



Published in final edited form as:

Anat Rec (Hoboken). 2014 October ; 297(10): 1839–1864. doi:10.1002/ar.22955.

The Need for Speed in Rodent Locomotion Analyses

Richard J. Batka^{1,2,*}, Todd J. Brown^{1,2}, Kathryn P. Mcmillan^{1,2}, Rena M. Meadows^{1,2,3}, Kathryn J. Jones^{1,2}, and Melissa M. Haulcomb^{1,2}

¹Department of Anatomy & Cell Biology, Indiana University School of Medicine, 635 Barnhill Drive, MS 5025 C, Indianapolis, Indiana

²R & D Services Richard L. Roudebush VA Medical Center, 1481 W. 10th Street, Mail Code, 151, Rm C-3074, Indianapolis, Indiana

³Stark Neurosciences Research Institute, Indiana University School of Medicine, Indianapolis, Indiana

Abstract

Locomotion analysis is now widely used across many animal species to understand the motor defects in disease, functional recovery following neural injury, and the effectiveness of various treatments. More recently, rodent locomotion analysis has become an increasingly popular method in a diverse range of research. Speed is an inseparable aspect of locomotion that is still not fully understood, and its effects are often not properly incorporated while analyzing data. In this hybrid manuscript, we accomplish three things: (1) review the interaction between speed and locomotion variables in rodent studies, (2) comprehensively analyze the relationship between speed and 162 locomotion variables in a group of 16 wild-type mice using the CatWalk gait analysis system, and (3) develop and test a statistical method in which locomotion variables are analyzed and reported in the context of speed. Notable results include the following: (1) over 90% of variables, reported by CatWalk, were dependent on speed with an average R^2 value of 0.624, (2) most variables were related to speed in a nonlinear manner, (3) current methods of controlling for speed are insufficient, and (4) the linear mixed model is an appropriate and effective statistical method for locomotion analyses that is inclusive of speed-dependent relationships. Given the pervasive dependency of locomotion variables on speed, we maintain that valid conclusions from locomotion analyses cannot be made unless they are analyzed and reported within the context of speed.

Keywords

mice; mouse; rodent; gait; locomotion; CatWalk; speed; velocity

INTRODUCTION

The scientific study of locomotion has existed for more than 200 years (Vincent and Goiffon, 1779), and was initiated to understand the biomechanics and physiology of

*Correspondence to: Mr. Richard Batka, Post 635 Barnhill Drive, MS 5025 C, Indianapolis, Indiana 46202. rbatka@iupui.edu.

mammalian movement. More recently, it is used clinically as a quantifiable measure to improve rehabilitation, assess treatment strategies, determine disease onset, and monitor disease progression, among others. Due to the wide range of applications and the parallels between rodent and human locomotion, rodent locomotion has become an increasingly popular method to study models of human disease.

Rodent locomotion has been used extensively in the context of disease models such as Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (Amende et al. 2005; Hampton and Amende, 2010; Vandeputte et al. 2010; Mancuso et al. 2011), assessment of pharmacological agents (Masocha and Parvathy 2009; Wang et al. 2011), genetic mutations (Bothe et al. 2004; Crone et al. 2009), and stroke (Encarnacion et al. 2011; Hetze et al. 2012). Much research has been devoted to understanding the effects of trauma on the brain, spinal cord, and peripheral nerves, as well as the assessment of the efficacy of treatments used for these types of injuries (Koopmans et al. 2005; Deumens et al. 2007; Mountney et al. 2013).

Two common types of rodent locomotion analyses can be broadly categorized as either forced or unforced. Forced analyses are those where speed is imposed on the subject, for example, DigiGait and TreadScan, both of which are treadmill-based (Vincelette et al. 2007; Beare et al. 2009). Unforced analyses can include open field testing, in which the animal is free to move at any velocity. The CatWalk gait analysis system is a widely used unforced locomotion analysis method that utilizes a restricted pathway to encourage unidirectional movement. Developed in 1996 by Frank Hamers as an alternative to/evolution of the Basso, Beattie, and Bresnahan scale (Basso et al., 1995; Hamers et al. 2006), CatWalk was made commercially available by Noldus Information Technology in 2005. Each type of rodent locomotion analysis (i.e., forced and unforced) has its proponents; however, it has been shown the two are not completely analogous (Herbin et al., 2004; Pereira et al. 2006; Herbin et al. 2007; Wooley et al. 2009).

Forced analyses offer the benefit of precisely controlling for speed, but are unlike natural over-ground movement regarding visual, vestibular, and sensory feedback (Herbin et al. 2007) and are, therefore, unsuitable for certain research paradigms. Unforced analyses are often preferred because they more closely mimic natural locomotion. However, the main disadvantage of unforced analyses is the inability to ensure the subjects move at exactly the same speed. Variations in speed are problematic for standardizing data, particularly when using quantitative, rather than qualitative, measures. Surprisingly wide ranges in both intraindividual and interindividual speed have been reported when using an unforced apparatus in normal rodents (Górska et al., 1998; Clarke and Still 1999; Koopmans et al. 2007; Cendelín et al., 2010). Expectedly, speed has been reported to change significantly following experimental conditions across many models and injury paradigms (Hamers et al. 2001; Starkey et al. 2005; Koopmans et al. 2005; Vlamings et al. 2007; Angeby-Möller et al. 2008; Neumann et al. 2009; Hoffmann et al. 2010; Faizi et al. 2011; Wang et al. 2012; Mignon et al. 2013; Mountney et al. 2013). The inherent variability of observed speeds in normal and experimental animals makes direct comparisons inordinately difficult.

In an attempt to mitigate this issue, some investigators have used restrictive analysis, a method that only includes data from runs that fell within defined ranges of speed or crossing time. For instance, Deumens et al. (2007) and Bozkurt et al. (2008) analyzed data from runs in which the rodents crossed the apparatus between 1 and 2 sec. Speed binning is a more inclusive method that compares data within nonoverlapping speed ranges (0 cm/sec–10 cm/sec, 11 cm/sec–20 cm/sec, etc.); these ranges can be equal or unequal. Another type uses a single, wide speed range to compare data in which the outcome variables are thought to be unaffected by speed. However, these approaches are hindered by a lack of consensus as to which speeds are similar. For instance, Koopmans et al. (2007) considered speeds from 50 cm/sec through 100 cm/sec to be similar. Gabriel et al. considered a speed range of 70 cm/sec–80 cm/sec to be similar in 2007, and expanded the range to 60 cm/sec–80 cm/sec in 2009. Other investigators consider speeds to be equivalent if they were not statistically different (Kloos et al. 2005; Gensel et al. 2006; Hendriks et al. 2006; Truin et al. 2009; Sucher et al. 2010; Ferland et al. 2011). This disagreement most likely results from a lack of understanding of the extent, nature, or degree of dependence of locomotion variables on speed.

A small set of variables have been well-established regarding their dependence on speed, and the most common among them are step cycle, stand, swing, stride length, and cadence. In 1974, Heglund et al., used a treadmill to establish that speed is interrelated to stride frequency (cadence) and stride length in many animals, including rats and mice. Using unforced gait analysis in rats, Hruska et al. (1979) confirmed the relationships between stride length and speed, and also added the interrelations between swing, stance, and stride time with speed. In 1986, Clarke and Parker confirmed some of these relationships in rats, although they showed stride length to gradually increase after a plateau, while Hruska found stride length increased linearly then plateaued. Since these foundational studies, there have been others confirming these speed-variable relationships in rats (Westerga and Gramsbergen 1990; Górska et al., 1998; Gillis and Biewener 2001; Thota et al. 2005; Koopmans et al. 2007) and in mice (Heglund and Taylor 1988; Clarke and Still 1999; Clarke and Still 2001; Leblond et al. 2003; Herbin et al., 2004; Herbin et al. 2007; Crone et al. 2009).

Despite a strong, although somewhat controversial, history of the relationship between locomotion variables and speed in rodents, the picture is far from complete. The speed-dependency status of many variables is currently either unknown or only partially characterized. Some recent studies report the existence of a relationship between locomotion variables and speed, but not the nature of that relationship (Cendelín et al., 2010; Balkaya et al. 2013). Still, others presumed that all relationships with speed were linear in their analyses, as evidenced by the use of Pearson correlations and linear regressions (Koopmans et al. 2007; Neumann et al. 2009). While it is important to identify whether a specific variable depends on speed, it is also important to determine the nature of the relationship (e.g., linear or curvilinear) and degree of dependence (e.g., strong or weak), as both affect the methods used to standardize and analyze the data.

To our knowledge, there are no studies that have thoroughly and comprehensively examined the relationships between locomotion variables and speed on an unforced apparatus in rats or

mice, and only one publication dedicated to the effects of speed and strain using the CatWalk system in rats (Koopmans et al. 2007). Over 100 publications have used the CatWalk system, yet relatively few investigators have attempted to control for speed. Interestingly, Cendelín et al. (2010) lost all significant differences between experimental and control mice after normalizing for speed. Thus, speed is an important confounding factor in gait analyses that has not yet received a thorough treatment regarding both the speed-dependent relationships of locomotion variables or statistical control thereof.

In this study, we determined that nearly all locomotion variables reported by the CatWalk system are uniquely dependent on speed, and we present a flexible and powerful statistical method that can incorporate speed and other confounding factors inherent in locomotion analyses. Although we used only wild-type (WT) mice on the CatWalk system, the information presented here is applicable to almost any locomotion study in which speed is not imposed on the subject.

MATERIALS AND METHODS

Animals

Sixteen female WT mice (B6SJLF1/J) were purchased from The Jackson Laboratory (stock # 100012; Bay Harbor, ME) at post-natal day (PND) 33. All behavioral testing and housing occurred in accordance with institutional and National Institutes of Health guidelines on the care and use of laboratory animals for research purposes, and was approved by the Institutional Animal Care and Use Committee. Mice were housed under a 12-hr light/dark cycle in autoclaved microisolator cages under social conditions (four mice per cage), and provided autoclaved pellets and autoclaved drinking water *ad libitum*. The facility that housed the mice was equipped with a laminar flow system to maintain a pathogen-free environment. All mice were permitted 5 days to acclimate to the environment prior to behavioral testing.

CatWalk Description

Gait analysis was performed using the CatWalk Automated Gait Analysis System (Noldus Information Technology; software version XT 9.1). All data acquisition were completed in a dark, silent room. A general explanation of the system is provided here, but detailed descriptions can be found in Hamers et al. 2001 and Hamers et al. 2006. The CatWalk apparatus consists of a glass plate, suspended approximately 1-m parallel to the floor. Green LEDs illuminate the glass plate; however, light above background is only visible when contact is made with the glass. Resting perpendicular to the glass plate are two black plastic panels that create a corridor along the length of the plate, called the “runway.” Above the runway is a lid containing dim red LED lights which create a silhouette of the animal as viewed from the animal’s underside. Below the glass plate is a high-speed camera that records the scattered-light paw prints as the mouse crosses the runway (each crossing is referred to as a run). The software digitizes the paw print information and, following manual and automatic classification of the paw prints, produces over 355 variables into eight broad categories (Supporting Information Video 1).

CatWalk—Settings and Run Criteria

The runway was set to a width of 7 cm, and a length of 60 cm was recorded for this study. The high-speed camera recorded video at 100 frames-per-second (model: GP-2360C, GEViCAM), with a 6 mm lens set at an aperture of 1.2 (model: DF6HA-1B, Fujifilm). Camera gain was set to 23.40 and the intensity threshold set to 0.20. The 16-V power supply was used to illuminate the green LEDs. Our specific modification to the CatWalk apparatus included a cage secured to the end of the runway at an angle such that the mice could easily enter it on completion of a run. This cage contained the same bedding used in the housing cages. No food restriction or reward was used.

Mice were allowed on the CatWalk on alternate days for the duration of this study. A minimum of three compliant runs, up to a maximum of 20 overall runs (compliant and noncompliant), were acquired per day. Compliant runs were initially defined as those lasting longer than 0.10 sec, but shorter than 13.0 sec, with a maximum allowed speed variation 60% (see CatWalk—Variable Explanations and Context). Following acquisition and classification of paw prints (i.e., the appropriate paw was selected within the CatWalk software), each run was examined in slow motion and the timing view was inspected (Supporting Information Video 1). Post-acquisition compliance criteria included discarding runs that displayed behavioral anomalies (e.g., sniffing, looking, rearing) and those in which there was noticeable deviation from steady-state locomotion (e.g., acceleration or deceleration). On average, six runs per animal per day were analyzed, with a minimum of 18 and maximum of 35 steps per run. The glass runway was cleaned with Sparkle™ glass cleaner after the completion of a trial, which is a series of runs for one animal, or as needed to remove debris.

A total of 1,922 individual runs were observed, however, 41% of those were excluded based on post-acquisition criteria; the remaining 1,137 runs were analyzed. The large post-acquisition loss of runs indicates the convention of collecting 3–5 runs during acquisition may be insufficient for accurate analysis. The minimum speed was 25 cm/sec, and the maximum was 125 cm/sec, and each discrete speed was observed within a 100 cm/sec range.

CatWalk—Training and Acquisition Regimen

Testing began at PND 38 and lasted through PND 113; data acquisition began at PND 45, but presented here are data from 12 consecutive experimental days starting at PND 89. PND 93 was omitted from analysis due to audible construction noise during acquisition. Data prior to PND 89 were excluded due to optimization of the software and physical setup, technical difficulties, and to minimize any training effects (Wooley et al. 2009). No formal training period was defined *a priori*, and animals were first allowed to move at will on the CatWalk. If mice stopped or displayed uneven speed, they were encouraged to move by any one of a variety of faint auditory motivators/cues. Some motivators include clicking noises made with the investigator's mouth and noises produced by rubbing the fingers of a gloved

This article includes AR WOW Videos, which can be viewed at <http://bcove.me/njry9ilc>; <http://bcove.me/fydsbdzs> and <http://bcove.me/3pow0kr1>.

hand back and forth (Supporting Information Video 2). For particularly curious or stubborn animals, the lid of the CatWalk was lifted and the animal was gently guided to the arrival cage by the investigators hand. This process was repeated, successively two-to-three times and the animal allowed to resume at will.

Skill acquisition was accomplished quickly and by two weeks most animals ran evenly with only the occasional need to be guided. By the eighth day of testing, we discovered the mice preferred to traverse the outside of the runway rather than be carried from end-to-end. Following a completed run, the mice would drop into the arrival cage, then climb on the glass plate, outside the field of view of the camera, and move to the starting point whereupon the investigators hand served as a bridge for the mouse to enter the runway. We called this maneuver “the loop” (Supporting Information Video 3), and this acquisition regimen was exclusively used for the data presented.

CatWalk—Software

The CatWalk software offers three types of data exportation: (1) run data, which is the raw data for a run, (2) run statistics, which is the average of all data for a particular run, and (3) trial statistics, which is the average of all runs within that trial. A full run statistics export provides means for 210 variables, 145 of which have standard deviations (SDs), totaling 355 variables. The software segregates variables into eight predefined categories: Paw Statistics, Step Sequence, Base of Support (BOS), Other Statistics, Print Positions, Phase Dispersions, Couplings, and Support. For simplicity, these were regrouped into the following categories: Paw Statistics, Other Statistics, and Inter-Paw Coordination. Paw Statistics remained unchanged, while the Other Statistics category encompassed BOS, Print Positions, Support, and Step Sequence; the Inter-Paw Coordination category encompassed Phase Dispersions and Couplings.

Variables requiring additional classification, beyond selecting the appropriate paw, were not included in our analyses; these exclusions are sciatic functional index, toe spread, intermediate toe spread, manual print length, paw angle body axis, and paw angle movement vector for each paw. Variables representing SDs and those related to run number, run comment and so forth were also excluded. In total, 162 of the potential 355 variables are presented here.

CatWalk reports average speed to many decimal places, and is considered continuous. However, we rounded these to the nearest whole number and they are referred to as discrete speeds. In the experiment design section of the software, units of centimeters were selected; therefore, all dimensional values are reported in: cm, cm², and cm/sec.

CatWalk—Variable Explanations and Context

Paw statistics—Variables in the Paw Statistics category are the most widely used in recent studies, and include the four most common sets of variables used in historical studies; namely Step Cycle, Stand, Swing, and Stride Length. This category comprises 22 sets of variables that cover both static (i.e., time-independent variables, such as paw dimensions) and dynamic (i.e., time-dependent variables, such as Duty Cycle) aspects of locomotion.

Step Cycle (s) is the duration between two placements of the same paw and is defined as the sum of Stand and Swing. Stand (s) is the duration of contact between the paw and glass plate, while Swing (s) refers to the duration of no contact between the paw and glass plate. Duty cycle (%) is Stand expressed as a percentage of Step Cycle. Stand Index reflects the speed, in arbitrary units, at which the paw is removed from the glass plate. Stand Index is used in studies of stroke, limb transplantation, and movement disorders, among others (Sucher et al. 2010; Vandeputte et al. 2010; Encarnacion et al. 2011). Swing Speed is the speed of the paw during Swing, and it is calculated as the quotient of Stride Length and Swing.

CatWalk defines Stride Length as the distance between successive placements of the same paw. It is calculated as the hypotenuse from the center of the paw, to account for placements that are not directly in-line. Single Stance is defined as the duration of a Step Cycle in which one hind paw is in contact with the glass, but the contralateral hind paw is not. Single Stance is often used as a quantifiable outcome measure in the context of pain (Coulthard et al. 2002; Coulthard et al., 2003). Dual Stance (s) is the duration of a Step Cycle in which both hind paws are simultaneously in contact with the glass. There are two variables for Dual Stance, Initial and Terminal, and they reflect the first and second instance of Dual Stance within a Step Cycle, respectively.

