Effects of Ankle Extensor Muscle Afferent Inputs on Hip Abductor and Adductor Activity

in the Decerebrate Walking Cat

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Running Head: LGS Nerve Stimulation During Decerebrate Walking
Abstract

Electrical stimulation of the lateral gastrocnemius-soleus (LGS) nerve at group I afferent strength leads to adaptations in the amplitude and timing of extensor muscle activity during walking in the decerebrate cat. Such afferent feedback in the stance leg might result from a delay in stance onset of the opposite leg. Concomitant adaptations in hip abductor and adductor activity would then be expected to maintain lateral stability and balance until the opposite leg is able to support the body. As many hip abductors and adductors are also hip extensors, we hypothesized that stimulation of the LGS nerve at group I afferent strength would produce increased activation and prolonged burst duration in hip abductor and adductor muscles in the premammillary decerebrate walking cat. LGS nerve stimulation during the extensor phase of the locomotor cycle consistently increased burst amplitude of the gluteus medius (GMd) and adductor femoris (AF) muscles, but not pectineus or gracilis. In addition, LGS stimulation prolonged the burst duration of both GMd and AF. Unexpectedly, long duration LGS stimulus trains resulted in two distinct outcomes on the hip abductor and adductor bursting pattern: a) a change of burst duration and timing similar to medial gastrocnemius (MG) or b) to continue rhythmically bursting uninterrupted. These results indicate that activation of muscle afferents from ankle extensors contributes to the regulation of activity of some hip abductor and adductor muscles, but not all. These results have implications for understanding the neural control of stability during locomotion as well as the organization of spinal locomotor networks.
Introduction

Most of our understanding of the neural control of locomotion relates to the flexion-extension movements associated with forward progression. By contrast, there is little understanding of the control of medial-lateral motions during walking. Frontal plane motions of the legs have important consequences for the maintenance of balance and stability. In studies of human locomotion, lateral stability has been shown to require active stabilization predominantly through hip abductor muscle torques during stance (MacKinnon and Winter 1993), and medial-lateral foot placement during swing (Bauby and Kuo 2000; Donelan et al. 2004; MacKinnon and Winter 1993; Redfern and Schumann 1994). Similar findings were also reported for walking cats, such that lateral stability and the medial-lateral placement of the hind paws were associated with differential activation of the hip abductors and adductors (Misiaszek 2006). Therefore, understanding balance control during walking requires knowledge of how abductors and adductors are controlled.

The basic locomotor rhythm of alternating flexion and extension is believed to be produced by neuronal networks in the spinal cord commonly referred to as a central pattern generator (CPG) for locomotion (Rossignol 1996). The rhythm generated by the CPG is regulated and adapted by descending influences arising from the brainstem, cerebellum, and cortex. However, afferent feedback from the legs also has powerful influences on the timing of the rhythm and the amplitude of muscle bursts (Pearson et al. 1998; Quevedo et al. 2005). It is reasonable to expect that many of the general principles of the control of flexion and extension will apply to the control of abduction-adduction. For example, Kriellaars et al. (1994) showed that afferent signals arising from hip muscle proprioceptors had powerful influences on the timing of the central rhythm as indicated by the electroneurogram from several muscle nerves,
including gracilis a hip adductor and extensor. Most hip adductor (e.g. adductor femoris, gracilis) and abductor (e.g. gluteals, tensor fascia latae) muscles anatomically are considered hip extensors (Crouch 1969). In the intact walking cat many of the hip abductor and adductor muscles are activated concomitantly with the other extensors of the leg, turning on at the end of swing just prior to ground contact and remaining active for most of the stance phase (Engberg and Lundberg 1969; Frigon et al. 2010; Misiaszek 2006; Pratt et al. 1991; Rasmussen et al. 1978). Therefore, the basic neural control of hip adductors and abductors during walking is likely similar to other extensors of the leg. However, the specific control of the adductors and abductors must be sufficiently different from each other, and from the other extensors in order to produce the torques in the frontal plane required for lateral stability (Misiaszek 2006).

The influence of afferent feedback on the regulation of the flexion-extension behaviour of walking is well established. Proprioceptors in muscles around the hip and ankle powerfully influence the timing of the stepping rhythm as well as the amplitude of the muscle activity in cats (Frigon et al. 2010; Lam and Pearson 2002a; Pearson 2004; Pearson et al. 1998; Stein et al. 2000). Adaptations to the locomotor cycle that are induced by afferent feedback must also be accompanied by active control of lateral stability. Thus, prolongation of the stance phase by stimulation of ankle extensor group I afferents, either electrically or due to some mechanical perturbation, must be accompanied by changes in the activity of muscles controlling lateral stability to prevent the animal from falling over. For example, if the paw of a walking cat enters a hole the opposite limb remains in contact with the ground for a prolonged period of time while the misplaced paw is retracted from the hole and repositioned on a stable surface (Gorassini et al. 1994). While the stance limb must support the weight of the hindquarters for an extended period of time, lateral stability of the pelvis must also be maintained to prevent the animal from falling
over during this corrective behavior. The sustained loading of the stance limb, detected by muscle afferents in extensors of the leg, has been suggested to be an important signal in generating the prolongation of the stance phase. Indeed, stimulation of group I muscle afferents from extensors in the leg leads to prolongation of the stance phase in decerebrate walking preparations (Whelan et al. 1995). A reasonable expectation is that the sensory cues that initiate step cycle adaptations will also generate the required changes in hip abductor and adductor activity to maintain stability during the prolongation of the stance phase.

