Modulation of phase durations, phase variations, and temporal coordination of the four limbs during quadrupedal split-belt locomotion in intact adult cats

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D’Angelo G, Thibaudier Y, Telonio A, Hurteau MF, Kuczynski V, Dambreville C, Frigon A. Modulation of phase durations, phase variations, and temporal coordination of the four limbs during quadrupedal split-belt locomotion in intact adult cats. J Neurophysiol 112: 1825–1837, 2014. First published July 16, 2014; doi:10.1152/jn.00160.2014.—Stepping along curvilinear paths produces speed differences between the inner and outer limb(s). This can be reproduced experimentally by independently controlling left and right speeds with split-belt locomotion. Here we provide additional details on the pattern of the four limbs during quadrupedal split-belt locomotion in intact cats. Six cats performed tied-belt locomotion (same speed bilaterally) and split-belt locomotion where one side (constant side) stepped at constant treadmill speed while the other side (varying side) stepped at several speeds. Cycle, stance, and swing durations changed in parallel in homolateral limbs with shorter and longer stance and swing durations on the fast side, respectively, compared with the slow side. Phase variations were quantified in all four limbs by measuring the slopes of the regressions between stance and cycle durations ($r_{STA}$) and between swing and cycle durations ($r_{SW}$). For a given limb, $r_{STA}$ and $r_{SW}$ were not significantly different from one another on the constant side whereas on the varying side $r_{STA}$ increased relative to tied-belt locomotion while $r_{SW}$ became more negative. Phase variations were similar for homolateral limbs. Increasing left-right speed differences produced a large increase in homolateral double support on the slow side, while triple-support periods decreased. Increasing left-right speed differences altered homologous coupling, homolateral coupling on the fast side, and coupling between the fast hindlimb and slow forelimb. Results indicate that homolateral limbs share similar control strategies, only certain features of the interlimb pattern adjust, and spinal locomotor networks of the left and right sides are organized symmetrically.

DURING TERRESTRIAL LOCOMOTION, stepping in a perfectly straight line for prolonged periods is an infrequent occurrence in everyday life (Reisman et al. 2005), as animals must often turn and step along curvilinear paths. A characteristic of stepping along a curvilinear path is that the left and right sides step at different speeds from one another, as the outer limb(s) must travel a greater distance than the inner one(s) (Courtine and Schieppati 2003; Dietz et al. 1994; Halbertsma 1983; Reisman et al. 2005; Zijlstra and Dietz 1995). Having one side step faster than the other can be achieved experimentally by using a split-belt treadmill composed of two independently controlled surfaces (Dietz et al. 1994; Forssberg et al. 1980; Frigon et al. 2013; Kulagin and Shik 1970; Reisman et al. 2005; Yang et al. 2005; Zijlstra and Dietz 1995). The overall aim of this study was to gain further insight into the control systems regulating adjustments in the locomotor pattern when the left and right sides step at different speeds from one another.

Split-belt locomotion produces predictable bilateral changes in phase durations in the hindlimbs of quadrupeds and in the legs of humans. For instance, the limb(s) stepping on the slow belt has relatively longer stance duration with shorter swing duration while the limb(s) stepping on the fast belt has relatively shorter stance duration with longer swing duration. Bilateral adjustments in phase durations during split-belt locomotion have been shown in intact, decerebrate, and chronic spinal-transected cats (Forssberg et al. 1980; Frigon et al. 2013; Halbertsma 1983; Kulagin and Shik 1970) as well as in humans (Dietz et al. 1994; Macellian et al. 2014; Prokop et al. 1995; Reisman et al. 2005; Yang et al. 2005; Zijlstra and Dietz 1995). Studies in chronic spinalized cats indicate that adjustments in hindlimb phase durations during split-belt locomotion can be mediated entirely at the level of the spinal cord (Forssberg et al. 1980; Frigon et al. 2013). To date, studies of adjustments in phase durations during quadrupedal split-belt locomotion have primarily focused on the hindlimbs. Thus the first objective of the present study was to evaluate whether forelimb phase durations adjust similarly to the hindlimbs during quadrupedal split-belt locomotion in intact adult cats. We hypothesize that phase durations of homolateral limbs change in parallel during quadrupedal split-belt locomotion, indicating similar control mechanisms at cervical and lumbar levels.

