Spatiotemporal control of interlimb coordination during transverse split-belt locomotion with 1:1 or 2:1 coupling patterns in intact adult cats

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DURING LOCOMOTION, the arms and legs of humans, or the forelimbs and hindlimbs of quadrupeds, perform rhythmic coordinated movements (interlimb coordination). Precise control of interlimb coordination is necessary to maintain dynamic stability and to transition smoothly between gait patterns. Although the four limbs perform coordinated rhythmic movements during locomotion, the arms/forelimbs and legs/hindlimbs must also be capable of acting independently from one another to perform a wide variety of tasks. Although this is evident in bipeds such as humans, quadrupedal mammals also require independent movement of the forelimbs and hindlimbs during locomotion when reaching, grasping, or swiping an object with the forepaw. The movements of the forelimbs must be precisely integrated with the movement of the hindlimbs to maintain dynamic stability. One way to experimentally simulate some aspects of this phenomenon is to have the forelimbs step at a different speed than the hindlimbs on a transverse split-belt treadmill where the animal must adapt to maintain stability (Akay et al. 2006; Cruse and Warnecke 1992; Thibaudier et al. 2013). Thus transverse split-belt locomotion can be used as a tool to challenge the system(s) regulating interlimb coordination in a dynamic task.

In a recent study, Thibaudier et al. (2013) showed in intact cats that when the forelimbs stepped at a slightly faster speed (i.e., 0.1 m/s) than the hindlimbs on a transverse split-belt treadmill, the forelimbs and hindlimbs could have unequal cycle periods, with the forelimbs taking two steps within one hindlimb cycle, or a 2:1 forelimb-hindlimb relationship. Although 2:1 forelimb-hindlimb relationships were intermixed with 1:1 relationships, as forelimb speed increased relative to hindlimb speed they became more frequent. In stark contrast, when the hindlimbs stepped faster (up to 3 times) than the forelimbs, a 1:1 forelimb-hindlimb relationship was always maintained (Thibaudier et al. 2013). It was proposed that the organization of the system(s) coordinating the forelimbs and hindlimbs is bidirectional, as faster forelimb or hindlimb speeds influenced the pattern at the other girdle, and asymmetric because the effects on cycle and phase durations were strikingly different if it was the forelimbs or hindlimbs that were stepping faster. An important question remains unanswered when the forelimbs take more steps than the hindlimbs: Are the forelimbs and hindlimbs still coupled? In other words, do the forelimbs remain phase locked with the hindlimbs?

The arms/forelimbs and legs/hindlimbs can also adopt a 2:1 relationship during locomotion under other conditions. For instance, at slow walking speeds (<2.7 km/h) in humans the arms swing at a frequency that is two times higher than the legs (Craik et al. 1976; Donker et al. 2001; Webb et al. 1994). Moreover, after partial spinal lesions in rats (Alluin et al. 2011; Cote et al. 2012) and cats (Barriere et al. 2010; English 1980; Gorska et al. 1996, 2013), a 2:1 forelimb-hindlimb relationship is often observed. The appearance of a 2:1 forelimb-hindlimb relationship after incomplete spinal cord injuries could indicate a loss of interlimb coordination or, alternatively, that a new form of coupling emerges to maintain dynamic stability, thus optimizing residual locomotor capacity. However, in quadrupeds, the changes that take place in the interlimb pattern with a 2:1 forelimb-hindlimb relationship in intact or injured systems are poorly understood.

As stated above, transverse split-belt locomotion in cats offers a substrate to evaluate adaptive changes when interlimb coordination is challenged in a dynamic task in an intact adult.
mammalian system. Although changes in cycle and phase durations during transverse split-belt locomotion have been well characterized (Akay et al. 2006; Cruse and Warnecke 1992; Thibaudier et al. 2013), the adjustments that take place in the interlimb pattern have not been investigated. To maintain dynamic stability during transverse split-belt locomotion with the forelimbs stepping faster or slower than the hindlimbs, there must be adjustments in spatial and temporal aspects of interlimb coordination. Therefore, the goal of the present study was to quantify spatiotemporal interlimb coordination during tied-belt (equal speed of the fore- and hindlimbs) and transverse split-belt (unequal speed of the fore- and hindlimbs) locomotion with 1:1 and 2:1 forelimb-hindlimb relationships. A loss of a 1:1 forelimb-hindlimb relationship could be indicative of a loss of interlimb coordination, or a new form of coupling could be established. Moreover, even with a 1:1 forelimb-hindlimb relationship, there could be no consistent phase interval values and no apparent coordinated behavior. We hypothesize that new forms of interlimb coordination, or coupling patterns, are established during transverse split-belt locomotion with the forelimbs or hindlimbs stepping slower or faster as a function of 1:1 or 2:1 forelimb-hindlimb relationships. The results provide additional information on the spatiotemporal coordination of the four limbs during locomotion and also serve as a basis to guide future studies assessing changes in the interlimb pattern following a variety of injuries to the sensorimotor system, as well as its control mechanisms.

