Published in final edited form as:

J Neurosci Methods. 2017 February 15; 278: 65–75. doi:10.1016/j.jneumeth.2017.01.002.

Characterization and validation of a split belt treadmill for measuring hindlimb ground-reaction forces in able-bodied and spinalized felines

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Abstract

BACKGROUND—The measurement of ground reaction forces (GRFs) in animals trained to locomote on a treadmill after spinal cord injury (SCI) could prove valuable for evaluating training outcomes; however, quantitative measures of the GRFs in spinal felines are limited.

NEW

METHOD—A split belt treadmill was designed and constructed to measure the GRFs of feline hindlimbs during stepping. The treadmill consists of two independent treadmill assemblies, each mounted on a force plate. The design allows measurements of the vertical (F_z), fore-aft (F_y) and mediolateral (F_x) ground-reaction forces for both hindlimbs while the forelimbs are resting on a platform.

RESULTS—Static and dynamic noise tests revealed little to no noise at frequencies below 6 Hz. Validation of the force plate measurements with a hand-held force sensor force showed good agreement between the two force readings. Peak normalized (to body mass) vertical GRFs for intact cats were $4.89 \pm 0.85 \text{ N/Kg}$ for the left hindlimb and $4.79 \pm 0.97 \text{ N/Kg}$ for the right. In comparison, trained spinalized cats peak normalized vertical GRFs were $2.20 \pm 0.94 \text{ N/Kg}$ for the left hindlimb and $2.85 \pm 0.99 \text{ N/Kg}$ for the right.

COMPARISON WITH OTHER EXISTING

METHODS—Previous methods of measuring GRFs used stationary single force plates or treadmill mounted to single force plate. Using independent treadmills for each hindlimb allows measurement of the individual hindlimb's GRFs in spinalized cats following body-weight supported treadmill training.

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Author disclosure: No competing financial interests exist.

CONCLUSIONS—The split belt force treadmill enables the simultaneous recording of ground-reaction forces for both hindlimbs in cats prior to spinalization, and following spinalization and body-weight-supported treadmill training (BWST).

Keywords

ground reaction forces; feline; locomotion; spinal cord injury; treadmill

Introduction

Spinal cord injury often causes deficits or complete loss of locomotor function depending on the severity of the injury. Body-weight supported training on a treadmill has been shown to promote recovery of locomotor function in spinal cats (Barbeau and Rossignol, 1987; Lovely et al., 1986) and rodents (Timoszyk et al., 2005), and to a lesser degree in individuals with spinal cord injury (Dietz and Harkema, 2004; Dobkin et al., 2006; Field-Fote and Roach, 2011; Fox et al., 2010). The animal or individual's paralyzed hindlimbs are initially moved in a gait pattern while weight is supported via the tail in cats or using a harness in humans. Automatic stepping returns over time and the amount of weight support is gradually decreased as locomotor capability increases. Although studies claim full-weight bearing capabilities for locomotor trained spinal cats, actual measured values of those capabilities have not been reported.

Ground reaction forces (GRFs) that occur during locomotion are typically measured using force plates (transducers that output a signal proportional to the force applied to the plate) mounted into a runway. The measurement of ground reaction forces aids in the assessment of locomotor function in humans and animals (Corbee et al., 2014; Cross, 1999; Fransz et al., 2013; Gregor et al., 2006; Lascelles et al., 2007; Schnabl and Bockstahler, 2015; Webb et al., 2011). Typically, force plates are mounted to capture a single step as the subject walks on a runway. Force plate mounted in a runway requires numerous trials in animals to ensure that sufficient steps where the animal steps on the force plate are acquired. Successive steps cannot be measured continuously using this method and walkways require large floor footprints, so force plates mounted under either the belt (Dingwell and Davis, 1996; Kram and Powell, 1989) or the entire treadmill (Kram et al., 1998) have been used to measure ground reaction forces during treadmill walking. The advantages of gathering force data on a treadmill as described in (Dingwell and Davis, 1996) include control of locomotor speed and continuous data collection. The ability to measure forces continuously allows for better assessment of the cyclical pattern seen in gait walking. Another advantage that many steps can be collected in a short period of time (Kram and Powell, 1989). While horizontal/fore-aft forces measured with force plates mounted under the belt suffer from error resulting from the belt friction, forces in all three directions (vertical, fore-aft and mediolateral) can be accurately obtained when the entire treadmill is mounted on the force plate (Kram et al., 1998; Willems and Gosseye, 2013). For spinal animals, instrumented treadmill present a distinct advantage since balance support must be provided by holding the tail, complicating the use of long walkway. In addition, since only the hindquarters are paralyzed and the forelimbs typically resting on a platform during evaluation of the lumbar locomotor center

ability, the moving treadmill belt is needed to provide the hip extension necessary to initiate the swing phase.