Maximum Contact is defined by CatWalk as the largest part of a paw print that contacts the glass. Calculations for several variables incorporate this measurement, including Max Contact At (%), Max Contact Area, Max Contact Max Intensity, and Max Contact Mean Intensity. Max Contact At refers to the duration, from the start of a run, until Maximum Contact occurs. Although Max Contact At is not included in a run statistics export, it is used to calculate the Max Contact At (%) set of variables, which have been studied in the context of Parkinsonism and myofascial inflammation (Miyagi et al. 2011; Baiguera et al. 2012). Max Contact Area (cm²) is the area of the paw during Maximum Contact. Print Area (cm²) is the product of Print Length and Print Width.

The Intensity set of variables are widely studied in the context of pain, peripheral nerve injury, and inflammation (Vrinten and Hamers 2003; Masocha and Parvathy 2009; Lin et al. 2010). They reflect the degree of contact between the paw and the glass plate and are expressed in arbitrary units. The Intensity set of variables are analogous to pressure such that weak contact with the glass produces low intensity light and forceful contact with the glass produces high intensity light. Max Contact Max Intensity is the maximum intensity of a paw during Maximum Contact. Max Contact Mean Intensity is the average intensity during Maximum Contact. Max Intensity At (%) is the duration, from the start of a run, until maximum intensity is detected for its respective paw and is expressed as a percentage of Stand. Maximum Intensity is the maximum intensity detected for a complete paw while Minimum Intensity is the minimum intensity detected for a complete paw. Mean Intensity of the 15 Most Intense Pixels is an alternative measure to Maximum Intensity that is less affected by aberrantly bright pixels.

Other statistics—Variables in the Other Statistics category are widely studied historically and contemporarily, and include variables representing coordination as well as those

commonly associated with gaits (e.g., walking, trotting, running). Run Duration (s) is the duration of a run, and is the total number of frames in which the animal's silhouette was in the camera's field of view. Cadence is the number of steps per second. The Average Speed (cm/sec) of a run is generated during data acquisition and is based on the animal's silhouette, excluding the tail. In CatWalk, time is determined using first and last frame in which the silhouette is found, and the distance moved between those frames is used instead of the camera's full field of view, which is determined by the investigator during experiment setup in the CatWalk software.

Maximum Variation (%) of a run reflects the maximum change in speed, such that a high value indicates a large change in speed while a low value indicates relatively constant speed. If the animal paused or stopped, for instance, the Maximum Variation would be a higher % in comparison to an animal that moved consistently and uninterrupted across the runway. Maximum Variation is most commonly used as a criterion for selecting compliant runs within the CatWalk software and can be thought of as the SD of Average Speed, expressed as a percentage.

The CatWalk manual does not suggest a specific value for Maximum Variation, and the software defaults to 60%. Most investigators do not report the Maximum Variation value, but those who do have used the default value (Hetze et al. 2012; Kopecky et al., 2012; Balkaya et al. 2013) or a more restrictive value (Mountney et al. 2013). Initially, a threshold value of 60% was our sole criterion for run compliance; however, on examination of individual runs, we found this to be insufficient. Several factors can alter Maximum Variation, including acceleration, deceleration, pausing, and rearing, and these events can occur at any point during the run (e.g., beginning, middle, or end). Because CatWalk provides data as an average of a run, what variation and when it occurred is not accessible with Maximum Variation alone. Post-acquisition screening revealed many instances of runs with a low Maximum Variation, which were excluded from analysis due to deviations from steady-state locomotion or for behavioral anomalies (see Cat-Walk—Settings and run criteria). Conversely, there were instances of Maximum Variation being relatively high (~30%), but no discernible acceleration or deceleration could be found in slow motion or in the timing view.

Print Positions describes the spatial relationships of the ipsilateral front and hind paws (Fig. 6A). BOS is the distance between the front paws or the hind paws and reflects stance width. The Support category of variables relays the percentage of a run that an animal was supported by a specific number or combination of paws. For example, Support Three indicates the percentage of the run that an animal had three paws simultaneously in contact with the glass runway. Instances where two paws were simultaneously in contact with the glass are categorized into Support Diagonal, Support Girdle, or Support Lateral (Fig. 7A).

There are six variables representing the sequential placement of paws which, as a whole, are called the normal step sequence patterns (NSSPs). Originally defined by Cheng et al. (1997) in the rat, the NSSPs are composed of Rotary (Ra and Rb), Cruciate (Ca and Cb), and Alternate (Aa and Ab) patterns (Fig. 8A).

Regularity index (RI) is often used as a measure of coordination, and refers to the percentage of the run that fell under the NSSPs (Klapka et al. 2005; Jeong et al. 2011). It is independent of the actual number or combination of patterns used, meaning if an animal used only one pattern or all six patterns during a run, the corresponding RI value would be 100%; anything less than 100% indicates use of anomalous patterns.

Number of Patterns is the total number of complete NSSPs in a run, and is also independent of the specific pattern. For instance, if an animal used the Aa pattern exclusively for a total of four complete Aa patterns in a run, then the number of patterns would equal four. Alternatively, if the animal used four complete patterns in the following order: Aa, Cb, Ab, Aa, the number of patterns would still equal four.

Inter-paw coordination—Couplings and Phase Dispersions describe the temporal relationship between the placement of two paws within a single Step Cycle, the anchor paw and the target paw, and are expressed as Anchor → Target. These sets of variables have been used as a measure of inter-paw coordination (Kloos et al. 2005; Mountney et al. 2013) and are part of the foundation of discriminating gaits, that is, walking, trotting, galloping and so forth. (Hildebrand, 1976). Phase Dispersions are expressed as the initial contact of the target paw as a percentage of Step Cycle time of the anchor paw. The values range from -50% to 75%, where a negative value reflects the target paw being placed before the anchor paw and a positive value indicates the target paw was placed after the anchor paw.

Couplings differ from Phase Dispersions in that the target cannot be placed before the anchor, and thus only have positive values ranging from 0% to 100%. Couplings also have reciprocal representations (Anchor → Target | Target → Anchor) which results in twice-as-many Couplings (36) than Phase Dispersions (18). CStat refers to the circular statistic for that variable and can range from 0 to 100. CStat R is the measure of variation in that circular statistic. Three categories of Inter-Paw Coordination are conserved between Couplings and Phase Dispersions: diagonal, girdle, and lateral (Fig. 7A). Both Phase Dispersions and Couplings have opposing representations, meaning there is a matching set for every Anchor → Target pair (e.g., right front (RF) → RH | LF → LH), for both normal means and for CStats. Two exceptions to this are in Couplings: LF → RF, because the left side is always an anchor in a girdle pair and RF → LH A, which is undefined in the CatWalk manual.

Statistics

Statistics used—Two independent statisticians were consulted for proper identification and execution of the tests and methods used in this investigation. All analyses were performed by the authors using SPSS version 20. Statistical significance set at P -value < 0.05 for all tests. Histograms depicting distributions are expressed as mean ± SD while all other data are presented as mean ± standard error of the mean (SEM). Speed distributions were tested for normality using a combination of the Kolmogorov–Smirnov (K–S test) statistic with a Lilliefors’ significance; skewness was also examined.

Both statisticians recommended the linear mixed model (LMM) for all analyses, due to its capability of including speed and other confounding variables inherent in locomotion studies. Time, subject, and intercept were added as random factors, and the “variance

components” covariance type was used. Sidak post hoc correction for multiple comparisons was used where appropriate.

Characterizing speed-dependent relationships—Three aspects were used to describe how a particular variable changed with speed, those are graphical, with increasing speed (WIS), and mathematical. The combination of these aspects fully describes the speed-variable relationship. Preliminary classifications were made visually, by describing the graphical relationships observed in scatterplots of each variable versus speed. Graphical relationships with respect to speed were classified as either: linear, curvilinear, or no dependence (ND). The directionality of each variable WIS was also performed visually, and defined as increases (\uparrow), decreases (\downarrow), ND ($-$), or complex, meaning they were a combination of \uparrow , \downarrow , and/or $-$. Mathematical characterization was performed subsequent to graphical and WIS.

Mathematical relationships share the same name as, and were derived from, the mathematical model tested in regression analyses. Criteria for model selection were as follows: highest F -score, highest coefficient of determination (R^2), lowest P -value, and graphical fit. The “curve estimation” function was used for regression analyses of each variable with respect to speed. Tests for each variable with respect to speed were performed one-at-a-time. Discrete speed was set as the independent variable (IV) and the remaining variables were set as the dependent variable (DV). Ten of the 11 mathematical models available in SPSS were tested (linear, logarithmic, inverse, quadratic, cubic, compound, power, S, growth, and exponential); the logistic model was excluded due to the necessity of defining an upper bound for every variable. Each mathematical model has associated with it a transformation to the IV, DV, or both. For example, the logarithmic transformation is the $\ln(\text{IV})$ and the power transformation is $\ln(\text{IV})$ and $\ln(\text{DV})$.

Linearizing the speed-variable relationships—Curvilinear relationships were linearized, while simultaneously checking ANOVA assumptions (i.e., normally-distributed residuals and a diffuse pattern of residuals vs. predicted). The transformations associated with the curve estimation function were exploited and we developed a simple procedure to linearize any nonlinear relationships. First, curve estimation was performed for all raw data using discrete speed as the IV. A combination of the output scatterplots and ANOVA results was used to determine the top three models. The first criteria for the models were ANOVA results, where the top models were those with the combination of the highest R^2 value, lowest P -value, and highest F -value.

If the linear model was one of the top three, it was deemed fit to add to the LMM, if not, the variables were transformed as specified by the model. A second set of curve estimations were run from transformed variables, and the top three models from these were selected, as previously described. At this point histograms of residuals and scatterplots of residuals versus predicted were compared to those from the first curve estimation. The model that best produced a combination of an output scatterplot with a linear relationship, a normal distribution of residuals, and a diffuse pattern of residuals versus predicted was selected as the appropriate model to add as a covariate in the LMM. A summary of this process is visualized in Figure 10. This process can be used to explore and linearize any confounding

factor and added to a LMM or other appropriate statistical test when its effects are to be considered.

Proof-of-concept experiment—To test the ability of the LMM to include speed, we created a situation in which speed was the only difference. The group of 16 WTs was split into the overall (12-day total) fastest and slowest six animals, called the “fast” and “slow” group, respectively; the remaining four were excluded from the analysis. Splitting the group in this manner was done because wide intraindividual and interindividual speed ranges are common on unforced apparatuses in normal animals (Fig. 2B; Clarke and Still 1999; Koopmans et al. 2007; Cendelín et al., 2010). More importantly, it is clear that significant speed differences exist following many types of experimental conditions including, but not limited to, disease onset, arthritis, and traumatic brain injury (Vlamings et al. 2007; Hoffmann et al. 2010; Mountney et al. 2013). Because these speed differences are realistic and expected, it is crucial for the statistical analysis to handle this type of situation without producing false-positives (i.e., finding differences in outcome variables that are exclusively the result of differences in speed).

Three sets of analyses were performed for this proof-of-concept experiment. The first was to examine the extent to which the fast and slow groups were different with respect to speed. The second was to setup the LMM such that it mimicked a way of analyzing locomotion data commonly used in many studies; that is, by comparing an outcome variable between two groups without considering the effect of speed. The third was to include speed as a covariate in the LMM and examine the results. Swing Speed for the RF paw was chosen as the outcome variable because its relationship with speed was already linear (Table 1), thus no linearization was needed (see previous subsection). For all parts of this experiment, the LMM was specified with subject as a random effect in an attempt to include as much natural variation as possible (Fig. 2B).

RESULTS

Speed Distributions Rarely Approximated Normal and were Always Negatively-Skewed

The total, 12-day, speed distribution approximated normal by the K–S test, but was negatively skewed (Fig. 1A; $P = 0.000$, skewness = -0.340); the mean speed was 78.73 cm/sec with a SD = 18.85 cm/sec. Speed distributions for five of the 12 testing days approximated normal by the K–S test (PNDs; 97, 101, 105, 109, and 113), three days did not approximate normal, but were within the skewness threshold of ± 0.500 (PNDs 99, 103, and 107), and four days did not approximate normal and were outside the skewness threshold (PNDs 89, 91, 95, and 111). Examples of these are displayed in Figs. 1B–D. Regardless of normality, each distribution was negatively skewed to some extent (minimum skewness = -0.149 , maximum skewness = -0.734 , average skewness = -0.422), indicating faster speeds were observed more than slower speeds. There was no discernible pattern regarding the normality of the distributions between individual days.

Daily Speed is Regularly Irregular Both as Individuals and as a Group

Mean speed per day for the entire group of WT mice was examined using a LMM with Sidak post hoc correction for multiple comparisons. The main effect for time was significant ($P = 0.000$), indicating an overall change in mean speed. The mean \pm SEM for each day are displayed in Fig. 2A, which shows a decline in mean speed across time and significant differences between certain days. Mean differences as small as 9.25 cm/sec were significant (PND 89 vs. PND 104, $P = 0.044$).

A marked intraindividual and interindividual difference in average speed can be seen between animals on a single day, within animals across time, and between animals across time (Fig. 2B), consistent with previous work. This is evidenced by the undulating trend lines per animal and intermittently large SEMs. The speed change between PND 89 and PND 113 was negative in 14 of the 16 animals; the results of a binomial test suggested the overall decline in speed with time was due to changes within the group and not to random intraindividual or interindividual differences $P(14|16, 0.50) = 0.004$. Taken together, one cannot expect to observe the same, or similar, speeds even from the same animal on the same day. Therefore, collecting many runs per animal per day is advantageous.

Over 90% of All Variables Tested were Uniquely Dependent on Speed, Most of Which Displayed a Curvilinear Relationship

Only 15 of the 162 variables (9.26%) showed no significant mathematical or graphical dependence on speed, while 98.74% of the remaining 147 variables were significant at $P = 0.000$. The R^2 values ranged from highly correlated ($R^2 = 0.998$ for Run Duration; Table 3; Fig. 5A) to minimally correlated ($R^2 = 0.093$ for RF Mean Intensity; Table 1; Fig. 4F). The mean R^2 value was 0.624 (SD = 0.275), however, the distribution was negatively skewed (-0.171), meaning a majority of variables were correlated at or above an R^2 value of 0.600. The linear mathematical model accounted for only 17.91% of the variables, while the remainder were curvilinear.

All 162 variables were successfully characterized, both graphically and mathematically. To simplify and streamline the results, we have included detailed tables that contain the mathematical relationship and the WIS designation for all variables within the three previously defined categories (Paw Statistics, Other Statistics, and Inter-Paw Coordination; see Materials and Methods). Table 1 includes results within the Paw Statistics category, while consolidated results illustrating inter-paw differences among these variables are displayed in Table 2. Detailed results for the Other Statistics category are displayed in Table 3, and those for the Inter-Paw Coordination category are located in Table 4.

Mathematical Classification of Each Variable is Necessary for Accurate Analysis

Out of the ten regression models assayed, eight were observed: inverse, linear, quadratic, logarithmic, power, compound, and S. Previously, investigators published each equation and respective values of the speed-dependent relationships (Hruska et al., 1979; Hruska and Silbergeld, 1979; Clarke and Parker 1986; Górska et al., 1998; Herbin et al., 2004; Herbin et al. 2007). We purposefully omitted the equations and values because each equation was unique to its variable. Also, equations and values will likely depend on the manner in which

they were analyzed (i.e., raw vs. speed-averaged) and the software program used for regression.

There were discrepancies between graphical and mathematical classifications such that a variable appeared to be linear, graphically, but was best described by a curvilinear equation. For instance, Swing Speed for three paws was best fit by a power model, although the scatterplot of Swing Speed versus Speed appears linear (Table 2; Fig. 3H). There were also many cases where differences regarding the speed-dependent relationship were detected by curve estimation, but not necessarily by the eye. For example, Stand for three paws was best fit by a power model, while one paw was best fit by an inverse model (Table 2), but the curves for all four paws appear to overlap completely in the scatterplot of Stand versus Speed (Fig. 3B). Therefore, it is imperative that the variables be individually characterized, both graphically and mathematically, prior to statistical analysis.

Paw Statistics Variables Exhibit Unique Speed-Dependent Relationships, Which were Often Different between Front/Hind and Left/Right

The WIS relationships for Mean Intensity, Minimum Intensity, Print Length, Print Width, and Print Area (Table 2; Fig. 4) revealed the mice shifted weight toward the front paws and away from the hind paws as they traveled faster. This weight shift is likely a strategy to increase speed, much in the same way humans shift weight forward to move from walking to running. A majority of variable sets within the Paw Statistics category shared the same WIS relationship regardless of front versus hind and left versus right (Table 2). Four of these variable sets were almost identical, graphically, across all paws: Step Cycle, Stand, Single Stance, and Stride Length (Fig. 3). This is in agreement with previous work in rats that described similar speed-dependent relationships between right, left, front, and hind in Stand, Step Cycle, and Stride Length (Hruska et al., 1979; Górska et al., 1998; Clarke and Still 1999; Thota et al. 2005).

No differences were detected between left versus right regarding the WIS relationships of the front or hind paws, respectively. That is, if a variable \uparrow WIS for the RF paw, it also \uparrow WIS for the LF paw, and likewise for the hind. However, there were five sets of variables in which the WIS direction was opposite for front versus hind: Max Contact Max Intensity, Max Contact Mean Intensity, Max Intensity, Mean Intensity, and Mean Intensity of the 15 Most Intense Pixels (Table 2). Further, the intersections between these lines did not occur within the same speed range during comparison of these variables (Fig. 4). This suggests the practice of averaging front and hind paws contralateral to an experimental condition is not appropriate for certain variables.

Seven variables showed a distinct separation between the right and left hind paws along the entirety of their curves: Stand Index, Max Intensity, Mean Intensity, Mean Intensity of the 15 Most Intense Pixels, Max Intensity At (%), Max Contact Mean Intensity, and Max Contact Max Intensity (Figs. 3, 4). Overall, it is indicated that more pressure was placed on the right hind paw than the left hind paw. We hypothesize this shift is an epiphenomenon of the clockwise loop (Supporting Information Video 3), given the near complete graphical overlap between front paws in all variables and symmetry between hind paws in the remaining variables.

