A Spinal Mechanism Related to Left–Right Symmetry Reduces Cutaneous Reflex Modulation Independently of Speed During Split-Belt Locomotion

Marie-France Hurteau and Alain Frigon

Department of Pharmacology-Physiology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Quebec J1H 5N4, Canada

Task- and phase-dependent reflex modulation during locomotion is well established, but we do not know the signals driving this modulation. To determine whether signals related to left–right symmetry of the locomotor pattern modulate cutaneous reflexes, we stimulated the superficial peroneal nerve in five intact female cats and in four spinal-transected cats (spinal cats, two males and two females) during split-belt locomotion at different left–right speeds. We compared cutaneous reflexes evoked in three ipsilateral and two contralateral hindlimb muscles during split-belt locomotion with those evoked during tied-belt (equal left–right speeds) locomotion at matched speeds of the slow and fast limbs. Our results showed similar phase-dependent modulation of cutaneous reflexes during tied-belt and split-belt locomotion in intact and spinal cats. During tied-belt locomotion in intact cats, an increase in speed significantly increased reflex modulation from minimum to maximum values, whereas in spinal cats, we observed a significant decrease. However, in all muscles of intact and spinal cats, split-belt locomotion significantly reduced reflex modulation compared with tied-belt locomotion independently of which limb was stepping on the slow or fast belt. Additionally, reflex modulation correlated more with spatial left–right symmetry, as opposed to a temporal one, in intact and spinal cats. Our results indicate that signals related to left–right symmetry reduce cutaneous reflex modulation independently of speed via a spinal mechanism. We propose that asymmetric sensory feedback from the left and right legs alters the state of the spinal network, thereby reducing cutaneous reflexes to prevent inputs from destabilizing a potentially unstable pattern.

Key words: locomotion; reflex; spinal cord; split-belt

Significance Statement

When we contact an obstacle during walking, receptors in the skin send signals to the CNS to alter the trajectory of the leg to maintain balance. This response, or reflex, is different when the leg is in the air and when it is contacting the ground. The reflex also differs when we walk at different speeds. Here, we investigated this reflex when the left and right legs were walking at different speeds on a split-belt treadmill in cats. We show that the reflex is smaller during split-belt locomotion compared with when both legs are walking at equal speeds. We propose that the spinal locomotor network controlling walking reduces the reflex response to optimize balance when gait is unstable.

Introduction

During locomotion, receptors located in the skin allow the body to interact with the environment (Panek et al., 2014). These in-

teractions are crucial to ensure dynamic balance and smooth forward progression. For example, in cats and humans, when the foot contacts an obstacle during the swing phase, cutaneous afferents send inputs to spinal neurons to modify the trajectory of the contacted limb and to reinforce contralateral limb support, which is termed the stumbling corrective reaction (Forssberg et al., 1977; Prochazka et al., 1978; Forssberg, 1979; Schillings et al., 1996; Van Wezel et al., 1997; Zehr and Stein, 1999; Quevedo et al., 2005a,b). The stumbling corrective reaction, or cutaneous reflexes consistent with it, is present in the hindlimbs of cats with a complete spinal transection (spinal cats), indicating that it is organized within the spinal cord (Forssberg et al., 1975, 1977;

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Correspondence should be addressed to Dr. Alain Frigon, Université de Sherbrooke, 3001, 12e Avenue Nord, Department of Pharmacology-Physiology, Faculty of Medicine and Health Sciences, Sherbrooke, Quebec J1H 5N4, Canada. E-mail: alain.frigon@usherbrooke.ca.

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Frigon and Rossignol, 2008a; Hurteau et al., 2017). Moreover, in cats and humans, reflexes evoked by stimulating the superficial peroneal (SP) nerve, which supplies the skin of the foot dorsum, are observed in the four limbs, indicating that the stumbling corrective reaction is a whole-body response (Haridas and Zehr, 2003; Haridas et al., 2006; Hurteau et al., 2018).

Several cat and human locomotion studies have shown that mechanisms modulate cutaneous reflexes according to task and movement phase (for review, see Burke, 1999; Zehr and Stein, 1999; Duysens et al., 2004; Rossignol et al., 2006). The flexible modulation of cutaneous reflexes reflects their functional importance for shaping locomotor output in response to external cues. We also recently showed that locomotor speed influences cutaneous reflex modulation in spinal cats (Hurteau et al., 2017). We observed the largest reflex modulation at intermediate speeds and the smallest modulation at the slowest and fastest speeds tested. Interestingly, the reduction in cutaneous reflex modulation correlated with an increase in the spatial asymmetry between the legs. Therefore, it appears that a signal related to left–right asymmetry reduces cutaneous reflex modulation via a spinal mechanism.

One approach to exploring this hypothesis is by evoking and recording cutaneous reflexes during split-belt locomotion, in which we can control the speed of each leg independently. Split-belt locomotion generates temporal and spatial asymmetries between the slow and fast limbs in cats and humans (Kulagin and Shik, 1970; Forssberg et al., 1980; Halbertsma, 1983; Dietz et al., 1994; Prokop et al., 1995; Reisman et al., 2005; Torres-Oviedo et al., 2011; Frigon et al., 2013, 2015, 2017; D'Angelo et al., 2014; Hoogkamer et al., 2014; Kuczynski et al., 2017), reproduces some aspects of stepping along a curved path (Courtine and Schieppati, 2003a,b), and simulates a limping gait, which can be considered an unstable form of locomotion (Duysens et al., 2004, 2012; McFadyen et al., 2009; Hoogkamer et al., 2015). During prolonged split-belt locomotion in humans, there is a return, or adaptation, of left–right symmetry in interlimb parameters such as double support periods and step lengths (Reisman et al., 2005). However, in intact and spinal cats, we did not observe adaptation of interlimb parameters during prolonged split-belt locomotion (Kuczynski et al., 2017), which makes the cat model particularly interesting to investigate reflex modulation in prolonged asymmetric conditions. The modulation of reflexes during split-belt locomotion, however, has received little attention. Duysens et al. (2004) used split-belt locomotion to activate the ankle flexor tibialis anterior during stance in humans and showed that suppressive responses evoked by sural nerve stimulation persisted during the support phase.