Current instrumented treadmill designs mount force plates under a single moving belt (Dingwell and Davis, 1996; Kram et al., 1998; Kram and Powell, 1989). This approach requires signal decomposition to differentiate the ground reaction forces of the left and right foot while stepping (Ballaz et al., 2013; Davis and Cavanagh, 1993). Decomposition of the signal may lead to loss of detail in data, which may be essential in gait analysis. In our study, a split belt treadmill was designed with two separate treadmill assemblies. Each assembly was mounted on a separate force plate allowing for collection of force data of the left and right feet separately. Designing such a treadmill enables the measurement of the ground reaction forces of adult spinal cats after treadmill training and comparison of the weight support before spinalization and after spinalization in body-weight supported trained animals.

Methods & Materials

Treadmill Design

A split belt treadmill was designed in AutoCAD software (Autodesk Inc., San Rafael, CA) and machined from stainless steel and aluminum. The entire assembly consists of two separate treadmills mounted side-by-side. Each treadmill has a footprint of 28 by 109 cm, with a belt walking surface approximately 11 cm wide by 69 cm long. Each treadmill assembly is bolted to a Kistler Type 9257BA (Kistler Instrument Corp., Winterthur, SUI) force plate mounted on a 30 wide by 76 long by 5 cm thick aluminum plate. Vibration transmission between the two treadmills was minimized by mounting the two aluminum plates supporting the treadmills on a 5 cm thick medium density fiberboard (MDF) mounted on a table (Little Giant model MTH1-2830-30, University Park, IL). When mounted on the MDF board, the distance between the aluminum plates holding the treadmill assemblies was 8 cm and the separation between treadmill belts was 0.6 cm. The table's legs sit on casters (WMI-60, WM Caster, Vernon CA) where the wheels can be lifted off the ground to couple the table to the ground via rubber spikes.

Each treadmill belt was powered via drive belt from an AC motor (Brother GL18N005-BWMYG2N, Brother International, Bridgewater, NJ). The motor was mounted underneath the walking belt top surface (see Figure 1). The entire walking surface formed by the two treadmill belts when mounted side-by-side is enclosed by a clear plexiglass box 17 cm wide by 67 cm long by 38 cm high to restrain the animal. The box assembly does not enter in contact with the treadmill belt at any point, and the forces applied to the enclosure are transmitted directly to the table or aluminum plates on which the treadmill is mounted (Figure 1, bottom panel). The speeds of the motors are controlled by two independent inverters (L100-007MFU2, Hitachi America Ltd, Charlotte, NC). The Kistler force plate underneath each treadmill has the ability to measure the vertical, fore-aft and mediolateral (F_z , F_y , F_x) ground reaction forces of each hindlimb separately (see Figure 1). The force sensitivity of the force plates (using Range 1/highest sensitivity setting of the force plate control unit, Type 5233A1, Kistler Instrument Corp., Winterthur, SUI) was 10 mV/N for the x and y axes and 5 mV/N for the z axis. The cutoff frequency of the built-in low-pass filter

of the force plate control unit was 200 Hz (2nd order Butterworth). Data from the force plates was recorded using a Vicon Motion Capture System (Vicon Motion System, Inc., Denver, CO, USA) that also allowed capture of the kinematics of locomotion.

Treadmill Validation

Static and Dynamic Noise Collection and Analysis—Both the right and left treadmill belts were off when recording the amount of static noise produced by the oscillations of the treadmill assembly in the laboratory environment. Dynamic measurements were recorded with both treadmill belts running at the same 0.3 m/s speed (cat's slow walking speed) to measure the additional mechanical vibrations induced by the motor and belt motion.

Voltage signals from both force plates were collected at 1000 Hz for each of the three axes of the force plates. The collected data was imported into MATLAB (Mathworks, Natick, MA) and converted from volts to Newtons using the manufacturer's calibration factor (z-axis calibration was confirmed using known weights applied to the belt surface under static condition). The force signal baseline offset deviation was then removed by calculating the mean for each axis and subtracting it from the original force vector. Finally, a Fast Fourier Transform (FFT) was performed on the force signals from the x, y, and z axes of each belt and the magnitude of the FFT was used to obtain the noise level distribution as a function of frequency. Plots of the FFT magnitude versus frequency revealed spectral peaks representing the frequency at which most of the noise in the force signal occurred, as well as the level of force noise within the frequency range relevant to gait (<10 Hz, 95% of power is below 10 Hz for the hindlimb joint marker positions in intact and trained spinal animals).