In the present study, the influence of inputs from group I muscle afferents from ankle extensors on the amplitude and timing of hip abductor and adductor activity was evaluated in the premammillary decerebrate walking cat preparation. We hypothesized that stimulation of the lateral gastrocnemius-soleus (LGS) nerve at group I afferent strength during the stance phase of walking would lead to prolongation of the activity of the hip abductor and adductor muscles, comparable to the prolongation of the activity seen in other extensors of the leg observed with this type of stimulation. We further hypothesized that stimulation of the LGS nerve at group I strength will lead to adaptations in the amplitude of the bursts of the hip adductors and abductors. As most hip abductors and adductors are also hip extensors, we hypothesized that stimulation of the LGS nerve at group I strength would lead to increased burst amplitude in both hip abductors and adductors, as is observed in other extensor muscles of the leg.

**Methods**

A total of 4 decerebrate cats were used for this study. All experimental procedures were approved by the University of Alberta Animal Care and Use Committee in accordance with the Canadian Council on Animal Care.
Cats were anaesthetized using isoflurane continuously provided through a cannula surgically placed inside the trachea. A carotid artery was cannulated to monitor blood pressure and a jugular vein was cannulated to allow the administration of drugs. The other carotid artery was ligated. A pair of Teflon-coated stainless steel wires (AS632, Cooner Wire Co.), with the insulation removed from a 3 mm length of the wire, was sutured into the bellies of the following right hindlimb muscles to record electromyographic (EMG) activity: medial gastrocnemius (MG, an ankle plantarflexor and knee flexor), iliopsoas (IP, a hip flexor), gluteus medius (GMd, a hip extensor and abductor), adductor femoris (AF, a hip extensor and adductor), gracilis (Grac, a hip extensor and adductor) and pectineus (Pect, a hip adductor). GMd and AF were also implanted on the left (contralateral, co) hindlimb. The muscles recorded in each animal are summarized in the figures. Electrode leads were passed subcutaneously to a connector attached to the back of the animal. To reduce the amount of sensory feedback contributing to the control of the right leg muscle activity, the right hindlimb was extensively denervated including the hamstrings branches of the sciatic nerve, femoral nerve, obturator nerve distal to AF, saphenous nerve (including medial articular nerve), common peroneal nerve, caudal cutaneous sural nerve, deep muscular branches of the tibial nerve (serving plantaris and the digital flexors), posterior articular nerve, the lateral gastrocnemius/soleus nerve and the distal portion of the tibial nerve. The combined nerve to the lateral gastrocnemius (LG) and soleus (S) muscles was exposed and cut close to the entry point into LG. The proximal end was then inserted into a cuff electrode for stimulation. To monitor the stimulation delivered from the LGS nerve, a recording nerve cuff was placed around the right sciatic nerve in 2 cats. In the other 2 cats, a cord dorsum potential was recorded to monitor the stimulus volley from the LGS nerve. The cord dorsum potentials were recorded from
the L4/5 level of the spinal cord following a laminectomy via recording wires (AS632, Cooner Wire Company) placed beneath the dorsal laminae.

Cats were then positioned over a treadmill using stereotaxic equipment to stabilize the head and a sling to support the hindlimb region of the animal. The right leg was secured by a clamp around the foot and rigid pins inserted into the femur just proximal to the knee joint. The leg was positioned such that the ankle, knee and hip were at 90°. The other three limbs were positioned on the treadmill belt and were free to step. A *premammillary decerebration* was performed by transecting the brainstem rostral to the superior colliculi. Following the decerebration, anaesthesia was discontinued. Bouts of spontaneous locomotion would occur after this point in response to a moving treadmill or in response to perineal stimulation.

**Protocol**

Threshold values for both the cord dorsum potential and the sciatic nerve potential were determined as the minimum voltage needed by a single stimulus pulse to the LGS nerve to generate the smallest detectable potential. Subsequent stimulus strength was then taken as a multiple of this threshold ($T$) value. In order to target our nerve stimulation to the group I muscle afferents (including Ia and Ib) we selected a stimulus strength of $2T$ (Jack 1978). A stimulation frequency of 150Hz was used along with train durations of 200 ms or 400 ms. The stimulation was delivered 0 ms or 200 ms following the onset of the right MG activity. All four combinations of these train durations and stimulation onset times were tested. (In one animal (Cat 2), data from the delayed stimulus onset trials were not available as this preparation discontinued stepping before reaching that point in the protocol.) The onset of MG burst activity was detected by the online monitoring of the rectified and filtered (50 Hz low pass with zero lag) EMG by an interactive program that permitted manual adjustment of the burst threshold. The
stimulus was programmed to trigger every 3rd MG burst during sequences of steady locomotor activity in the hindlimbs. EMG activity was amplified and filtered (30-3,000 Hz, P511 amplifier, Grass Instruments) prior to acquisition using a custom written LabView data acquisition routine and 12-bit data acquisition card (DAQ card AI-16E-4, National Instruments).