A consistent feature of overground locomotion in a straight or normal treadmill locomotion is that the stance phase varies more than the swing phase as a function of cycle duration (reviewed in Frigon 2012; Gossard et al. 2011). This can be quantified by measuring and comparing the slopes of the regressions between stance duration and cycle duration ($r_{STA}$) and between swing duration and cycle duration ($r_{SW}$). In a recent study, Frigon et al. (2013) showed that phase variations in the left and right hindlimbs of intact and chronic spinalized cats were similarly modulated during split-belt locomotion. In these experiments, one side stepped at a constant treadmill speed while the other side stepped at several different treadmill speeds. On the “constant” side, $r_{STA}$ was decreased compared with the tied-belt condition (i.e., equal treadmill speed on left and right sides) while $r_{SW}$ was increased. In fact, $r_{STA}$ was not significantly different from $r_{SW}$ on the constant side, indicating that both the stance and swing phases varied in approximately equal proportion with cycle duration. On the “varying” side,
rSTA was increased compared with the tied-belt condition while rSW became more negative. Similar results were found in intact and chronic spinalized cats, indicating that the regulation of phase variations was mediated within the spinal cord. The regulation of phase variations has been used to model some features of the hindlimb central pattern generator (CPG) in mammals (Spardy et al. 2011; Yakovenko 2011; Yakovenko et al. 2005). It is unclear whether the forelimbs share similar control strategies in regulating phase variations when the left and right sides step at different speeds from one another. Therefore, the second objective of the present study was to determine whether the regulation of phase variations is similar in the forelimbs and hindlimbs during quadrupedal split-belt locomotion, which could provide some insight as to the organization of forelimb CPGs. We hypothesize that phase variations of homolateral limbs change in parallel during quadrupedal split-belt locomotion, again indicating similar control strategies at cervical and lumbar levels.

The ability to step along curvilinear paths while maintaining stability requires precise and flexible coordination of the four limbs in quadrupeds and of the left and right legs in bipeds. As stated above, split-belt locomotion reproduces some features of curvilinear path stepping, such as different speeds for the inner and outer limbs. As such, split-belt locomotion offers a substrate to systematically manipulate interlimb coordination to study its neural and biomechanical control (Reisman et al. 2005). Increasing the speed difference between the left and right treadmill belts (L-R speed \( \Delta \)) can also be used to assess the adaptive capacity of interlimb coordination. To date, studies of split-belt locomotion have almost exclusively focused on the coordination between the hindlimbs or legs, whereas adjustments between the forelimbs or arms, as well as between girdles, have been largely unexplored. One study investigated the effects of bipedal split-belt locomotion on the movement of the arms in humans (MacLellan et al. 2013). In that study, increasing the speed difference between the left and right legs increased the trajectory of the fast leg in the antero-posterior direction, with the slower leg having smaller movement amplitudes. At the same time, both the left and right arms showed greater movement amplitudes. The bilateral increase in arm swing could be a mechanism to stabilize the torso due to the overall increase in walking speed or because of the more unstable gait. Evidently, in quadrupeds the forelimbs play an important role in weight support and in propulsion. As a result, the coordination between the forelimbs and hindlimbs probably requires a much tighter control than in humans. Therefore, the third objective of the present study was to assess the temporal coordination between the four limbs during quadrupedal split-belt locomotion with an increasing difference in the treadmill speeds between the slow and fast sides.

It is well known that the control of movement within the central nervous system of mammals is organized asymmetrically, both functionally and anatomically (Galaburda et al. 1978; Mutha et al. 2012; Peng and Charron 2013). For example, lateralization of brain function leads to some people being right- or left-handed. Left-right asymmetries are found throughout the nervous system, including spinal reflexes (Hultborn and Malmsen 1983), dorsal root ganglia (Ygge et al. 1981), and in the number of muscle spindles of homologous muscles (Barker and Chin 1960). However, it was previously reported that temporal adjustments to split-belt locomotion in humans were symmetric if it was the left or right side that was walking faster (Zijlstra and Dietz 1995). To determine whether this is also the case in quadrupeds, the fourth objective of the present study was to determine whether adjustments to split-belt locomotion were symmetric by evaluating changes in several parameters of the pattern using both the left and right sides as the slow and fast sides in intact cats.

**MATERIALS AND METHODS**

**Animals and ethical considerations.** All procedures were approved by the Animal Care Committee of the Université de Sherbrooke and were in accordance with policies and directives of the Canadian Council on Animal Care. Before and after experiments, animals were housed and fed within designated areas. Six adult cats (2 males, 4 females) weighing between 3.5 and 6.0 kg were used. Five of these cats were used in other studies (Frigon et al. 2013, 2014; Thibaudier et al. 2013) to provide answers to other scientific questions. This is part of our effort to maximize the scientific output of each animal. Kinematic data from five of the six cats were obtained after surgical implantation of electrodes for electromyography (Frigon et al. 2013, 2014). However, the surgical procedures are not described in detail here as the present report does not refer further to electromyography.