Force Plate Readings of the Forces Applied at the Belt Validation Utilizing Nano17 Force Sensor—The Kistler force plate readings of the forces applied at the treadmill belt were validated by conducting right and left gait simulation trials in order to ensure that the vertical, fore-aft, and mediolateral ground-reaction force recordings were accurate during actual treadmill operation. A Nano17 Force Sensor (ATI Industrial Automation, Apex, NC) was used to produce and simultaneously measure the forces applied to the treadmill belt. Before using the Nano17 to apply forces to the treadmill belts, its calibration was verified by loading the sensor with known weights (0.01–0.5 kg) while the sensor was mounted horizontally (vertical/z-axis force) or vertically. The Nano17 was then mounted on a 5 by 5 cm 5 mm thick piece of plexiglass held by the experimenter. A 3.8 cm diameter aluminum disk with three rubber half-balls glued to the disk was attached to the tool end of the Nano17. Forces were applied by pressing the tool end of the Nano force sensor to the left or right belt with both belts off or running at 0.3 m/s. Forces were applied to the treadmill belt by pressing the Nano17 on the belt and moving with the belt (if on) from the front of the treadmill to the rear, at which point the Nano17 was lifted off the belt and re-pressed on the belt at the front of the treadmill. Approximately 20 presses were applied with varying forces in the vertical, fore-aft and mediolateral directions. Care was taken to ensure that the 3 half-balls were in contact with the belt and that no slippage occurred between the tool-end and the belt. This guaranteed that the z-axes of the treadmill force plate and Nano17 were aligned throughout the contact period.

The data analysis process used to correlate the Nano17 readings with the force plate readings was the same for both left and right treadmill belts. The first two steps, which included scaling and zeroing the force signals, were identical to those done on the force plate output during the noise tests. Next, a fourth-order, low-pass Butterworth filter was used to filter out signals above 60Hz. Since forces were produced by pressing the Nano17 force sensor tool-end's face on the treadmill belt, the z axes of the two sensors were co-linear. Thus the vertical forces recorded by the Nano17 and the Kistler force plates were directly compared. While alignment of the z axes was guaranteed, the x and y axes of the two sensors had to be aligned. All presses with respect to the x and y directions were examined individually by separately analyzing each of the individual press on the belt. This was done since the angle of rotation between the two sensors was slightly different for every press, and every one of those angles needed to be precisely calculated in order to align the Nano17's x and y axes to the force plate's x and y axes. We correlated the angles obtained with our axes alignment method with measurements obtained by our Vicon Motion Analysis system (using reflective markers mounted on the plexiglass plate holding the force sensor) and found the two measurements to be in agreement. The angle of rotation for each simulated step was obtained by least-square fit between the x and y forces on the force plate and the rotated x and y forces of the Nano17 sensor. Once the angle for a specific press/simulated step was found, it was substituted in for the angle of rotation of the Nano17 force sensor's x and y forces. The following two equations were used to rotate the x and y axes of the Nano17 forces:

$$F_{x-rot} = (\cos\theta \times F_x) - (\sin\theta \times F_y)$$

$$F_{y-rot} = (\sin\theta \times F_x) + (\cos\theta \times F_y)$$

where F_x and F_y are the Nano 17 force sensor output in the x and y directions respectively, θ is the angle of rotation providing the best alignment between the x and y axes of the two sensor, and F_{x-rot} and F_{y-rot} are the Nano 17 forces in the x and y axes following the rotation.

After the rotations had been completed for each of the simulated steps, the Nano17 F_x and F_y were combined into two vectors, and compared with the force plate's F_x and F_y .

The final step in the validation process now consisted of seeing how well the Nano17 recorded forces correlated with those collected by either the right or left force plate. This was done by running a linear fit of the force plate *versus* Nano17 force in each direction in order to obtain the slope values of the fits and the Pearson correlation coefficient (r) of the fit. The RMS errors between the Nano17 and force plate measured forces were also calculated.

