Video Article

Asymmetric Walkway: A Novel Behavioral Assay for Studying Asymmetric Locomotion

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Abstract

Behavioral assays are commonly used for the assessment of sensorimotor impairment in the central nervous system (CNS). The most sophisticated methods for quantifying locomotor deficits in rodents is to measure minute disturbances of unconstrained gait overground (e.g., manual BBB score or automated CatWalk). However, cortical inputs are not required for the generation of basic locomotion produced by the spinal central pattern generator (CPG). Thus, unconstrained walking tasks test locomotor deficits due to motor cortical impairment only indirectly. In this study, we propose a novel, precise foot-placement locomotor task that evaluates cortical inputs to the spinal CPG. An instrumented peg-way was used to impose symmetrical and asymmetrical locomotor tasks mimicking lateralized movement deficits. We demonstrate that shifts from equidistant inter-stride lengths of 20% produce changes in the forelimb stance phase characteristics during locomotion with preferred stride length. Furthermore, we propose that the asymmetric walkway allows for measurements of behavioral outcomes produced by cortical control signals. These measures are relevant for the assessment of impairment after cortical damage.

Video Link

The video component of this article can be found at <http://www.jove.com/video/52921/>

Introduction

Post-stroke morbidity in the surviving population includes gross motor impairments that pose a challenge for quantitative evaluation in both humans post stroke and animal models of neurologic impairment¹. In the clinical setting, these motor impairments are measured using subjective criteria which are more sensitive to severe rather than moderate impairment exhibited by the majority of patients. Similarly, such subjective assessments of post-injury motor behavior in animals are common, e.g., the Basso, Beattie, and Bresnahan (BBB) locomotor scale method^{2,3}. While these subjective evaluation methods are helping translation between gait rehabilitation studies in quadruped animal models and humans, the details of motor deficits associated with activity of separate muscle groups are not assessed. Moreover, the assessment of motor cortical contribution to locomotion, as the putative culprit of motor deficit in cerebrovascular accident, can only be obtained indirectly even using the most novel automated quantitative methods^{4,5}, as they rely on open-field or linear walking tasks. These tasks do not require cortical contribution and can be performed by the neural mechanisms of the spinal cord, i.e., the central pattern generator (CPG) network which is spared in most animal models of neural damage, e.g., spinalized animals⁶⁻⁸. Essential cortical contribution to these spinal mechanisms has been experimentally implicated in tasks that require anticipated postural adjustments⁹ and reaching¹⁰, as well as precise stepping¹⁰.

Moreover, most neurological damage is asymmetric; for example, stroke causes hemiparesis, i.e., weakness on one side of the body, which results in an asymmetric gait¹¹⁻¹⁴. The asymmetry of hemiplegic gait is produced by asymmetric spatiotemporal muscle activation most significantly manifested in the shortening of the extensor-associated stance phase and the lengthening of the flexor-associated swing phase of the step cycle on the paretic side^{15,16}. This trend has not yet been explored across a range of locomotor speeds in healthy or paretic animals. In the current study, we employed the analysis of phase duration characteristics¹⁷ that describes the relationship between the duration of swing or stance phases as a function of cycle duration in each step. The obtained linear regression model was then further described with an analysis of asymmetry across all limbs.

We report a novel low-cost method for assessing the activity of descending cortical inputs in the motor system of quadruped animals based on a precise stepping locomotor task. This task is designed to challenge the motor cortex by imposing demands on foot placement over a natural range of walking speeds. In addition, foot-placement requirements are manipulated to preferentially challenge the left or right side of the motor system. In a similar locomotor task, Metz & Whishaw (2009) examined the rates of failure, the number of missed steps on irregular rung walkway, in rats. Our method is complimentary to this previous study, and it details the quality of phase control in "successful" steps¹⁸.

Protocol

The following training paradigm employs the analysis of phase adjustments of the average adult Sprague-Dawley rat. Please ensure that the protocol described herein is in accordance with your institutional animal care guidelines. All procedures in this study were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) and Office for Laboratory Animal Welfare (OLAW) at West Virginia University School of Medicine and abides by the National Institutes of Health guidelines for the use of experimental animals.