**Data analysis**

Custom written software (Labview 8.0, National Instruments) was used to analyze selected sequences of EMG activity. Only those step sequences showing regular, consistent stepping were used for analysis. Stepping sequences were excluded from analysis if visual inspection suggested large variability in the unstimulated cycle duration or if the rhythm was erratic or inconsistent. Stepping sequences with fewer than 20 continuous steps were not analyzed. Selected EMG traces were then digitally full-wave rectified and low-pass filtered (50Hz, 2nd-order dual-pass Butterworth filter). EMG burst amplitude was measured as the average rectified amplitude over the entire stimulation interval (200 ms or 400 ms) for each muscle. This value was compared to the average amplitude over the same time interval of the control burst which immediately preceded the stimulated burst. Burst amplitudes were expressed as a percentage of the average control value. The amplitude differences between stimulated and control bursts for all trials were then grouped and compared using paired t-tests (significance level set at 0.05). All comparisons were performed on an individual animal basis. For muscles that are active during the extensor phase of the step cycle (MG, GMd, AF, Grac, Pect) EMG burst durations were measured in ms from the onset until the offset of a continuous burst. Cycle durations for IP, Pect, coGMd and coAF were measured from the onset of the burst immediately preceding the stimulus to the onset of the subsequent burst. The stimulated burst and cycle durations were compared with the control burst and cycle durations immediately preceding
stimulation. The burst and cycle durations for control and stimulated steps were compared using paired $t$-tests (significance level set at 0.05) for each animal. Descriptive statistics are reported as the mean and standard deviation.

Results

Effect of LGS stimulation on hip abductor and adductor EMG amplitude

Stimulation of the right LGS nerve at 2T during the extensor phase of the step cycle of the right leg in the decerebrate walking cat consistently resulted in an increase in the EMG burst amplitudes of the right MG muscle. This effect can be observed in the data traces of all five figures while Figs. 1 and 2 show summary graphs with the effect quantified. Stimulation of the right LGS nerve with durations of both 200 ms and 400 ms qualitatively had comparable effects on the amplitudes of the recorded bursts, as can be seen from the data traces in each of the figures. However, as the longer stimulus duration delivered 200 ms after the onset of the right MG burst would often outlast the normal burst duration and because the 400 ms stimulus duration resulted in prolongation of the ongoing bursts (see below), the effect of the stimulus on burst amplitudes is only presented for the 200 ms duration stimuli.

The effect of right LGS nerve stimulation on the right MG burst amplitude was similar whether the stimulus occurred at the onset of the MG burst (Fig. 1A, Cat 1) or 200 ms later (Fig. 2A, Cat 3). As can be seen in the summary graphs of Fig. 1B and 2B, this significant increase ($p<0.05$) in MG burst amplitude was consistently observed in all cats. The stimulation of the right LGS nerve also resulted in an increase in the burst amplitude of the right GMd, a hip abductor, regardless of whether the stimulus occurred at MG burst onset (Fig. 1A, Cat 1) or 200
ms later (Fig. 2A, Cat 3). Significant increases (p<0.05) in right GMd burst amplitudes were observed in all cats with stimulation at both time points (Figs. 1B and 2B).

Stimulation of the right LGS nerve also resulted in an increase in burst amplitude of the right AF, a hip adductor. This is seen in the data traces for a single animal (Cat 3) for stimuli delivered 200 ms after MG burst onset in Fig. 2A. Significant increases (p<0.05) in right AF burst amplitude were observed in both animals with AF recordings and for stimuli delivered at both time points in the step cycle (Figs. 1B and 2B). In contrast, neither right Pect nor right Grac were affected by the stimulus to the right LGS nerve. The lack of effect on burst amplitude in these two adductor muscles can be seen in the data traces from one animal (Cat 1) in Fig. 1A for stimuli delivered at right MG burst onset, when both muscles are active during the step cycle.

As shown in Fig. 1B, the lack of effect on Pect burst amplitude was observed in all three animals with Pect recordings. Stimuli delivered to the LGS nerve 200 ms after the MG burst onset also did not alter the burst amplitude of the right Grac or Pect muscles (Fig. 2B).