Variables Commonly used as Quantitative Outcome Measures

The following variables within the Other Statistics category are routinely used as stand-alone measures and/or as criteria for run compliance. BOS reflects stance width for the front or hind paws, and is commonly studied in the context of spinal cord injury (Dai et al. 2011; Petrosyan et al. 2013; Streijger et al. 2013). BOS for the hind paws ↑ slightly WIS, while the BOS for the front paws ↓, suggesting a narrowing of front stance and a widening of hind stance WIS (Fig. 5C). These results are in contrast to Hruska and Silbergeld (1979), who described BOS as independent of speed in male Sprague-Dawley rats.

RI (%) ↓ WIS in a linear fashion (Table 3; Fig. 5D), and the lowest observed value was 94%. Previous work in rats (Koopmans et al. 2005; Hendriks et al. 2006; Angeby-Möller et al. 2008) and in mice (Neumann et al. 2009; Cendelin et al., 2010; Cho et al. 2012) suggest values less than 100% are expected in normal animals. Our results further support the concept of a normative range. Maximum Variation (%), which is primarily used as a criterion for run compliance, was found to ↓ WIS in a linear fashion. Because it is speed-dependent, it also has potential to be used as an outcome measure (Wang et al. 2012)

Run Duration (s) was highly dependent on speed in a curvilinear manner ($R^2 = 0.998$). It ↓ WIS and was best fit by the power model. Some investigators set run duration limits between 1 and 2 sec to control for speed. In this study, this corresponded to a speed range from approximately 35 cm/sec to 70 cm/sec and, as we have shown, differences as small as 9.25 cm/sec can be significant (Fig. 2A). Run Duration not only depends on speed but also on the recorded length of the runway, which was undeclared in most studies. Given that over 90% the variables were strongly dependent on speed, often in a unique, curvilinear manner, this method of speed control is most likely not appropriate.

Variables used to Define Gaits Displayed Complex Relationships With Respect to Speed

Print Positions reflects the relative placement of a hind paw to the ipsilateral front paw (Fig. 6A). The speed-dependency described here is in contrast to previous work utilizing both rats (Hruska and Silbergeld 1979; Koopmans et al. 2007) and mice (Neumann et al. 2009). However, Neumann et al. (2009) reported Print Positions to be independent of speed using data from a speed range of 10–40 mm/s in mice, whereas we observed changes on the cm/sec scale (25–125 cm/sec), suggesting the small speed range may explain the reported lack of speed-dependency. Additionally, our ongoing work in Wistar rats has revealed speed-dependency of Print Positions consistent with that of the current study (data not shown).

Unique to Print Positions were three distinct graphical regions that occurred about a nonarbitrary value (Fig. 6B); positive values were almost exclusively observed below 60 cm/sec, between 60 cm/sec and 70 cm/sec values fluctuated near zero, and negative values were exclusively seen above 70 cm/sec. Print Positions also has strong biomechanical connotations. For instance, a negative value indicates the hind paw was recorded in front of the preceding front paw (Fig. 6A), and one can imagine the animal hunching its body to accomplish this. These data led us to hypothesize that Print Positions may be a novel, singular variable by which to discriminate gaits (walking, trotting, galloping, etc.). However,

as CatWalk is limited to acquiring information from paw prints only, there is no way to determine the biomechanical aspect without a view of the animal's body as it moves.

The Support variables represent the percentage of a run that a single paw, or combination of paws, was in contact with the glass (Fig. 7A). Four of these variables displayed expected results based on their definitions (see Materials and Methods; Table 2; Fig. 7B): (1) Support Three ↓ WIS, and was effectively not observed past 50 cm/sec (–) as it was always less than 1%, (2) Support Single and Support Zero ↑ WIS (Górska et al., 1999), and (3) no instances of Support Four were observed, as it is seldom seen during forward motion (Abourachid et al. 2007). Interestingly, Support Lateral and Support Girdle were rarely seen and comprised 8.62% and 2.44% of a run, respectively (Fig. 7B; Table 3). Conversely, Support Diagonal always accounted for >40% of a run across all speeds; this agrees with previous work in rats (Górska et al., 1999). The range between 35 cm/sec and 45 cm/sec was the most dynamic, where there was an approximately equal chance of observing Support Single, Support Three, and Support Lateral (Fig. 8B).

Variables representing the NSSPs relay the percentage of an entire run that particular sequence of footfalls was used (Fig. 8A). No instances of the rotary sequences were observed (Ra or Rb), which supports previous work done in rats (Koopmans et al. 2005; Deumens et al. 2007), and in mice (Neumann et al. 2009; Wang et al. 2012), but is in contrast to Cheng et al. (1997), who originally defined the sequences. The complete absence of rotary sequences (Ra or Rb) is explained by the infrequent occurrence of Lateral and Girdle Support variables, on which these sequences depend (Fig. 7B; 8A). Cruciate (Ca and Cb) sequences also necessitate lateral placement of paws, which explains why they were less commonly observed than alternate (Aa and Ab) sequences. Both cruciate and alternate sequences use diagonal placement of paws; this, in part, accounts for the predominance of diagonal support (Fig. 8A; 7B). Based on the graphical representation of the combination of these variables (Fig. 8B), the range from approximately 40 cm/sec to 60 cm/sec was the most dynamic, in which each step sequence had a nearly equal chance of being observed. The three sets of variables considered here (Print Positions, Support, and NSSPs) each displayed distinct graphical regions, alone or in combination, which may be indicative of a particular gait or gait transition.

Several distinct methods exist to define gaits and gait transitions in mammals (Vilensky et al., 1997). From a foot placement perspective, the timing and sequence of footfalls is a popular determinant of gaits, and much contemporary work is an extension of the criteria proposed by Hildebrand in 1976 (Abourachid 2003; Herbin et al., 2004). Variables commonly used to assess gait in CatWalk and other locomotion analysis systems include the Support variables, NSSPs, Phase Dispersions, and Couplings. As we have shown here, the transition points or dynamic ranges within Print Positions, Support, and NSSPs do not completely agree (60–70 cm/sec, 35–45 cm/sec, and 40–60 cm/sec, respectively), and transitions in graphs of Phase Dispersions and Couplings are less obvious due to the multiple ways of examining them (Fig. 9). When examined independently, Print Positions, Support, NSSPs, Phase Dispersions, and Couplings, each display a unique graphical region that may be suggestive of a biomechanical change or potentially mark a gait transition along the speed continuum. However, these transition points are inconsistent between variable

sets, and therefore, depend on which set is examined. The absence of consistency between different gait-determining methods reveals more research is required to establish a unifying theory of gait.

Additionally, the study of gait from a footfall perspective is complicated by any method, including CatWalk, in which data is reported as an average of a run or a trial. Run/trail averages only provide a static picture, and gait is necessarily a dynamic entity. In order for this picture to be accurate, rodents would need to move at precisely the same speed for the entirety of the run and have a maximum variation of 0%; both of which are highly unlikely. This is not to say accurate gait analysis is inaccessible through CatWalk, only that the necessary methods are not automated (Hildebrand 1989; Abourachid et al. 2007).

Inter-Paw Coordination Variables Show Striking Similarities or Differences When Examined in the Context of Speed

The Inter-Paw Coordination category of variables reflects the timing of placement between two paws, the Anchor and the Target (expressed as Anchor → Target), and are the basis of some gait-discriminating methods (Hildebrand, 1976). Conceptualizing the Anchor → Target can be aided with NSSP definitions (Fig. 8B). Unsurprisingly, a Spearman's rho test revealed correlations between NSSPs and the Inter-Paw Coordination variables (data not shown). Inter-Paw Coordination is split into Phase Dispersions and Couplings, both of which have slightly different definitions. Because they have conserved aspects (see Materials and Methods), it is easy to see they can be mistaken as interchangeable.

Variables representing the girdle pair LF → RF for normal mean, CStat, and CStat R (both Phase Dispersions and Couplings), and RF → LH A, and RF → LH CStat A (Couplings only) were independent of speed (Table 4). These results support Hamers et al. (2006), who reported the girdle pairs were independent of speed and were always at 50%. This can only be confirmed in part for all girdle variables, as LH → RH and LH → RH Cstat (Phase Dispersions and Couplings) and RH → LH and RH → LH CStat (Couplings only) were significantly related to speed in a quadratic fashion (Table 4). Overall, our results agree with previous work in that girdle variables were observed between 45% and 55% throughout the majority of our speed range. However, at the extremes of our observed speeds there was a divergence from 50% (data not shown).

Interestingly, the normal mean and CStat mean for diagonal pairs in Phase Dispersions revealed completely different graphical and WIS results across the entire speed range (Fig. 9A). In contrast, the same diagonal pairs in Couplings (normal mean and CStat means) display identical graphical and WIS results (Table 4; Fig. 9B). With respect to girdle and lateral pairs, Phase Dispersion, and Coupling variables revealed the same graphical and WIS relationship, thus the distinct differences between normal mean and CStat mean were unique to the diagonal pairs (Table 4; Fig. 9F).

For the entire Couplings category, the normal mean and CStat mean for the same Anchor → Target pair always shared the same mathematical model, approximate R^2 value, WIS relationship, and were graphically indistinguishable (Table 4; Fig. 9B). While this suggests redundancy within this category, differences may be revealed under certain experimental

conditions. Couplings, unlike Phase Dispersions, include reciprocal Anchor \rightarrow Target representations (RF \rightarrow LH I LH \rightarrow RF), and when graphed together their curves are complementary (Fig. 9C–D); the complementary curves were present in both normal mean and CStat mean (data not shown). Complementary curves for diagonal reciprocals were not identical (Fig. 9C) and is likely due to scaling as negative values for Couplings do not exist (see Materials Methods).

In comparisons between Couplings and Phase Dispersions (36 and 18 variables, respectively), only three variables displayed different mathematical relationships for the same Anchor \rightarrow Target pair (Table 4). Therefore, the similarities and disparities within the Inter-Paw Coordination category illustrate two things: (1) mathematical characterization alone is inadequate to fully describe speed-variable relationships, and (2) Phase Dispersions and Couplings are not interchangeable. To our knowledge these two sets of variables have not been studied in parallel, and future studies adopting a more comprehensive analysis may lead to better understanding of experimental paradigms.

Speed-Dependent Relationships Described in the Current Study Corroborate Previous Work, Regardless of Species, Strain, or Apparatus

Four sets of variables (one for each paw) and one discrete variable have received the most attention regarding their relationships to speed, those are: Step Cycle, Stand, Swing, Stride Length, and Cadence. To place our work in the context of the literature, the results of the current study were compared with previously published work; a summary can be found in Table 5. Additionally, these variables can be visualized in Figs. 6A–C, 6G, and 5B. The previous studies compared here include both treadmill (forced) and restricted pathway apparatuses (unforced), and span different species and strains of rodents. Some studies analyzed speed-averaged data while others analyzed raw data. Importantly, our results regarding graphical and WIS relationships agree with a majority of the previous work. Differences between mathematical models were mixed, which could be due to: (1) unequal and/or nonoverlapping speed ranges between these studies, (2) differences in run criteria and acquisition regimen, and (3) increasing sophistication of computer-aided statistical analysis with time.

The LMM is a Statistical Method that can Effectively Incorporate Speed, and Many Other Confounding Factors

The LMM was identified as the most comprehensive statistical test for longitudinal locomotion studies for three reasons. First, the LMM can accommodate an uneven number of runs per animal per day, which is an unavoidable circumstance arising from the need to screen runs for behavioral and steady-state compliance. Second, the LMM can be setup to handle the significant intra- and inter-animal variations in speed and performance (Fig. 2B). Third, and most importantly, the LMM has the option of including anything that covaries with the DV in a linear manner when those effects are to be considered.

Adding speed as a covariate does not control for speed in the sense that it normalizes to a common, shared speed. Rather, it allows the LMM to do three things in addition to the analyses it can perform in the absence of a covariate. First, it can calculate and compare the

slope of the variable versus speed. Second, it can calculate and compare the intercept of the variable versus speed. Third, it can compare the variable between and within groups at a common speed via estimated marginal means. It is important to note that the LMM can compare the DV even if shared speeds do not exist. This is due to the required linear nature of the DV and speed; the LMM calculates the linear equation for observed data and interpolates values for missing data. Because most of the variables were curvilinear with respect to speed, we developed a way to linearize those relationships while keeping some ANOVA assumptions intact (see Materials and Methods; Fig. 10).

Differences in Speed Alone Resulted in a False-Positive for an Outcome Measure; the False-Positive was Abolished by Including Speed as a Covariate in the LMM

We created a proof-of-concept experiment to test the ability of the LMM to include speed. The six fastest and six slowest animals were split into a “fast” and “slow” group, respectively, and three analyses were performed. For the first analysis, a LMM was run comparing the mean speed between groups; as expected, the main effects for group and for time were significant ($P = 0.005$ and $P = 0.001$, respectively), and the interaction of group \times time was not (Fig. 11A). These results demonstrated four things: (1) the fast group was significantly faster than the slow group overall (12-day average), (2) the average speed of both groups declined with time, (3) the fast group was significantly faster than the slow group on each of the 12 days, and (4) the smallest significant difference in speed between the groups was 12.79 cm/sec (Fig. 11A).

In the second analysis, a LMM was setup to examine RF Swing Speed between the fast and slow groups, excluding speed as a covariate, which is similar to way locomotion data is commonly analyzed. The LMM revealed the main effect for group and for time was significant ($P = 0.001$ and $P = 0.000$, respectively), while the interaction of group \times time was not. Statistical analysis revealed significant differences between groups on every day ($P = 0.047$). Figure 11B depicts the mean RF Swing Speed \pm SEM of each group per day across time, showing that RF Swing Speed for the fast group was significantly different from the slow group on each day. These results are false-positives, and the similarities between Fig. 11A, B illustrate the covariance between speed and RF Swing Speed.

The final analysis used the method proposed in the current study, in which speed was included as a covariate to the LMM and its interactions with time and with subject were included as random effects. The model was specified such that it allowed for each animal to have a different slope of RF Swing Speed versus speed, and each animal to have a different intercept per day and across days. The results indicated the main effects for group and for time were not significantly different ($P = 0.291$ and $P = 0.462$), nor were any of the combinations of interactions between speed, group, and time. There were also no significant differences among the slopes or intercepts of Swing Speed versus speed overall or on individual days. Figure 12A depicts slope \pm SEM for each group across all days, while Figure 12B displays a scatterplot depicting RF Swing Speed versus speed for both groups on PND 99. Thus, including speed as a covariate in the LMM abolished the false-positive results from the second analysis in which speed-dependent effects were ignored.

Clearly, the data points for the slow group were observed throughout the entire speed range, but more concentrated below 80 cm/sec, and vice versa for the fast group. Linear regression lines for the two groups were identical for this day and completely overlap, showing the relationship between RF Swing Speed and speed was the same between groups. The data points for each group across all 12 days are displayed as a scatterplot in Figure 12C. As on PND 99 (Fig. 12B), the data points for the slow group were concentrated at slower speeds, while the data points for the fast group were concentrated at faster speeds. The relationship between RF Swing Speed and speed were indistinguishable between groups as evidenced by the overlapping linear regression lines. The results of this three-part proof-of-concept experiment demonstrated that differences in speed alone resulted in a false-positive for RF Swing Speed when common types of analyses were performed, and that this false-positive can be eliminated by including speed as a covariate to the LMM.

DISCUSSION

When using locomotion analysis for quantifiable outcome measures, the benefits of unforced over forced systems can be offset by the inability to regulate speed. Current methods to control for speed often require various forms of restrictive analysis or speed binning in which data are only analyzed if they fall within predefined speed or crossing time ranges. Exclusion of data is obviously not ideal, but the main issue with restrictive analysis is that ranges are idiosyncratic and lack standardization. This is likely due to the incomplete understanding of the extent, nature, and degree of dependence of locomotion variables on speed.

Consistent with previous work, we observed a wide range of speeds among our group of WT mice. Individual mouse speed was regularly irregular both on a daily basis and across time. This relatively large group of WT mice provided a realistic scenario to evaluate the relationships between speed and locomotion variables in depth, and to assess the LMM as a statistical method capable of incorporating speed.

Speed-Dependent Relationships

We characterized each speed-variable relationship graphically, WIS, then mathematically. All three aspects (graphical, WIS, and mathematical) were necessary to sufficiently describe the speed-dependent relationships as a variable could appear to be linear but be best described by a curvilinear mathematical model. It is important to properly linearize speed-variable relationships because many statistical analyses require linear covariates, and we provided a method here to accomplish this (see Materials and Methods). While other statistical tests can analyze nonlinear relationships between the DV and IV, they are less accessible than linear methods.

An overwhelming majority of variables reported by CatWalk, and common to other systems, were uniquely dependent on speed. Furthermore, most of those variables displayed curvilinear relationships. These facts present a clear problem if restrictive analyses are used because they are susceptible to false-positives or false-negatives, depending on the size and location of defined speed/crossing time ranges. Additionally, the speed-variable relationships are dynamic and using singular speed ranges precludes understanding of

changes to the relationship itself. Speed binning addresses the issue of excluding usable data and is, therefore, cognizant of the speed-variable relationship. However, the unique speed-dependent relationships would mean defining equal (for linear) and unequal (for curvilinear) bins on a variable-by-variable basis.

