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To cite this article: Fei San Lee, Carlos J Cruz, Kyle D Allen & Rebecca A Wachs (2024) Gait assessment in a female rat Sprague Dawley model of disc-associated low back pain, Connective Tissue Research, 65:5, 407-420, DOI: [10.1080/03008207.2024.2395287](https://doi.org/10.1080/03008207.2024.2395287)

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# Gait assessment in a female rat Sprague Dawley model of disc-associated low back pain

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## ABSTRACT

**Purpose:** Gait disturbances are common in human low back pain (LBP) patients, suggesting potential applicability to rodent LBP models. This study aims to assess the influence of disc-associated LBP on gait in female Sprague Dawley rats and explore the utility of the open-source Gait Analysis Instrumentation and Technology Optimized for Rodents (GAITOR) suite as a potential alternative tool for spontaneous pain assessment in a previously established LBP model.

**Materials and Methods:** Disc degeneration was surgically induced using a one-level disc scrape injury method, and microcomputed tomography was used to assess disc volume loss. After disc injury, axial hypersensitivity was evaluated using the grip strength assay, and an open field test was used to detect spontaneous pain-like behavior.

**Results:** Results demonstrated that injured animals exhibit a significant loss in disc volume and reduced grip strength. Open field test did not detect significant differences in distance traveled between sham and injured animals. Concurrently, animals with injured discs did not display significant gait abnormalities in stance time imbalance, temporal symmetry, spatial symmetry, step width, stride length, and duty factor compared to sham. However, comparisons with reference values of normal gait reported in prior literature reveal that injured animals exhibit mild deviations in forelimb and hindlimb stance time imbalance, forelimb temporal symmetry, and hindlimb spatial symmetry at some time points.

**Conclusions:** This study concludes that the disc injury may have very mild effects on gait in female rats within 9 weeks post-injury and recommends future in depth dynamic gait analysis and longer studies beyond 9 weeks to potentially detect gait.

## ARTICLE HISTORY

Received 22 February 2024

Revised 11 August 2024

Accepted 18 August 2024

## KEYWORDS

Gait; low back pain; disc degeneration; in vivo assays; rat model

## Introduction

Low back pain (LBP) affects a large proportion of the population, leading to disability and work absenteeism.<sup>1–4</sup> The pain and limited mobility associated with LBP contribute to disability,<sup>5,6</sup> particularly in instrumental activities of daily living.<sup>5</sup> LBP is also linked to lower extremity physical function decline, increasing the likelihood of falls among older individuals.<sup>7,8</sup> Gait disturbances are common in LBP patients, including altered kinematics, reduced walking speeds and shorter stride length,<sup>9–11</sup> asymmetrical gait patterns,<sup>10</sup> and larger step width<sup>9</sup> compared to healthy individuals. Given the strong correlation between gait disturbance and LBP, gait analysis offers an indirect and objective measure of a patient's pain level and disability.<sup>12</sup>

Since gait abnormalities are observed in human patients with LBP, it is thought that gait abnormalities

may also exist in animal models of LBP. Many *in vivo* rodent models of disc-associated LBP have been developed to study the underlying pain mechanisms, assess potential treatments, and advance our understanding of the complex relationship between disc pathology and pain. Nevertheless, only a few of these studies have implemented gait analysis to describe gait abnormalities in LBP models.<sup>13–15</sup> Our lab has established a model of disc-associated LBP in female Sprague Dawley rats that mimics the human representation of pathological discs.<sup>16</sup> In our model, the rats responded to evoked pain-like behavior assay including the grip strength assay and pressure algometry, which signifies the development of axial hypersensitivity.<sup>16</sup> However, our open field test results were inconclusive between sham and injured.<sup>16</sup> Thus, gait analysis in this disc-associated LBP model could serve as an alternative spontaneous pain-like behavior assay.

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 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/03008207.2024.2395287>

