

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/361982106>

Left-Right Locomotor Coordination in Human Neonates

Article in *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* · July 2022

DOI: 10.1523/JNEUROSCI.0612-22.2022

CITATIONS

0

READS

87

10 authors, including:



Arthur Dewolf

Université Catholique de Louvain - UCLouvain

41 PUBLICATIONS 250 CITATIONS

[SEE PROFILE](#)



Valentina la scaleia

Foundation Santa Lucia

13 PUBLICATIONS 647 CITATIONS

[SEE PROFILE](#)



Francesca Sylos Labini

Foundation Santa Lucia

41 PUBLICATIONS 1,178 CITATIONS

[SEE PROFILE](#)



Vito Mondì

Policlinico Casilino, Rome, Italy

22 PUBLICATIONS 690 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Early development of human locomotion [View project](#)



MindWalker [View project](#)

Left–Right Locomotor Coordination in Human Neonates

Arthur H. Dewolf,¹ Valentina La Scaleia,² Adele Fabiano,^{3,4} Francesca Sylos-Labini,² Vito Mondì,³ Simonetta Picone,³ Ambrogio Di Paolo,⁴ Piermichele Paolillo,³ Yuri Ivanenko,² and Francesco Lacquaniti^{1,2}

¹Department of Systems Medicine and Center of Space Biomedicine, University of Rome Tor Vergata, 00133 Rome, Italy, ²Laboratory of Neuromotor Physiology, Istituto di Ricovero e Cura a Carattere Scientifico Santa Lucia Foundation, 00179 Rome, Italy, ³Neonatology and Neonatal Intensive Care Unit, Casilino Hospital, 00169 Rome, Italy, and ⁴Neonatology and Neonatal Intensive Care Unit, Ospedale San Giovanni, 00184 Rome, Italy

Terrestrial locomotion requires coordinated bilateral activation of limb muscles, with left–right alternation in walking or running, and synchronous activation in hopping or skipping. The neural mechanisms involved in interlimb coordination at birth are well known in different mammalian species, but less so in humans. Here, 46 neonates (of either sex) performed bilateral and unilateral stepping with one leg blocked in different positions. By recording EMG activities of lower-limb muscles, we observed episodes of left–right alternating or synchronous coordination. In most cases, the frequency of EMG oscillations during sequences of consecutive steps was approximately similar between the two sides, but in some cases it was considerably different, with episodes of 2:1 interlimb coordination and episodes of activity deletions on the blocked side. Hip position of the blocked limb significantly affected ipsilateral, but not contralateral, muscle activities. Thus, hip extension backward engaged hip flexor muscle, and hip flexion engaged hip extensors. Moreover, the sudden release of the blocked limb in the posterior position elicited the immediate initiation of the swing phase of the limb, with hip flexion and a burst of an ankle flexor muscle. Extensor muscles showed load responses at midstance. The variable interlimb coordination and its incomplete sensory modulation suggest that the neonatal locomotor networks do not operate in the same manner as in mature locomotion, also because of the limited cortical control at birth. These neonatal mechanisms share many properties with spinal mammalian preparations (i.e., independent pattern generators for each limb, and for flexor and extensor muscles, load, and hip position feedback).

Key words: early development; human locomotion; interlimb coordination; neonatal stepping

Significance Statement

Bilateral coupling and reciprocal activation of flexor and extensor burst generators represent the fundamental mechanisms used by mammalian limbed locomotion. Considerable progress has been made in deciphering the early development of the spinal networks and left–right coordination in different mammals, but less is known about human newborns. We compared bilateral and unilateral stepping in human neonates, where cortical control is still underdeveloped. We found neonatal mechanisms that share many properties with spinal mammalian preparations (i.e., independent pattern generators for each limb, the independent generators for flexor and extensor muscles, load, and hip-position feedback). The variable interlimb coordination and its incomplete sensory modulation suggest that the human neonatal locomotor networks do not operate in the same manner as in mature locomotion.

Introduction

Limb locomotion requires coordinated bilateral muscle activation, with left–right alternation in walking or running, and synchronous activation in hopping or skipping. The rhythm and pattern of muscle contractions are determined by spinal interneuronal networks controlling motoneuron activity (Kiehn, 2016; Grillner and El Manira, 2020; Maxwell and Soteropoulos, 2020; Rancic and Gosgnach, 2021). These spinal networks include ipsilaterally projecting interneurons for intralimb muscle coordination and contralaterally projecting commissural interneurons for interlimb coordination. In adults, the strength of the spinal connections is constantly adjusted by intrinsic changes in spinal

Received Mar. 28, 2022; revised May 13, 2022; accepted May 28, 2022.

Author contributions: V.L.S., Y.I., and F.L. designed research; A.H.D., V.L.S., A.F., F.S.-L., V.M., S.P., A.D.P., P.P., Y.I., and F.L. performed research; A.H.D., V.L.S., and Y.I. analyzed data; A.H.D. and F.L. wrote the paper.

This work was supported by the Italian Ministry of Health (Ricerca Corrente, Istituto di Ricovero e Cura a Carattere Scientifico Fondazione Santa Lucia, Ricerca Finalizzata Grant RF-2019-12370232), the Italian Space Agency (Grants I/006/06/0 and 2019-11-U.O.), and the Italian University Ministry (Progetti di Ricerca di Interesse Nazionale Grants 2017CBF8NJ_005 and 2020EM9A8X_003).

The authors declare no competing financial interests.

Correspondence should be addressed to Arthur H. Dewolf at arthur.dewolf@uclouvain.be or Francesco Lacquaniti at francesco.lacquaniti@uniroma2.it.

<https://doi.org/10.1523/JNEUROSCI.0612-22.2022>

Copyright © 2022 the authors

excitability, sensory feedback, and supraspinal signals, allowing animals to adapt stance-to-swing transition and limbs kinematics to speed and direction changes or external perturbations (Butt et al., 2002; Pearson, 2004; Rossignol, 2006; Grillner and El Manira, 2020; Frigon et al., 2021).

How does interlimb coordination develop? Considerable progress has been made in deciphering the underpinnings of early development of the spinal networks in different mammalian species (Branchereau et al., 2000; Clarac et al., 2004; Kudo et al., 2004; Goulding, 2009; Arber, 2012; Bernhardt et al., 2013). Bilateral rhythmic bursting patterns of motoneurons are revealed in embryonic and fetal rodent preparations, progressively evolving from mainly synchronous to mainly alternating patterns between the limbs (Bernhardt et al., 2013). Kittens spinalized soon after birth adjust hindlimb locomotion so that the left and right hindlimbs can step at different speeds on a split-belt treadmill (Forssberg et al., 1980). Spinal cords isolated from neonatal rodents produce left–right alternating neuronal activity on bath application of neurotransmitters (Kudo and Yamada, 1987; Kremer and Lev-Tov, 1998; Jiang et al., 1999; Hägglund et al., 2013). Importantly, locomotor-like rhythmic bursting can be induced unilaterally (Kudo and Yamada, 1987; Hägglund et al., 2013) and independently in flexor or extensor networks (Hägglund et al., 2013), consistent with a distributed, modular organization of central pattern generators (Grillner, 1985; Kiehn, 2016).

As for the development of left–right coordination in humans, it is hypothesized that it shares many similarities with that of other mammals (Forssberg, 1992; Yang et al., 2004; Lacquaniti et al., 2012). Human fetuses exhibit a rich repertoire of leg movements, including single-leg or double-leg kicks, and interlimb alternation with variable phase (Einspieler et al., 2021). Neonatal kicking movements exhibit both synchronous and alternating left–right coordination (Thelen and Fisher, 1982; Piek, 2002). In addition, newborn infants can step on the ground if supported (Forssberg, 1985; Yang et al., 1998b; Dominici et al., 2011), also demonstrating episodes of synchronous or alternating left–right coordination (Thelen and Fisher, 1982; Siekerman et al., 2015).

Although the basic spinal infrastructure of locomotor control probably is similar across mammals and other vertebrates (Grillner and El Manira, 2020), cortical control plays a much more significant role in human adult locomotion than in other animals (Nielsen, 2003; Yang and Gorassini, 2006). Accordingly, the neural substrates of left–right coordination may be difficult to disentangle when cortical signals start overruling other signals during development (Ritterband-Rosenbaum et al., 2017). In this respect, newborns represent an ideal model system to study the role of subcortical and sensory contributions, with very limited contribution of cortical control. Neonate stepping mainly reflects spinal and brainstem control, as shown by stepping anencephalic neonates (Peiper, 1961). During normal development, the corticospinal system (CST) reaches the lumbosacral cord at 29 gestational weeks, but the pattern of motoneuron innervation and conduction velocities of CST start converging toward mature values only ~6 months after birth, and CST myelination is completed at ~1–2 years of age (Eyre, 2007; Ten Donkelaar et al., 2014; Williams et al., 2017).

Here we describe a series of experiments in neonates performing both bilateral and unilateral stepping. Unilateral stepping was obtained by holding one lower limb stationary in different positions, while the other limb stepped on the treadmill belt. By comparing bilateral and unilateral stepping, we aimed at uncovering the potential role of unilateral rhythm generators, as

well as the interlimb coordination that mainly depends on subcortical mechanisms and sensory inputs.

Materials and Methods

Participants

We studied stepping responses in 46 full-term neonates, 25 females, and 21 males, 1–20 d of age (median age, 2 d postpartum; interquartile range, 6 d), 50.7 ± 2.2 cm body (crown-to-heel) length, and 3.24 ± 0.42 kg in weight. Their Apgar (1953) score (a clinical index ranging from 0 to 10 for the evaluation of the newborn based on five items: heart rate, respiration, muscle tone, reflex reaction, and skin color) was >7 at 1 and 5 min. All neonates had an uneventful delivery and perinatal history. These children had been preselected by the neonatologist a few minutes before the recording as those able to perform some stepping movements. The experiments were performed at the well infant maternity ward of the Casilino Hospital and San Giovanni Hospital. The experimental procedures described below, conducted in accordance with the World Medical Association Declaration of Helsinki for medical research involving human subjects, were approved by the Research Ethics Committees of ASL Roma C (protocol CEI/15843 study no. 609, and protocol 27593 study no. 38.15) and the Santa Lucia Foundation (protocol CE-PROG.273–22). Both parents (when available) provided informed consent to participate in the study after the nature of the study was explained, and one parent always provided written informed consent. Deidentified data from the participants were used in all analyses and figures.

Experimental design

Barefoot neonates stepped on a treadmill, with the belt moving at 0.05 m/s. We used a motorized pediatric treadmill (Carlin's Creations), with a walkable surface of 51×31 cm (length $\times$ width). Limb kinematics was recorded at 100 Hz by means of a motion capture system (SIMI) with three video cameras (resolution, 640×480 pixels) positioned around the treadmill. Reflective markers (diameter, 9 mm) were attached on each side of the child to the skin overlying the following landmarks: greater trochanter (GT), lateral femur epicondyle, lateral malleolus (LM), and fifth metatarso-phalangeal joint (VM). EMG activity was recorded by means of surface electrodes from the following muscles from each side of the body: biceps femoris (BF), tibialis anterior (TA), rectus femoris (RF), and gastrocnemius lateralis (LG). Because of the small size of these muscles in neonates and the possibility of local electrical cross talk, we could not determine the specific compartment from which we were recording. All EMGs were recorded at 2000 Hz using the wireless Zerowire system (Aurion), with a bandwidth of 20–1000 Hz. To minimize cross talk, we used miniature (recording diameter, 2 mm), reusable Ag-AgCl surface EMG disk electrodes (Beckman Instruments). To minimize movement artifacts, preamplified EMG sensor units were attached at the wrist of the neonatologist holding the child, and twisted pairs of wires ~25 cm length (between electrodes and units) were fixed along the neonate's limb using elastic gauze. The sampling of kinematic and EMG data was synchronized.

After the markers and the EMG electrodes had been positioned, the neonates were held upright under the arms by the neonatologist, with the feet soles touching the treadmill surface. The neonatologist slightly tilted the child forward to facilitate the stepping response and supported the minimum possible amount of the child weight. To study unilateral stepping, while the child was stepping on the treadmill, his/her left limb was suddenly blocked by an experimenter (different from the person holding the child) in a middle (approximately vertical), anterior (hip flexion), or posterior (hip extension) position. To this end, the experimenter held the left foot of the child in the hand, gently but firmly (Fig. 1A). After a variable amount of time of unilateral stepping, the blocked limb was suddenly released. Because of the erratic nature of neonatal stepping, we could not control the position or phase of the stepping limb at the time of release of the blocked limb. The neonatologist tried to provide the same handling of the child across all conditions.