In the present study, we used split-belt locomotion to determine whether asymmetric stepping modulates cutaneous reflexes in muscles ipsilateral and contralateral to the stimulation in both intact and spinal cats compared with matched speeds in tied-belt conditions. We hypothesize that split-belt locomotion reduces cutaneous reflex modulation compared with matched tied-belt speeds in both intact and spinal cats, consistent with a spinal mechanism. We also hypothesize that this reduction correlates more importantly with a spatial left–right asymmetry in the locomotor pattern, as opposed to a temporal one.

Materials and Methods

Ethical approval. The Animal Care Committee of the Université de Sherbrooke approved the experimental procedures (Protocol 273–15) and were in accordance with policies and directives of the Canadian Council on Animal Care. We obtained the current dataset from 5 adult (>9 months) female intact cats and 4 adult spinal cats (2 females and 2 males), weighing between 2.5 and 5.0 kg. As part of our effort to reduce the

number of research animals, we used all cats in other projects to answer other scientific questions (D'Angelo et al., 2014; Frigon et al., 2014, 2017; Dambreville et al., 2015; Hurteau et al., 2015, 2017, 2018; Dambreville et al., 2016; Kuczynski et al., 2017).

General surgical procedures. We performed the implantation and spinal transection surgeries under aseptic conditions in an operating room with sterilized equipment, as described previously (Hurteau et al., 2017, 2018). Before surgery, we sedated and induced the animals with an intramuscular injection of butorphanol (0.4 mg/kg), acepromazine (0.1 mg/kg), and glycopyrrolate (0.01 mg/kg) and an intramuscular injection of ketamine/diazepam (0.11 ml/kg in a 1:1 ratio), respectively. We then shaved the cats and cleaned their skin with chlorhexidine soap. We then anesthetized the cats with isoflurane (1.5–3%) by mask followed by endotracheal intubation. We adjusted isoflurane concentration by monitoring cardiac and respiratory rates and limb withdrawal with pressure applied to the paw and jaw tone. We monitored body temperature with a rectal thermometer and maintained it between 35°C and 37°C during surgery with a water-filled heating pad placed under the animal and an infrared lamp ~50 cm over it. At the end of surgery, we administered an antibiotic (Convenia, 0.1 ml/kg) subcutaneously. For analgesia, we taped a transdermal fentanyl patch (25 µg/h) to the back of the animal 2–3 cm rostral to the base of the tail and subcutaneously injected buprenorphine (0.01 mg/kg), a fast-acting analgesic, at the end of the surgery and ~7 h later. Until they regained consciousness, we kept the animals in an incubator under close supervision. We removed the fentanyl patch 5 d after surgery. At the end of the experiments, we injected a lethal dose of pentobarbital through the animal's cephalic vein.

Implantation procedure. To record muscle activity (EMG), we directed pairs of Teflon-insulated multistrand fine wires (AS633; Cooner Wire) subcutaneously from a head-mounted 34-pin connector (Omnetics) and sowed them into the belly of selected hindlimb muscles. During implantation, we electrically stimulated each muscle through the appropriate head connector channel to verify electrode placement. The current data include EMG from the following muscles: semitendinosus (St, knee flexor/hip extensor), vastus lateralis (VL, knee extensor), and lateral gastrocnemius (LG, ankle extensor/knee flexor). During experiments, EMGs were preamplified (10×, custom-made system), band-pass filtered (30–1000 Hz), and amplified (100–5000×) using a 16-channel amplifier (AM Systems 3500). EMG data were digitized (5000 Hz) with a National Instruments card (NI 6032E) acquired with custom-made acquisition software, and stored on a computer. For bipolar nerve stimulation, we directed pairs of Teflon-insulated multistrand fine wires (AS633; Cooner Wire) subcutaneously from a head-mounted four-pin connector (Omnetics). We then fixed the wires inside silicon tubes (~1 cm) and placed them around the left and right SP nerves just proximal to the ankle joint. The SP nerve is purely sensory (cutaneous) at this level (Bernard et al., 2007).

Spinal transection and locomotor training. In four cats, we transected the spinal cord at low thoracic levels. We first made a small incision of the skin over the 12th and 13th thoracic vertebrae, which correspond to the same spinal segments in cats (Eldridge, 1984). After carefully setting aside muscle and connective tissue, we performed a small dorsal laminectomy. We applied lidocaine (xylocaine) topically and injected it within the spinal cord before transecting the spinal cord with surgical scissors. We inserted hemostatic material (Spongostan) within the gap before sewing back muscles and skin in anatomical layers. After spinal transection, we manually expressed the bladder of spinal cats one to two times daily. Experienced personnel closely monitored spinal cats on a daily basis for the duration of the study.

A week after spinal transection, we started training cats on a treadmill so that they recovered hindlimb locomotion. We performed five training sessions per week that lasted 20–30 min each. At first, two experimenters moved the hindlimbs over the moving treadmill belt to reproduce a locomotor pattern, with one of the experimenters holding the tail for support. The forelimbs were positioned on a fixed platform located 1 cm above the belt and a Plexiglas separator was placed between the hindlimbs to prevent crossing. After a few days of training, we elicited stepping movements by stimulating the skin of the perineum. Over a few additional weeks, cats recovered full weight-bearing hindlimb locomotion with

consistent plantar placement. During data collection, an experimenter held the tail to provide equilibrium.

Experimental design. Cats performed experiments on a treadmill with two independently controlled running surfaces 120 cm long and 30 cm wide (Bertec). We initially trained intact cats to step on the treadmill at various tied-belt and split-belt speeds using food and affection as reward. During experiments, we stimulated the left or right SP nerve while cats performed one of four locomotor conditions: Tied-slow, Tied-fast, Split-slow, and Split-fast. The terms “slow” and “fast” always refer to the ipsilateral limb (the stimulated limb). Intact cats performed tied-belt locomotion at 0.4 m/s (Tied-slow) and 0.7 m/s (Tied-fast), whereas spinal cats performed it at 0.4 m/s (Tied-slow) and 0.6 m/s (Tied-fast). We used the same speeds during split-belt locomotion for the slow and fast limbs. In the Split-slow condition of intact cats, the ipsilateral and contralateral limbs stepped at 0.4 m/s and 0.7 m/s, respectively, while in the Split-fast condition the ipsilateral and contralateral limbs stepped at 0.7 m/s and 0.4 m/s, respectively. In the Split-slow condition of spinal cats, the ipsilateral and contralateral limbs stepped at 0.4 m/s and 0.6 m/s, respectively, while in the Split-fast condition the ipsilateral and contralateral limbs stepped at 0.6 m/s and 0.4 m/s, respectively. We used 0.6 m/s for the fast speed in spinal cats because we found the pattern inconsistent during split-belt locomotion at faster speeds, with extra steps taken by the fast limb.