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. Five adult cats (1 male, 4 females) weighing between 3.5 and 7.7 kg were used. Cats were trained to step on a transverse split-belt treadmill composed of four independent running surfaces 120 cm long and 30 cm wide (Bertec, Columbus, OH). In the present study, only the front and back surfaces on one side of the treadmill were used, which were separated by a 0.5-cm gap (Fig. 1A). An encasement composed of three panels held by a metal frame was used to train the animals to step with the forelimbs and hindlimbs on the front and rear belts, respectively. The two sides of the encasement were Plexiglas separators (120 cm long, 50 cm high) placed 30 cm from one another to prevent the animals from moving laterally, while the back of the encasement was closed by a wood plank. Thus the encasement was open at the top and at the front. Recording sessions started once animals could step comfortably with the fore- and hindlimbs on the front and rear belts, respectively, requiring approximately four additional weeks of training. After training, the animals performed bouts of transverse split-belt locomotion without contacting the sides of the encasement. Cats were given food and affection as reward during training and experimental sessions.

Fig. 1. Experimental setup, locomotor conditions, and types of coupling patterns. The experimental setup used in the present study is shown schematically in A. B: cats were trained to step with the forelimbs (FL) and hindlimbs (HL) on 2 separate belts in 4 different conditions: tied-belt conditions with fore- and hindlimbs stepping at 0.4 m/s (Tied4) or 0.8 m/s (Tied8) and split-belt conditions with forelimbs stepping at 0.8 m/s or 0.4 m/s and hindlimbs stepping at 0.4 m/s (8F4H) or 0.8 m/s (4F8H). C. in the 8F4H condition, 1:1 (8F4H-1:1) and 2:1 (8F4H-2:1) forelimb-hindlimb relationships could be observed within an episode. With a 2:1 forelimb-hindlimb relationship, the first (8F4H-2:1\textsuperscript{1st}) and second (8F4H-2:1\textsuperscript{2nd}) forelimb steps were divided for various measurements. LF, left forelimb; RF, right forelimb; LH, left hindlimb; RH, right hindlimb.
Experimental protocol. Five cats performed one session of several locomotor episodes in two tied-belt (equal speeds of the front and rear belts) and two transverse split-belt (unequal speeds of the front and rear belts) conditions (Fig. 1B). In the tied-belt conditions, the fore- and hindlimbs stepped at 0.4 m/s (Tied4 condition) or 0.8 m/s (Tied8 condition). In the transverse split-belt conditions, the forelimbs stepped at 0.8 m/s or 0.4 m/s with the hindlimbs stepping at 0.4 m/s (8F4H condition) or 0.8 m/s (4F8H condition), respectively. Each episode consisted of 6–15 cycles, and only steps where the animal had its forelimbs and hindlimbs on their respective belts were retained for analysis.

Data acquisition and analysis. Videos of the left and right sides were captured with two cameras (Basler AG) at 60 frames/s. A custom-made LabVIEW program acquired the images and synchronized the cameras. Analysis was done off-line. Cycle duration was measured from successive foot contacts. Stance duration corresponded to the interval of time from foot contact to the most caudal displacement of the toe relative to the hip (Halbertsma 1983), while swing duration was measured as cycle duration minus stance duration. Stride length was measured for the right forelimb and right hindlimb as the distance between contact and liftoff added to the distance traveled by the treadmill during the swing phase, obtained by multiplying swing duration by treadmill speed (Courtine et al. 2005; Goetz et al. 2012). Step length was measured as the distance between the leading and trailing homologous limbs at paw contact of the leading limb (Hoogkamer et al. 2014). The left and right limbs were the leading and trailing limbs, respectively.

Interlimb coordination was quantified temporally and spatially. Temporal interlimb coordination was evaluated by measuring support periods and the phase intervals between pairs of limbs. During a normalized cycle (from successive right hindlimb contacts), there are eight different epochs that can be determined based on the pattern of limb supports (footfall patterns) (Frigon et al. 2014; Gray 1968; Wetzel and Stuart 1976). Each support period was calculated for the group. Phase intervals were calculated by measuring the absolute amount of time between contacts of two limbs divided by the cycle duration of the reference limb (English 1979; English and Lennard 1982; Frigon et al. 2014; Orsal et al. 1990). These values were then multiplied by 360 and expressed in degrees to illustrate their continuity. Phase intervals were calculated by measuring the absolute difference between steps during an episode of locomotion.

As another measure of step-by-step variability for homolateral and diagonal coupling, the absolute deviation from the mean was measured for phase intervals by calculating the absolute difference between the phase interval value obtained in each cycle and the mean of the episode (El Amir 2012; Frigon et al. 2014). The mean absolute deviation was then calculated by averaging the values for all cycles during an episode.

