Short-latency crossed inhibitory responses in extensor muscles during locomotion in the cat

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Abstract

During locomotion, contacting an obstacle generates a coordinated response involving flexion of the stimulated leg and activation of extensors contralaterally to ensure adequate support and forward progression. Activation of motoneurons innervating contralateral muscles (i.e. crossed extensor reflex) has always been described as an excitation but the present paper shows that excitatory responses during locomotion are almost always preceded by a short period of inhibition. Data from 7 cats chronically implanted with bipolar electrodes to record electromyography (EMG) of several hindlimb muscles bilaterally were used. A stimulating cuff electrode placed around the left tibial (Tib) and left superficial peroneal (SP) nerves at the level of the ankle in 5 and 2 cats, respectively, evoked cutaneous reflexes during locomotion. During locomotion, short-latency (~13 ms) inhibitory responses were frequently observed in extensors of the right leg (i.e. contralateral to the stimulation), such as gluteus medius and triceps surae muscles, which were followed by excitatory responses (~25 ms). Burst durations of the left Srt, a hip flexor, and ankle extensors of the right leg increased concomitantly in the mid- to late-flexion phases of locomotion with nerve stimulation. Moreover, the onset and offset of Srt and ankle extensor bursts bilaterally were altered in specific phases of the step cycle. Short-latency crossed inhibition in ankle extensors appears to be an integral component of cutaneous reflex pathways in intact cats during locomotion, which could be important in synchronizing EMG bursts in muscles of both legs.

Keywords: spinal reflex, locomotion, crossed inhibition
Introduction

Bilateral postural adjustments mediated by ipsilateral and crossed spinal reflex pathways are important to adapt to the environment during walking (Zehr and Stein 1999; Burke 1999; McCrea 2001; Rossignol et al. 2006). For instance, mechanical stimulation of the foot dorsum during the swing phase of locomotion generates a coordinated reflex, the stumbling corrective reaction, in several leg muscles bilaterally allowing the perturbed limb to progress over the obstacle (Prochazka et al. 1978; Forssberg 1979; Wand et al. 1980; Buford and Smith 1993; Zehr and Stein 1999). In the stumbling corrective reaction the ipsilateral leg is lifted over the obstacle while the contralateral leg supports the stimulated limb (i.e. crossed extensor reflex). The locomotor program must be capable of adjusting both hindlimbs when one limb is perturbed so that progression and equilibrium is preserved (Saltiel and Rossignol 2004b) but pathways involved in this bilateral coupling are unclear. Recent work in cats has delineated interneuronal spinal pathways involved in stumbling corrective reactions in the ipsilateral limb during fictive locomotion (Quevedo et al. 2005a; Quevedo et al. 2005b) but pathways to the contralateral leg were not studied.

Electrically stimulating cutaneous afferents from the foot has been used as an alternative to mechanical stimulation to evaluate responses in several leg muscles (Duysens and Pearson 1976; Duysens 1977; Duysens and Loeb 1980; Abraham et al. 1985; Pratt et al. 1991; Buford and Smith 1993; Loeb 1993). During swing, electrical stimulation of cutaneous afferents from the paw evokes, in the ipsilateral limb, short- (P1) and longer- (P2) latency excitatory responses in flexors in the swing phase whereas during stance, flexors are typically silent, and responses in extensors are characterized by short-latency inhibition (N1) followed by longer-latency (P2-P3) excitation (Duysens and Loeb 1980; Abraham et al. 1985; Pratt et al. 1991; Loeb 1993). Similar
responses are also evoked in the forelimbs during locomotion (Drew and Rossignol 1987; Zehr and Duysens 2004). In the contralateral hindlimb, excitatory responses (P2) are observed in extensors at a latency of 20-25 ms (Duysens and Loeb 1980) and it was proposed that crossed excitatory pathways coordinate activity between limbs during locomotion (Sherrington 1910a; Lundberg 1979; Gauthier and Rossignol 1981; Lundberg et al. 1987; Rossignol et al. 2006).