Gait Data Collection and Analysis

The ground reaction forces for four intact/able-bodied cats and four spinal cats were recorded on the split belt treadmill during bipedal walking at a speed of 0.4 m/s. Two of the cats were recorded pre- and post-SCI. Pre-transection the cats' masses ranged from 2.36–

4.18 Kg (mean 3.41 ± 0.79 Kg), and from 2.71–3.60 Kg (mean 3.13 ± 0.47 Kg) post-transection. The forelimbs stood above the treadmill assembly on a mounted platform sitting ~2 cm above the belts, and a 6 mm thick, 7 cm in height plexiglass divider was mounted between the hindlimbs to prevent the hindlegs from crossing over one another due to increased adductor activity (Belanger et al., 1996). The platform was mounted to the aluminum plates mounted under the force plates to avoid transmission of the forelimb forces to the force plates, and the divider was mounted to the front and back panels of the animal enclosure area. The transection and body-weight supported training procedures are detailed in (Boyce et al., 2007). Briefly, the animals were acclimated to quadrupedal and bipedal treadmill walking from 1–4 weeks; once acclimated, kinematic/force recordings of the cat's gait were collected. The animal's spinal cord was then fully transected at the T11–T12 level. The cats were allowed to recover for 1–2 weeks and bipedal body-weight supported treadmill training was conducted for 15 minutes/day, 5 days/week for 9–21 weeks prior to the recording sessions in this report. The hindlimbs were supported by the tail for balance and perineal or tail stimulation was used in the beginning to initiate stepping. By the time of the recordings (10–23 weeks post-SCI), holding the tail for balance and the hip extension brought upon by the belt movement were sufficient to initiate stepping in the animals tested.

Gait Data Analysis

All data was analyzed using custom scripts written in MATLAB (The Mathworks, Inc., Natick, MA) or Python (Python Software Foundation, www.python.org) language. Discrete wavelet transform (DWT) SureShrink denoising method was applied to remove treadmill resonance frequencies from the locomotion data. DWT decomposes a signal into wavelet coefficients or levels, the wavelet coefficient are then thresholded to remove the noise component from the underlying signal. SureShrink thresholding is adaptive and provides a more accurate representation of the original signal (Donoho and Johnstone, 1995). The SureShrink method used a soft threshold, wavelet Daubechies 4 or 'db4', and 5–8 decomposition levels depending on the amount of noise in the data. Baseline correction was applied to the data after denoising by acquiring the mean of force data points during the swing phase of the gait cycle or when no forces were acting on the treadmill, and subtracting it from the entire data set. The stance onset and offset of each step in the data were then identified from the vertical ground reaction forces by manual user selection. Stance time was normalized from 0 (onset) to 1 (offset) and ground reaction force in all three axes during stance was normalized to the mass of the cat at the time of the recording session. The normalized forces were then averaged across all steps for each cat, and then averaged across cats to produce a mean force profile across stance time for each of the three Cartesian directions (see Figure 6 and Table 1 for number of steps from each animal). The peak normalized forces in each of the three directions were then taken from the mean peak normalized forces (averaged across steps for each cat, and then across cats (see Table 2).

Results

Static and Dynamic Noise Analysis

Figure 2 shows the frequency distribution of the force plate sensor noise in each axis. Under static conditions (i.e. belts not moving), very little noise is present at any frequency, less than

0.3 N (i.e. 30 g), and almost none ($<0.05\text{N}$) below 10Hz. Noise increases under dynamic conditions but the peaks are at high frequencies (peaks around 60, 100 and 130 Hz) compared to the frequency of interest for ground reaction forces during gait. For frequencies below 10Hz, noise level is below 0.1N. The RMS value of the noise below 10Hz was less than 0.05N in all three axes under static conditions, and less than 0.1 N under dynamic conditions.

Force Plate Readings Validation Utilizing Nano17 Force Sensor

Figure 3 shows the results of the linear fits between the forces measured by the hand-held Nano17 pressing on the treadmill belt, and the forces measured by the Kistler force plate mounted underneath the treadmill when the belt motors were off. The linear fits were excellent in all cases with Pearson's correlation coefficient >0.98 for all forces (vertical (F_z), fore-aft (F_y) and mediolateral (F_x) for both the right and left treadmill belts). In addition, the slopes of the linear fits between the Nano17 and force plate readings were a perfect 1.0 for all three axes for both treadmills. The RMS values of the differences between Nano17 and force plate readings were similar for the right and left treadmill. RMS reading differences in the vertical and mediolateral force directions were $\sim 0.5\text{ N}$, and the value dropped to 0.3 N for the fore-aft direction.