The LMM

The LMM is a type of ANOVA that is ideal for longitudinal, repeated-measures studies and is it commonly used in medical and psychological analyses in humans (Norman and Streiner 2008; Knafl et al., 2012). To our knowledge, this is the first study to utilize the LMM for rodent locomotion; it was effective not only in speed-variable situations but also in general analyses such as evaluating mean speed with time. This method proved to be powerful and flexible due to its ability to identify subjects, include repeated measures, random and fixed effects, and it offered many covariance types. Most importantly, it is able to incorporate speed and other confounding factors as linear covariates. Adding speed as a covariate is not a form of control in which the DV is normalized to a common, shared speed. Instead, it allows the LMM to calculate a slope and intercept for the observed values of DV versus speed. This means the LMM can detect changes in three different ways: (1) parallel lines with differing intercepts revealing a shift along the entire speed continuum (unpublished data), (2) converging lines reflecting differences only in certain speed ranges (Hruska and Silbergeld 1979), and (3) completely different curves (i.e., linear vs. curvilinear) showing a disruption of the entire speed-dependent relationship (Herbin et al. 2007). In this way, the predominant drawback of unforced locomotion analyses (i.e., uncontrolled speed variation) is actually advantageous.

Limitations of the LMM

Speed is not the only confounding factor that should be considered in locomotion studies. We, and others, have shown there are many sources of variance in rodent behavioral and locomotion analyses that can lead to quantifiable changes in outcome measurements including species, strain, sex, age, weight, experience, and testing interval (Parker and Clarke 1990; Dellon and Dellon 1991; McIlwain et al. 2001; Bothe et al. 2004; Bothe et al. 2005; Paylor et al. 2006; Koopmans et al. 2007; Wooley et al. 2009). With the exception of weight, these factors cannot be added as covariates in statistical tests. However, these effects can be minimized with the addition of a baseline group of similar age and weight, and to which no experimental condition is applied. These animals should be tested in parallel with experimental group, including simultaneous testing at equivalent intervals. Ideally, comparisons to the longitudinal baseline should be made at the appropriate post-condition time point, rather than creating one large set of normative data as the sum of all longitudinal data. Creating normative baseline data at each individual postcondition time point will minimize the effects of age, experience, testing interval, and weight. This leaves only speed, and intra- inter-animal variability in speed and performance to be handled by the LMM.

Analysis of Locomotion

We explored every variable reported by CatWalk in the interest of being thorough, such that it would provide a foundation for other investigators regardless of research interest. However, the large number of variables reported by CatWalk and other systems can be

daunting to approach, and many investigators analyze only a segment of available data. There are statistical strategies to detect salient changes and reduce the number of variables to be analyzed, including exploratory analysis and factor analysis (Cendelín et al., 2010; Boeltz et al. 2013). We advocate a more comprehensive approach to locomotion analysis rather than selecting variables with the greatest change. Many statistical programs allow for automation of processes such that one can examine multiple variables, or even all variables, simultaneously. This decreases the labor-intensiveness of statistical analysis while increasing yield, and facilitating a more broad characterization of locomotion within the context of speed. By examining global changes, one is better able to determine which variables provide meaningful results regarding paradigmatic and biological relevance.

Concluding Remarks

We have demonstrated an overwhelming majority of variables reported by CatWalk are significantly related to speed, and often in a curvilinear manner. The extent of this speed-dependency requires that speed, as an IV, remain inclusive in data analysis and presentation. Common methods of analyzing locomotion data either ignore the effects of speed or subjectively restrict data, and are, therefore, exceptionally susceptible to false-positives or false-negatives. The LMM offers a new way to analyze locomotion in which variables are examined as they change with speed, rather than through the simple normalization of speed. Including speed as a covariate in the LMM eliminated the false-positives seen in our proof-of-concept experiment and, therefore, it is an appropriate method for future studies. Finally, many of our results compared directly to previous work regardless of species, strain, or apparatus, and the methods considered here are applicable to a wide range of studies, not only to mice on the CatWalk.

Acknowledgments

Grant sponsor: NIH NINDS; Grant number: RO1 NS40433.

The authors would like to thank Stephanie Dickinson from the Indiana University Statistical Consulting Center and George Eckert from the Indiana University School of Medicine Department of Biostatistics; without their statistical guidance, this publication would not have been possible.

LITERATURE CITED

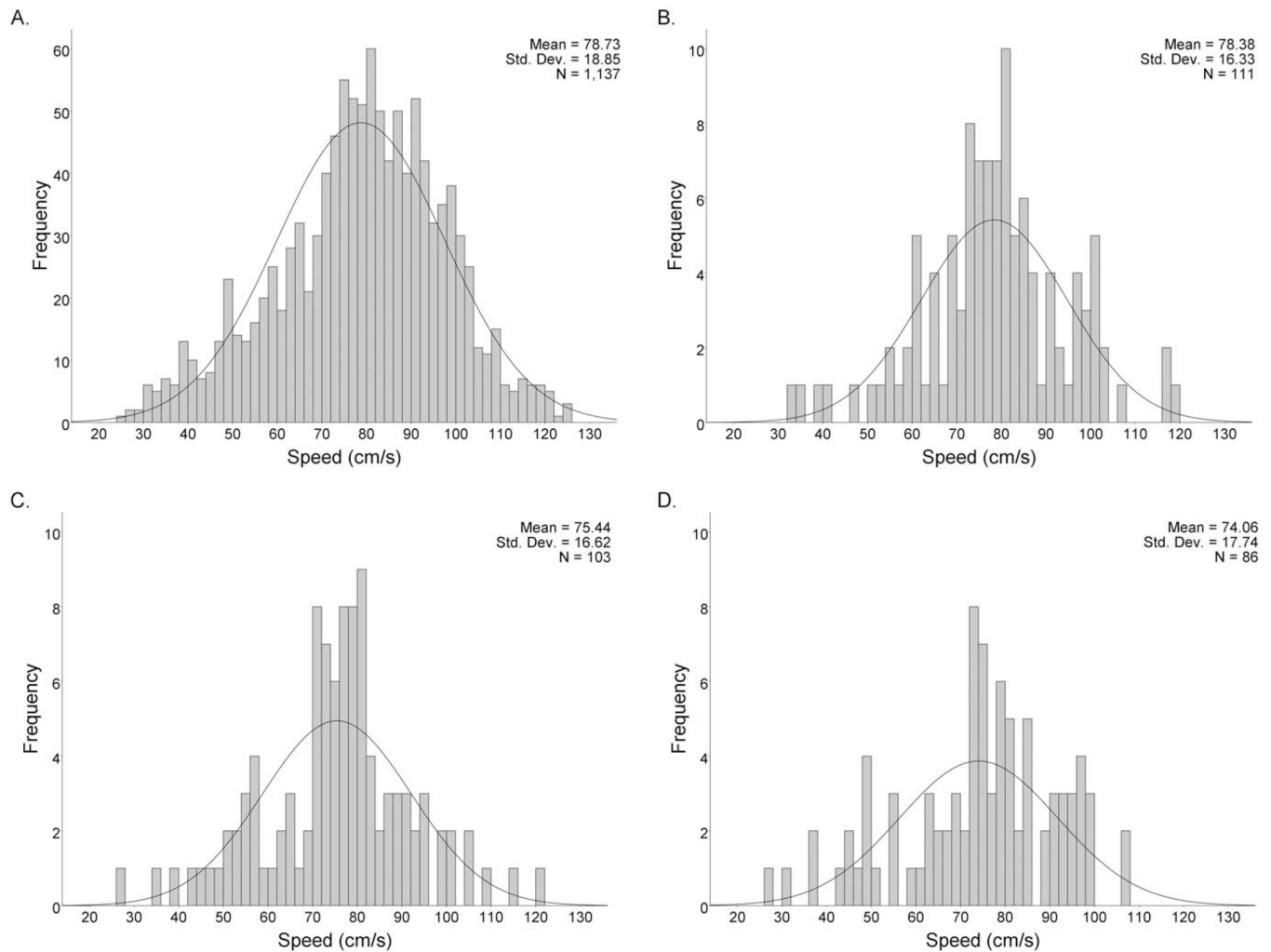
- Abourachid A. A new way of analysing symmetrical and asymmetrical gaits in quadrupeds. *C R Biol.* 2003; 326:625–630. [PubMed: 14556381]
- Abourachid A, Herbin M, Hackert R, Maes L, Martin V. Experimental study of coordination patterns during unsteady locomotion in mammals. *J Exp Biol.* 2007; 210:366–372. [PubMed: 17210971]
- Amende I, Kale A, McCue S, Glazier S, Morgan JP, Hampton TG. Gait dynamics in mouse models of Parkinson's disease and Huntington's disease. *J Neuroeng Rehabil.* 2005; 2:20. [PubMed: 16042805]
- Angeby-Möller K, Berge OG, Hamers FP. Using the CatWalk method to assess weight-bearing and pain behaviour in walking rats with ankle joint monoarthritis induced by carrageenan: effects of morphine and rofecoxib. *J Neurosci Methods.* 2008; 174:1–9. [PubMed: 18634823]
- Baiguera C, Alghisi M, Pinna A, Bellucci A, De Luca MA, Frau L, Morelli M, Ingrassia R, Benarese M, Porrini V, Pellitteri M, Bertini G, Fabene PF, Sigala S, Spillantini MG, Liou HC, Spano PF, Pizzi M. Late-onset Parkinsonism in NFKB/c-Rel-deficient mice. *Brain: A J Neurol.* 2012; 135:2750–2765.

- Balkaya M, Krober J, Gertz K, Peruzzaro S, Endres M. Characterization of long-term functional outcome in a murine model of mild brain ischemia. *J Neurosci methods*. 2013; 213:179–187. [PubMed: 23291083]
- Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma*. 1995; 12:1–21. [PubMed: 7783230]
- Beare JE, Morehouse JR, DeVries WH, Enzmann GU, Burke DA, Magnuson DS, Whittemore SR. Gait analysis in normal and spinal contused mice using the TreadScan system. *J Neurotrauma*. 2009; 26:2045–2056. [PubMed: 19886808]
- Boeltz T, Ireland M, Mathis K, Nicolini J, Poplavski K, Rose SJ, Wilson E, English AW. Effects of treadmill training on functional recovery following peripheral nerve injury in rats. *J Neurophysiol*. 2013; 109:2645–2657. [PubMed: 23468390]
- Bothe GW, Bolivar VJ, Vedder MJ, Geistfeld JG. Genetic and behavioral differences among five inbred mouse strains commonly used in the production of transgenic and knockout mice. *Genes Brain Behav*. 2004; 3:149–157. [PubMed: 15140010]
- Bothe GW, Bolivar VJ, Vedder MJ, Geistfeld JG. Behavioral differences among fourteen inbred mouse strains commonly used as disease models. *Comp Med*. 2005; 55:326–334. [PubMed: 16158908]
- Bozkurt A, Deumens R, Scheffel J, O'Dey DM, Weis J, Joosten EA, Fuhrmann T, Brook GA, Pallua N. CatWalk gait analysis in assessment of functional recovery after sciatic nerve injury. *J Neurosci Methods*. 2008; 173:91–98. [PubMed: 18577402]
- Cendelín J, Voller J, Vožeh F. Ataxic gait analysis in a mouse model of the olivocerebellar degeneration. *Behav Brain Res*. 2010; 210:8–15. [PubMed: 20122968]
- Cheng H, Almstrom S, Gimenez-Llort L, Chang R, Ove Ogren S, Hoffer B, Olson L. Gait analysis of adult paraplegic rats after spinal cord repair. *Exp Neurol*. 1997; 148:544–557. [PubMed: 9417831]
- Cho N, Nguyen DH, Satkunendrarajah K, Branch DR, Fehlings MG. Evaluating the role of IL-11, a novel cytokine in the IL-6 family, in a mouse model of spinal cord injury. *J Neuroinflammation*. 2012; 9:134. [PubMed: 22715999]
- Clarke KA, Parker AJ. A quantitative study of normal locomotion in the rat. *Physiol Behav*. 1986; 38:345–351. [PubMed: 3786515]
- Clarke KA, Still J. Gait analysis in the mouse. *Physiol Behav*. 1999; 66:723–729. [PubMed: 10405098]
- Clarke KA, Still J. Development and consistency of gait in the mouse. *Physiol Behav*. 2001; 73:159–164. [PubMed: 11399307]
- Cohen AH, Gans C. Muscle activity in rat locomotion: movement analysis and electromyography of the flexors and extensors of the elbow. *J Morphol*. 1975; 146:177–196. [PubMed: 1152066]
- Coulthard P, Pleuvry BJ, Brewster M, Wilson KL, Macfarlane TV. Gait analysis as an objective measure in a chronic pain model. *J Neurosci Methods*. 2002; 116:197–213. [PubMed: 12044669]
- Coulthard P, Simjee SU, Pleuvry BJ. Gait analysis as a correlate of pain induced by carrageenan intraplantar injection. *J Neurosci Methods*. 2003; 128:95–102. [PubMed: 12948552]
- Crone SA, Zhong G, Harris-Warrick R, Sharma K. In mice lacking V2a interneurons, gait depends on speed of locomotion. *J Neurosci*. 2009; 29:7098–7109. [PubMed: 19474336]
- Dai H, Macarthur L, McAtee M, Hockenbury N, Das P, Bregman BS. Delayed rehabilitation with task-specific therapies improves forelimb function after a cervical spinal cord injury. *Restor Neurol Neurosci*. 2011; 29:91–103. [PubMed: 21701061]
- Dellon ES, Dellon AL. Functional assessment of neurologic impairment: track analysis in diabetic and compression neuropathies. *Plastic Reconstr Surg*. 1991; 88:686–694.
- Deumens R, Jaken RJ, Marcus MA, Joosten EA. The CatWalk gait analysis in assessment of both dynamic and static gait changes after adult rat sciatic nerve resection. *J Neurosci Methods*. 2007; 164:120–130. [PubMed: 17532474]
- Encarnacion A, Horie N, Keren-Gill H, Bliss TM, Steinberg GK, Shamloo M. Long-term behavioral assessment of function in an experimental model for ischemic stroke. *J Neurosci Methods*. 2011; 196:247–257. [PubMed: 21256866]
- Faizi M, Bader PL, Tun C, Encarnacion A, Kleschevnikov A, Belichenko P, Saw N, Priestley M, Tsien RW, Mobley WC, Shamloo M. Comprehensive behavioral phenotyping of Ts65Dn mouse model

- of Down syndrome: activation of beta1-adrenergic receptor by xamoterol as a potential cognitive enhancer. *Neurobiol Dis.* 2011; 43:397–413. [PubMed: 21527343]
- Ferland CE, Laverty S, Beaudry F, Vachon P. Gait analysis and pain response of two rodent models of osteoarthritis. *Pharmacol Biochem Behav.* 2011; 97:603–610. [PubMed: 21111752]
- Gabriel AF, Marcus MA, Honig WM, Walenkamp GH, Joosten EA. The CatWalk method: a detailed analysis of behavioral changes after acute inflammatory pain in the rat. *J Neurosci Methods.* 2007; 163:9–16. [PubMed: 17382401]
- Gabriel AF, Marcus MA, Walenkamp GH, Joosten EA. The CatWalk method: assessment of mechanical allodynia in experimental chronic pain. *Behav Brain Res.* 2009; 198:477–480. [PubMed: 19146883]
- Gensel JC, Tovar CA, Hamers FP, Deibert RJ, Beattie MS, Bresnahan JC. Behavioral and histological characterization of unilateral cervical spinal cord contusion injury in rats. *J Neurotrauma.* 2006; 23:36–54. [PubMed: 16430371]
- Gillis GB, Biewener AA. Hindlimb muscle function in relation to speed and gait: in vivo patterns of strain and activation in a hip and knee extensor of the rat (*Rattus norvegicus*). *J Exp Biol.* 2001; 204:2717–2731. [PubMed: 11533122]
- Górska T, Majczy ski H, Zmyslowski W. Overground locomotion in intact rats: contact electrode recording. *Acta Neurobiol Exp.* 1998; 58:227–237.
- Górska T, Zmyslowski W, Majczy ski H. Overground locomotion in intact rats: interlimb coordination, support patterns and support phases duration. *Acta Neurobiol Exp.* 1999; 59:131–144.
- Hamers FP, Koopmans GC, Joosten EA. CatWalk-assisted gait analysis in the assessment of spinal cord injury. *J Neurotrauma.* 2006; 23:537–548. [PubMed: 16629635]
- Hamers FP, Lankhorst AJ, van Laar TJ, Veldhuis WB, Gispens WH. Automated quantitative gait analysis during overground locomotion in the rat: its application to spinal cord contusion and transection injuries. *J Neurotrauma.* 2001; 18:187–201. [PubMed: 11229711]
- Hampton TG, Amende I. Treadmill gait analysis characterizes gait alterations in Parkinson's disease and amyotrophic lateral sclerosis mouse models. *J Mot Behav.* 2010; 42:1–4. [PubMed: 19906638]
- Heglund NC, Taylor CR. Speed, stride frequency and energy cost per stride: how do they change with body size and gait? *J Exp Biol.* 1988; 138:301–318. [PubMed: 3193059]
- Heglund NC, Taylor CR, McMahon TA. Scaling stride frequency and gait to animal size: mice to horses. *Science.* 1974; 186:1112–1113. [PubMed: 4469699]
- Hendriks WT, Eggers R, Ruitenbergh MJ, Blits B, Hamers FP, Verhaagen J, Boer GJ. Profound differences in spontaneous long-term functional recovery after defined spinal tract lesions in the rat. *J Neurotrauma.* 2006; 23:18–35. [PubMed: 16430370]
- Herbin M, Gasc JP, Renous S. Symmetrical and asymmetrical gaits in the mouse: patterns to increase velocity. *J Comp Physiol A.* 2004; 190:895–906.
- Herbin M, Hackert R, Gasc JP, Renous S. Gait parameters of treadmill versus overground locomotion in mouse. *Behav Brain Res.* 2007; 181:173–179. [PubMed: 17521749]
- Hetz S, Romer C, Teufelhart C, Meisel A, Engel O. Gait analysis as a method for assessing neurological outcome in a mouse model of stroke. *J Neurosci Methods.* 2012; 206:7–14. [PubMed: 22343052]
- Hildebrand M. Analysis of tetrapod gaits: general considerations and symmetrical gaits. *Neural Control Locomotion.* 1976; 18:203–206.
- Hildebrand M. The quadrupedal gaits of vertebrates. *Bio-Science.* 1989; 39:766–774.
- Hoffmann MH, Hopf R, Niederreiter B, Redl H, Smolen JS, Steiner G. Gait changes precede overt arthritis and strongly correlate with symptoms and histopathological events in pristane-induced arthritis. *Arthritis Res Ther.* 2010; 12:R41. [PubMed: 20222952]
- Hruska RE, Kennedy S, Silbergeld EK. Quantitative aspects of normal locomotion in rats. *Life Sci.* 1979; 25:171–179. [PubMed: 491843]
- Hruska RE, Silbergeld EK. Abnormal locomotion in rats after bilateral intrastriatal injection of kainic acid. *Life Sci.* 1979; 25:181–193. [PubMed: 491844]