However, by conditioning monosynaptic reflexes to examine motoneuron excitability it was shown that crossed inhibitory pathways also exist (Lloyd 1944; Curtis et al. 1958; Holmqvist and Lundberg 1959). In another study, inhibitory post-synaptic potentials mediated by a disynaptic pathway were recorded in motoneurons of the sacral cord following stimulation of low-threshold fibers of the contralateral dorsal root of the same segment (Curtis et al. 1958). Crossed disynaptic inhibition of sacral motoneurons was shown to be mediated by group Ia muscle spindle afferents (Jankowska et al. 1978). Stimulation of group II or cutaneous afferents in non-locomotor anesthetized cats also evokes short-latency inhibition in contralateral ankle extensor motoneurons (Arya et al. 1991; Aggelopoulos et al. 1996; Edgley and Aggelopoulos 2006). During locomotion, inhibition of contralateral extensors while the ipsilateral limb is perturbed during swing would tend to destabilize the animal. Reflex pathways during locomotion are often thought as adjusting phase onset and offset by terminating stance and initiating swing, for example, but rarely within the context of adjusting the coordination between limbs.

A few studies have suggested that probably several interlimb coordination mechanisms exist (Forssberg et al. 1980; Saltiel and Rossignol 2004a; Saltiel and Rossignol 2004b) and it is possible that crossed inhibition plays a critical role. Therefore, because crossed inhibitory responses are elicited by stimulating group II or cutaneous afferents in reduced non-locomotor
preparations (Arya et al. 1991; Aggelopoulos et al. 1996; Edgley and Aggelopoulos 2006) we hypothesized that these responses could also be present in walking cats.

Methods

Animals and general procedures

Data were obtained from 7 adult cats (6 males, 1 female) weighing between 3.0 and 7.0 kg. Cats in the present study were used in other experiments (Frigon and Rossignol 2007) but specific observations have not been published previously. Cats were first selected based on their ability to walk for prolonged periods on a treadmill and trained for approximately 2 weeks at their preferred speed (0.3-0.5 m/s). Cats were subsequently implanted with chronic electrodes for EMG recordings and nerve stimulation, allowed to recover from the implantation, and baseline values of EMGs and reflexes were recorded for 33-60 days.

The experimental protocol was in accordance with the guidelines of the animal Ethics Committee of the Université de Montréal. All surgical procedures were performed under general anesthesia and aseptic conditions. Prior to surgery, cats were injected with an analgesic (Anafen 2 mg/kg; subcutaneously) and pre-medicated (Atravet 0.1 mg/kg, Glycopyrrolate 0.01 mg/kg, Ketamine 0.01 mg/kg; intramuscularly). Cats were then intubated and maintained under gaseous anesthesia (isoflurane 2%) while heart rate and respiration were monitored. After surgery, an analgesic (Buprenorphine 0.01 mg/kg) was administered subcutaneously. An oral antibiotic (cephatab or apo-cephalex, 100 mg/day) was given for 10 days following surgery.

EMG

Chronic electromyographic (EMG) electrodes were implanted bilaterally in the following hindlimb muscles for all cats: semitendinosus (St: knee flexor/hip extensor), anterior part of
sartorius (Srt: hip flexor/knee extensor), vastus lateralis (VL: knee extensor), lateral gastrocnemius (LG: ankle extensor/knee flexor) and tibialis anterior (TA: ankle flexor). The medial gastrocnemius (MG: ankle extensor/knee flexor), soleus (Sol: ankle extensor) and gluteus medius (GM: hip extensor) were also implanted in 5, 3, and 2 cats, respectively. A pair of Teflon-insulated multistrain fine wires (AS633; Cooner wire, Chatsworth, CA) was directed subcutaneously from head-mounted fifteen pin connectors (Cinch Connectors; TTI Inc., Pointe-Claire, Canada) and sewn into the belly of each muscle for bipolar EMG recordings. EMG recordings were bandpass filtered (100-3000 Hz) and amplified (gains of 0.5-50K) using two Lynx-8 amplifiers (Neuralynx, Tucson, Arizona). EMG data were digitized (5000 Hz) using custom-made acquisition software.