**Experimental protocol.** Cats were trained to step on an animal treadmill with two independently controlled running surfaces 120 cm long and 30 cm wide (Bertec, Columbus, OH). The treadmill setup is shown schematically in Fig. 1A. A Plexiglas separator (120 cm long, 3 cm high, 0.5 cm wide) was placed between the left and right belts to ensure that the limbs on the left and right sides stepped on separate belts. To prevent the cat from moving laterally on the treadmill, two Plexiglas separators (120 cm long, 50 cm high) were also placed 30 cm apart from one another. The Plexiglas separators were held in place ~0.5 cm above the treadmill belts by a metal frame (not shown). After a few days of training, cats could easily step with the left and right sides on their respective belts without making contact with the centrally or laterally placed Plexiglas separators. Cats were given food and affection as reward. Experiments began after a minimum of 2 wk of familiarization in which cats performed tied-belt locomotion at multiple speeds and split-belt locomotion at various L-R speeds \( \Delta s = 3-5 \) times a week, with sessions lasting 15–30 min.

Each cat performed one session that consisted of several locomotor episodes with the left and right belts operating at the same speed (tied-belt locomotion) or at different speeds (split-belt locomotion). The locomotor conditions used are shown in Fig. 1B. Tied-belt locomotion was performed at eight treadmill speeds from 0.3 to 1.0 m/s in 0.1-m/s increments. In the split-belt conditions, one side stepped at a treadmill speed of 0.4 m/s, referred to as the constant side, while the other side (the varying side) stepped at treadmill speeds from 0.3 to 1.0 m/s in 0.1-m/s increments, with the exception of 0.4 m/s (tied-belt condition). The left and right sides were used as the constant and varying sides in equal proportion \( n = 7 \) episodes per side. The L-R speed \( \Delta s \) is text and figures are expressed in absolute terms, and the slow and fast sides are indicated as left speed smaller than right speed \( (L < R) \) or left speed greater than right speed \( (L > R) \). The different tied-belt and split-belt conditions were presented randomly, and \( \sim 30 \) s of rest was given between episodes. The objective was to obtain \( \sim 15 \) cycles per episode, and a session took 30–45 min to get enough cycles in each of the 22 episodes (8 tied-belt episodes + 14 split-belt episodes; see Fig. 1B).

**Data acquisition and analysis.** Videos of the left and right sides were captured with two cameras (Basler AcA640–100 gm) at 60 frames/s with a spatial resolution of 640 × 480 pixels. A customized LabVIEW program acquired the images and synchronized the cameras. Videos were analyzed off-line at 60 frames/s with custom-made software. Paw contact and the most caudal displacement of the
The 2 belts are separated by a low separator of the treadmill with the left and right sides of an intact cat on 2 separate belts. Fig. 1. Experimental setup and locomotor conditions.

To the linear regressions between stance duration and cycle duration function of cycle duration for each limb to measure the slopes of minus stance duration. Durations for each limb were averaged for paw contacts while stance duration corresponded to the interval of limbs. For a given limb, cycle duration was measured from successive to toe. Cycle, stance, and swing durations were measured for all four of the limb was the frame with the most caudal displacement of the contact with the treadmill surface, while the most caudal displacement contact was defined as the first frame in which the paw made visible limb were determined for all four limbs by visual inspection. Paw arbitrarily because cycle duration remained approximately equal in split-belt conditions. Right hindlimb cycle duration was used split-belt locomotion with cycle duration obtained during tied-belt closer to the expected value of the fast or slow sides, we measured determine whether cycle duration during split-belt locomotion was distributions (English and Lennard 1982). Phase intervals were then expressed in degrees to illustrate their continuous nature and possible distributions (English and Lennard 1982). Phase intervals were measured for the six different limb pairings.

Statistical analyses. Statistical tests were performed with IBM SPSS Statistics 20.0 or with Statgraphics Centurion XVI (StatPoint Technologies). Statistical tests were performed for the group (n = 6 cats). Repeated-measures analyses of variance (ANOVAs) were performed across cats with IBM SPSS Statistics 20.0 (Analyze, General Linear Model, Repeated Measures). A two-factor (L-R speed Δ, limb) repeated-measures ANOVA was performed on cycle, stance, and swing durations to determine the effect of L-R speed Δ and to compare homolateral limbs. A two-factor (L-R speed Δ, side) repeated-measures ANOVA was performed on the absolute difference from tied belt for RH cycle duration. A one-factor (L-R speed Δ) repeated-measures ANOVA was performed on each of the support periods and on the mean phase intervals for each of the six limb pairings. A Greenhouse-Geisser correction was made if Mauchly’s test of sphericity was significant. For individual episodes, tSTA and tSW were compared with one another with a multiple regression analysis (Comparison of Regression Lines in Statgraphics). An ANOVA was used to determine whether the regressions were significant (Simple Regression in Statgraphics). For the group, paired t-tests were performed on mean tSTA and tSW values with IBM SPSS Statistics 2.0 (Analyze, Compare Means, Paired Samples T Test). Statistical significance for all tests was set at P < 0.05.