Statistical analysis. Statistical tests were performed with IBM SPSS Statistics 20.0. For each parameter, a one-factor (condition) repeated-measures analysis of variance (ANOVA) was performed. Pairwise comparisons were then performed if there was a main effect of condition with no adjustments for multiple comparisons (Rothman 1990). Significance level was set at P < 0.05. The results in Figs. 3, 4, 5, 7, 9, and 10 are mean ± SD values from five cats. Rayleigh’s test was performed to determine whether the data points shown in the circular plots of Figs. 6 and 8 were randomly distributed (Kjaerulf and Kiehn 1996).

RESULTS

Footfall patterns and periods of support during tied-belt and transverse split-belt locomotion. Figure 2 shows the locomotor pattern in the four different conditions for a single cat. All panels are arranged in the same way, with the stance phases of the four limbs and limb support patterns shown below; black circles represent the limbs contacting the surface. The footfall pattern during tied-belt locomotion remained the same with a sequence of right hindlimb, right forelimb, left hindlimb, and left forelimb contacts (Fig. 2, A and B). This is termed a lateral sequence, where contact of the hindlimb is followed by contact of the homolateral hindlimb (Hildebrand 1967; Lemelin et al. 2003; Stevens 2006; Thibaudier et al. 2013). A lateral sequence was also observed during transverse split-belt locomotion in the 4F8H condition, where the hindlimbs stepped faster than the forelimbs (Fig. 2C). However, in the 8F4H condition, where forelimb speed was faster than hindlimb speed, the forelimbs could perform two steps within a hindlimb cycle, or a 2:1 forelimb-hindlimb relationship (Fig. 2D). As a result, in the 8F4H condition the sequence of events became more variable and the footfall pattern could change (see also Thibaudier et al. 2013).

A locomotor cycle normally contains eight distinct time phases, each characterized by the pattern of limbs that are supporting the body (reviewed in Gray 1968; Wetzel and Stuart 1976). Starting with right hindlimb contact, the number of limbs contacting the surface followed a sequence of 3–4–3–2–3–4–3–2 during tied-belt locomotion at 0.4 m/s (Fig. 2A). This type of sequence was found during tied-belt locomotion at
treadmill speeds of 0.4 – 0.8 m/s (Frigon et al. 2014). During tied-belt locomotion at 0.8 m/s (Fig. 2B) and in the 4F8H condition (Fig. 2C), there were sequences of 3-4-3-2-3-4-3-2 and 3-2-3-2-3-2-3-2. The 3-2-3-2-3-2-3-2 pattern was observed during tied-belt locomotion at treadmill speeds of 0.9 and 1.0 m/s (Frigon et al. 2014). In the 8F4H condition, there could be 8–12 distinct time phases within a hindlimb cycle because of the extra steps taken by the forelimbs (Fig. 2D). Despite the presence of a 2:1 forelimb-hindlimb relationship, the animal maintained a smooth stable locomotor pattern without stumbling.

Figure 3 shows normalized support periods in the different conditions across cats. To accurately evaluate the different patterns in the 8F4H condition, the cycles with a 2:1 forelimb-hindlimb relationship (8F4H-2:1) were analyzed separately from those with a 1:1 forelimb-hindlimb relationship (8F4H-1:1). In the 8F4H condition, all cats showed a mixture of 1:1 and 2:1 forelimb-hindlimb relationships. Of the 60 hindlimb cycles analyzed, 36 (60%) had a 2:1 forelimb-hindlimb relationship. Across cats, 60.9 ± 15.3% of cycles were classified as 2:1 forelimb-hindlimb relationships. The numbers above the panels in Fig. 3 indicate the number of limbs contacting the surface during the different time phases. Across cats, the sequence of limbs contacting the surface was 3-4-3-2-3-4-3-2 in the Tied4, Tied8, and 8F4H-1:1 conditions. Patterns of 3-2-3-2-3-2-3-2 and 3-4-3-2-3-4-3-2-3-2 were found in the 4F8H-1:1 and 8F4H-2:1 conditions, respectively. Therefore, the sequence of limbs contacting the ground is modifiable if it is the forelimbs or hindlimbs that are stepping slower or faster.

To provide a more precise evaluation of changes in support periods, the relative duration of each of the eight support periods was measured across cats in the different conditions with a 1:1 forelimb-hindlimb relationship (Fig. 4). In the 8F4H condition, only cycles with a 1:1 forelimb-hindlimb relationship were included in this analysis. There was a significant effect of condition on the duration of support periods 2, 3, 4, 6, 7, and 8. Support periods 2 and 6 were periods of quadruple support during tied-belt locomotion and in the 8F4H condition, whereas they were periods of diagonal double support in the 4F8H condition. Support periods 3 and 7 were periods of triple support with both forelimbs contacting the surface along with one hindlimb. Support periods 4 and 8 were periods of
homolateral double support. Figure 4 shows that when the hindlimbs stepped faster than the forelimbs (4F8H condition), the cycle was reorganized by replacing periods of quadruple support with periods of diagonal support (Fig. 4, B and F), by increasing periods of triple support where the two forelimbs were contacting the surface (Fig. 4, C and G), and by reducing periods of homolateral double support (Fig. 4, D and H). When the forelimbs stepped faster than the hindlimbs (8F4H condition) with a 1:1 forelimb-hindlimb relationship, the cycle was reorganized primarily by increasing one period of quadruple support (Fig. 4F), by reducing periods of triple support where the two forelimbs were contacting the surface (Fig. 4, C and G), and by increasing the period of homolateral support on one side (Fig. 4H).