Step cycle duration was measured as the time between two successive Srt bursts. Burst duration was determined as the time from onset to offset. The effects of Tib nerve stimulation, evoked at different phases of the step cycle, on durations of the step cycle and selected EMG bursts (Srt and ankle extensors bilaterally) were assessed in 5 cats and expressed as the difference from the control (i.e. non-stimulated) values in ms. Stimulated cycles were grouped in one of 10 bins according to the time stimulation was delivered during the step cycle while non-stimulated cycles were averaged to provide control values. The mean control value was then subtracted from the mean stimulated values in each of the 10 bins providing a difference from control in ms in each phase. Correlation coefficients (r) were calculated (Sigmaplot 9.0) to determine the strength of the linear association between burst durations of the left Srt and right ankle extensors and between the right Srt and left ankle extensors with Tib nerve stimulation during different phases of the step cycle.

Nerve stimulation
A chronic stimulating electrode composed of bipolar wires (AS633; Cooner wire, Chatsworth, CA) embedded in a polymer (Denstply International) cuff (Julien and Rossignol 1982) was placed around the left tibial (Tib) nerve at the ankle adjacent to the Achilles’ tendon in 5 cats and around the left superficial peroneal (SP) on the dorsum of the foot in 2 cats. Both nerves were stimulated (Grass S88 stimulator) at varying intensities during locomotion with a single 1 ms pulse at a constant time (100 ms after onset of left St burst) to determine the threshold for obtaining a small yet consistent short-latency (~10 ms) response in TA. Stimulation current was then set at 1.2-1.5 times this threshold. During testing sessions, stimuli were given once every three cycles. The time of the stimulus was varied pseudo-randomly to evoke responses at different times during the step cycle for a total of approximately 120-200 stimulations. In one session left SP nerve stimulation was triggered at 100 ms for ~100 stimulations following left St burst onset.

Reflexes were measured as detailed previously (Frigon and Rossignol 2007) and only responses evoked in extensors bilaterally will be described. Figure 1 provides a detailed description of the methodology used to quantify reflex responses. Briefly, the EMGs were grouped into stimulated or control (non-stimulated) trials. The step cycle was divided into 10 phases by synchronizing the cycle to the onset of the left St burst. At least 50 control cycles (i.e. without stimulation) were averaged and separated into these 10 bins according to the time they were evoked in the cycle to provide a template of baseline locomotor EMG (bEMG) during the step cycle (dotted line in Fig. 3). From the 120-200 stimulations, approximately 10-20 reflex responses were grouped in each of the 10 bins superimposed on the bEMG for that bin. Onset and offset of reflexes in extensors, delineated as a prominent negative or positive deflection away from the bEMG, were determined manually using pre-defined latencies as guidelines.
(Duysens and Stein 1978; Abraham et al. 1985; Pratt et al. 1991; Loeb 1993). We used previously described nomenclature (Duysens and Loeb 1980) where N and P respectively denote negative (inhibitory) and positive (excitatory) responses. The numbered suffix indicates response onset where 1 is ~10 ms and 2 is ~25 ms. Excitatory responses in ipsilateral extensors beginning at ~ 35 ms are sometimes termed P3 (Duysens and Loeb 1980) but for simplicity will be referred here as P2. EMG from onset to offset was rectified and integrated and the bEMG was subtracted from this value. The subtracted value was then divided by a 10 ms portion of the bEMG in the corresponding bin to provide a measure of reflex amplitude. Correlation coefficients (r) were calculated (Sigmastat 9.0) to determine the strength of the linear association between the amplitude of N1 and P2 in ankle extensors of the same limb evoked by Tib nerve stimulation during the step cycle.

Statistics

A one-way analysis of variance (ANOVA) was used to determine the effects of Tib nerve stimulation when given at different times during the step cycle on the duration of the step cycle and selected EMG bursts across 5 cats. If significant, Dunnet’s post-hoc test was performed against control (non-stimulated) values. An ANOVA was also used to determine significant differences between the latency and duration of ipsilateral and contralateral responses of the same nature (e.g. inhibition or excitation) evoked by Tib nerve stimulation across 5 cats. The duration and latency of inhibitory and excitatory responses in extensors on the ipsilateral and contralateral sides were grouped separately to evaluate differences between both hindlimbs. For example, inhibitory responses in extensors of the ipsilateral limb were compared to inhibitory responses in extensors of the contralateral limb. Significance level was set at p ≤ 0.05. Descriptive statistics are mean values ± the standard error of measurement (SEM).
Results