Effect of LGS stimulation on hip abductor and adductor EMG burst duration

The influence of right LGS stimulation on the duration of the right extensor phase of the decerebrate walking cycle was dependent upon the duration of the stimulus train. As can be seen in Fig. 1A, stimulus trains of 200 ms with a 0 ms delay had no discernible effect on the durations of the MG or the GMd bursts for Cat 1. The average right MG burst duration for the data depicted in Fig. 1A was 424.8 ± 87.33 ms for the stimulated steps and 414.8 ± 65.84 ms for the control steps, which was not significantly different (p=0.40). The GMd burst durations were 406.3 ± 49.24 ms and 403.1 ± 51.02 ms for the unstimulated and stimulated steps, respectively, and these were not different (p=0.80). Similarly, 200 ms duration stimuli delivered 200 ms after MG burst onset had no observable effect on MG burst duration, as shown in the data traces for
one cat (Fig. 2A, Cat 3). For the data shown in Fig. 2A the duration of the MG burst was 632.4 ± 32.20 ms, which was not different (p=0.54) from the unstimulated steps with a burst duration of 636.0 ± 42.18 ms. The GMd burst durations were also not significantly different (p=0.69) with durations of 465.7 ± 40.02 ms and 462.3 ± 30.50 ms for the unstimulated and stimulated steps, respectively. Comparable results were observed in all cats, and the influence on AF burst duration was similar to that of GMd for the animals described here. The short duration 200 ms stimulus trains had little influence on the duration or timing of the EMG activity.

In contrast, the 400 ms stimulus trains consistently resulted in prolongation of the MG burst and a concomitant delay in the onset of the subsequent IP burst. Examples of this effect are depicted in Figs. 3, 4 and 5. In Fig. 3, the stimulus was delivered at the onset of the MG burst. Data traces from two cats are shown in Fig. 3A. Cat 4 represents data that were typical of 3 of the 4 animals tested showing an increase in MG burst duration of about 150 ms (602.3 ± 48.56 ms and 751.4 ± 44.79 ms for control and stimulated steps respectively). This difference, summarized in the plots in Fig. 3B, was significant (p<0.05) and was observed in all cats. The GMd (611.1 ± 39.43 ms and 740.5 ± 44.80 ms for control and stimulated steps respectively) and AF (592.1 ± 26.4 ms and 729.0 ± 34.88 ms for control and stimulated steps respectively) burst durations were also prolonged. In contrast, Pect burst duration, which was active at the time of stimulus delivery, was not affected (p=0.64) with burst durations of 448.1 ± 41.13 ms and 458.6 ± 24.85 ms for control and stimulated steps respectively. The lack of effect on Pect burst duration was observed in all 3 cats for which Pect was recorded. In addition, in Cat 1 the duration of the Grac burst was also not affected (p=0.13) with burst durations of 621.6 ± 55.21 ms and 596.6 ± 53.09 ms for control and stimulated steps respectively. Concomitant with the prolongation of the burst durations of some extensor muscles, the IP cycle duration was
increased by the 400 ms stimulus trains. For Cat 4, the stimulation significantly increased (p<0.05) the IP cycle duration to 1010.3 ± 53.23 ms from the control cycle durations of 871.0 ± 39.55 ms. This increase in IP cycle duration was observed in all cats (Fig. 3C).

In Fig. 3A the data traces from Cat 2 are also shown. Stimulus trains of 400 ms duration delivered at MG burst onset also increased the MG and GMD burst durations, while also increasing the IP and Pect cycle durations. However, the effect of the stimulation was much more pronounced than in the other 3 cats. In this cat, MG burst durations increased from 331.5 ± 33.10 ms during control steps to 997.9 ± 53.10 ms for the stimulated steps. This was mirrored in GMD with burst durations of 392.2 ± 38.88 ms and 992.2 ± 57.18 ms for control and stimulated steps respectively. While the MG and GMD muscles maintained prolonged bursts, the IP and Pect bursts were delayed, reflected by increased cycle durations for both IP (701.8 ± 31.55 ms and 1304.7 ± 71.84 ms for control and stimulated steps respectively) and Pect (691.9 ± 39.60 ms and 1306.3 ± 65.95 ms for control and stimulated steps respectively).

Delaying the onset of the 400 ms duration stimulus trains by 200 ms resulted in a more pronounced prolongation of the MG burst, with a concomitant delay in the IP burst, in 2 of the 3 cats for which this was tested (Fig. 4). Data traces from Cat 4 are shown in Fig. 4A, while those from Cat 1 are shown in Fig. 5A, showing that 400 ms duration trains occurring 200 ms after the onset of the MG burst usually resulted in a substantially prolonged extensor phase of the step cycle. As shown in the summary plots in Fig. 4B and 4C, this pronounced effect of the stimulus was observed in the burst durations of MG, GMD and AF and the cycle durations of IP and Pect in both Cats 1 and 4. It is also apparent that the stimulus had no effect (p=0.93) on the duration of the Grac burst in Cat 1. The data for Cat 3 are also shown in Fig. 4A, with the summary data depicted in the plots of Fig. 4B and 4C. In this cat, delaying the stimulus train also significantly
prolonged (p<0.05) the burst durations of MG, GMd and AF, while significantly delaying the onset of the IP burst, however the effect was less pronounced than in the other two cats.

