. Multiple regression yielded $p<0.01$, thus statistically proving that these populations are distinctly different. Of the 6 experiments exhibiting autogenic excitation from MG, 3 exhibited this force-dependent trend in the autogenic excitation for the dynamic response, 1 displayed greater excitation at lower and higher background forces

a.



b.

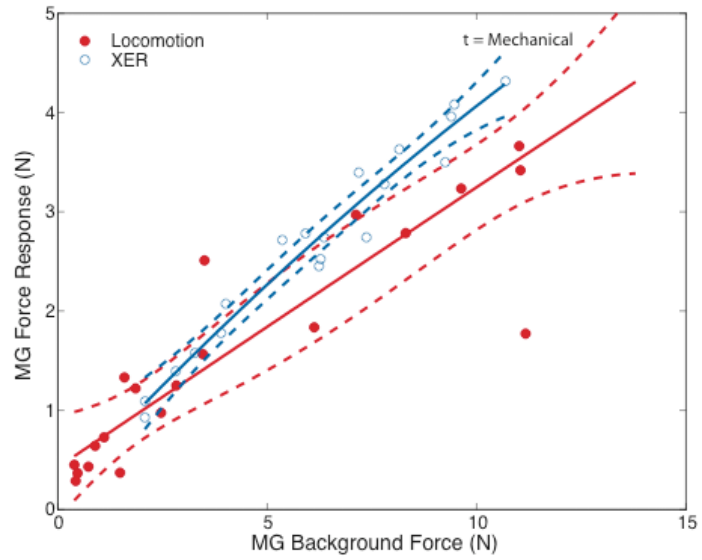
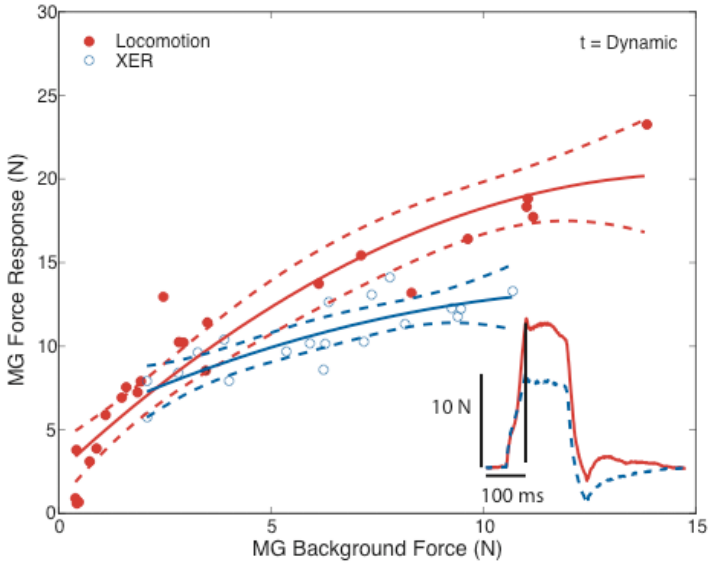


Figure 3.8 (a) Autogenic responses for MG during locomotion and XER for the mechanical phase. (b) Expanded view of the autogenic responses of MG during locomotion and XER for the mechanical phase. (c) Autogenic excitation of MG during locomotion and XER for the dynamic phase and (d) the static phase. The same conventions as Figure 3.3 apply. Two traces matched at 8 N background force in MG from locomotion (solid line) and XER (dashed line) have been superimposed to illustrate the magnitude of excitation from MG, and the vertical line indicates the sample time. (e) Two traces matched at a background force of 3 N for locomotion (solid line) and XER (dotted line) expanded in time to illustrate that the latency of autogenic excitation. (f) Two traces matched at a background force of 10 N for locomotion (solid line) and XER (dotted line) expanded in time to illustrate that the latency of autogenic excitation.

c.



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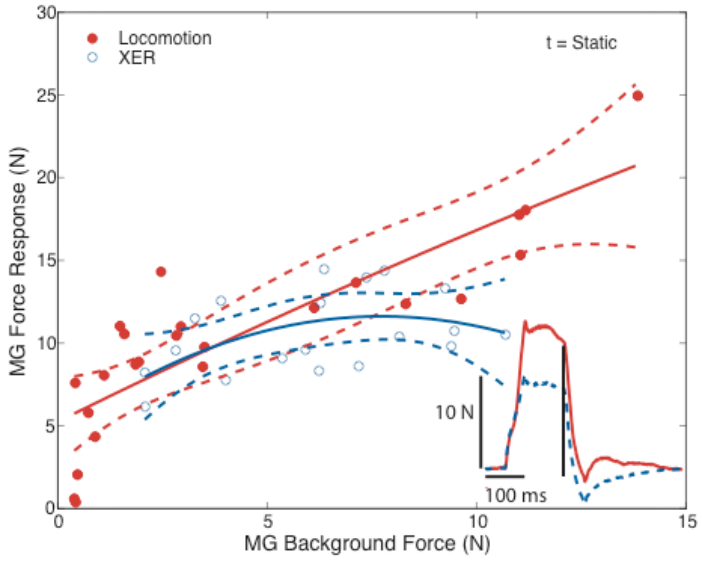
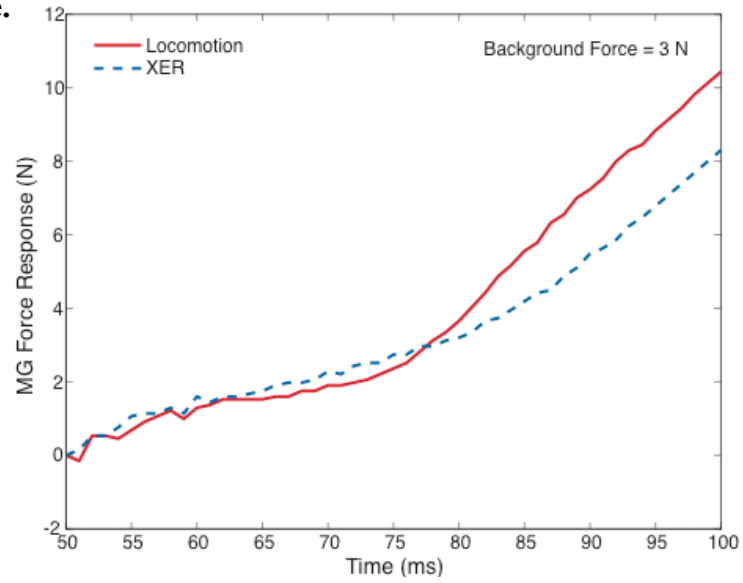


Figure 3.8 (continued)

e.



f.

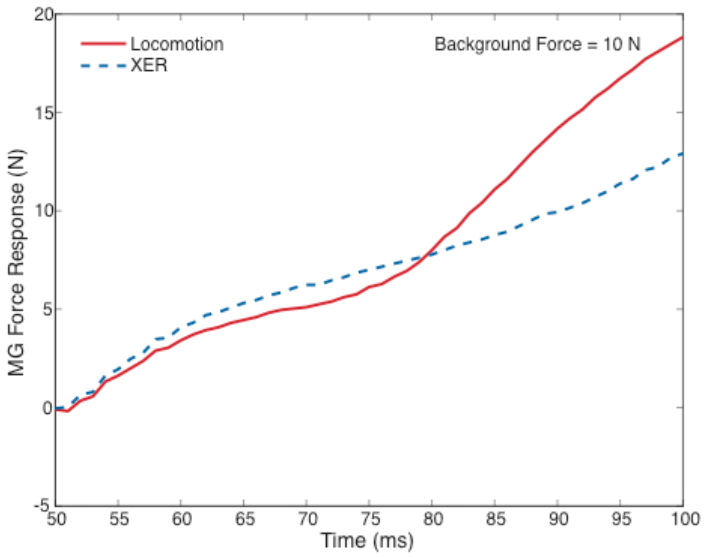


Figure 3.8 (continued)

(parabolic), 1 exhibited excitation that remained constant with increasing background force, and 1 decreased excitation with increasing background force.

Force responses in the recipient muscle, MG, during locomotion (solid line) and during XER (dashed line) are inset in Figure 3.8c and Figure 3.8d to illustrate the magnitude and timing of autogenic excitation from MG. Each trace was chosen at the mean MG background force of 8 N, and the baselines were subtracted to better visualize the excitation. The autogenic excitation from MG was force-dependent over the 100 ms hold period. Figure 3.8d depicts force responses in the static phase of autogenic feedback and illustrates the increase in variability when compared to the dynamic phase (Figure 3.8c). The same trends as presented for the dynamic phase, as described above, applied for the autogenic gain increases in MG during the static phase.

To evaluate the time course of the autogenic excitation, individual traces from locomotion and XER matched at 3 N and 10 N, Figures 3.8e and 3.8f respectively, have been plotted at the beginning of the stretch. Traces matched at 3N correspond to stretches occurring in the swing phase of locomotion, and illustrate that there is a mechanical effect that results in decreased force responses during this phase of locomotion (Figure 3.8e). However, the excitatory reflex response overcomes this mechanical effect with a latency of 27 ms following the beginning of the stretch, which is slightly longer than that of the entire G. Traces matched at 10N represent the stance phase of locomotion, as shown in Figure 3.8f. The mechanical response is similar in magnitude to the swing

phase, yet the reflex response has a slightly longer latency following the beginning of stretch.

To assess the magnitude and time-course of the autogenic excitation from MG during locomotion, a three-dimensional difference plot was created as previously described, as shown in Figure 3.9. The surface represents the difference between the force responses obtained during locomotion and XER as a function of time and MG background force. The maximum response difference is approximately 7 N, and remains force-dependent during the hold period.

In contrast to the autogenic excitation exhibited by MG during locomotion, LG demonstrated no increase in autogenic gain (Figure 3.10). Of the 6 experiments evaluating the strength and sign of autogenic feedback from LG, 2 were eliminated due to poor LG activation. Of the 4 remaining experiments, all exhibited stepping behavior, and only 1 experiment yielded an increase in autogenic excitation, compared to XER, from LG. The remaining experiments yielded no statistical significance between responses obtained during locomotion and XER. Figure 3.10a demonstrates that there is no statistical difference between the two populations of data during the mechanical phase. Statistical tests yield a p-value<0.01, thus suggesting that these data populations are not separate. Figure 3.10b depicts responses acquired 50 ms following the beginning of the ramp, representing the dynamic phase of the autogenic feedback from LG during locomotion.

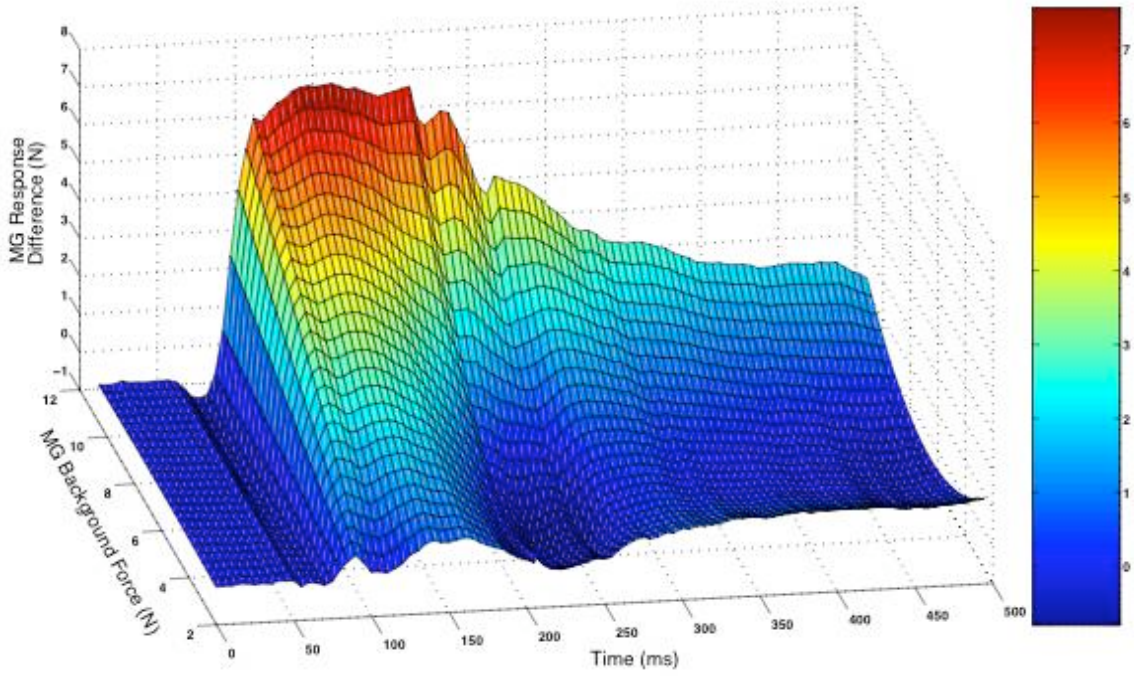


Figure 3.9 A three-dimensional surface that quantifies the difference in the magnitude of autogenic excitation from MG during locomotion compared to XER as a function of force and time. The same conventions as Figure 3.4 apply.

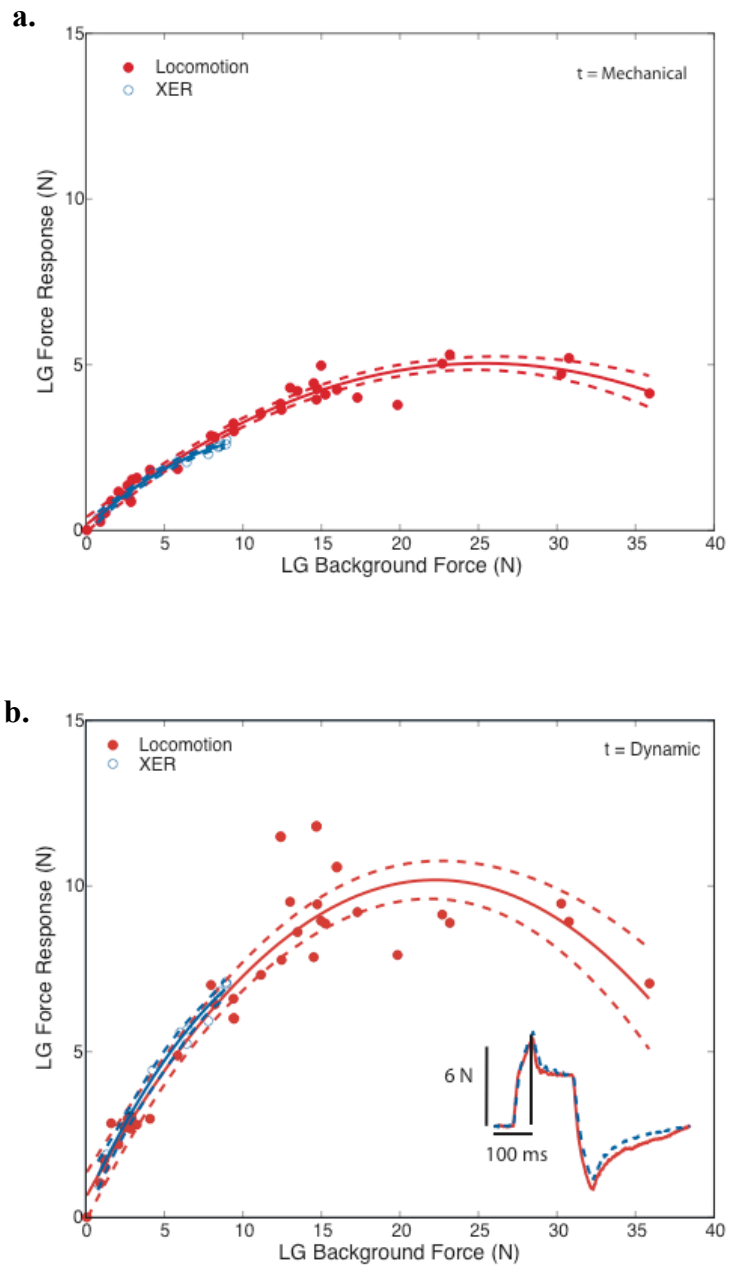


Figure 3.10 (a) Autogenic responses for LG during locomotion and XER for the mechanical phase, (b) the dynamic phase, and (c) the static phase. The same conventions as Figure 3.3 apply. Two traces matched at 9 N background force in LG from locomotion (solid line) and XER (dashed line) have been superimposed to illustrate the lack of excitation from LG, and the vertical line indicates the sample time.

c.

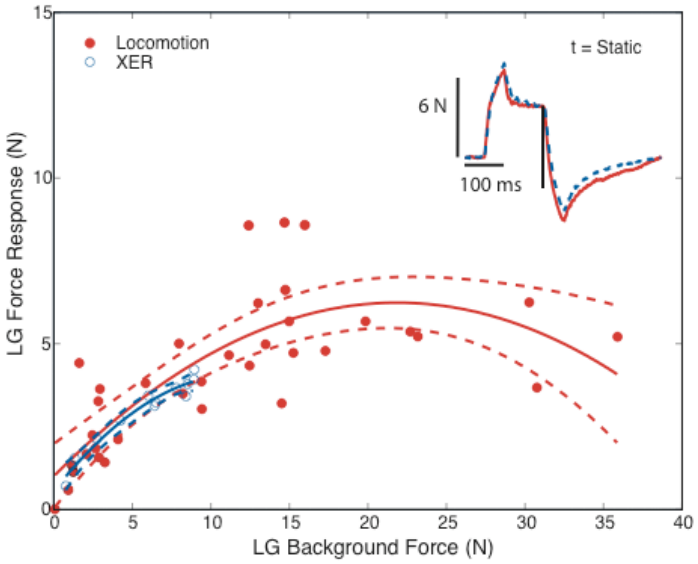


Figure 3.10 (continued)

Force responses in LG during locomotion (solid line) and during XER (dashed line) are inset in Figures 3.10b and 3.10c to illustrate that the traces are indistinguishable. Each trace was chosen at the mean LG background force of 9 N, and the baselines were subtracted. Figure 3.10c depicts force responses in the static phase of LG autogenic feedback and illustrates the completely overlapping populations and decrease in force responses when compared with the dynamic phase (Figure 3.10b).

To contrast the magnitude and time-course of the autogenic feedback from LG with the feedback in MG during locomotion, a three-dimensional difference plot was created as previously described, as shown in Figure 3.11. The surface represents the difference between the force responses obtained during locomotion and XER as a function of time and LG background force. The maximum response difference during the ramp and hold stretch in LG is approximately 1 N (Figure 3.11) when compared to 7 N in MG (Figure 3.9), both of which occur at the highest background force. Both three-dimensional plots have been placed on the same scale for comparison.

3.3.3 Autogenic feedback decreases slightly in PLAN during locomotion

The decrease in autogenic feedback in PLAN during locomotion was examined in eighteen total experiments, all of which exhibited stepping behavior. Of these experiments, 5 were eliminated due to poor PLAN activation during XER. Of the remaining 13 experiments evaluating the force responses in PLAN both during locomotion and XER, 7 experiments demonstrated a decrease in autogenic gain in PLAN, 1 experiment exhibited an increase in autogenic gain, and 4 preparations did not exhibit

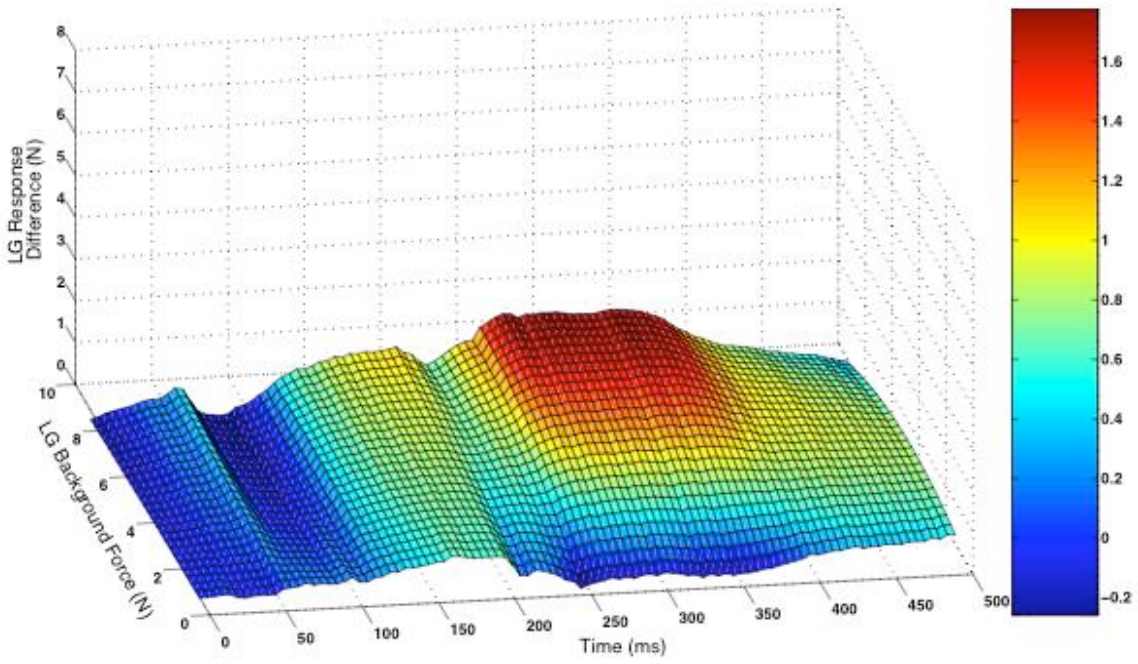


Figure 3.11 A three-dimensional surface that quantifies the difference in the magnitude of autogenic excitation from LG during locomotion as compared to XER as a function of force and time. The same conventions as Figure 3.5 apply.

increases in autogenic gain during locomotion, but rather demonstrated no significant difference in the force responses between the two behavioral states. Additionally, 1 experiment demonstrated a mixed response with a decrease in gain at lower background force and an increase in gain at higher background forces. Figure 3.12 depicts a representative example of the decrease in autogenic feedback in PLAN during stepping. This depression is not due to a purely mechanical event, as demonstrated by the overlapping force responses shown in Figure3.12a. There is a small but significant decrease in autogenic gain between locomotion and XER in the dynamic phase that is slightly force-dependent, as depicted in Figure 3.12b. Of the 7 experiments exhibiting autogenic depression in PLAN, 4 exhibited a force-dependent trend for the dynamic response, 2 displayed greater decreases at lower and higher background forces (parabolic), and 1 possessed little overlapping background force for the two populations, therefore a clear relationship could not be determined. For the static response, 1 experiment that showed no statistical difference for the dynamic phase exhibited a depression that decreased with increasing background force.