During experiments, we evoked cutaneous reflexes by delivering electrical stimuli to the SP nerve with a Grass S88 stimulator using a single pulse of 0.2 ms duration. At the beginning of each experiment, we determined the motor threshold, defined as the lowest intensity required for a small short latency (8–10 ms) excitatory response in the ipsilateral St muscle 50 ms following its burst onset. We then set stimulation intensity at 1.2 times the motor threshold for the rest of the experiment. In each locomotor condition, we delivered 120–150 stimuli pseudorandomly every two to four locomotor cycles at varying delays relative to an ipsilateral hindlimb extensor burst onset to evoke responses at different times during the step cycle. The detection of the extensor burst to trigger the stimulation is based on a threshold and as the EMG activity varies slightly from burst to burst, the specified delays can also vary. This affects the number of stimuli, or responses, that fall in each bin. We obtained a minimum of two stimuli per bin in each condition for all cats. Each locomotor condition tested lasted 4–8 min. For a given nerve stimulation, we collected data for all four locomotor conditions within a single session. At the end of each session, we confirmed that the motor threshold remained stable.

Reflex analysis. We recently described the procedure to quantify reflexes (Hurteau et al., 2017, 2018). Briefly, we synchronized the step cycle to an extensor burst onset (VL or LG) in the stimulated limb (the ipsilateral limb). We tagged cycles as stimulated or control (nonstimulated) and divided them into 10 subphases (bins) of equal duration. We removed sections where the cat stepped irregularly, based on EMG and video data. In cycles with stimulation, we used the onset of the stimulus as the reference for the start of the cycle. We averaged responses in each bin. The control cycles provided a baseline locomotor EMG (bEMG) in each bin. We superimposed the averaged responses on bEMG to show how they deflect from the normal background of EMG. To quantify reflex responses, we used predefined latencies as guidelines (Duysens and Stein, 1978; Duysens and Loeb, 1980; Pratt et al., 1991; Loeb, 1993) and 95% confidence intervals to set a window at the onset and offset of evoked responses, defined as prominent negative or positive deflections away from the bEMG.

Based on the terminology introduced by Duysens and Loeb (1980), we termed short-latency (~8–10 ms) excitatory and inhibitory responses as P1 and N1 responses, respectively, and midlatency (~20–25 ms) excitatory responses as P2 responses. We also classified responses in the contralateral VL as P1 because they had onsets <18 ms, which is the minimal latency for spino-bulbo-spinal reflexes in cats (Shimamura and Livingstone, 1963). We rectified and integrated the EMG of reflex responses from onset to offset and then subtracted the bEMG within the same window, thus providing a net value. We then divided this net value by the bEMG to provide a normalized measure of reflex amplitude. The division is required to determine whether changes in reflex responses across the

cycle are independent of changes in bEMG activity (Matthews, 1986; Duysens et al., 1993; Frigon and Rossignol, 2008a; Frigon et al., 2009; Hurteau et al., 2017). To illustrate the modulation of reflexes by phase and condition (tied-belt and split-belt conditions), we normalized reflex response in each muscle to the maximal value obtained in one of the four conditions. We also calculated a modulation index by measuring the difference between the largest and smallest responses out of the 10 bins for each condition (Hurteau et al., 2017). Because we observed inhibitory (N1) responses in some phases, which we considered negative values, and excitatory (P1) responses in other phases, the modulation index could exceed 100% in some muscles.

Data acquisition and analysis. We used two cameras (Basler AcA640–100 gm) to capture the videos of the left and right sides of the animals at 60 frames/s with a spatial resolution of 640 × 480 pixels. A custom-made Labview program acquired images and synchronized them with EMG data. We analyzed videos offline at 60 frames/s using custom-made software. We determined paw contact and liftoff by visual inspection for the four limbs in intact cats and for the hindlimbs in spinal cats. We defined paw contact as the first frame where the paw made visible contact with the treadmill surface and liftoff as the most caudal displacement of the limb. We measured cycle duration from successive paw contacts of the same limb; stance duration corresponded to the interval of time from paw contact to liftoff. Swing duration corresponded to cycle duration minus stance duration.

To determine how the locomotor pattern deviates from perfect left–right symmetry on a step-by-step basis, we measured a temporal and a spatial phasing index (Hurteau et al., 2017). We measured the temporal phase interval as the interval of time between stance onsets of the ipsilateral and contralateral hindlimbs divided by ipsilateral hindlimb cycle duration (Fig. 1A). We then measured the temporal phasing index by calculating the absolute deviation of the temporal phase interval from a perfect symmetry of 0.5. For the spatial phasing index, we first measured stride length as the horizontal distance traveled from stance offset to onset plus the distance traveled by the opposite treadmill belt during the swing phase, which corresponds to swing duration multiplied by treadmill speed (Fig. 1B) (Goetz et al., 2012; Thibaudier and Frigon, 2014; Harnie et al., 2018). We then measured step length as the distance between the leading and trailing limb at stance onset of the leading limb (Hoogkamer et al., 2014). We measured stride and step lengths for the ipsilateral hindlimb. We then measured a spatial analog of the temporal phase interval, the gap interval, by dividing step length by stride length (Abourachid et al., 2007; Thibaudier and Frigon, 2014). We measured the spatial phasing index by calculating the absolute deviation of the gap interval from a perfect symmetry of 0.5.