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In rodents, gait analysis is widely used to study movement disorders<sup>17</sup> and is employed in models featuring sensory-motor dysfunction such as Parkinson's disease,<sup>18,19</sup> spinal cord injury,<sup>20,21</sup> and stroke.<sup>22,23</sup> Gait has also been analyzed in rodent models of neuropathic pain,<sup>24–27</sup> inflammatory pain,<sup>25,27</sup> and osteoarthritis pain.<sup>28</sup> Commercially available methods of rodent gait assessment that have been used in animal models of pain include automated systems such as CatWalk<sup>TM</sup> (Noldus),<sup>29–31</sup> DigiGait<sup>TM</sup> (Mouse Specifics),<sup>27</sup> or TreadScan<sup>TM32</sup> and GaitScan<sup>TM</sup> (CleverSys).<sup>33</sup> The specific features of each system have been comprehensively reviewed and described elsewhere.<sup>34</sup> A major limitation of automated gait systems is that they are expensive, and their analysis software is proprietary and difficult to modify. This makes the software less adaptable and the detection of other gait measurements of interest more difficult. Further, systems like DigiGait and TreadScan use a belt treadmill floor to drive gait, which may cause stress-like responses during testing that mask pain-associated gait compensations.<sup>32</sup> Custom built arenas for gait analysis such as the Gait Analysis Instrumentation and Technology Optimized for Rodents (GAITOR) system are a cost-effective option and are designed to be adaptable to multiple movement disorder models.<sup>35</sup> The GAITOR suite also comes with open-source code for video analysis and gait calculations that can be customized if needed for specific models.<sup>35</sup> This study aims to utilize the GAITOR suite to evaluate if disc injury affects gait and whether gait analysis can be used as an alternative spontaneous pain-like assessment tool in our *in vivo* LBP model.

## Methods

### Animals

All animal experiments were conducted in accordance with the National Institutes of Health (NIH) guidelines following the Public Health Safety (PHS) policy on Humane Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Nebraska–Lincoln. Forty adult female Sprague Dawley rats at 16-weeks of age were purchased from Envigo and housed in pairs with a 12-h light/dark cycle and ad libitum access to food and water. The animals in this study were a subset of another ongoing study to evaluate the therapeutic efficacy of a drug in alleviating disc-associated pain. The animals were randomly assigned to sham or three injured groups, and the first 10 animals assigned to each group were selected

for gait analysis. Therefore, 10 animals in the sham control group and total 30 animals from the three injured groups ( $n = 10$  per group) were selected for gait analysis in this current work. All animals were acclimated to personnel handling for 3 weeks and gait arena for 2 weeks prior to data collection. All surgical procedures and behavioral testing performed on the animals are described herein.

### Surgical procedure

Surgical procedures were performed as previously published in Sprague Dawley rats to induce disc-associated LBP.<sup>16</sup> On the day of surgery, animals were anesthetized with 2–3% isoflurane in oxygen and administered subcutaneously with Buprenorphine SR (0.75 mg/kg) for post-operative analgesia. The ventral abdomen was shaved, and the iliac crest was used to demarcate the approximate level of the L5-L6 disc. A midline incision is made along the ventral surface of the abdomen skin perpendicular to the iliac crest markings. After exposing the abdominal cavity, the abdominal organs were gently retracted with a gauze soaked in saline toward the lateral and cranial wall of the peritoneal space to visualize the retroperitoneum. The L5-L6 disc was approached with care by avoiding any major blood vessels and punctured bilaterally with a strong point dissecting needle (Roboz, RS-6066) set to 3 mm depth and then swept back and forth along a 90° arc six times in the transverse plane [Figure 2\(a\)](#). A continuous subcuticular suture closure pattern was used to close the abdominal wall and skin incision. All animals were monitored every 12 h for 3 days after surgery for signs of pain and distress and wound closure. Animals were allowed to rest and heal for 2 weeks after surgery before beginning gait data collection. Animals in the sham group underwent the same surgical procedure except that the discs were only visualized and not punctured with the needle.

### Microcomputed tomography and disc volume analysis

Microcomputed tomography ( $\mu$ CT) scans of the L5-L6 disc were acquired at weeks 0 and 10 utilizing a Quantum Gx2 $\mu$ CT Imaging System (PerkinElmer, Waltham, MA). The animals were anesthetized under isoflurane and positioned supine in the scanner, a 2-min scan with 90 kV power, 88  $\mu$ A tube current, 45 mm FOV, 90 mm voxel size, and a Cu 0.06 + Al 0.5  $\times$ -ray filter was obtained. The collected data, exported as a VOX file, was then transferred to Analyze 14.0 (Analyze Direct) for disc volume analysis.

A previously developed method from our lab was employed for disc volume quantification,<sup>36</sup> involving a 700 Hounsfield units (HU) threshold to identify bony objects in each scan.<sup>37,38</sup> Briefly, after locking the bony objects to prevent modification, the L5-L6 disc space was manually colored on each slice, followed by the use of semi-automatic tools (coronal smoothing and propagating objects) to enhance consistency between slices. The completed drawings were saved as an object map, and the built-in software analyzed the object maps to quantify disc volume. Disc volume of each animal was normalized to its own baseline to account for animal variation for statistical analysis.