Crossed inhibition evoked by SP or Tib nerve stimulation

Short-latency inhibitory responses were observed in extensors of the left leg during stance of the left leg and in the right leg during stance of the right leg with stimulation of the Tib or SP nerves of the left leg, which were followed in both cases by longer-latency excitatory responses. For instance, figure 2 shows the effects of Tib nerve stimulation on selected EMG bursts (Srt and triceps surae muscles bilaterally) during locomotion in one cat. In the same session but at different times, the left Tib nerve was stimulated during stance of the left (Fig. 2A) and stance of the right (Fig. 2B) leg. In both instances stimulation evoked a very brief period of silence, or inhibition, of the ongoing EMG in ankle extensors of both legs. A closer examination in the right LG clearly shows that stimulating the left Tib nerve during stance of the right leg produces a period of inhibition starting approximately 12 ms following the stimulus (Fig. 2C).

In one session for one cat the left SP nerve was stimulated repeatedly at a fixed interval following the onset of the left St burst to elicit a large of number of responses while contralateral extensors were active. Figure 3 illustrates cutaneous reflex responses at short- and longer-latency in the right LG evoked by stimulating the left SP nerve approximately 100 times in one cat while the right hindlimb was in the latter half of stance. A short-latency crossed inhibition at ~13 ms of the ongoing EMG lasting for ~14 ms is observed (grey area) followed by a longer-latency excitatory response at ~26 ms (black area), which lasts for ~14 ms. Crossed inhibitory responses were consistently observed in ankle and hip extensors but not in vastus lateralis, a knee extensor. Although preliminary results showed that Tib nerve stimulation evoked short-latency inhibitory responses during the hip flexor burst of the contralateral Srt, a bifunctional muscle, the
presence of crossed inhibition in flexors and other muscles requires further investigation before a conclusive statement can be made.

To compare reflex responses evoked in extensors of the left and right leg, muscles were recorded bilaterally and stimulation was evoked at different times during the step cycle. Figure 4 shows the average of ~10 responses in each of the 10 phases of the step cycle, synchronized to the left St burst, to stimulation of the left Tib nerve in the left (Fig. 4A) and right (Fig. 4B) MG of the same cat. Stimulation of the left Tib nerve evoked a short-latency inhibition (~10 ms) and a longer-latency excitation (~35-40 ms) in the left MG. Stimulation of the same nerve likewise evoked a short-latency inhibition (~13 ms) and longer-latency excitation (~25 ms) in the right MG during locomotion. As can be seen, inhibitory responses in the right MG appear at a slightly longer latency and are of shorter duration than responses in the left MG. Response amplitudes in MG of both hindlimbs were modulated according to the phase of the step cycle. The background locomotor EMG is given on the far right for each muscle to illustrate the activity of these muscles during the step cycle. Responses were qualitatively similar in LG, soleus, and GM (not shown).

For 5 cats, the mean latency of crossed inhibitory response evoked by Tib nerve stimulation, averaged across all ankle extensors muscles, was 13 ms with a duration of 11 ms. Crossed excitatory responses had a mean latency of 24 ms with a duration of 19 ms. On the left side, for these same muscles, inhibitory responses had a mean latency of 11 ms with a duration of 29 ms. Excitatory responses of the left leg had a mean latency of 38 ms with a duration of 20 ms. On average, inhibitory and excitatory responses in the right leg had a significantly longer and shorter latency of 2 ms and 15 ms, respectively, compared to the left side. Furthermore, the
duration of inhibitory responses on the left side was significantly greater than on the right side by 18 ms but the duration of excitatory responses was not significantly different (p = 0.524).

To assess the relationship between the amplitude of short-latency inhibition with the amplitude of longer-latency excitation, N1 and P2 responses were plotted and correlations were made. Upper panels of figure 5 show group data of N1 and P2 responses evoked in ankle extensors of the left (Fig. 5A) and right (Fig. 5B) hindlimbs with stimulation of the left Tib nerve at different times during the step cycle for 5 cats. Response amplitude was modulated throughout the step cycle. Bottom panels show by regression analyses that N1 and P2 amplitudes correlated strongly (r = -0.85) on the right side (Fig. 5D) but that there was no correlation (r = -0.22) on the left side (Fig. 5C). In other words, a large N1 amplitude will be associated with a large P2 amplitude and vice-versa in contralateral ankle extensors.