- Jeong MA, Plunet W, Streijger F, Lee JH, Plemel JR, Park S, Lam CK, Liu J, Tetzlaff W. Intermittent fasting improves functional recovery after rat thoracic contusion spinal cord injury. *J Neurotrauma*. 2011; 28:479–492. [PubMed: 21219083]
- Klapka N, Hermanns S, Straten G, Masannek C, Duis S, Hamers FP, Muller D, Zuschratter W, Muller HW. Suppression of fibrous scarring in spinal cord injury of rat promotes longdistance regeneration of corticospinal tract axons, rescue of primary motoneurons in somatosensory cortex and significant functional recovery. *Eur J Neurosci*. 2005; 22:3047–3058. [PubMed: 16367771]
- Kloos AD, Fisher LC, Detloff MR, Hassenzahl DL, Basso DM. Stepwise motor and all-or-none sensory recovery is associated with nonlinear sparing after incremental spinal cord injury in rats. *Exp Neurol*. 2005; 191:251–265. [PubMed: 15649480]
- Knafelz GJ, Beeber L, Schwartz TA. A strategy for selecting among alternative models for continuous longitudinal data. *Res Nurs Health*. 2012; 35:647–658. [PubMed: 22911221]
- Koopmans GC, Deumens R, Brook G, Gerver J, Honig WM, Hamers FP, Joosten EA. Strain and locomotor speed affect overground locomotion in intact rats. *Physiol Behav*. 2007; 92:993–1001. [PubMed: 17959205]
- Koopmans GC, Deumens R, Honig WM, Hamers FP, Steinbusch HW, Joosten EA. The assessment of locomotor function in spinal cord injured rats: the importance of objective analysis of coordination. *J Neurotrauma*. 2005; 22:214–225. [PubMed: 15716628]
- Kopecky B, Decook R, Fritsch B. Mutational ataxia resulting from abnormal vestibular acquisition and processing is partially compensated for. *Behav Neurosci*. 2012; 126:301–313. [PubMed: 22309445]
- Leblond H, L'Esperance M, Orsal D, Rossignol S. Treadmill locomotion in the intact and spinal mouse. *J Neurosci*. 2003; 23:11411–11419. [PubMed: 14673005]
- Lin KL, Yang DY, Chu IM, Cheng FC, Chen CJ, Ho SP, Pan HC. DuraSeal as a ligature in the anastomosis of rat sciatic nerve gap injury. *J Surg Res*. 2010; 161:101–110. [PubMed: 19482304]
- Mancuso R, Oliván S, Osta R, Navarro X. Evolution of gait abnormalities in SOD1(G93A) transgenic mice. *Brain Res*. 2011; 1406:65–73. [PubMed: 21733494]
- Masocha W, Parvathy SS. Assessment of weight bearing changes and pharmacological antinociception in mice with LPS-induced monoarthritis using the Catwalk gait analysis system. *Life Sci*. 2009; 85:462–469. [PubMed: 19683012]
- Mcllwain KL, Merriweather MY, Yuva-Paylor LA, Paylor R. The use of behavioral test batteries: effects of training history. *Physiol Behav*. 2001; 73:705–717. [PubMed: 11566205]
- Mignon L, Magat J, Schakman O, Marbaix E, Gallez B, Jordan BE. Hexafluorobenzene in comparison with perfluoro-15-crown-5-ether for repeated monitoring of oxygenation using 19F MRI in a mouse model. *Magn Reson Med*. 2013; 69:248–254. [PubMed: 22442096]
- Miyagi M, Ishikawa T, Kamoda H, Orita S, Kuniyoshi K, Ochiai N, Kishida S, Nakamura J, Eguchi Y, Arai G, Suzuki M, Aoki Y, Toyone T, Takahashi K, Inoue G, Ohtori S. Assessment of gait in a rat model of myofascial inflammation using the CatWalk system. *Spine*. 2011; 36:1760–1764. [PubMed: 21673621]
- Mountney A, Leung LY, Pedersen R, Shear D, Tortella F. Longitudinal assessment of gait abnormalities following penetrating ballistic-like brain injury in rats. *J Neurosci Methods*. 2013; 212:1–16. [PubMed: 22981945]
- Neumann M, Wang Y, Kim S, Hong SM, Jeng L, Bilgen M, Liu J. Assessing gait impairment following experimental traumatic brain injury in mice. *J Neurosci Methods*. 2009; 176:34–44. [PubMed: 18805438]
- Norman, GR.; Streiner, DL. *Biostatistics: The Bare Essentials*. 3rd. Shelton, Connecticut: People's Medical Pub. House; 2008.
- Parker AJ, Clarke KA. Gait topography in rat locomotion. *Physiol Behav*. 1990; 48:41–47. [PubMed: 2236277]
- Paylor R, Spencer CM, Yuva-Paylor LA, Pieke-Dahl S. The use of behavioral test batteries, II: effect of test interval. *Physiol Behav*. 2006; 87:95–102. [PubMed: 16197969]
- Pereira JE, Cabrita AM, Filipe VM, Bulas-Cruz J, Couto PA, Melo-Pinto P, Costa LM, Geuna S, Mauricio AC, Varejao AS. A comparison analysis of hindlimb kinematics during overground and treadmill locomotion in rats. *Behav Brain Res*. 2006; 172:212–218. [PubMed: 16777243]

- Petrosyan HA, Hunanyan AS, Alessi V, Schnell L, Levine J, Arvanian VL. Neutralization of inhibitory molecule NG2 improves synaptic transmission, retrograde transport, and locomotor function after spinal cord injury in adult rats. *J Neurosci*. 2013; 33:4032–4043. [PubMed: 23447612]
- Starkey ML, Barritt AW, Yip PK, Davies M, Hamers FP, McMahon SB, Bradbury EJ. Assessing behavioural function following a pyramidotomy lesion of the corticospinal tract in adult mice. *Exp Neurol*. 2005; 195:524–539. [PubMed: 16051217]
- Strejiger F, Beernink TM, Lee JH, Bhatnagar T, Park S, Kwon BK, Tetzlaff W. Characterization of a cervical spinal cord hemi-contusion injury in mice using the infinite horizon impactor. *J Neurotrauma*. 2013; 30:869–883. [PubMed: 23360150]
- Sucher R, Lin CH, Zanoun R, Atsina KK, Weinstock M, Pulikkotil B, Schneeberger S, Zheng XX, Pratschke J, Lee WP, Brandacher G. Mouse hind limb transplantation: a new composite tissue allotransplantation model using nonsuture supermicrosurgery. *Transplantation*. 2010; 90:1374–1380. [PubMed: 21076369]
- Thota AK, Watson SC, Knapp E, Thompson B, Jung R. Neuromechanical control of locomotion in the rat. *J Neurotrauma*. 2005; 22:442–465. [PubMed: 15853462]
- Truin M, van Kleef M, Verboeket Y, Deumens R, Honig W, Joosten EA. The effect of Spinal Cord Stimulation in mice with chronic neuropathic pain after partial ligation of the sciatic nerve. *Pain*. 2009; 145:312–318. [PubMed: 19619944]
- Vandeputte C, Taymans JM, Casteels C, Coun F, Ni Y, Van Laere K, Baekelandt V. Automated quantitative gait analysis in animal models of movement disorders. *BMC Neurosci*. 2010; 11:92. [PubMed: 20691122]
- Vilensky JA, Libii JN, Moore AM. Trot-gallop gait transitions in quadrupeds. *Physiol Behav*. 1991; 50:835–842. [PubMed: 1775560]
- Vincelette J, Xu Y, Zhang LN, Schaefer CJ, Vergona R, Sullivan ME, Hampton TG, Wang YX. Gait analysis in a murine model of collagen-induced arthritis. *Arthritis Res Ther*. 2007; 9:R123. [PubMed: 18036238]
- Vincent, A-F.; Goiffon, G-C. *Mémoire Artificielle Des Principes Relatifs à la Fidelle Représentation Des Animaux, Tant En Peinture Qu'en Sculpture*. Paris, France: Premier partie, Concernant le cheval; 1779.
- Vlamings R, Visser-Vandewalle V, Koopmans G, Joosten EA, Kozan R, Kaplan S, Steinbusch HW, Temel Y. High frequency stimulation of the subthalamic nucleus improves speed of locomotion but impairs forelimb movement in Parkinsonian rats. *Neuroscience*. 2007; 148:815–823. [PubMed: 17706885]
- Vrinten DH, Hamers FF. 'CatWalk' automated quantitative gait analysis as a novel method to assess mechanical allodynia in the rat; a comparison with von Frey testing. *Pain*. 2003; 102:203–209. [PubMed: 12620612]
- Wang XH, Lu G, Hu X, Tsang KS, Kwong WH, Wu FX, Meng HW, Jiang S, Liu SW, Ng HK, Poon WS. Quantitative assessment of gait and neurochemical correlation in a classical murine model of Parkinson's disease. *BMC Neurosci*. 2012; 13:142. [PubMed: 23151254]
- Wang Y, Neumann M, Hansen K, Hong SM, Kim S, Noble-Haesslein LJ, Liu J. Fluoxetine increases hippocampal neurogenesis and induces epigenetic factors but does not improve functional recovery after traumatic brain injury. *J Neurotrauma*. 2011; 28:259–268. [PubMed: 21175261]
- Westerga J, Gramsbergen A. The development of locomotion in the rat. *Brain Res Dev Brain Res*. 1990; 57:163–174. [PubMed: 2073717]
- Wooley CM, Xing S, Burgess RW, Cox GA, Seburn KL. Age, experience and genetic background influence treadmill walking in mice. *Physiol Behav*. 2009; 96:350–361. [PubMed: 19027767]

**Fig. 1.**

Variations in speed distributions. **A:** Total speed distribution across 12 time points $n=1,137$, mean = 78.73 cm/sec, and SD = 18.85 cm/sec; this distribution approximated normal (K-S $P = 0.000$, skewness = -0.340). **B:** Speed distribution for PND 105, $n = 111$, mean = 78.38 cm/sec, SD = 16.33 cm/sec; this distribution approximated normal (K-S $P = 0.195$). **C:** Speed distribution for PND 103, $n = 103$, mean = 75.44 cm/sec, SD = 16.62 cm/sec; this distribution approximated normal (K-S $P = 0.006$, skewness = -0.159). **D:** Speed distribution for PND 111, $n = 86$, mean = 74.06 cm/sec, SD = 17.75 cm/sec; this distribution did not approximate normal (K-S $P = 0.008$, skewness = -0.553).

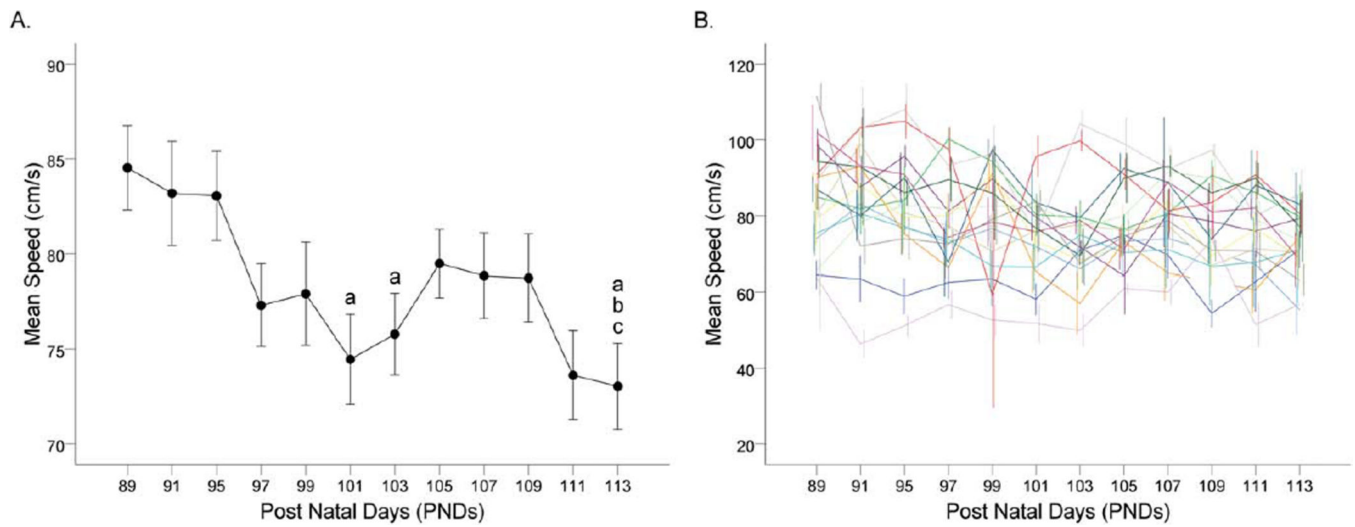


Fig. 2.

Average speed \pm SEM per day, overall and per animal. **A:** Average speed for the group of 16 WT animals across 12 time points. Each day is expressed as mean \pm SEM. (a) = significantly different from PND 89 ($P = 0.044$). (b) = significantly different from PND 91 ($P = 0.005$). (c) = significantly different from PND 95 ($P = 0.023$). **B:** Average speed \pm SEM of each of the 16 subjects across 12 time points. Each colored line represents a different animal.

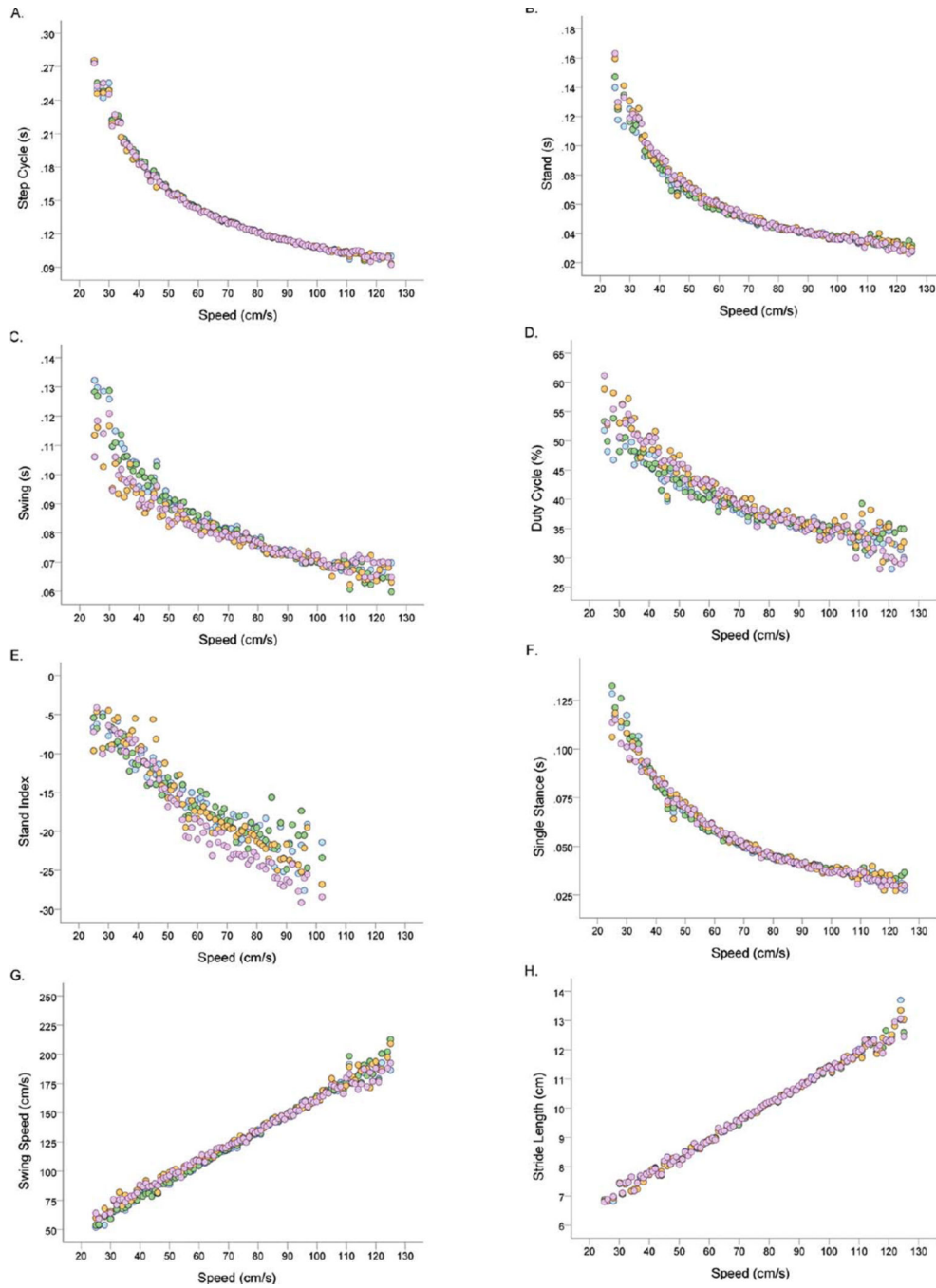


Fig. 3. Scatterplots of speed-averaged data depicting the relationship between discrete speed (cm/sec) and Paw Statistics variables. Data are color coded: Blue = left front (LF) paw, Green = RF paw, Orange = left hind (LH) paw, and Pink = right hind (RH) paw. **A:** Step cycle versus speed. LF, RF, LH, RH = inverse, ↓ WIS. **B:** Stand versus speed. LF, LH, RH = power, ↓ WIS; RF = inverse, ↓ WIS. **C:** Swing versus speed. LF = inverse, ↓ WIS; RF, LH, RH = power, ↓ WIS. **D:** Duty cycle versus speed. LF, RH = logarithmic, ↓ WIS; RF, LH = power, ↓ WIS. **E:** Stand index versus speed. LF, RF, LH, RH = logarithmic, ↓ WIS. **F:** Single

stance versus speed. LF, RF = inverse, ↓ WIS; LH, RH = power, ↓ WIS. **G:** Swing speed versus speed. LF, RF, RH = power, ↑ WIS; LH = linear, ↑ WIS. **H:** Stride length versus speed. LF, RF, LH, RH = linear, ↑ WIS.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

