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Gait analysis of locomotory impairment in rats before and after neuromuscular injury

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Abstract

We used a gait analysis system (GASTM) to measure the changes in locomotion parameters of adult Sprague–Dawley rats after neuromuscular injury, induced by repeated large-strain lengthening contractions of the dorsiflexors muscles. We developed a logistic regression model from test runs of control and permanently impaired (denervation of the dorsiflexor muscles) rats and used this model to predict the probabilities of locomotory impairment in rats injured by lengthening contractions. The data showed that GASTM predicts the probability of locomotory impairment with very high reliability, with values close to 100% immediately after injury and close to 0% after several weeks of recovery from injury. The six transformed locomotion parameters most effective in the model were in three domains: frequency, force, and time. We conclude that application of the GASTM instrument with our predictive model accurately identifies locomotory changes due to neuromuscular deficits. Use of this technology should be valuable for monitoring the progression of a neuromuscular disease and the effects of therapeutic interventions.

Keywords

Neuromuscular injury; Denervation; Locomotion; Logistic regression

1. Introduction

Rats and mice have become increasingly important for modeling human diseases and have provided many insights into pathogenic mechanisms (Durbeej and Campbell, 2002), but assessment of the phenotypic changes in rodent models is sometimes difficult. In many cases, severe measures, often involving terminal experiments, are required to detect phenotypical changes including harvesting of tissue to assess histological changes or for in vitro studies (DelloRusso et al., 2001; Durbeej and Campbell, 2002; Hamer et al., 2002). Since many diseases of the nervous and musculoskeletal systems result in impaired locomotion, non-invasive measurement of locomotion parameters (LP) could provide new insights into these diseases as well as facilitating longitudinal studies of their progression and treatment.

We have described a system that uses load cells and video technology to assess quantitatively the gait of rats. The gait analysis system (GASTTM) (Tasch et al., 2008) is capable of measuring 20 different parameters of locomotion and provides a non-invasive measure of locomotion in individual rats over time. We recently used it to distinguish differences in gait between control and SOD1-G93A rats, a model of amyotrophic lateral sclerosis in man, at pre-symptomatic stages (Tang et al., 2009). Here we test the hypothesis that our quantitative measurements can detect changes in gait that are induced by permanent impairment of function of a single lower hindlimb, and further, that it can detect subtle changes in gait, linked to the partial and temporary loss of muscle function.

2. Methods and materials

2.1. Gait analysis system

A gait analysis system GASTTM (Fig. 1) measures a wide range of locomotion parameters (LPs) of rats that walk through a compartment in which measurements are conducted, from the entrance, via the sensor module, to the exit platform, where a toy and food are placed. The sensor module has left and right transparent floor-plates, each of which is supported by an array of 7 load cells, including 4 Omega LCLB-5 load cells, 2 Omega LCLB-2 load cells and a single Transducer Techniques load cell (150 g #206757), which measure the vertical (F_z), transverse (F_x), and longitudinal (F_y) ground reaction forces (GRF), respectively (Tasch et al., 2008). In addition, a digital video camera (Sony, DMK31BF03) records images of the walking rats from below and, as a result, can be used to display time histories of 3 GRF components, and 2 (X and Y) limb positions (Fig. 2). Lighting is controlled by 2 arrays of LEDs, and the load cell data as well as the video recording are sampled at 200 Hz and 30 fps, respectively.

2.2. Locomotion parameters (LPs)

From the signals of 14 load cells and recorded video images the values of 20 LPs per limb were evaluated for the left fore (LF), right fore (RF), left hind (LH), and right hind (RH) limbs. The 20 LPs, listed in Table 1, are non-dimensional except for stance time, F_{z0} , and F_{y0} that have dimensions of seconds (s), 1/s, and 1/s, respectively.

2.3. Associating segments of load cell signals and limbs

Fig. 3 shows load cell signals during the time period of 0.63 to 0.79 s generated when the RF limb is in contact with the right floor plate. We can associate the other limbs with the signals from the load cell signals that they generate similarly. By designating each fore and hind limb with dashed and solid lines, respectively (Fig. 4), we can calculate numerous LPs that characterize the locomotion of a rat through each limb for every test run.