The force responses are somewhat lower in the static phase (Figure 3.12c) than in the dynamic phase (Figure 3.12b), and both populations have relatively tight 95% confidence intervals, indicating that the polynomial fits effectively represent the data. Multiple regression yielded $p<0.01$ for both the dynamic and static phases, thus suggesting that these populations are not statistically different. Traces matched at a background force of 6 N inset in Figures 3.12b and 3.12c illustrate the similar responses for both behavioral states.

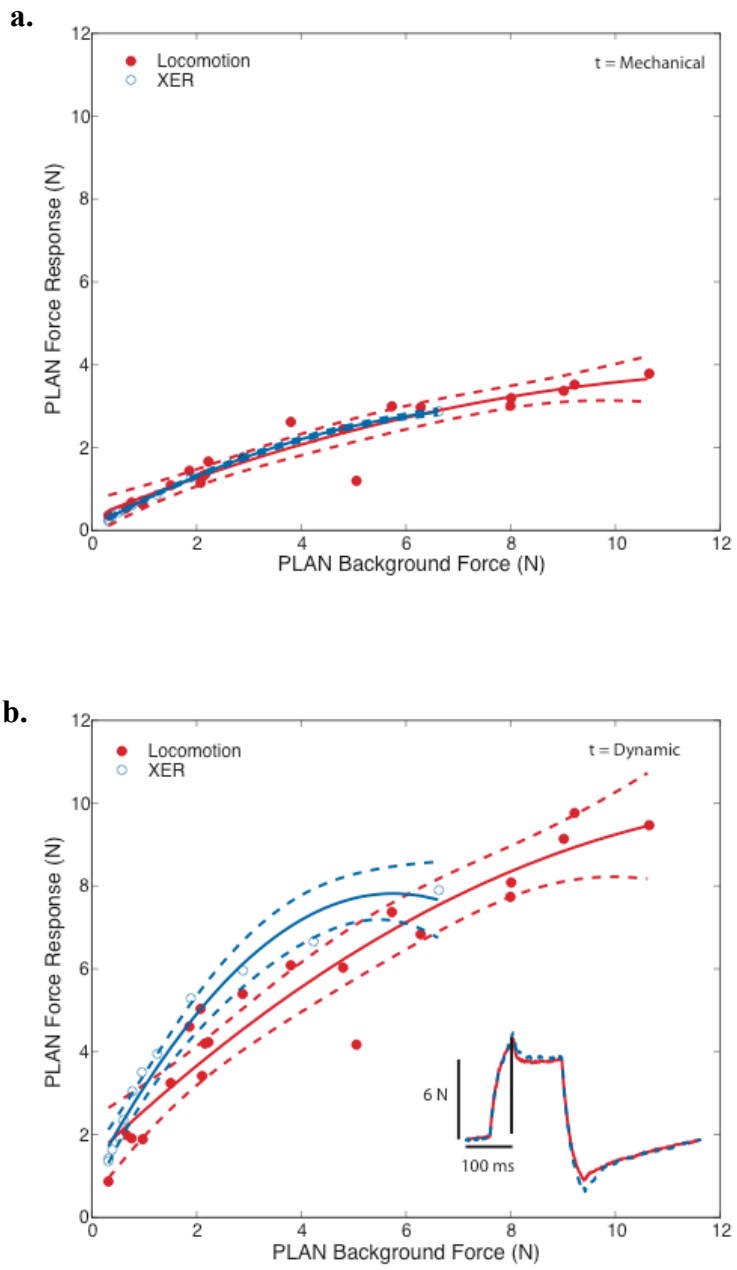


Figure 3.12 (a) Autogenic responses for PLAN during locomotion and XER for the mechanical phase, (b) the dynamic phase, and (c) the static phase. The same conventions as Figure 3.3 apply. Two traces matched at 6 N background force in PLAN from locomotion (solid line) and XER (dashed line) have been superimposed to illustrate the lack of excitation from PLAN, and the vertical line indicates the sample time.

c.

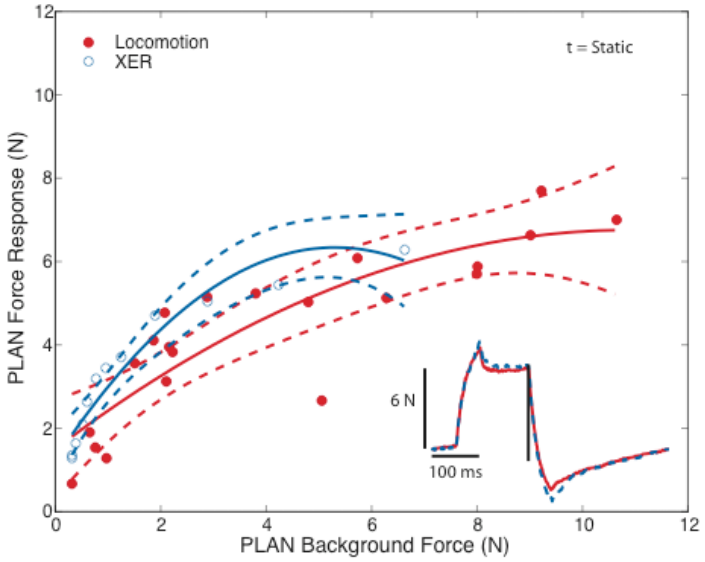


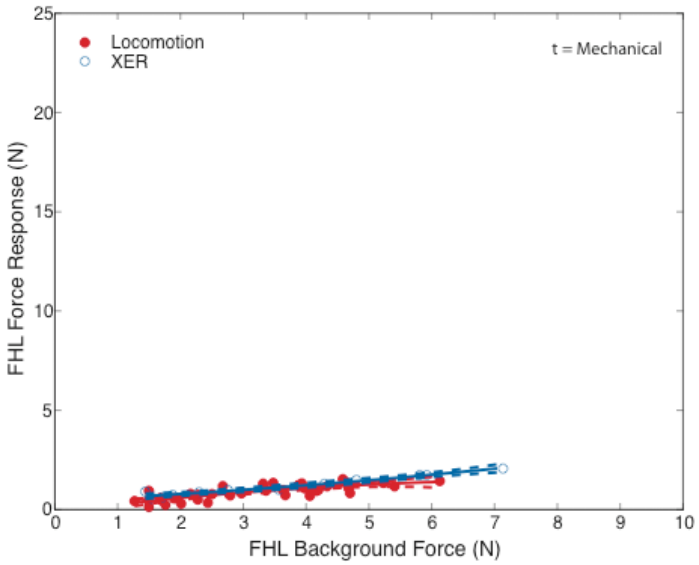
Figure 3.12 (continued)

3.3.4 Autogenic feedback decreases in FHL during locomotion

The decrease in autogenic feedback in FHL during locomotion was examined in twenty-five total experiments, all of which exhibited stepping behavior. Seven experiments were eliminated due to poor FHL activation during XER. Of these remaining eighteen experiments evaluating the force responses in FHL both during locomotion and XER, eleven demonstrated decreased autogenic responses in FHL. The remaining 7 preparations demonstrated no significant difference in the force responses between the two behavioral states. Figure 3.13 depicts a representative example of the decrease in autogenic feedback in FHL during stepping. This is not due to a purely mechanical event, as demonstrated by the overlapping force responses shown in Figure 3.13a. There is a decrease in autogenic gain for the dynamic timepoint that is slightly force-dependent, as depicted in Figure 3.13b. Of the eleven experiments exhibiting decreases in FHL autogenic gain, 10 exhibited a force-dependent trend in the decrease for the dynamic response and a clear relationship could not be determined for the remaining experiment. One experiment that exhibited no statistical difference between the two populations yielded force-dependent depression for the static phase. Force responses are less force-dependent in the static phase (Figure 3.13c) than in the dynamic phase (Figure 3.13b). In this example, multiple regression yielded $p<0.01$ for both the dynamic and static phases, thus statistically proving that these populations are distinctly different.

Traces inset in Figures 3.13b and 3.13c are force responses during locomotion (solid line) and during XER (dashed line). The background force for both conditions was

a.



b.

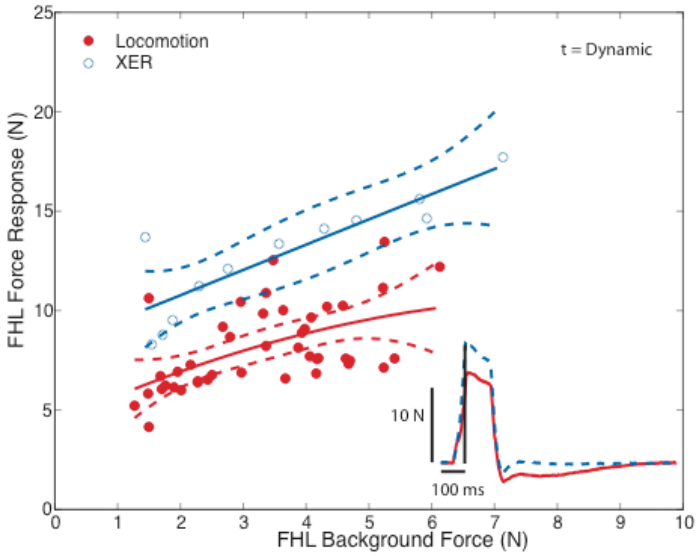
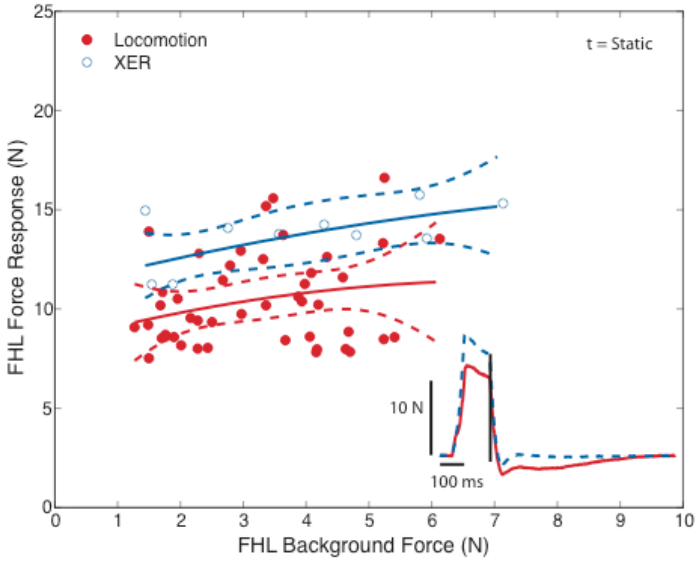


Figure 3.13 (a) Autogenic responses for FHL during locomotion and XER for the mechanical phase, (b) the dynamic phase, and (c) the static phase. The same conventions as Figure 3.3 apply. Two traces matched at 6 N background force in FHL from locomotion (solid line) and XER (dashed line) have been superimposed to illustrate the magnitude of depression in FHL, and the vertical line indicates the sample time. (d) Two traces matched at a background force of 3 N for locomotion (solid line) and XER (dotted line) expanded in time. (e) Two traces matched at a background force of 6 N for locomotion (solid line) and XER (dotted line) expanded in time.

c.



d.

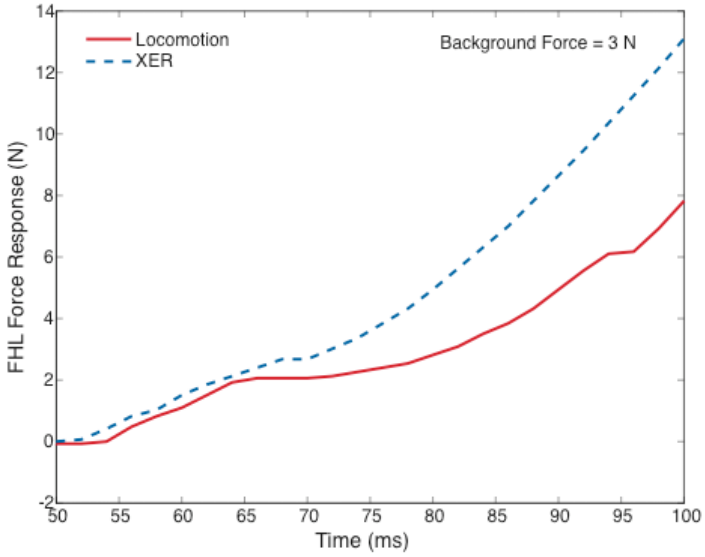


Figure 3.13 (continued)

e.

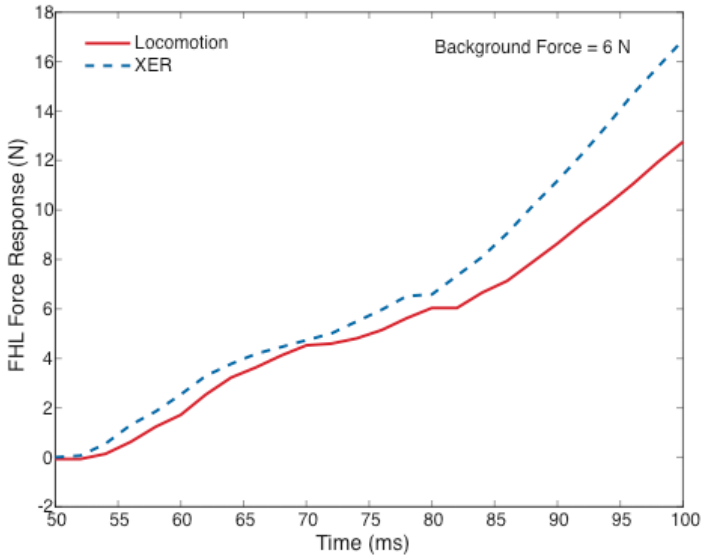


Figure 3.13 (continued)

matched at the mean background force of approximately 6 N. Baselines were subtracted from both traces to illustrate the magnitude of the autogenic depression in FHL.

Individual traces from locomotion and XER matched at 3 N and 6 N, Figures 3.13d and 3.13e respectively, have been plotted at the beginning of the stretch. Traces matched at 3N illustrate that there is a significant mechanical effect that results in decreased force responses, while an additional depression occurs at approximately 15 ms, causing the two traces to diverge (Figure 3.13d). Traces matched at 6N illustrate an initial mechanical response of a similar magnitude (Figure 3.13e), yet the depression has a slightly longer latency of approximately 20 ms

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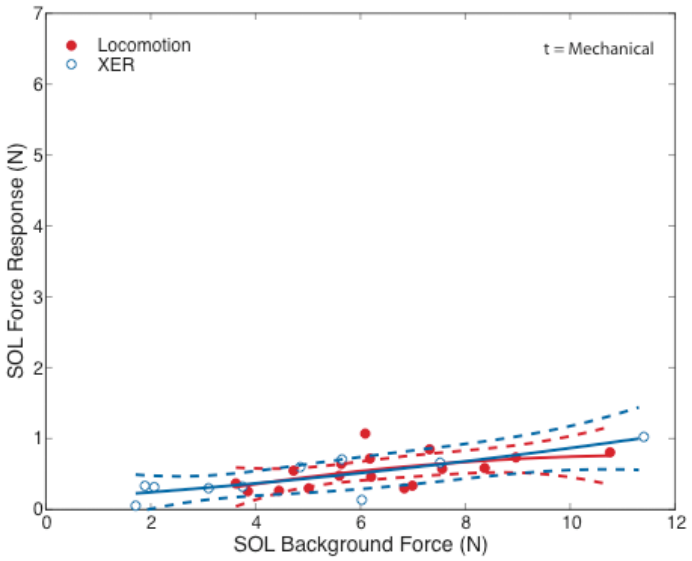
3.3.5 Autogenic feedback decreases in SOL during locomotion

The decrease in autogenic feedback in SOL during locomotion was examined in 6 total experiments, all of which exhibited stepping behavior. One experiment was eliminated due to non-overlapping background forces for the two behavioral conditions, locomotion and XER. Of the 5 remaining experiments evaluating the force responses in SOL both during locomotion and XER, 4 demonstrated autogenic depression in SOL. The remaining preparation demonstrated no significant difference in the force responses between the two behavioral states. Figure 3.14 depicts a representative example of the decrease in autogenic feedback in SOL during stepping. This decrease in autogenic gain is not due to a purely mechanical event, as demonstrated by the overlapping force responses shown in Figure 3.14a. There is a clear decrease in autogenic gain between

a.



b.

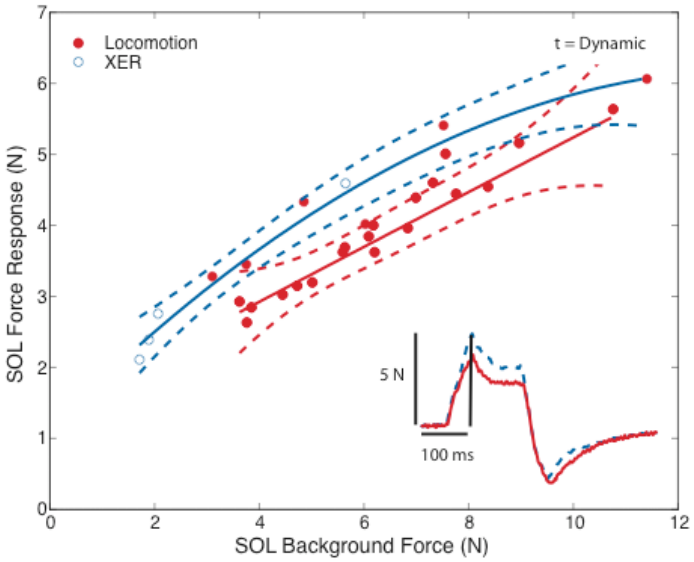
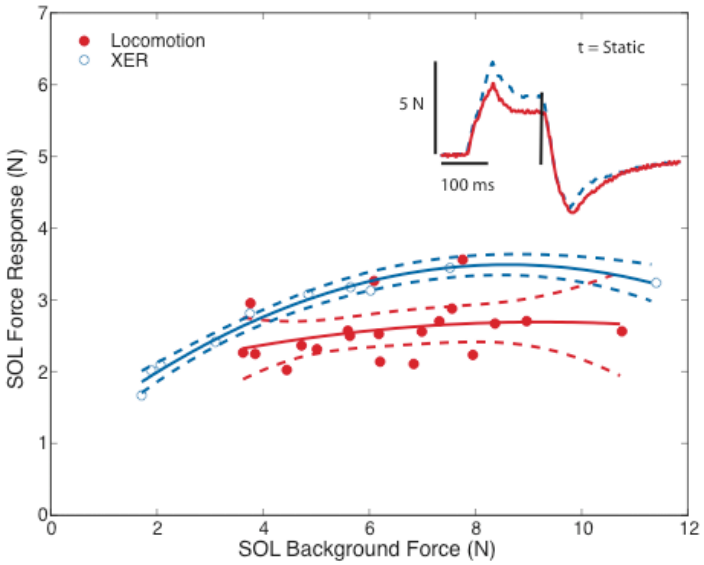


Figure 3.14 (a) No statistical difference in autogenic responses for SOL during locomotion and XER for the mechanical phase, (b) the dynamic phase, and (c) the static phase. The same conventions as Figure 3.3 apply. Two traces matched at 6 N background force in SOL from locomotion (solid line) and XER (dashed line) have been superimposed to illustrate the magnitude of depression from SOL, and the vertical line indicates the sample time. (d) Two traces matched at a background force of 4 N for locomotion (solid line) and XER (dotted line) expanded in time. (e) Two traces matched at a background force of 10 N for locomotion (solid line) and XER (dotted line) expanded in time.

c.



d.