Stepping was more easily elicited when the neonates were alert (Thelen et al., 1982). Accordingly, no recording was conducted if the

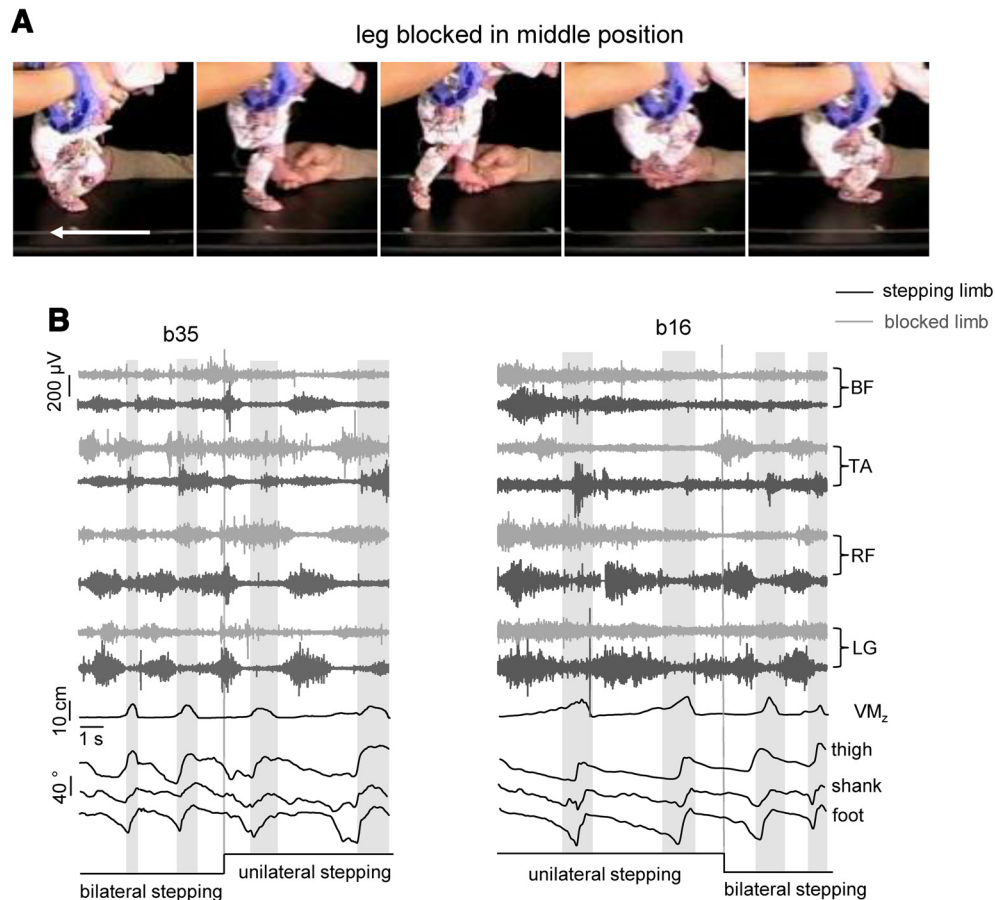


Figure 1. *A*, Illustration of one neonate performing unilateral stepping with one limb manually blocked in the middle position. *B*, Examples of raw EMGs and limb kinematics during bilateral and unilateral stepping with the limb blocked in the middle position in two different neonates. VM_z, Vertical coordinate of the fifth metatarsal marker. Traces include the transition from bilateral to unilateral stepping or the contrary (vertical line marks transition). Notice that a brief segment of RF activity of the stepping limb of neonate b16 was corrupt and has been replaced by a zero line.

child was drowsy or asleep. Each testing session, including markers and electrode placements, lasted ~30 min. A total of 322 and 571 steps was recorded during bilateral and unilateral stepping, respectively (Table 1). Not all children performed all tests (bilateral stepping, and unilateral stepping with anterior, middle, or posterior block). As shown in Table 1, the number of steps typically performed by the neonates was low, because of the well known difficulty of eliciting sustained stepping in neonates (Forssberg, 1985; Thelen and Ulrich, 1991; Yang et al., 1998b).

Data analysis

Data analysis was performed using custom-written programs in MATLAB (MathWorks).

Selection of stepping movements and spatiotemporal gait parameters. Stepping movements were identified from video recordings. Both bilateral and unilateral stepping were determined using the same criterion, namely a contact of the right foot with the treadmill belt starting with the fifth metatarso-phalangeal joint ahead of the greater trochanter and ending with the fifth metatarso-phalangeal joint posterior to the greater trochanter, followed by a swing phase during which the foot was brought ahead of the hip for the next contact with the belt. The steps during which the transition from bilateral to unilateral stepping occurred (and vice versa) were not included in the analysis, except when explicitly stated. The start and end of the stance phase were determined based on the timing of the local minima of the vertical displacement of the foot marker (VM), assisted by visual inspection of video recordings (Dominici et al., 2007; Ivanenko et al., 2013; La Scaleia et al., 2018). The stride duration was defined as the time interval between two successive foot touch-down (TD) events of the right limb. The stance and swing phases were defined as the time intervals between TD and the successive

Table 1. Number of steps per neonates

	Number of neonates	Number of steps	Median (interquartile range) steps per neonate
Bilateral stepping	37	322	6 (7)
Unilateral stepping			
Anterior	32	161	4 (3)
Middle	43	253	4 (5)
Posterior	30	157	5 (4)

ipsilateral toe-off (TO), and between TO and the successive ipsilateral TD, respectively. The interstride variability of stride and swing duration was computed by averaging the SD of each phase duration of every child.

Several previous studies of neonates selected for analysis a few consecutive strides involving alternating (left–right) foot placements (Yang et al., 1998b; Dominici et al., 2011; Ivanenko et al., 2013; Sylos-Labini et al., 2017, 2020). Importantly, here we included in the analysis all successful steps (as defined above) regardless of whether or not there was left–right alternation, since we were interested in the patterns of interlimb coordination regardless of the phase.

Kinematic parameters. The acquired kinematic data were low-pass filtered at 10 Hz with a zero-lag Butterworth fourth-order filter. The kinematic data were then time interpolated over individual step cycles to fit a normalized 200-point time base. To characterize the kinematics of the right limb during either bilateral or unilateral stepping in the sagittal plane, we calculated the following parameters: the range of motion of the thigh, shank, and foot angle relative to the vertical, the values of these

segment angles at TD and TO, and the anteroposterior (10), lateral (y), and vertical (z) excursion of the fifth metatarso-phalangeal joint trajectories normalized to limb length (defined as the distance between GT and LM). As a measure of similarity between bilateral and unilateral stepping, we computed the cross-correlations among thigh, shank, and foot angles of individual steps, and the average traces of bilateral stepping. The cross-correlation provides information about similarities or dissimilarities in temporal characteristics (time lag) and shape characteristics (r value). We used a Fisher Z -transformation to compute the average coefficient of correlation in each condition.

EMG parameters. Raw digitized EMG data were first inspected visually to detect artifacts and remove the corrupted data segment from further analysis. Muscles within the steps containing parts of traces that were removed, were not analyzed. Over a total of 7144 recorded EMGs (eight muscles per 893 steps), 864 traces were not considered. The remaining EMG data were high-pass filtered at 60 Hz, full-wave rectified, and low-pass filtered at 10 Hz to obtain the envelope time series. All filters were zero-lag fourth-order Butterworth filters. As in the case of the kinematic data, the processed EMG data were time interpolated over a normalized 200-point time base. For each muscle of both limbs, we computed the mean activation over each step cycle. In addition, we calculated the center of activity (CoA) over each step cycle as the angle of the vector (circ_moment.m function in the CircStat MATLAB toolbox 8), which points to the center of mass of the circular distribution using the following formulas:

$$A = \sum_{t=1}^{200} (\cos \theta_t \times \text{EMG}_t), \quad (1)$$

$$B = \sum_{t=1}^{200} (\sin \theta_t \times \text{EMG}_t), \quad (2)$$

$$\text{CoA} = \tan^{-1}(B/A). \quad (3)$$

The CoA has been previously used to characterize the temporal shifts of EMG activity (Yakovenko et al., 2002; Ivanenko et al., 2006; Sylos-Labini et al., 2011, 2014; Dewolf et al., 2021) when one cannot reliably identify a single peak of activity, as was the case here. The CoA was considered only for those cycles in which the EMG waveform was not uniformly distributed along the cycle (Rayleigh test for nonuniformity of circular data, $p < 0.05$). The duration of the muscle activation was estimated using the full-width at half-maximum (FWHM), defined as the duration during which the EMG activity exceeded half of its maximum (Martino et al., 2014; Dewolf et al., 2020).

Interlimb EMG coupling. To characterize the interlimb coupling of EMG activity in bilateral and unilateral stepping, we computed the difference between the CoA (ΔCoA) of the EMG envelopes of the two limbs. Given the high variability of interlimb coupling, we defined the interlimb coupling as alternating when the phase (ΔCoA) was $>25\%$ and $<75\%$ of the step cycle. Interlimb coupling was defined as synchronous when the phase was between -25% and 25% of the step cycle.

Frequency analysis. We decomposed the time series of LG envelopes of both limbs in their Fourier series components (fast Fourier transform; fft.m built-in MATLAB function) for bilateral stepping and unilateral stepping. For this analysis, we required that at least five consecutive steps were performed. We considered the frequency with the highest magnitude as the dominant frequency of each limb. These frequencies were considered similar between limbs if they did not differ by $>50\%$. In these cases, we computed the phase difference between the dominant frequency of the LG activities of the two limbs. If the frequencies were considered as different, we compared the LG activation intervals of both limbs, using the methods of Staude and Wolf (1999) and Staude et al. (2001). The onset times of each burst of LG activity were determined. Episodes of different left–right coordination were determined by visual inspection of burst activation by two different experimenters independently. One burst on the blocked limb during each step of the stepping limb was considered as 1:1 interlimb coordination, two bursts on the blocked limb during one step of the stepping limb or one burst of the blocked limb every two steps of the stepping limb were considered as 2:1

interlimb coordination, and no or tonic activity on the blocked limb was considered as 0:1 coordination. The inter-rater concordance was 92.5%. For the steps without agreement, the opinion of a third independent experimenter was asked.

Release of the blocked limb. The start and end of perturbations were determined by visual inspection of the video. We considered the TA activation elicited by the sudden release of the blocked limb. The onset time of a burst of TA activity closest to the release was determined (Staude and Wolf, 1999; Staude et al., 2001). TA burst onsets occurring during the first 400 ms after the release were considered in the analysis. In the Results, we report the statistics of TA burst onsets in milliseconds (resolution of EMG signals), but the actual temporal accuracy was limited by the video recording at 100 Hz (see above) and the visual detection of release time.

Statistical analysis

Descriptive statistics included the mean $\pm$ SD values across all strides of all participants of a given condition (ensemble average) for normally distributed samples, or the median and interquartile range values for non-normally distributed samples. Sample normality was assessed using the Kolmogorov–Smirnov test. To evaluate whether the parameters from bilateral and unilateral stepping with the leg blocked in middle position had a statistically significant difference in mean or median values, we used either the Student's t test (for normally distributed samples) or the Mann–Whitney U test (for non-normally distributed samples). The effect of blocked limb position was compared using either the one-way ANOVA (for normal samples) or the Kruskal–Wallis test (for non-normal samples). The *post hoc* Tukey–Kramer multiple-comparison test was used. We used circular statistics for the CoA of each muscle. In particular, we used the Watson–Williams test to characterize the mean CoA, and the Watson's U^2 test to compare the distribution of CoA in each condition (Landler et al., 2021). The *post hoc* Tukey–Kramer multiple-comparisons test was also used. We used the Rayleigh test for non-uniformity of circular data to check whether EMG waveforms or CoA samples were distributed uniformly around the cycle or had a narrow distribution with a mean direction. A nonuniform CoA distribution indicates that one type of interlimb coordination was more frequently performed. If significant, the mean vector angle relative to the step cycle (referred to as the circular mean) and the mean vector length were calculated. The mean vector length ($0 < r < 1$) is a measure of angular variation, with $r = 0$ indicating a random distribution (Batschelet, 1981). All statistical hypotheses were rejected at $\alpha \leq 5\%$. Statistical analyses were performed with the statistical software package SPSS (version 23.0; IBM).

Data availability

All data generated or analyzed during this study are included in this published article. Additional data are available from the corresponding authors on reasonable request. All analysis codes have been published, and appropriate references are given in the article. Implementation details will be provided from the corresponding authors on reasonable request.