1. Equipment Setup

- Construct the asymmetric walkway as an open-top plastic box braced with aluminum supports at each corner measuring 155 cm x 104 cm (**Figure 1**). Brace the top edges of the box with aluminum bars grooved on both sides to allow for alternate peg placement, along the perimeter of the box, so that each consecutive peg on the same side defines the stride length.
- Place a 20 cm x 20 cm platform on each corner (four total) separating the conditions represented on each side. This distance should be sufficient for the inclusion of the distance traversed by a single rat step cycle.
 - Use pegs made of aluminum with dimensions of 20 cm x 1 cm x 0.5 cm. Bend the top of each peg 2.5 cm from the tip to produce a foot placement platform.
 - Secure the pegs to the grooved bars using sliding inside brackets through machined holes at the same distance to ensure level horizontal placement. Adjust positions using a screwdriver and a ruler. Use a 1 cm peg width that corresponds approximately to the average rat paw size; thinner or wider pegs are either uncomfortable or increase the foot placement variability.
- Manipulate the peg placement on each side to produce one of three precise stepping challenge conditions.
 - Produce a symmetric locomotor task with a 15 cm stride length (SL15) by setting the left inter-stride length (lSL) and right inter-stride length (rSL) to the half of stride length (7.5 cm).
 - Impose an additional symmetric condition (SL12) by changing lSL and rSL lengths to 6.0 cm.
 - Produce the asymmetric tasks by changing the distance between pegs on the left and right sides, termed the inter-stride length. To challenge the motor system asymmetrically, change the lSL and rSL by 20% to impose short inter-stride lengths either on the left (L6R9 condition) or on the right (L9R6) side. The 1.5 cm perturbations impose an lSL of 6 cm and rSL of 9 cm for the L6R9 condition, or an lSL of 9 cm and a rSL of 6 cm for the L9R6 condition.
- For rats, keep the stride length for all conditions except for SL12 at a preferred 15 cm.
- For convenience, assign each long side of the walkway an asymmetric condition favoring either the left or the right side of the subject, while reserving the two short sides for the symmetric control condition.
- Setup a high definition camera with a sampling rate of at least 60 Hz so that the placement of limbs on pegs is unobstructed with camera pointing perpendicularly to the walkway with the field of view covering about 7 steps. The first and last steps in proximity to platforms are ignored.

2. Training on Apparatus

- Please use standard training resources, e.g., NIH Training in Basic Biotechnology for Laboratory Rats, to familiarize with general behavioral training of rodents.
- In the beginning of training, acclimate subjects by placing and rewarding them on the 20 x 20 cm platform for at least 5 min. Then, guide the animals across a peg arrangement with a 1 cm inter-stride length to the next platform by the presentation of a food reward. Reward animals verbally and with petting for reaching the platform.
- After 5 training runs, space the pegs an extra 1 - 2 cm apart and perform the next 5 training runs. The number of repetitions listed herein is sufficient to produce statistically appropriate sample size (20 - 35 steps).
 - If the animal acquires the task more slowly as judged by consistency of stepping (no stopping) and posture (arched back), then focus training on the strengthening of these skills at the short stride lengths (S12) before resuming training on the long strides (S15) eventually approaching the desired stride length.
 - If the new spacing induces anxiety or discomfort with the task, readjust the pegs to the previous setting and repeat the training paradigm.
 - Proceed with this training until the appropriate inter-stride lengths are achieved for the four conditions and locomotor standards are met. In our experience, the rats respond well to vocal encouragement as cues for initiating a trial. The testing can be done on the same day as training provided the subjects are motivated to perform the task.

Note: The locomotor standards are as follows: walking is consistent and does not involve stops or missteps; head-bobbing is minimal; the back is arched and the tail is raised during locomotion; each limb is clearly visible from an orthogonal view of the walkway at the onset and offset of the stance phase. This selection process is essential as the present study focuses only on walking rather than other gaiting behavior.

3. Testing and Data Analysis

- Test animals on S12, S15, L9R6, and L6R9 tasks (described in section 1.3) using randomized session design. Use breaks to avoid adaptation within a task.
- Record sessions with high definition camera with a sampling rate of at least 60 Hz. Import video recordings without re-sampling into video editing software and select only the walking bouts for further analysis.
- Mark onsets and offsets of kinematic phases in video recordings from each subject.