Effects of Tib nerve stimulation on step cycle and EMG burst durations

The effects of stimulating the Tib nerve on selected EMG bursts were assessed in 5 cats to determine if nerve stimulation produced concomitant increases in muscle bursts that are simultaneously active bilaterally. Across 5 cats (Figure 6), stimulation of the left Tib nerve prolonged the burst durations of the left Srt and right ankle extensors in phases 0.15 and 0.25, when these muscles were both active (Fig. 6A). The burst durations of ankle extensors of the right leg were unchanged from phases 0.75-0.95, when these muscles are active but the left Srt is silent. Thus, prolongation of the burst in ankle extensors of the right leg occurs only in phases where the burst or activity of the left Srt is also increased. There was no effect of Tib nerve stimulation on burst durations of the right Srt and left ankle extensors at any point during the step cycle (Fig. 6B). For the group, there was a strong correlation (r = 0.91) between the durations of the left Srt and right ankle extensors with Tib nerve stimulation at different times during the step
cycle (Fig. 6C) whereas for the right Srt and left ankle extensors (Fig. 6D) the correlation was less strong ($r = 0.65$).

Across cats there were no significant effects of Tib nerve stimulation on step cycle duration ($p \geq 0.05$). However, there were significant shifts in the onset and offset of certain muscles relative to the onset of the left Srt but only in two specific parts of the step cycle, with the first and second parts corresponding to phases 0.15-0.35 (e.g. mid- to late flexion of the left leg) and 0.85 (early extension of the right leg), respectively (not shown). For instance, the left Srt burst had a delayed offset (16-44 ms) during phases 0.15-0.35 due to the increased burst duration of this muscle, which was accompanied by a delayed offset (17-22 ms) of right ankle extensors in the same phases. Onsets of left ankle extensors and right Srt were also delayed (4-10 ms) in phases 0.15-0.25 but their offsets were unchanged in these phases. In phase 0.85, the offset (28 ms) and onset (24 ms) of left and right ankle extensors, respectively, occurred earlier. The right Srt burst also finished earlier (3 ms). Therefore, stimulation can influence the timing and durations of hip flexors and ankle extensors bilaterally in specific phases of the step cycle.

**Discussion**

In the present study, short-latency inhibitory responses in extensors of the limb contralateral to SP and Tib nerve stimulation were evoked during locomotion. To the best of our knowledge no crossed inhibitory responses, evoked by stimulating cutaneous afferents, have been described in intact walking cats, showing that crossed inhibitory pathways described in anesthetized non-locomotor cats (Curtis et al. 1958; Arya et al. 1991; Edgley and Aggelopoulos 2006) operate during normal locomotion. Duysens and Loeb (1980) described in detail responses evoked in contralateral muscles by stimulating cutaneous nerves of the foot during locomotion in the cat. The reason for the absence of crossed inhibition in that paper is unclear.
but an inspection of their figure 3 would seem to indicate that a period of inhibition precedes the excitatory response in the contralateral MG. The lack of a template of background locomotor activity (e.g. non-stimulated trials) probably prevented a clear distinction of the crossed inhibition that was observed in the present study. The crossed pathways responsible for these responses and their putative functions during locomotion are discussed.

**Afferents mediating the responses**

Stimulation of both SP and Tib nerves just above motor threshold evoked qualitatively similar short-latency crossed inhibitory responses in extensors indicating that some of these effects were mediated by large diameter cutaneous afferents. Although the Tib nerve innervates intrinsic muscles of the foot and contains group I and II muscle afferents the SP nerve at the level of the ankle is entirely cutaneous. Stimulating the Tib nerve at an intensity of 1.2-1.5 x the threshold for evoking small but consistent short-latency responses in the ipsilateral TA could have recruited group I muscle afferents and group II muscle and cutaneous afferents. Edgley and Aggelopoulos (2006) reported that IPSPs in contralateral extensor motoneurons evoked by stimulating SP or sural nerves appeared near the threshold of the most excitable fibers, suggesting that large diameter Aβ fibers, most likely mechanoreceptor afferents, were responsible. Smaller diameter afferents can also contribute to responses because increasing stimulus intensity evoked larger crossed inhibitory responses in anesthetized cats, indicating a convergence between large and small diameter afferents (Edgley and Aggelopoulos 2006). Irrespective of which afferent type mediated responses, it is clear that crossed inhibition can be evoked in the intact cat during locomotion and forms part of the crossed pathway (i.e. inhibition followed by excitation) for certain muscles.
Central pathways