Results

General movement characteristics

We monitored bilateral stepping and unilateral stepping in neonates held upright on a treadmill. During unilateral stepping, the left lower limb was blocked in anterior, middle, or posterior position, while the right lower limb was free to move. Exemplary recordings of limb kinematics and bilateral raw EMG activities are plotted in Figures 1–3. All neonates who produced bilateral steps also stepped unilaterally when one limb was blocked. After blocking the left limb, the right limb generally continued stepping on the treadmill (Fig. 1B, neonate b35). After the release of the blocked limb, in most cases both limbs stepped on the treadmill (Fig. 1B, b16). Details of the responses after the release will

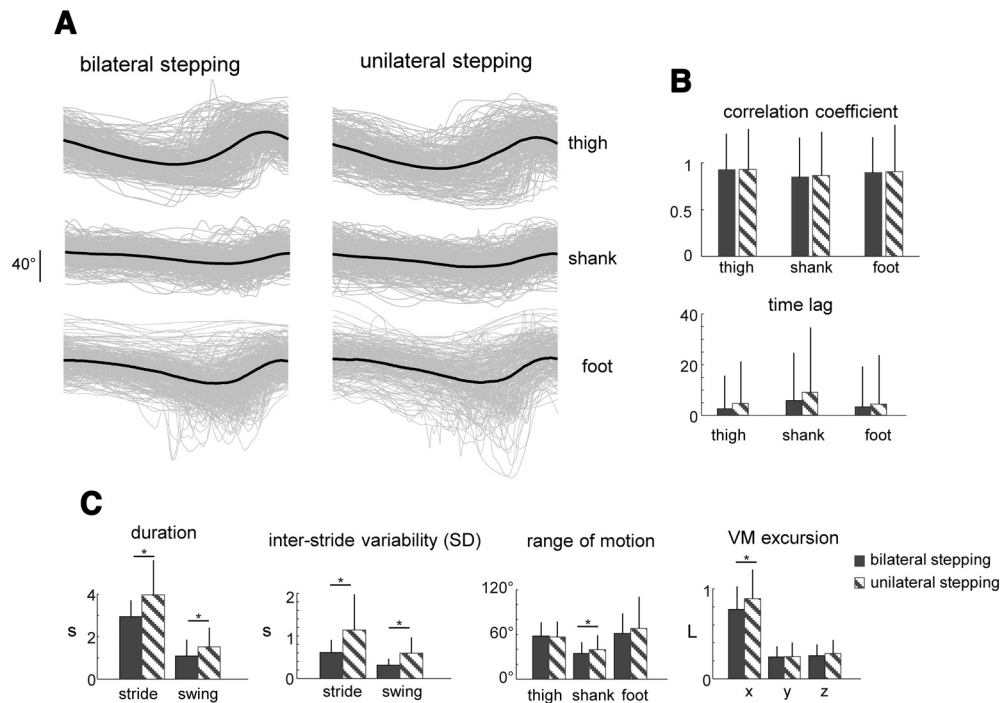


Figure 2. Kinematics of the stepping limb. **A**, Thigh, shank, and foot elevation angles of all steps of bilateral and unilateral stepping with the limb blocked in middle position in gray, ensemble-average traces (i.e., average across steps in all neonates) in black. **B**, Coefficient of correlation and time lag of the cross-correlations between each individual trace and the ensemble-average trace of bilateral stepping. **C**, Average stride and swing duration; interstride variability; range of motion of the thigh, shank, and foot angles; and the excursion of the fifth metatarsophalangeal joint in horizontal (x), lateral (y), and vertical (z) direction. Asterisks denote significant differences.

be reported in a subsequent section (Release of the blocked limb).

Figure 2A shows individual traces and ensemble averages of the segment angles relative to the vertical of the stepping limb during both bilateral and unilateral stepping. Notice that, in contrast with the anatomic hip angle, these segment angles (including the thigh angle) do not depend on the tilt of the body. Despite the considerable interstep variability, it can be seen that the kinematics of the right limb was similar during unilateral and bilateral stepping. In both cases, backward movements of the thigh, shank, and foot segments during stance were followed by a triple forward movement during swing (Fig. 2A). The average cross-correlations of all individual steps with the ensemble average traces of bilateral stepping (Fig. 2B) were not significantly different between bilateral stepping and unilateral stepping with the left limb blocked in the middle position (Mann–Whitney *U* test; thigh: $Z = -0.62$, $p = 0.533$; shank: $Z = -1.78$, $p = 0.074$; foot: $Z = -0.81$, $p = 0.421$). The time lag of the cross-correlations also was not significantly different between the two conditions (Mann–Whitney *U* test; thigh: $Z = -0.13$, $p = 0.898$; shank: $Z = -1.36$, $p = 0.173$; foot: $Z = -0.21$, $p = 0.833$).

Some differences between bilateral and unilateral stepping emerged when considering the stride parameters (Fig. 2C). When the left limb was blocked in the middle position, on average the duration of the stride was 1.03 s longer than that during bilateral stepping (Mann–Whitney *U* test; $Z = -9.74$, $p < 0.001$), and the duration of the swing phase was 0.43 s longer than during bilateral stepping (Mann–Whitney *U* test; $Z = -6.31$, $p < 0.001$). These two increments were proportional to each other, so that the ratio between swing and stance duration was not significantly different between the two conditions (Mann–Whitney *U* test; $Z = -1.12$, $p = 0.262$). In addition, the variability of stride and swing duration across individual cycles was significantly

greater during unilateral than bilateral stepping (Mann–Whitney *U* test; stride: $Z = -3.62$, $p < 0.001$; Student's *t* test; swing: $t = -4.23$, $p < 0.001$).

Foot displacement (estimated from the VM range of motion) was significantly greater in the anteroposterior direction, but not in the vertical and lateral directions during unilateral stepping compared with bilateral stepping (Student's *t* test; anteroposterior: $t = -4.71$, $p < 0.001$; Mann–Whitney *U* test; lateral: $Z = -1.50$, $p = 0.133$; vertical: $Z = -1.18$, $p = 0.236$). The range of shank segment motion was also significantly greater during unilateral stepping, whereas the thigh and foot ranges of motion were similar in both conditions (Student's *t* test; thigh: $t = 1.17$, $p = 0.242$; Mann–Whitney *U* test; shank: $Z = -3.85$, $p < 0.001$; foot: $Z = -1.53$, $p = 0.236$).

Interlimb EMG coupling

Figure 3 reports the raw EMG traces for different neonates during bilateral (Fig. 3A) and unilateral (Fig. 3B) stepping. Time-varying muscle activities shared many qualitative features between bilateral and unilateral stepping, but there were also some important quantitative differences. During both bilateral and unilateral stepping, BF, RF, and LG muscles of the right limb tended to be coactivated over most of the stance phase, while TA was mainly active during the swing phase. The EMG activity of the left limb was modulated approximately out of phase with the corresponding activity of the right limb in several strides of both bilateral stepping (e.g., b35, b21, b3) and unilateral stepping (e.g., b27e2, b35), resulting in quasi-alternating coordination. In other cases, however, the EMG activity of the left limb was modulated approximately in phase with the right limb activity in both bilateral stepping (e.g., b40) and unilateral stepping (e.g., b27e1, b35), resulting in quasi-synchronous coordination. Notice that both modes of interlimb coordination,

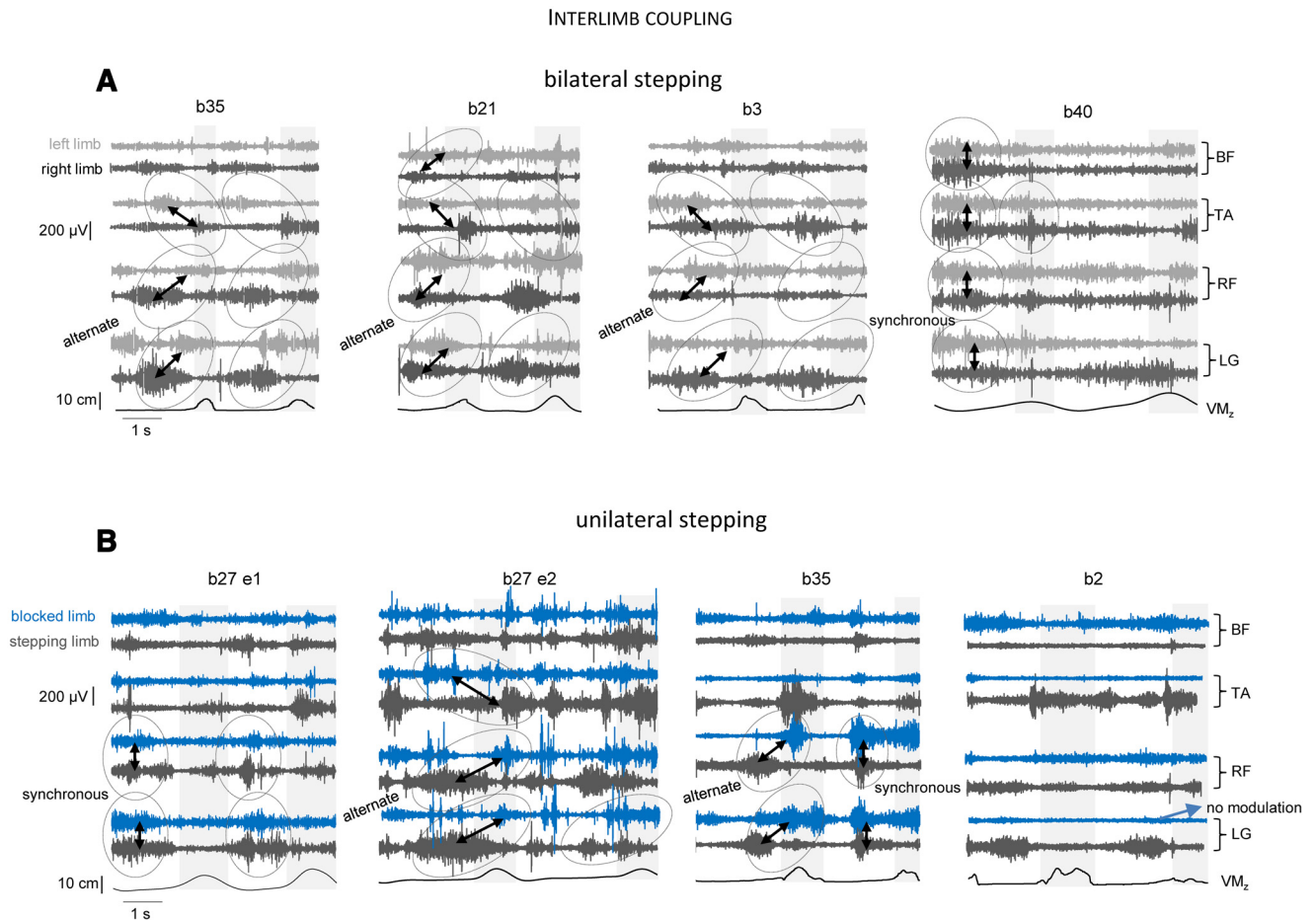


Figure 3. Recorded bilateral EMG profiles during bilateral and unilateral stepping with the limb blocked in the middle position. **A**, Examples of raw EMGs of two consecutive steps in four different neonates during bilateral stepping. Note that alternations between left (light gray) and right (dark gray) limb muscles are mostly observed. **B**, Examples of raw EMGs of two consecutive steps in three different neonates during bilateral stepping. Both alternate and synchronous muscle activation of the stepping limb (light gray) and the blocked limb (blue) are observed. VM₂, Vertical coordinate of the fifth metatarsal marker.

alternating and synchronous, could occur in the same child during separate stepping episodes (e.g., b27e1 vs b27e2) or even during consecutive strides of the same episode (e.g., b35).

We quantified the timing of rectified, low-pass filtered EMG activity in terms of its CoA over all step cycles in which the EMG waveform was not uniformly distributed along the cycle (Rayleigh test). Figure 4 reports the polar histograms of the CoA of the recorded muscles of the right limb versus the normalized step cycle during bilateral (Fig. 4A) and unilateral (Fig. 4B) stepping. The distribution of these CoAs was narrowly tuned (Rayleigh test, $p < 0.001$), indicating a relatively consistent timing of peak activation. In bilateral stepping (Fig. 4A), the circular mean of the CoA (Fig. 4A, red arrow) was $25.4 \pm 18.9\%$ (circular SD), $80.3 \pm 17.2\%$, $30.3 \pm 15.9\%$, and $31.9 \pm 12.1\%$ of the step cycle for the BF, TA, RF, and LG, respectively. During unilateral stepping (Fig. 4B), the circular mean of the CoA was $34.1 \pm 18.7\%$, $81.6 \pm 15.2\%$, $30.8 \pm 16.4\%$, and $33.0 \pm 13.3\%$ of the step cycle for the BF, TA, RF, and LG of the stepping limb, respectively. The mean CoA of BF was timed slightly but significantly later in unilateral stepping (Watson–Williams test; $F_{(1,457)} = 10.8$, $p = 0.001$). However, the mean CoA of the other muscles did not differ significantly between unilateral and bilateral stepping (Watson–Williams test; TA: $F_{(1,520)} = 0.5$, $p = 0.464$; RF: $F_{(1,503)} = 0.06$, $p = 0.798$; LG: $F_{(1,508)} = 0.6$, $p = 0.423$).