2.4. Logistic regression model

In earlier work, we modeled bovine lameness and used logistic regression to evaluate predictions of lameness in dairy cattle (Rajkondawar et al., 2002). Here we use logistic regression models (Hosmer and Lemeshaw, 2000) to predict the probability that a rat has a permanent locomotory impairment, as assessed with the LPs. This is expressed mathematically as:

$$P(\text{a rat} \in \text{Permanent impairment group}) = \frac{\exp(\beta_0 + \sum \beta_i \text{LP}_i)}{1 + \exp(\beta_0 + \sum \beta_i \text{LP}_i)} \quad (1)$$

where the β_i is the i -th coefficient of the logistic regression model and is estimated by appropriate statistical methods. Liu et al. (2009) demonstrated that the accuracy of the

predictions of such models is significantly improved when the LPs are transformed via spline transformations. An implementation of these transformations is available in SAS (SAS Institute Inc., Cary, NC). The probability that a rat has a permanent locomotory impairment is hence evaluated as:

$$P(\text{a rat} \in \text{Permanent impairment group}) = \frac{\exp(\beta_0 + \sum \beta_i \text{TLP}_i)}{1 + \exp(\beta_0 + \sum \beta_i \text{TLP}_i)} \quad (2)$$

where TLP_i is the transformed value of LP_i .

2.5. Obtaining gait measurements

Ten male, Sprague–Dawley rats (46 days old) from Charles River Laboratories (Shrewsbury, MA) were used in the study. Each rat was housed in a separate cage with darkness/lighting cycle, 12 h on and 12 h off, and food and water *ad libitum*. The animals were weighed and placed individually at the system entrance (Fig. 1). Upon opening of the gate, the rat crossed the system, moving towards its toy and food, placed at the system's exit. This procedure was repeated up to 3 times for each rat on a single day. After 7 days of training and adaptation to the system, each animal was tested twice per week. Data were recorded when the rats reached 55 days of age and for the next 87 consecutive days.

Permanent neuromuscular damage was introduced to the left hind limb in 5 of the 10 rats, at the age of 102 days, while the biweekly data recording continued. Rats were anesthetized with vaporized isoflurane (2% with an oxygen flow rate of 0.5 L/min) and general anesthesia was confirmed by lack of response to a normally painful stimulus (pinching the foot). The surgical procedure consisted of cutting the fibular nerve, which innervates the ankle dorsiflexors muscles and is easily accessible due to its subcutaneous location at the neck of the fibula (lateral knee). Under sterile conditions, the skin was shaved, a small (less than 1.5 cm) incision was made, and the nerve was identified and cut. To ensure that nerve growth did not ensue, we removed a small section (~5 mm) of the nerve. The incision was sutured closed and topical antibiotics and analgesics were applied. A record of 264 test runs (112 test runs of rats with permanent locomotory impairment, and 152 test runs of control rats) established the training database that we used to derive the logistic regression model for rats with locomotory impairment.

Under general anesthesia as described above, transient neuromuscular injury was introduced to the left hind limbs at 172 days of age in 3 of the 5 remaining rats, used previously as controls, by inducing 8 large-strain lengthening contractions to the ankle dorsiflexor muscles based on methods previously described, with some modifications (Lovering et al., 2007). This protocol resulted in a $44 \pm 6\%$ loss of maximal isometric tetanic torque in the dorsiflexors muscles immediately after injury. To compare pre- and post-injury locomotion, we recorded data from 1 week prior to injury through 5 weeks after injury. A total of 123 test runs were recorded to establish the testing database, which we used to assess the severity of locomotory impairment of the injured rats.

2.6. Assessing the severity of locomotory impairment

The logistic regression model obtained for control rats and rats with permanent neuromuscular damage (denervation) was applied to test the locomotion of rats that sustained a transient impairment of muscle function caused by lengthening contractions. Using the probability value, we were able to identify deterioration of locomotion after the transient injury, as well as improvement during recovery from the injury.

2.7. Animal protocols

All protocols described in this study were approved by IACUC at the University of Maryland, Baltimore County and the University of Maryland, School of Medicine. At the end of our experiments, animals were euthanized by sodium pentobarbitol injection (150 mg/kg IP).

3. Results

We used the GASTM system (Tasch et al., 2008) to measure 20 different parameters of the gait of walking rats, and present vertical (F_z), longitudinal (F_y), and transverse (F_x) components of the ground reaction forces (GRF), as well as longitudinal (Y) and transverse (X) limb positions, plotted versus time (s) for both left and right floor plates, in Fig. 4. We used images of the walking rat, recorded from below by a video camera, to associate particular signals generated by the system with the position and activity of individual limbs. Each fore and hind limb is designated with dashed and solid lines, respectively (Fig. 4), enabling us to calculate the locomotion parameters (LPs) associated with the movements of individual limbs for every test run.