The differences in latencies of inhibitory responses on the left and right sides were similar to those following stimulation of cutaneous afferents in anesthetized cats (Edgley and Aggelopoulos 2006). For instance, contralateral inhibition of EMG on average started ~2 ms later than ipsilateral inhibitory responses whereas Edgley and Aggelopoulos (2006) reported a difference of ~1 ms. The small difference between the two preparations could simply be due to the conduction distance from the spinal cord to recording sites and because in the present study we recorded EMG activity whereas Edgley and Aggelopoulos (2006) recorded post-synaptic potentials in motoneurons.

Central pathways responsible for crossed inhibitory responses are probably the same described for anesthetized preparations (Jankowska et al. 2005b; Bannatyne et al. 2006; Edgley and Aggelopoulos 2006). For example, inhibitory interneurons in the dorsal horn of mid-lumbar spinal segments have wide ranging ipsilateral and contralateral projections to many regions of the spinal grey matter including connections with large cholinergic neurons in the ventral horn, most likely motoneurons (Bannatyne et al. 2006). Excitatory connections from primary cutaneous afferents to these inhibitory interneurons in the dorsal horn could mediate crossed inhibition during locomotion. Another pathway mediating crossed inhibition in anesthetized cats includes activation of contralateral Ia inhibitory interneurons by excitatory commissural interneurons (Jankowska et al. 2005b). Commissural interneurons, inhibitory and excitatory, can be excited by various afferents, including group II and cutaneous afferents (Jankowska et al. 2005a; Jankowska et al. 2005b; Edgley and Aggelopoulos 2006; Jankowska 2007).

Short-latency crossed inhibition was not present in the right VL following stimulation of the SP or Tib nerves of the left leg. The absence of crossed inhibition in VL could be due to the
fact that motor pools of VL are located at L5-L6 whereas those of glutei and triceps surae muscles, which exhibited crossed inhibition, are found at L7-S1 (Vanderhorst and Holstege 1997; Yakovenko et al. 2002). This could suggest that relay neurons in the crossed inhibitory pathway observed in the present study are located more caudally within the spinal cord.

The strong correlation between the amplitude of crossed inhibition and excitation (Fig. 5) could mean that the short-latency inhibition controls the excitability of the longer-latency excitatory pathway without requiring inputs from supraspinal levels. It is likely that part of the excitatory response is mediated by post-inhibitory rebound (Abraham et al. 1985). However, because short- and longer-latency excitatory responses can be evoked during the swing phase of locomotion in ipsilateral ankle extensors without preceding inhibition a longer latency reflex pathway must also be involved (Duysens and Loeb 1980). Therefore, excitatory responses are probably mediated by reflex pathways, which can be supplemented via post-inhibitory rebound.

*Effects of stimulation and bilateral cycle adjustments*

During fictive locomotion, stimulating the Tib nerve during ipsilateral stance increased the duration of the activity of ipsilateral extensors, whereas stimulation during the ipsilateral flexion phase terminated flexion and initiated extension (Guertin et al. 1995). In intact cats stimulation can advance or delay phases but abrupt terminations and initiations are rarely observed because this would disrupt ongoing locomotion. Instead, the activation of sensory pathways ensures proper interlimb coordination by adjusting the timing and durations of specific bursts. In a situation where speed is enforced by the treadmill, step cycle duration remains largely unaffected, although sub-components of the step cycle can be altered. For example, as shown previously (Duysens and Stein 1978) and in this study stimulating the Tib nerve during ipsilateral flexion prolonged ipsilateral hip flexor and contralateral ankle extensor bursts whereas
stimulation during ipsilateral stance had little or variable (Duysens and Stein 1978) effect. Crossed pathways coupling both hindlimbs from cutaneous or muscle afferents could ensure that step cycle duration remains constant while at the same time varying the sub-phases (e.g. flexion and extension phases bilaterally) to ensure proper interlimb coordination.