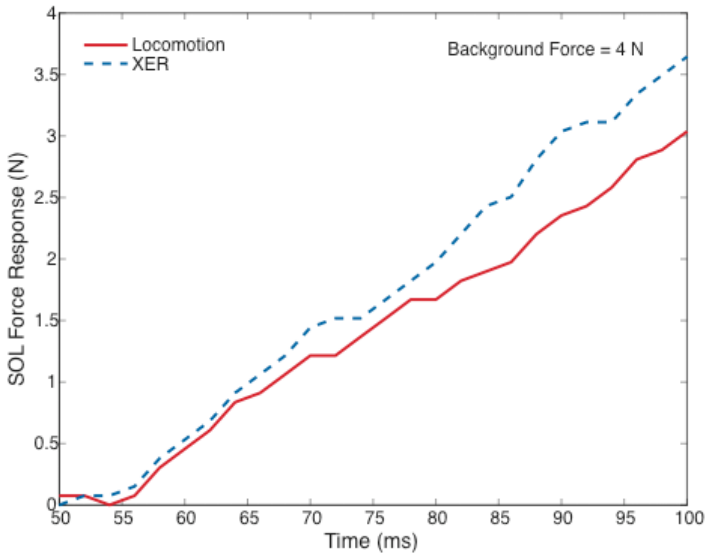


Figure 3.14 (continued)

e.

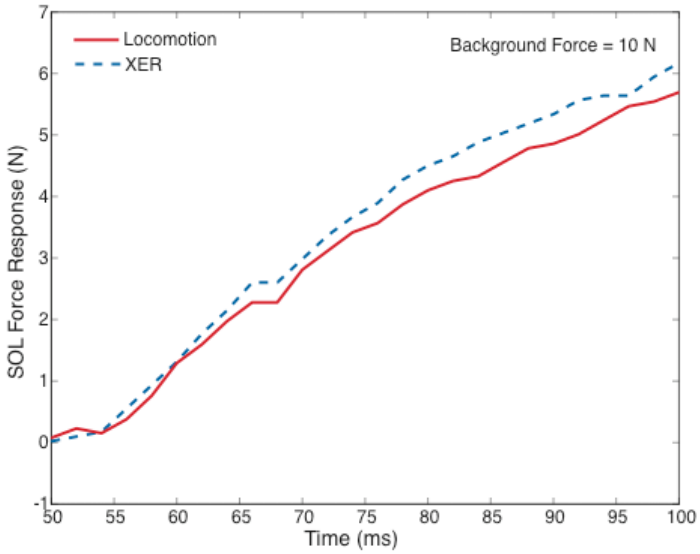


Figure 3.14 (continued)

locomotion and XER in the dynamic phase that is slightly force-dependent, as depicted in Figure 3.14b. Of the 4 experiments exhibiting autogenic depression in SOL, 3 exhibited a force-dependent trend in the depression for the dynamic response and 1 experiment contained no significant trend in the depression.

The force responses are less force-dependent in the static phase (Figure 3.14c) than in the dynamic phase (Figure 3.14b), this also corresponds to non-overlapping confidence intervals. Statistical test demonstrate that force responses obtained for both the dynamic and static timepoints are distinct. Traces matched at 6 N and baseline subtracted (Figures 3.14b and 3.14c) are force responses during locomotion (solid line) and during XER (dashed line); these illustrate the magnitude of the autogenic depression in SOL.

Individual traces from locomotion and XER matched at 4 N and10 N, Figures 3.14d and 3.14e respectively, further evaluate SOL autogenic responses. Traces matched at 4N illustrate a significant mechanical effect (Figure 3.14d). Additionally, the traces begin to diverge at approximately 15 ms (Figure 3.14d). Traces matched at 10N, representing the stance phase of locomotion (Figure 3.14e) illustrate a mechanical effect of similar magnitude.

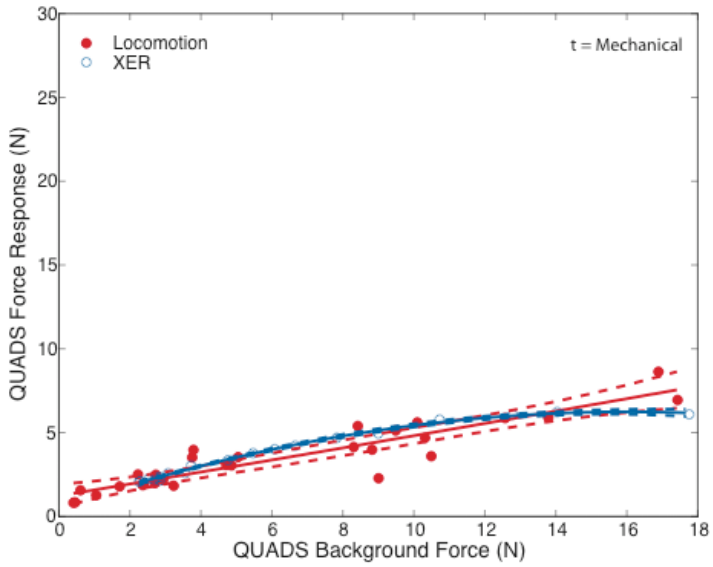
3.3.6 Autogenic feedback decreases in QUADS during locomotion

The decrease in autogenic feedback in QUADS during locomotion was examined in 6 total experiments, all of which exhibited spontaneous stepping behavior. One

experiment was eliminated due to poor QUADS activation during XER. Of the remaining 5 experiments evaluating the force responses in QUADS both during locomotion and XER, 3 demonstrated autogenic depression in QUADS. The remaining 2 preparations demonstrated no significant difference in the force responses between the two behavioral states. Figure 3.15 depicts a representative example of the decrease in autogenic feedback in QUADS during stepping. This autogenic depression could be partially due to a mechanical event, as demonstrated by the slight decrease in locomotion force responses as shown in Figure 3.15a. There is a decrease in autogenic gain between locomotion and XER in the dynamic phase, as depicted in Figure 3.15b. Of the 3 experiments exhibiting autogenic depression in QUADS, the trends were variable; 1 exhibited increasing depression with increasing background force, 1 decreased depression with increasing background force, and 1 did not change with changing background force. An additional experiment that showed no significant difference in the two populations during the dynamic phase, yielded a depression that was independent of changing background force for the static phase.

The force responses during locomotion are much lower in the static phase (Figure 3.15c) than in the dynamic phase (Figure 3.15d) Statistical tests illustrate that responses for both the dynamic and static phases are distinct. Traces matched at 10 N inset in Figures 3.15b and 3.15c are force responses during locomotion (solid line) and during XER (dashed line) that illustrate the magnitude of the autogenic depression in QUADS.

a.



b.

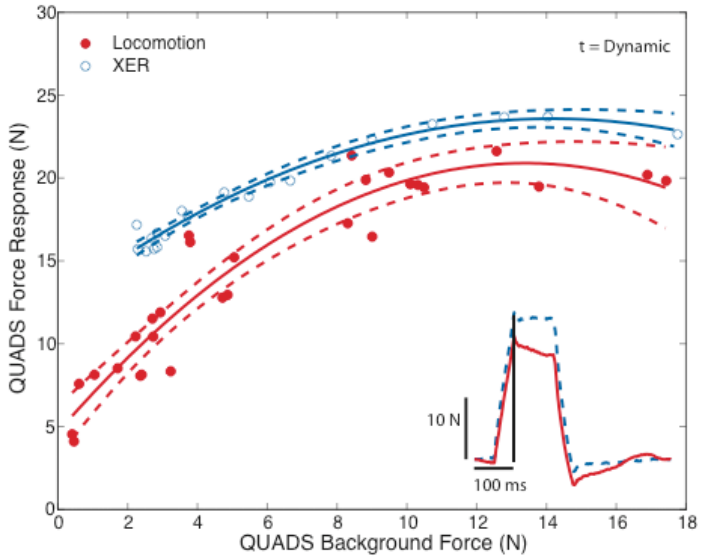
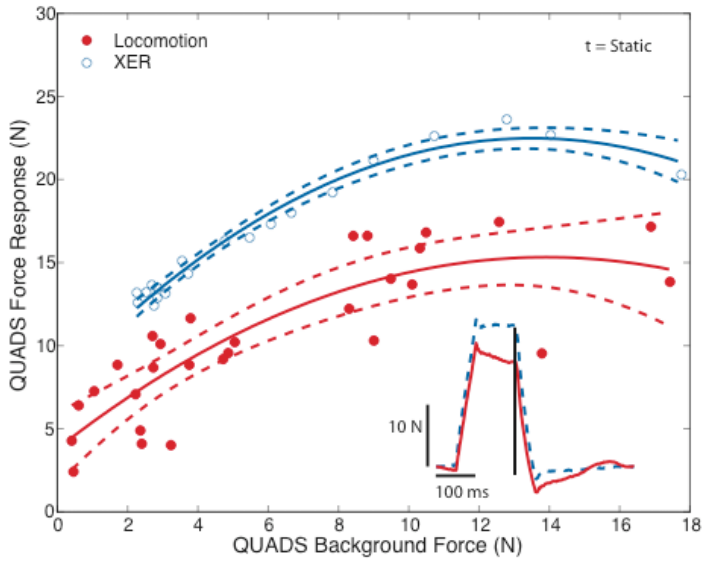


Figure 3.15 (a) Autogenic responses for QUADS during locomotion and XER for the mechanical phase, (b) the dynamic phase, and (c) the static phase. The same conventions as Figure 3.3 apply. Two traces matched at 10 N background force in QUADS from locomotion (solid line) and XER (dashed line) have been superimposed to illustrate the magnitude of depression from QUADS, and the vertical line indicates the sample time. (d) Two traces matched at a background force of 3 N for locomotion (solid line) and XER (dotted line) expanded in time. (e) Two traces matched at a background force of 18 N for locomotion (solid line) and XER (dotted line) expanded in time.

c.



d.

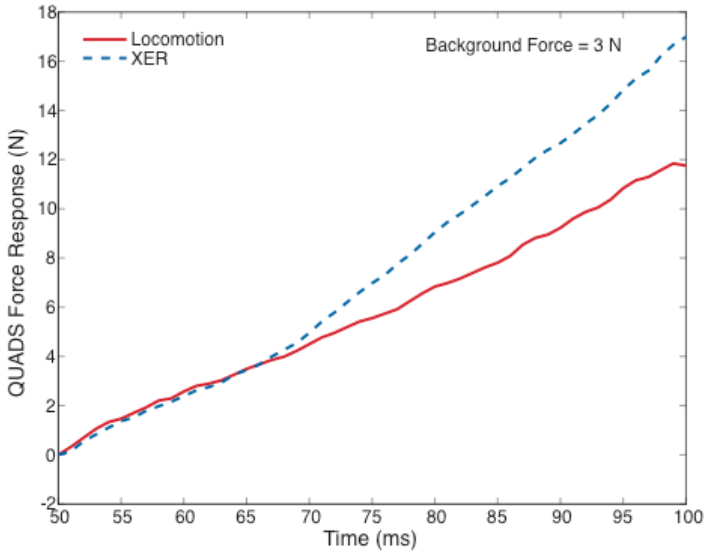


Figure 3.15 (continued)

e.

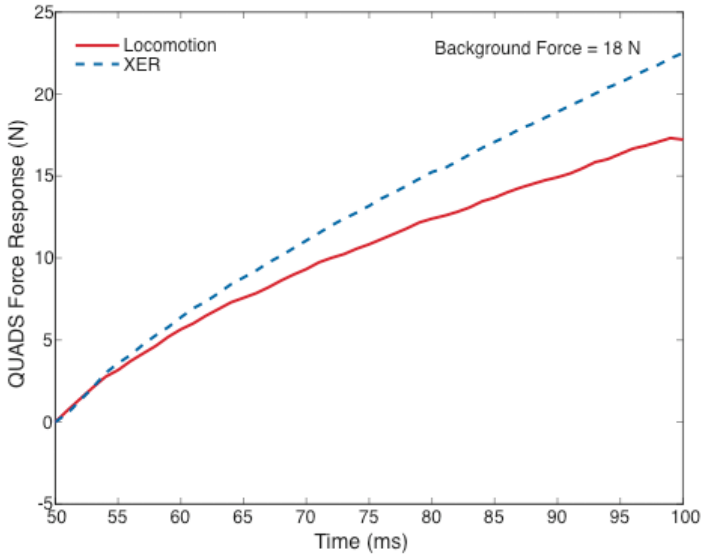


Figure 3.15 (continued)

Individual traces from locomotion and XER matched at 3 N and 18 N, Figures 3.15d and 3.15e respectively, have been plotted at the beginning of the stretch. Traces matched at 3N illustrate that there is not a significant mechanical (Figure 3.15d). The two traces begin to diverge at approximately 15 ms (Figure 3.15d). Traces matched at 18 N (Figure 3.15e) illustrate the mechanical response and a gradual divergence of the two force responses.

3.4 Discussion

In this study, we have attempted to address the organization and functional implications of autogenic feedback among ankle extensors, namely G, MG, LG, PLAN, FHL, SOL, and knee extensors, QUADS, during spontaneous locomotion in premammillary decerebrate cats. Recent studies in our laboratory suggest that rather than a global change in force feedback from inhibition to excitation during locomotion, heterogenic pathways between ankle extensor muscles remain inhibitory (Ross and Nichols Submitted). Furthermore, we found that positive force feedback manifested itself as an increase in autogenic gain, particularly in MG, during all phases of stance during locomotion. The remaining muscles exhibited a depression in autogenic feedback between the two behavioral states. Despite the variability in locomotion, from spontaneous stepping with oscillations in the background force to aided stepping with stimulation, the results remained consistent across preparations. The following discussion addresses these results from both a neural and biomechanical perspective, and evaluates the possible functions of selective autogenic positive force feedback during locomotion.

The finding of reduced autogenic responses during locomotion was unexpected, especially in view of evidence that negative force feedback is supposed to be suppressed. Both neural and mechanical factors can contribute to the decreased autogenic force responses in hindlimb extensors during locomotion. Mechanically, the muscles are held isometrically, yet the internal length of the muscle undergoes shortening and lengthening during locomotion. This constant changing length increases the rate of cross-bridge detachment and turnover, which decreases the stiffness, and therefore force output of the muscle (Joyce et al. 1969; Rack and Westbury 1969; Kirsch et al. 1994). This effect was insufficient to explain the depression, and in some cases, the onset of depression had a latency similar to that of a reflexive effect. In addition to this mechanical effect, the fusimotor system is altered during locomotion. Static gamma activation is increased during all phases of locomotion, thus decreasing the muscle spindle's sensitivity to stretch (Taylor et al. 1985; Bennett et al. 1996; Murphy et al. 2002). The combined effect of decreased stiffness and sensitivity to stretch could enhance the inhibitory responses exhibited during the mechanical, dynamic, and static phases. Despite this phenomenon, MG overcomes the mechanical effects to exhibit increases in autogenic gain during locomotion (Ross et al. 2002; Ross et al. 2005).

While positive force feedback is exhibited in MG during locomotion, our findings suggest that an increase in autogenic gain is not widespread among all hindlimb extensors. Previous studies in fictive locomotion preparations utilizing electrical stimulation of the mesencephalic locomotor region (MLR) or drugs, such as clonidine or L-DOPA, to initiate stepping have supported the theory that positive force feedback is

widespread (Guertin et al. 1995; McCrea et al. 1995; Donelan and Pearson 2004). Recent studies have observed the emergence of an excitatory pathway during locomotion between muscles known to exchange predominately force-dependent inhibition, such as MG and PLAN, by utilizing electrical nerve stimulation at group I strength (Pearson and Collins 1993; Guertin et al. 1995). It is plausible that the mode of activation, whether electrical or natural, could partially explain the variation in the expression of positive force feedback in the current study (Enriquez-Denton et al. 2002). Another factor could be the method of inducing stepping in a given preparation. Spontaneously locomoting cats might yield a different distribution in positive force feedback when compared to cats requiring drugs or MLR stimulation to evoke stepping.

The distribution of autogenic force feedback remains largely unexplored in both locomoting and non-locomoting cat preparations. Electrophysiological studies in anesthetized cats do suggest that homonymous projections from Golgi tendon organs onto extensor motoneurons are weak (Eccles et al. 1957). However, this autogenic inhibition has not been well documented in decerebrate cats under conditions of XER. Studies in either spontaneously or fictively locomoting cats have evaluated the strength and sign of heterogenic feedback pathways by stimulating nerves at group-I strength (Pearson and Collins 1993; Gossard et al. 1994; Guertin et al. 1995). However, autogenic responses were not reported. One study that does provide some insight into increases in autogenic gain during locomotion was completed in the spontaneously locomoting cat using muscle stretch. Stretching the entire triceps surae prolonged the extensor phase of locomotion, as measured by an increase in the magnitude of MG EMG (Duysens and

Pearson 1980). Incidentally, MG is the same muscle that exhibits the increase in autogenic gain in the present study.

The distribution of positive force feedback during locomotion could vary with the required motor task. Treadmill locomotion yields muscle activation patterns that closely resemble those observed during level walking (Smith et al. 1998; Gottschall et al. 2005). In this study, positive force feedback occurs specifically in MG during level walking. If positive force feedback reinforces contractile force during load-bearing, one might expect more widespread positive force feedback during up-slope walking, when antigravity muscles produce larger forces (Gregor et al. 2006). The reversal of force-dependent inhibition to excitation is documented in fictively locomoting cats (Gossard et al. 1994; McCrea et al. 1995) where there is a lack of sensory feedback modulation, and the specific motor task is unknown. Therefore, the differences in results could be due to differences in behavioral state.

Analyzing the relative anatomical differences among ankle extensors could provide a rationale for MG serving as the major source of excitatory autogenic feedback during locomotion. Muscle stretch activates both length and force dependent feedback from muscle spindles and Golgi tendon organs, respectively. It is difficult to ascertain whether or not the increase in autogenic gain is due to an increase in drive to excitatory length feedback, or if the excitation is due to the expression of positive force feedback. Anatomically, MG possesses twice as many muscle spindles as LG. This theory is contradicted by the fact that FHL actually possesses significantly more length receptors

than the remaining muscles, including MG (Chin et al. 1962). The relative numbers of Golgi tendon organs for each of the muscles is unknown.

Biomechanics could provide an alternative explanation for the distribution of autogenic excitation among hindlimb extensors. During level walking, MG produces a greater force and peak moment than LG, as measured by implanted force buckles in intact cats (Fowler et al. 1993). Variability in the peak moment and force in PLAN is significantly higher than MG, yet the overall moment curve mimics that of the metatarsophalangeal joint, due to the insertion onto the flexor digitorum brevis tendon (Fowler et al. 1993). Additionally, the hindlimb extensors in this study, G, PLAN, SOL, and FHL, all generate torques about the ankle joint (Lawrence and Nichols 1999). MG generates the largest non-sagittal moment about the ankle, and this could increase the stability of the limb during locomotion.

While G is the greatest source of autogenic excitation during locomotion, it also serves as the greatest source of heterogenic inhibition (Ross and Nichols Submitted). It is known from electrophysiological studies in anesthetized cats and in decerebrate cats using muscle stretch that force dependent feedback from Golgi tendon organs is weak autogenically and distributed widely to muscles crossing joints (Eccles et al. 1957; Nichols 1989; Nichols 1999). A localized increase in autogenic gain in MG could serve as a loading reflex that serves multiple joints. The increase in force would, in turn, activate heterogenic force-dependent pathways, thus spreading inhibition across multiple joints and axes of rotation. This widespread inhibition, in combination with the localized

positive feedback, could contribute to the regulation of whole-limb stiffness (Nichols et al. 1999).

CHAPTER 4

MECHANISMS OF AUTOGENIC GAIN INCREASE

4.1 Introduction

The strength and role of positive force feedback during locomotion has largely been explored in reduced preparations using drugs or electrical stimulation to evoke stepping (Gossard et al. 1994; Guertin et al. 1995; McCrea et al. 1995). Previous studies in this laboratory have revealed that the greatest source of heterogenic inhibition and autogenic excitation during locomotion is MG (Ross et al. 2005). Evaluating stretch-evoked heterogenic responses is somewhat simplified because the neural connections among hindlimb extensors are known (Eccles et al. 1957; Eccles et al. 1957; Nichols 1989; Bonasera and Nichols 1994; Bonasera and Nichols 1996; Nichols 1999; Wilmink and Nichols 2003). Autogenic force responses are complicated because they contain both reflexive and intrinsic components. Reflex contributions from muscle spindles and Golgi tendon organs can be difficult to distinguish in stretch-evoked force responses due to similar latencies for both rapid pathways. Despite this, there are several methods to selectively activate either length-dependent or force-dependent pathways during both locomotion and XER. While one method alone may not be entirely conclusive, the combination of several methods can provide converging evidence for a mechanism of increased autogenic gain during locomotion.

One such method for distinguishing length and force mechanisms exploits existing heterogenic pathways among hindlimb extensors. While G, PLAN, and FHL exchange predominately force-dependent inhibition, MG and LG exchange

predominately length-dependent excitation (Eccles et al. 1957; Nichols 1989). In Chapter 3, we have demonstrated that MG serves as the major source of autogenic excitation during locomotion (Ross et al. 2005), however it has not been documented how this increase in gain affects synergists, namely LG. It has been widely documented that force feedback from Golgi tendon organs exhibits a reflex reversal with the initiation of locomotion, whereby locomotion depresses Ib inhibition and activates an alternate, excitatory pathway that receives input at group-I strength (Pearson and Collins 1993). While we found evidence for inhibition between muscles with known force-dependent connections, we investigated the expression of positive force feedback between muscles where no such pathways were known to exist. Evidence that the initiation of locomotion enhances not only the MG autogenic response, but also the heterogenic response from MG onto LG supports this theory of an alternate positive force feedback pathway.