Stride and step lengths during tied-belt and transverse split-belt locomotion. To quantify spatial adjustments in the locomotor pattern in the different conditions, stride and step lengths were measured. The main comparisons of interest here were to determine whether forelimb and hindlimb stride lengths and step lengths were different in the transverse split-belt conditions from what would be expected based on the speed of the treadmill (values obtained during tied-belt locomotion). As such, the 4F8H and 8F4H conditions were compared with the Tied4 and Tied8 conditions for the forelimbs, respectively, whereas the 8F4H and 4F8H conditions were compared with the Tied4 and Tied8 conditions for the hindlimbs, respectively. In the 8F4H condition, cycles with a 1:1 forelimb-hindlimb relationship (8F4H-1:1) were separated from the cycles with a 2:1 forelimb-hindlimb relationship where the first forelimb step (8F4H-2:11st) was separated from the second forelimb step (8F4H-2:12nd) (see Fig. 1C).

Stride length measures the horizontal distance traveled by a limb between successive contacts. On a treadmill, because the animal remains in place relative to the surface, the distance traveled by the treadmill during the swing phase was added (see MATERIALS AND METHODS). Stride length was measured for the right forelimb and right hindlimb. Forelimb stride length in the 4F8H condition (29.9 ± 1.9 cm) was significantly shorter than in the Tied4 condition (34.3 ± 2.0 cm) (Fig. 5A). When compared with the Tied8 condition, forelimb stride length was significantly shorter by 6.5 cm (−14%) and 4 cm (−9%) for the first and second steps, respectively, with a 2:1 coupling pattern (8F4H-2:1). Hindlimb stride length in the 4F8H condition (60.3 ± 2.4 cm) was significantly longer than in the Tied8 condition (46.4 ±

Fig. 3. Footfall patterns and periods of support during tied-belt and transverse split-belt locomotion across animals. All panels are organized in the same way, with horizontal bars representing the average ± SD stance phase duration of the 4 limbs in the 4 different conditions. In the 8F4H condition, cycles with a 1:1 forelimb-hindlimb relationship (8F4H-1:1; D) were separated from cycles with a 2:1 forelimb-hindlimb relationship (8F4H-2:1; E). Stance durations were normalized to right hindlimb contact. Intervals between vertical lines are used to identify support periods. Numbers above the panels indicate the number of limbs contacting the treadmill surface during each support period. Horizontal bars are the average ± SD from 5 cats.
2.6 cm) (Fig. 5B). From these results, it is clear that forelimb and hindlimb stride lengths are not solely determined by treadmill speed. Altogether, the hindlimbs take longer strides when the forelimbs take shorter strides. This phenomenon can be seen in the 8F4H-2:1 and 4F8H conditions.

Step length is the distance between the leading and trailing homologous limbs at paw contact of the leading limb. Forelimb step length in the 4F8H condition (13.5 ± 2.7 cm) was significantly shorter than in the Tied4 condition (16.2 ± 1.5 cm) (Fig. 5C). Moreover, forelimb step length in the second

Fig. 4. Modulation of support periods during tied-belt and transverse split-belt locomotion across animals. Each panel shows 1 of the 8 support periods in the 4 conditions with a 1:1 forelimb-hindlimb relationship. The limbs contacting the surface are shown in black in the footfall diagram at top of each panel. Each vertical bar is the average ± SD from 5 cats. Note that support periods 2 (B) and 6 (F) have black and gray bars representing periods of quadruple and double support, respectively. When 1 condition was significantly different from 2 or more other conditions, the comparison starts with the longer and thicker vertical line. For support periods 2 and 6, quadrupedal support periods were not compared with diagonal support periods. *Significant differences between conditions (P < 0.05, pairwise comparisons).

Fig. 5. Modulation of stride and step lengths during transverse split-belt locomotion compared with tied-belt locomotion across animals. Panels show stride lengths for right forelimb (A) and right hindlimb (B) as well as step lengths measured for forelimbs (C) and hindlimbs (D) in the 4 conditions. In the 8F4H condition, cycles with a 1:1 forelimb-hindlimb relationship (8F4H-1:1) were separated from those with a 2:1 forelimb-hindlimb relationship (8F4H-2:1). Moreover, with a 2:1 forelimb-hindlimb relationship, the first forelimb cycle (8F4H-2:11st) was separated from the second forelimb cycle (8F4H-2:12nd). Each vertical bar is the average ± SD of 1 session from 5 cats. When 1 condition was significantly different from 2 or more other conditions, the comparison starts with the longer and thicker vertical line. *Significant differences between conditions (P < 0.05, pairwise comparisons).
step of the 8F4H condition (19.8 ± 2.6 cm) was significantly shorter than in the Tied8 condition (21.5 ± 2.0 cm) (Fig. 5C). The results indicate that the forelimbs adjusted mostly by reducing the distance between the two limbs at contact in order to maintain a 1:1 forelimb-hindlimb relationship or to allow sufficient time to perform two steps during one step of the hindlimbs. On the other hand, hindlimb step length in the 4F8H condition (28.6 ± 2.1 cm) was significantly longer than in the Tied8 condition (22.7 ± 1.5 cm) (Fig. 5D). These results indicate that the hindlimbs adjust primarily by increasing their distance from one another at contact.