Table 1 lists the 20 LPs that we evaluated for every limb, for every test run of control and permanently impaired (denervated) rats. The numerical values of LPs, recorded for a permanently impaired rat and for a control rat, are presented in Table 2. Note the generally lower LP force values of the LH limb, which had been denervated by fibular nerve section 12 days prior to the test.

In an effort to rank the effectiveness of the 20 LPs in detecting defects in locomotion, we examined the effectiveness of each individual LP to classify the rats correctly into permanently impaired and control groups. We transformed the LPs by following the procedures introduced in Liu et al. (2009). The classification rate improved remarkably when the LPs were transformed using spline transformation (Schumaker, 2007), such that the probability of misclassification by the model when the top 6 individual transformed LPs (TLPs) were used, ranged from 26.5% to 28.8%. Table 3 prioritizes the 6 most useful TLPs, one TLP at a time. F_{20} was the best predictor; and the propulsion duration period (NP) was the worst.

When we derived a logistic regression model based on the transformed values of the best 1–6 TLPs listed in Table 3, our ability to identify control versus denervated animals from the data alone improved with the number of these variables that we used (Fig. 5). The cutoff value of the probability predictions for the control and denervated groups was 0.5. Incorporating a total of 6 variables into the model and selecting a probability cutoff value of 0.5 provide specificity and sensitivity of at least 90% in analyses of control and denervated rats (Fig. 5).

We used the 6-variable model of control and permanently impaired rats to calculate the probability of impairment in the locomotion of 3 rats (Fig. 6), injured by a series of large-strain lengthening contractions of their ankle dorsiflexors in one hindlimb. In Fig. 6, mean probability values of two or more daily test runs per animal are depicted, whereas a single daily test run was omitted. Our functional measurements indicate that the ankle dorsiflexors lose ~45% of the contractile torque after this form of injury (Lovering et al., 2007). Prior to injury, all of the rats, except for one occasion, demonstrated a probability of impairment in locomotion of less than 0.5 (Fig. 6). Immediately after injury, all 3 injured rats showed a probability of impairment in locomotion of about 1. Furthermore, our analysis of probabilities indicated that all 3 rats recovered fully, i.e., their probabilities of impairment approached 0 over time, although at different rates (6, 20, and 34 days for complete

recovery). We conclude that the 6 LPs, determined by our apparatus and subjected to spline transformation, can reliably identify rats that have been subjected to an injury that partially reduces the efficacy of only one muscle group (the ankle dorsiflexors) in a single hindlimb.

4. Discussion

Analysis of locomotion in rodents can be useful in assessing normal or altered neuromuscular function (Clarke, 1991). Measurements of the ground forces applied by the limbs during locomotion provide a non-invasive way to assess gait and monitor functions of individual limbs in locomoting rats (Muir and Whishaw, 1999). Rats with lesions similar to those seen in Huntington's disease show spontaneous motor symptoms that include gait abnormalities (Guvot et al., 1979). These abnormalities are not easily detected by eye, but were readily identified and quantified by computerized video analysis. Quantitative methods to assess temporal and spatial locomotion parameters have been reported (Hamers et al., 2001). Non-quantitative video-based methods to characterize the effects of nerve injury are also available (Walker et al., 1994). These methods assess multiple locomotion parameters, but the data they produce have not been used to determine whether a certain type of disease or locomotion impairment is present. The GASTM records locomotion parameters, associates the signals with the placement of individual limbs, and assesses the probability that certain locomotion impairment is present. Here we use the GASTM to establish the parameters associated with gait abnormalities caused by denervation of a single limb and to assess, with a high degree of reliability, changes in gait that are linked to much milder and reversible injuries.

Our results show that GASTM is an effective tool for evaluating permanent or temporary defects in gait consequent to neuromuscular injury. To develop a model that could be used effectively to identify changes in gait associated with mild and reversible injuries, we first prepared a dataset from rats that we trained to walk through the GASTM system. Comparisons of locomotion records of control rats and of rats with permanent locomotory impairment caused by denervation of a single hindlimb revealed clear differences. After analyzing these differences using spline transformation, we generated datasets that we then applied to examine the effects of injuries caused by several large-strain lengthening contractions in a single hindlimb. In particular, we selected 6 parameters from this dataset that were most effective in identifying denervated and control rats, which we then applied to the data we obtained from the injured animals.