While the G head separation experiments provide evidence for positive force-dependent feedback, there are experimental paradigms that selectively activate either length or force dependent pathways. The use of vibration is a method to activate length-dependent pathways from muscle spindles without generating much force. Vibrating the muscle-tendon unit at a frequency of 150 Hz activates muscle spindles in a 1:1 ratio (Matthews and Watson 1981). The comparison can then be made of vibration responses obtained during locomotion and XER to assess whether or not increases in autogenic gain are present during stepping. This, in addition to stretch-evoked autogenic responses within the same preparation yields further insight into the source of an increase in autogenic gain during locomotion.

Evaluating the muscle responses to stretch versus release, or movement prior to stretch, also provides a piece of evidence for the contribution of length or force feedback to an increase in autogenic gain during locomotion. Length feedback functions to linearize the response of a muscle to stretch (Nichols and Houk 1976). In the absence of length feedback, the intrinsic response of muscle is highly nonlinear. Introducing prior movement to a reinnervated, or areflexive, muscle prior to stretch linearizes the stretch-evoked response, similar to the function of the stretch reflex (Huyghues-Despointes et al. 2003; Huyghues-Despointes et al. 2003). Similarly, the stretch reflex linearizes the response to release. Should an increase in autogenic gain be mediated by length-dependent mechanisms, this might result in a response that would then induce an exaggerated asymmetry in stretch versus release responses. However, maintained symmetry between stretch and release responses would suggest force-dependent mechanisms.

Previous studies have evaluated the existence of positive force feedback using electrical stimulation of sensory afferents originating from extensor muscles (Pearson and Collins 1993; Gossard et al. 1994; Guertin et al. 1995). Evidence suggests that electrical stimulation of the sensory nerve elicits excitatory postsynaptic potentials (EPSPs) in extensor motoneurons following administration of LDOPA or stimulation of the MLR in fictively locomoting preparations (Gossard et al. 1994; McCrea et al. 1995). Also, nerve stimulation has demonstrated excitatory EMG in non-fictive preparations (Duysens and Pearson 1980). Studies suggest that there are significant differences between

synchronous, electrical stimulation and natural stimulation (Enriquez-Denton et al. 2002). In this study, we compare the force responses obtained using natural stimulation, muscle stretch, and electrical nerve stimulation as an input to muscle. We hypothesize that the presence of positive force feedback is exaggerated with the use of artificial stimulation.

The distribution of excitatory force feedback might be dependent on the motor task. This is evident in studies evaluating muscle activation patterns during different motor tasks: uphill and downhill walking (Smith et al. 1998; Gottschall et al. 2005). It has been proposed that positive force feedback could be more widespread during walking up a slope when larger forces are required of antigravity muscles (Gregor et al. 2006). That is, increases in autogenic gain might be preferential to muscles other than G during uphill walking. As a first step to testing this theory, we evaluated autogenic responses during head tilt down and up, which has been shown to exhibit similar muscle activation patterns to uphill and downhill walking respectively (Gottschall et al. 2005). If, in fact autogenic responses differentiate due to head tilt, this would be in support of our theory that the array of experimental paradigms used to study positive force feedback results in a spectrum of behaviors.

The purpose of this study was twofold. First, we explored the source of afferents responsible for the increase in autogenic gain during locomotion. Second, we sought to reconcile the spectrum of experimental results from previous studies investigating positive force feedback. We utilized an actively stepping preparation with the natural stimulus of muscle stretch. Our experiments addressed the following central question: are

increases in autogenic feedback during stepping mediated by length or force mechanisms? We found evidence for positive feedback autogenically in Chapter 3 (Ross et al. 2002; Ross et al. 2005) that appeared to be mediated by Golgi tendon organs. Additionally, the increase in autogenic feedback is selective for MG, yet is expressed heterogenically onto LG during stepping. We propose that these autogenic gain increases provide a localized loading reflex. We also found evidence that there was a decrease in the heterogenic inhibition when using nerve stimulation when compared to stretch-evoked responses. This could explain differences between results from this laboratory when compared to previous studies. Preliminary accounts of these results have been published (Ross et al. 2002; Ross et al. 2003; Ross and Nichols 2004; Ross et al. 2005).

4.2 Methods

4.2.1 Preparation

Several methods were used to further investigate the origin of an increase in autogenic gain during locomotion. The surgical preparation remained relatively unchanged, and has been previously described (see Chapter 2). Briefly, fifteen cats ranging from 3 to 6 kilograms were deeply anesthetized using isoflurane gas. A tracheotomy was performed, the carotid arteries were looped for ligation, and the external jugular vein was cannulated for fluid delivery throughout the experiment. Withdrawal responses were monitored, and the level of anesthetic was adjusted accordingly.

The right hindlimb was immobilized, bone pins were inserted into the femur and tibia, and the leg was clamped to maintain the knee at a 110^{o} angle. The animal was

placed in the stereotaxic frame, supported above a variable-speed treadmill. The core temperature was maintained at 37^{o} C. Reference sutures inserted around the tendons of the peroneus muscles and sutures inserted into the muscle tendons were used to determine the initial lengths of the muscles.

The G, PLAN, and FHL of the right, immobilized hindlimb were dissected. Both PLAN and FHL were cut near their insertion onto FDB and FDL respectively. In 8 experiments, the G was separated into its respective heads, MG and LG. Each muscle was attached to the myographs and linear motors via their tendons. Mineral oil was used to ensure that the muscles stayed moist.

A premammillary decerebration was performed, whereby the brainstem was transected rostral to the superior colliculus while preserving the mammillary bodies and subthalamic nucleus. All brain matter rostral to the transection was removed. Gelfoam and cotton were placed on the base of the cranium to minimize bleeding. Anesthesia was then titrated down and withdrawn.

Three limbs were free to step on the treadmill while the right hindlimb remained immobilized. Stimulation of the skin beneath the tail was used to initiate stepping when spontaneous locomotion did not occur. Once locomotion data were obtained, the XER was elicited with electrical stimulation of the left posterior tibial nerve at 2 times threshold. At the end of each experiment, the animal was euthanized with an overdose of Nembutal followed by a pneumothorax.

4.2.2 Data Acquisition

The Parker 406LXR linear motors used in this study were mounted on a custom-built aluminum frame and could be adjusted in the horizontal, vertical, and diagonal directions so that the motor could be properly aligned with the appropriate muscle. Data was acquired digitally through the *dSPACE* board at a sampling rate of 1000 Hz. Data acquisition software in *Simulink* and a graphical interface in *Controldesk* were used to specify the change in length, velocity, and hold time. The typical paradigm was a 2 mm stretch at a velocity of 0.04 m/s, 100 ms hold period, and 2 mm release. All muscles were maintained at their referenced length (see above) when the knee was fixed at 110^{o} and the ankle was at 90^{o}.

Once initiation of stepping from the three unfixed limbs commenced, random stretches were manually triggered so as to capture a significant number of trials in all phases of the step cycle. Oscillations in the background force of MG in the right hindlimb and force responses due to stretch during stepping are shown in Figure 4.1a. Figure 4.1b depicts the length input to the muscle during locomotion, which corresponds in time to the force responses depicted in Figure 4.1a.

The stretching paradigm in the right hindlimb was then repeated during XER so that we could determine how autogenic feedback was reorganized with the initiation of stepping. As described below, force responses obtained during locomotion (filled circles) were compared to those obtained in the XER (open circles) to evaluate the change in

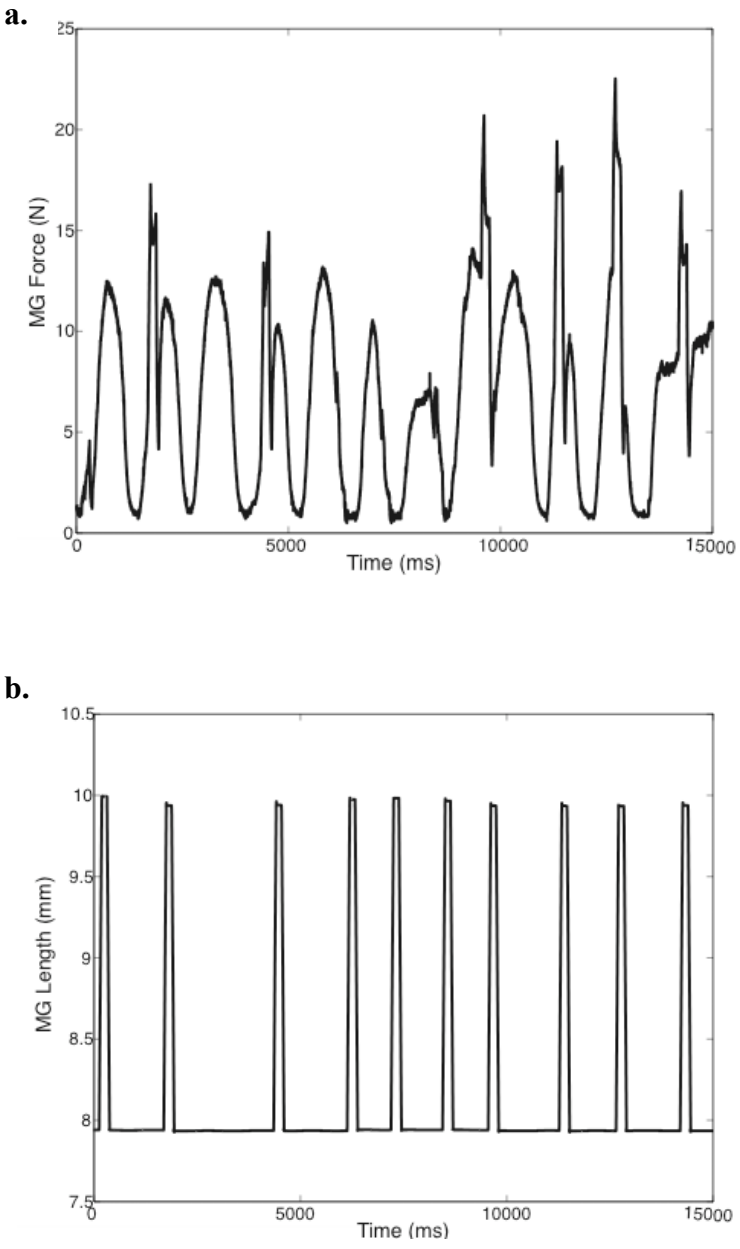
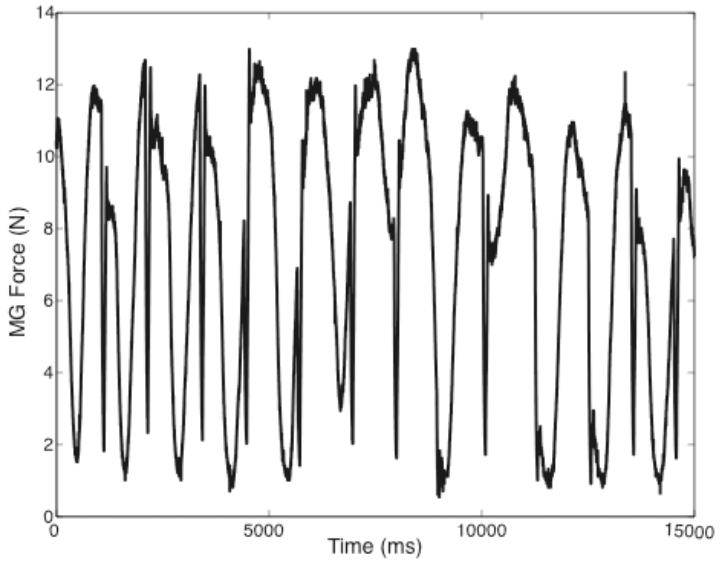


Figure 4.1 (a) Ramp and hold stretches delivered on top of the oscillating background force in MG. (b) Ramp and hold length input to MG during locomotion. (c) Prior releases delivered on top of the oscillating background force in MG. (d) Prior release length input to MG during locomotion.

c.



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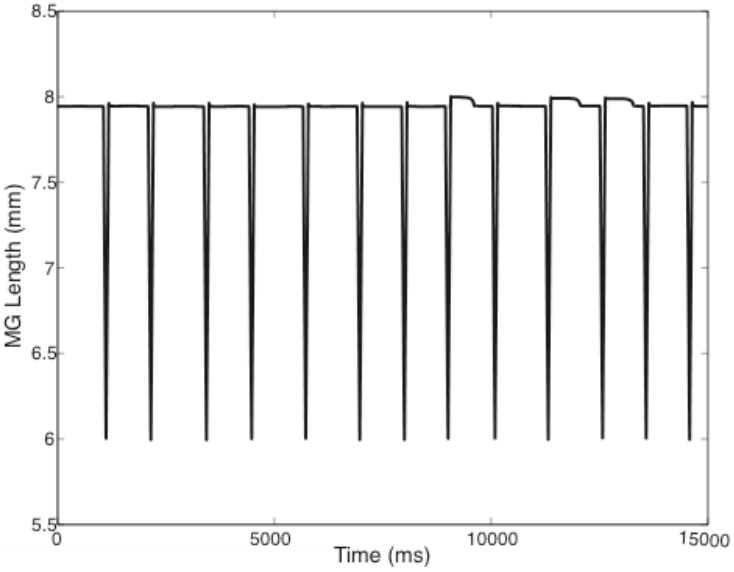


Figure 4.1 (continued)

autogenic gain with the initiation of locomotion. Finally, individual myographs were calibrated using a two-point calibration.

4.2.2.1 Stretch vs. Release

In addition to stretch, prior release was used to evaluate the source of an increase in autogenic gain in MG, during locomotion. Releases were manually triggered randomly so as to capture a significant number of trials in all phases of the step cycle. Varying amplitudes of prior release were administered prior to the 2 mm stretch at 0.04 m/s: 0.5 mm, 1.0 mm, 1.5 mm, and 2.0 mm, corresponding to 0.01 m/s, 0.02 m/s, 0.03 m/s, and 0.04 m/s respectively. Oscillations in the background force of MG in the right hindlimb and force responses due to a 2.0 mm release during stepping are shown in Figure 4.1c. Figure 4.1d depicts the length input to the muscle during locomotion, which corresponds in time to the force responses depicted in Figure 4.1c.

4.2.2.2 Vibration

Muscle spindles were selectively activated using muscle vibration to determine the mechanism of the increase in MG autogenic gain during locomotion. Muscles were dissected and attached to the linear motors via tendon clamps. The motors administered episodes of vibration at a frequency of 75 Hz, amplitude of 200 μm, and duration of 500 ms during both locomotion and XER. Vibration episodes were manually triggered so as to capture a significant number of trials in all phases of the step cycle. Force responses to vibration were compared during both locomotion and XER and contrasted with stretch responses in the same experiment, as shown in Figure 4.5.

4.2.2.3 Head Tilt

Head tilt was used to simulate different behavioral tasks and assess the alteration in background force of hindlimb extensors as well as strength and sign of autogenic feedback in G, PLAN, and FHL. The head was rotated about the ear bars up at a 50% grade (26.7 ^{o}), down at a 50% grade, and level with respect to the treadmill. Rotating the head up simulates walking downhill and rotating the head down simulates walking uphill (Gottschall et al. 2005). The head tilt was performed during both locomotion and XER, and force responses were analyzed as described below.

4.2.2.4 Nerve Stimulation

Cutaneous reflexes were selectively activated by stimulating the ipsilateral sural nerve during locomotion and XER. During the leg dissection, the sural nerve was carefully dissected from the distal innervation on the lateral dorsum of the foot to the proximal innervation near MG. The nerve was sutured and ligated near the distal end, and draped on a bipolar hook electrode. Care was taken not to stretch or damage the nerve during the dissection or stimulation. The stimulus input was controlled using a Master 8. The nerve was stimulated at 2 times threshold (2T) with a 5-pulse train stimulus, pulse duration of 1 ms, and frequency of 0.5 Hz. Threshold corresponds to the minimum stimulation required to elicit a force response in the dissected muscles. The stimulation was performed during both locomotion and XER to compare responses during both behavioral conditions. The MG nerve was also stimulated during locomotion and XER to selectively activate force-dependent pathways to PLAN. The MG nerve was carefully dissected, ligated, and cut near its muscle innervation, and draped on a bipolar electrode.

Care was taken not to stretch or damage the nerve during the dissection or stimulation. The nerve was stimulated at 1-2 T during both locomotion and XER.

4.2.3 Data Analysis:

Force measurements were used as an output measure for each of the various inputs to determine the mechanism of increases in autogenic gain. Data acquired, including those acquired during the head tilt experiments, were organized by state, whereby state one corresponded to data obtained when the recipient muscle was stretched alone (filled circles), while state two represented those from stretching the donor and recipient muscles together (open triangles). Comparing recipient muscle responses during state one with those during state two reveals the heterogenic contribution. Force output and length input of G, FHL, and PLAN were recorded for each stretch. Similarly, comparing recipient muscle responses during locomotion (filled circles) with those during XER (open circles) reveals the autogenic gain between the behavioral states. Force output and length input to the muscles were recorded for each stretch. The remaining experimental paradigms, including stretch versus release, vibration, and nerve stimulation, were performed during locomotion and XER. Force responses for each experimental paradigm were analyzed using methods outlined below.

Software in Matlab version 7.01 was used to analyze the data. For those methods utilizing muscle stretch, including head tilt and stretch versus release, the background force of the muscle was calculated as an average of the force during the interval 10 ms prior to the beginning of the stretch, during the isometric hold period. A baseline was

then constructed by performing a linear interpolation from the mean force response just prior to the stretch to the mean force after the end of the release. The entire baseline was then subtracted from the overall force response. A similar baseline calculation was performed for nerve stimulation experiments, whereby a baseline was fit to the 10 ms interval just prior to stimulation and the 10 ms interval following the return to the initial force.

To evaluate the strength and sign of heterogenic feedback during either locomotion or XER, individual force responses at specific time points were obtained from the baseline subtracted force data, and background force was obtained from the original force trace. Figure 4.2 depicts the typical analysis, whereby force responses for a specific timepoint, 50 ms following the beginning of the stretch (dynamic phase), are plotted as a function of background force. Each data point represents a response of the recipient muscle obtained when the muscle was either stretched alone (filled circles) or response of the recipient muscle when it was stretched with another muscle (open triangles), and individual responses represent an individual stretch. Polynomial fits and 95% confidence intervals were fit to each population of data for a given time point. A similar analysis was performed during XER, as shown in Figure 4.2b. Individual traces from both behavioral conditions were matched at similar background forces to visualize the magnitude and timecourse of the reflexive effects.

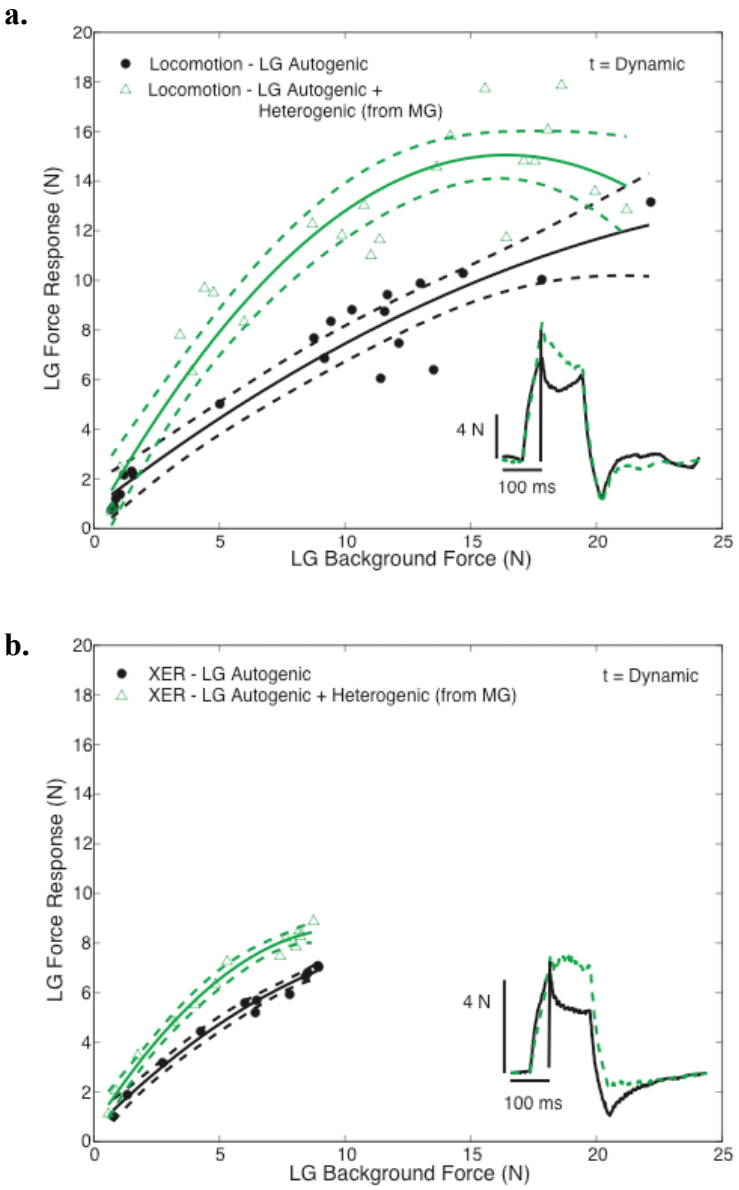


Figure 4.2 (a) Heterogenic excitation from MG onto LG, where MG is the donor muscle and LG is the recipient muscle during locomotion for the dynamic phase. (b) Heterogenic excitation from MG onto LG during XER for the dynamic phase. Filled circles and open triangles represent LG force responses from stretches occurring in state one and state two, respectively. Polynomials and 95% confidence intervals are fit to each population of data, and statistical tests reveal that the populations for the dynamic phase are distinctly separated ($p<0.01$). Two traces matched at 10 N (a) and 4 N (b) background force in LG from state one (solid line) and state two (dashed line) have been superimposed to illustrate the magnitude of excitation from MG onto LG during locomotion and XER respectively. The vertical line indicates the sample time.