### **Grip strength axial hypersensitivity assay**

Evoked pain-like behavior assays, such as the grip strength assay, are useful measures for hypersensitivity in animals. Lowered grip strengths indicate axial hypersensitivity, which is indicative of disc-associated LBP in rodent models.<sup>16,39,40</sup> To evaluate signs of disc-associated LBP, a grip strength assay was conducted using a Columbus Instruments 1027SR apparatus (Columbus Instruments, Columbus, OH). To perform this assay, the animals were positioned to naturally grip a wire mesh connected to a force sensor, then stretching the animal caudally from the base of its tail. The maximum force of the animal's grip strength measured on the force sensor was recorded. The average from three trial recordings were log-transformed to achieve normality and normalized as a fraction of baseline values. Assessments were performed biweekly post-disc injury (week 2, 4, 6, 8, 10). Two blinded female experimenters, each handling a set of 20 animals, ensured consistent, unbiased data collection throughout the study.

### **Open field test**

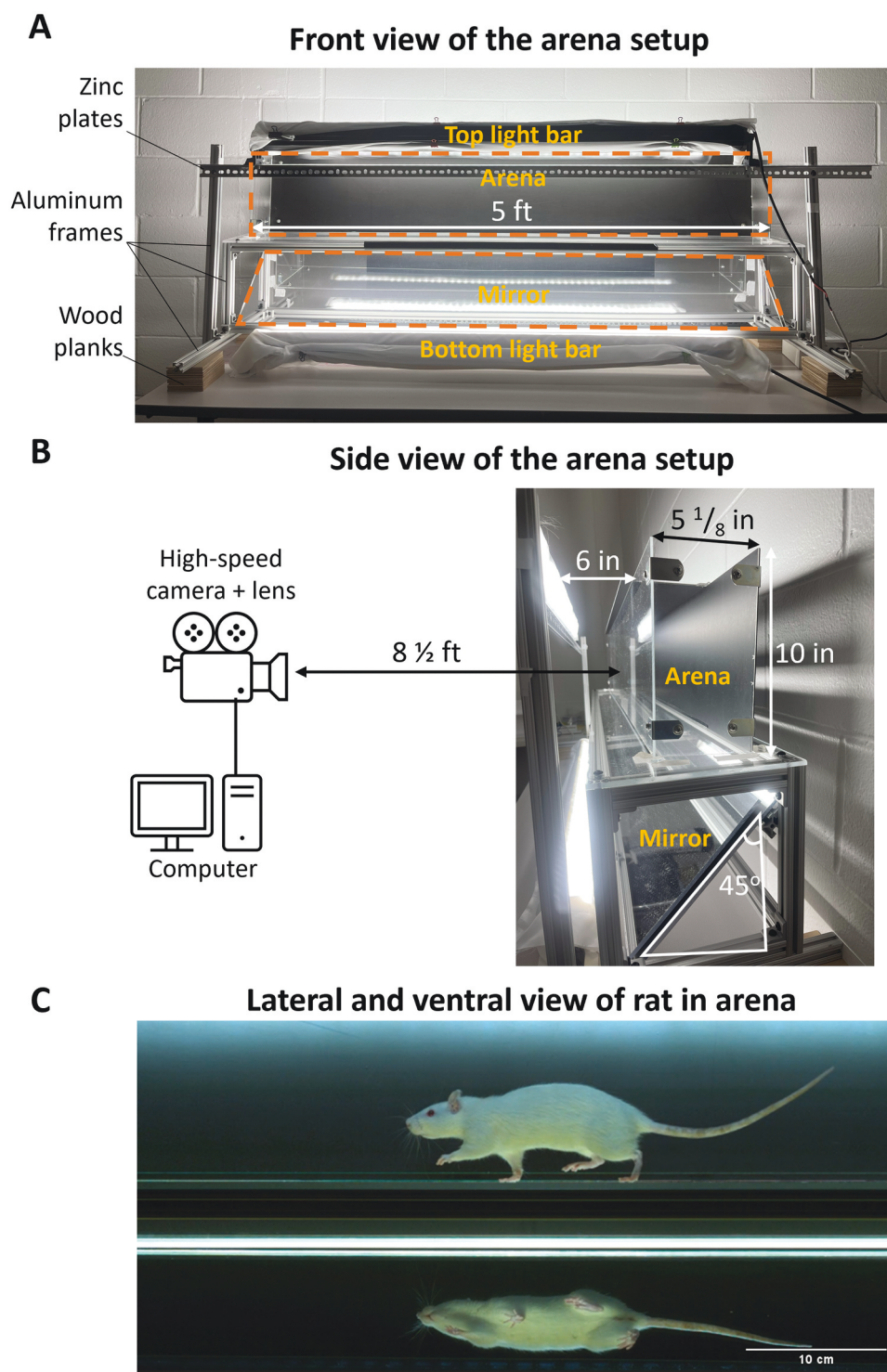
Non-stimulus dependent assays are valuable for detecting spontaneous pain-like behavior in rodents with reduced subjectivity. The open-field test utilized a custom-built acrylic 2' × 2' × 2' (L × W × H) black, opaque arena. The open field test was performed in a darkroom with red fluorescent lighting. In addition, a diffuser fabric was placed overhead of the arena to prevent glares and shadows from direct lighting. Animals were not acclimated to the arena before data collection to ensure the novelty effect of animal exploration in the arena.<sup>41</sup> During the test, each animal was allowed to explore the open field arena for 30 min and video recordings were collected using an overhead low-illumination 2MP HD varifocal web camera