From this dataset we generated a logistic regression model that predicted the probability of locomotory impairment and reliably identified those animals that had been mildly and reversibly injured by lengthening contractions. We also used these parameters to follow the recovery of gait in individual animals for 5 weeks after injury. The ability of the GASTM to identify injured animals and to determine when they have recovered from the injury, which causes only a ~45% loss of function of a single group of muscles in one hind limb, suggests that the instrument and the analytic methods we have developed are both highly sensitive and reliable.

Interestingly, despite using an injury model that produces a stereotypical response in terms of loss and recovery of contractile torque (Lovering et al., 2007), each animal that we tested with the GASTM showed a unique time course for recovery of gait. This suggests that, similar to humans, factors such as pain and the ability to compensate for injury may affect gait in rats. Future studies with the GASTM will test if therapeutic interventions that reduce pain and inflammation improve gait during the days that follow injury from lengthening contractions.

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Fig. 1.

A schematic of the gait analysis systemTM for rodents. The system measures locomotion parameters in 4 domains: force, space, time and frequency. The measured forces are in vertical (z), longitudinal (y) and transverse (x) directions. The locomotion parameters feed a statistical model that calculates the probability that a rodent displays locomotion impairment.

Fig. 2.

The outputs generated by the gait analysis system in a typical test run. The signatures of the left and right limbs are depicted on the left and right sides, respectively. The transverse (F_x), longitudinal (F_y), and vertical (F_z) are normalized with respect to the rat's body weight and plotted as a function of time (s). The longitudinal (Y) and transverse (X) limb positions are normalized with respect to the length and width of the floor plates, respectively. A video image of the rat from below is also recorded to enable associating load cell signals with limb placements (see Figs 3 and 4).

Fig. 3.

To associate load cell signatures with a limb the computer mouse is placed on a selected signature (vertical marker) and the most recent video image is displayed. The load cell signature on the right floor-plate from 0.63 to 0.79 s is associated with the right fore limb.

Fig. 4.

The load signatures generated by a single limb are identified and the fore and hind limbs are marked with dashed and solid heavy lines, respectively.

Fig. 5.

The range of predicting probabilities that the examined rat has locomotory impairment when 1, 2, 3, ...and, 6 top performing TLPs (listed in Table 3) are used. C is control and D is denervated or permanently impaired groups. The cutoff value of the probability predictions for the control and denervated groups was 0.5. Note the improved specificity and sensitivity of the control (C) and denervated (D) groups, as the number of TLPs increases from 1 to 6.

Fig. 6.

The probability of locomotory impairment of the three injured rats. Large-strain injury was induced on day 0. The alteration in gait after injury is reflected by the high probability of locomotion impairment at day 0. Despite, animal to animal variability in gait during the 5 weeks after injury, the probability of locomotion impairment for all three animals returned to pre-injury levels by the 34th day. The cutoff value of the probability predictions for locomotory impairment was 0.5.

Table 1

Definitions of the 20 locomotion parameters (LPs) evaluated in this manuscript.

No.	Variable	Units	Definition
1	F_{zmax}	Non-dimensional	Maximum value of the vertical GRF component of a selected limb
2	Stance time	s	Time duration that a selected limb is in contact with the floor
3	T_F_{zmax}	Non-dimensional	Time of F_{zmax} normalized by the stance time of a selected limb
4	F_{zmean}	Non-dimensional	$\left[\frac{\int_{S\ time} F_z dt}{\text{Stance time}} \right]$ The mean value of the vertical GRF component of a selected limb
5	$F_{z\omega}$	1/s	$\left[\int_{50} F_z d\omega \right]$ The Fourier transform of F_z summed over the first 50Hz for a selected limb
6	Stride	Non-dimensional	Stride length of a selected limb calculated as the difference between two consecutive contact positions; the contact positions are normalized by the floor length, which is 13.25 inch
7	F_{ymax}	Non-dimensional	Maximum value of the longitudinal GRF component of a selected paw
8	T_F_{ymax}	Non-dimensional	Time of F_{ymax} divided by Stance time
9	F_{ymin}	Non-dimensional	Minimum value of the longitudinal GRF component of a selected paw
10	T_F_{ymin}	Non-dimensional	Time of F_{ymin} divided by Stance time
11	F_{ymean}	Non-dimensional	$\left[\frac{\int_{S\ time} F_y dt}{\text{Stance time}} \right]$ The average value of the longitudinal GRF component of a selected paw
12	$F_{y\omega}$	1/s	$\left[\int_{50} F_y d\omega \right]$ The Fourier transform of F_y summed over the first 50Hz for a selected paw
13	F_{xmax}	Non-dimensional	Maximum value of the transverse GRF component of a selected paw
14	F_{xmin}	Non-dimensional	Minimum value of the transverse GRF component of a selected paw
15	F_{xmean}	Non-dimensional	$\left[\frac{\int_{S\ time} F_x dt}{\text{Stance time}} \right]$ The average value of the transverse GRF component of a selected paw
16	F_{jP}	Non-dimensional	The mean value of the propelling (positive) longitudinal force of a selected limb
17	F_{jB}	Non-dimensional	The mean value of the braking (negative) longitudinal force of a selected limb
18	NP	Non-dimensional	The number of samples in which the longitudinal force is propelling (positive in value); the sampling rate is 200Hz
19	NB	Non-dimensional	The number of samples in which the longitudinal force is braking (negative in value); the sampling rate is 200Hz
20	NPB	Non-dimensional	The number of times in which the longitudinal force switches sign from braking to propelling and vice versa