It was also reported that most timing adjustments occurred around mid- or late swing (Forssberg et al. 1980), which in our preparation corresponds approximately to phases 0.25-0.35 where effects of stimulation on locomotor bursts were most evident. For example, stimulation of the left Tib nerve delivered during phases 0.25-0.35 delayed the offset of the left Srt and of right ankle extensors. The onsets of left ankle extensors and of the right Srt were also delayed. Thus, there are critical points during locomotion in which peripheral inputs can influence the step cycle, as shown previously during fictive locomotion (Saltiel and Rossignol 2004a; Saltiel and Rossignol 2004b). Additionally, when the cat was in a double support phase stimulation of the left Tib nerve did not increase the burst duration of ankle extensors of the right leg, most likely because the left leg was also being supported. Therefore, biomechanical events can modify or ‘override’ some bilateral cycle adjustments (Saltiel and Rossignol 2004a; Saltiel and Rossignol 2004b). In a functional context during locomotion, a prolongation of swing of the left leg during a perturbation would require a concomitant increase in stance of the right leg so that progression and equilibrium are maintained.

**Functional considerations**

It has been proposed that decomposing the step cycle into several sub-phases, with each requiring the activation of a specific set of modules would simplify how descending systems modify limb activity (Grillner 1981; Grillner and Wallen 1985; Stein and Smith 1997; Lafreniere-Roula and McCrea 2005; Krouchev et al. 2006; Ivanenko et al. 2007). Although
only a concept, these modules would undoubtedly be coupled by feedback from the periphery through various ipsilateral and crossed pathways. As such, inhibition in addition to excitation would provide more flexibility to this system. The burst durations of the left Srt and right ankle extensors (Fig. 6) were strongly correlated with stimulation of the left Tib nerve while these muscles were active suggesting that crossed pathways interconnect left hip flexor and right ankle extensor ‘modules’.

Sherrington (Sherrington 1910b; Sherrington 1913) long ago suggested that inhibition in reflex pathways is an integral component of various reflex pathways in several behaviors, including locomotion, but the precise function of inhibition remains poorly understood. In particular, crossed inhibition of extensors while the ipsilateral leg is in flexion would tend to destabilize the animal during walking. Although we can only speculate as to the function of crossed inhibition during locomotion, when considered during the forward progression of stepping, crossed inhibition may serve to temporarily and very briefly "halt" or slow down forward progression. After all, crossed extension is only going to be useful if the ipsilateral flexion actually frees the limb from the perturbation. Moreover, short-latency inhibition of extensors, ipsilaterally or contralaterally, could serve to delay the onset of excitatory responses to enable supraspinal structures sufficient time to influence these pathways. Therefore, even though the precise function of short-latency inhibition of contralateral extensors remains unclear what is certain is that crossed extensor reflexes are more complex than originally thought.

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References


**Figure legends**

**Figure 1.** Methodology for analysing reflex responses during locomotion in the cat. A) Raw EMG burst recordings of several hindlimb muscles during locomotion (an 8 second sequence is shown). A cycle is defined as the period between onsets of two successive St bursts. Stimuli (vertical lines in first trace) were given approximately once every three cycles at different delays following St burst onset using a pre-programmed sequence to evoke responses in different parts of the step cycle. Up and down arrows respectively
indicate onset and offset of left St bursts during locomotion. Cycles are tagged as stimulated (S) if a stimulus was given whereas the preceding burst is generally tagged as control (C) cycle provided it did not follow a stimulated cycle. Stimulated cycles are grouped in one of 10 bins according to the time stimulation was given during the step cycle. For example, in a cycle lasting 1000 ms 10 equal bins of 100 ms would be generated. A stimulus given from 0-99 ms following St burst onset would be placed in the first bin and so on for each stimuli. B) A template of locomotor EMGs was generated from 81 control cycles beginning at left St burst onset and normalized to 1. Each template is separated into 10 bins and provides the background level of EMG (bEMG) in each phase of the step cycle. C) Stimulated cycle are grouped and averaged into one of 10 bins with the corresponding bEMG superimposed. Onsets and offsets (short vertical lines) of short- and longer-latency responses are determined manually for extensors. D) In each phase the bEMG occurring in the same time window as the response is subtracted from the response in the stimulated cycles illustrated by the integrated areas (short- and longer-latency responses are shown in grey and black, respectively). The subtracted value is then divided by a 10 ms block of bEMG in the same bin giving N1 and P2 response amplitudes. The division is necessary because inhibitory responses are a function of bEMG; meaning that subtracted values are larger if there is a greater level of bEMG and vice-versa. A fixed time window is used for all bins because if the same time window as the response was used the duration of the inhibition or excitation would be taken out of the equation. E) N1 and P2 responses are expressed as a percentage of the maximum response in one of the 10 bins. For example, the largest P2 response in this case was in the 4th bin and every response is
expressed as a percentage of that value. The black rectangle represents the activity of the muscle during the step cycle.