(ALPCAM, China company location). Open field test videos were collected in week 0 (baseline), week 10, and 15. The middle 20-min section of the video recordings were analyzed on EthoVision (Noldus, Leesburg, VA) for parameters including total distance traveled, maximum velocity, acceleration, mean turn angle, and the total duration of unsupported rearing, supported rearing, grooming, and frequency in the center zone. Ethanol-soaked paper towels were used to clean each arena after each test to eliminate biological traces before introducing the next animal.

### **Gait recording**

The Gait Analysis Instrumentation and Technology Optimized for Rodents (GAITOR) Suite consists of a custom-built arena and an open-source software (<https://github.com/OrthoBME/GAITORsuite>) for the utility of rodent gait video analysis developed by Dr Kyle Allen's group at the University of Florida (<https://www.gaitor.org/>).<sup>35</sup> The GAITOR suite has been applied to rodent models of orthopedic injury, sciatic nerve injury, spinal cord injury, and elbow joint contracture.<sup>28,42–44</sup> The arena has a black acrylic back, a lid with three transparent acrylic sides and a transparent floor on top of an aluminum frame. The arena floor can be replaced with an instrumental force panel to measure dynamic gait,<sup>35</sup> but only spatiotemporal gait was assessed in this study. Underneath the arena floor, a mirror is mounted at 45° angle to capture the animal's ventral view (Figure 1).<sup>35</sup> The detailed dimensions and lighting setup of the arena are described elsewhere and illustrated in Figure 1.<sup>35,45</sup> The arena wall is tilted backwards slightly to leave about a 6–7 mm gap from the arena floor to allow clear visualization of paws in contact with the arena floor. The only light sources in the room are two 52-inch LED light bars (Nilight) mounted in front of the arena and a computer screen that is facing away from the arena (Figure 1). LED light bars with diffuser fabric to diffuse light and the bottom light bar were wrapped with orange cellophane wrap (Michaels) to shine orange filtered light on the bottom of the arena, which provides better contrast of the rat paws.