Figure 4 shows the results of the linear fits between the forces measured by the hand-held Nano17 pressing on the treadmill belt, and the forces measured by the Kistler force plate with the belts moving at 0.3 m/s. Again, the linear fits were excellent in all cases with Pearson's correlation coefficient >0.98 for all force directions. In addition, the slopes of the linear fits were a perfect 1.0 for both the vertical and mediolateral forces (F_z and F_x respectively) for both treadmills. The slope of the linear fit for the fore-aft (F_y) force was slightly lower at 0.9, although errors in this direction are more likely due to measurement error on the part of the Nano17 than on the part of the force plates. For the y direction, the Nano17 was operating under non-inertial conditions (sensor moving along with the belt in that direction, associated with acceleration and deceleration), which are known to affect force sensor output (Pagnacco et al., 2000).

The RMS values of the differences between the force plate and Nano17 readings were around 0.5 N for the vertical forces (left and right treadmills) for ~ 20 simulated "steps" (see Figure 5). The RMS value of the vertical force over the same period was 10.7 N for "steps" on the left belt and 10.9 N for steps on the right belt. This translates to a 5% error between the vertical force applied to the treadmill belt and the readings of the force plate mounted under the treadmill (see Figure 5). For the mediolateral forces, the RMS differences were 0.4 N for both left and right treadmills and force signal RMS values were 4.4 N and 2.9 N for the left and right belts respectively, indicating an 9–14% error between the reading of the forces applied on the belt and that of the force plate. Largest RMS difference occurred for the fore-aft forces, but those errors were largely due to the movement of the Nano17 moving with the belt. In this case the RMS differences were $\sim 0.3\text{ N}$ for both belts, and mostly indicative of an error in the Nano17 readings caused by the motion of the force sensor in that direction.

Ground reaction forces in intact and locomotor trained spinal animals

The mass of the animals used in the study at the time of the recording sessions and the number of steps analyzed for each animal are indicated in Table 1. The mean normalized peak vertical ground reaction forces for the animals in this study are presented in Table 2. The values in Table 2 represent the mean and standard deviations of the ground reaction forces for the four intact and for spinal cats used. The individual ground reaction forces for each cat were averaged over a minimum of 29 steps (range 29–448 steps). The mean profiles of the ground reaction forces during stance across all steps are shown in Figure 6.

Vertical ground reaction force showed a single peak at around 20% of the stance time, with the peak being more sharply defined in intact cats. Vertical forces in spinal cats also peaked around the same time and stayed at or near that top value until about 60% of the stance time. Normalized vertical ground reaction forces were significantly lower in the spinal animals, although all of the animals in the study had been trained for at least 8 weeks and required no perineal stimulation to initiate locomotion by that point. Fore-aft forces in intact animals were positive (indicative of a braking force) for the initial 35% of stance, and then negative/propulsive for the remainder of stance. Mean fore-aft forces were positive for the whole duration of stance for the spinal animals, suggesting that the forces applied via the tail might have actually been pushing the animals forward. In the intact animals, the mediolateral forces were pushing outwards as observed for overground walking (Corbee et al., 2014). In the spinal cats, mediolateral forces did not show a consistent pattern for both the right and left hindlimbs, likely due to the lack of balance control in these animals. Differences in vertical forces between the left and right hindlimbs were greater than for intact animals, indicative of the lack of balance control in the spinal animals.

Figure 7 shows the mean vertical and fore-aft ground reaction forces for the spinal animal with the highest weight support (averaged across all steps for that animal). Since the hindlimbs support 40% of body weight during stance or locomotion (Macpherson, 1988; Manter, 1938) the required peak vertical force needed for full hindlimb weight support is around 4 N/Kg. The mean peak vertical force for this animal (right and left hindlimbs) was exactly 4.0 N/Kg (cat 4, down from 4.9 N/Kg pre-transection). The means in the other three animals were 2.1 (cat 1), 2.5 (cat 2, down from 5.1 N/Kg pre-transection) and 3.0 (cat 3) N/Kg, all below the theoretical minimum for full weight-bearing capability.

Although three of the animals had mean peak vertical ground reaction forces insufficient to provide full weight support, two still showed bouts of locomotion where the normalized peak vertical forces were greater than 4.0 N/Kg, demonstrating that most trained spinal cats are capable of full weight bearing stepping. Figure 8 shows examples of the right hindlimb normalized ground reaction vertical forces during locomotor bouts for the three trained spinal cats with mean normalized peak vertical forces below 4.0 N/kg (i.e. cats 1, 2 and 3). The normalized peak vertical forces rise above 4.0 N/Kg over 6–8 steps throughout the trial for two of the three cats.