Table 2

Numerical values of the 20 measured LPs for a permanently impaired and control rats. Data of the permanently impaired rat were recorded when the denervated rat was 114 days old and 12 days after introducing the permanent locomotory impairment. Data of the control rat, recorded at 94 days of age.

Locomotion Parameter (LP)	Denervated rat – 12 days after being introduced to a permanent locomotory impairment				Control rat			
	LF	RF	LH	RH	LF	RF	LH	RH
F_{max}	0.6330	0.6316	0.3628	0.8133	0.7263	0.6801	0.4944	0.5138
Stance time (s)	0.1500	0.1650	0.1750	0.3050	0.2950	0.3000	0.8650	0.4350
$T_{-}F_{\text{max}}$	0.4000	0.3939	0.3143	0.2295	0.3220	0.2667	0.7052	0.1609
F_{mean}	0.4015	0.4163	0.2161	0.4614	0.3722	0.4157	0.3541	0.4008
F_{ao} (1/s)	0.3946	0.4117	0.2452	0.6158	0.6051	0.6505	0.3941	0.3876
Stride	0.4927	0.6649	0.4582	0.5608	0.2381	0.4114	0.0669	0.0676
F_{ymax}	0.0631	0.0405	0.0555	-0.0050	0.2079	0.3047	0.2303	0.1440
$T_{-}F_{\text{max}}$	0.9000	0.6970	0.6857	0.0492	0.3729	0.9500	0.7168	0.0345
F_{ymin}	-0.1906	-0.0012	-0.1433	-0.3142	-0.3030	-0.3995	-0.0887	0.0283
$T_{-}F_{\text{ymin}}$	0.2333	0	0.0286	0.2787	0.4576	0.3000	0.9653	1.0000
F_{ymean}	-0.0490	0.0232	-0.0351	-0.0668	0.0046	-0.0180	0.0233	0.0823
F_{yao} (1/s)	0.1127	0.0247	0.0953	0.1758	0.2529	0.2345	0.0822	0.1084
F_{max}	0.0010	0.1322	0.0192	0.1925	0.2225	0.1779	0.0421	0.1726
F_{amin}	-0.0892	-0.0150	-0.0534	-0.0025	-0.2698	-0.1282	-0.1132	0.0444
F_{ymean}	-0.0001	0.0626	0.0006	0.0913	0.0156	0.0691	0.0079	0.1180
F_{p}	0.0375	0.0152	0.0338	0	0.1058	0.0956	0.0502	0.0685
F_{yB}	-0.1115	-1.0030	-0.0548	-0.0248	-0.0841	-0.0897	-0.0367	0.0000
NP	13	32	8	0	28	31	120	88
NB	18	2	28	62	32	30	54	0
NPB	6	1	2	0	10	3	14	0

Note the generally reduced force capacity of the LH limb of the denervated rat. Except for stance time, F_{Zao} , and F_{yao} all other variables are non dimensional.

Table 3

Ranking the transformed LPs based on their misclassification performance when a single TLP is used at a time. The misclassification rate of the Fourier transform of the vertical reaction force, $F_{z\omega}$, is the best with misclassification rate of 26.5%, and the time duration during which the longitudinal reaction is propelling, NP, has the worst performance with misclassification rate of 28.8%.

TLP	Misclassification (%)
$F_{z\omega}$	26.5
$F_{y\text{mean}}$	26.9
$F_{x\text{min}}$	28.0
NB	28.0
$F_{z\text{mean}}$	28.4
NP	28.8