Statistical analysis. We quantified reflex responses in the ipsilateral St, VL, and LG, as well as in the contralateral VL and LG. In three of five intact cats and in two of four spinal cats, both the left and right SP nerves evoked responses. We treated responses evoked by left and right nerves separately and pooled them for statistical analysis ($n = 8$ and $n = 6$ nerves stimulated for five intact and four spinal cats, respectively). To assess phase-dependent modulation, we performed a one-factor (phase) repeated-measures ANOVA on short- and midlatency responses for pooled data. To compare cycle and stance durations across conditions and between limbs, we performed a two-factor (condition, limb) repeated-measures ANOVA for pooled data. We also determined whether locomotor condition affected the temporal and gap intervals, the temporal and spatial phasing indexes, as well as reflex modulation indexes using a one-factor (condition) repeated-measures ANOVA for pooled data. For temporal and spatial measures and modulation indexes, we used pairwise comparisons if there was a significant main effect and/or interaction of the repeated-measures ANOVAs. We also measured Pearson's correlation coefficient (r) between the modulation index of P1 responses of the ipsilateral St and the temporal and spatial phasing indexes. We considered an α -error of $p < 0.05$ as significant for all tests.

We did not adjust the α level for multiple comparisons. We followed the recommendation of Rothman (1990) for not correcting for multiple comparisons, in contrast to other views (Greenland and Robins, 1991; Poole, 1991; Greenland, 2008). However, even proponents of adjusting for multiple comparisons agree with Rothman's recommendation when

Figure 1. Measurements of temporal and spatial phase intervals. **A**, Measurement of the temporal phase interval using the onsets of the ipsilateral (iST) and contralateral (cST) stance phases. **B**, Measurement of the gap interval using step length and stride length. The figure was modified with permission from Harnie et al. (2018).

the objective of the analysis is to scientifically report and interpret the data (Greenland and Robins, 1991). Implicit in the assumption that a correction for multiple corrections is needed is that the associations between variables in the dataset are manifestations of random processes, or chance (Rothman, 1990; Greenland and Robins, 1991). Although this presumption may hold true for certain associations (Greenland and Robins, 1991), we do not believe it is valid when considering the association between spatiotemporal variables or reflex amplitude and speed/left–right speed differences. In this study, we believe that it was more important to avoid false negatives (type II errors) than false positives (type I errors). We could have applied the recommendation of lowering the p threshold to 0.005 (Ioannidis, 2018) and, indeed, many of our reported differences would have remained significant; however, other potentially important physiological relationships in the data would have been obscured.

Results

Modulation of the pattern during tied-belt and split-belt locomotion

In the present study, we evoked and compared cutaneous reflexes at two speeds during tied-belt locomotion (Tied-slow, Tied-fast) and in the slow (Split-slow) and fast (Split-fast) limbs during split-belt locomotion in intact and spinal cats. Note again that “slow” and “fast” refer to the speed of the ipsilateral limb. To address the physiological role of cutaneous reflex responses, it was first important to characterize the pattern during tied-belt and split-belt locomotion.

Figure 2 shows EMG waveforms of the muscles studied in the present study along with the stance phases of the hindlimbs in a representative intact (Fig. 2A) and spinal (Fig. 2B) cat during tied-belt and split-belt locomotion. Note that the EMGs for a given muscle are at the same vertical scale in all four panels. In the intact and spinal cat, increasing treadmill speed during tied-belt locomotion reduced stance phase and extensor burst durations bilaterally. During split-belt locomotion, ipsilateral stance and extensor burst durations were longer and shorter when the ipsilateral limb was on the slow (Split-slow) and fast (Split-fast) belts, respectively. As expected, contralateral stance phase and extensor burst durations showed an opposite pattern.

Figure 2. Modulation of the locomotor pattern during tied-belt and split-belt locomotion in intact and spinal cats. The figure shows EMG waveforms from ipsilateral (i) and contralateral (c) hindlimb muscles along with hindlimb stance phases (ST, thick black bars) in the four locomotor conditions in an intact (**A**) and spinal (**B**) cat. The stimulation (STIM) of the ipsilateral SP nerve is shown below the stance phases.

For pooled data, the locomotor condition significantly affected cycle duration in intact ($p = 1.05 \times 10^{-8}$, ANOVA) and spinal ($p = 0.02$, ANOVA) cats (Fig. 3A). As expected, cycle duration was significantly shorter in the Tied-fast condition compared with the Tied-slow condition, whereas during split-belt locomotion it was intermediate to the tied-belt conditions. We found no significant differences between cycle durations of the ipsilateral and contralateral limbs ($p = 0.09$ and $p = 0.19$ for intact and spinal cats, respectively, ANOVA), and no significant interactions ($p = 0.10$ and $p = 0.35$ for intact and spinal cats, respectively, ANOVA). The locomotor condition also significantly affected stance duration in intact ($p = 3.61 \times 10^{-8}$, ANOVA) and spinal ($p = 0.0004$, ANOVA) cats (Fig. 3B). Stance durations were shorter bilaterally in the Tied-fast condition compared with the Tied-slow condition. Although we found no significant differences between limbs for intact ($p = 0.19$, ANOVA) and spinal ($p = 0.11$, ANOVA) cats because the split-belt conditions were mirror images, we found significant interactions between condition and limb ($p = 2.76 \times 10^{-6}$ and $p = 0.00006$ for intact and spinal cats, respectively, ANOVA). The ipsilateral stance phase was significantly longer ($p = 0.00008$ and $p = 0.0004$ for intact and spinal cats, respectively, ANOVA) and shorter ($p = 0.0002$ and $p = 0.0002$ for intact and spinal cats, respectively, ANOVA) when it was on the slow (Split-slow) and fast (Split-fast) belts, respectively.

The locomotor condition significantly affected the temporal ($p = 0.0003$ and $p = 0.00004$ for intact and spinal cats, respectively, ANOVA) and spatial ($p = 2.23 \times 10^{-10}$ and $p = 0.00007$ for intact and spinal cats, respectively, ANOVA) phasing between hindlimbs. Speed did not affect temporal phasing because we observed no difference between tied-belt conditions ($p = 0.95$ and $p = 0.11$ for intact and spinal cats, respectively, ANOVA) (Fig. 3C). Having the ipsilateral limb on the slow and fast belts significantly increased and reduced temporal phasing, respectively, compared with one or both tied-belt conditions. One notable difference was that the change in temporal phasing during split-belt locomotion was visibly greater in intact cats compared with spinal cats. Speed also did not affect spatial phasing because we observed no difference between the tied-belt conditions ($p = 0.43$ and $p = 0.86$ for intact and spinal cats, respectively, ANOVA) (Fig. 3D). Having the ipsilateral limb on the slow and fast belts significantly increased and reduced spatial phasing, respectively, compared with both tied-belt conditions.