Bilateral coordination during tied-belt and transverse split-belt locomotion. To quantify temporal bilateral coordination, phase intervals between homologous limbs were measured in the four conditions. For temporal phasing, values of 0° or 360° indicate a strict in-phase coupling while a value of 180° indicates a strict out-of-phase coupling. Previous studies have used values between 270° and 90° to denote an in-phase coupling and values between 90° and 270° for out-of-phase coupling (English and Lennard 1982). Spatial phasing between homologous limbs was measured in the four conditions by expressing step length as a function of stride length. For spatial phasing, values of 0° and 360° indicate that the two limbs contact the surface at the same point on the horizontal axis while a value of 180° indicates that the two limbs contact the surface at a distance corresponding to half of the stride length. Figure 6 shows temporal and spatial phasing for homologous limbs in a single cat, where each data point represents a locomotor cycle. In all conditions, Rayleigh’s test was significant, with r values ranging from 0.79 to 0.99, indicating that phase and gap intervals were not randomly distributed. In the tied-belt conditions and in the 4F8H condition, temporal and spatial phasing values between forelimbs (Fig. 6, A and B) and between hindlimbs (Fig. 6, C and D) were clustered around 180°, indicating a strict out-of-phase coupling. The phasing was slightly more variable on a step-by-step basis in the Tied4 condition. In the 8F4H condition, the temporal phasing between forelimbs was tightly clustered around 180° (Fig. 6A) while the spatial phasing between forelimbs was more variable, with values dispersed between 128° and 220° (Fig. 6B). The temporal (Fig. 6C) and spatial (Fig. 6D) phasing between hindlimbs were considerably more variable, with values dispersed between 128° and 220° and between 125° and 237°, respectively. This shows that the hindlimbs adjust their temporal and spatial coordination more than the forelimbs on a step-by-step basis with a 2:1 forelimb-hindlimb relationship.

Fig. 6. Temporal and spatial coordination of homologous pairs of limbs during tied-belt and transverse split-belt locomotion in one cat. Phase (i.e., interval of time between contacts) and gap (i.e., step length divided by stride length) intervals were calculated for homologous forelimbs and hindlimbs to quantify temporal and spatial coordination, respectively. Phase and gap interval values were then normalized to cycle duration and stride length of the reference limbs (first limb used in calculation), respectively, and multiplied by 360. In the circular plots, phase and gap intervals are expressed in degrees around the circumference while cycle durations (A and C) or stride length (B and D) are plotted in radii. Each data point represents a locomotor cycle. The limb pairs in each panel are shown in black in the cat diagram at top right. To measure temporal and spatial phasing, cycles with a 1:1 forelimb-hindlimb relationship were separated from those with a 2:1 forelimb-hindlimb relationship. With a 2:1 forelimb-hindlimb relationship, the first forelimb step (2:1–1st step) was separated from the second forelimb step (2:1–2nd step).
To evaluate the accuracy and consistency of bilateral coordination (i.e., between homologous limbs) for the group, PCI and GCI were measured (see MATERIALS AND METHODS). Larger PCI and GCI values reflect a less accurate and more variable step-by-step temporal and spatial coordination, respectively. In the forelimbs, there were no significant differences between conditions for PCI (Fig. 7A) and significant differences in GCI only between the Tied8 condition (6.04 ± 2.18) and the 4F8H condition (13.91 ± 3.29) and 8F4H:2:1 condition (9.33 ± 2.62) conditions (Fig. 7B). In the hindlimbs, PCI and GCI values were significantly larger in the 8F4H-2:1 condition compared with the other conditions (Fig. 7, C and D). Thus the bilateral coordination between hindlimbs was less accurate and more variable with a 2:1 forelimb-hindlimb relationship.

Coordination between fore- and hindlimbs during tied-belt and transverse split-belt locomotion. As expected, the coordination between homolateral limbs and between diagonal limbs was considerably altered during transverse split-belt locomotion. To determine whether the appearance of a 2:1 forelimb-hindlimb relationship represented a loss of intergirdle coordination or the establishment of a new form of coordination, it was important in the 8F4H condition to separate cycles with 1:1 and 2:1 forelimb-hindlimb relationships and to separately evaluate the phasing for the first and second steps with a 2:1 forelimb-hindlimb relationship.