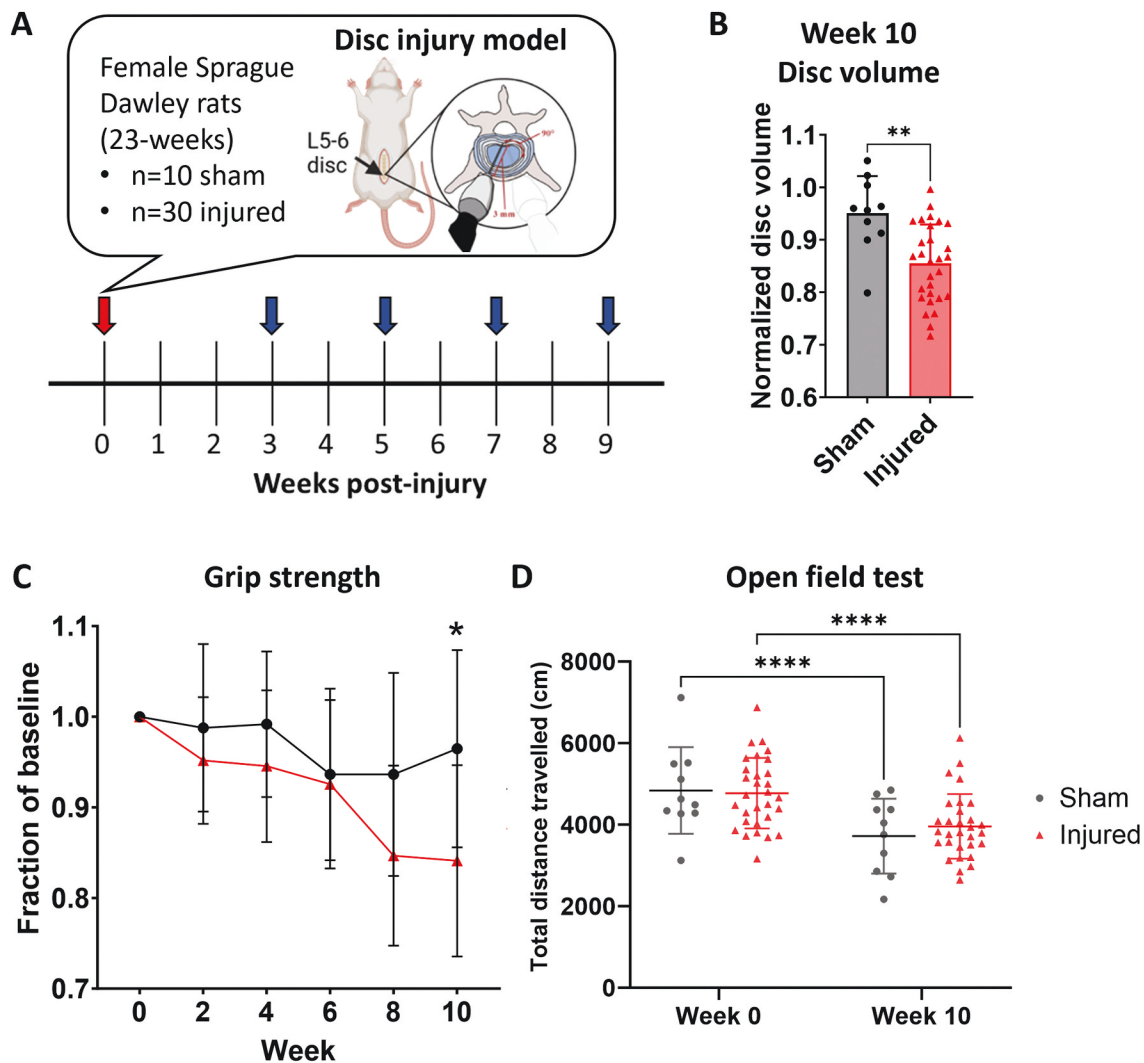
Video clips of the animal walking back and forth in the arena were recorded using a ZEISS Milvus 1.4/50 lens attached to a high-speed camera (Phantom Miro C321) and Phantom Camera Control Version 3.8 software (Phantom) using the following settings: 1280 × 720 resolution, 500 fps sample rate, and 300 μs exposure. The lens aperture f-stop was set to 2.8 to 5.6. The camera is placed 8 foot 8 inches perpendicular to the front of the arena wall. At the start of each week, the camera angle



**Figure 1.** Overview of the GAITOR system. (A) Front view of the Experimental Dynamic Gait Arena for Rodents (EDGAR), a custom-built arena for gait analysis. (B) Side view of EDGAR. (C) Screenshot of a video recording where the animal is walking across the arena. Top: lateral view showing paw contacting floor. Bottom: ventral view showing animal fore and hind paws.

and position were calibrated so that it is level to the floor of the arena. A 6 × 24 inch sewing ruler was placed in the empty arena after the camera was calibrated, before beginning the recording session to capture a calibration video for scaling the videos during analysis.

Two weeks prior to surgery, animals were acclimated to the arena two times for 10 min each, 5 days apart, then once for 30 min. Following disc injury surgery, gait video recording sessions were held every 2 weeks in weeks 3, 5, 7, and 9 [Figure 2\(a\)](#). Data collection each



**Figure 2.** Disc injury leads to disc degeneration and lowered grip strength.

(a) Overview of model and study timeline. The red arrow indicates the week of disc injury. Blue arrows show the preceding gait recording sessions on weeks 3, 5, 7, 9. (b) Disc volume in week 10 normalized to baseline was significantly lower in injured group compared to sham indicating disc degeneration after disc injury. (c) Injured animal's grip strength decreased over time and was significantly lower than sham in week 10 suggesting the axial hypersensitivity due to disc injury. (d) Distance traveled in open-field test was not significantly different between sham and injured groups but reduced significantly in week 10 compared to baseline (week 0) in both groups, suggesting that disc injury did not have any effects on animal exploration behavior. The markers and bars represent the mean and error bars represent the standard deviation. Asterisks (\*) symbolize statistical significance ( $p < 0.05$ ) between sham and injured groups for (B) disc volume (unpaired t-test) and (c) grip strength (Mann-Whitney test with Holm-Sidak test) and between groups or weeks for (d) open field test (2-way ANOVA with Fisher's LSD test).

week consisted of 3 days of recording sessions with 12 to 14 animals per session. During each recording session, the animals were removed from their home cage, weighed, and placed on one end of the arena. Each animal remained in the arena for up to 30 min or until a minimum of nine successful trials containing at least two complete gait cycles were obtained. Successful trials consist of at least two complete gait cycles starting from the left hind foot, according to the

classic version of the Hildebrand plot.<sup>46</sup> At the end of each recording session before placing the next animal in the arena, the arena was cleaned with water-soaked KimWipes to remove biological traces. A total of 2277 trials were acquired. The video files were saved as CINE files and cropped from the time the animal's nose enters the video frame until the last paw leaves the frame. The cropped videos were batch saved as AVI files for analysis.