Although the stimulation of the LGS nerve with a 400 ms train usually resulted in prolongation of the GMd and AF burst, with a concomitant delay in the onset of the subsequent Pect burst, there were several instances when the hip abductor and adductor muscles continued to burst rhythmically. As shown in Fig. 5B, during these events the MG burst would maintain a sustained contraction and IP bursting would be absent until after the sustained MG burst subsided. Despite this disruption in the rhythmic alternating pattern of extensor-flexor activity, the GMd and Pect muscles continued to burst rhythmically with little apparent disruption to the timing of the bursts. It is important to note that the data in Fig. 5B are from the same animal as the data in Fig. 5A. Events such as that depicted in Fig. 5B were observed in all cats, except for Cat 3. Moreover, these events were only observed if the 400 ms stimulus train produced pronounced prolongation of the extensor and delay of the flexor phase. Thus, these events were observed in Cat 1 and 4 when the stimulus was delivered 200 ms after the onset of the MG burst. In Cat 2, these events were observed during trials with a 0 ms delay. In Cat 1, 5 of these events were elicited from 46 stimuli and in Cat 4, 3 events were triggered from 72 stimuli. Cat 2, which exhibited the events with stimuli delivered with a 0 ms delay, 2 events were observed from a total of 38 stimuli. The stimuli that produced these rare events were excluded as outliers from the analyses described above.

**Effect of LGS nerve stimulation on contralateral hip muscle activity**

Stimulation of the right LGS nerve did affect the activity of the muscles recorded in the left, contralateral leg. As can be seen in the example data traces shown in all five figures, the amplitude and timing of the bursts in coAF and coGMd were largely unaffected by the delivery
of the stimulus. As shown in Figs. 1B and 2B, the 200 ms duration stimuli did not alter (p>0.05) the coAF or coGMd amplitudes for any of the animals during the period of the stimulation, even if the stimulus was delivered at a time when the muscle was normally active. This was also true for the longer 400 ms duration stimuli as can be seen from the sample data traces in Figs. 3 through 5 (summary data not presented).

Given the pronounced effect of the 400 ms duration stimuli on the duration and timing of muscle activity in the right leg it might be expected that the timing of the activity of the muscles of the left leg would also be altered. However, this was not the case. As can be seen in Figs. 3C and 4C the cycle durations of the contralateral muscles were unaffected (p>0.05) by the 400 ms duration stimuli, regardless of the magnitude of the influence on the duration and timing of the muscle activity of the right leg.

Discussion

The principle finding of this study is that stimulation of the LGS nerve at group I afferent strength during the extensor phase of the stepping cycle in the decerebrate cat leads to increased activity in extensor muscles throughout the leg, including those that also act as abductors and adductors of the hip. However, neither Grac, a hip extensor and adductor, nor Pect, a hip adductor, were affected by the same stimulation, even though these muscles were both active during the delivery of the stimulation. Combined, these findings demonstrate that group I afferent inputs from the ankle extensors LGS contribute to the regulation of some proximal muscles about the hip, including muscles that act to abduct and adduct the hip, which might then contribute to the control of medial-lateral stability during walking. Another important observation is that normally the rhythmic bursting pattern of the hip abductors and adductors was closely linked with that of the more traditional extensor-flexor bursting pattern demonstrated by
MG and IP. However, we frequently observed that hip abductors and adductors would continue bursting rhythmically while the MG and IP rhythm was suspended following LGS nerve stimulation. This dissociation of the bursting pattern of the abductor and adductor muscles from the more traditional extensor-flexor muscles of the legs has implications for understanding how the neural systems controlling the timing and sequencing of the rhythmic pattern of activity are organized.

Effects of stimulating the LGS nerve on hip muscle activity

Previous studies have demonstrated that ankle extensor group I afferents have a strong influence on the activity of extensor muscles throughout the leg of decerebrate cats during walking (Frigon et al. 2010; Guertin et al. 1995; Whelan et al. 1995; Whelan and Pearson 1997). Therefore, in the present study it was expected that LGS nerve stimulation would lead to excitation and prolongation of GMd, AF and Grac activity during the extensor phase of the step cycle as these muscles are typically considered to be extensors. However, these muscles also provide abductor and adductor actions at the hip. Our results provide conclusive evidence that sensory cues that regulate extensor muscle activity during the stance phase of the locomotor cycle can also contribute to the regulation of activity of GMd and AF, consistent with observations for other extensors of the leg. However, in the one cat for which we recorded Grac this same stimulation had no effect on the activity of this muscle. Moreover, the activity of Pect, also an adductor at the hip, was also unaffected by the LGS nerve stimulation even if the stimulus was delivered when Pect was active during the early extensor phase. Therefore, LGS nerve stimulation did not provide a generalized excitation to all hip adductors, or to all muscles with both extensor and adductor actions. The implication is that this distal source of afferent
feedback has differential effects on the activity of the proximal muscles of the hip, which might therefore have important consequences in the regulation of stability during walking.