**Fig. 7.** Phase (PCI) and gap (GCI) coordination indexes of homologous limbs pairs during tied-belt and transverse split-belt locomotion across animals. PCI and GCI values are shown for the forelimbs (A and B) and the hindlimbs (C and D) in the 4 different conditions. In the 8F4H condition, cycles with a 1:1 forelimb-hindlimb relationship (8F4H-1:1) were separated from those with a 2:1 forelimb-hindlimb relationship (8F4H-2:1). Moreover, with a 2:1 forelimb-hindlimb relationship, the first forelimb cycle (8F4H-2:1-1st) was separated from the second forelimb cycle (8F4H-2:1-2nd). Each vertical bar is the average ± SD of 1 session from 5 cats. When 1 condition was significantly different from 2 or more other conditions, the comparison starts with the longer and thicker vertical line. *Significant differences between conditions (P < 0.05, pairwise comparisons).

DISCUSSION

The present study quantified spatiotemporal adjustments in interlimb coordination during tied-belt and transverse split-belt locomotion in intact adult cats with 1:1 and 2:1 forelimb-hindlimb relationships. The results showed reorganization of the support pattern, as well as both spatial and temporal coordination during transverse split-belt locomotion where the...
forelimbs or hindlimbs were stepping slower or faster. These adjustments allowed the animals to maintain dynamic stability, as no stumbling was observed, even with a 2:1 forelimb-hindlimb relationship where a new form of interlimb coordination, or coupling pattern, was established. The insight obtained from these findings pertaining to the control of interlimb coordination during locomotion is discussed in the following sections.

**Modulation of limb support patterns.** Early gait studies proposed that the relative duration of the eight support periods within a normalized cycle were equal (Gray 1968). However, it was later shown that the duration of support periods was greatly influenced by speed and by the pattern of locomotion (Wetzel and Stuart 1976). In the present study, quadruple support decreased while double support increased with an increase in speed during tied-belt locomotion, as shown previously (Frigon et al. 2014). In a recent study, Frigon et al. (2014) showed sequences of 3-4-3-2-3-4-3-2 and 3-4-3-2-3-2-3-2 during tied-belt locomotion at 0.4 m/s and 0.8 m/s, respectively. Interestingly, during transverse split-belt locomotion with the hindlimbs stepping faster than the forelimbs (4F8H condition), there was a consistent sequence of 3-2-3-2-3-2-3-2, a pattern observed during tied-belt locomotion at speeds of 0.9–1.0 m/s (Frigon et al. 2014). Thus the sequence of limb supports did not adjust by taking a sequence expected at 0.4 m/s or 0.8 m/s. Instead, it switched to a sequence normally found at higher speeds. An analysis of changes in the relative duration of individual support periods showed that periods of triple support where the two forelimbs were contacting the surface increased in the 4F8H condition (Fig. 4, C and G). When the forelimbs stepped faster than the hindlimbs (8F4H condition), the sequence of limb support patterns across cats was 3-4-3-2-3-4-3-2 when there was a 1:1 coupling pattern (Fig. 3). Therefore, when there is a 1:1 coupling pattern, the adjustment in the sequence of limb support patterns can be differentially regulated if it is the forelimbs or hindlimbs that are stepping faster or slower. Differential or asymmetric adjustments were also observed in phase durations during trans-
verse split-belt locomotion with the forelimbs stepping slower or faster than the hindlimbs (Thibaudier et al. 2013). Reorganization of the support pattern was also observed after a dorsolateral or ventrolateral lesion at T_{10}-T_{11}, with a switch from a 3-2-3-2-3-2-3-2 to a 3-4-3-2-3-4-3-2 sequence in cats (Gorska et al. 1996). Therefore, limb support patterns can flexibly adjust when interlimb coordination is challenged.

**Spatiotemporal control of interlimb coordination.** Plotnik et al. (2007) introduced the PCI to measure the accuracy and consistency of temporal interlimb coordination during locomotion. This measure has been used in clinical studies to evaluate the accuracy and consistency of temporal interlimb coordination during locomotion in intact cats and dogs, no difference between conditions (Plotnik et al. 2013), where changes in stride length to adapt to speed are negligible. Although changes in hindlimb PCI and GCI values showed similar directional changes (increase or decrease) in different conditions, GCI values were significantly larger in the 8F4H condition, cycles with a 1:1 forelimb-hindlimb relationship (8F4H-1:1) were separated from those with a 2:1 forelimb-hindlimb relationship (8F4H-2:1). With a 2:1 forelimb-hindlimb relationship, the first forelimb cycle (8F4H-2:1<sup>1st</sup>) was separated from the second forelimb cycle (8F4H-2:1<sup>2nd</sup>). Each vertical bar is the average ± SD from 5 cats. When 1 condition was significantly different from 2 or more other conditions, the comparison starts with the longer and thicker vertical line. *Significant differences between conditions (P < 0.05, pairwise comparisons).