For stretch versus release data, individual traces for each condition were matched at similar background forces. From that subset of data, force responses at a specific time point were obtained from the baseline subtracted data. These responses were plotted versus the change in length of the muscle as shown in Figure 4.4c. Force responses acquired during the head tilt experiments were pooled over a range of background force and plotted as a mean and standard deviation as shown in Figure 4.6a. Additionally, the first 5 force traces that were acquired within the first 10 seconds after stepping commenced and the head was tilted were baseline subtracted and averaged for each of the conditions: head level, head down, and head up. In contrast to the mean responses, this average technique revealed the transient responses due to head tilt.

Statistics were performed using Statistica 6.0 and Excel to test the separation of the data populations. Two regression models were fit to the data. In the full model, force response at the specified time point was the dependent variable (Y). The predictors in the full model included the grouping variable (X_2), representing state one or state two, background force (x_1), background force squared (x_1^2), the grouping and background force crossed term (x_1X_2), and the grouping variable multiplied by the squared background force term ($x_1^2X_2$). The reduced model lacked all terms containing the grouping variable, thus pooling the data into one population. An F statistic was then calculated by obtaining the sum of squared errors (SSE) and degrees of freedom (DF) from both the full and reduced models, as well as the mean squared errors (MSE) from the full model. A p-value < 0.01 from the statistical test stated above rejects the null hypothesis, and statistically proves that the two populations are distinctly different.

4.3 Results

The purpose of these studies was to determine the source of the selective increase in autogenic feedback in G during stepping. It has been previously shown in Chapters 2 and 3 that during locomotion, G, specifically MG, serves as the main source of heterogenic inhibition and autogenic excitation (Ross and Nichols Submitted) This study contains results from fifteen experiments, and examines responses in G, including MG and LG, PLAN, and FHL to various perturbations used to investigate the mechanisms of preferential autogenic gain increases in MG. There was evidence of not only an increase in autogenic excitation in MG, but also an increase in heterogenic excitation from MG onto LG during locomotion, suggesting that positive force feedback could be projecting to its close synergist. Force responses in MG due to stretch and release were linear, suggesting that positive force feedback preserves linearity in the muscle responses. Furthermore, the increase in autogenic gain exhibited during stretch could not be replicated by administering vibration to the muscle, further supporting that the increase in autogenic gain could be due to force-dependent mechanisms and not solely explained by length-dependent responses. Altering head position with respect to the treadmill altered activation and stretch-evoked force responses differentially in hindlimb extensors, simulating task specificity. Finally, nerve stimulation studies revealed that MG force responses increased during XER with sural nerve stimulation, and heterogenic inhibition decreased from G onto PLAN with MG nerve stimulation during locomotion when compared to responses obtained during muscle stretch.

4.3.1 Heterogenic excitation from MG onto LG increases during locomotion

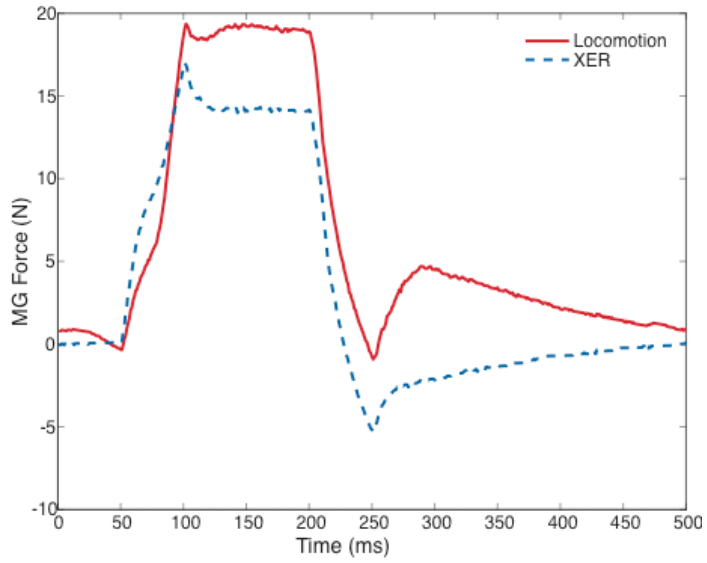
The heterogenic feedback from MG onto LG during locomotion was examined in 5 total experiments, 4 of which exhibited stepping behavior. Of these remaining experiments evaluating the interaction between MG and LG, all demonstrated excitation from MG onto LG. Figure 4.2a depicts a representative example of the heterogenic excitation for the dynamic timepoint, 50 ms following the beginning of the stretch, from MG onto LG during stepping. This is in contrast to the excitation exhibited during XER, as shown in Figure 4.2b. Multiple regression yielded $p<0.01$, thus statistically confirming that there is heterogenic excitation from MG onto LG during locomotion. Traces inset in Figure 4.2 are force responses for state one (solid line) and state two (dashed line). In either example, traces were obtained at the approximate mean of the recipient muscle background force; this corresponded to 10 N during locomotion (Figure 4.2a) and 4 N during XER (Figure 4.2b). Baselines were subtracted from both traces to better illustrate the magnitude and time course of the excitation from MG onto LG. It is evident that the magnitude of separation of the populations in state one versus state two increases threefold for the dynamic time point during locomotion when compared to XER. While the static responses are not illustrated, these are actually greater in XER than in locomotion, in part due to the mechanical effects of stepping.

In addition to evaluating heterogenic excitation from MG onto LG using the two-state analysis method described above, we performed a series of experiments using one-state analysis techniques. These techniques were employed in 6 experiments, 5 of which exhibited stepping behavior, whereby MG is stretched alone and LG is held isometric

during both locomotion and XER. Similar to evaluating the strength and sign of autogenic feedback, this method requires adequate activation during both locomotion and XER for an accurate comparison between the behavioral states. Of the 5 remaining experiments, only 2 experiments exhibited adequate responses during XER. Both of these successful experiments exhibited heterogenic excitation from MG on LG using the one-state method.

To evaluate the strength and sign of heterogenic feedback from MG onto LG, individual traces for state one during locomotion (solid line) and XER (dashed line) were matched at similar background forces for stretch-evoked responses in MG and isometric responses in LG. These isometric responses in LG essentially reflect the heterogenic feedback without the complication of intrinsic mechanical responses in the muscle due to stretch. Therefore, these responses are purely reflexive. Figure 4.3 illustrates results from this technique. Stretch-evoked responses in MG, as shown in Figure 4.3a, were matched at a background force of 10 N, and baselines were subtracted from both traces to better illustrate the magnitude and time course of the autogenic excitation of MG during locomotion. Isometric responses in LG corresponding to these stretches in MG occurring at a background force of approximately 8 N have been baseline subtracted and superimposed to illustrate the purely reflexive heterogenic excitation from MG onto LG during locomotion when compared to XER (Figure 4.3b).

a.



b.

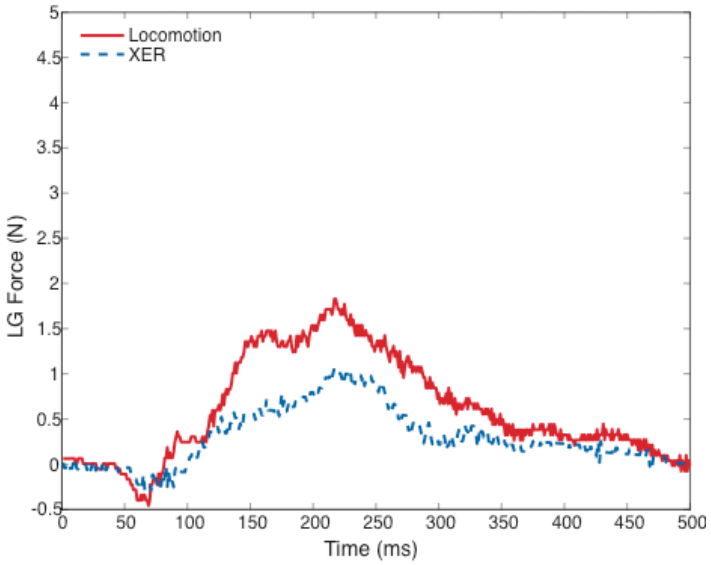


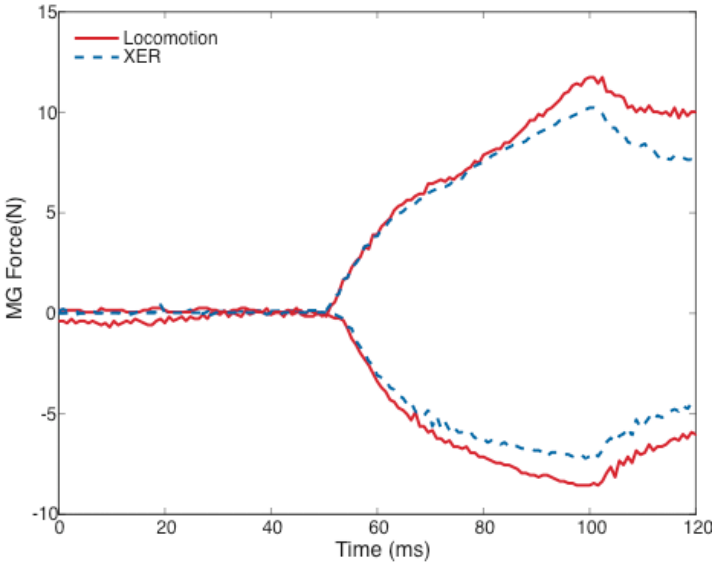
Figure 4.3 (a) Stretch-evoked autogenic excitation in MG during locomotion. (b) Isometric responses in LG due to MG stretch illustrate heterogenic excitation from MG onto LG during locomotion and XER. Two traces matched at 10 N background force in MG from state one (solid line) during locomotion and XER (dashed line) have been superimposed to illustrate the magnitude of autogenic excitation of MG during locomotion (a). Isometric responses in LG due to MG stretch have been matched at 8 N background force in LG during locomotion (solid line) and XER (dashed line) and superimposed to illustrate the magnitude of heterogenic excitation from MG onto LG during locomotion and XER (b).

4.3.2 Positive force feedback preserves linearity to release and stretch during locomotion

The autogenic responses in MG due to varying amplitudes of stretch and release were evaluated in 5 experiments, 4 of which exhibited stepping. Of these experiments, 2 contained only release data with varying amplitudes of 2 mm, 1.5 mm, 1.0 mm, and 0.5 mm release prior to the 2 mm stretch. Of the remaining 2 experiments, 1 contained only 2 mm stretch data while the second contained data varying the same amplitudes as described above with stretch and release. All data was acquired in one-state files.

Autogenic forces in MG exhibited symmetrical responses to both stretch and release, as demonstrated in Figure 4.4a. Individual traces matched at a background force of 10 N in MG for state one (locomotion) and state two (XER) for 2 mm stretch and 2 mm release have been baseline subtracted and superimposed to illustrate the relative symmetry of MG autogenic responses. These traces have been expanded to better visualize the autogenic responses during the dynamic phase of the stretch. Figure 4.4b contains traces also matched at a background force of 10 N. Baselines have been subtracted from responses for 2 mm release (solid line), 1.5 mm release (dashed line), 1.0 mm release (dotted line), and 2 mm stretch (dash-dotted line), and traces have been superimposed to illustrate the relative responses to varying amplitudes of stretch and release.

a.



b.

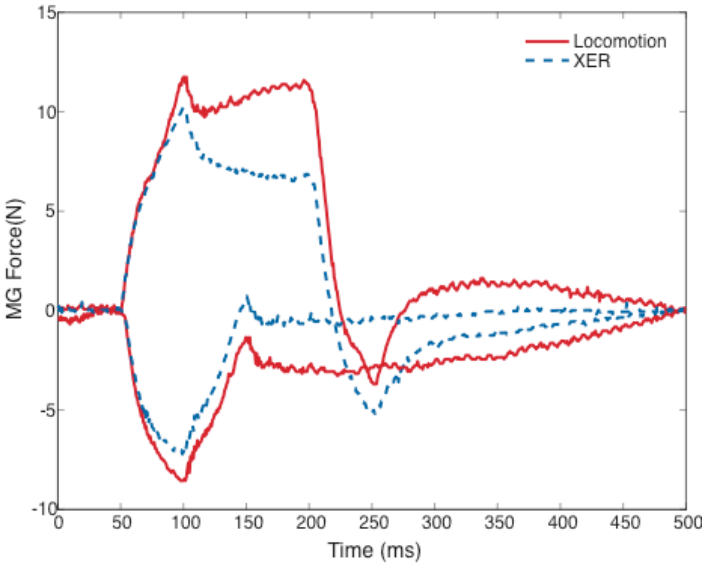


Figure 4.4 (a) Traces matched at 10 N background force in MG from state one (solid line) during locomotion and XER (dashed line) for 2 mm stretch and 2 mm release superimposed to illustrate the symmetry of responses to opposing inputs. (b) Force responses due to various magnitudes of release preceding the 2 mm stretch (dash-dot): 2 mm release (solid line), 1.5 mm release (dashed line), and 0.5 mm (dotted line). (c) Linearity plots constructed from stretch and release data from several experiments exhibited linearity.

c.

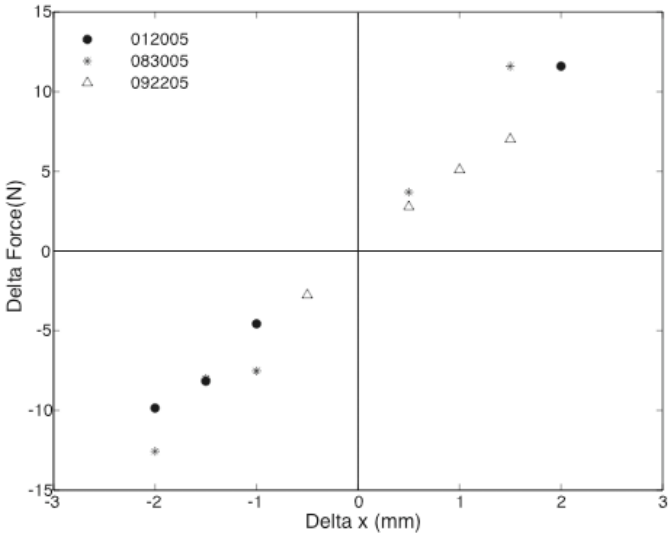


Figure 4.4 (continued)

The response in MG to stretch versus release has been further quantified across experiments by constructing a linearity plot, as shown in Figure 4.4c. In each experiment, traces were matched at a background force of approximately 12 N. This background force was chosen so as to include the maximum number of experiments and ensure that release responses achieved minimum force responses that exceeded passive force levels so as to alleviate errors due to saturation effects. Baselines were subtracted from each trace, and responses were calculated 50 ms following the beginning of either the stretch or release. The initial force was calculated as an average of the 10 ms just prior to movement, and delta force equated to the initial force subtracted from the response. This value was plotted versus the change in length, which corresponded to the magnitude of either the stretch or release. Data from 3 experiments have been superimposed, and responses from each experiment are illustrated by a different symbol, as shown in Figure 4.4c. MG responses obtained from a given experiment yield very linear responses, suggesting that positive force feedback in MG maintains the linearized response to stretch and release that is typically present in reflexive muscles.

4.3.3 Vibration does not cause an increase in G autogenic gain during locomotion

The effect of vibration on autogenic responses in G was evaluated in 3 experiments, all of which exhibited stepping. During both locomotion and XER, 500 ms of vibration at 75 Hz and amplitude of 0.2 mm were administered to each of the muscles in a 4-state file. That is, each of the 4 muscles attached to the linear motors and myographs were vibrated individually, and force responses were acquired. Figure 4.5a depicts the length input to G during these episodes of vibration. Despite the small drift in

the baseline and the slight cross-talk between the motor encoders, the motors are still able to deliver the rapid vibration pulse to the muscles individually. A single 500 ms vibration episode has been expanded, as shown in Figure 4.5b. While the overall amplitude is approximately 0.2 mm, there is some variation in the amplitude of each individual pulse. Despite this, the given input should be sufficient to selectively activate the length-dependent muscle spindle receptors with little or no effect on force-dependent Golgi tendon organs (Matthews and Watson 1981).

To evaluate the autogenic response in G due to vibration, force responses during single episodes of vibration were matched at similar background forces and superimposed. Figure 4.5c illustrates force responses matched a background force of approximately 22 N during both locomotion (solid line) and XER (dashed line) for a 500 ms episode of vibration. To better illustrate the magnitude of the responses during each behavioral condition, the force traces have been filtered using a fourth order Butterworth notch filter with a cutoff frequency of 50 to 80 Hz. The resulting traces have been superimposed, as shown in Figure 4.5d, and reveal that there is a slight decrease in the response to vibration during locomotion when compared to XER. This is in contrast to the stretch-evoked responses obtained in the same experiment also at a background force of 22 N. The traces depicted in Figure 4.5e are force responses in G due to a ramp and hold stretch. The baselines have been subtracted, and the traces have been superimposed to illustrate the magnitude of G autogenic excitation due to stretch during locomotion when compared to XER. These findings suggest that the stretch-evoked autogenic excitation exhibited in G during locomotion cannot be solely explained by length-

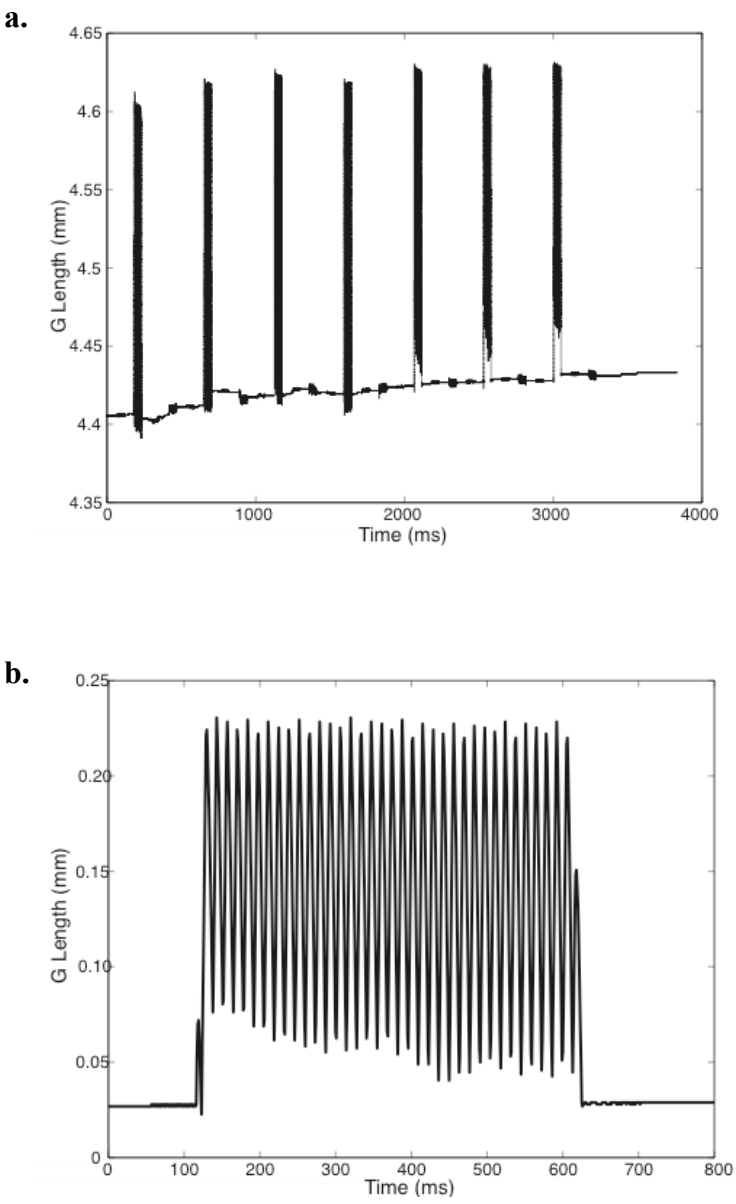
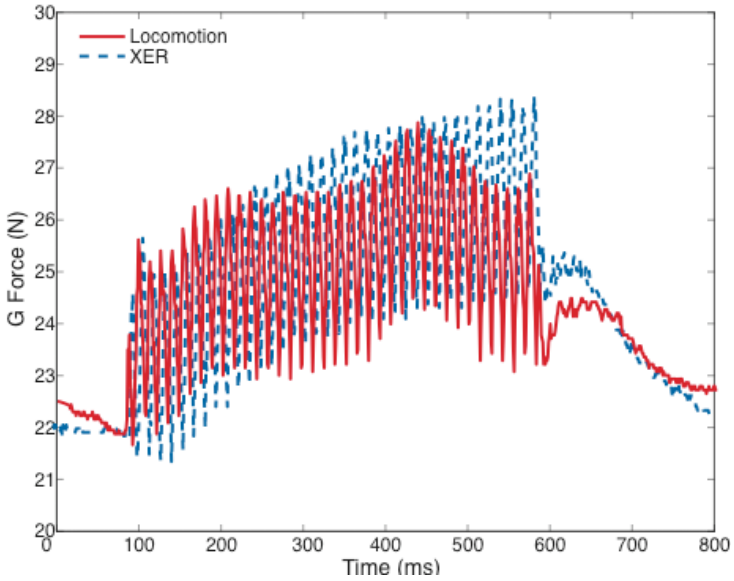


Figure 4.5 (a) Vibration episodes (500 ms duration) at a frequency of 75 Hz and amplitude of 200 μm administered to G during locomotion. (b) The length input to G expanded in time to illustrate a single episode. (c) Superimposed force responses during locomotion (solid line) and XER (dashed line) matched at a background force of 22 N. (d) Filtered G force responses (notch: 50-80 Hz) to illustrate that responses to vibration are greater during XER. (e) Stretch-evoked responses in G matched at a background force of approximately 22 N and superimposed to demonstrate autogenic excitation in G during locomotion (solid line) when compared to XER (dashed line).

c.