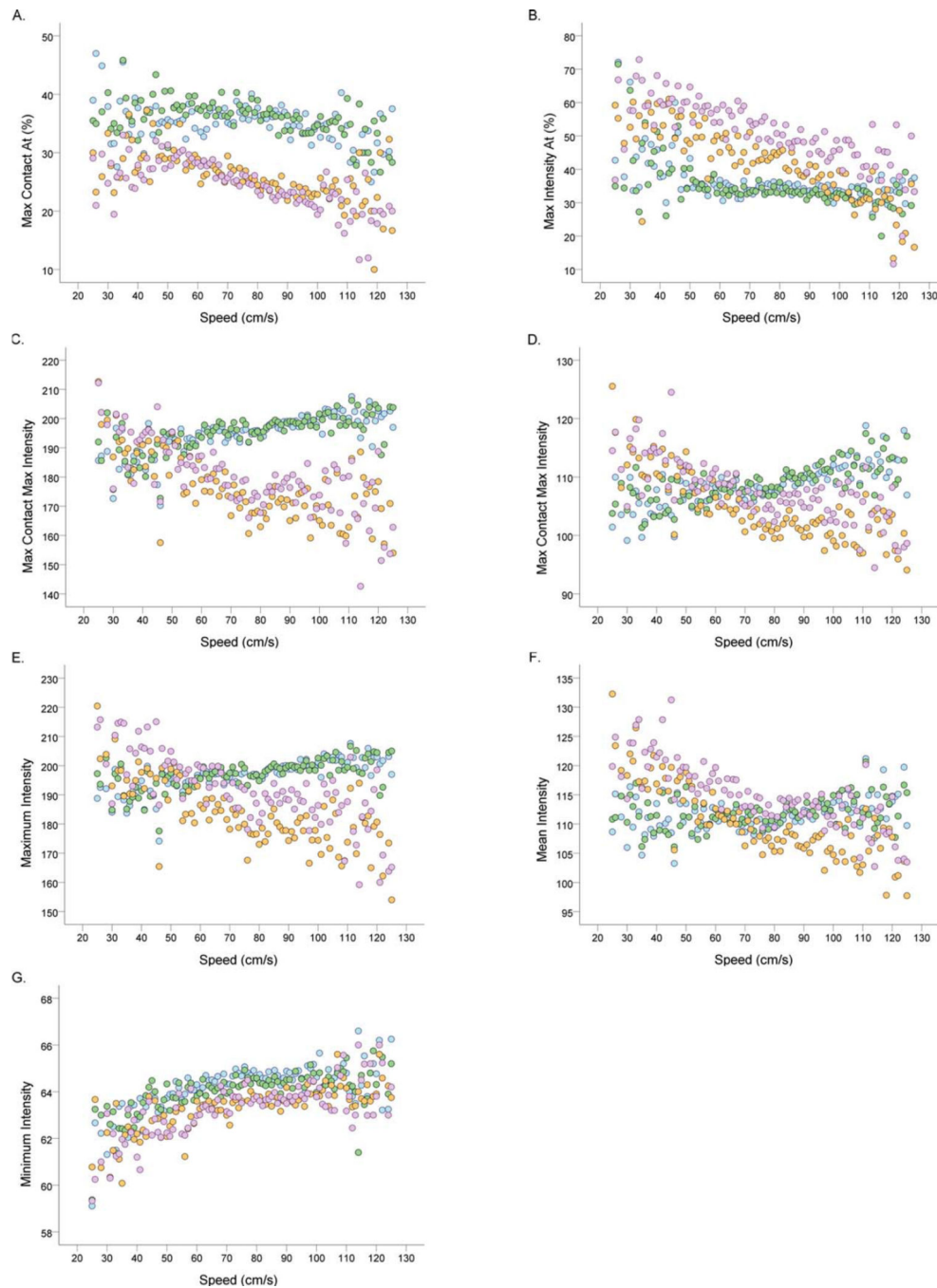


Fig. 4. Scatterplots of speed-averaged data depicting the relationship between discrete speed (cm/sec) and Paw Statistics variables. Data is color coded: Blue = left front (LF) paw, Green = RF paw, Orange = left hind (LH) paw, and Pink = right hind (RH) paw. **A:** Max contact at (%) versus speed. LF, LH, RH = linear, ↓ WIS; RF = compound, ↓ WIS. **B:** Max intensity at (%) versus speed. LF = S, ↓ -WIS; RF = inverse, ↓ -WIS; LH, RH = linear, ↓ WIS. **C:** Max contact max intensity versus speed. LF, RF = logarithmic, ↑ WIS; LH = inverse ↓ WIS; RH = logarithmic, ↓ WIS. **D:** Max contact mean intensity versus speed. LF = linear, ↑ WIS; RF

= compound, \uparrow WIS; LH = inverse, \downarrow WIS; RH = compound, \downarrow WIS. **E:** Maximum intensity versus speed. LF, RF = linear, \uparrow WIS; LH = inverse, \downarrow WIS; RH = linear, \downarrow WIS. **F:** Mean intensity versus speed. LF, RF = compound, \uparrow WIS; LH = inverse, \downarrow WIS; RH = compound, \downarrow WIS. **G:** Minimum intensity versus speed. LF, LH = S, \uparrow WIS; RF = inverse, \downarrow WIS; LH = logarithmic, \uparrow WIS.

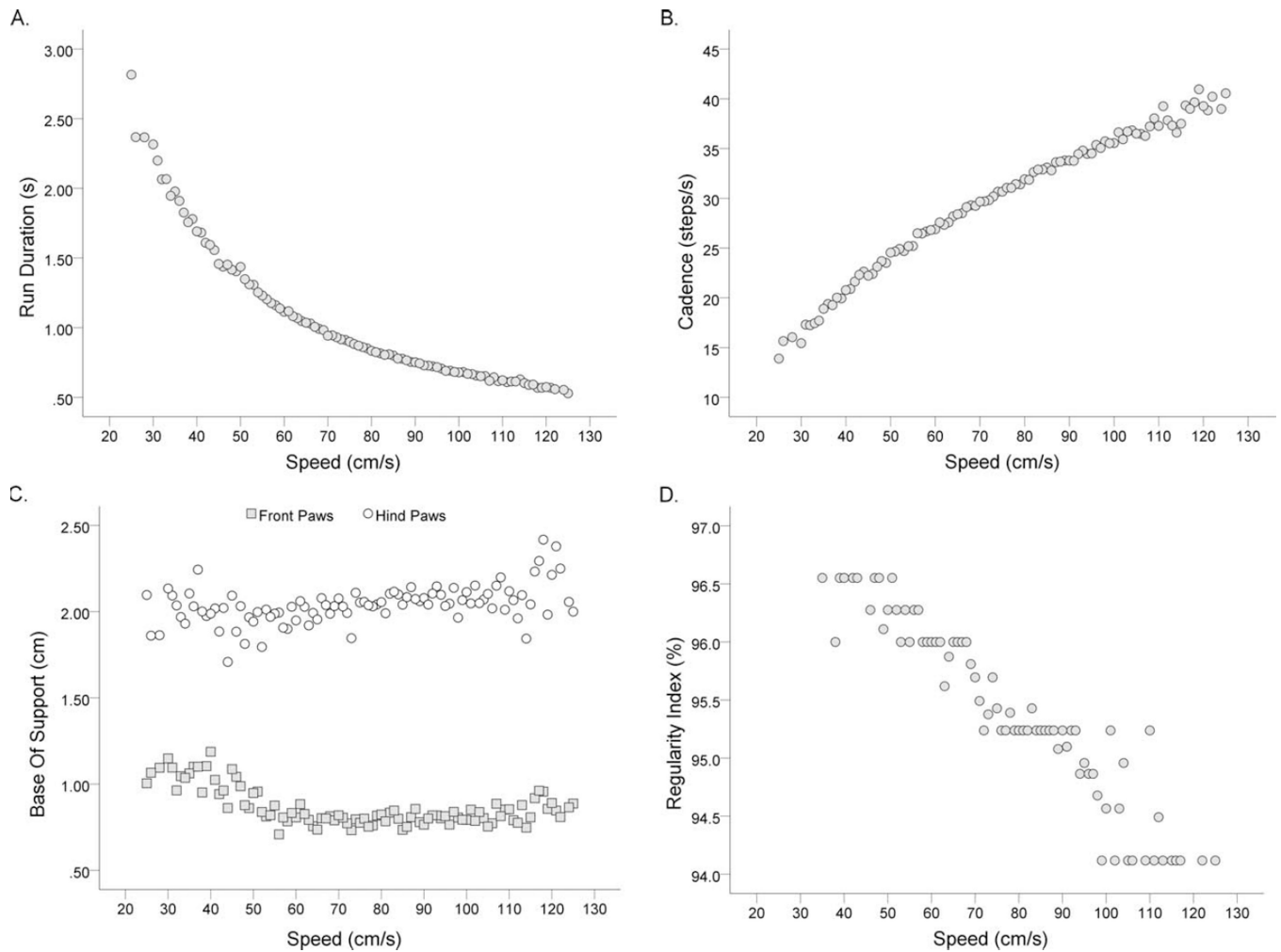


Fig. 5. Scatterplots of speed-averaged data depicting relationships between discrete speed (cm/sec) and Other Statistics. **A:** Run duration versus speed. Power, \downarrow WIS. **B:** Cadence versus speed. Logarithmic, \uparrow WIS. **C:** Base of support (BOS) versus speed. Gray square represents front paws (F), open circle represents hind paws (HP). F = quadratic, $\downarrow\uparrow$ WIS. HP = linear, \uparrow WIS. **D:** Regularity index versus speed. Linear, \downarrow WIS.

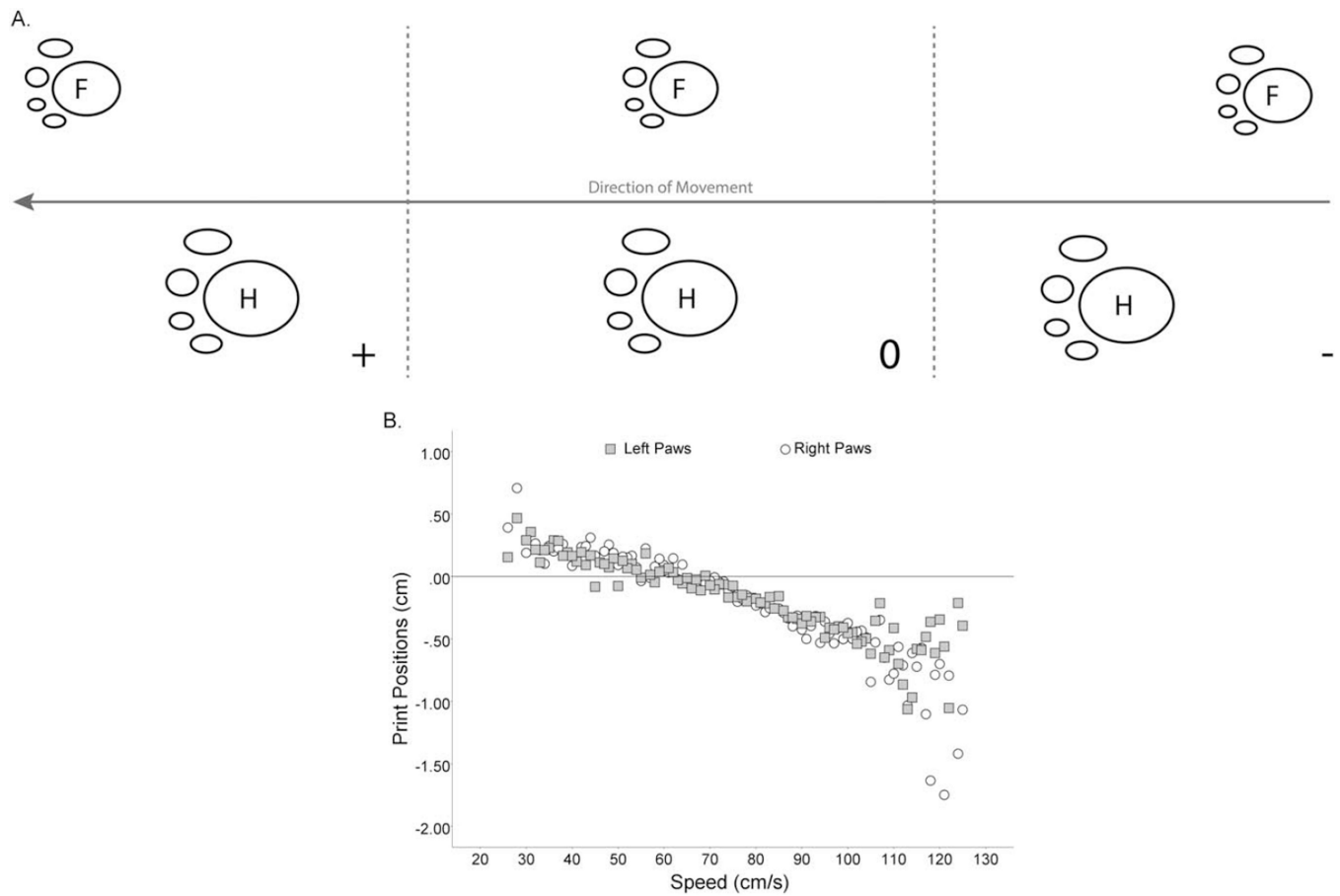


Fig. 6. Diagrammatic depiction of the print positions parameter and scatterplot of speed-averaged data depicting relationships between discrete speed (cm/sec) and print positions. **A:** Print positions describes the distance between the hind paw (H) and the previously placed front paw (F) ipsilaterally. (+) indicates H was placed behind F, (0) indicates H was placed at F, and (-) indicates H was placed in front of F. **B:** Print positions versus speed. Reference line at 0 cm. Gray square represents left paws (L), open circle represents right paws (R). R, L = quadratic, ↓ WIS.

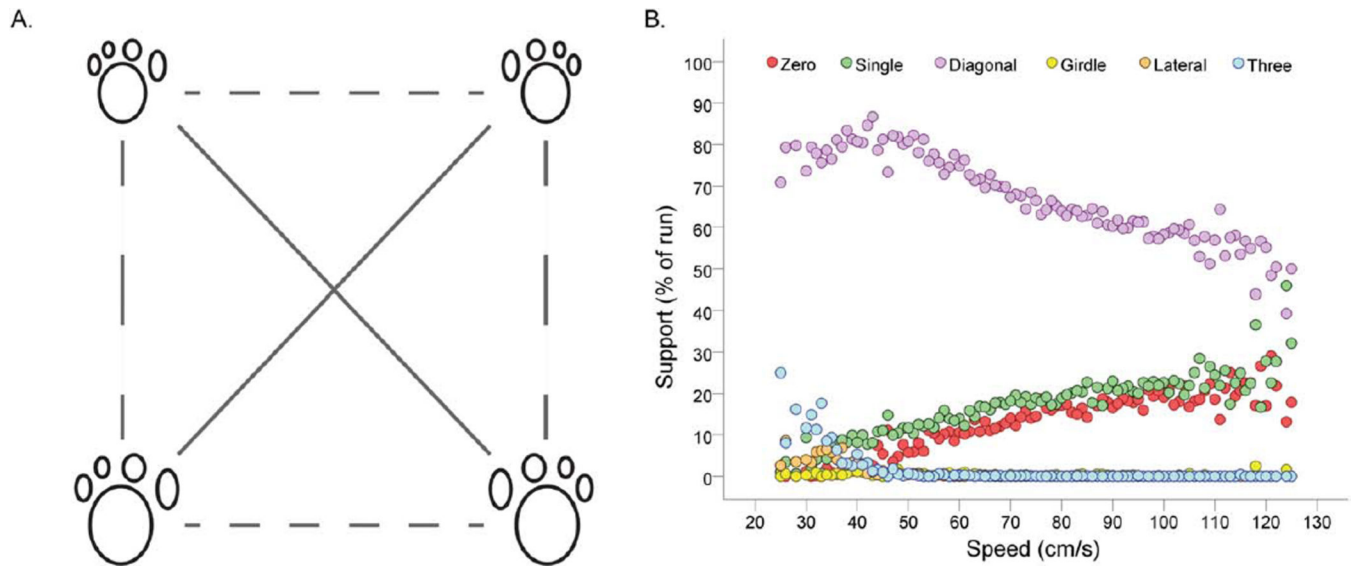


Fig. 7. Graphical depiction of two-paw combination categories and scatterplot of speed-averaged data depicting the relationship between discrete speed (cm/sec) and support variables. **A:** Graphical depiction of the categories used to describe pairs of paws. Solid lines = diagonal, small dashed lines = girdle, and medium dashed lines = lateral. **B:** Support (%) versus speed, which relays the percentage of a run that a particular number and/or combination of paws were in contact with the glass. Zero = logarithmic, \uparrow WIS. Single = logarithmic, \uparrow WIS. Three = inverse, \downarrow WIS. Diagonal = quadratic, $\uparrow\downarrow$ WIS. Girdle = logarithmic, \downarrow WIS. Lateral = inverse \downarrow WIS.