Discussion and Conclusions

Treadmill Validation

Three-axes force measuring instrumented treadmills have become commercially available for human gait study, but are still largely unused/rare for studies in small animals. Most ground reaction forces are collected either with three-axes force plates mounted in a walkway [Corbee et al., 2014; Kaya et al., 2006; Manter, 1938], vertical force only pressure sensitive walkways [Lascelles et al., 2007; (Corbee et al., 2014; Kaya et al., 2006; Manter, 1938; Schnabl and Bockstahler, 2015) or single-axis force transducer mounted under the treadmill belt (Bockstahler et al., 2007; Musienko et al., 2012). Our split-belt design offers the advantages of requiring a smaller foot print than walkway and facilitates the attachment of fixed physiology transducers such as EMG cables or pneumograph. In addition, kinematic measurements can be obtained in a smaller working volume and with lower resolution cameras. Finally, the split-belt design allows independent measurement of each hindlimb's ground reaction forces in all three Cartesian directions, and permits experiments testing the coupling between ipsi- and contralateral spinal locomotor centers (Frigon et al., 2013).

The natural oscillation frequency of our treadmill was above the frequency content of gait, facilitating filtering of the noise due to force plate oscillations. The match between the static forces applied to the treadmill surface and those recorded at the force plates were excellent. The match under dynamic conditions (i.e. with the belts moving) were again excellent for the force directions where the Nano17 was not moving (vertical and lateral) while small error in the fore-aft direction occurred as a result of the Nano17 sensor movement in that direction. Overall, our validation measurements confirm modeling studies showing that a treadmill assembly mounted on a force plate is insensitive to belt noise and friction (Willems and Gosseye, 2013).

Comparisons Between Ground Reaction Forces During Overground and Treadmill Walking

Studies in humans show good correlations between the ground reaction forces measured during overground walking on a walkway and ground reaction forces measured on a treadmill (Riley et al., 2007). Results in large quadrupeds (horses) also show similarity between walkway and treadmill walking ground reaction forces although a number of differences have been noted. Vertical force profiles in horses demonstrate similar peaks of 5–6 N/Kg during walking for both walkway and treadmill (Biknevicius et al., 2004; Weishaupt et al., 2010), but vertical ground reaction forces increased with speed on the treadmill (Weishaupt et al., 2010), while they did not for walkway running (Khumsap et al., 2001) or trotting (Dutto et al., 2004). We are not aware of any other studies on measuring ground reaction forces in all three Cartesian directions during treadmill walking in cats, but our results are in line with the ground reaction forces reported during walkway walking in cats (Corbee et al., 2014; Gregor et al., 2006; Kaya et al., 2006; Lascelles et al., 2007; Manter, 1938; Schnabl and Bockstahler, 2015).

Manter showed peak vertical forces of about 4.5 N/Kg for the hindlimb (one cat, his figure 7), and fore-aft forces that were initially braking and switched to propulsive at 25–30% of the stance time with peak braking forces of 0.9–1.3 N/Kg (Manter, 1938). Similar results are

reported by (Kaya et al., 2006) for level walkway walking, with the vertical ground reaction forces at around 15 N (specific cat masses not specified, but mean mass of the six cats in the study was 5.3 ± 1.2 Kg) and the horizontal/fore-aft force showing an initial braking phase switching to propulsion at 35% of the stance time with a peak braking force of ~ 2 N. Gregor's group reported higher value for average peak vertical ground reaction force (5.3 ± 1.2 N/Kg), but similar braking forces as Manter and Kaya et al. (peak braking force of 0.8 ± 0.4 N/Kg and peak propulsive force of 1.2 ± 0.4 N/Kg, switch from braking to propulsion occurring at around 35% of the stance phase). Other studies in intact cats report similar values, e.g. 4.8 N/Kg (Corbee et al., 2014), 3.8 N/Kg (38.3% BM) (Lascelles et al., 2007), and 4.0 N/Kg (40.6% BM) (Verdugo et al., 2013) for peak vertical forces, and 0.81 N/Kg (Corbee et al., 2014) peak braking force with braking occupying 36% of the stance time. In comparison, our values were 4.6 N/Kg for the peak vertical forces, and 0.2–0.4 N/Kg for the peak braking force with the braking force occupying $\sim 30\%$ of the stance period. While our braking forces are somewhat lower than the values reported in the literature, it must be noted that the other studies involved cats walking quadrupedally (as opposed to bipedally in our case) and at higher velocities (0.54 m/s (Gregor et al); 0.55 m/s (Manter); 0.7 m/s (Corbee et al); not reported in Kaya et al) which require higher braking and propulsive forces. Overall, our results tend to confirm studies in humans that ground reaction forces are similar for overground and treadmill walking, although additional studies at larger speed and for quadrupedal walking would be necessary to confirm our initial results at one speed.