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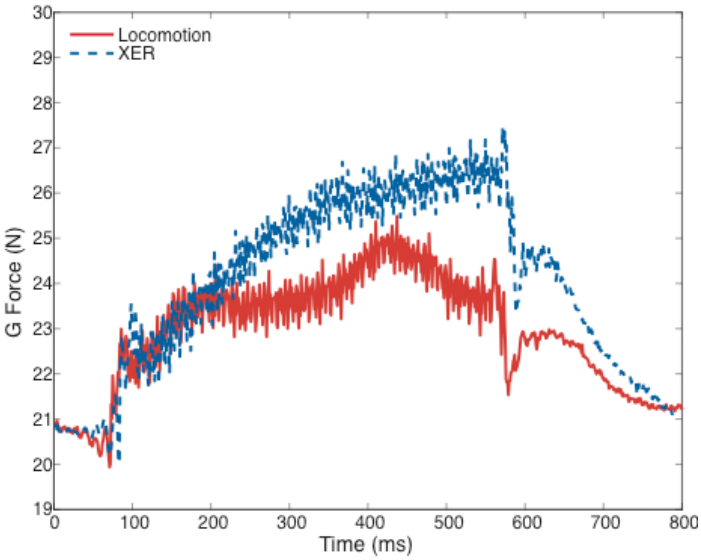

Figure 4.5 (continued)

e.

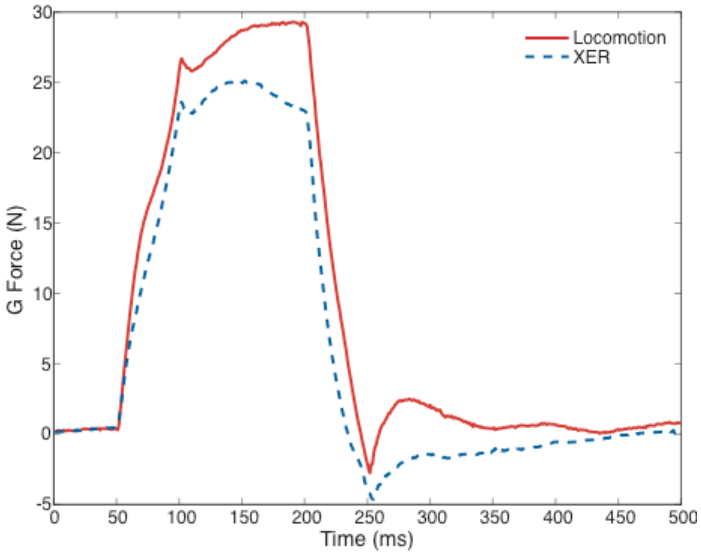

Figure 4.5 (continued)

dependent mechanisms. Rather, this suggests that an increase in G autogenic gain during locomotion could be evidence for positive force feedback.

4.3.4 Head tilt results in differential gain changes among hindlimb extensors

Three preliminary experiments, 2 of which exhibited stepping, were devoted to exploring the effects of head tilt on hindlimb extensor activation and stretch-evoked response. During stepping, the head was tilted up at a 50% grade (26.7^{o}), down at a 50% grade, and level with respect to the treadmill. For a given trial, stepping was initiated, the head was tilted, and muscles were stretched in a two-state configuration, as previously described (see 4.2 Methods). To evaluate the effect of head tilt on autogenic gain, force responses were calculated for state one in each of the head tilt conditions. Force responses 50 ms following the beginning of stretch were plotted as a function of the recipient muscle's background force (not shown).

Due to the lack of force dependency in the force responses of G, FHL, and PLAN, dynamic responses were pooled together, and the mean and standard deviations for each population of data were calculated for the head tilt conditions. The mean and standard deviations for G, FHL, and PLAN are plotted for the head level (black), head down 50% (dark grey), and head up 50% (light grey) in Figures 4.6a, 4.6c, and 4.6f respectively. As shown, there is no statistical difference in the force responses for G, FHL, or PLAN obtained for either the head up 50% or head down 50% condition when compared to responses obtained in the head level condition.

Previous data in this laboratory suggest that the effect of tilting the head down or up simulates uphill or downhill walking, respectively. Specifically, the patterns of muscle activation when tilting the head up mimic the patterns of muscle activation when the animal is walking downhill, and patterns when tilting the head down mimic patterns of muscle activation when the animal is walking uphill. While these effects are robust, they are restricted in time to approximately 10 seconds (Gottschall et al. 2005). Due to this short-lived effect, it is conceivable that any head tilt effects on force responses would not be detected using the analysis method previously described. Instead, the individual traces corresponding to the first 5 ramp and hold stretches following the head tilt were baseline subtracted and averaged. The average traces for the head level (solid), head down (dashed), and head up (dashed-dot) conditions have been superimposed for G, FHL, PLAN in Figures 4.6b, 4.6d, and 4.6e. The initial background forces for each condition are not statistically different, therefore any differences in the responses are due to the head tilt condition. While all 3 hindlimb extensors express differential responses to head tilt, FHL exhibits the stereotypical response whereby force responses increase with the head tilted down and decrease with the head tilted up when compared to head level responses, as shown in Figure 4.6d. These preliminary results have implications for motor task-specificity.

4.3.5 Cutaneous and proprioceptive pathways are modulated differently during locomotion

The modulation of cutaneous pathways during locomotion was evaluated in 2 experiments, both of which exhibited stepping. The sural nerve, a purely cutaneous nerve,

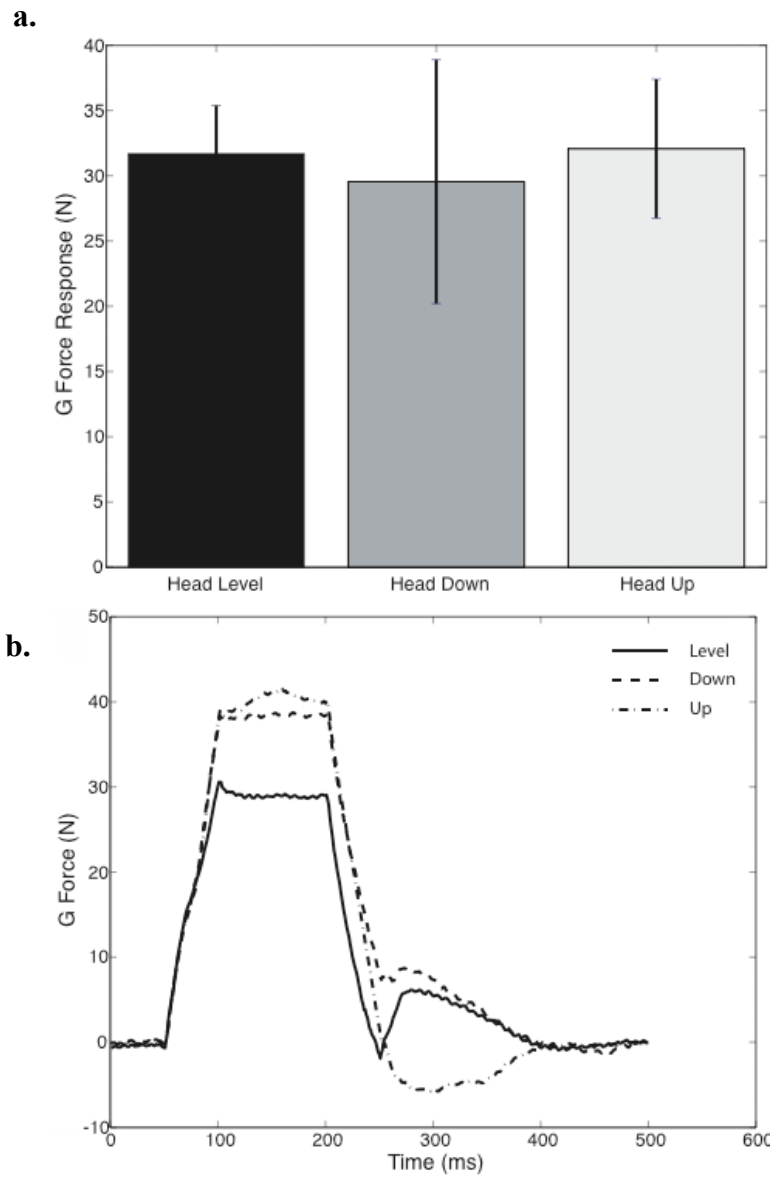

Figure 4.6 (a) Mean, autogenic force responses in G for the head level (black), head tilted down at a 50% grade (grey), and head tilted up at a 50% grade (white), and standard deviations for each condition. (b) Average of first 5 stretch-evoked responses in G for head level (solid line), head down (dashed line), and head up (dash-dot line) conditions. (c) Mean, autogenic force responses in FHL for the head level, head tilted down at a 50% grade, and head tilted up at a 50% grade, and standard deviations for each condition. (d) Average of first 5 stretch-evoked responses in FHL for head level (solid line), head down (dashed line), and head up (dash-dot line) conditions. (e) Mean, autogenic force responses in PLAN for the head level, head tilted down at a 50% grade, and head tilted up at a 50% grade, and standard deviations for each condition. (f) Average of first 5 stretch-evoked responses in PLAN for head level (solid line), head down (dashed line), and head up (dash-dot line) conditions.

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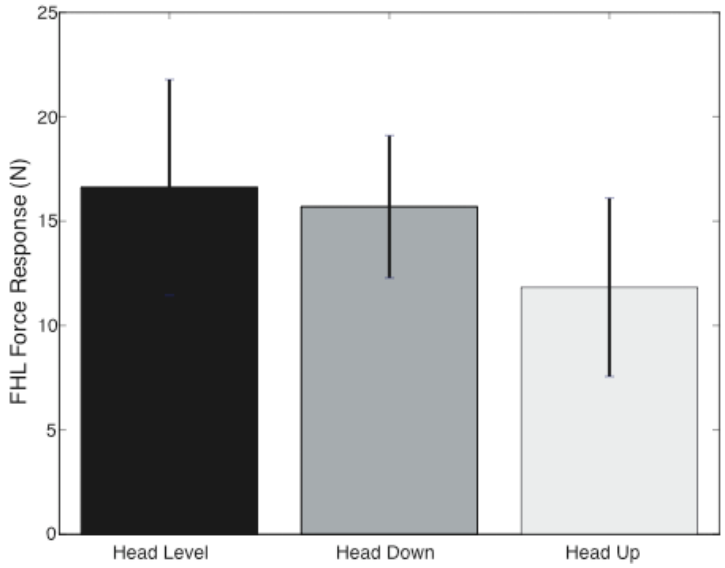

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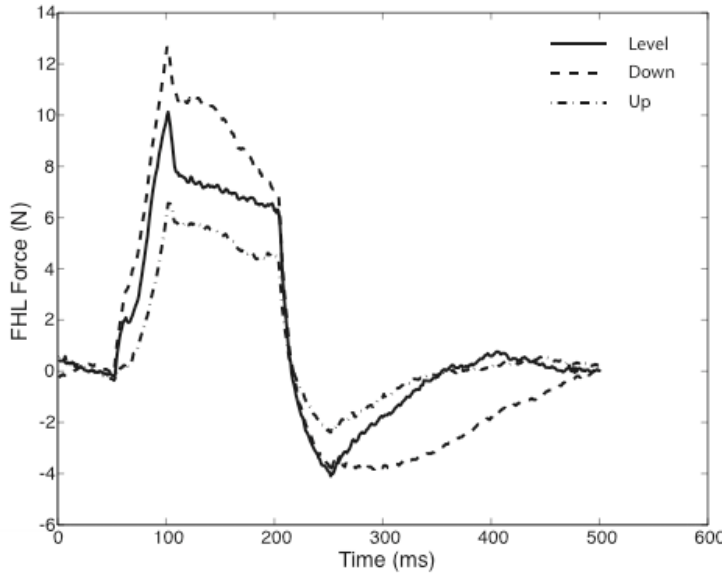

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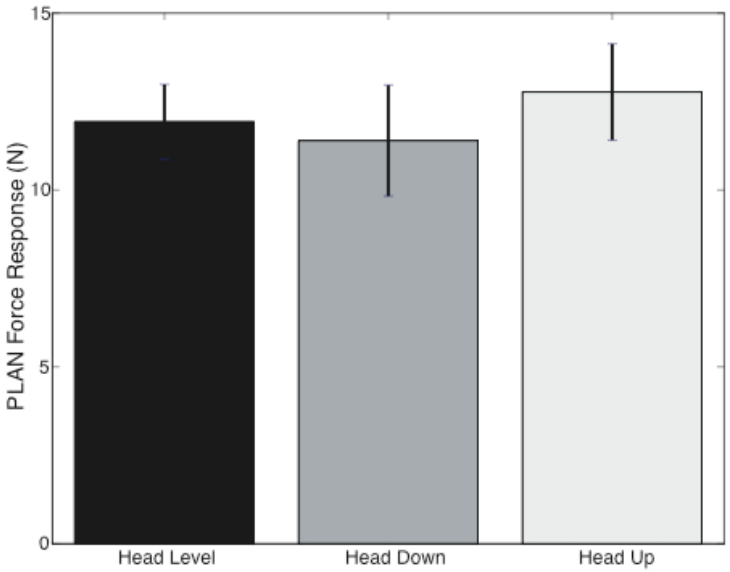

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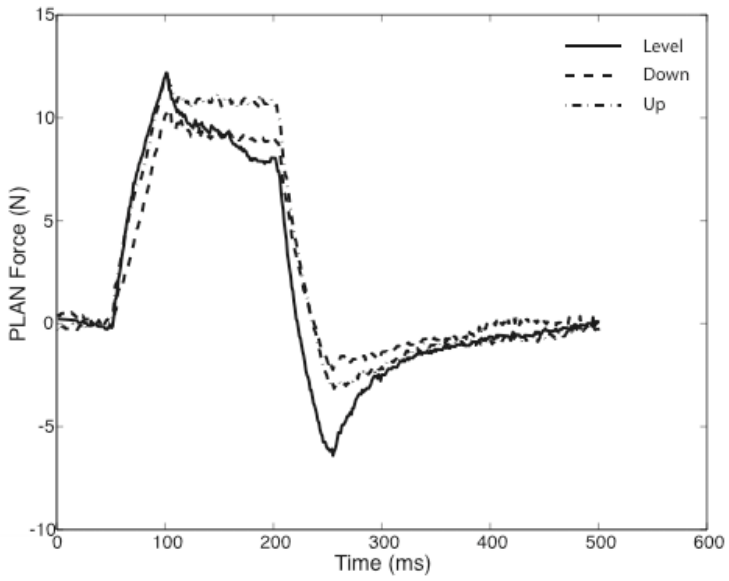


Figure 4.6 (continued)

was stimulated at threshold for either a single pulse or 5-pulse train at a frequency of 0.5 Hz. Force responses to sural nerve stimulation were obtained 100 ms following the initiation of the stimulus pulse. This also corresponded to the peak force response. Figure 4.7a depicts force responses obtained during both locomotion (filled circles) and XER (open circles) due to a 5-pulse stimulation train at 0.5 Hz to the sural nerve. Individual responses correspond to successive stimulation trains, and each population of data is plotted with polynomial fits and 95% confidence intervals. In this example, the confidence intervals are clearly separate for the two populations of data in both behavioral conditions, confirming that rather than an increase in autogenic gain in MG during locomotion, sural nerve stimulation induces a decrease in MG autogenic force responses. Additionally, multiple regression yielded $p<0.01$, thus statistically proving that these populations are distinctly different. Traces matched at an MG background force of 8 N during locomotion (solid) and XER (dashed) have been baseline subtracted and superimposed inset to illustrate the magnitude of this depression, as shown in Figure 4.7b. This is in contrast to stretch-evoked data, which illustrates that there is an increase in MG autogenic gain during locomotion, possibly due to positive force feedback.

4.3.6 MG nerve stimulation causes a decrease in heterogenic inhibition from G onto PLAN during locomotion

The comparison of stretch-evoked responses to those obtained during MG nerve stimulation was made in 2 experiments. Of these experiments, 1 exhibited robust stepping while the other exhibited sporadic stepping. Prior to MG nerve stimulation, heterogenic feedback pathways were evaluated using the two-state stretch paradigm previously

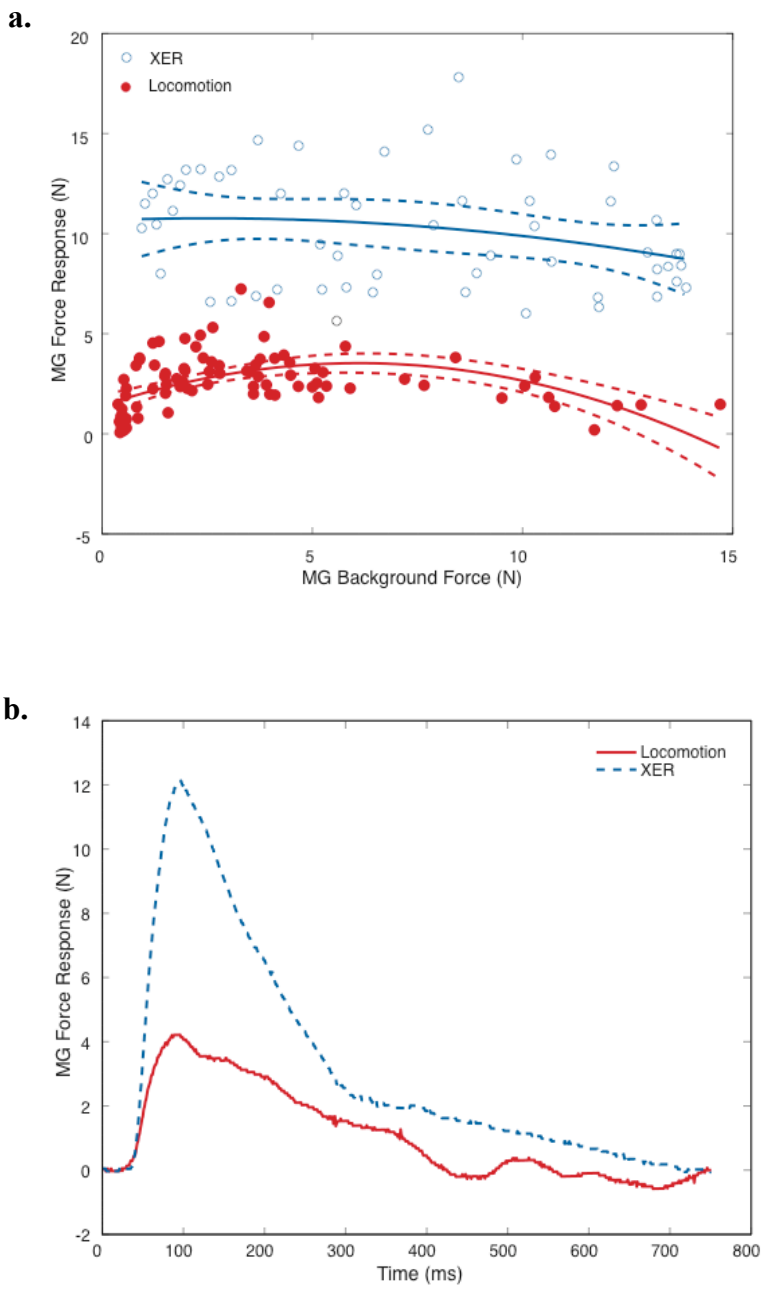


Figure 4.7 (a) Force responses in MG due to ipsilateral sural nerve stimulation at 2 times threshold for a 5-pulse train at 0.5 Hz. (b) Two traces matched at 8 N background force in MG from locomotion (solid line) and XER (dashed line) superimposed to illustrate the magnitude of autogenic excitation from MG with sural nerve stimulation during XER when compared to locomotion.

described. Figure 4.8a depicts a representative example of the heterogenic inhibition for the dynamic timepoint, 50 ms following the beginning of the stretch, from G onto PLAN during stepping. This is comparable to the heterogenic inhibition exhibited during XER, as shown in Figure 4.8b. In this example, the confidence intervals are clearly separate for the two populations of data in both behavioral conditions, confirming that there is heterogenic inhibition from G onto PLAN. Additionally, multiple regression yielded $p<0.01$, thus statistically proving that these populations are distinctly different.