To estimate the phase of interlimb coupling of EMG activities, we computed the difference between the CoA (Δ CoA) of a given muscle of the left limb and that of the right limb. Figure 4 reports the results clustered in two main subsets, alternating and synchronous, based on the Δ CoA of LG muscle. We chose to refer to this muscle because the CoA dispersion (circular SD) across steps was significantly smaller for LG than for the other muscles (Watson's U^2 test; LG vs BF: $U^2 = 1.73$, $p < 0.001$; LG vs TA: $U^2 = 6.52$, $p < 0.001$; LG vs RF: $U^2 = 0.44$, $p < 0.001$). We classified the steps as alternating when Δ CoA of LG was between 25% and 75% of the step cycle, and as synchronous when Δ CoA of LG was between -25% and $+25\%$ of the step cycle. The pie charts in the insets report the percentages of these two classes. A further class (no EMG modulation) of the pie charts includes the steps in which the LG envelope of one or both limbs was distributed uniformly around the cycle (Rayleigh test, $p > 0.05$).

The percentage of alternating steps was higher during bilateral stepping (77%) than unilateral stepping (40%). Moreover, in these alternating steps, the modulation of EMG activities was more consistently out of phase ($\sim 50\%$ of the phase shift along the cycle) in the former than the latter condition. Thus, in bilateral stepping, the mean Δ CoA values were $48.4 \pm 1.7\%$, $49.8 \pm 9.8\%$, $31.8 \pm 11.7\%$, and $43.2 \pm 2.9\%$ for LG, BF, TA, and RF, respectively. All these values were statistically significant ($r = 0.76$, $p < 0.001$; $r = 0.18$, $p = 0.004$; $r = 0.15$, $p = 0.014$; $r = 0.52$, $p < 0.001$, for LG, BF, TA, and RF, respectively). The circular

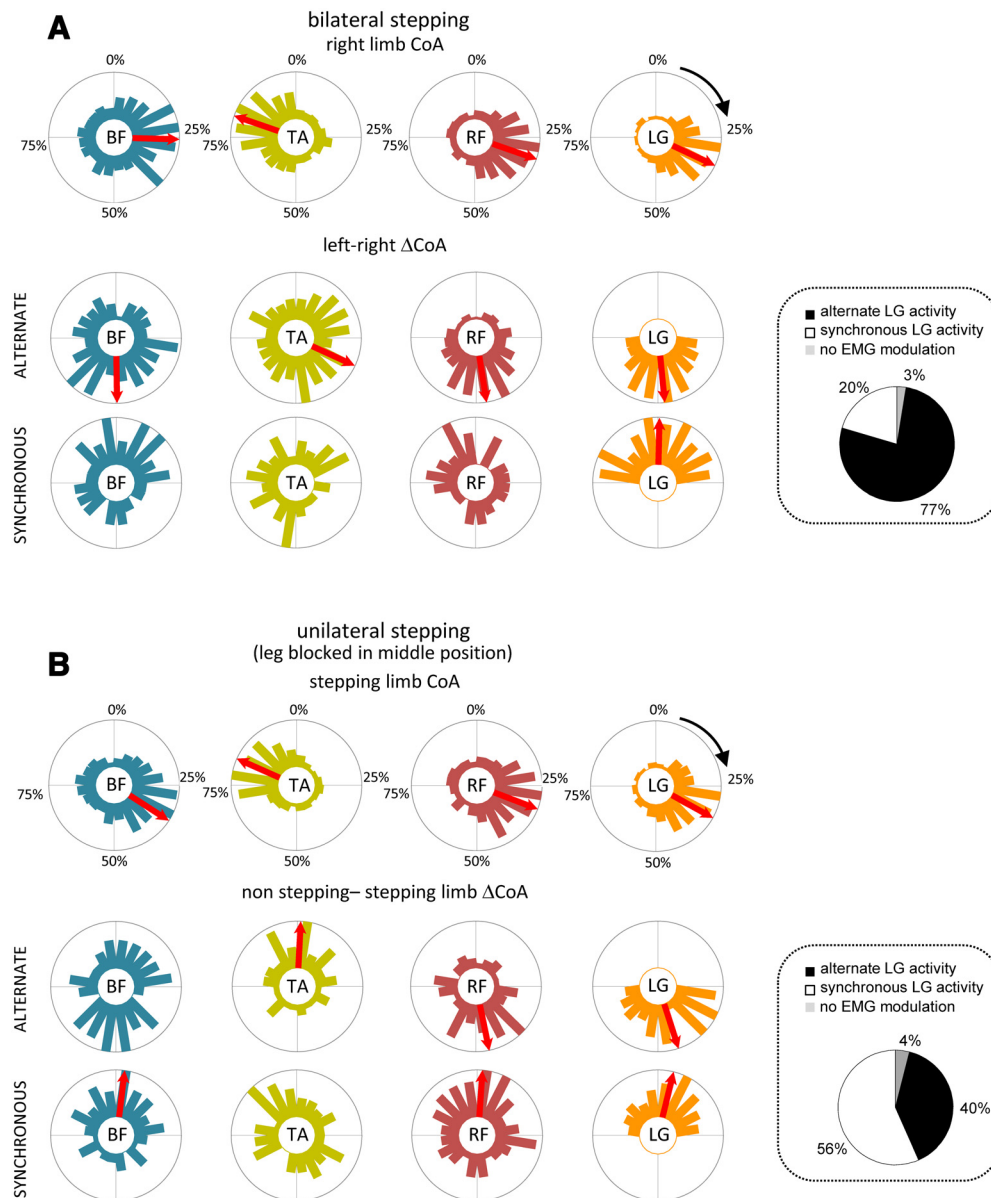


Figure 4. *A, B*, Interlimb coupling during bilateral (*A*) and unilateral (*B*) stepping with the blocked limb in middle position. *A, B*, First line, Polar histograms of center of activity for bilateral stepping versus normalized cycles discretized in 20 sectors; second and third lines, polar histograms of the phase lag between the center of activity of both limbs (Δ CoA) for bilateral stepping versus normalized cycles discretized in 20 sectors; black arrows, progression time, with angle that varies from 0° to 360° corresponding to a 0% and 100% cycle. Bar height denotes the percentage of cycles whose center of activity (or Δ CoA) is located in the corresponding sector. Red arrows, Resultant (circular mean) center of activity (Rayleigh test for nonuniform circular distributions, $p < 0.05$). The pie chart presents the percentage of steps with alternate or synchronous LG bilateral activation, or with no modulation of LG EMG activity (i.e., where the center of activity of one of the limbs was not identifiable; Rayleigh test, $p > 0.05$).

mean of CoA over the alternating steps was $77.3 \pm 4.8\%$, $24.6 \pm 9.8\%$, $77.1 \pm 2.1\%$, and $79.6 \pm 1.6\%$ of the step cycle for the BF, TA, RF, and LG of the left limb, respectively.

In unilateral stepping, the mean Δ CoA was $45.1 \pm 3.3\%$ ($r = 0.66$, $p < 0.001$), $0.8 \pm 8.5\%$ ($r = 0.34$, $p < 0.001$), and $46.7 \pm 7.7\%$ ($r = 0.35$, $p < 0.001$) for LG, TA, and RF, respectively. Thus, while LG and RF were approximately out of phase between the stepping limb and the blocked limb, TA was approximately in phase. Indeed, the circular mean of CoA was $76.3 \pm 4.2\%$, $82.1 \pm 5.2\%$, and $82.5 \pm 8.9\%$ of the step cycle for the LG, RF, and TA of the blocked limb, respectively. Moreover, the distribution of TA Δ CoA was more narrowly tuned during unilateral than bilateral stepping (Watson's U^2 test; $U^2 = 0.44$, $p < 0.001$), while the Δ CoA distributions of the other muscles were not significantly different (Watson's U^2 test; $p > 0.27$). Δ CoA of BF

activity was uniformly distributed along the cycle (Rayleigh test, $p = 0.476$), indicating a highly variable coupling of this muscle between the limbs.

We found the opposite pattern of results for the steps classified as synchronous. The percentage of synchronous steps was higher during unilateral stepping (56%) than the bilateral stepping (20%). Moreover, in these steps, the modulation of EMG activities was more consistently in phase ($\sim 0\%$ of phase shift along the cycle) in the former than the latter condition. Thus, in unilateral stepping, the mean Δ CoA was $3.8 \pm 2.4\%$ ($r = 0.73$, $p < 0.001$), $1.9 \pm 7.1\%$ ($r = 0.33$, $p < 0.001$), and $1.1 \pm 10.6\%$ ($r = 0.24$, $p = 0.007$) for LG, BF, and RF, respectively. The Δ CoA of TA activity was uniformly distributed along the cycle (Rayleigh test, $p = 0.321$). In bilateral stepping, the mean Δ CoA of LG was $0.4 \pm 4.1\%$ ($r = 0.64$, $p < 0.001$). However, in this

condition, the ΔCoA of the other muscles was uniformly distributed along the cycle (Rayleigh test; BF: $r = 0.26$, $p = 0.072$; TA: $r = 0.14$, $p = 0.354$; RF: $r = 0.18$, $p = 0.246$), indicating a highly variable coupling of these muscles between the limbs.

As noticed above, we performed the classification of the steps using the ΔCoA of LG muscle, the most narrowly tuned among the recorded muscles. The classification was only modestly affected by using the ΔCoA of RF or BF, whereas the use of TA ΔCoA yielded greater differences given the idiosyncratic modulation of this muscle described above.

The classification of alternating and synchronous EMG activations we reported above relied on broad time windows (50% of the step cycle). If we used narrower time windows, the percentage of steps that did not fall in either class was considerable. Thus, the percentage of alternating steps based on LG ΔCoA between 45% and 55% of the step cycle was 20% and 8% for bilateral and unilateral stepping, respectively. The percentage of synchronous steps based on LG ΔCoA between -5% and 5% of the step cycle was 4% and 11% for bilateral and unilateral stepping, respectively. These results underscore the highly variable nature of interlimb coupling in neonates.

Oscillation frequencies of EMG activity

The analysis of interlimb coordination reported in the previous section involved all single steps considered individually. Additional information can be gained by considering sequences of consecutive steps. Indeed, interlimb coordination also may be affected by intrinsic rhythmic activations of the muscles of the two limbs maintained over a sequence of steps. In theory, rhythmicity may be similar or different between the two limbs. To assess rhythmicity, we first selected the cases with at least five consecutive steps, as in the examples of Figure 5A. The data of nine neonates complied with this requirement for bilateral stepping. For unilateral stepping, the data of 5, 13, and 6 neonates with the leg blocked in the anterior, middle, and posterior position, respectively, complied with this requirement. In all of these cases, we conducted a Fourier analysis of the LG envelope (Fig. 5B). The dominant frequency of oscillation of the stepping limb was not affected by the position of the blocked limb (Kruskal–Wallis test, $p > 0.261$). We found that the dominant frequency of oscillation of the stepping limb was significantly smaller in unilateral stepping (all data pooled together) than in bilateral stepping (0.25 ± 0.07 vs 0.34 ± 0.07 Hz; Mann–Whitney U test: $Z = 9.7$, $p = 0.002$), consistent with the longer stride duration in the former than in the latter condition (Fig. 2C).

During bilateral stepping, in eight neonates (89%) the dominant frequency on the left side differed by $<50\%$ of that on the right side (Fig. 5B). In these cases, the phase difference between the dominant frequencies was between 25% and 75% of the step cycle. On average, the phase difference between the dominant frequencies was $43.2 \pm 14.9\%$ (circular mean $\pm$ SD) of the step cycle, showing approximately alternating activation of LG muscles of the two sides. During unilateral stepping with the leg blocked in the middle position, in nine neonates (69%) the right and left dominant frequencies differed by $<50\%$. The average phase difference between these dominant frequencies was between 25% and 75% of step cycle for five of these neonates ($48.6 \pm 7.0\%$) and between -25% and 25% for four others ($-7.6 \pm 14.2\%$), showing both alternating and synchronous activation of bilateral LG muscle (Fig. 5B). With the leg blocked in the

anterior position, in four neonates (80%) the right and left dominant frequencies differed by $<50\%$, whereas with the leg blocked in the posterior position, in four neonates (66%) the right and left dominant frequencies differed by $<50\%$. Among these eight neonates, the average phase difference between the dominant frequencies was between 25% and 75% of step cycle for four neonates ($59.7 \pm 4.5\%$) and between -25% and 25% for four others ($12.1 \pm 11.4\%$), showing both alternating and synchronous activation of bilateral LG muscle.