**Table 1.** Summary of spatiotemporal gait patterns.<sup>28</sup>

| Parameter                   | Explanation                                                                                                                                            | Observations                                                                                                                                                                                                                           |
|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Spatial patterns</b>     |                                                                                                                                                        |                                                                                                                                                                                                                                        |
| Step width                  | <b>Distance between the limbs</b> perpendicular to direction of travel                                                                                 | Velocity-dependent                                                                                                                                                                                                                     |
| Stride length               | Distance from foot strike to subsequent foot strike of the <b>same foot</b>                                                                            | Velocity-dependent                                                                                                                                                                                                                     |
| Spatial symmetry            | $\frac{\text{Right step length}}{\text{Stride length}}$                                                                                                | Values near 50% indicate the right foot hits the ground approximately halfway between left foot strikes                                                                                                                                |
| <b>Temporal patterns</b>    |                                                                                                                                                        |                                                                                                                                                                                                                                        |
| Duty factor (DF)            | $\frac{\text{Stance time of limb}}{\text{Stride time of limb}}$                                                                                        | Velocity-dependent                                                                                                                                                                                                                     |
| Stance time imbalance (L-R) | $\text{LeftlimbDF} - \text{RightlimbDF}$                                                                                                               | >0: right limb injury<br><0: left limb injury<br>= 0: equal stance times between the left and right limbs                                                                                                                              |
| Temporal symmetry           | $\frac{(\text{Time of right foot strike1} - \text{Time of left foot strike1})}{(\text{Time of left foot strike2} - \text{Time of left foot strike1})}$ | >50%: More rapid right-to-left, delayed left-to-right (right limb injury)<br><50%: More rapid left-to-right, delayed right-to-left (left limb injury)<br>= 50%: right foot-strike occurs halfway between two left foot-strikes in time |

### Gait video analysis

Gait videos were analyzed using a MATLAB generated video processing script called Automated Gait Analysis Through Hues and Areas (AGATHAv2) as previously described by Kloefkorn et al.<sup>43</sup> and Jacobs et al.<sup>35</sup> AGATHAv2 (<https://github.com/OrthoBME/GAITORSuite>) is an automated process that isolates and identifies the animal's body and paws from the videos. Fundamentally, the colorThresholder tool in MATLAB is used to create filters that isolate the animal from the arena's background in the lateral and ventral view. Additionally, colorThresholder also allows for paw prints to be isolated from the ventral view. The velocity of the animal is calculated based on the center point of the animal's body in the ventral view. AGATHAv2 detects the time and location of when the animal's paw strikes the arena floor in the lateral view, generating a foot-strike and toe-off (FSTO) map. The FSTO objects and the paw print images were used to calculate velocity-independent variables such as temporal symmetry, spatial symmetry, and duty factor imbalance. Velocity-dependent variables were also calculated such as left and right duty factor (hind and fore), stride length, and step width (hind and fore). Table 1 summarizes the calculated gait patterns and equations used in this study.<sup>28</sup>

### Trial inclusion and exclusion criteria

Acceptable trials met three inclusion criteria to be considered for analysis: 1) Velocity range between 25 and 75 cm/s. Any trials outside of this range were excluded because the animal was either running or had intermittent stops. 2) At least two gait cycles per trial. Two gait cycles are necessary to assure the animal is not appreciably accelerating or decelerating during the trial (<10% variation in velocity over the trial). 3)

No unrelated health concerns. Animals with other health concerns unrelated to the study may have different gait patterns that are not a result of disc injury and therefore were excluded. A total of 1522 trials did not meet these three criteria and were excluded from statistical analysis. The remaining 1095 trials were analyzed as described below.

### Study design

The results presented herein are a subset of data presented in another study. The degeneration of the disc in the injured animals progressed for 10 weeks post-injury before intervention with the test compounds. Therefore, it is important to note that all the animals in the injured group were treated identically and did not receive any test drugs during this gait analysis period. It should also be noted that these data were analyzed independently of the analysis presented previously. In this analysis, trials from the injury groups were combined herein to analyze the gait difference in injured groups ( $n = 29$ ) compared to sham ( $n = 10$ ). All animals remained healthy without any health concerns and no animals were excluded from gait analysis except for one animal from the injured group was removed due to disc-level mispuncture as confirmed by  $\mu$ CT.