It was somewhat surprising that the stimulation of the LGS nerve appeared to have comparable effects on the activity of GMd and AF. Previously, it was demonstrated that the GMd and AF muscles were differentially regulated in response to balance disturbances imposed during walking in the intact, behaving cat (Misiaszek 2006) or during locomotion in a decerebrate preparation (Musienko et al. 2012). Therefore, one would expect that if LGS afferent feedback contributes to the control of frontal plane stability that the influence of this input would differentially influence the activity of these muscles. However, the lack of distinct influences of the LGS stimulation on these two muscles presumably reflects the fact that both are also extensors of the hip. The increased co-activation of these muscles would therefore presumably provide increased extensor torque at the hip, but a relatively neutral effect in the frontal plane. This suggests that the LGS nerve stimulation produces a relatively generalized increase in extensor activity throughout the leg, which would be important for the regulation and control of stepping actions of the leg (Pearson 2004), but does not appear to provide an input that specifically regulates abduction or adduction actions at the hip. Nevertheless, increased co-activation in these muscles might play an important role in stabilizing the hip during the stance phase of walking by increasing the stiffness of the joint in the frontal plane and preventing or minimizing the impact of an unexpected disturbance. Functionally, group I muscle afferents from ankle extensors have been argued to be important in signaling the loading of the leg during the stance phase of walking and thereby indicate that extensor activity is required to maintain support of the body until such time as the contralateral leg is in contact with the ground and able to assume the load of the body’s mass (Pearson 2004). Therefore, if the leg continues to bear
load it will also be critical to not only support the mass vertically, but also control and stabilize the mass in the frontal plane. For example, when a walking cat steps into a hole the stance leg maintains weight support, without falling over, until such time as the foot that entered the hole is replaced on a supporting surface (Gorassini et al. 1994). It seems logical then that the same afferent cues that signal the continued need for weight support would also contribute to the regulation of the frontal plane stability by increasing the stiffness of the hip joint. The specific differential control of the GMd and AF muscles observed in response to balance disturbances (Misiaszek 2006; Musienko et al. 2012) would presumably then be initiated by other sensory cues, such as cutaneous inputs from the paws (Bolton and Misiaszek 2009; Honeycutt and Nichols 2010) or muscle afferents from the hip muscles themselves (Musienko et al. 2012).

The differential effect of the LGS nerve stimulation on the activity of Pect and Grac from that of AF indicates that there is differential control of the hip adductor muscles. This presumably reflects differences in the functional roles of these muscles. For instance, AF is typically active during the stance phase of the step cycle when the other extensors of the leg are also active. Therefore, it would be expected that activation of AF with the concomitant activation of GMd during stance would provide torques about the hip that stabilize the body over the stance limb in the frontal plane. In contrast, Pect is typically active when IP is active, indicating this muscle assists in controlling the medial-lateral swing trajectory of the leg. It therefore stands to reason that group I afferents from ankle extensor muscles, which will presumably signal events associated with the stance phase of the step cycle, will influence the activity of AF but not Pect. It is noteworthy that Grac, which is both a hip extensor and adductor akin to AF, was unaffected by the stimulation of the LGS nerve. In the one animal for which data were recorded from Grac this muscle became active prior to the onset of the MG burst and frequently overlapped activity...
in IP. Therefore, Grac was active prior to the other extensors of the leg and for a portion of the flexor phase of the step cycle. This suggests that Grac has a functional role that is unique from that of AF. Consequently, the contribution of somatosensory feedback to the regulation of abductor and adductor muscle activity during walking appears to be specific to the functional requirements of the muscle studied within the context of the ongoing task.

It is important to note that in our preparation the right leg was extensively denervated and immobilized, while the left leg remained intact and was free to step on the treadmill. The right leg was denervated in this study in an attempt to minimize the influence of other somatosensory inputs to the control of the muscle activity of interest and thereby isolate the effects of the right LGS nerve stimulation. However, the denervation is necessarily incomplete as the nerves serving the recorded muscles remain intact, in particular the hip abductors and adductors. Therefore, the rhythmic contraction of the muscles during the stepping sequences and the stepping of the left leg could provide sensory feedback that interacts with the feedback derived from the LGS nerve stimulation. Consequently, some of the effects observed in this study might arise from feedback secondary to the direct effects of the LGS nerve stimulation per se, such as arising from the increased contraction of the MG, GMd or AF muscles. It should also be noted that in the reduced preparation the influence of the LGS nerve stimulation might be overstated when compared to the intact leg. In the intact animal the feedback from all of the sources that were eliminated in this preparation might also contribute to the regulation of the muscles that were recorded. Therefore, the weight of the contribution from the group I afferents from the LGS nerve might be muted in comparison. Nevertheless, this study demonstrates that stimulation of the LGS nerve at group I strength leads to clear changes in the activity of some hip muscles,
while not affecting others. Therefore, this source of somatosensory feedback will likely contribute to the control of these muscles in the intact behaving animal.