Notice that one neonate (11%) in bilateral stepping and seven neonates (29.2%) in unilateral stepping had oscillation frequencies differing by $>50\%$ between left and right LG activation (Fig. 5A, examples). In these neonates, we compared the onset times of LG bursts of activation between the two limbs. For unilateral stepping, in most cases (55% of 40 steps), there was one burst on the blocked limb during each step of the stepping limb (Figs. 3B, b27e1, 5A). Despite the different oscillation frequencies, this can still be considered a 1:1 interlimb coordination. In other cases (20%), however, there were two bursts on the blocked limb during one step of the stepping limb (i.e., 2:1 interlimb coordination; Fig. 5C). In still other cases (25%), we observed activity on the stepping limb only, whereas the blocked limb was either silent or displayed tonic activity (i.e., 0:1 coordination; Figs. 3B, b2, 5A). For bilateral stepping, the neonate with different left–right dominant frequency also displayed episodes of 2:1 coordination.

Effects of different positions of the blocked limb

Blocking the left limb in different positions (anterior, middle, or posterior) during unilateral stepping had minor effects on the contralateral stepping limb, but major effects on the EMG activity of the blocked limb.

Stepping limb characteristics

Figure 6A compares different kinematic parameters across the three conditions of limb block. Stride and swing durations were slightly but significantly shorter with the limb blocked in the posterior position than in the middle block (Fig. 6A; Kruskal–Wallis test; stride: $\chi^2 = 69.9$, $p = 0.001$; swing: $\chi^2 = 42.6$, $p = 0.033$) and anterior block (Kruskal–Wallis test; stride: $\chi^2 = 69.9$, $p < 0.001$; swing: $\chi^2 = 62.4$, $p = 0.002$). While the overall range of motion of limb segments was not significantly affected by the position of the blocked limb (Kruskal–Wallis test; thigh: $\chi^2 = 1.9$, $p = 0.381$; foot: $\chi^2 = 2.1$, $p = 0.341$; ANOVA – shank: $F_{(2,502)} = 1.8$, $p = 0.160$), the limb posture at TD and TO was significantly different. Thus, the thigh angle relative to the vertical was rotated more anteriorly by $\sim 20^\circ$ at TD and TO with the limb blocked in anterior than middle and posterior position (ANOVA; TD: $F_{(2,500)} = 43.9$, $p < 0.001$; TO: $F_{(2,500)} = 25.6$, $p < 0.001$). There was no significant difference of the thigh angle at TD and TO between the middle and posterior positions (Tukey–Kramer *post hoc* test; TD, $p = 0.395$; TO, $p = 0.999$). The shank angle at TD was closer to the vertical with the limb blocked in the anterior than in the middle and posterior position (Tukey–Kramer *post hoc* test, $p < 0.001$), but no significant differences existed at TO (ANOVA; $F_{(2,500)} = 2.1$, $p = 0.129$). The foot at TD was more dorsiflexed in the anterior position (Kruskal–Wallis test; $\chi^2 = 70.5$, $p < 0.001$) and more extended in the posterior position (Kruskal–Wallis test; $\chi^2 = 43.4$, $p = 0.014$) compared with the middle position. At TO, the foot was slightly more extended in the posterior compared with the anterior position (Kruskal–Wallis test; $\chi^2 = 46.5$, $p = 0.022$).

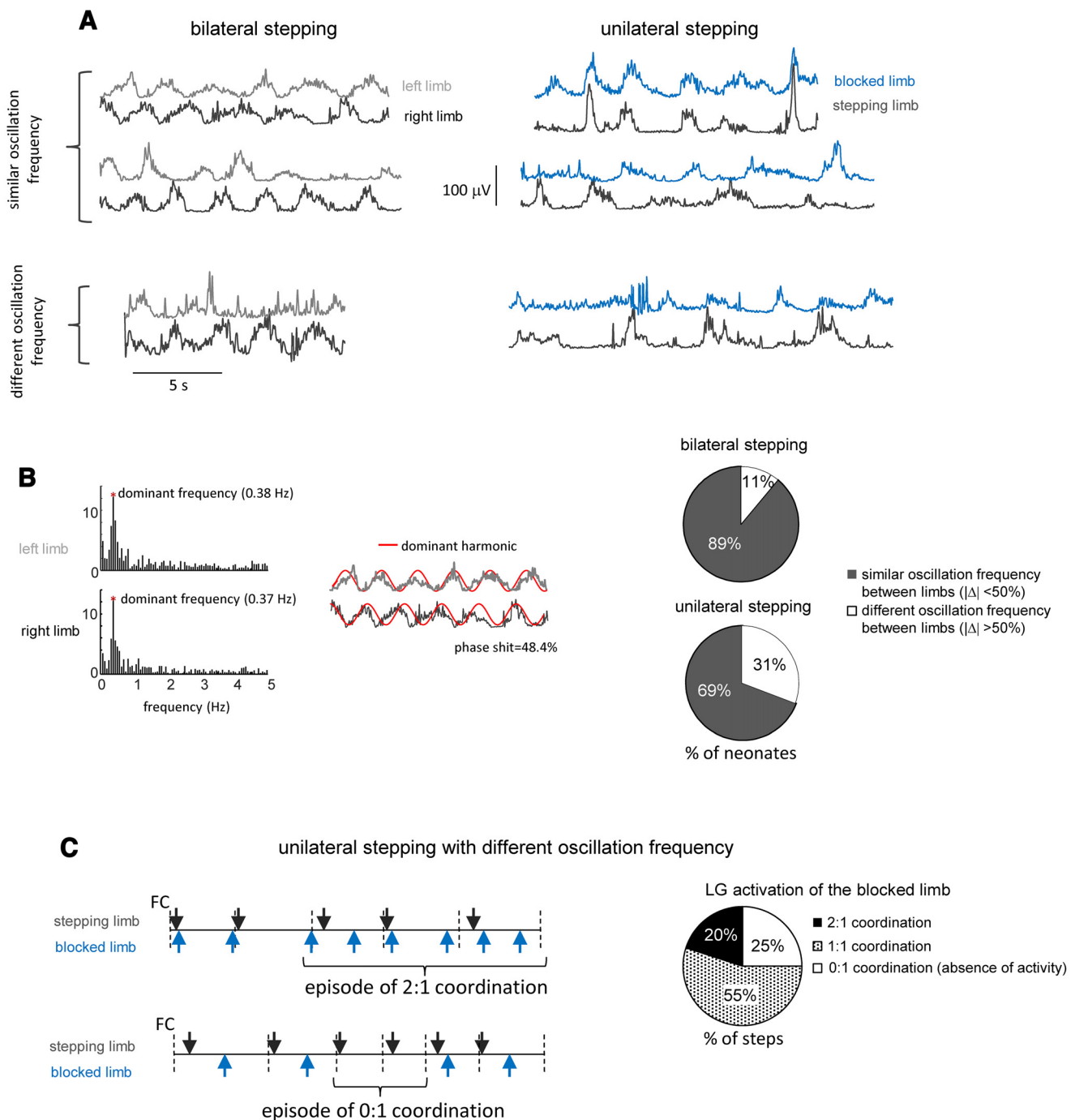


Figure 5. Decomposition of LG envelope time series into their Fourier series components during bilateral stepping ($n = 9$) and unilateral stepping ($n = 13$) with the leg blocked in the middle position. **A**, Examples of bilateral LG envelope during several consecutive steps with similar (top) or different left and right oscillation frequencies (bottom). **B**, Illustration of the fast Fourier transform analysis and the percentage of neonates with different and similar left and right oscillation frequencies during bilateral and unilateral stepping with leg blocked in the middle position. **C**, Two examples of the bilateral onset of LG bursts of activation during unilateral stepping with different limb oscillation frequencies. In most cases, there was one burst onset of the blocked limb during each step of the stepping limb (1:1 coordination). In some cases, an episode with two burst onsets of the blocked limb during one step of the stepping limb were observed (2:1 coordination). In other cases, an episode without LG activation of the blocked limb (0:1 coordination) occurred. The pie chart presents the quantification of the different episodes of coordination in unilateral stepping.

Figure 6B compares different EMG parameters across the three conditions of limb block. The duration of BF activation, estimated as the FWHM, was significantly longer with the limb blocked in the posterior position (Kruskal–Wallis test; $\chi^2 = -61.7$, $p < 0.001$). The CoA of RF and LG occurred significantly later in the stance with the blocked limb in the anterior position (Watson–Williams test; RF: $F_{(2,500)} = 6.5$, $p = 0.011$; LG: $F_{(2,484)} =$

8.7, $p = 0.003$). However, the mean EMG activation of all muscles was not significantly different across the blocking conditions ($p > 0.08$).

Blocked limb characteristics

Blocking the left limb in different positions affected the relative percentages of steps classified as alternating or synchronous

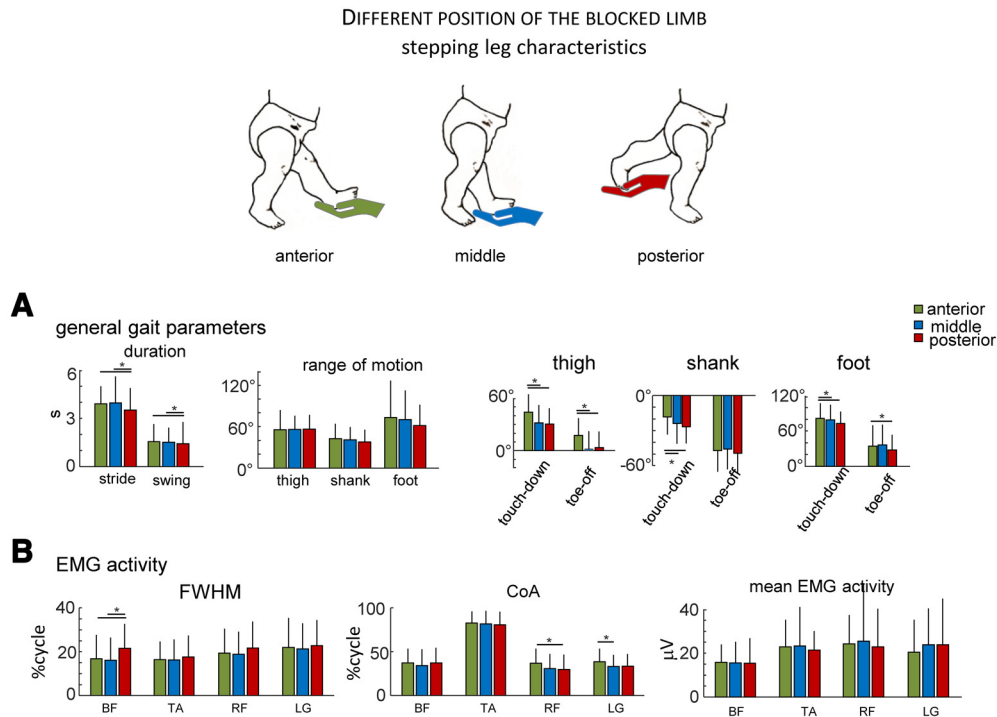


Figure 6. Effect of blocked limb position on stepping limb characteristics. **A**, Average (across steps in all neonates) stride and swing duration; range of motion of the thigh, shank, and foot angles; and orientation of the thigh, shank, and foot at touch-down and toe-off during unilateral stepping with the limb blocked in the anterior, middle, and posterior positions. **B**, Average FWHM, CoA, and mean EMG activity across one step of the four stepping limb muscles. Asterisks denote significant differences.

(Fig. 7A). As in Figure 4, we used the criterion of the Δ CoA of LG muscle falling in one of the two broad time windows (each 50% of the step cycle). Moreover, the muscle activations of the blocked limb were more affected by its position than those of the contralateral stepping limb (Fig. 7B). For steps classified as alternating, blocking the limb in the anterior position resulted in a shift of the CoA of TA later in the cycle than with the limb blocked in the posterior position (Watson–Williams test; $F_{(1,111)} = 7.1$, $p = 0.009$). In addition, the CoA of BF occurred later with the limb in the posterior position compared with the middle position (Watson–Williams test; $F_{(1,124)} = 9.5$, $p = 0.002$) and anterior position (Watson–Williams test; $F_{(1,93)} = 13.3$, $p < 0.001$). For steps classified as synchronous, blocking the limb in the anterior position resulted in a shift of the CoA of TA and RF earlier in the cycle than with the limb blocked in the middle position (Watson–Williams test; TA: $F_{(1,158)} = 17.4$, $p < 0.001$; RF: $F_{(1,154)} = 62.8$, $p < 0.001$) and the posterior position (Watson–Williams test; TA: $F_{(1,101)} = 20.6$, $p < 0.001$; RF: $F_{(1,104)} = 7.4$, $p = 0.007$).