The temporal and spatial changes that we observed in the pattern during tied-belt and split-belt locomotion were consistent with previous studies in intact or spinal cats (Forssberg et al., 1980; Halbertsma, 1983; Frigon et al., 2013, 2015, 2017; D'Angelo et al., 2014; Kuczynski et al., 2017). During split-belt locomotion, these changes included temporal and spatial asymmetries between the hindlimbs.

Cutaneous reflex responses in ipsilateral hindlimb muscles

We compared cutaneous reflexes evoked in ipsilateral and contralateral hindlimb muscles by stimulating the SP nerve during tied-belt and split-belt locomotion in intact and spinal cats. To facilitate comparisons between conditions and muscles, we organized the figures in the same way (Figs. 4, 5, 6, 7, 8). In these figures, panels A and B show responses evoked in intact and spinal cats, respectively, divided into the four conditions: Tied-slow, Tied-fast, Split-slow, and Split-fast. Each subdivision shows averaged waveforms in the 10 bins of the cycle in a window of 65 ms that starts 5 ms before the stimulation for a single cat (same cat for all four conditions). To show how evoked responses deviated

Figure 3. Temporal and spatial parameters during tied-belt and split-belt locomotion for pooled data. Top, cycle (A) and stance (B) durations of the ipsilateral and contralateral hindlimbs. Bottom, temporal (C) and spatial (D) intervals between the hindlimbs for pooled data. Each bar/data point is the mean $\pm$ SD of eight trials in five intact cats and six trials in four spinal cats. The p -values were obtained with pairwise comparisons.

Figure 4. Cutaneous reflexes in the ipsilateral semitendinosus (iSt) during tied-belt and split-belt locomotion. SP nerve stimulation evoked responses in the iSt of intact ($n = 8$ trials in 6 cats; **A**) and spinal ($n = 6$ trials in four cats; **B**) cats in 4 locomotor conditions. In each condition, we show averaged waveforms in the 10 bins of the cycle with a window of 65 ms that starts 5 ms before stimulation in a representative intact and spinal cat. Black traces represent averaged cycles that received a stimulation (i.e., reflex responses, $n = 5$ –27 stimuli per bin) and gray traces represent averaged cycles without stimulation ($n = 82$ –266 cycles). Scale bars, shown in arbitrary units (a.u.), are the same across bins and conditions for a given cat. Aligned vertically to the right is the average rectified EMG trace of the iSt muscle over the cycle normalized to the ipsilateral VL burst onset. Scatter plots represent reflex amplitudes (mean $\pm$ SD) in each bin of the cycle expressed as a percentage of the maximal response found in one of the bins of one of the conditions for pooled data. Horizontal bars above the scatter plots show the stance phases of the ipsilateral (Ipsi) and contralateral (Contra) limbs and the period of EMG activity from 20 to 30 cycles. Error bars indicate mean $\pm$ SD. p -values at the top of the scatter plots are the result of the one-factor (phase) repeated-measures ANOVA.

from the normal pattern of EMG, averaged cycles that received a stimulation (black traces) are superimposed on averaged cycles without stimulation (the bEMG). An average rectified EMG trace of the muscle normalized to step cycle onset (onset of the ipsilateral VL or LG) is aligned vertically to the right of the waveforms. The scatter plots below the waveforms show normalized reflex amplitudes of short-latency (P1/N1) and midlatency (P2) responses for pooled data. Ipsilateral and contralateral stance phases, as well as the period of activity of the muscle, are shown above the scatter plots. Because P2 responses are small or absent in spinal cats, they were not analyzed (LaBella et al., 1992; Duysens et al., 2004; Frigon and Rossignol, 2008a; Hurteau et al., 2017).

We evaluated cutaneous reflexes in the ipsilateral St, a knee flexor and hip extensor that displays a brief period of activity around swing onset and sometimes a second short burst during early stance (Fig. 2). In the present study, stimulating the SP nerve evoked P1 and P2 responses in intact cats that were phase modulated (Fig. 4A). In spinal cats, we observed a similar (not statistically compared) phase-dependent modulation of P1 responses

and a reduction or absence of P2 responses (Fig. 4B). These results are consistent with previous findings in intact and spinal cats (Forssberg et al., 1977; Forssberg, 1979; Duysens and Loeb, 1980; Pratt et al., 1991; Loeb, 1993; Bretzner and Drew, 2005; Frigon and Rossignol, 2007, 2008a; Frigon et al., 2009; Hurteau et al., 2017, 2018). We found a significant phase-dependent modulation of P1 and P2 (intact cats only) response amplitudes in all conditions for pooled data of intact and spinal cats that peaked in bins 8 or 9 toward the end of the main burst (p -values are indicated on scatter plots, ANOVA), which corresponds to swing onset/early swing. However, we observed notable differences in response amplitudes between tied-belt and split-belt locomotion. Indeed, P1 and/or P2 responses were approximately reduced by half from tied-belt to split-belt locomotion in both the slow and fast limbs in intact and spinal cats.

We next evaluated the modulation of cutaneous reflexes in the ipsilateral VL, a knee extensor that is active throughout most of the stance phase (Fig. 2). In the present study, stimulating the SP nerve in intact cats evoked N1 responses followed by P2 responses

Figure 7. Cutaneous reflexes in the contralateral VL (cVL) during tied-belt and split-belt locomotion. SP nerve stimulation evoked responses in the cVL of intact ($n = 4$ trials in 4 cats; **A**) and spinal ($n = 5$ trials in 3 cats; **B**) cats in 4 locomotor conditions. In each condition, we show averaged waveforms in the 10 bins of the cycle with a window of 65 ms that starts 5 ms before stimulation in a representative intact and spinal cat. Black traces represent averaged cycles that received a stimulation (i.e., reflex responses, $n = 4$ –19 stimuli per bin) and gray traces represent averaged cycles without stimulation ($n = 82$ –242 cycles). Scale bars, shown in arbitrary units (a.u.), are the same across bins and conditions for a given cat. Aligned vertically to the right is the average rectified EMG trace of the cVL muscle over the cycle normalized to the ipsilateral VL burst onset. Scatter plots represent reflex amplitudes (mean $\pm$ SD) in each bin of the cycle expressed as a percentage of the maximal response found in one of the bins of one of the conditions for pooled data. Horizontal bars above the scatter plots show the stance phases of the ipsilateral (Ipsi) and contralateral (Contra) limbs and the period of EMG activity from 20 to 30 cycles. Error bars indicate mean $\pm$ SD. p -values at the top of the scatter plots are the result of the one-factor (phase) repeated-measures ANOVA.

tion, with the largest responses found at the end of the contralateral VL burst/late stance, when the ipsilateral limb was in mid- to late swing (bins 10 and 1). In spinal cats, responses peaked in the early to middle part of the contralateral VL burst, when the ipsilateral limb was in late stance or early swing (bins 7 and 8). In both intact and spinal cats, peak P1 response amplitudes during split-belt locomotion were approximately half those observed during tied-belt locomotion.