RESULTS

Modulation of the locomotor pattern during quadrupedal split-belt locomotion. Figure 2 shows the locomotor pattern of a single cat during quadrupedal split-belt locomotion. All panels are arranged in the same way, with the stance phase of the four limbs and the pattern of limb supports below. The first row of limb support patterns represents periods of triple support, while the second row shows intervening periods of quadruple or double support. Each support period is delimited by vertical lines. A complete cycle (i.e., from successive RH contacts) normally contains eight distinct time periods that can be determined on the basis of the pattern of limb supports (Frigon et al. 2014; Gray 1968; Hildebrand 1989; Wetzel and Stuart 1976). Individual support periods were calculated for each locomotor episode and expressed as a percentage of cycle duration (Frigon et al. 2014). In quadrupeds, coordinated behavior must be realized for six different limb pairings: 1) homologous coupling between the left (LF) and right (RF) forelimbs and 2) between the left (LH) and right (RH) hindlimbs; 3) homolateral coupling between LF and LH and 4) between RF and RH; and 5) diagonal coupling between RH and LF and 6) between LH and RF. Phase intervals were calculated by measuring the absolute interval of time between contacts of two limbs and expressed as a function of cycle duration (English 1979; English and Lennard 1982; Frigon et al. 2014; Orsal et al. 1990). Phase intervals were then expressed in degrees to illustrate their continuous nature and possible pairings.
where contact of the hindlimb is followed by contact of the homolateral forelimb (Hildebrand 1967; Lemelin et al. 2003; Patrick et al. 2009; Stevens 2006; Thibaudier et al. 2013).

On the slow side, the stance phases of the homolateral forelimb and hindlimb were longer while the swing phases were shorter than in the homologous limbs on the fast side. This left-right asymmetry in stance and swing durations increased with L-R speed \( \Delta \) (compare Fig. 2, A and E, or Fig. 2, B and F). At a given L-R speed \( \Delta \), despite the large differences in stance and swing durations between limbs on the slow and fast sides, cycle duration remained equal in all four limbs. In other words, a 1:1 rhythm was maintained for the slow and fast sides. As a result, because of adjustments in phase durations, the pattern of limb supports and the phasing between limb contacts was altered. For instance, at the smallest L-R speed \( \Delta \) of 0.1 m/s, the number of limbs contacting the surface was variable, with sequences of 3-4-3-2-3-4-3-2 (Fig. 2A), 3-4-3-2-3-2-3-2 (Fig. 2A), and 3-2-3-2-3-2-3-2 (Fig. 2B). As the L-R speed \( \Delta \) increased to 0.6 m/s (Fig. 2, E and F), the pattern of limb supports became more consistent, with sequences of

![Diagram of limb coordination during quadrupedal split-belt locomotion](image-url)
3-4-3-2-3-4-3-2 primarily. The most noticeable adjustment with an increase in L-R speed $\Delta$ was an increase in the period of homolateral double support on the slow side.

Cycle and phase durations during quadrupedal split-belt locomotion. Figure 3 shows cycle and phase durations for the group at six L-R speed $\Delta$s in the L < R (Fig. 3, left) and L > R (Fig. 3, right) split-belt conditions; the limb shown in a given panel is in black in the cat diagram illustrated in the top right corner of each panel. Previous studies have shown adjustments in cycle and phase durations during quadrupedal split-belt locomotion (Frigon et al. 2013; Halbertsma 1983; Kulagin and Shik 1970). Here we wanted to determine whether the adjustments were similar in the homolateral forelimb and hindlimb.

A two-factor (L-R speed $\Delta$, limb) repeated-measures ANOVA was performed on the cycle and phase durations of homolateral limbs in the two split-belt conditions (L < R and L > R). There was a significant effect of L-R speed $\Delta$ ($P < 0.001$) on cycle, stance, and swing durations for the left and right homolateral limbs in both split-belt conditions, except for swing duration in the L < R condition. There was no significant effect of limb on cycle, stance, and swing durations ($P > 0.05$). These data indicate that forelimb and hindlimb cycle and phase durations change in parallel during quadrupedal split-belt locomotion.

Cycle duration during split-belt locomotion was always intermediate to the cycle durations obtained during tied-belt locomotion at the treadmill speeds of the slow and fast limbs (data not shown). In other words, the limbs on the fast belt had longer cycle durations while the limbs on the slow belt had shorter cycle durations than what would be expected based on belt speed. Figure 4 shows the absolute difference between RH cycle duration obtained during split-belt locomotion and RH cycle duration obtained during tied-belt locomotion at the speeds of the slow and fast belts with the left side stepping slower (Fig. 4A) or faster (Fig. 4B) than the right side. There was a significant effect of L-R speed $\Delta$ ($P < 0.001$) on the absolute difference from tied-belt locomotion in both split-belt conditions. In the L < R condition, there was a significant effect of side ($P < 0.05$), indicating that cycle duration during split-belt locomotion was closer to the cycle duration obtained at the same belt speeds as the fast side. The tendency was similar in the L > R condition, although not significant ($P = 0.16$).