We also found significant effects of the blocked limb position on the duration (estimated as FWHM) and mean muscle activation. For alternating steps, the duration of activation of RF and LG was more prolonged in the posterior than the anterior position (Kruskal–Wallis test; RF: $\chi^2 = -34.1$, $p = 0.004$; LG: $\chi^2 = -39.4$, $p = 0.001$). The activation duration of BF and TA was not significantly different across limb positions (Kruskal–Wallis test; BF: $\chi^2 = 2.2$, $p = 0.329$; TA: $\chi^2 = 0.8$, $p = 0.663$). Similarly, for synchronous steps the FWHM of RF and LG were significantly longer in the posterior position than in the middle position (Kruskal–Wallis test; RF: $\chi^2 = -34.6$, $p = 0.002$; LG: $\chi^2 = -28.7$, $p = 0.029$) and the anterior position (Kruskal–Wallis test; RF: $\chi^2 = -58.7$, $p < 0.001$; LG: $\chi^2 = -36.6$, $p = 0.01$). The FWHM of BF and TA was not significantly different across limb positions (ANOVA; – BF: $F_{(2,463)} =$

1.2, $p = 0.312$; Kruskal–Wallis test; TA: $\chi^2 = 0.7$, $p = 0.680$). For both alternating and synchronous steps, the mean activation of BF significantly increased from the posterior to the anterior position (Kruskal–Wallis test; alternating: $\chi^2 = 44.6$, $p < 0.001$; synchronous: $\chi^2 = 34.8$, $p < 0.001$), whereas the mean activation of RF and LG significantly increased from the anterior to the posterior position (Kruskal–Wallis test; alternating: $\chi^2 > 16.9$, $p < 0.001$; synchronous: $\chi^2 > 16.0$, $p < 0.001$). This differential gradient of activation of BF and RF activations as a function of limb position is schematically depicted in Figure 7C. TA activation was not significantly affected by the position of the ipsilateral limb, whatever the interlimb coupling (Kruskal–Wallis test; alternating: $\chi^2 = 5.4$, $p = 0.065$; synchronous: $\chi^2 = 1.4$, $p = 0.474$).

Release of the blocked limb

We found another important effect at the release of the blocked limb, occurring mainly when the limb was blocked in the posterior position. In total, there were 30 neonates who underwent this perturbation (Table 1). After unilateral stepping with the limb blocked in this position, in 17 of 22 neonates (77%), the sudden release of the blocked limb elicited the immediate initiation of the swing phase of the limb now free to move, associated with hip joint flexion (Fig. 8). The remaining five neonates did not move the leg after the release and started stepping later with this limb. In the other eight neonates whose left limbs had been blocked in the posterior position, the posterior perturbation was followed by unilateral stepping with the blocked limb in the middle or anterior position. With the limb blocked in the middle position, 5 of 21 neonates (24%) initiated a swing phase after the release, whereas only 1 of 10 neonates (10%) started a swing phase with the limb previously blocked in the anterior position. In the other neonates who underwent these perturbations (Table 1), the perturbation was followed by unilateral stepping with the blocked limb in a different position.

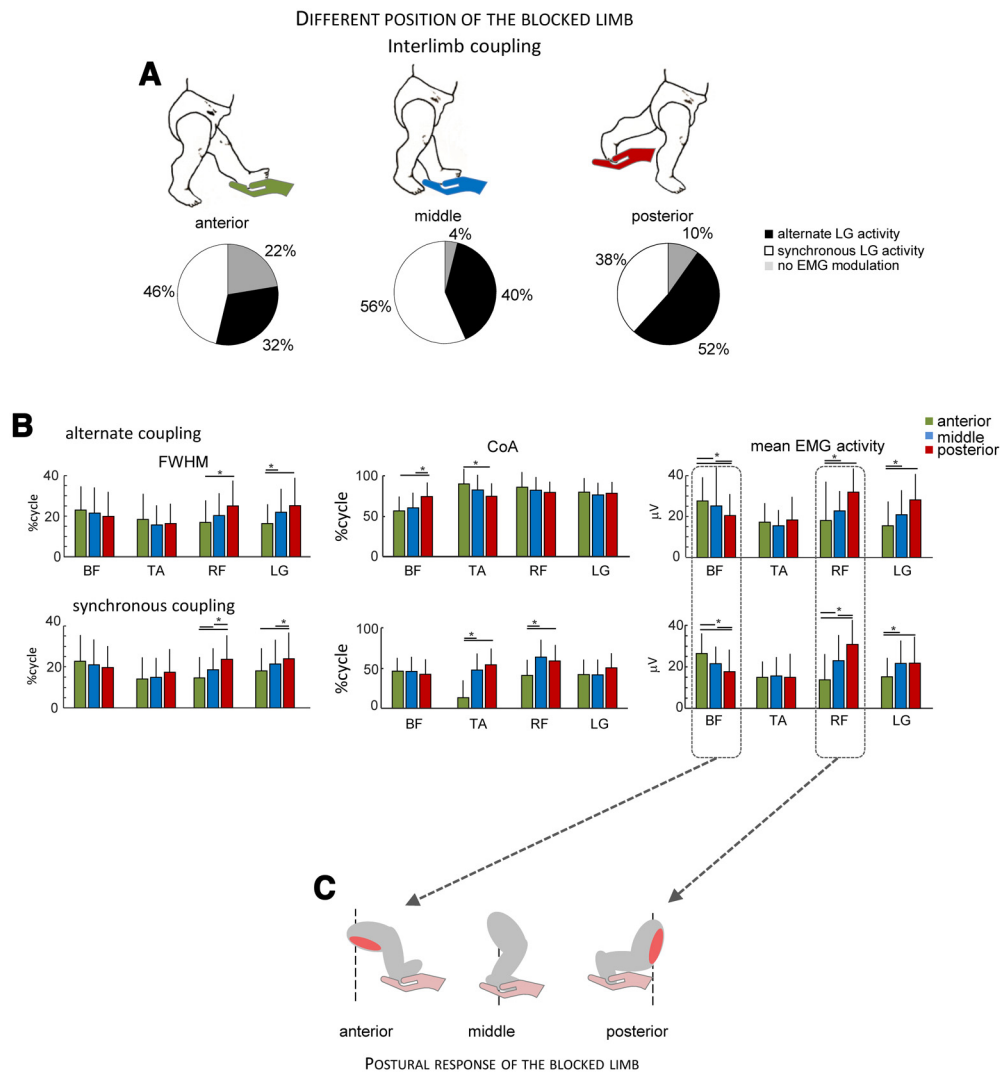


Figure 7. Effect of blocked limb position on blocked limb characteristics. **A**, Percentage of steps with alternate or synchronous LG bilateral activation, or with no modulation of LG EMG activity (i.e., where the center of activity of one of the limbs was not identifiable; Rayleigh test, $p > 0.05$). **B**, Average FWHM, CoA, and mean EMG activity of the four blocked limb muscles of the alternated (top) or synchronous steps (bottom). Asterisks denote significant differences.

Because the swing phase is accompanied by the activation of the TA muscle in neonate stepping (Fig. 4; Dominici et al., 2011; Sylos-Labini et al., 2020), we computed the burst onset of TA after the limb release. Over the 23 neonates who initiated the swing phase after the sudden release (see above), the activation of TA of the blocked limb was absent in 5 of them. Over the remaining 18 neonates, a burst of TA activation occurred, on average, 127 ± 90 ms after the release of the blocked limb (Fig. 8).

Loading responses of the stepping limb

We frequently observed loading responses of the right limb during both unilateral and bilateral stepping (Fig. 9A). Starting from touch-down, the initially flexed knee underwent a large extension (by $27.4 \pm 16.1^\circ$ and $26.6 \pm 16.3^\circ$ in bilateral and unilateral stepping, respectively) that lasted over the first part of the stance, raising the hip joint above the foot. Knee extension was associated with the activation of limb extensor muscles (RF and LG) during midstance, with their CoA occurring at $30.8 \pm 2.8\%$ (RF) and $32.2 \pm 2.0\%$ (LG) in bilateral stepping and at $31.0 \pm 3.2\%$ (RF) and $34.1 \pm 2.4\%$ (LG) in unilateral stepping. This behavior was reliably detected in most steps (90.3% of steps in bilateral

stepping and 77.7% of steps in unilateral stepping with the limb blocked in the middle position).

Discussion

All neonates who produced bilateral steps also stepped unilaterally when one limb was blocked. We observed distinct episodes of alternating or synchronous coordination between the limbs (Fig. 3). The former were more frequent during bilateral stepping, while the latter were more frequent during unilateral stepping (Fig. 4). In general, however, the phase relationship of left–right coordination was quite variable, as assessed from the EMG activity profiles. By considering sequences of five consecutive steps, we found that the dominant frequency of EMG oscillations of the gastrocnemius lateralis was approximately comparable between the two sides in several cases (for both alternating and synchronous modes), but it was considerably different in other cases, more often during unilateral than bilateral stepping (Fig. 5). When the oscillation frequency was different, the sequences exhibited various interlimb coordination ratios, with one, two, or no EMG bursts on the blocked limb for each step of the stepping limb (i.e., 1:1, 2:1, or 0:1 coordination,

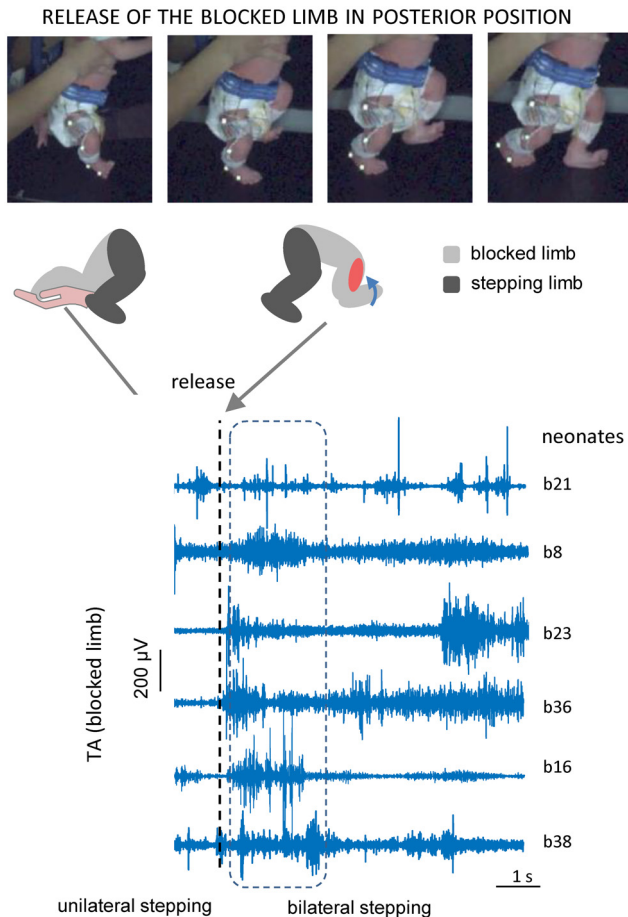


Figure 8. Top, Illustration of one neonate producing a dorsiflexion of the ankle after the release of the blocked limb in posterior position. Bottom, Examples of raw TA EMGs after the release of the blocked limb in posterior position in six different neonates.

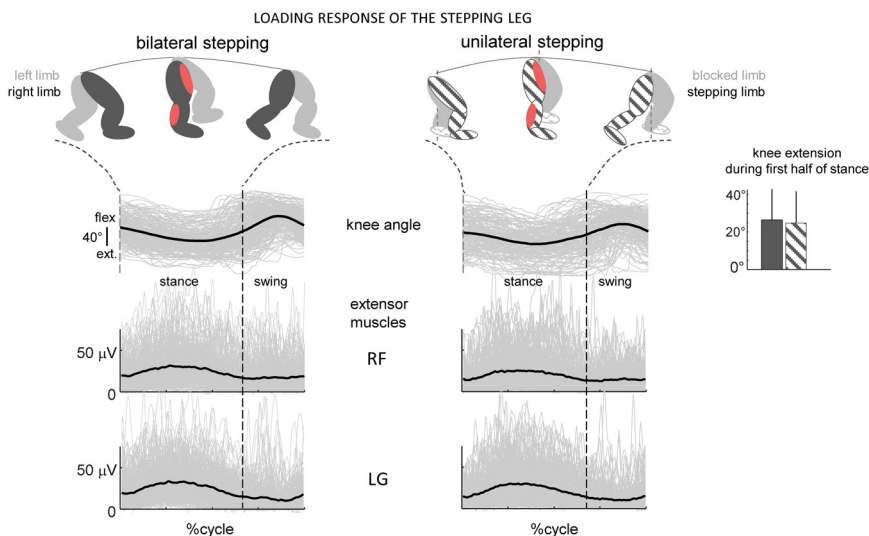


Figure 9. Loading response of the stepping limb. Knee joint angle, RF and LG EMG envelope of all steps in which an extension of the knee was present during the first half of stance; black, the ensemble-average traces (average across steps in all neonates). The bar plot on the right presents the average knee extension (average across steps in all neonates) during the first 50% of stance.

respectively). In the 0:1 mode, EMG activity on the blocked limb was either silent or tonic. Episodes of 2:1 coordination were also found during bilateral stepping.