In intact cats (Fig. 8A), stimulating the SP nerve evoked P2 responses in the contralateral LG that were sometimes preceded by N1 responses (Frigon and Rossignol, 2008b), whereas in spinal cats (Fig. 8B), we only observed P1 responses. The lack of N1 responses in spinal cats could explain the shorter latency of excitatory responses. To facilitate comparisons between intact and spinal cats, we only analyzed excitatory responses (P2 and P1 in intact and spinal cats, respectively). Due to the small number of nerves that evoked responses in the crossed LG in intact cats ($n = 2$), we did not find a significant phase-

dependent modulation of P2 responses, whereas in spinal cats ($n = 3$), P1 responses were significantly modulated by phase in the Tied-slow and Split-fast conditions (p -values are indicated on scatter plots, ANOVA). In intact cats, we observed the largest responses toward the end of the contralateral LG burst, when the ipsilateral limb was in mid-swing (bin 10). In spinal cats, responses peaked in the middle part of the contralateral LG burst, when the ipsilateral limb was in late stance or early swing (bins 7 and 8). In both intact and spinal cats, peak P2 or P1 response amplitudes during split-belt locomotion were approximately half those observed during tied-belt locomotion.

In summary, cutaneous reflex responses in contralateral extensors (VL and LG) maintained a similar (not statistically compared) pattern of phase modulation during tied-belt and split-belt locomotion in intact and spinal cats. However, in both groups, response amplitudes were smaller in both the slow and fast limbs during split-belt locomotion.

Figure 8. Cutaneous reflexes in the contralateral LG (cLG) during tied-belt and split-belt locomotion. SP nerve stimulation evoked responses in the cLG of intact ($n = 2$ trials in 2 cats; **A**) and spinal ($n = 3$ trials in 2 cats; **B**) cats in 4 locomotor conditions. In each condition, we show averaged waveforms in the 10 bins of the cycle with a window of 65 ms that starts 5 ms before stimulation in a representative intact and spinal cat. Black traces represent averaged cycles that received a stimulation (i.e., reflex responses, $n = 5$ –27 stimuli per bin) and gray traces represent averaged cycles without stimulation ($n = 68$ –266 cycles). Scale bars, shown in arbitrary units (a.u.), are the same across bins and conditions for a given cat. Aligned vertically to the right is the average rectified EMG trace of the cLG muscle over the cycle normalized to the ipsilateral VL burst onset. Scatter plots represent reflex amplitudes (mean $\pm$ SD) in each bin of the cycle expressed as a percentage of the maximal response found in one of the bins of one of the conditions for pooled data. Horizontal bars above the scatter plots show the stance phases of the ipsilateral (Ipsi) and contralateral (Contra) limbs and the period of EMG activity from 20 to 30 cycles. Error bars indicate mean $\pm$ SD. p -values at the top of the scatter plots are the result of the one-factor (phase) repeated-measures ANOVA.

Asymmetric stepping reduces cutaneous reflex amplitude independently of speed

To compare reflex responses evoked in the different conditions, we measured a modulation index for each condition by subtracting the smallest response from the largest response observed in the 10 bins (Hurteau et al., 2017). For short-latency responses in VL and LG, because both N1 and P1 responses were present, the modulation indexes could exceed 100%. The modulation indexes for all muscles, ipsilateral and contralateral, were strikingly similar (not statistically compared) across conditions. In intact cats, we observed the largest modulation indexes for P1/N1 and P2 responses in the Tied-fast condition followed by the Tied-slow condition and the two split-belt conditions (Fig. 9A). For the majority of responses in all muscles, we found a significant increase in the modulation indexes from the Tied-slow to the Tied-fast conditions. Conversely, we found significantly smaller modulation indexes for the majority of responses in all muscles in the two split-belt conditions compared with the tied-belt conditions, with no significant differences between split-belt condi-

tions for all responses. Note that we observed only one significant change in P2 responses of the contralateral LG because of the small number of evoked responses ($n = 2$ from eight nerves stimulated). In spinal cats, we observed the largest modulation indexes for P1/N1 responses in the Tied-slow condition followed by the Tied-fast condition and the two split-belt conditions (Fig. 9B). We found a significant decrease in the modulation indexes from the Tied-slow to the Tied-fast conditions for all muscles with the exception of the ipsilateral VL ($p = 0.09$, ANOVA). We found significantly smaller modulation indexes in the two split-belt conditions of all muscles compared with one or both tied-belt conditions.

In summary, split-belt locomotion reduced cutaneous reflex modulation compared with tied-belt locomotion in both intact and spinal cats. Although we observed significant differences in cutaneous reflex modulation between the two tied-belt speeds (i.e., a speed-dependent effect), we found no significant differences between the two split-belt conditions even though in one condition evoked responses were in the slow limb and in the

A Intact cats **B Spinal cats**

Figure 9. Cutaneous reflex modulation across locomotor conditions for pooled data. The figure shows the modulation indexes for P1/N1 and/or P2 responses for all muscles obtained in each condition for intact (**A**) and spinal (**B**) cats. Each bar represents the mean $\pm$ SD of pooled data (i.e., nerves stimulated, see legends of Figs. 3, 4, 5, 6, and 7 for the number of trials and cats). p -values were obtained with pairwise comparisons.

other condition they were in the fast limb. This indicates a reflex modulation independent of speed during split-belt locomotion.