### Statistical analysis

For disc volume, grip strength and open field test data, statistical analyses were performed using GraphPad PRISM (version 10.1.0). Normality of data was analyzed using the Shapiro-Wilk test. Normal data (disc volume) was analyzed for unpaired t-test. Grip strength analysis was performed using a repeated 2-way ANOVA method with Sidak's test to compare groups at each week. Open field test data was normally distributed and

analyzed using a 2-way ANOVA with Fisher's multiple comparisons test.

For gait data, statistical analyses were performed using R studio (version 2023.06.0). Throughout analysis, linear mixed-effects models were used, where week and group were treated as fixed effects and animal was treated as a random effect to account for repeated measures within subjects. For velocity-dependent data (duty factor, stride length, and step width), velocity was also included as a fixed-effect. This model also accounted for the longitudinal nature of the data, with repeated measures across time. The normality of the residuals in the linear mixed effect model was assessed using density plots. If indicated by a significant ANOVA result in group and week effects, a post hoc Tukey's HSD test was used to identify specific differences in the least square means between all combinations of groups and weeks. Asterisks symbols indicate p-value <0.05 and statistical significance between weeks or groups. To evaluate the shifts from a constant, a nonpaired least square means of the linear mixed-effects model were calculated to evaluate whether DF imbalance met the numerical definition of zero, and whether temporal and spatial symmetry met the numerical definition of 0.5. These numerical definitions are derived from the equations in Table 1, where theoretical balance and symmetry yield a value of zero and 0.5, respectively. These values also align with symmetric and balanced, non-pathologic walking gait patterns in rodents reported in prior literature.<sup>28,42,43</sup> Asterisks symbols indicate p-value <0.05 and statistically significant deviation from numerical definitions.

## Results

### *Disc injury results in a loss of disc volume and axial hypersensitivity*

Injured animals presented significant disc volume loss ( $p = 0.0011$ ) indicating disc degeneration by week 10 post-injury Figure 2(b). Injured animals also developed axial hypersensitivity as measured by the significant reduction in normalized grip strength compared to sham in week 10 ( $p = 0.00346$ ) Figure 2(c). In the open field test, the total distance traveled did not differ significantly between groups, but both sham and injured groups had significantly reduced total distance traveled in week 10 compared to week 0 baselines Figure 2(d). These data indicate that our disc injury model significantly induced disc volume loss and axial hypersensitivity by week 10 post-injury.