Altogether, these observations indicate that, in newborns, locomotor pattern generators on the left and right sides of the spinal cord exhibit a coupling that can vary from moment to moment. Sometimes the two sides are coupled together, albeit loosely, with a coupling that can be out of phase or in phase. However, at other times, the two sides are uncoupled, bursting independently of one another or generating tonic or no activity when the ipsilateral limb is motionless.

Central neural substrates

Our findings argue for the neonatal presence of autonomous pattern generators for each limb. Pattern generators for flexor and extensor muscles may also burst independently. Indeed, during alternating episodes of unilateral stepping, while the activity of extensor muscle LG was approximately out of phase between the stepping limb and the blocked limb, the activity of flexor muscle TA was approximately in phase between the two sides.

Considering that cortical control contributes little to neonatal locomotion (Peiper, 1961), the present results can be compared with those in adult patients with complete spinal cord lesions during unilateral aided stepping. Depending on the context, these patients may exhibit rhythmic EMG activity or low, tonic activity in the blocked limb (Dietz et al., 2002; Ferris et al., 2004; Kawashima et al., 2005). Our results are also in line with observations in spinal-transected cats (i.e., “spinal cats”) showing that their interlimb coupling can be weaker and more variable than in cats with intact supraspinal control. In a seminal report, when one hindlimb of a spinal cat was held stationary, the other hindlimb continued stepping, but the extensor and flexor bursts in the blocked limb were replaced by tonic activity (Grillner and Rossignol, 1978). However, in other instances, the nonstepping hindlimb of spinal cats displays rhythmic activity and even performs forward steps if let go (A. Frigon, personal communication). Moreover, episodes of 2:1 coordination (or even higher interlimb ratios) have been described in low spinal cats stepping on a split-belt treadmill (Forssberg et al., 1980; Frigon et al., 2017).

In addition to the lack or limited influence of cortical signals, several other factors may theoretically contribute to the variable interlimb coordination in human neonates, as they do in spinal cats. Low speed is one such factor, because the interlimb pattern (spatial and temporal) is quite variable at slow speeds in spinal cats (Dambreville et al., 2015). Another factor is that an asymmetric pattern might reduce the influence of sensory feedback onto spinal motor circuits (Hurteau et al., 2017; Hurteau and Frigon, 2018). For example, when the hindlimb locomotor pattern is asymmetric during split-belt locomotion, cutaneous feedback is reduced in spinal cats (Hurteau and Frigon, 2018). In the present experiments, the asymmetric locomotor pattern resulted from blocking one limb. Still another potential factor is the low muscle strength of neonates. Indeed, the loss of power of ankle muscles (because of unilateral denervation) led to the appearance of

2:1 coordination patterns between the hindlimbs at the slowest tested speeds in spinal cats (Harnie et al., 2018), again reminiscent of some of the present results.

Notice that transient episodes with no EMG activity in some muscles such as those we sporadically found during both bilateral and unilateral stepping have also been described during fictive locomotion in spinal cats (Grillner and Zangger, 1979) and treadmill locomotion in decerebrate cats (Duysens, 1977). They have been interpreted as spontaneous errors or deletions of bursts of motoneuron activity that can occur within otherwise rhythmic flexor and extensor activity (Lafreniere-Roula and McCrea, 2005), and presented as evidence for a two-layer organization of spinal locomotor networks, with a rhythm-generating layer on top of a pattern-generating layer (McCrea and Rybak, 2007). However, in our case the absence of activity could also be because of biomechanical factors. Thus, limb muscle activity (e.g., hamstring muscles) can be very low at slow speeds and when the limb does not travel far enough forward or backward (Ivanenko et al., 2013; Sylos-Labini et al., 2017).

Finally, our results are closely reminiscent of those obtained in the isolated spinal cord of neonatal rodents. In this preparation, spontaneous rhythmic bursting can be observed (Branchereau et al., 2000; Whelan et al., 2000) or it can be induced unilaterally in each hemicord on bath application of neurotransmitters, (Kudo and Yamada, 1987; Kiehn and Kjaerulff, 1996; Hägglund et al., 2013) and independently in flexor or extensor networks (Häggglund et al., 2013; Machado et al., 2015), consistent with a distributed, modular organization of central pattern generators (Grillner, 1985; Kiehn, 2016). Moreover, using intersectional mouse genetics, different modules of commissural interneurons (V0) have been demonstrated as a function of speed and gait mode (Talpalar et al., 2013). Each module regulates the pattern of interlimb coordination, generating alternating or synchronous left–right activities. Notice that the rhythms recorded in the isolated neonatal spinal cords are often irregular, with cycle durations overlapping the step cycle durations of our neonates.

Based on these analogies, we surmise that left–right coordination of human newborn stepping depends on subcortical mechanisms similar to those discovered in other mammals, including multiple autonomous pattern generators distributed in the lumbosacral cord, ipsilaterally projecting interneurons for intralimb muscle coordination, and contralaterally projecting commissural interneurons for interlimb coordination. In addition, brainstem descending systems (e.g., the serotonergic projections) may contribute to neonatal stepping, since they innervate the spinal cord and myelinate much earlier than the corticospinal system in humans (Ten Donkelaar et al., 2014). Their influence can be inferred by the strong effect of alertness on the possibility of eliciting stepping in neonates (Thelen et al., 1982).

Role of sensory signals

In addition to intraspinal and supraspinal connections between the central pattern generators of each limb, sensory signals play a crucial role in interlimb coordination (Duysens et al., 2000; Pang and Yang, 2002; Pearson, 2004; Rossignol et al., 2006; Grillner and El Manira, 2020; Frigon et al., 2021). Two main sources of feedback have been uncovered: load signals and hip position signals. During stance, the load signals increase the extensor activity and suppress the onset of swing (Duysens and Pearson, 1980). At the end of stance, loading decreases and swing can start. Here, evidence for loading responses in neonates was provided by the observation that limb extensor muscles were activated at midstance, and the initially flexed knee underwent a large

extension during both unilateral and bilateral stepping (Fig. 9). The other important sensory signal is related to hip position (Andersson and Grillner, 1981). At the end of stance, the start of the swing phase is triggered by hip extension, as signaled by the stretch of hip flexor muscles (Hiebert et al., 1996). Here, we found evidence for the modulation of EMG activity dependent on hip position in the blocked limb during unilateral stepping. Thus, the mean amplitude and duration of activation of the hip flexor RF was significantly greater with the limb blocked in the posterior position and extended at the hip than in the other positions (Fig. 7). The opposite trend was observed for the hip extensor BF. Moreover, the sudden release of the blocked limb in the posterior position elicited the immediate initiation of the swing phase of the limb now free to move, associated with hip flexion and a burst of ankle flexor TA (Fig. 8). Again, this is reminiscent of the behavior of chronic spinal cats (Grillner and Rossignol, 1978). When the hindlimb of these animals is manually brought to an extended position and then released, a flexor burst is immediately generated, consistent with the role of hip extension receptors in the initiation of the swing phase (Grillner and Rossignol, 1978). Here, the hip position of the blocked limb affected the limb posture of the contralateral stepping limb (Fig. 6), but this effect might be partly because of biomechanical coupling between the limbs via the trunk.

Comparison with more mature locomotion

The highly variable interlimb coordination and its incomplete sensory modulation suggest that in human newborns the locomotor networks do not operate in the same manner as in mature locomotion. In fact, the automatic stepping of newborns differs from mature walk in several aspects, such as the digitigrade, rather than the plantigrade, foot contact, hyperflexion of the lower limbs during swing, high interstep variability, and the presence of simple flexion–extension patterns of muscle activity compared with the more complex profiles with multiple activity bursts in adults (Forssberg, 1985; Yang et al., 1998b; Dominici et al., 2011). At birth, the full four-phase locomotor pattern (support, lift up, swing, and touch down) is not yet developed, and the stepping movements consist simply of an alternation between flexors and extensors (Forssberg, 1985; Yang et al., 1998b; Sylos-Labini et al., 2020). By tracking the development of ground stepping in 4- to 48-month-old infants, it was found that the number of temporal patterns of muscle activation increased progressively after the age of 6 months, reaching adult-like conformation only after independent, unsupported walking was established (Sylos-Labini et al., 2020). Also the patterns of interlimb coordination of older infants are more similar to the adult than those of neonates. Thelen and Ulrich (1991) conducted a longitudinal study on children 1–7 months of age stepping on a treadmill. They found that the percentage of alternating relative to synchronous steps increased with age. Moreover, extensive work on infants ~6 months old showed that transient perturbations to one stepping limb produce highly coordinated, bilateral responses (Yang et al., 1998a, 2005; Pang and Yang, 2001; Musselman and Yang, 2008). However, interlimb coupling remains variable even in these infants, and at 14 months still is far from the tight alternation at 50% of the gait cycle of adults (Yang et al., 2015).

References

- Andersson O, Grillner S (1981) Peripheral control of the cat's step cycle I. Phase dependent effects of ramp-movements of the hip during "fictive locomotion". *Acta Physiol Scand* 113:89–101.