Cutaneous reflex modulation correlates with left–right symmetry

We recently showed that cutaneous reflex modulation in spinal cats as a function of speed correlated with spatial left–right symmetry, as opposed to temporal symmetry (Hurteau et al., 2017). Similar to that study, we measured the temporal (Fig. 10A) and spatial (Fig. 10B) phasing indexes, an indication of how the locomotor pattern deviates from perfect left–right symmetry (a zero value) on a step-by-step basis. Locomotor condition significantly affected the temporal index of intact cats ($p = 0.001$, ANOVA), but not of spinal cats ($p = 0.21$, ANOVA). As stated earlier, the change in the temporal phasing between the hindlimbs induced by split-belt locomotion was visibly greater in intact cats compared with spinal cats, which could be due to a supraspinal/volitional effect (see Discussion). In intact cats, an increase in speed during tied-belt locomotion significantly reduced the temporal index ($p = 0.01$, ANOVA), indicating a more symmetric pattern. In contrast, both split-belt conditions significantly increased the

temporal index compared with both tied-belt conditions, consistent with a more asymmetric temporal pattern. In both intact and spinal cats, locomotor condition significantly affected the spatial index ($p = 0.00007$ and $p = 0.0006$ for intact and spinal cats, respectively, ANOVA). In intact cats, an increase in speed during tied-belt locomotion significantly reduced the spatial index ($p = 0.00009$, ANOVA), indicating a more symmetric pattern, whereas we observed no such change in spinal cats ($p = 0.19$, ANOVA). Conversely, both split-belt conditions significantly increased the spatial index compared with both tied-belt conditions, consistent with a more asymmetric spatial pattern.

To determine whether the modulation of reflexes in the different conditions correlated with changes in temporal and spatial symmetry, we measured Pearson's correlation coefficient (r) between the modulation index of P1 responses of the ipsilateral St and the temporal and spatial indexes. We selected P1 responses of the ipsilateral St because they were present in all intact and spinal cats. The modulation index of the ipsilateral St correlated negatively and significantly with the temporal index of intact cats ($r = -0.643$, $p = 0.0007$, bivariate correlation), but not in spinal cats ($r = -0.361$, $p = 0.083$, bivariate correlation). Conversely, the

Figure 10. Temporal and spatial symmetry of the locomotor pattern across conditions for pooled data. The figure shows temporal (**A**) and spatial (**B**) phasing indexes for pooled data ($n = 8$ trials in 5 intact cats and $n = 6$ trials in 4 spinal cats). Each data point is the mean $\pm$ SD of pooled data. p -values were obtained with pairwise comparisons. Note that pairwise comparisons were not performed for the temporal phasing index of spinal cats because there was no significant main effect of the conditions (repeated-measures ANOVA).

modulation index of the ipsilateral St correlated negatively and significantly with the spatial index in both intact ($r = -0.723$, $p = 2.93 \times 10^{-6}$, bivariate correlation) and spinal ($r = -0.602$, $p = 0.002$, bivariate correlation) cats. These results indicate that, as spatial asymmetry increases, cutaneous reflex modulation decreases.

Discussion

We characterized cutaneous reflexes in three ipsilateral and two contralateral hindlimb muscles of intact and spinal cats during tied-belt locomotion at two speeds and during split-belt locomotion at one left–right speed difference with the ipsilateral limb stepping on the slow and fast belts. We showed similar phase-dependent reflex modulation in all muscles during tied-belt and split-belt locomotion. However, in all muscles, split-belt locomotion reduced reflex modulation compared with tied-belt locomotion. Moreover, although speed significantly affected reflex modulation during tied-belt locomotion, with a differential change in intact and

spinal cats, we observed no differences between the slow and fast limbs during split-belt locomotion. Our results indicate that a signal related to left–right symmetry reduces cutaneous reflex modulation via a spinal mechanism. In the following sections, we consider the functional role of this spinal-mediated task-dependent modulation and potential mechanisms involved.

Functional considerations

The main questions are as follows: why is cutaneous reflex modulation smaller during split-belt locomotion and what function does this serve? To answer these questions, we must consider how split-belt locomotion alters the pattern. Specifically, it creates temporal and spatial asymmetries between left and right sides, or slow and fast limbs, simulating some aspects of stepping along a curved path, such as a faster speed for the outer leg that must travel a greater distance in the same amount of time as the inner leg (Courtine and Schieppati, 2003a,b). Stepping on a split-belt treadmill also simulates a limping gait (Duysens et al., 2004, 2012; McFadyen et al., 2009; Hoogkamer et al., 2015), particularly in cats, in which the pattern does not adapt to prolonged exposure (Kuczyński et al., 2017), contrary to humans (Prokop et al., 1995; Reisman et al., 2005). We consider the limping gait induced by split-belt locomotion an unstable form of locomotion, as observed in some pathologies (Harris-Love et al., 2001; Duysens et al., 2012; Handžić and Reed, 2015). Here, we define stability as the body's resistance to disruption of dynamic balance (equilibrium). In other words, during limping, as occurs with split-belt locomotion, the body is relatively less resistant to disruptions of its dynamic balance compared with symmetrical gaits, such as tied-belt locomotion.

We propose that reducing cutaneous reflexes during split-belt locomotion decreases the risk of destabilizing the pattern, thus maximizing dynamic balance when gait is unstable or detected as such.

However, as a trade-off, reduced cutaneous reflexes would also mean a diminished capacity in responding to obstacles. To compensate, walking in an environment with a greater perceived risk of falling or threat to postural stability increases the gain of cutaneous reflexes (Haridas et al., 2005, 2006). In other words, when the system anticipates disruptions to stability, it increases the effect of cutaneous feedback when perturbed, whereas when already destabilized, such as limping during split-belt locomotion, it reduces cutaneous reflexes to avoid further destabilizing the pattern. Precision walking on a narrow beam also reduces the amplitude of another type of reflex, the soleus H-reflex, by $\sim 40\%$ compared with treadmill locomotion at matched speeds in healthy human subjects (Llewellyn et al., 1990). Some patholo-

gies that induce spasticity, such as spinal cord injury and multiple sclerosis, in which we assume gait is unstable, also reduced reflex modulation (H-, stretch, and cutaneous reflexes) in humans (Yang et al., 1991; Jones and Yang, 1994; Sinkjaer et al., 1995, 1996). These studies concluded that impaired interactions between afferent inputs from the legs and central mechanisms contributed to locomotor deficits. However, they did not investigate symmetry between the legs, which could be a main factor driving the modulation.