Phase variations during quadrupedal split-belt locomotion. To more closely evaluate the relationships between the phases (i.e., stance, swing) and cycle as a function of left-right coordination, we measured the slopes of the linear regressions between the phases and cycle duration in all four limbs during quadrupedal split-belt locomotion. We recently performed this

Fig. 3. Cycle and phase durations of the forelimbs and hindlimbs during quadrupedal split-belt locomotion across cats. Left (A–D) and right (E–H) panels show cycle, stance, and swing durations as a function of the difference in treadmill speed between the left and right sides (L-R speed $\Delta$). In A–D, the left side was stepping at a constant treadmill speed of 0.4 m/s while the right side stepped at treadmill speeds of 0.5–1.0 m/s in 0.1-m/s increments. In E–H, the right side was stepping at a constant treadmill speed of 0.4 m/s while the left side stepped at treadmill speeds of 0.5–1.0 m/s in 0.1-m/s increments. Each panel shows cycle, stance, and swing duration for 1 of the 4 limbs. The limb shown in each panel is filled in black in the cat diagram at top right. Each data point is the average ± SD obtained at 1 L-R speed $\Delta$ of 6 cats.
analysis in the hindlimbs of intact and chronic spinalized cats (Frigon et al. 2013). Here we wanted to determine whether similar changes in r_{STA} and r_{SW} occurred in the homolateral forelimb and hindlimb. Figure 5 shows examples from one cat with the left side stepping at a constant treadmill speed of 0.4 m/s across episodes (n = 7) while the right side stepped at treadmill speeds ranging from 0.3 m/s to 1.0 m/s in 0.1-m/s increments; the limb shown is illustrated in the cat diagram in the top left corner of each panel. When the left side held a constant treadmill speed, r_{STA} and r_{SW} had similar values in LF (Fig. 5A) and LH (Fig. 5B). In the LF, r_{SW} was significantly greater than r_{STA} (Fig. 5A). On the other hand, r_{STA} was significantly greater than r_{SW} in RF (Fig. 5C) and RH (Fig. 5D), where the durations of the stance and swing phases increased and decreased with cycle duration, respectively.

Figure 6 shows r_{STA} and r_{SW} values across cats in the two split-belt conditions. In each split-belt condition, eight comparisons were made (paired t-tests). For each limb, r_{STA} and r_{SW} were compared to one another and the r_{STA} in the homolateral limbs. The same comparison was also done for r_{SW} between homolateral limbs. Figure 6 shows that r_{STA} was significantly greater than r_{SW} in the homolateral limbs where treadmill speed was varied (i.e., RF and RH in Fig. 6A, LF and LH in Fig. 6B). There was no significant difference in r_{STA} or r_{SW} between homolateral limbs in the two split-belt conditions. Thus the regulation of phase variations during split-belt locomotion is similar in the fore- and hindlimbs. It is also clear that the two split-belt conditions are mirror images of one another in terms of changes in phase variations.

Footfall patterns and periods of support during quadrupedal split-belt locomotion. As shown in Fig. 2, a change in phase durations on the slow and fast sides altered the pattern of limb supports and the relative duration of support periods within a locomotor cycle. Figure 7 shows the averaged support periods normalized to cycle duration across cats when the left (Fig. 7, A–F) or right (Figs. 7, G–L) side was the slow side; the numbers above the panels indicate the number of limbs contacting the ground during the eight time phases of the cycle. The footfall pattern remained consistent proceeding with RH, RF, LH, and LF contacts. When the slow side was the left side, the sequence of limbs contacting the surface was 3-2-3-2-3-2-3-2 at the smallest L-R speed Δ of 0.1 m/s (Fig. 7A) before becoming a 3-4-3-2-3-2-3-2 pattern at all other L-R speed Δs (Figs. 7, B–F). When the slow side was the right side, the sequence of limbs contacting the surface was consistent, with a pattern of 3-2-3-2-3-4-3-2 at all L-R speed Δs (Figs. 7, G–L). Thus the adjustments in support patterns are approximately mirror images that depend on the side (i.e., left or right) that is stepping slower or faster.