- Apgar V (1953) A proposal for a new method of evaluation of the newborn infant. *Curr Res Anesth Analg* 32:260–267.
- Arber S (2012) Motor circuits in action: specification, connectivity, and function. *Neuron* 74:975–989.
- Batschelet E (1981) Circular statistics in biology. London, New York: Academic.
- Bernhardt NR, Memic F, Kullander K (2013) Genetic analysis of left-right coordination of locomotion. *Front Biosci (Landmark Ed)* 18:21–35.
- Branchereau P, Morin D, Bonnot A, Ballion B, Chapron J, Viala D (2000) Development of lumbar rhythmic networks: from embryonic to neonate locomotor-like patterns in the mouse. *Brain Res Bull* 53:711–718.
- Butt SJB, Lebreton JM, Kiehn O (2002) Organization of left-right coordination in the mammalian locomotor network. *Brain Res Brain Res Rev* 40:107–117.
- Clarac F, Brocard F, Vinay L (2004) The maturation of locomotor networks. *Prog Brain Res* 143:57–66.
- Dambreville C, Labarre A, Thibaudier Y, Hurteau M-F, Frigon A (2015) The spinal control of locomotion and step-to-step variability in left-right symmetry from slow to moderate speeds. *J Neurophysiol* 114:1119–1128.
- Dewolf AH, Mesquita RM, Willems PA (2020) Intra-limb and muscular coordination during walking on slopes. *Eur J Appl Physiol* 120:1841–1854.
- Dewolf AH, Sylos-Labini F, Cappellini G, Zhvansky D, Willems PA, Ivanenko Y, Lacquaniti F (2021) Neuromuscular age-related adjustment of gait when moving upwards and downwards. *Front Hum Neurosci* 15:749366.
- Dietz V, Müller R, Colombo G (2002) Locomotor activity in spinal man: significance of afferent input from joint and load receptors. *Brain* 125:2626–2634.
- Dominici N, Ivanenko YP, Lacquaniti F (2007) Control of foot trajectory in walking toddlers: adaptation to load changes. *J Neurophysiol* 97:2790–2801.
- Dominici N, Ivanenko YP, Cappellini G, d'Avella A, Mondì V, Cicchese M, Fabiano A, Silei T, Di Paolo A, Giannini C, Poppele RE, Lacquaniti F (2011) Locomotor primitives in newborn babies and their development. *Science* 334:997–999.
- Duysens J (1977) Reflex control of locomotion as revealed by stimulation of cutaneous afferents in spontaneously walking prenatally cats. *J Neurophysiol* 40:737–751.
- Duysens J, Pearson KG (1980) Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. *Brain Res* 187:321–332.
- Duysens J, Clarac F, Cruse H (2000) Load-regulating mechanisms in gait and posture: comparative aspects. *Physiol Rev* 80:83–133.
- Einspieler C, Prayer D, Marschik PB (2021) Fetal movements: the origin of human behaviour. *Dev Med Child Neurol* 63:1142–1148.
- Eyre JA (2007) Corticospinal tract development and its plasticity after perinatal injury. *Neurosci Biobehav Rev* 31:1136–1149.
- Ferris DP, Gordon KE, Beres-Jones JA, Harkema SJ (2004) Muscle activation during unilateral stepping occurs in the nonstepping limb of humans with clinically complete spinal cord injury. *Spinal Cord* 42:14–23.
- Forssberg H (1985) Ontogeny of human locomotor control I. Infant stepping, supported locomotion and transition to independent locomotion. *Exp Brain Res* 57:480–493.
- Forssberg H (1992) Evolution of plantigrade gait: is there a neuronal correlate? *Dev Med Child Neurol* 34:920–925.
- Forssberg H, Grillner S, Halbertsma J, Rossignol S (1980) The locomotion of the low spinal cat. II. Interlimb coordination. *Acta Physiol Scand* 108:283–295.
- Frigon A, Desrochers É, Thibaudier Y, Hurteau M-F, Dambreville C (2017) Left-right coordination from simple to extreme conditions during split-belt locomotion in the chronic spinal adult cat. *J Physiol* 595:341–361.
- Frigon A, Akay T, Prilutsky BI (2021) Control of mammalian locomotion by somatosensory feedback. *Compr Physiol* 12:2877–2947.
- Goulding M (2009) Circuits controlling vertebrate locomotion: moving in a new direction. *Nat Rev Neurosci* 10:507–518.
- Grillner S (1985) Neurobiological bases of rhythmic motor acts in vertebrates. *Science* 228:143–149.
- Grillner S, El Manira A (2020) Current principles of motor control, with special reference to vertebrate locomotion. *Physiol Rev* 100:271–320.
- Grillner S, Rossignol S (1978) On the initiation of the swing phase of locomotion in chronic spinal cats. *Brain Res* 146:269–277.
- Grillner S, Zangger P (1979) On the central generation of locomotion in the low spinal cat. *Exp Brain Res* 34:241–261.
- Häggglund M, Dougherty KJ, Borgius L, Itoharu S, Iwasato T, Kiehn O (2013) Optogenetic dissection reveals multiple rhythmic modules underlying locomotion. *Proc Natl Acad Sci U S A* 110:11589–11594.
- Harnie J, Côté-Sarrazin C, Hurteau M-F, Desrochers E, Doelman A, Amhis N, Frigon A (2018) The modulation of locomotor speed is maintained following partial denervation of ankle extensors in spinal cats. *J Neurophysiol* 120:1274–1285.
- Hiebert GW, Whelan PJ, Prochazka A, Pearson KG (1996) Contribution of hind limb flexor muscle afferents to the timing of phase transitions in the cat step cycle. *J Neurophysiol* 75:1126–1137.
- Hurteau M-F, Frigon A (2018) A spinal mechanism related to left-right symmetry reduces cutaneous reflex modulation independently of speed during split-belt locomotion. *J Neurosci* 38:10314–10328.
- Hurteau M-F, Thibaudier Y, Dambreville C, Chraïbi A, Desrochers E, Telonio A, Frigon A (2017) Nonlinear modulation of cutaneous reflexes with increasing speed of locomotion in spinal cats. *J Neurosci* 37:3896–3912.
- Ivanenko YP, Poppele RE, Lacquaniti F (2006) Motor control programs and walking. *Neuroscientist* 12:339–348.
- Ivanenko YP, Dominici N, Cappellini G, Di Paolo A, Giannini C, Poppele RE, Lacquaniti F (2013) Changes in the spinal segmental motor output for stepping during development from infant to adult. *J Neurosci* 33:3025–3036a.
- Jiang Z, Carlin KP, Brownstone RM (1999) An in vitro functionally mature mouse spinal cord preparation for the study of spinal motor networks. *Brain Res* 816:493–499.
- Kawashima N, Nozaki D, Abe MO, Akai M, Nakazawa K (2005) Alternate leg movement amplifies locomotor-like muscle activity in spinal cord injured persons. *J Neurophysiol* 93:777–785.
- Kiehn O (2016) Decoding the organization of spinal circuits that control locomotion. *Nat Rev Neurosci* 17:224–238.
- Kiehn O, Kjaerulff O (1996) Spatiotemporal characteristics of 5-HT and dopamine-induced rhythmic hindlimb activity in the in vitro neonatal rat. *J Neurophysiol* 75:1472–1482.
- Kremer E, Lev-Tov A (1998) GABA-receptor-independent dorsal root afferents depolarization in the neonatal rat spinal cord. *J Neurophysiol* 79:2581–2592.
- Kudo N, Yamada T (1987) Morphological and physiological studies of development of the monosynaptic reflex pathway in the rat lumbar spinal cord. *J Physiol* 389:441–459.
- Kudo N, Nishimaru H, Nakayama K (2004) Developmental changes in rhythmic spinal neuronal activity in the rat fetus. *Prog Brain Res* 143:49–55.
- Lacquaniti F, Ivanenko YP, Zago M (2012) Development of human locomotion. *Curr Opin Neurobiol* 22:822–828.
- Laffreniere-Roula M, McCrea DA (2005) Deletions of rhythmic motoneuron activity during fictive locomotion and scratch provide clues to the organization of the mammalian central pattern generator. *J Neurophysiol* 94:1120–1132.
- Landler L, Ruxton GD, Malkemper EP (2021) Advice on comparing two independent samples of circular data in biology. *Sci Rep* 11:20337.
- La Scaleia V, Ivanenko Y, Fabiano A, Sylos-Labini F, Cappellini G, Picone S, Paolillo P, Di Paolo A, Lacquaniti F (2018) Early manifestation of arm-leg coordination during stepping on a surface in human neonates. *Exp Brain Res* 236:1105–1115.
- Machado TA, Pnevmatikakis E, Paninski L, Jessell TM, Miri A (2015) Primacy of flexor locomotor pattern revealed by ancestral reversion of motor neuron identity. *Cell* 162:338–350.
- Martino G, Ivanenko YP, Serrao M, Ranavolo A, d'Avella A, Draicchio F, Conte C, Casali C, Lacquaniti F (2014) Locomotor patterns in cerebellar ataxia. *J Neurophysiol* 112:2810–2821.
- Maxwell DJ, Soteropoulos DS (2020) The mammalian spinal commissural system: properties and functions. *J Neurophysiol* 123:4–21.
- McCrea DA, Rybak IA (2007) Modeling the mammalian locomotor CPG: insights from mistakes and perturbations. *Prog Brain Res* 165:235–253.
- Musselman KE, Yang JF (2008) Interlimb coordination in rhythmic leg movements: spontaneous and training-induced manifestations in human infants. *J Neurophysiol* 100:2225–2234.
- Nielsen JB (2003) How we walk: central control of muscle activity during human walking. *Neuroscientist* 9:195–204.

- Pang MYC, Yang JF (2001) Interlimb co-ordination in human infant stepping. *J Physiol* 533:617–625.
- Pang MYC, Yang JF (2002) Sensory gating for the initiation of the swing phase in different directions of human infant stepping. *J Neurosci* 22:5734–5740.
- Pearson KG (2004) Generating the walking gait: role of sensory feedback. *Prog Brain Res* 143:123–129.
- Peiper A (1961) Cerebral function in infancy and childhood. New York: Springer.
- Piek JP (2002) The role of variability in early motor development. *Infant Behav Dev* 25:452–465.
- Rancic V, Gosgnach S (2021) Recent insights into the rhythmogenic core of the locomotor CPG. *Int J Mol Sci* 22:1394.
- Ritterband-Rosenbaum A, Herskind A, Li X, Willerslev-Olsen M, Olsen MD, Farmer SF, Nielsen JB (2017) A critical period of corticomuscular and EMG–EMG coherence detection in healthy infants aged 9–25 weeks. *J Physiol* 595:2699–2713.
- Rossignol S (2006) Plasticity of connections underlying locomotor recovery after central and/or peripheral lesions in the adult mammals. *Philos Trans R Soc Lond B Biol Sci* 361:1647–1671.
- Rossignol S, Dubuc R, Gossard J-P (2006) Dynamic sensorimotor interactions in locomotion. *Physiol Rev* 86:89–154.
- Siekerman K, Barbu-Roth M, Anderson DI, Donnelly A, Goffinet F, Teulier C (2015) Treadmill stimulation improves newborn stepping. *Dev Psychobiol* 57:247–254.
- Staude G, Wolf W (1999) Objective motor response onset detection in surface myoelectric signals. *Med Eng Phys* 21:449–467.
- Staude G, Flachenecker C, Daumer M, Wolf W (2001) Onset detection in surface electromyographic signals: a systematic comparison of methods. *EURASIP J Adv Signal Process* 2001:867853.
- Sylos-Labini F, Ivanenko YP, Cappellini G, Gravano S, Lacquaniti F (2011) Smooth changes in the EMG patterns during gait transitions under body weight unloading. *J Neurophysiol* 106:1525–1536.
- Sylos-Labini F, La Scaleia V, d'Avella A, Pisotta I, Tamburella F, Scivoletto G, Molinari M, Wang S, Wang L, van Asseldonk E, van der Kooij H, Hoellinger T, Cheron G, Thorsteinsson F, Ilzkovitz M, Gancet J, Hauffe R, Zanov F, Lacquaniti F, Ivanenko YP (2014) EMG patterns during assisted walking in the exoskeleton. *Front Hum Neurosci* 8:423.
- Sylos-Labini F, Magnani S, Cappellini G, La Scaleia V, Fabiano A, Picone S, Paolillo P, Di Paolo A, Lacquaniti F, Ivanenko Y (2017) Foot placement characteristics and plantar pressure distribution patterns during stepping on ground in neonates. *Front Physiol* 8:784.
- Sylos-Labini F, La Scaleia V, Cappellini G, Fabiano A, Picone S, Keshishian ES, Zhvansky DS, Paolillo P, Solopova IA, d'Avella A, Ivanenko Y, Lacquaniti F (2020) Distinct locomotor precursors in newborn babies. *Proc Natl Acad Sci U S A* 117:9604–9612.
- Talpalár AE, Bouvier J, Borgius L, Fortin G, Pierani A, Kiehn O (2013) Dual-mode operation of neuronal networks involved in left-right alternation. *Nature* 500:85–88.
- Ten Donkelaar HJ, Lammens M, Hori A (2014) Clinical neuroembryology: development and developmental disorders of the human central nervous system. New York: Springer.
- Thelen E, Fisher DM (1982) Newborn stepping: an explanation for a “disappearing” reflex. *Dev Psychol* 18:760–775.
- Thelen E, Ulrich BD (1991) Hidden skills: a dynamic systems analysis of treadmill stepping during the first year. *Monogr Soc Res Child Dev* 56:1–98.
- Thelen E, Fisher DM, Ridley-Johnson R, Griffin NJ (1982) Effects of body build and arousal on newborn infant stepping. *Dev Psychobiol* 15:447–453.
- Whelan P, Bonnot A, O'Donovan MJ (2000) Properties of rhythmic activity generated by the isolated spinal cord of the neonatal mouse. *J Neurophysiol* 84:2821–2833.
- Williams PTJA, Jiang Y-Q, Martin JH (2017) Motor system plasticity after unilateral injury in the developing brain. *Dev Med Child Neurol* 59:1224–1229.
- Yakovenko S, Mushahwar V, VanderHorst V, Holstege G, Prochazka A (2002) Spatiotemporal activation of lumbosacral motoneurons in the locomotor step cycle. *J Neurophysiol* 87:1542–1553.
- Yang JF, Gorassini M (2006) Spinal and brain control of human walking: implications for retraining of walking. *Neuroscientist* 12:379–389.
- Yang JF, Stephens MJ, Vishram R (1998a) Transient disturbances to one limb produce coordinated, bilateral responses during infant stepping. *J Neurophysiol* 79:2329–2337.
- Yang JF, Stephens MJ, Vishram R (1998b) Infant stepping: a method to study the sensory control of human walking. *J Physiol* 507:927–937.
- Yang JF, Lam T, Pang MYC, Lamont E, Musselman K, Seinen E (2004) Infant stepping: a window to the behaviour of the human pattern generator for walking. *Can J Physiol Pharmacol* 82:662–674.
- Yang JF, Lamont EV, Pang MYC (2005) Split-belt treadmill stepping in infants suggests autonomous pattern generators for the left and right leg in humans. *J Neurosci* 25:6869–6876.
- Yang JF, Mitton M, Musselman KE, Patrick SK, Tajino J (2015) Characteristics of the developing human locomotor system: similarities to other mammals. *Dev Psychobiol* 57:397–408.