4. Here, use the custom software called videoa written in Matlab to manually identify the time of stance onset and offset for each limb on a frame-by-frame basis, where stance onset is indicated by the loss of motion blur associated with the limb placement on a peg, and stance offset, occurring at the onset of limb lift-off, is indicated by the first evidence of motion blur.
5. Calculate the duration of swing phase as the time remaining between two consecutive kinematic stance onsets. Exclude any behavior not consistent with overground quadrupedal walking, e.g., when gait contains a double swing phase (both forelimbs or hindlimbs off the ground), from proceeding analyses.
6. Plot the duration of each phase as a function of the corresponding step cycle duration. Capture the relationship with the linear regression model ($T_{\text{phase}} = B1 + B2 \cdot T_c$) obtained for each limb, where T_c is cycle duration, T_{phase} is either T_e extensor-related stance or T_f , which is the flexor-related swing, and $B1$ and $B2$ are empirical constants (offset and slope) of the regression model.
Note: The slope ($B2$) represents the amount of change in phase duration with the change in speed of locomotion.
7. Use Equations 1 and 2 (**Figure 2C**) for each limb to calculate asymmetry index (AI). Both equations have the same form of a simple ratio that normalizes the difference of two values to their sum.
 1. Using Equation 1, calculate the horizontal difference (AI_h) that uses the difference between slopes of stance modulation left (l) and right (r) limbs. Similarly, calculate the vertical asymmetry (AI_v) using the slopes from front/anterior (a) and back / posterior (p) limbs. The result of applying these two equations is the dataset of 4 x-y points corresponding to 1) forelimb asymmetry, aAI_h ; 2) hindlimb asymmetry, pAI_h ; 3) left forelimb-hindlimb asymmetry, lAI_v ; 4) right forelimb-hindlimb asymmetry, rAI_v .
 2. Plot these values as a patch (**Figure 2B**) for the visual representation of asymmetry across all limbs.
8. Calculate diagonality indices (DI) to assess diagonal coupling between parameters of a forelimb and its contralateral hindlimb (Equation 3, **Figure 2C**).
9. Test the DI, as well as the difference of four AIs between conditions of opposing asymmetry ($\Delta AI = |AIL9R6 - AIL6R9|$) for statistical significance using a one-way ANOVA with the post-hoc comparison of means analysis¹⁹

Representative Results

Figure 2 shows the analysis of asymmetry during the locomotor tasks for a single representative subject. The values were calculated for all conditions using Equation 1 and 2 from all subjects individually (**Figure 2**) and from composite data of 8 female Sprague-Dawley rats (250 - 400 g, **Figure 3**). Generally, the modulation of the forelimb stance phase was lesser for the side to which the locomotion condition was favored (short ISL), consistent with the notion that the stance phase on the preferred side (long ISL) tended to occupy a greater portion of the step cycle as compared to the favored limb as the speed of locomotion decreases.

The difference between corresponding asymmetry indices obtained from conditions L9R6 and L6R9 (ΔAI) were tested with a one-way ANOVA ($\alpha = 0.05$) and post-hoc t-tests with conservative Bonferroni correction (adjusted $\alpha = 0.0125$) using *anova1* and *multcompare* functions in Matlab. Overall, the difference between groups was significant ($p = 0.002$). The anterior horizontal asymmetry index (ΔaAI_h) corresponding to the asymmetry between forelimbs was significantly different ($p = 0.006$) between the left-favored (L6R9) and the right-favored (L9R6) conditions (**Figure 4A**). The difference between the conditions for the right vertical asymmetry index (ΔrAI_v) showed a trend, but it was not significantly different from zero ($p = 0.031$, $\alpha = 0.0125$). Similarly, we found a significant difference ($p = 0.020$, $\alpha = 0.05$) in the diagonality index between two asymmetric conditions (**Figure 4B**). ANOVA testing found no differences between DI in different tasks, but there was only a single post-hoc t-test, which required no additional alpha correction.

As this method relies on the animals' natural ability to solve the asymmetric foot placement, some animals may exhibit gallop-like behavior where the posterior limbs were simultaneously in swing. This gait was observed in 3 animals, and the behavior was excluded from further analyses.

Figure 1. Walkway Model. (A) Schematic of the walkway used for the symmetric and asymmetric gait tasks. (B) Peg arrangement setting the right ($rISL$) and left ($lISL$) inter-stride lengths in relation to the stride length (SL). The four conditions include a symmetrical control locomotor task of stride length (SL) of 15 cm (S15), a symmetrical locomotor task representing a 20% reduction in SL and preferred speed (S12), a left limb preferred (L9R6) and a right limb preferred (L6R9) locomotor task. [Please click here to view a larger version of this figure.](#)

Figure 2. Calculation of Asymmetry and Diagonality Indices. (A) The relationship between stance or swing phase duration (y-axis) and cycle duration (x-axis) for left-limb favored gait (L6R9) is represented by the regression analysis and the heat map of data point density. The phase characteristics were represented with the stance phase linear regressions using the slope-intercept equations. The insets correspond to the left forelimb (LF), right forelimb (RF), left hindlimb (LH) and right hindlimb (RH) heat maps. (B) Asymmetry index calculated as shown in Equations (1) and (2), where r , l , a and p -slopes of the stance phase linear regressions, as shown in (A) for the right, left, anterior and posterior limbs, respectively. lAI_v , rAI_v , aAI_h and pAI_h - left-vertical, right vertical, fore-horizontal and hind-horizontal asymmetry indices, respectively, calculated for all four conditions described in Figure 1. (C) Diagonality indices (DIs) calculated as shown in Equation (3) for all four conditions described in Figure 1. lF , rF , lH and rH -left forelimb, right forelimb, left hindlimb and right hindlimb stance phase linear regression slopes. [Please click here to view a larger version of this figure.](#)