Traces inset in Figure 4.8 are force responses for state one (solid) and state two (dashed). In either example, traces were obtained at the approximate mean of the recipient muscle background force during XER, 8 N. Baselines were subtracted from both traces to better illustrate the magnitude and time course of the inhibition from G onto PLAN. It is evident that the magnitude of separation of the populations in state one versus state two remains similar for the dynamic time point during locomotion when compared to XER. While the static responses are not illustrated, these exhibit the same trend.

Once stretch-evoked responses had been obtained during both locomotion and XER, the MG nerve was cut near its muscle innervation. Stimulation trains of 150 Hz for a duration of 500 ms at 2 times threshold were administered both during stepping and XER. Individual responses to MG nerve stimulation have been matched at similar background forces and the baselines subtracted. Figure 4.8c depicts individual force

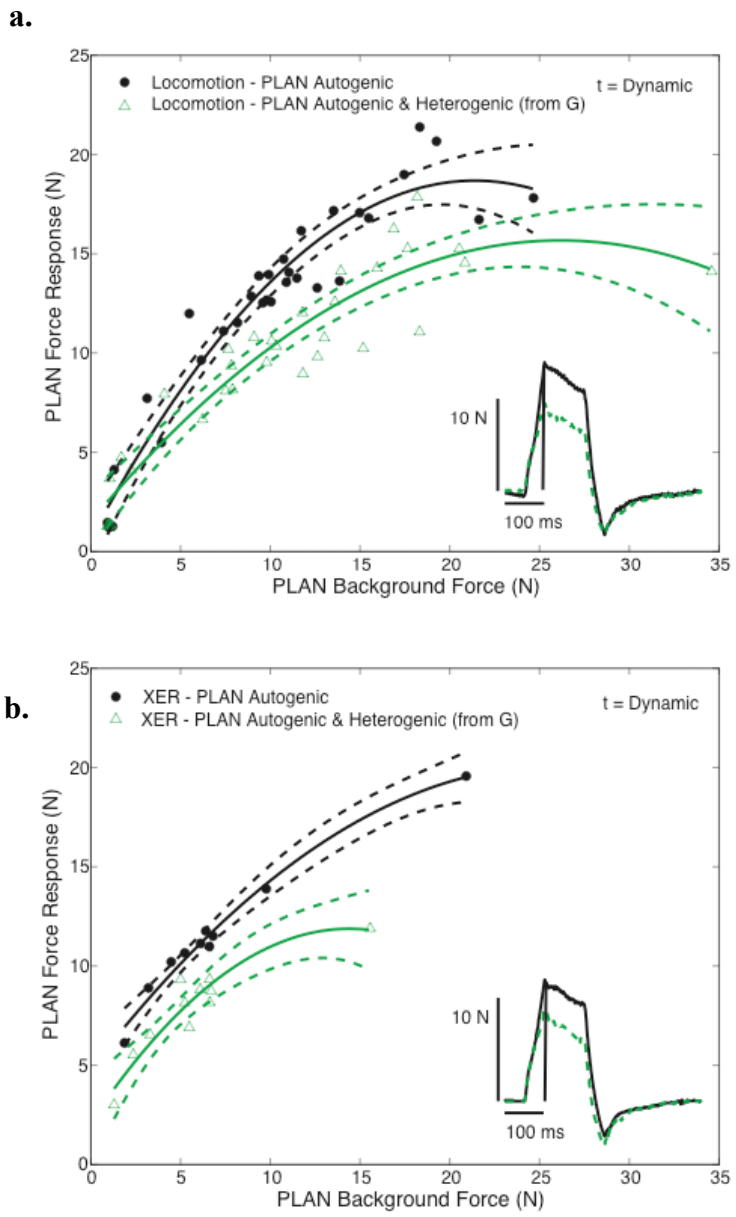
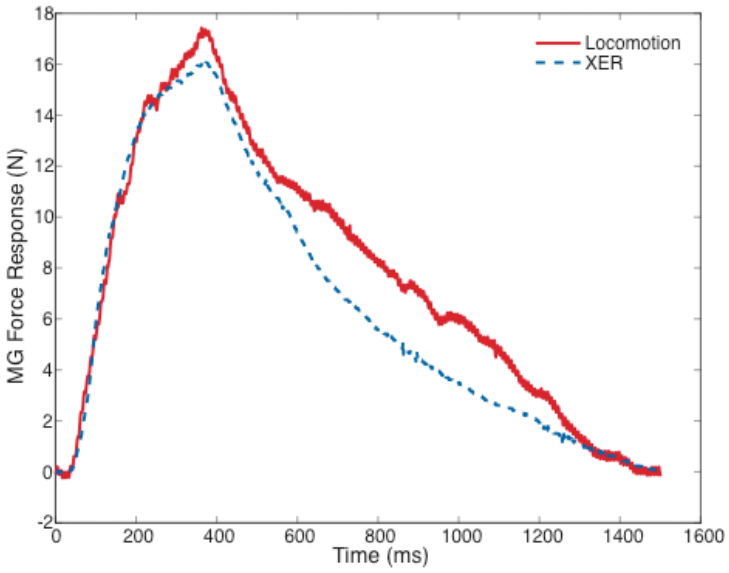


Figure 4.8 (a) Heterogenic inhibition from G onto PLAN for the dynamic phase during locomotion. (b) Heterogenic inhibition from G onto PLAN for the dynamic phase during XER. Similar conventions to Figure 4.2 apply. Two traces matched at 8 N background force in PLAN from state one (solid line) and state two (dashed line) have been superimposed to illustrate the magnitude of inhibition from G onto PLAN during either behavioral state. The vertical line indicates the sample time. (c) Autogenic responses in G due to MG nerve stimulation (2T, 150 Hz., 500 ms) during locomotion (solid line) and XER (dashed line). (d) Heterogenic responses in PLAN due to MG nerve stimulation during locomotion and XER. Individual force responses in G and PLAN have been matched at background force of 22 N and 12 N respectively during locomotion (solid line) and XER (dashed line) and superimposed.

c.



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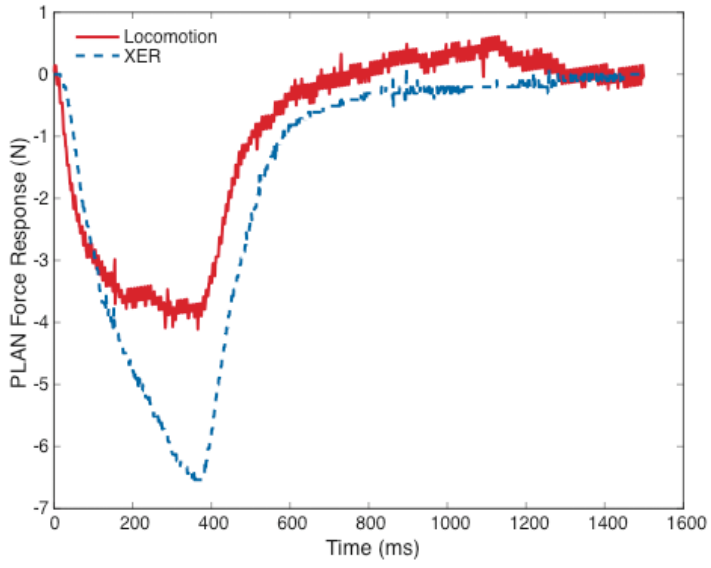

Figure 4.8 (continued)

responses due MG nerve stimulation during locomotion (solid) and XER (dashed) matched at a background force of approximately 22 N. Figure 4.8d represents the subsequent heterogenic responses in PLAN due to the MG nerve stimulation. While there is not a significant increase in MG autogenic force response due to nerve stimulation, the heterogenic response from MG onto PLAN is significantly reduced during locomotion when compared to XER. Interestingly, the stretch-evoked responses from G to PLAN depicted in Figures 4.8a and 4.8b are of similar magnitude for either behavioral condition. This example contains both the force-dependent pathway from MG onto PLAN and the slight length-dependent pathway from LG onto PLAN. In the nerve stimulation example, only the force-dependent pathway from MG onto PLAN is stimulated, yet the magnitude of heterogenic inhibition is still decreased during locomotion when compared to XER. This is likely due to positive force feedback that is expressed during locomotion and selectively activated with nerve stimulation.

4.4 Discussion

In this study, we have addressed the source of the selective increase in autogenic gain during spontaneous locomotion in premammillary decerebrate cats, and we have attempted to reconcile our experimental results with previous studies investigating positive force feedback. In this laboratory, we have previously found that heterogenic feedback between ankle extensors, namely G, PLAN, FHL, and QUADS, remains inhibitory with the initiation of, and during locomotion (Ross and Nichols Submitted). The expression of positive feedback during locomotion is mainly autogenic, particularly in G or MG (Ross et al. 2002; Ross et al. 2005). Evidence suggests that this increase in

MG autogenic gain is most likely due to force-dependent mechanisms. Specifically, stretch-evoked responses of MG exhibit an increase in autogenic gain during stepping, yet force responses to vibration do not exhibit the increase, but rather a decrease. This autogenic gain increase in MG also spreads to its synergist, LG during stepping. Furthermore, MG responses to stretch and release maintain a linear profile during locomotion, suggesting that the receptor mediating the increase in autogenic gain also exhibits a symmetric response to release and stretch; this is indicative of Golgi tendon organs rather than muscle spindles. The following discussion addresses the results from this study in both a neural and biomechanical context, and supports the theory that there is a behavioral spectrum among preparations and task specificity to the distribution and strength of positive force feedback.

The neural connections between both heads of G have been verified in various experimental preparations. It has been demonstrated using intracellular motoneuron recording in anesthetized cats, and verified with stretch-evoked responses in decerebrate cats under conditions of XER, that MG and LG exchange exclusively length-dependent excitation (Eccles et al. 1957; Nichols 1989). Previous studies suggest that increases in autogenic gain are restricted to MG, rather than both heads of G. In addition to this gain increase, this study reveals that the increase is also expressed heterogenically from MG onto LG. The opening of an alternate, force-dependent pathway with the initiation of locomotion has been documented in previous studies (Pearson and Collins 1993; Guertin et al. 1995; McCrea et al. 1995). Since there is no known force-dependent feedback exchanged between these muscles (Eccles et al. 1957), it is logical to assume that the

increase in isometric and stretch-evoked heterogenic force responses in LG during locomotion when compared to XER is due to a new, excitatory pathway. While alone inconclusive, these data are evidence for positive force feedback during locomotion.

Exploiting the mechanical properties of the sensory receptors provides another method for determining the mechanism of the increase in autogenic gain. It is known that the purely mechanical response of muscle responds asymmetrically to stretch and release. Intrinsically, the muscle exhibits short-range stiffness and abrupt yielding with stretch and a continuous, yet larger decrease in force due to release (Joyce et al. 1969; Nichols and Houk 1973; Nichols and Houk 1976). In contrast, a reflexive muscle displays symmetrical force responses to stretch and release, suggesting that the stretch reflex functions to linearize the muscles' response to stretch and release. Linearity plots reveal the symmetrical responses of reflexive muscle under conditions of XER (Nichols and Houk 1976). Unlike muscle spindles, Golgi tendon organs respond symmetrically to stretch and release. If the selective increase in autogenic gain in MG during locomotion were due to length-dependent mechanisms, the result would be an asymmetry in the muscles' response to stretch and release due to the already asymmetrical response of the muscle spindle. However, if the increase in gain were due to positive force feedback, the responses to stretch and release would remain linear. In fact, results from the current study reveal that MG maintains the symmetrical response to both stretch and release, suggesting that a force-dependent pathway is responsible for increases in autogenic gain during locomotion.

Vibration to the muscle tendon unit was another mechanical manipulation performed during both locomotion and XER. Previous studies have shown that vibration at 150 Hz with an amplitude of 50 micrometers elicits spindle Ia afferent response in 1:1 ratio (Clark et al. 1981; Matthews and Watson 1981), however Golgi tendon organs remain unaffected (Brown et al. 1967). In the current study, the actual frequency of vibration was 75 Hz, however this range of frequency has also been proven to be sufficient to activate muscle spindle afferents selectively (Matthews 1966). In these experiments, the muscle possessed a background tension, which is required to ensure muscle spindle activation (Clark et al. 1981), though amplitudes of 50 micrometers were insufficient to induce a force response in this experimental preparation. Therefore, vibration amplitudes of 100 – 200 micrometers (Matthews 1966; Brown et al. 1967) were applied to the muscle during both behavioral states. The force responses to vibration exhibited by MG during locomotion did not exceed those obtained during XER. On the contrary, the vibration responses during locomotion were actually depressed, most likely due to the fibers' increase in internal lengthening and shortening during stepping. Muscle stretch performed in the same experiment under both behavioral conditions revealed that the stretch-evoked autogenic responses in MG exhibited the increase in autogenic gain during locomotion. This suggests that length-dependent mechanisms are not responsible for the selective increase in MG autogenic gain present during locomotion.

It has been proposed that motor task specificity may play a role in the expression of positive force feedback. The current study begins to address this issue by tilting the head and evaluating the changes in autogenic force responses during locomotion.

Previous studies have measured an increase in hindlimb extensor activity (EMG), particularly in MG, with increasing slope and speed (Pierotti et al. 1989; Carlson-Kuhta et al. 1998). Similarly, the extensor activity decreases significantly during downslope walking, when compared to level walking (Smith et al. 1998). Despite this decrease in activity, MG experiences increased stretch during downslope walking. Likewise, there is decreased stretch and increased activity during upslope walking (Gregor et al. 2006), suggesting that length-dependent mechanisms are not solely responsible for the activity modulation. Data from this laboratory suggest that this modulation in muscle activity during upslope and downslope walking can be recreated by simply tilting the head with respect to the treadmill down and up respectively (Gottschall et al. 2005). Results from the current study also suggest that the autogenic force responses are modulated during head tilt, particularly in FHL. This suggests that the distribution of positive force feedback might, in fact, be more widespread during different behavioral tasks, such as upslope walking. Further studies will need to evaluate the strength, sign, and distribution of positive force feedback during different motor tasks.

Proprioceptive and cutaneous pathways are modulated differently during locomotion. Previous studies have evaluated the effect of cutaneous and muscle nerve stimulation on the timing of the step cycle and reflex activation in hindlimb extensors (Duysens and Pearson 1976; Duysens 1977; Duysens and Stein 1978; Duysens and Loeb 1979; Duysens and Loeb 1980). Stimulation of the sural nerve, which consists of purely cutaneous afferents innervating the lateral plantar surface of the foot, produced little or no effect on the extensor or flexor burst duration in unrestrained walking cats (Duysens and

Stein 1978). Low-threshold stimulation of the sural nerve resulted in excitatory reflex activation of hindlimb extensors, yet stimulation at higher intensities reversed this effect (Duysens and Stein 1978). In contrast, the tibial nerve, while predominately cutaneous, also contains muscle afferents. Studies have shown that stimulating the tibial nerve even at low thresholds results in prolongation of the flexion phase (Duysens and Stein 1978). Similar results have also been found in human locomotion. The current study reveals decreased MG force response to sural nerve stimulation during locomotion when compared to XER. This is in contrast to the stretch-evoked MG autogenic response that is enhanced during locomotion when compared to XER. It is conceivable that the stimulation of the tibial and sural nerves could provide powerful, excitatory input signals to MG that compete during locomotion. This has important implications for the organization of force feedback and the interaction with the central pattern generator (CPG).

Data obtained from intercollicular to premammillary preparations suggests an alternative explanation for the varying distribution of force feedback in the different studies. The restriction of positive force feedback to a single muscle in this study might be explained by the experimental preparation. Studies have utilized electrical nerve stimulation at group I strength to evaluate force feedback for pathways lacking Ia connections (Pearson and Collins 1993; Guertin et al. 1995), or electrical stimulation of the mesencephalic locomotor region (MLR) to evoke stepping (Guertin et al. 1995; McCrea et al. 1995; Donelan and Pearson 2004). The differences in activation between synchronous electrical stimulation and natural stimulation have been previously

documented (Enriquez-Denton et al. 2002). The natural stimulation of muscle stretch utilized in this study could explain the restricted expression of positive force feedback. To reconcile the strength and distribution of positive force feedback in the spontaneously locomoting premammillary cat, it was essential to attempt to replicate previously published results. While the limb was not extensively denervated, MG nerve stimulation did reveal a decrease in the heterogenic inhibition from MG onto PLAN during locomotion when compared to muscle stretch. Interestingly, in the case of muscle stretch, the slightly excitatory pathway from LG onto PLAN was intact whereas it was not in the stimulation, yet the heterogenic inhibition was greater from MG onto PLAN during muscle stretch. We propose that this array of experimental paradigms, from intercollicular, to spontaneously stepping preparations, to paralyzed fictive preparations, represents a behavioral spectrum that ranges from predominately inhibitory force feedback to more widespread excitatory force feedback.

CHAPTER 5

DISCUSSION

The focus of this study has been on mapping the distribution of heterogenic and autogenic feedback among ankle extensors, including G (MG, and LG), PLAN, FHL, SOL, and knee extensors, QUADS, during spontaneous locomotion in premammillary decerebrate cats. Furthermore, we have addressed the mechanisms underlying this organization. Stretch-evoked force responses were compared between locomotion and XER to ascertain whether feedback was reorganized during different behavioral states. We found that heterogenic feedback between hindlimb extensors, particularly that emanating from G, specifically MG, onto PLAN and FHL remained inhibitory with the initiation of, and during, locomotion. Inhibition, though weaker, still remained from FHL and PLAN onto G, between FHL and PLAN, and between G and QUADS. We did, indeed, find evidence for positive force feedback in the autogenic force responses of G, particularly in MG rather than both heads of G, during stepping. The remaining muscles in this study exhibited force responses during locomotion that were slightly lower than those obtained during XER. Figure 5.1 graphically depicts the organization of the pathways found in this study. In this section, I discuss these results and their importance for motor control from both a neural and biomechanical perspective.

When evaluating the organization of feedback, and its functional implications for motor control and locomotion, it is important to first consider its organization and roles in

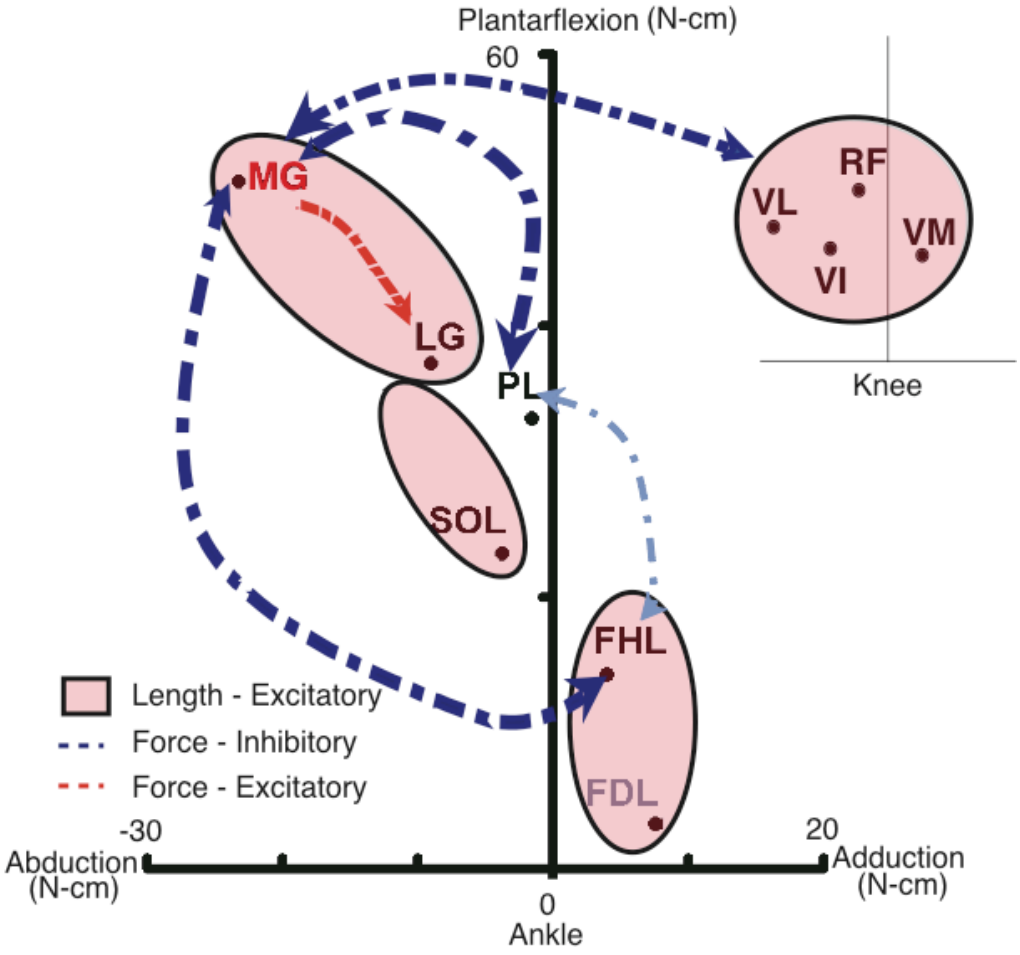

Figure 5.1 Organization of autogenic and heterogenic pathways among hindlimb extensors during locomotion found in this study.

quiescent preparations. Through the years, several methods have been used to measure the strength and sign of heterogenic feedback pathways in non-locomoting preparations. Intracellular recordings of extensor motoneurons in anesthetized cats determined the monosynaptic connections between these muscles (Eccles et al. 1957; Eccles et al. 1957).