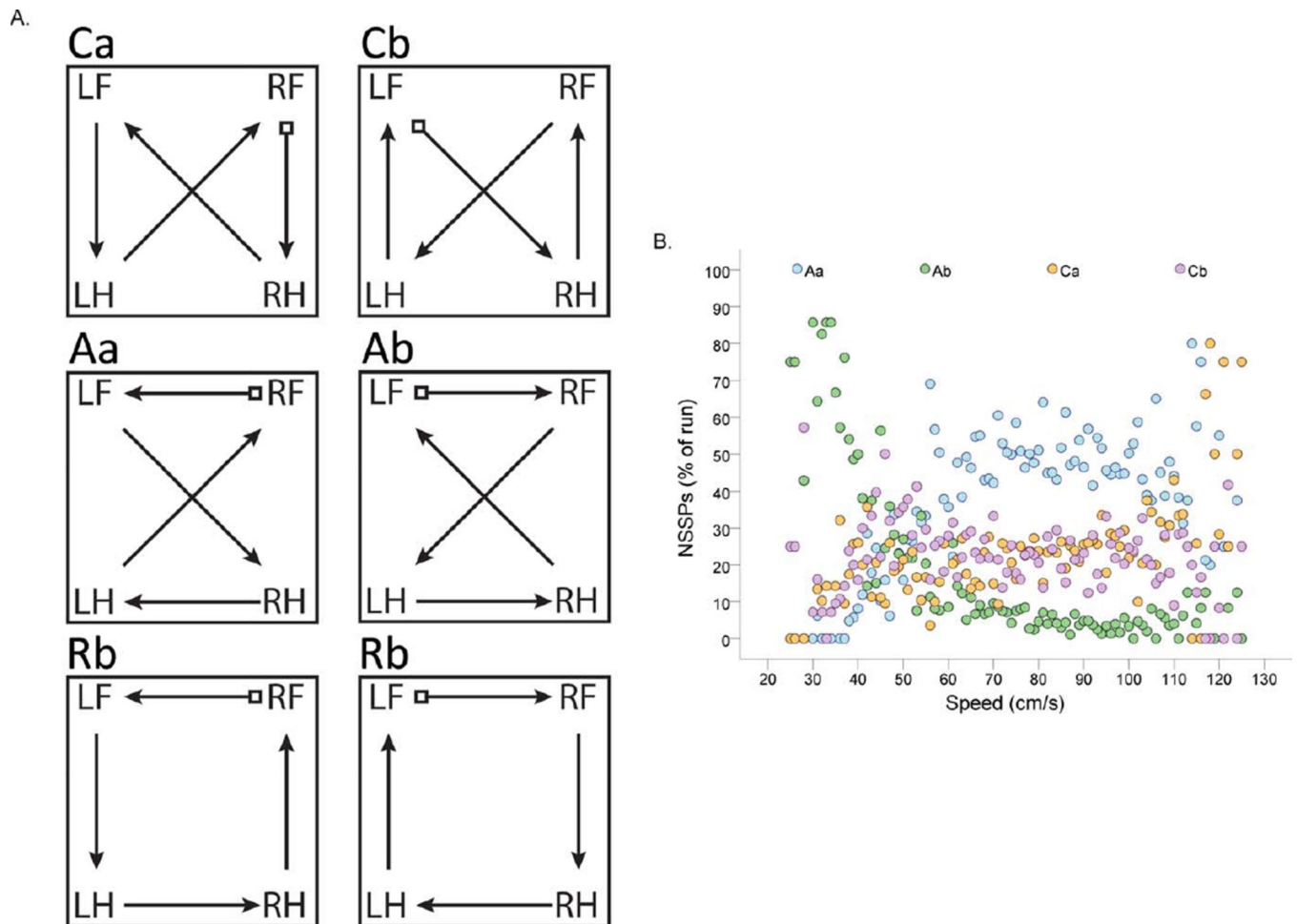


Fig. 8. Graphical representation of the six normal step sequence patterns (NSSPs) and scatterplot of speed-averaged data depicting relationships between discrete speed (cm/sec) and NSSPs. **A:** The boxed arrow indicates the first paw placed within the sequence. From top left to bottom right: Ca, Cb, Aa, Ab, Ra, and Rb. **B:** NSSPs (%) versus speed, which relays the percentage of a run that a particular sequential placement of paws was used. All data points are color coded according to the legend. Aa = quadratic, $\uparrow\downarrow$ WIS. Ab = inverse, \downarrow -. Ca = linear, \uparrow WIS. Cb = no dependence (ND), - WIS.

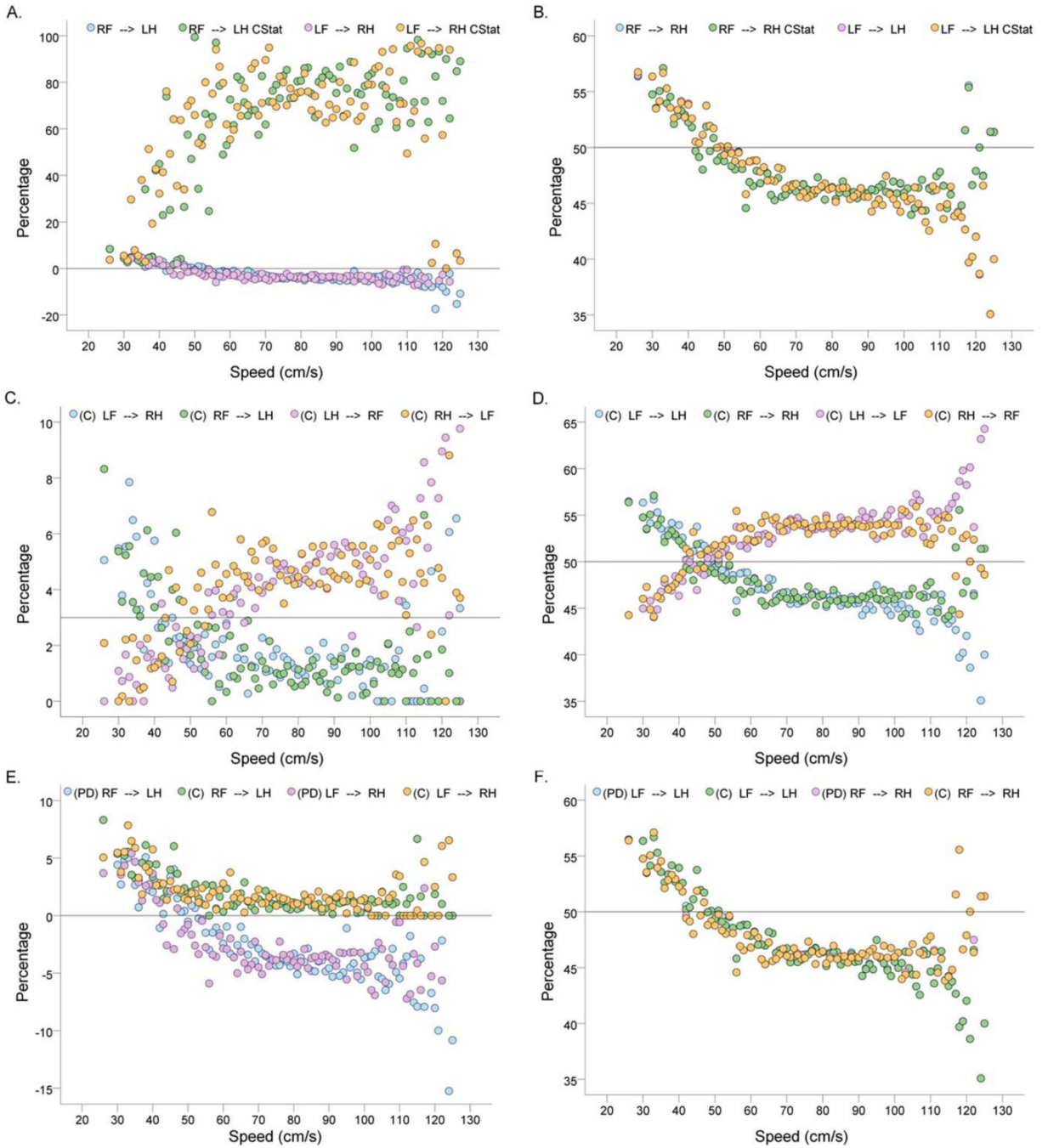


Fig. 9. Scatterplots of speed-averaged data depicting the relationships between Phase Dispersions (PD), Couplings (C), and discrete speed (cm/sec). All points are color coded according to respective legends. **A:** Normal mean versus CStat Mean for diagonal pairs in Phase Dispersions, reference line at 0%. **B:** Normal mean versus CStat Mean for lateral pairs in Phase Dispersions, reference line at 50%. **C:** Reciprocal representations for diagonal pairs in Couplings, reference line at 3.1%; Blue and pink are reciprocals, green and orange are reciprocals. **D:** Reciprocal representations for lateral pairs in Couplings, reference line at

50%. **E:** Comparison of diagonal pairs between Phase Dispersions and Couplings versus speed, reference line at 0%. **F:** Comparison of lateral pairs between Phase Dispersions and Couplings versus speed, reference line at 50%.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

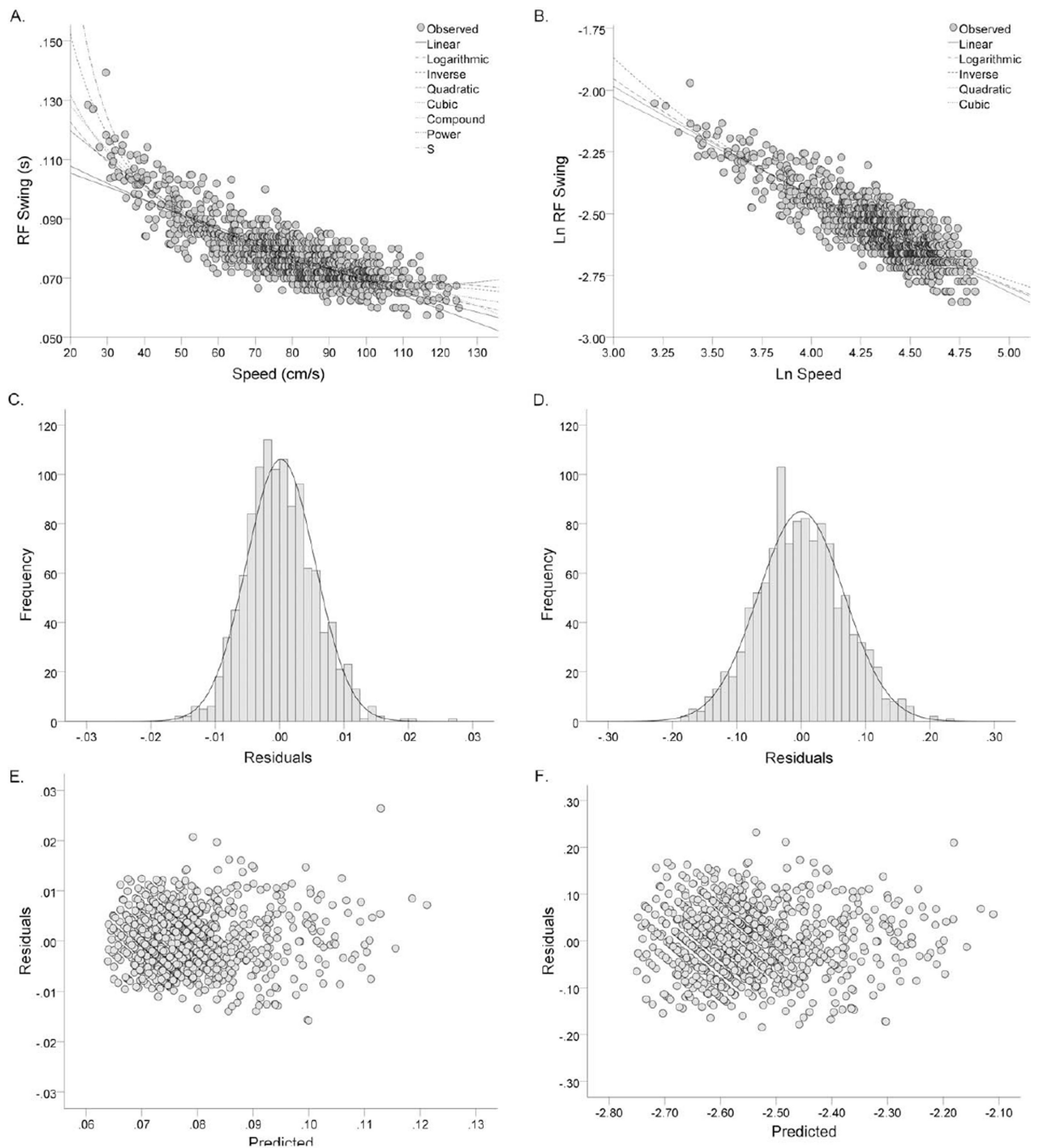


Fig. 10.

Visual guide to linearizing relationships with speed. **A:** Scatterplot of curve estimation from raw data of RF swing versus speed; the power model was selected ($R^2 = 0.721$, $P = 0.000$). **B:** Scatterplot of curve estimation from raw data transformed according to the power model [$\ln(y)$ and $\ln(x)$]; the linear model was selected ($R^2 = 0.721$, $P = 0.000$). **C:** Histogram of residuals from the power model of raw data. **D:** Histogram of residuals from linear model of the transformed data. **E:** Scatterplot of residuals versus predicted for the power model of raw

data. **F**: Scatterplot of residuals versus predicted for the linear model of the transformed data.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

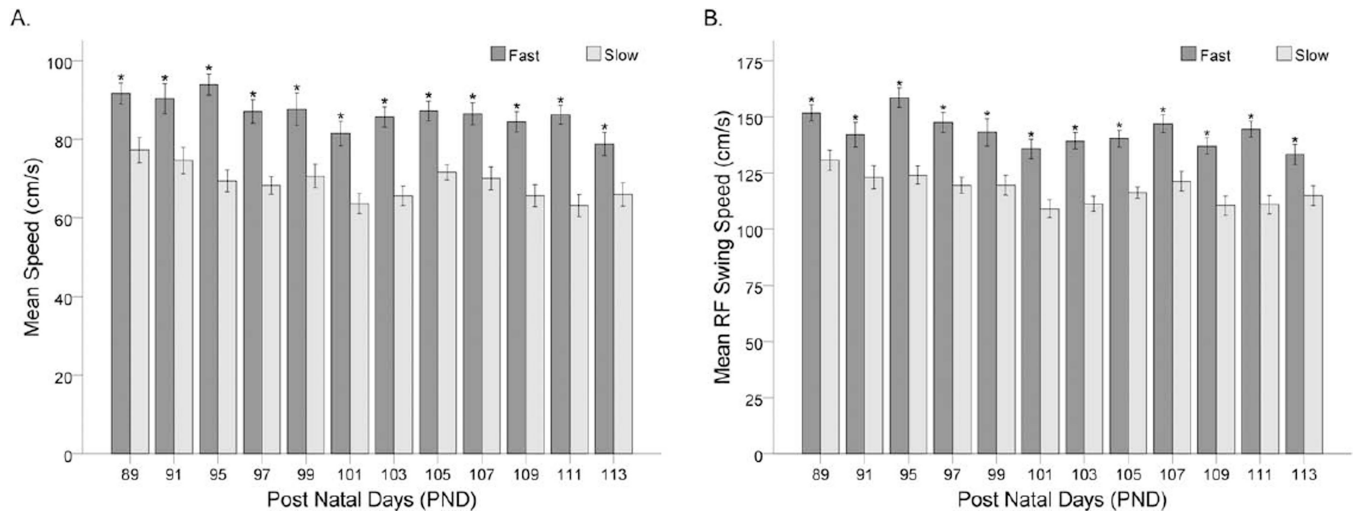


Fig. 11.

Comparison between fast and slow groups, with speed excluded as a covariate in the linear mixed model (LMM). **A:** Mean speed \pm SEM per day between groups. Asterisks denote significance at $P < 0.035$. **B:** Mean RF swing speed \pm SEM per day between groups. Asterisks denote significance at $P < 0.047$.