From the data shown in Fig. 7, it is clear that some support periods are more affected than others with a change in L-R speed Δ. Figure 8 shows the relative duration (i.e., as % of cycle duration) of each of the eight support periods across cats when the left (Fig. 8, A–H) and right (Fig. 8, I–P) sides were the slow side. In the L < R condition (Fig. 8, A–H), support periods 1 (Fig. 8A), 2 (Fig. 8B), 5 (Fig. 8E), 7 (Fig. 8G), and 8 (Fig. 8H) were significantly affected by L-R speed Δ (1-
factor repeated-measures ANOVA). In the L > R condition (Fig. 8, A–P), support periods I (Fig. 8I), 2 (Fig. 8J), 3 (Fig. 8K), 4 (Fig. 8L), 5 (Fig. 8M), and 7 (Fig. 8O) were signficantly affected by L-R speed Δ. The most pronounced change was a large increase in the period of homolateral double support on the slow side with increasing L-R speed Δ (Fig. 8, H and L).

**Interlimb coordination during quadrupedal split-belt locomotion.** To quantify changes in interlimb coordination as a function of L-R speed Δ, we used an approach similar to previous studies to calculate the phasing between pairs of limbs (English 1979; English and Lennard 1982; Frigon et al. 2014; Orsal et al. 1990). The time interval between contacts of two limbs was measured and divided by cycle duration. This value was then multiplied by 360 and expressed in degrees. Interlimb phasing was calculated for the six possible limb pairings.

Values of 0° or 360° indicate a strict in-phase coupling, while a value of 180° indicates a strict out-of-phase coupling. Figure 9 shows the phasing between the six limb pairings in the L < R split-belt condition in one cat. For clarity, the three lowest L-R speed Δs are separated from the three highest. Cycle durations are shown as radii and are indicated in Fig. 9A, left. Cycle duration increases from the center out, and each data point represents a phase interval from a single locomotor cycle. The homologous coupling between forelimbs (Fig. 9A) and between hindlimbs (Fig. 9B) was ~180° at a L-R speed Δ of 0.1 m/s, an out-of-phase coupling, and shifted toward 120° as the L-R speed Δ increased. Left homolateral coupling did not visibly change as a function of L-R speed Δ, remaining mostly between values of 30° and 60° (Fig. 9C). Right homolateral coupling showed values between 30° and 60° at low L-R speed Δs, whereas values between 0° and 60° were found at high L-R speed Δs (Fig. 9D). The diagonal coupling between RH and LF shifted from values around 210° at the lowest L-R speed Δ to 150° at the highest L-R speed Δ (Fig. 9E). The diagonal coupling between LH and RF showed a small shift from values between 210° and 270° at low L-R speed Δs to values mostly between 240° and 270° at high L-R speed Δs. Therefore, the most notable changes in interlimb phasing as a function of L-R speed Δ were found for homologous couplings (forelimbs and hindlimbs) and for the diagonal coupling between RH and LF.

Figure 10 summarizes interlimb phasing for the six limb pairings across cats in the L < R (Fig. 10, left) and L > R (Fig. 10, right) split-belt conditions. In the L < R and L > R split-belt conditions, there was a significant effect of L-R speed Δ on the homologous coupling between forelimbs (Fig. 10, A and C) and between hindlimbs (Fig. 10, B and D). Although homologous coupling decreased and increased with an increase in L-R speed Δ in the L < R and L > R split-belt conditions, respectively, the change was similar because phase intervals were calculated relative to contact of the right side. Thus, in the L < R split-belt condition, contact of the left side (i.e., slow side) occurs relatively sooner in the cycle that starts with contact of the right side (i.e., fast side) with an increase in L-R speed Δ. In the L > R split-belt condition, contact of the right side (i.e., fast side) occurs relatively later in the cycle that starts with contact of the right side (i.e., slow side) with an increase in L-R speed Δ.

DISCUSSION

The present findings showed novel data on changes in the locomotor pattern when the left side stepped at different treadmill speeds than the right side during quadrupedal split-belt locomotion in intact adult cats. Increasing left-right speed differences with split-belt locomotion altered phase durations and phase variations in all four limbs. The modulation of phase durations and phase variations was similar in homolateral limbs, indicating similar control strategies at cervical and
The step cycle was reorganized so that the duration of the homolateral double-support period on the slow side increased considerably with increasing L-R speed. Moreover, the coordination between certain limb pairs was affected by increasing L-R speed, indicating that specific features of the interlimb pattern are modified by increasing the speed difference between the left and right sides. Finally, adjustments in the locomotor pattern during quadrupedal split-belt locomotion were similar with the left and right sides as the slow or fast side, indicating symmetric organization of left and right locomotor networks. The neurophysiological insight obtained from these findings is discussed in the following sections.