**Figure 2.** Effects of Tib nerve stimulation on the duration of selected locomotor bursts (Srt and triceps surae muscles bilaterally). Stimulation of the left Tib nerve was delivered at different times during stance of the left (Fig. 2A) and right (Fig. 2B) hindlimb in the same cat. A closer look at the crossed inhibition in the right triceps surae muscles is provided in Fig. 2C.

**Figure 3.** Cutaneous reflexes evoked by stimulating the left SP nerve in the right lateral gastrocnemius (LG) during the latter half of the extension phase of the right hindlimb. Stimulation of SP evoked a short-latency inhibition (~ 3 ms) followed by a longer-latency excitation (~26 ms). Onsets and offsets of both responses were determined and the areas of EMG activity were integrated in stimulated cycles (solid black line) and the locomotor template (dotted black line) to provide a measure of N1 (grey area) and P2 (black area) responses. Responses were then divided by a 10 ms block of the baseline locomotor EMG (blEMG), during the same phase of the step cycle, to give amplitudes of N1 and P2. Each line (template and stimulated cycles) is the average of approximately 100 cycles.

**Figure 4.** Cutaneous reflexes evoked in the left (Fig. 4A) and right MG (Fig. 4B) of the same cat by stimulating the left Tib nerve. Averaged reflex responses were separated and grouped into 10 phases according to the time they were evoked in the step cycle. Each line is the average of approximately 10 stimulations. On the far right of each panel is the rectified single EMG burst of the corresponding muscle during the step cycle. Each EMG burst line is the average of approximately 60 bursts.
**Figure 5.** Upper panels show reflex amplitudes of N1 and P2 in ankle extensors of the left (Fig. 5A) and right (Fig. 5B) legs as a function of blEMG expressed as a % of the maximal value during the step cycle at the same scale. Each data point is the mean ± SEM of approximately 10 responses. Black horizontal rectangles represent the period of activity of each muscle during the normalized step cycle. Bottom panels show the relationship between these N1 and P2 amplitudes in ankle extensors of the left (Fig. 5C) and right (Fig. 5D) legs with corresponding correlation coefficients (r). Note that only those points where the muscle was active are included in the regression analyses.

**Figure 6.** Upper panels show the effects of stimulating the left Tib nerve at different times during the step cycle on burst durations of the left Srt and right ankle extensors (Fig. 6A) and of the right Srt and left ankle extensors (Fig. 6B), expressed as the difference from control (i.e. non-stimulated bursts), across five cats at the same scale. Black and grey bars at the bottom of each graph show the period of activity for Srt and ankle extensors, respectively. Bottom panels show the linear relationship and coefficient of correlation (r) between burst durations of the left Srt and right ankle extensors (Fig. 6C) and of the right Srt and left ankle extensors (Fig. 6D) during locomotion. Each data point is the mean ± SEM where * is p ≤ 0.05 and *** is p ≤ 0.001.
A

Left Tib nerve stim

Left St

Left Srt

Left Sol

Right St

Right Srt

Right Sol

Right TA

0.35 m/s

1 sec

B

Control: n = 81

Stimuli: n = 12

N1

P2

C

D

E

% of maximum response
Phase of step cycle (relative to left St onset)

Left ankle extensors

Right ankle extensors

N1 amplitude

P2 amplitude

r = -0.22

r = -0.85
Phase of stim (relative to left Srt onset)

Left Srt burst duration

$r = 0.91$

Left ankle extensors

Right Srt burst duration

$r = 0.65$