*Insights into the organization of the central pattern generator*

Stimulation of the LGS nerve with a 400 ms train duration resulted in prolonged activation of the ankle extensor muscle MG, which was accompanied by a suspension of activity in the hip flexor IP. This sort of suspension in the bursting activity of flexors and extensors is well documented and has led to insights into how the CPG for locomotion might be organized (Rybak et al. 2006a; Rybak et al. 2006b). In our study, the bursting activity of GMd and AF usually mirrored the events observed in MG, while the bursting activity of Pect and Grac (which overlapped activity in IP and MG) usually mirrored the events in IP (Fig. 5A). This would tend to suggest that the activity of these muscles is controlled in much the same way as other muscles active during extensor or flexor phases of the locomotor cycle. However, we also frequently observed GMd, AF, Pect and Grac to continue to burst rhythmically during periods of suspended activity in MG and IP (Fig.5B). The implication is that muscles that abduct and adduct the hip have flexibility in their pattern of activity and are not strictly governed by the controls for the more traditional flexors and extensors of the leg. This appears to be related to their function as abductors and adductors and not related to the joint they cross as IP is a hip flexor and consistently stopped bursting for the duration of the prolonged MG bursts.

In our study, the contralateral leg of the cat was intact and free to step on the moving treadmill belt. Therefore, there was considerable afferent input from the moving left leg and this leg consistently maintained its rhythm during the LGS stimulation events in the right leg. Indeed, the LGS stimulation of the right leg appeared to have no influence on the amplitude or timing of the activity of the left GMd or AF muscles. For this reason, when the bursting of the MG and IP
muscles resumed after a period of suspended activity the bursting pattern was well timed with
the pattern observed before the stepping pattern was suspended. Therefore, the fact that GMd,
AF, Pect and Grac bursting during the suspended activity in MG and IP maintained the same
timing as the control periods of stepping is explained by the strong influence from the activity
and feedback from the contralateral leg. It is our opinion that this is best explained by a CPG
model with a bipartite structure such as that described by Lafreniere-Roula and McCrea (2005)
and Rybak et al. (2006a; 2006b). In this model the timing of the rhythm is controlled by a
rhythm generating (RG) network, separate from the coordination and activation of multiple
motoneurone populations by a pattern formation (PF) network. The flexibility in the appearance
of bursting in the abductors and adductors during suspended activity in MG and IP we observed
suggests that the hip abductors and adductors are more closely coordinated with each other than
they are with the other flexors and extensors of the leg. For this to be the case then the control of
abductors and adductors would require an increased degree of complexity in the PF network to
allow for the flexibility of coordination with the extensors and flexors we observed.

Alternatively, the dissociation of the abductor and adductor bursting patterns from the
MG and IP activity could be explained by a model of control involving unit burst generators such
as described by Grillner (1981). In this model, separate oscillators would coordinate the timing
of a limited number of motoneurone pools. These separate oscillators can then be linked to allow
synchronized activity of the groups of motoneurone pools. Thus, that GMd, Pect and Grac
continue bursting while MG and IP are suspended in Fig. 5B could be explained by separate unit
burst generators for the two sets of muscles. Indeed, Lafreniere-Roula and McCrea (2005)
suggest that results such as we show in Fig. 5 would be evidence in support of the unit burst
generator proposal as GMd sometimes mirrors the prolonged activity in MG and sometimes does
not. However, it is unclear why the hip abductor and adductor muscles would be coordinated by a separate unit burst generator from the hip flexor IP, which appears to be more tightly coordinated with the ankle extensor MG in our preparation. In the scenario whereby the GMd, Pect and Grac activity shown in Fig. 5B is governed by a separate unit burst generator from that of IP then there is the distinct possibility that the temporal relationships of the flexors and extensors of the hip could become uncoordinated. Therefore, a more reasonable explanation for the occasional dissociation of activity of GMd and Pect from that of MG and IP depicted in Fig. 5B is that there is a common rhythm generator that oversees the timing of the step-related bursting, but that the specific activation of groups of motoneurones within that rhythm is coordinated by separate networks, such as that proposed by the pattern formation layer of the bipartite model (Lafreniere-Roula and McCrea 2005; Rybak et al. 2006a; Rybak et al. 2006b).

It was surprising that stimulation of the right LGS nerve did not affect the timing of the EMG activity of the left, contralateral leg (Figs 3 and 4). We had expected that adaptations to the step cycle of one leg would be reflected in the step cycle of the contralateral leg to ensure the maintenance of a coordinated bilateral stepping pattern. For example, Lam and Pearson (2002b) showed bilateral adaptations to the stepping pattern of decerebrate walking cats with stimulation of the nerves serving the sartorius muscles. However, in that study, the hind legs of the cats were intact except for the nerves serving the sartorius muscles and both legs were free to step on the treadmill belt. In our study, only the contralateral (left) leg was intact and free to step on the treadmill, whereas the afferent feedback from the ipsilateral (right) leg was reduced. Therefore, the lack of an effect on the stepping pattern of the contralateral leg in our study might be a consequence of the extensive denervation and immobilization of the ipsilateral leg such that the
afferent feedback from the intact, stepping contralateral leg dominates the control of the stepping
of that leg.