Comparisons Between SCI and Able-Bodied Cats' Ground Reaction Forces

The minimal peak vertical force needed for full hindlimb weight support in cats is around 4 N/Kg since the hindlimbs support 40% of body weight during stance or locomotion (studies cited above). Our mean normalized peak vertical force in intact animals was above this theoretical minimum, but it was only around 2.5 N/Kg in spinal animals. In our animal with the largest weight support (Fig 7) we did obtain 100% weight support. Fore-aft forces in that animal still showed braking throughout stance suggesting that the weight support provided by the experimenter added a small forward push to the animal's hindquarter so that the hindlimbs were exerting a forward force on the belts during the entire stance period.

The data from the other animals would suggest that the experimenter provided as much as 4.5 N of support. This estimate comes from the difference between the peak normalized ground reaction force needed for full weight support (4 N/Kg) and the mean peak normalized vertical ground reaction force obtained in the non-full weight supporting animals (2.5 N/kg) multiplied by a mean mass of 3.0 Kg for spinal cats (actual mean mass was 3.3 Kg for the three animals that did not show full weight support). This amount of force would correspond to holding a mass of 450 g for the extent of the training session (15–20 minutes). It appears unlikely that the experimenter actually provided that amount of weight support as no fatigue or significant sense of force exertion was reported by the experimenter (MAL in all cases), and in two of the animals the tail was actually held horizontally with no apparent tension (video recording).

We also considered that the lower peak normalized vertical GRF may be due to the reduced load that needs to be supported in spinal animals since hindquarters' muscles atrophy

following spinalization (Fung and Macpherson, 1999). Two of our animals (cats 1 and 4, our worst and best performers in terms of weight bearing capability) actually gained weight following spinalization. If we assume that weight loss occurring following transection was due to hindquarters' atrophy in the two animals that lost weight, the normalized vertical peak forces needed in those two animals for full weight support is reduced to 3.0 N/Kg in cat 2 and 3.6 N/Kg in cat 3. The mean (left and right hindlimbs) normalized peak vertical forces in these two animals were 2.5 (cat 2) and 3.0 (cat 3) N/Kg, still below the required normalized peak vertical forces for full weight support after assuming that all weight loss is occurring in the hindquarters.

The most likely explanation for the mean vertical peak forces being below the values needed for full weight support is that the animal's weight support varies considerably over the course of a trial, and that full weight support occurs on a number of steps, as suggested by the data of figure 8, even though the mean peak force is below the required 4.0 N/Kg.

Overall, it seems most likely that trained spinal cats can perform full weight bearing steps with regularity, although the average peak vertical force over a recording session may not amount to full weight support for the duration of the entire session. We are currently evaluating ground reaction forces in a larger spinal animals group to further answer this question.

Conclusions

The instrumented treadmill developed allows for direct measurements of the ground reaction forces in all three Cartesian coordinates even during the period of double stance support. Validation of the treadmill was conducted and demonstrated that the noise was above the frequency of interest for gait analysis, and that the forces measured at the force plates mounted under the treadmills were a good reflection of the forces applied to the surface belts in the three Cartesian coordinates. Ground reaction forces during treadmill walking in intact animals were similar to the forces during overground walking over the narrow range of speed and conditions tested. Ground reaction forces in trained spinal animals showed a range of weight-bearing capability, with only one animal out of four demonstrating the ability to constantly support the full weight of the hindquarters.

Acknowledgments

This study was funded by NIH Grants NS055976 and EB012855. The funding sources had no involvement in study design, data collection, analysis, interpretation or reporting.

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Highlights

- We developed and validated a split belt instrumented treadmill to measure the three components of the ground reaction forces from the left and right hindlimbs in cats
- Force plates mounted under each of the treadmill assembly faithfully capture the forces applied to the treadmill belt and are insensitive to the treadmill internal forces
- The split-belt design allows capture of each hindlimb's ground reaction forces even during period of double stance support
- Ground reaction forces for treadmill walking are similar to ground reaction forces during walkway walking in cats
- Treadmill provides the first quantitative measure of the weight-bearing capabilities of locomotor trained spinal cats

fully assembled treadmill

Figure 1.

Top panel: 3D rendering of one treadmill. A second mirror-image treadmill is mounted next to the one shown and the two assemblies allow independent capture of the left and right hindlimbs ground reaction forces. The treadmill belt is belt-driven by an AC motor, and each treadmill assembly is bolted to a 6-axis force plate. Bottom panel: Photograph of the actual treadmill showing both assemblies mounted side-by-side (note the separation between the two belts), the enclosure used to restrain the animal and the forelimb platform on the left side of the photograph.