Reflex modulation correlates with left–right symmetry

We recently showed that speed modulated cutaneous reflexes nonlinearly in spinal cats, with the largest amplitudes observed at intermediate speeds of 0.4 and 0.6 m/s and the smallest responses observed at the slowest and fastest speeds of 0.2 and 0.8 m/s, respectively (Hurteau et al., 2017). In the present study, cutaneous reflex modulation increased from 0.4 to 0.7 m/s in intact cats, whereas it decreased from 0.4 to 0.6 m/s in spinal cats (Fig. 9). Could this be a difference in reflex modulation as a function of locomotor speed between intact and spinal cats? Although we cannot completely exclude this possibility, we think that speed-dependent changes in left–right symmetry instead explain this differential modulation. Indeed, the pattern of intact cats became more symmetric, both temporally and spatially, with increasing speed during tied-belt locomotion, whereas we observed a small nonsignificant decrease in spatial symmetry in spinal cats with no temporal changes (Fig. 10). The step-by-step consistency of left–right symmetry increases with increasing speed in intact cats, at least up to 1.0 m/s (Frigon et al., 2014), and in spinal cats from 0.1 to 0.4 m/s (Dambreville et al., 2015). However, in spinal cats, the step-by-step consistency of left–right symmetry starts breaking down at ~0.6 m/s and we observed a loose coupling between hindlimbs at faster speeds (Frigon et al., 2017). A notable difference between intact and spinal cats is activation of hip flexors before knee flexors at the stance-to-swing transition, which is thought to contribute to paw drag during early swing (Jiang and Drew, 1996; Rossignol and Frigon, 2011). As speed increases, impaired phase transitions produce greater locomotor instabilities in spinal cats. Here, we manipulated left–right symmetry with split-belt locomotion to simulate limping. The modulation of P1 responses in the ipsilateral St correlated with spatial and temporal asymmetry in intact cats and with spatial asymmetry in spinal cats. Therefore, overall, reflex modulation correlated more with spatial asymmetry, consistent with our hypothesis that signals related to left–right spatial asymmetry reduced cutaneous reflex modulation. As stated, split-belt locomotion changed the temporal phasing index in intact cats, consistent with a more asymmetric temporal pattern, with no effect in spinal cats. The main difference is that intact cats can consciously perceive the altered pattern generated by split-belt locomotion, whereas spinal cats cannot. We propose that the greater change in temporal phasing in intact cats during split-belt locomotion is due to supraspinal/volitional factors.

Figure 11. Schematic illustration of the potential mechanisms modulating cutaneous reflexes. Afferents from the SP nerve project to spinal neurons to elicit reflex responses in the ipsilateral and contralateral hindlimbs. Sensory feedback arising from the left and right legs project to left and right central pattern generators (yin and yang symbols) and/or to a neuron that uses or compares inputs from the left and right legs. The modulatory mechanism controls presynaptic inhibition of primary afferents from the SP nerve according to task.

Spinal mechanism modulates cutaneous reflexes during split-belt locomotion

Our results indicate that a spinal mechanism reduced cutaneous reflex modulation during split-belt locomotion compared with tied-belt locomotion because we observed similar results in intact and spinal cats. These results suggest that spinal networks have the ability to recognize left–right asymmetries in the locomotor pattern, potentially indicating gait instability. Conversely, perceived threats to stability and increased cutaneous feedback gain reported in humans (Haridas et al., 2005, 2006) is most likely mediated by cerebral cortical mechanisms (Pijnappels et al., 1998; Christensen et al., 1999).

What signals the reduction in cutaneous reflex modulation and by what spinal mechanism? Although we can only speculate, sensory feedback signaling left–right asymmetry most likely triggers the reduction in cutaneous reflex modulation during split-belt locomotion. This feedback signal can come from different sources. Because reflex modulation more closely correlated with spatial symmetry in intact and spinal cats compared with temporal symmetry, the source of sensory feedback probably comes from leg position/loading signals. Position signals could relate to whole leg position, such as limb orientation angles (Shen and Poppele, 1995), or to more localized specific joints/segments, such as hip angle (Grillner and Rossignol, 1978). We recently showed how sensory feedback related to hip position/limb loading differed between slow and fast limbs during split-belt locomotion and how these can regulate phase transitions in spinal cats (Frigon et al., 2017).

Presynaptic inhibition of SP nerve afferents is a likely mechanism for the decreased cutaneous reflex modulation, because it was observed in all muscles ipsilateral and contralateral to the stimulation during split-belt locomotion. The simplest explanation for this generalized decrease is reduced neurotransmitter released from primary afferent terminals (Burke, 1999; Rudomin

and Schmidt, 1999). We propose that a mechanism determines whether the locomotor pattern is symmetric by using or comparing sensory feedback from the left and right legs, thus altering the state of the spinal network. The modulatory mechanism could be controlled by left and right central pattern generators and/or more directly from sensory afferents providing position/loading feedback from the legs. We also cannot exclude that the mechanism is part of the spinal locomotor central pattern generator, which then sets the tonic level of presynaptic inhibition task dependently. **Figure 11** illustrates potential mechanisms involved in cutaneous reflex modulation during split-belt locomotion via signals related to leg position/loading. We also cannot exclude that the state of spinal neurons differs between tied-belt and split-belt locomotion. For instance, higher synchronous activity of spinal neurons would lead to larger reflex responses to a given sensory input.

Concluding remarks

In conclusion, we have shown a spinal-mediated task-dependent modulation of cutaneous reflexes during locomotion. Specifically, split-belt locomotion reduced cutaneous reflex modulation in ipsilateral and contralateral hindlimb muscles of intact and spinal cats compared with matched tied-belt speeds. We proposed that sensory feedback from the limbs signals spinal networks of a left–right asymmetry and a potentially unstable gait. In turn, the spinal networks reduce incoming inputs to prevent them from perturbing or destabilizing an already precarious gait. From a clinical perspective, the results suggest that restoring left–right symmetry in pathological conditions could normalize interactions between afferents inputs from the legs and spinal networks controlling locomotion, potentially improving functional recovery.

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