Summary and conclusions

Electrical stimulation of the LGS nerve at group I strength leads to adaptations in the
amplitude and timing of extensor muscle activity in the walking decerebrate cat. The resultant
facilitation of GMd and AF was consistent with the role of these muscles as extensors at the hip;
however these muscles also contribute to movement about the frontal plane. Therefore, in
addition to weight support and step propulsion this afferent feedback could play a role in
controlling stability during the stance phase of walking by increasing the stiffness of the hip joint
in the frontal plane. However, the LGS nerve stimulation does not appear to provide a signal that
specifically regulates abduction or adduction actions at the hip per se. An important result from
this investigation is that the sensory link between ankle extensor afferents and hip abductors and
adductors is not strictly coupled with the control of other leg muscles. The disparate influence of
stimulation on different muscles acting at the same joint, such as GMd and IP, indicates that
these muscles may be recruited into different synergies. This could allow for flexible adaptation
to stepping and postural challenges in multiple planes. Overall, these results have implications
for understanding the neural control of lateral stability during locomotion and the organization of
spinal locomotor networks.

Acknowledgements

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References


Figure Legends

Figure 1: A, Average EMG traces (n=10) from control (thin traces) and stimulated steps (thick traces) from one cat (Cat 1). Stimulation was for 200 ms duration, initiated at the onset of the MG burst. B, The average EMG amplitudes for a window defined by the duration of the stimulus train is depicted for each animal for each muscle tested. The average amplitudes of stimulated trials are expressed as a percentage of the average control amplitude. The horizontal dashed line represents the average unstimulated trials, or 100%. Error bars indicate standard deviations. The * denotes significant difference between the control and stimulated average amplitudes (paired t-test, p<0.05).

Figure 2: A, Average EMG traces (n=10) from control (thin traces) and stimulated steps (thick traces) from one cat (Cat 3). Stimulation was for 200 ms duration, initiated 200 ms after the onset of the MG burst. B, The average EMG amplitudes for a window defined by the duration of the stimulus train is depicted for each animal for each muscle tested. The average amplitudes of stimulated trials are expressed as a percentage of the average control amplitude. The horizontal dashed line represents the average unstimulated trials, or 100%. Error bars indicate standard deviations. The * denotes significant difference between the control and stimulated average amplitudes (paired t-test, p<0.05).

Figure 3: The effect of a 400 ms stimulus train delivered to the LGS nerve at MG burst onset on the burst and cycle durations. A, EMG traces for a sequence of steps with an individual stimulation trial from two cats, with data from Cat 4 on the left and Cat 2 on the right. B,
Average burst durations for the stimulated (filled bars) and control (empty bars) steps are depicted for each animal for each muscle tested. C, Average cycle durations for the stimulated (filled bars) and control (empty bars) steps are depicted for each animal for each muscle tested. Error bars indicate standard deviations. The * denotes significant difference between the control and stimulated average burst durations (paired t-test, p<0.05).

**Figure 4:** The effect of a 400 ms stimulus train delivered to the LGS nerve 200 ms after MG burst onset on the burst and cycle durations. A, EMG traces for a sequence of steps with an individual stimulation trial from two cats, with data from Cat 4 on the left and Cat 3 on the right. B, Average burst durations for the stimulated (filled bars) and control (empty bars) steps are depicted for each animal for each muscle tested. C, Average cycle durations for the stimulated (filled bars) and control (empty bars) steps are depicted for each animal for each muscle tested. Error bars indicate standard deviations. The * denotes significant difference between the control and stimulated average burst durations (paired t-test, p<0.05).

**Figure 5:** Two sequences of data from one cat (Cat 1) using a stimulus train of 400 ms with a 200 ms delay after the onset of the MG burst. A, The stimulus resulted in sustained activity in MG and an absence of IP bursting during this time. Concomitantly, GMd activity was also sustained, while Pect and Grac bursting was absent. Subsequently, the rhythmic bursting resumed. B, The same stimulus also generated a response in which Pect, Grac and GMd all continued bursting, despite the sustained activity in MG and pause in activity of IP.
A

MG
IP
GMd
Grac
Pect
coAF
Slin

B

MG
Gmd
AF
coAF
IP
Grac
Pect
coGMd

500 ms

EMG Amplitude (% control)
A

Cat 4

MG
IP
GMd
AF
Pect
coGMd

500 ms

Cat 2

MG
IP
GMd
Pect
coGMd
coAF

500 ms

B

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C

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* Control
* Stimulated