Figure 2.

Frequency distribution of the force plate sensor noise in each axis under static (motors off; top row) and dynamic (both belts running at 0.3 m/s) conditions. Noise content is above the frequencies of interest for kinetic analysis (>10Hz).

Figure 3.

Validation of the force plate measurements of the forces applied at the treadmill belt using a hand-held Nano17 force sensor (ATI Industrial Automation, Apex, NC). Left column shows the linear fits between the forces measured in the x (mediolateral), y (fore-aft) and z (vertical) directions by the hand-held Nano17 pressing on the left treadmill belt, and the forces measured by the left Kistler force plate mounted underneath the treadmill when the belt motors were off. Right column shows the same for the right treadmill. Pearson's correlation

coefficient was >0.98 between the two sets of readings with intercepts less than 0.2 N and slopes of 1.0 for the linear fits.

Figure 4.

Validation of the force plate measurements with the treadmill belts running at 0.3 m/s. Left column shows the linear fits between the forces measured by the hand-held Nano17 pressing on the left treadmill belt, and the forces measured by the left Kistler force plate with the belt moving at 0.3 m/s. Right column shows the same for the right treadmill. The linear fits showed Pearson's correlation coefficient >0.98 for all force directions. Intercepts were small (<0.3 N) and the slopes were at 1.0 except for the fore-aft direction where inertial forces due to the Nano17 motion introduced a small error in that reading.

Figure 5.

Example of the fit between the vertical force readings of hand-held Nano17 and Kistler force plate for a series of presses on the treadmill belt with the belt running at 0.3 m/s. RMS (root-mean-squared) values of the differences between the force plate and Nano17 readings were ~0.5 N for the vertical forces (left and right treadmills) for 17 simulated "steps".

Figure 6.

Ground reaction forces during the stance of gait in intact and spinalized cats. The values represent the means and 95% confidence intervals of the ground reaction forces for all the steps from the four intact (left column) and four spinal (right column) cats used. Vertical ground reaction forces (1st row) peaked at around 20% of the stance phase in both intact and spinal cats. Fore-aft forces (2nd row) show initial braking (positive or in the fore/anterior direction) and then propulsion (negative forces) in intact animals, but forces are strictly positive for the spinal animals indicating that the hindlimbs were actually exerting a braking

force on the treadmill. Mediolateral forces (3rd row) are pushing laterally in intact animals, lateral direction being positive for the left hindlimb/treadmill and negative for the right hindlimb/treadmill. In spinal animals the mediolateral force is outward for the left hindlimb, and near zero for the right hindlimb indicative of the lack of balance in those animals and an increased adductor tone.

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Figure 7.

Mean vertical and fore-aft ground reaction forces for the spinal animal with the highest weight support (cat 4). Mean peak vertical force reached 4.0 N/Kg between 20–40% of the stance phase period, while the fore-aft forces were positive (braking) throughout stance.

Figure 8.

Normalized vertical ground reaction forces of the right hindlimb over multiple steps for the three spinal animals whose mean peak vertical forces were lower than 4.0 N/Kg. Two of those animals were capable of full weight bearing stepping over some of the stepping period, although the means for both right and left hindlimbs for the whole period of stepping were lower than 4.0 N/Kg, as shown in Figure 6. The third animal (cat 1) displayed a crouched position on the forelimbs, thus shifting more weight support to the forelimbs, and did not

show normalized peak vertical forces equal or greater than 4.0 N/Kg over the entire recording session.

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Table 1

Masses of the animals used in the study at the time of the recording sessions and number of steps analyzed.

cat id	Intact		Spinal	
	mass (Kg)	# of steps	mass (Kg)	# of steps
cat 1			2.74	64
cat 2	4.18	61	3.48	448
cat 3			3.6	57
cat 4	2.36	112	2.71	77
cat 5	3.8	29		
cat 6	3.3	154		

Summary of cats used in the study

Table 2

Mean normalized peak GRFs of left and right hindlimbs for pre-spinalized and spinalized cats. All GRFs are normalized to the mass of the cat at the time of the recording session.

		Normalized Peak GRFs	
		Intact (N/Kg)	Spinal (N/Kg)
<i>Vertical</i>	Left	4.89±0.85	2.20±0.94
	Right	4.79±0.97	2.85±0.99
<i>Fore-aft</i>	Left	-0.35±0.60	0.62±0.54
	Right	-0.35±0.68	0.83±0.67
<i>Lateral</i>	Left	0.43±0.51	0.37±0.54
	Right	-0.44±0.54	-0.01±0.49