Additional experiments utilizing muscle stretch in the activated (XER) decerebrate cat not only confirmed the existence of these pathways, but also incorporated the population response and muscle mechanics (Nichols 1989; Nichols 1999). The mechanographic technique, whereby the force of the muscle in response to stretch is recorded, was used to confirm the organization of these pathways (Nichols 1987). For the purposes of this discussion, it is important to note that in the intercollicular decerebrate cat, strong, force-dependent inhibition exists from G onto PLAN and FHL (Bonasera and Nichols 1994; Nichols 1994) and bi-directional, though asymmetrical heterogenic inhibition exists between QUADS and the triceps surae (Wilmink and Nichols 2003). With the exception of the feedback between SOL and VI, few, if any, heterogenic, excitatory (length-dependent) feedback pathways between synergistic groups exist in non-locomoting conditions among the muscles used in this study.

How, if at all, does the organization of heterogenic feedback change with the initiation of locomotion? Since heterogenic inhibition is thought to play an important role in coordinating and stabilizing the limb during movement, it would make sense, from a design standpoint, that one would want to maintain that stability during such an important and potentially destabilizing movement as locomotion. In the premammillary decerebrate

cat, using muscle stretch to evaluate the strength and sign of heterogenic feedback, heterogenic feedback remains inhibitory during locomotion. Furthermore, MG serves as the major source of inhibition, particularly to FHL and PLAN. The expression of force-dependent inhibition in this study does contradict results from previous studies, which suggest that positive force feedback is more widespread during locomotion. It is worth noting, however, that heterogenic inhibition was observed in one previous experiment. Guertin and colleagues describe an asymmetric relationship between G and the QUADS using nerve and MLR stimulation in the fictive locomoting cat preparation, similar to the results obtained in this study (Guertin et al. 1995).

While the distribution of heterogenic feedback has been mapped in XER and now, to some extent, during locomotion, the distribution of autogenic feedback remains largely unexplored in both locomoting and non-locomoting cat preparations. It is known from electrophysiological studies that homonymous projections from Golgi tendon organs onto extensor motoneurons are weak (Eccles et al. 1957). Many of the heterogenic projections have been verified using muscle stretch in the decerebrate cat (Nichols 1989), yet autogenic feedback remains largely unexplored in this preparation. Furthermore, many initial studies demonstrating positive force feedback during locomotion specifically cite examples where heterogenic feedback exhibits reversal (Pearson and Collins 1993; Gossard et al. 1994; Guertin et al. 1995). Perhaps with the exception of one critical study, the effects on autogenic feedback are not reported. In this particular study, one of the first studies documenting positive force feedback in the spontaneously locomoting cat, muscle stretch was used as an input to the system. Stretching the entire triceps surae prolonged

the extensor phase of locomotion, as measured by an increase in the magnitude of MG EMG (Duysens and Pearson 1980).

We found no evidence for excitatory heterogenic feedback between extensor muscles, except for between MG and LG. These two muscles comprise a synergistic group. However, we do, in fact, observe increases in autogenic gain, specifically in MG, during locomotion that could be evidence for positive force feedback. Incidentally, MG is the same muscle that exhibits the increase in autogenic gain in the study previously discussed (Duysens and Pearson 1980). Interestingly, despite the muscles being held isometric, the internal lengthening and shortening of the muscle fibers experienced during stepping likely increases cross-bridge detachment and turnover rate, and therefore should depress the overall muscle stiffness and stretch-evoked force response (Joyce et al. 1969; Rack and Westbury 1969; Kirsch et al. 1994). The increase of static gamma activation during locomotion also potentially decreases the muscle spindle's sensitivity to stretch (Taylor et al. 1985; Bennett et al. 1996; Murphy et al. 2002). The combination of decreased stiffness and decreased sensitivity to stretch could further depress force responses obtained during locomotion for the mechanical, dynamic, and static phases. This suggests that force responses obtained during locomotion should be lower than those obtained during XER, a behavioral state in which overall activation and, therefore, the internal muscle length remains constant. In fact, this decrease in force response is observed during locomotion for all muscles, including in MG prior to any influence of length or force dependent feedback. Additionally, MG overcomes this initial mechanical

depression by exhibiting an increase in autogenic gain at latencies comparable to group I feedback during locomotion.

It is important to evaluate the properties of MG, or of the entire system, that would dictate why this muscle would be such a powerful source of heterogenic inhibition and autogenic excitation during locomotion. Among the ankle extensors used in this study, the combination of both heads of G possess the largest physiological cross-sectional area and the largest pinnation angle (Sacks and Roy 1982). This suggests that G should be capable of generating the greatest force. However, it is actually MG that is the greatest source of heterogenic inhibition; while MG does have a slightly larger pinnation angle than LG, MG actually possesses a slightly smaller physiological cross-sectional area (Sacks and Roy 1982), causing this purely anatomical argument to breakdown. Along the same lines as muscle architecture, the number of sensory receptors, namely muscle spindles and Golgi tendon organs, in each muscle might possibly explain the organization of feedback. In particular, the distribution of Golgi tendon organs among the hindlimb extensors could influence the amount of inhibition present during locomotion. That is, the strong heterogenic inhibition emanating from MG during locomotion could possibly be explained if MG possessed the greatest number of Golgi tendon organs. However, the relative numbers of Golgi tendon organs for MG and LG are not known, however the ratio of muscle spindles to Golgi tendon organs may be preserved across muscles (Eldred et al. 1974).

Biomechanics might also play a significant role in determining why MG spreads the largest magnitude of inhibition and exhibits autogenic excitation during locomotion. During level walking, MG produces a greater force and peak moment than LG, as measured by implanted force buckles in intact cats (Fowler et al. 1993). Furthermore, each of the muscles used in this study have unique linkages that influence the pulling direction and mechanical actions of the limb and individual joints. For instance, G and SOL, while differing in the number of spanned joints, each insert onto the calcaneus to form the Achilles tendon. However, PLAN and FHL both have mechanical actions on the toes due to their insertions into FDB and FDL respectively. Classically, each of these muscles was classified as an ankle extensor, yet each has a unique action about the ankle. For instance, abduction torques about the ankle are generated by MG, LG, PLAN, and SOL, however the greatest abduction torque, and in fact, the greatest non-sagittal moment generated among these muscles, is generated by MG. FHL is the only muscle among these that generates an adduction torque about the ankle (Lawrence and Nichols 1999).

While the increases in gain certainly suggest evidence for positive force feedback, several experimental paradigms were completed to verify this source, since it can be difficult to distinguish length and force dependent mechanisms. The goal is to either preferentially activate muscle spindles or Golgi tendon organs, or perform a manipulation in which the receptors respond differently, to determine the source of increases in autogenic gain. One such experiment involves a mechanical manipulation that affects muscles spindles and Golgi tendon organs differently.

Both extrafusal and intrafusal fibers of muscle are affected by prior movement. The intrinsic response of muscle to stretch and release is asymmetrical, as is the response of muscle spindles (Nichols and Houk 1976). The combined asymmetries suggest that the stretch reflex functions to linearize the muscles' response to stretch and release (Nichols and Houk 1976). Linearity plots, measuring the force response in relation to the change in length, verify that reflexive muscles exhibit a linear, and therefore, symmetrical response to stretch and release (Nichols and Houk 1976; Haftel et al. 2004). On the other hand, Golgi tendon organs are not affected by prior history (Haftel et al. 2004), and presumably respond symmetrically to stretch and release.

In this study, autogenic MG force responses obtained during locomotion were evaluated to determine if they were or were not symmetrical for stretch and release. This serves as a test for length versus force feedback as a source for the increase in MG gain observed during locomotion. If the increase in gain were due to length-dependent mechanisms, this would provide an additive effect of the asymmetrical intrinsic and reflexive responses, leading to an asymmetry in the responses. Conversely, if the increase in gain were due to force-dependent mechanisms, the symmetry would be preserved. Because autogenic MG force responses remained linear, and therefore symmetrical, positive force feedback is implicated as a source for the increase in autogenic gain observed during locomotion.

While suggestive of a mechanism, these results are certainly not enough evidence to conclude that positive force feedback is definitely the source of increases in autogenic

gain. Experiments involving selective activation of the muscle spindle receptor provide additional support. Vibration applied to the muscle tendon unit during both locomotion and XER has been shown to selectively activate muscle spindles while not generating much force in the muscle, therefore not activating Golgi tendon organs (Brown et al. 1967). In this study, force responses in MG to vibration at 75 Hz and 0.2 mm were slightly depressed during locomotion when compared to XER, most likely due to the effects of prior movement, as previously described. Conversely, MG force responses to stretch clearly demonstrated the increase in autogenic gain. This, in combination with the history dependence data previously described, suggests that the increases in gain observed in MG during locomotion are evidence for positive force feedback.

Since MG and LG are close synergists, it is important to compare the stretch-evoked autogenic and heterogenic force responses in these muscles to gain further insight into the expression of positive force feedback during locomotion. It has been verified both in anesthetized and decerebrate cats that MG and LG exchange exclusively length-dependent excitation (Eccles et al. 1957; Nichols 1989). In this study, MG autogenic force responses exhibit increases in gain during locomotion, whereas LG autogenic force responses do not. Interestingly, the increase in MG gain spreads to LG via heterogenic feedback. This would be indicative of a previously unexamined, excitatory force-dependent pathway opening with the initiation of locomotion, as described in previous studies (Pearson and Collins 1993; Guertin et al. 1995; McCrea et al. 1995). This provides additional evidence for the expression of positive force feedback in this preparation.

We have found evidence for positive force feedback, yet why is the distribution of positive force feedback different than what has been described in previous studies? One possible difference involves how locomotion is induced in the various preparations. Previously, stepping in non-immobilized preparations or rhythmic motor output in fictively locomoting preparations has been induced with either electrical stimulation of the MLR or the administration of drugs such as clonidine (Gossard et al. 1994; Guertin et al. 1995; McCrea et al. 1995; Donelan and Pearson 2004). Our use of the spontaneously locomoting preparation with some natural stimulation to evoke stepping, when necessary, might explain the different patterns of force feedback from previous studies.

While previous studies have used spontaneously locomoting preparations, the methods also involve more artificial means of activating Ib afferents (Pearson and Collins 1993). Therefore, this is another factor that might influence the expression or even distribution of positive force feedback. Studies have utilized electrical nerve stimulation at group I strength to evaluate force feedback for pathways lacking Ia connections (Pearson and Collins 1993; Guertin et al. 1995). It is known that electrical stimulation, which is synchronous in nature, provides an input significantly different to that produced by natural stimulation (Enriquez-Denton et al. 2002). Instead, we utilize muscle stretch at a magnitude similar to that observed *in vivo* (Goslow et al. 1973) and muscle force as a means to measure reflex strength. It is conceivable that electrical stimulation could be recruiting interneurons that are not recruited by this natural stimulation of muscle stretch,

and that force feedback could be modulated differently during stepping with electrical versus natural stimulation (Morita et al. 1998).

Still another explanation for differences seen between preparations is the lack of sensory feedback present in these experiments. Often, these limbs are extensively denervated with the exception of the specific interaction studied. Or, in the case of the fictive preparations, there is no ongoing sensory input during stepping. In initial experiments evaluating the heterogenic feedback from PLAN onto MG, only the MG and proximal hip muscles remained innervated (Pearson and Collins 1993), whereas, the nerve supply remains completely intact in the experimental paradigm presented in this study. Furthermore, electrical stimulation, as described above, was the method of activating Ib feedback between these two muscles (Pearson and Collins 1993). It is entirely plausible that the combined effect of the preparation and mode of activation could alter the expression and distribution of positive force feedback. In the present study, the hindlimb is immobilized and muscles are held isometrically. Therefore, it is possible that the sensory information emanating from the immobilized hindlimb is not equivalent to that coming from an intact, stepping limb. Despite this possible mismatch, one muscle, MG, exhibits positive force feedback during stepping in this reduced preparation. Furthermore, positive force feedback is found in both the fictive preparation, where there are no forces, and in the spontaneously locomoting preparation, where presumably forces may be exaggerated. Therefore, it is plausible that positive force feedback would exist in the intact animal where forces are between these extremes.

In this study, we attempted to reconcile the differences in the expression of positive force feedback in different preparations by comparing responses to stretch versus electrical stimulation within the same animal. The limb was not extensively denervated, yet PLAN force responses due to electrical stimulation of the MG nerve were significantly lower than those in response to a two-state stretch paradigm evaluating heterogenic feedback pathways from MG onto PLAN. Interestingly, in the case of muscle stretch, the slightly excitatory pathway from LG onto PLAN (Nichols 1994) was intact whereas it was not in the case of nerve stimulation. Despite this, the heterogenic inhibition was greater from MG onto PLAN during muscle stretch. While this is not a reflex reversal, it does provide evidence that there are different outcomes with natural versus electrical activation of these force-dependent pathways.

Among decerebrate preparations, the specific level of transection might also influence the interpretation of the data obtained in the current study. It has been documented, though not extensively, that patterns of heterogenic feedback are not changed for either intercollicular or premammillary decerebrations, yet the overall gain of the system is slightly depressed in the premammillary decerebrate cat when compared to the intercollicular decerebrate preparation (Nichols 1989). Higher background forces and slightly larger muscle stiffnesses have been observed in intercollicular animals when compared to premammillary decerebrate animals (Nichols and Steeves 1986). Interestingly, it is data obtained in the intercollicular during XER that serves as an important comparison for the reorganization of pathways during locomotion. One obvious difference involves the heterogenic interactions between the triceps surae,

specifically G, and the QUADS. It has been documented in previous studies using the intercollicular decerebrate cat, that the feedback between these muscles is bi-directionally strong and force-dependent (Wilmink and Nichols 2003). However, the findings in the current study are that these interactions are relatively weak in comparison. Fortunately, the experiments in this study do serve as individual controls, because responses obtained during locomotion are compared with those obtained during XER within the same animal. However, it is conceivable that the difference in responses obtained in either behavioral state would be more striking in the intercollicular decerebrate preparation when compared to the premammillary decerebrate cat. In 1 experiment, we did perform the premammillary decerebration, initiate locomotion, collect data, and then attempt to perform a second decerebration slightly caudal to the first, in attempts to make an intercollicular decerebrate animal. This proved to be a difficult task that was simply not repeatable. Therefore, we were not able to directly address the issue of transection level on the relative gains of feedback pathways.

Obviously, the preparation, from the level of transection to the method of initiating stepping (rhythmic behavior) or even activating necessary pathways, clearly influences the strength and sign of force feedback during locomotion. We propose that this array of experimental paradigms, from intercollicular, to spontaneously stepping preparations, to paralyzed fictive preparations, represents a behavioral spectrum that ranges from predominately inhibitory force feedback to more widespread excitatory force feedback. Perhaps the underlying rationale for this is that the distribution of positive force feedback is dependent on the motor task. This is reasonable since it is known that the

patterns of muscle activation during either treadmill locomotion or level walking are significantly different than those observed during upslope or downslope walking (Carlson-Kuhta et al. 1998; Smith et al. 1998; Gregor et al. 2006). Specifically, muscle activity is shifted to the hindlimbs, particularly extensors, during upslope walking and to the forelimbs during downslope walking. Since it has been shown that hindlimb extensors undergo significant length changes during downslope rather than upslope walking and feedback among hindlimb extensors is predominately force-dependent, upslope walking might be a motor task in which positive force feedback would become more important (Gregor et al. 2006). In level walking, MG is the muscle exhibiting positive force feedback, however this effect might spread to other hindlimb extensors during upslope walking.

We attempted to address the issue of task specificity in this study by inducing different behaviors in this preparation. It has been previously shown in this laboratory that the patterns of muscle activation for upslope and downslope walking, as previously described, can be transiently replicated by simply tilting the head (Gottschall et al. 2005). Specifically, tilting the head down simulates the patterns observed during upslope walking, while tilting the head up simulates the patterns observed during downslope walking. The question is: do forces change in a similar manner? And, could this be a method used to induce a more widespread distribution of positive force feedback? While these results are preliminary, this study evaluates the changes in autogenic force responses during locomotion with the head level, the head tilted up (downslope), and the head tilted down (upslope). Similar to those obtained previously in this laboratory, the

changes in activation due to head tilt are transient; therefore, typical analysis methods used in this study are not valid. However, comparing autogenic force responses obtained within the first 10 seconds of a given trial revealed that autogenic force responses are modulated during head tilt, particularly in FHL. As expected, autogenic force responses were greatest in the head down condition and lowest in the head up condition, when compared to those in the head level condition. Data obtained in these experiments did not have the heads of G separated; therefore, it was unclear whether or not MG was similarly modulated. This suggests that this would be an acceptable model to investigate further the strength, sign, and distribution of positive force feedback during different motor tasks.

Why is force-dependent inhibition among extensors maintained during locomotion? What role, if any, would these pathways play in regulating limb biomechanics and stability? Let us think of it in terms of the stiffness of the limb. We know that length feedback contributes to the regulation of stiffness. This occurs particularly about an individual joint, since the distribution of length feedback is strong autogenically yet weak heterogenically. However, force dependent feedback from Golgi tendon organs is weak autogenically and distributed widely to muscles crossing joints and axes of rotation. In the case of this study where heterogenic inhibition is maintained, an increase in central drive or a perturbation to the system would not only function to stiffen a muscle and individual joints due to length feedback, but it would also coordinate and enhance the compliance of the limb by distributing inhibition across joints. For this

reason, force-dependent feedback is believed to play a large role in interjoint coordination and limb stability.

What evidence is there that the enhancement in autogenic gain is, in fact, due to positive force feedback? At first glance, it might seem that the expression of positive force feedback in MG could be mediated by an increase in muscle spindle gain. However, this increase in gamma fusimotor drive during stepping would most likely result in nonlinearities of force responses due to stretch versus release (Nichols and Cope 2004). On the contrary, MG forces exhibited linear responses to both stretch and release. Likewise, it is possible that there is an overall increase in extensor drive during stepping due to group I interneurons communicating with the CPG. However, this would result in excitation at a latency consistent with polysynaptic pathways. The excitatory effect shown in this study exhibited a latency consistent with a monosynaptic effect.

What is the proposed organization of the network of interneurons involved in mediating this gain change during locomotion? It has been shown that the expression of positive force feedback is confined to a single muscle, yet it remains unclear how exactly this is achieved. For simplicity, it is likely that the circuitry involved in these preferential gain changes remains exactly the same. The question is then how does the system alter the activation of muscles in such a specific pattern. It is possible that the preferential gain change is achieved centrally. Therefore, descending signals would predetermine the gain of specific motor pools. Or, perhaps a more attractive possibility is that the overall organization is dictated by the system itself. For instance, the biomechanical demands of

the limb may increase the drive preferentially to a muscle, such as MG. However, when the demands change, such as when the animal is walking uphill, the drive to other muscles may be required, and therefore the distribution of positive force feedback would be widened. It is possible that this redistribution could occur due to decreased thresholds among interneurons serving specific muscles. Or, it may be more likely that the redistribution depends upon the muscles that are developing the largest forces. This latter hypothesis would allow for an online flexibility for redistributing positive force feedback in response to different motor tasks.

So, why does the distribution of both positive and negative force feedback found in this study make sense physiologically? It has been proposed that one possible function of positive force feedback is to provide a loading reflex, such that the force in the load bearing extensor muscles increases to ensure the completion of a step (Pearson 1995). In this study, MG serves as not only the greatest source of increases in autogenic gain, but also the greatest source of heterogenic inhibition (Ross and Nichols Submitted). Functionally, the autogenic increase in MG, a biarticular muscle, would therefore serve as the localized loading reflex serving multiple joints. This localized increase would also activate heterogenic force-dependent pathways, thus spreading inhibition across multiple joints and axes of rotation. In fact, this distribution of heterogenic force feedback serves as a neural equivalent to the already established biomechanical connections between muscles. These parallel systems function to reinforce the coordination of the entire limb. Furthermore, this widespread inhibition, in combination with the localized positive feedback, would therefore regulate whole-limb stiffness, and promote stability.

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VITA

KYLA TURPIN ROSS

Kyla Turpin Ross was born in Baton Rouge, Louisiana. She received a B.S. in Biological and Agricultural Engineering from Louisiana State University in Baton Rouge, LA in 2000 before joining the inaugural class of the Joint Biomedical Engineering program at Georgia Institute of Technology and Emory University. In addition to research, Kyla's true passions are adventure travel and music. Her quest for adventure has led her and her husband to such destinations as Europe, the Middle East, and Africa. Her hope is to experience new thrills on each continent. Kyla also has a deep love for music. While she is partial to Simon and Garfunkel, opera and Broadway musicals are also true loves. In her spare time, Kyla is often dreaming and planning for the next big adventure.