Control of phase durations and phase variations: similar control mechanisms regulating forelimb and hindlimb locomotion. In a recent study, we showed that hindlimb phase durations and phase variations were similarly modulated in intact and chronic spinalized adult cats during split-belt locomotion, indicating that the modulation was mediated within the spinal cord (Frigon et al. 2013). The present findings showed that the modulation of phase durations (Fig. 3) and phase variations (Fig. 6) on the slow and fast sides was similar for homolateral limbs, indicating that control strategies regulating phase durations and phase variations are similar for the forelimbs and hindlimbs. On the basis of experimental evidence, such as reflex modulation, it has been suggested that rhythmic movements of the forelimbs/arms and hindlimbs/legs during quadrupedal or bipedal locomotion are governed by similar spinal circuitry (i.e., locomotor CPGs) at cervical and lumbar levels, respectively (reviewed in Zehr et al. 2004; Zehr and Duysens 2004). Similar modulation of phase durations and phase variations in the forelimbs and hindlimbs of intact cats and in the hindlimbs of chronic spinalized adult cats are consistent with this hypothesis.
Although phase durations and phase variations can be controlled entirely at the level of the spinal cord by locomotor CPGs, under normal circumstances they are also modulated by sensory feedback from the periphery and by inputs from supraspinal structures. For instance, a slight dorsiflexion of the ankle in decerebrate cats during fictive locomotion increased the slope of the regression between extensor burst duration and cycle duration ($r_{\text{EXT}}$) while the slope of the regression between flexor burst duration and cycle duration ($r_{\text{FLEX}}$) went toward 0 (see Fig. 4 of Frigon and Gossard 2010). In hindlimb-attached neonatal rodent in vitro preparations, hindlimb $r_{\text{EXT}}$ and $r_{\text{FLEX}}$ were similar during fictive locomotion without phasic sensory feedback (Juvin et al. 2007). However, when the hindlimbs were loaded, $r_{\text{EXT}}$ increased considerably while $r_{\text{FLEX}}$ went toward 0. 

![Figure 8](http://jn.physiology.org/)

Fig. 8. Effect of increasing the difference in speed between the left and right sides on the 8 support periods during quadrupedal split-belt locomotion across cats. Each panel represents 1 of the 8 support periods when left treadmill speed is slower than right treadmill speed ($L < R$; A–H) or when left treadmill speed is faster than right treadmill speed ($L > R$; I–P). The limbs contacting the surface are shown in black in the footfall diagram in each panel. Top left, top right, bottom left, and bottom right circles represent LF, RF, LH, and RH, respectively. Each vertical bar is the average ± SD of 6 cats. Note that support period 2 in the $L < R$ split-belt condition could be a period of diagonal support or quadruple support. Asterisks indicate a significant effect of L-R speed $\Delta$ on a given support period (1-factor repeated-measures ANOVA): *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. 

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toward 0. Similar results were found in human infants when loading was applied to the legs in a variety of actual rhythmic movements (Musselman and Yang 2007). Thus stance-related inputs (i.e., force, stretch of ankle extensors, and/or pressure to the plantar surface) can considerably modify the regulation of phase durations and variations. We posit that the simultaneous differential modulations of phase variations in the forelimbs and hindlimbs on the left and right sides during quadrupedal split-belt locomotion (Figs. 5 and 6) are mediated by asymmetric stance-related sensory feedback from the slow and fast sides.

Undoubtedly, the similar behavior of homolateral limbs results from the fact that both limbs are moving on the same belt at the same treadmill speed. However, the limbs were rarely locked in identical phases. This would have been observed with true pacing or homolateral coupling of 0° or 360°.

As such, it is unlikely that the specific mechanisms regulating phase durations and phase variations in the forelimbs are temporally coupled to those of the hindlimbs. Forssberg et al. (1980) proposed that several neuronal mechanisms could be involved in maintaining bilateral coordination in a “push or pull” manner due to sensory feedback from the slow and fast sides. Studies in human infants (Yang et al. 2005) and adults (Dietz et al. 1994; Zijlstra and Dietz 1995) have proposed that sensory feedback from the fast limb drives bilateral changes in phase durations because cycle duration during split-belt locomotion in infants was closer to the value obtained on the fast side during tied-belt locomotion. Although some of our results are consistent with this hypothesis (see Fig. 4), the stance phase of homolateral limbs on the slow side occupied an increasingly greater proportion of the cycle with increasing L-R speed Δ (Fig. 3). Moreover, in terms of support periods, the largest
change was an increase in the homolateral double-support period of the slow side with increasing L-R speed (see Figs. 7 and 8). Thus it is likely that sensory feedback from both the slow and fast moving limbs drives the adjustments to split-belt locomotion.