**Fig. 9.** Mean phase intervals for homolateral and diagonal limb pairs during tied-belt and transverse split-belt locomotion across animals. The mean phase interval between limb contacts is shown for homolateral (A) and diagonal (B) limb pairs in the 4 different conditions. In the 8F4H condition, cycles with a 1:1 forelimb-hindlimb relationship (8F4H-1:1) were separated from those with a 2:1 forelimb-hindlimb relationship (8F4H-2:1). With a 2:1 forelimb-hindlimb relationship, the first forelimb cycle (8F4H-2:1<sup>1st</sup>) was separated from the second forelimb cycle (8F4H-2:1<sup>2nd</sup>). Each vertical bar is the average ± SD from 5 cats. When 1 condition was significantly different from 2 or more other conditions, the comparison starts with the longer and thicker vertical line. *Significant differences between conditions (P < 0.05, pairwise comparisons).

**Fig. 10.** Absolute deviation of phase intervals for homolateral and diagonal limb pairs during tied-belt and transverse split-belt locomotion across animals. The absolute deviation of the phase interval obtained during a cycle and the mean phase interval of the episode. In the 8F4H condition, cycles with a 1:1 forelimb-hindlimb relationship (8F4H-1:1) were separated from those with a 2:1 forelimb-hindlimb relationship (8F4H-2:1). With a 2:1 forelimb-hindlimb relationship, the first forelimb cycle (8F4H-2:1<sup>1st</sup>) was separated from the second forelimb cycle (8F4H-2:1<sup>2nd</sup>). Each vertical bar is the average ± SD from 5 cats. When 1 condition was significantly different from 2 or more other conditions, the comparison starts with the longer and thicker vertical line. *Significant differences between conditions (P < 0.05, pairwise comparisons).
temporal and spatial aspects of bilateral coordination was observed during symmetric gaits (Abourachid et al. 2007; Maes et al. 2008). However, during obstacle avoidance, a mismatch between temporal and spatial bilateral coordination was reported (Abourachid et al. 2007; Maes et al. 2008). Thus temporal and spatial coordination can change independently of one another when interlimb coordination is challenged (e.g., transverse split-belt locomotion, obstacle avoidance).

An elegant series of experiments showed dissociation between temporal and spatial measures when adapting to left-right split-belt locomotion in healthy adults and children (Malone et al. 2012; Malone and Bastian 2010; Vasudevan et al. 2011), and it was proposed that temporal and spatial features of bilateral coordination could be controlled by different anatomical structures. For instance, spatial coordination is thought to require conscious efforts as opposed to temporal coordination, which is thought to develop at an earlier stage in life compared with spatial adaptation. As such, it was proposed that spatial coordination is controlled by cerebellar interactions with cerebral structures while temporal coordination is mediated at lower levels involving the cerebellum, brain stem, and spinal cord (Malone et al. 2012).

A new form of interlimb coordination. During transverse split-belt locomotion with the forelimbs stepping faster than the hindlimbs, a 2:1 forelimb-hindlimb relationship was often observed. However, when this occurred, a new form of interlimb coordination, or 2:1 coupling pattern, was established (Fig. 8). In other words, the phasing for the first and second forelimb steps was observed around certain values and not randomly distributed. Dissociation of arm and leg rhythms also occurs in humans walking at slow speeds (<2.7 km/h) (Craig et al. 1976; Donker et al. 2001; Webb et al. 1994). Moreover, it was proposed that coordination between arm and leg movements is characterized not only by specific forms of frequency locking (1:1 vs. 2:1 coupling) but also by specific forms of phase locking, defined by phase intervals clustered around specific values (Donker et al. 2001). Indeed, with a 2:1 coupling pattern between the arms and legs, the arm swings both in phase and out of phase with the homolateral leg (Craig et al. 1976). In the present study, the coordination was also phase locked during 2:1 coupling, with the forelimb contacting the surface in phase or out of phase with the homolateral hindlimb (Fig. 8). Previous studies also showed that a 2:1 coupling pattern between the arms and leg (Donker et al. 2001; Serrien and Swinnen 1997) or between the two arms (Vasudevan and Zehr 2011) was less stable than a 1:1 pattern. In a similar vein, the absolute deviation of phase intervals of homolateral and diagonal coupling was two times higher with a 2:1 coupling pattern in the 8F4H condition (Fig. 10). These different forms of coupling between the arms/hindlimbs and legs/hindlimbs could be useful to provide independent control of the arms/hindlimbs while maintaining dynamic stability.

Several studies have also shown 2:1, 3:1, and 4:1 relationships between the left and right sides on a split-belt treadmill that can independently control the speed and/or direction of the left and right sides (Choi and Bastian 2007; Forsberg et al. 1980; Frigon et al. 2013; Halbertsma 1983; Kulagin and Shik 1970; Thelen et al. 1987; Yang et al. 2005). Yang et al. (2005) showed in human infants that, although the relationship between the left and right legs during split-belt locomotion was 2:1 or 3:1, phase intervals were maintained around certain values and were not randomly distributed. Although not quantified, similar results have been observed during split-belt locomotion in chronic spinalized cats (Forsberg et al. 1980), indicating that such interlimb coordination can be mediated entirely at the level of the spinal cord. At present, it is unclear whether new forms of coordination between the forelimbs and hindlimbs during transverse split-belt locomotion are mediated primarily at the level of the spinal cord or whether supraspinal signals are also involved. In turtles with a spinal transection at the first cervical vertebra, stimulation of the dorsolateral funiculus could produce a 2:1 forelimb-hindlimb relationship during swimming movements (Stein 1978). When a 2:1 forelimb-hindlimb relationship was observed, protraction of the hindlimb coincided with every second retraction of the forelimb, indicating that forelimb and hindlimb movements were phase locked or coupled.