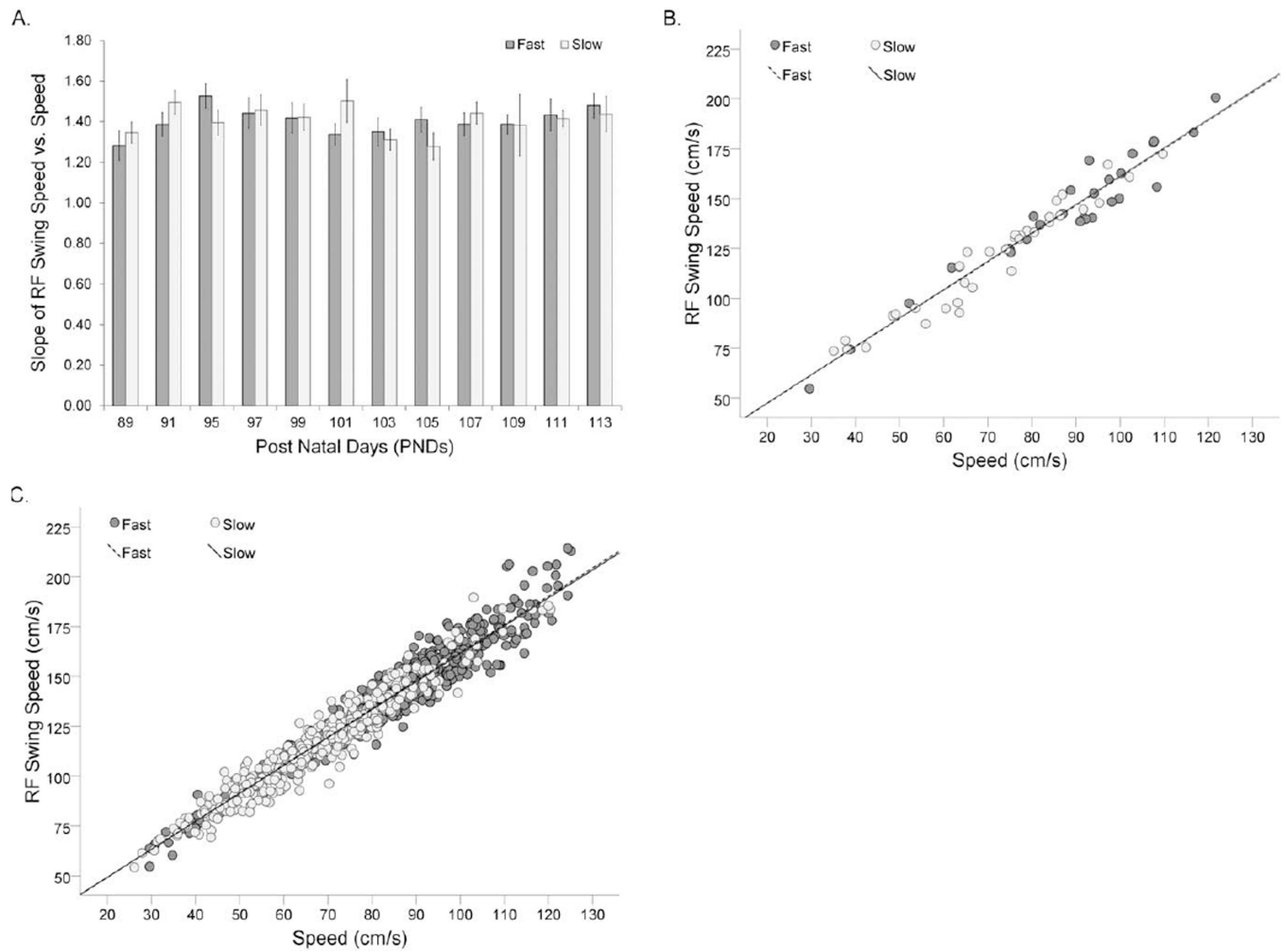


Fig. 12.

Comparison between fast and slow groups, with speed included as a covariate in the linear mixed model (LMM). **A:** Mean slope of RF swing speed versus speed per day between groups. No difference existed between groups overall or on any day. **B:** Scatter-plot depicting group averages of RF swing speed versus speed for PND 99. No significant differences existed between groups regarding slope or intercept. **C:** Scatterplot depicting the total raw data of RF swing speed versus speed between groups. No significant differences existed between groups regarding slope or intercept.

TABLE 1

Mathematical and WIS relationships for all paw statistics

Variable	Left front			Right front		
	Relationship	R ²	P-value	Relationship	R ²	P-value
Step Cycle (s)	Inverse	0.994	0.000	Inverse	0.995	0.000
Stand (s)	Power	0.988	0.000	Inverse	0.984	0.000
Swing (s)	Inverse	0.976	0.000	Power	0.970	0.000
Duty Cycle (%)	Logarithmic	0.937	0.000	Power	0.923	0.000
Stand Index	Logarithmic	0.918	0.000	Logarithmic	0.877	0.000
Swing Speed (cm/s)	Power	0.994	0.000	Power	0.993	0.000
Stride Length (cm)	Linear	0.992	0.000	Linear	0.994	0.000
Single Stance (s)	Inverse	0.991	0.000	Inverse	0.992	0.000
Initial Dual Stance (s)	Inverse	0.412	0.000	Inverse	0.551	0.000
Terminal Dual Stance (s)	Inverse	0.526	0.000	Inverse	0.438	0.000
Max Contact At (%)	Linear	0.173	0.000	Compound	0.273	0.000
Max Contact Area (cm ²)	ND			ND		
Print Area (cm ²)	ND			ND		
Print Length (cm)	Linear	0.725	0.000	Linear	0.696	0.000
Print Width (cm)	Linear	0.250	0.000	Linear	0.104	0.001
Max Contact Max Intensity	Logarithmic	0.578	0.000	Logarithmic	0.487	0.000
Max Contact Mean Intensity	Linear	0.571	0.000	Compound	0.534	0.000
Max Intensity At (%)	S	0.427	0.000	Inverse	0.405	0.000
Maximum Intensity	Linear	0.533	0.000	Linear	0.410	0.000
Mean Intensity	Compound	0.154	0.000	Compound	0.093	0.002
Mean Intensity Of The 15 Most Intense Pixels	Linear	0.279	0.000	Compound	0.213	0.000
Minimum Intensity	S	0.655	0.000	Inverse	0.528	0.000

Variable	Left hind			Right hind		
	Relationship	R ²	P-value	Relationship	R ²	P-value
Step Cycle (s)	Inverse	0.993	0.000	Inverse	0.994	0.000
Stand (s)	Power	0.983	0.000	Power	0.989	0.000

Variable	Left front				Right front			
	Relationship	R ²	P-value	WIS	Relationship	R ²	P-value	WIS
Swing (s)	Power	0.943	0.000	↓	Power	0.942	0.000	↓
Duty Cycle (%)	Power	0.930	0.000	↓	Logarithmic	0.959	0.000	↓
Stand Index	Logarithmic	0.876	0.000	↓	Logarithmic	0.942	0.000	↓
Swing Speed (cm/s)	Linear	0.990	0.000	↑	Power	0.992	0.000	↑
Stride Length (cm)	Linear	0.994	0.000		Linear	0.993	0.000	
Single Stance (s)	Power	0.981	0.000	↓	Power	0.989	0.000	↓
Initial Dual Stance (s)	Inverse	0.645	0.000	↓	Inverse	0.583	0.000	↓
Terminal Dual Stance (s)	Inverse	0.600	0.000	↓	Inverse	0.639	0.000	↓
Max Contact At (%)	Linear	0.405	0.000	↓	Linear	0.517	0.000	↓
Max Contact Area (cm ²)	Inverse	0.244	0.000	↓	Inverse	0.376	0.000	↓
Print Area (cm ²)	Inverse	0.208	0.000	↓	Inverse	0.351	0.000	↓
Print Length (cm)	ND				ND			
Print Width (cm)	Inverse	0.322	0.000	↓	Inverse	0.548	0.000	↓
Max Contact Max Intensity	Inverse	0.565	0.000	↓	Logarithmic	0.557	0.000	↓
Max Contact Mean Intensity	Inverse	0.739	0.000	↓	Compound	0.570	0.000	↓
Max Intensity At (%)	Linear	0.729	0.000	↓	Linear	0.571	0.000	↓
Maximum Intensity	Inverse	0.593	0.000	↓	Linear	0.574	0.000	↓
Mean Intensity	Inverse	0.768	0.000	↓	Compound	0.649	0.000	↓
Mean Intensity Of The 15 Most Intense Pixels	Inverse	0.619	0.000	↓	Linear	0.573	0.000	↓
Minimum Intensity	Logarithmic	0.607	0.000	↑	S	0.629	0.000	↑

ND = no dependence, WIS = with increasing speed, ↑ = increases, ↓ = decreases, – = no change.

TABLE 2

Consolidated mathematical and WIS relationships for all paw statistics

Variable	Mathematical relationships				Graphical relationships			
	Left front	Right front	Left hind	Right hind	LF	RF	LH	RH
					WIS	WIS	WIS	WIS
Step Cycle (s)	Inverse	Inverse	Inverse	Inverse	↑	↑	↑	↑
Stand (s)	Power	Inverse	Power	Power	↑	↑	↑	↑
Swing (s)	Inverse	Power	Power	Power	↑	↑	↑	↑
Duty Cycle (%)	Logarithmic	Power	Power	Logarithmic	↓	↓	↓	↓
Stand Index	Logarithmic	Logarithmic	Logarithmic	Logarithmic	↓	↓	↓	↓
Swing Speed (cm/s)	Power	Power	Linear	Power	↑	↑	↑	↑
Stride Length (cm)	Linear	Linear	Linear	Linear				
Single Stance (s)	Inverse	Inverse	Power	Power	↓	↓	↓	↓
Initial Dual Stance (s)	Inverse	Inverse	Inverse	Inverse	↓	↓	↓	↓
Terminal Dual Stance (s)	Inverse	Inverse	Inverse	Inverse	↓	↓	↓	↓
Max Contact At (%)	Linear	Compound	Linear	Linear	↓	↓	↓	↓
Max Contact Area (cm ²)	ND	ND	Inverse	Inverse	-	-	↓	↓
Print Area (cm ²)	ND	ND	Inverse	Inverse	-	-	↓	↓
Print Length (cm)	Linear	Linear	ND	ND	↑	↑	-	-
Print Width (cm)	Linear	Linear	Inverse	Inverse	↓	↓	↓	↓
Max Contact Max Intensity	Logarithmic	Logarithmic	Inverse	Logarithmic	↑	↑	↓	↓
Max Contact Mean Intensity	Linear	Compound	Inverse	Compound			↓	↓
Max Intensity At (%)	S	Inverse	Linear	Linear	↓	↓	↓	↓
Maximum Intensity	Linear	Linear	Inverse	Linear			↓	↓
Mean Intensity	Compound	Compound	Inverse	Compound	↑	↑	↓	↓
Mean Intensity Of The 15 Most Intense Pixels	Linear	Compound	Inverse	Linear	↑	↑	↓	↓
Minimum Intensity	S	Inverse	Logarithmic	S	↑	↑	↑	↑

Left front = LF, right front = RF, left hind = LH, right hind = RH, ND = no dependence, WIS = with increasing speed, ↑ = increases, ↓ = decreases, - = no change. **Bold** text indicates differences in mathematical relationship, WIS relationship or both between the paws.

TABLE 3

Mathematical and WIS relationships for all other statistics

Variable	Relationship	R^2	P -value	WIS
Run Duration (s)	Power	0.998	0.000	↓
Number Of Steps	Logarithmic	0.992	0.000	↓
Cadence (Steps/s)	Logarithmic	0.993	0.000	
Maximum Variation (%)	Linear	0.141	0.000	↓
Print Positions Left Paws (cm)	Quadratic	0.826	0.000	↓
Print Positions Right Paws (cm)	Quadratic	0.873	0.000	↓
Base of Support Front Paws (cm)	Quadratic	0.714	0.000	↑↓
Base of Support Hind Paws (cm)	Linear	0.225	0.000	↑
Regularity Index (%)	Linear	0.904	0.000	↓
Number Of Patterns	Logarithmic	0.956	0.000	↓
Step Sequence Aa (%)	Quadratic	0.707	0.000	↑↓
Step Sequence Ab (%)	Inverse	0.812	0.000	↓-
Step Sequence Ca (%)	Linear	0.333	0.000	↑
Step Sequence Cb (%)	ND			-
Support Lateral(%)	Inverse	0.658	0.000	↓
Support Girdle(%)	Logarithmic	0.102	0.001	↓-
Support Zero (%)	Logarithmic	0.893	0.000	↑
Support Single (%)	Logarithmic	0.822	0.000	↑
Support Diagonal (%)	Quadratic	0.881	0.000	↑↓
Support Three(%)	Inverse	0.646	0.000	↓

ND = no dependence, WIS = with increasing speed, ↑ = increases, ↓ = decreases, - = no change.

TABLE 4

Mathematical and WIS relationships for all couplings and phase dispersions

Variable (Anchor → Target)	Couplings			Phase dispersions				
	Relationship	R ²	P-value	WIS	Relationship	R ²	P-value	WIS
RF→LH	Inverse	0.560	0.000	↓	Logarithmic	0.739	0.000	↓
RF→LH CStat	Inverse	0.561	0.000	↓	Inverse	0.671	0.000	↑
RF→LH CStat R	S	0.407	0.000	↑	Linear	0.239	0.000	↓
LF→RH	Quadratic	0.397	0.000	↓↑	Quadratic	0.571	0.000	↓↑
LF→RH CStat	Quadratic	0.399	0.000	↓↑	Quadratic	0.509	0.000	↓↑
LF→RH CStat R	Quadratic	0.161	0.000	↑↓	Compound	0.231	0.000	↓
LH→RH	Quadratic	0.453	0.000	↓↑	Quadratic	0.440	0.000	↓↑
LH→RH CStat	Quadratic	0.454	0.000	↑↓	Quadratic	0.445	0.000	↓↑
LH→RH CStat R	Linear	0.401	0.000	↓	Linear	0.268	0.000	↓
LF→RF	ND	-	-	-	ND	-	-	-
LF→RF CStat	ND	-	-	-	ND	-	-	-
LF→RF CStat R	ND	-	-	-	ND	-	-	-
RF→RH	Quadratic	0.753	0.000	↓↑	Quadratic	0.758	0.000	↓↑
RF→RH CStat	Quadratic	0.755	0.000	↓↑	Quadratic	0.760	0.000	↓↑
RF→RH CStat R	Compound	0.139	0.000	↓	Compound	0.153	0.000	↓
LF→LH	Logarithmic	0.843	0.000	↓	Logarithmic	0.841	0.000	↓
LF→LH CStat	Logarithmic	0.843	0.000	↓	Logarithmic	0.842	0.000	↓
LF→LH CStat R	Quadratic	0.226	0.000	↑↓	Quadratic	0.226	0.000	↓↑
LH→RF	Logarithmic	0.634	0.000	↑				
LH→RF CStat	Logarithmic	0.631	0.000	↑				
LH→RF CStat R	Linear	0.497	0.000	↓				
RH→LF	Inverse	0.435	0.000	↑-				
RH→LF CStat	Inverse	0.438	0.000	↑-				
RH→LF CStat R	Logarithmic	0.332	0.000	↓				
RH→LH	Quadratic	0.421	0.000	↓↑				
RH→LH CStat	Quadratic	0.420	0.000	↓↑				
RH→LH CStat R	Linear	0.242	0.000	↓				

Variable (Anchor → Target)	Couplings			Phase dispersions				
	Relationship	R ²	P-value	WIS	Relationship	R ²	P-value	WIS
RF → LH A	ND			-				
RF → LH CStat A	ND			-				
RF → LH CStat R A	Quadratic	0.222	0.000	↓				
RH → RF	Quadratic	0.751	0.000	↓				
RH → RF CStat	Quadratic	0.751	0.000	↓				
RH → RF CStat R	Quadratic	0.308	0.000	↓				
LH → LF	S	0.830	0.000	↑				
LH → LF CStat	S	0.832	0.000	↑				
LH → LF CStat R	Quadratic	0.229	0.000	↑				

Left front = LF, right front = RF, left hind = LH, right hind = RH. ND = no dependence, WIS = with increasing speed, ↑ = increases, ↓ = decreases, - = no change.

TABLE 5

Summary of WIS, graphical, and/or mathematical relationships

Publication	Step cycle	Stand	Swing	Stride length	Cadence
Herbin et al. 2004 (M, T)	↓ WIS, curvilinear	↓ WIS, curvilinear	↓ WIS, linear	↓ WIS, curvilinear	↓ WIS, curvilinear
Leblond et al. 2003 (M, T)	↓ WIS, curvilinear	j. WIS, curvilinear	ND		
Crone et al. 2009 (M, T)	↓ WIS, curvilinear	↓ WIS, curvilinear	↓ WIS, curvilinear	↓ WIS, linear	↓ WIS, curvilinear
Herbin et al. 2007 (M, T, U)	j. WIS, curvilinear	j. WIS, curvilinear	↓ WIS, linear	↓ WIS, curvilinear	↓ WIS, curvilinear
Heglund et al. 1974 (M, T)				↓ WIS	↓ WIS, curvilinear
Heglund and Taylor 1988 (M, T)					↓ WIS, curvilinear
Clarke and Still 2001 (M, U)	↓ WIS, curvilinear	↓ WIS	↓ WIS		
Clarke and Still 1999 (M, U)	j. WIS, curvilinear				
Gillis and Biewener 2001 (R, T)	↓ WIS, curvilinear	↓ WIS, curvilinear	ND		
Hruska and Silbergeld 1979 (R, U)	j. WIS, curvilinear	j. WIS, curvilinear	ND	↓ WIS, curvilinear	
Cohen and Gans 1975 (R, U)	↓ WIS, curvilinear			↓ WIS, linear	
Westerga and Gramsbergen 1990 (R, U)	↓ WIS, curvilinear, power	↓ WIS, curvilinear, power	↓ WIS	↓ WIS, linear	↓ WIS, curvilinear, power
Górska et al., 1998 (R, U)	↓ WIS, curvilinear, power	↓ WIS, curvilinear, power	↓ WIS, curvilinear, power		
Hruska et al. 1979 (R, U)	↓ WIS, curvilinear, power	↓ WIS, curvilinear, power	ND	↓ WIS, curvilinear, quadratic	
Koopmans et al. 2007 (R, U)		↓ WIS	↓ WIS (Wistar only)	↓ WIS	
Clarke and Parker 1986 (R, U)				↓ WIS, curvilinear	↓ WIS, curvilinear, quadratic
Thota et al. 2005 (R, T, U)				↓ WIS, linear	
Current study (M, U)	↓ WIS, curvilinear, inverse	↓ WIS, curvilinear, power for LF, LH, RH, inverse for RF	↓ WIS, curvilinear, power for RF, RH, LH, inverse for LF	↓ WIS, linear	↓ WIS, curvilinear, logarithmic

ND = no dependence, WIS = with increasing speed, ↑ = increases, ↓ = decreases, M = mouse, R = rat, T = treadmill, U = unforced