Figure 3. Composite Data for Asymmetry and Diagonality using Phase Characteristics from All 8 Subjects. (A) Heat map representing the distribution of stance or swing versus cycle duration for left-limb favored gait (L9R6). The phase characteristics of the stance phase linear regression were calculated as in Figure 1A, and are represented by the slope-intercept formula inset. (B) Asymmetry index calculated as shown in Figure 1B. ΔlAI_v , ΔrAI_v , ΔaAI_h and ΔpAI_h - left-vertical, right vertical, anterior-horizontal and posterior-horizontal asymmetry index differences, respectively, calculated for all four conditions as described in Equation 3 by subtracting the corresponding asymmetry indices of the right-favored gait (L6R9) from the left-favored gait (L9R6) conditions. Asterisk - statistical significance as calculated by the Bootstrap method. [Please click here to view a larger version of this figure.](#)

Figure 4. Analysis of Asymmetric Measures. (A). Absolute difference in asymmetric indices (AI) between conditions L9R6 and L6R9 was tested with one-way ANOVA with post-hoc t-test analysis adjusted with the Bonferroni correction for multiple tests. The change in forelimb asymmetry (ΔAI_h) between L9R6 and L6R9 was significant. (B) Analysis of distribution of diagonality indices (DI) of conditions S15, S12, L9R6 and L6R9 using one-way ANOVA with the post-hoc t-test of the difference between asymmetric tasks (black). [Please click here to view a larger version of this figure.](#)

Discussion

The rationale for this study was to develop a behavioral task that quantitatively assesses the changes in precise control of asymmetric locomotor behaviors. The existence of the spinal CPG has been functionally demonstrated for some time²⁰, but the anatomical and functional characteristics that describe its mechanism as well as its modulatory inputs from descending or sensory feedback pathways have not been characterized until the past decade^{6,21,22}. The current consensus is that the intrinsic spinal, sensory feedback, and descending commands are tightly integrated in the generation of locomotor behavior¹.

The asymmetric precise foot placement task presented herein is designed to functionally challenge the control systems responsible for the dexterous asymmetric control of stepping known to require cortical inputs^{23,24}. This performance was assessed relative to the symmetric tasks that are less reliant on the descending cortical and brainstem control. Thus, we have an opportunity to discern the contributions of the spinal and descending pathways. Since the motor cortex is directly involved in the modulation of muscle phases during locomotion, reaching and postural adjustments^{9,10,25}, the analysis of phase modulation in response to imposed asymmetric precise stepping tasks provides a basis for describing changes in volitional control. This is evident in the significant lateralized phase modulation between left- and right-favored tasks, characterized by the differences in asymmetry indices. We have also observed changes in whole body coordination that required diagonal coupling between contralateral forelimbs and hindlimbs, characterized by differences in the diagonal index.

Both focal stroke^{26,27} and spinal cord hemilection^{28,29} animal models cause mild to moderate movement deficits akin to those observed clinically. In animal models, cortical lesioning of the corticospinal tract impedes or prevents precise stepping^{30,31}. The application of our methodology to the characterization of cortical impairment in stroke models is yet to be described, though some of our preliminary data on rats with middle cerebral artery occlusions showed increased AI, and even a negative slope of the stance phase with increasing cycle duration for the limb on the side contralateral to stroke. This may correspond to a delay in the onset of consecutive locomotor phases, which is consistent with an asymmetry in both the step length ratio and the single limb support time observed in post-stroke patients^{15,32}.

One limitation of this method is that it is inappropriate for the analysis of severely affected animals. However, this subgroup is not necessarily the focus of attention in studies of hemiparetic animals. Furthermore, subjective tracking of this type of deficit requires additional sub scales that may also be associated with high inter-rater variability, creating demand for gross computational methodology³³. Thus, the challenge remains not in the assessment of deficits in the severely affected animals, but in the assessment of the mild to severe subgroup. Moreover, the ability to distinguish damage to specific hierarchical areas has been virtually impossible in a non-invasive method. The experimental setup presented herein is an effective tool for the evaluation of moderate impairment by monitoring modulatory activity of the motor antagonistic phases that drive the CPG with different speed demands, presumably contributed by higher order factors of the motor control hierarchy⁶.

Disclosures

The authors have nothing to disclose.

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