The control of phase durations and phase variations has been used in efforts to model hindlimb CPG circuitry (Spardy et al. 2011; Yakovenko 2011; Yakovenko et al. 2005). In these models, the durations and variations of the extensor and flexor phases are generated by a set of coupled oscillators with reciprocal connections. Phase variations are determined by increased excitability within either oscillator. Commands from the oscillators are then transmitted to other neurons that coordinate the activity of motor pools. During locomotion, modulation of the excitability within the extensor or flexor oscillator can come from peripheral sensory feedback, descending inputs, and/or intrinsic mechanisms within spinal locomotor CPGs. In a recent study, phase variations were altered in cats after a lateral spinal hemisection at low thoracic levels (T10) on the left side (Martinez et al. 2012). Specifically, rSTA and rSW were decreased and increased on the lesioned side, respectively, compared with values obtained in the intact state. On the nonlesioned side, rSTA and rSW were increased and decreased, respectively, compared with values obtained in the intact state. Although this was taken as evidence for intrinsic changes within the spinal locomotor CPGs of the left and right hindlimbs, it is more likely that changes in phase variations were mediated by asymmetric sensory feedback from the reduced stance phase and increased swing phase on the lesioned side and/or from asymmetric descending inputs to the left and right spinal locomotor CPGs.

**Modulation of interlimb coordination during quadrupedal split-belt locomotion.** Across L-R speed Δs, cats maintained a lateral sequence whereby contact of the hindlimb was followed by contact of the homolateral forelimb. This type of sequence

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Fig. 10. Effect of increasing the difference in treadmill speed between the left and right sides on interlimb phasing during quadrupedal split-belt locomotion across cats. The mean phase interval between limb contacts is shown for the 6 different limb pairs, which are shown in black in the cat diagram at top right of each panel. For each cycle, the phase interval was measured between limb contacts and then normalized to the cycle duration of the reference limb (i.e., the first limb used in the calculation). It was then multiplied by 360 and expressed in degrees. Each bar is the mean ± SD phase interval of 6 cats. Asterisks indicate a significant effect of L-R speed Δ on a given limb pair (1-factor repeated-measures ANOVA): *P < 0.05, **P < 0.01, ***P < 0.001.
is used preferentially in the majority of terrestrial quadrupeds (Hildebrand 1976), with the exception of nonhuman primates that primarily use a diagonal sequence whereby contact of the hindlimb is followed by contact of the contralateral forelimb (Hildebrand 1976; Stevens 2006; Vilensky and Larson 1989). Crawling in human infants and adults is also characterized by a lateral sequence (Patrick et al. 2009). However, Thibaudier et al. (2013) recently showed that cats could adopt a diagonal sequence if the forelimbs stepped faster than the hindlimbs on a transverse split-belt treadmill. Thus the type of sequence can be altered under certain circumstances. The lateral sequence offers more stability than the diagonal sequence (Hildebrand 1989), and in the present study it was preferentially used by cats across L-R speed Δs.

Cats tend to adopt a pacing gait on a treadmill (Blaszczyk and Loeb 1993), as opposed to a trotting gait during over-ground locomotion (English 1979). In a counterclockwise direction, values of homolateral coupling between 315° and 45° are generally considered as a pacinglike gait with values of 0° or 360° considered true pacing (English and Lennard 1982). In the present study, the coupling between homolateral limbs on the fast side showed a significant modulation with increasing L-R speed Δ (Fig. 10). At the lowest L-R speed Δ of 0.1 m/s, homolateral coupling on the fast side across cats was around 60°, which is considered a low lateral couplet (English and Lennard 1982). With increasing L-R speed Δ, there was a tendency toward a pacinglike gait on the fast side, with values around 40° at the highest L-R speed Δ of 0.6 m/s. Frigon et al. (2014) also showed that homolateral coupling values went toward a pacing form of gait with increasing speed during tied-belt locomotion. Although pacing offers less stability than trotlike gaits, it prevents interference (i.e., contact) between homolateral limbs (Hildebrand 1989; Patrick et al. 2009). Moreover, Frigon et al. (2014) showed that each of the eight support periods was significantly affected by increasing treadmill speed from 0.3 m/s to 1.0 m/s in 0.1-m/s increments, all with a P < 0.001 (see their Fig. 8). Here only certain support periods were significantly affected by increasing L-R speed Δ (Fig. 8). In particular, periods of triple support and the homolateral double support on the slow side were affected. While periods of triple support decreased with increasing L-R speed Δ, homolateral support on the slow side increased.

Concluding remarks. In summary, the present findings showed similar control strategies of the forelimbs and hindlimbs in the regulation of phase durations and phase variations during quadrupedal split-belt locomotion. Zijlstra and Dietz (1995) showed that adjustments to split-belt locomotion in humans were similar if it was the left or right leg that was stepping slower or faster. The present study confirms these results in intact cats during quadrupedal split-belt locomotion, again highlighting the similarities between human and cat studies. This suggests that spinal locomotor networks on the left and right sides are organized symmetrically, at least from a functional perspective. In conclusion, split-belt locomotion in an animal model provides a useful tool to systematically probe and challenge the capacity for interlimb coordination in the intact system and after various types of injuries or in disease. A better understanding of the mechanisms and pathways that contribute to interlimb coordination in health and disease could provide important insights for gait rehabilitation in a variety of patient populations (Thompson 2012).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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