2:1 Coupling patterns during locomotion after incomplete spinal cord injuries. Several studies have reported the emergence of a 2:1 forelimb-hindlimb relationship during locomotion after incomplete spinal lesions. Barriere et al. (2010) observed a 2:1 forelimb-hindlimb relationship following a lateral thoracic hemisection only in some cats, particularly those with the largest lesions. Similar results have been reported in cats after ventral/ventrolateral (Brustein and Rossignol 1998) or dorsal/dorsolateral (Gorska et al. 1996; Jiang and Drew 1996) thoracic lesions. Thus disrupting ventral or dorsal spinal pathways can lead to a 2:1 forelimb-hindlimb relationship. It could be that disrupting propriospinal pathways that project to and from forelimb and hindlimb spinal locomotor networks are responsible for the appearance of a 2:1 forelimb-hindlimb relationship. However, a 2:1 forelimb-hindlimb relationship can also occur after an incomplete spinal cord injury at cervical levels (Cote et al. 2012), which spares propriospinal pathways that connect cervical and lumbar regions. Therefore, it does not appear that any one pathway is crucial in maintaining a 1:1 forelimb-hindlimb relationship, and several different pathways, originating supraspinally or contained within the spinal cord, could be involved.

In locomotor performance scales, the appearance of a 2:1 forelimb-hindlimb relationship is generally taken as evidence of a loss of coordination between the fore- and hindlimbs. For instance, the most widely used scale is the Basso, Beattie, Bresnahan (BBB) scale (Basso et al. 1995). In this scale, a score of 12 (out of 21) is defined by “plantar stepping with frequent to consistent weight bearing and occasional forelimb-hindlimb coordination.” A 2:1 forelimb-hindlimb relationship is categorized as occasional forelimb-hindlimb coordination. However, as shown in the present study, a 2:1 forelimb-hindlimb relationship is not necessarily indicative of a loss of coordination between the forelimbs and hindlimbs. Instead, a new form of coordination or coupling pattern can be established. It is possible that new coupling patterns are also established after incomplete spinal lesions in animal models. For instance, Gorska et al. (1996) observed a 2:1 forelimb-hindlimb relationship in cats with a bilateral dorsolateral lesion of the spinal cord. When this occurred, hindlimb cycle duration increased and forelimb cycle duration decreased, as the forelimbs could take two steps within one hindlimb cycle. This is similar to what occurs during transverse split-belt locomotion with the forelimbs stepping faster than the hindlimbs in cats with intact spinal cords (Akay et al. 2006; Thibaudier et al. 2016 INTERLIMB COORDINATION IN TRANSVERSE SPLIT-BELT LOCOMOTION

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2013). It is recommended that performance scales incorporate more rigorous analyses of interlimb coordination, such as phase and gap intervals, in their assessment of interlimb coordination to determine whether new forms of interlimb coordination or coupling patterns are established after incomplete spinal lesions.

Concluding remarks. In summary, the step cycle is reorganized and interlimb coordination adjusts during transverse split-belt locomotion in intact adult cats. However, the adjustments to transverse split-belt locomotion are differentially modulated if the forelimbs step faster or slower than the hindlimbs, indicating that the system(s) coordinating the fore- and hindlimbs is organized asymmetrically, as previously proposed (Thibaudier et al. 2013; Thibaudier and Hurteau 2012). An asymmetric organization in the system(s) coordinating the forelimbs/arms and hindlimbs/legs has also been proposed based on experimental evidence in the in vitro neonatal rat preparation (Juvin et al. 2012) and in humans during combined arm and leg cycling (Sakamoto et al. 2014). In addition, the present results showed that the appearance of a 2:1 forelimb-hindlimb relationship was not evidence of a loss of coordination between the forelimbs and hindlimbs. Instead, a new form of coordination was established. It is possible that this new 2:1 coupling pattern is also present after incomplete spinal lesions in animal models. A better understanding of interlimb coordination before and after spinal cord injury or with other movement disorders could promote therapeutic strategies aimed at strengthening interlimb coordination, thus facilitating locomotor recovery.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: Y.T. and A.F. conception and design of research; Y.T. performed experiments; Y.T. analyzed data; Y.T. and A.F. interpreted results of experiments; Y.T. and A.F. prepared figures; Y.T. and A.F. drafted manuscript; Y.T. and A.F. edited and revised manuscript; Y.T. and A.F. approved final version of manuscript.

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