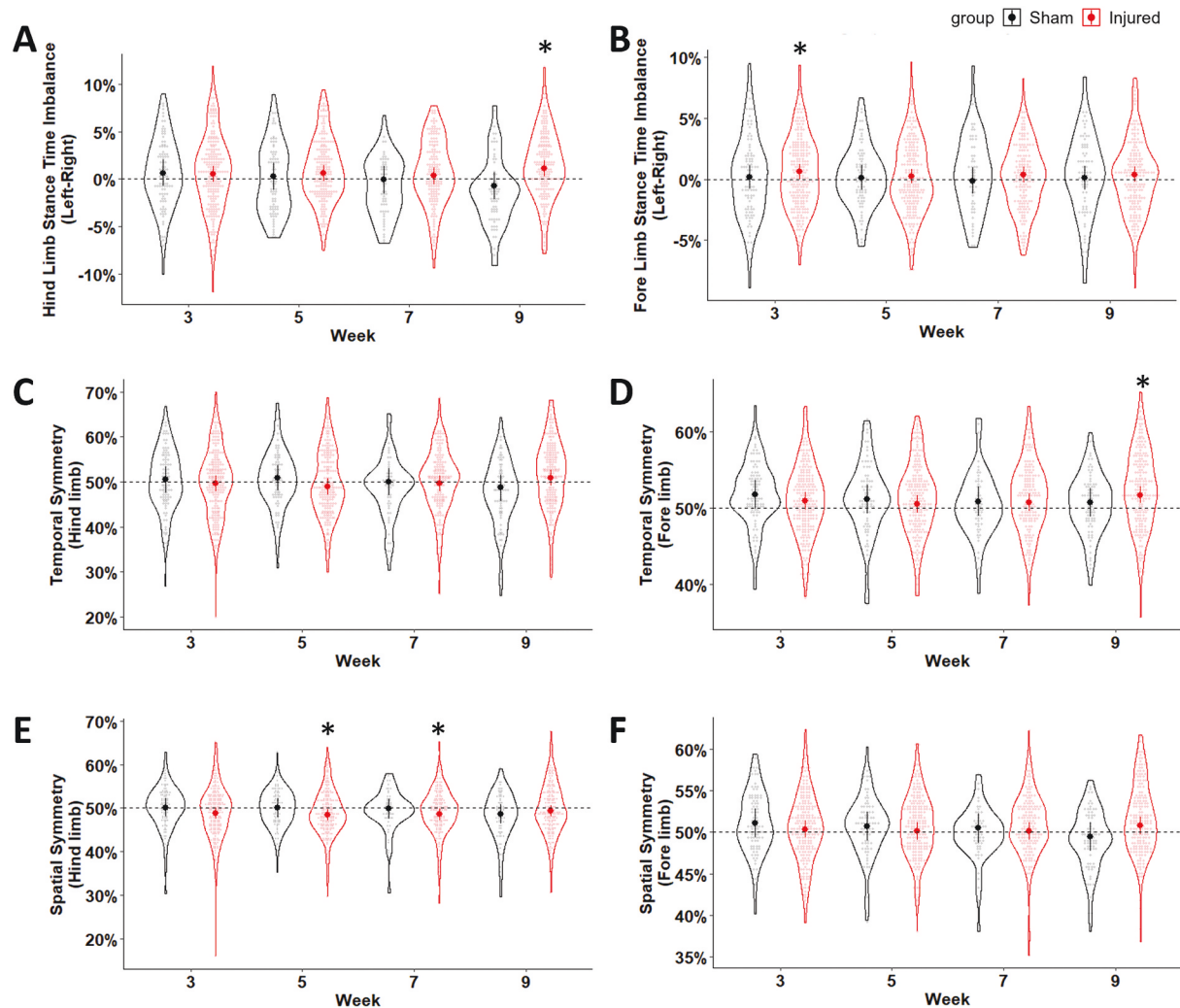
### *Disc injury did not result in gait abnormalities, but stride length and duty factor changed over time in each group*

Despite injured discs and the development of axial hypersensitivity in our female Sprague Dawley rats, no significant differences were detected in spatiotemporal gait measurements between sham and injured animals throughout all weeks (Table 2). Stance time imbalance (Figure 3(a-b); Table 2), temporal and spatial symmetries (Figure 3(c-f); Table 2) and step width Figure 4(b), were unaffected by both injury and time. However, the walking velocity (Figure 4(a); Table 2), hindlimb and forelimb stride length (Figure 4(c); Table 2) and duty factor for all limbs (Figure 5; Table 2) changed significantly over time in both sham and

**Table 2.** Summary of p-values from 2-way ANOVA (LMER model).

| Gait measurements            | velocity  | week      | group   | week:group |
|------------------------------|-----------|-----------|---------|------------|
| Step width (hind)            | 0.18690   | 0.33440   | 0.90400 | 0.15610    |
| Step width (fore)            | 0.19941   | 0.04975   | 0.11007 | 0.24617    |
| Stride length (hind)         | <2.2E-16* | 4.43E-05* | 0.58800 | 0.62250    |
| Stride length (fore)         | <2.2E-16* | 1.17E-04* | 0.69432 | 0.57271    |
| DF right hind                | <2.2E-16* | 2.81E-08* | 0.20805 | 0.03391*   |
| DF left hind                 | <2.2E-16* | 2.57E-05* | 0.80410 | 0.20150    |
| DF right fore                | <2.2E-16* | 9.64E-06* | 0.94670 | 0.39270    |
| DF left fore                 | <2.2E-16* | 2.74E-06* | 0.64110 | 0.41890    |
| Stance time imbalance (hind) | N/A       | 0.32401   | 0.35747 | 0.00764*   |
| Stance time imbalance (fore) | N/A       | 0.62780   | 0.58850 | 0.87030    |
| Velocity                     | N/A       | 2.89E-05* | 0.19265 | 0.03301*   |
| Temporal symmetry (hind)     | N/A       | 0.99306   | 0.93674 | 0.00308*   |
| Temporal symmetry (fore)     | N/A       | 0.30377   | 0.88661 | 0.05917    |
| Spatial symmetry (hind)      | N/A       | 0.65134   | 0.47905 | 0.02719*   |
| Spatial symmetry (fore)      | N/A       | 0.22469   | 0.92945 | 0.00137*   |

\*Asterisks symbolize statistical significance ( $p < 0.05$ ) from ANOVA F-tests and significant parameters affecting each gait measurements.



**Figure 3.** Non-velocity dependent spatiotemporal gait patterns show symmetric and balanced walking gait patterns at most timepoints.

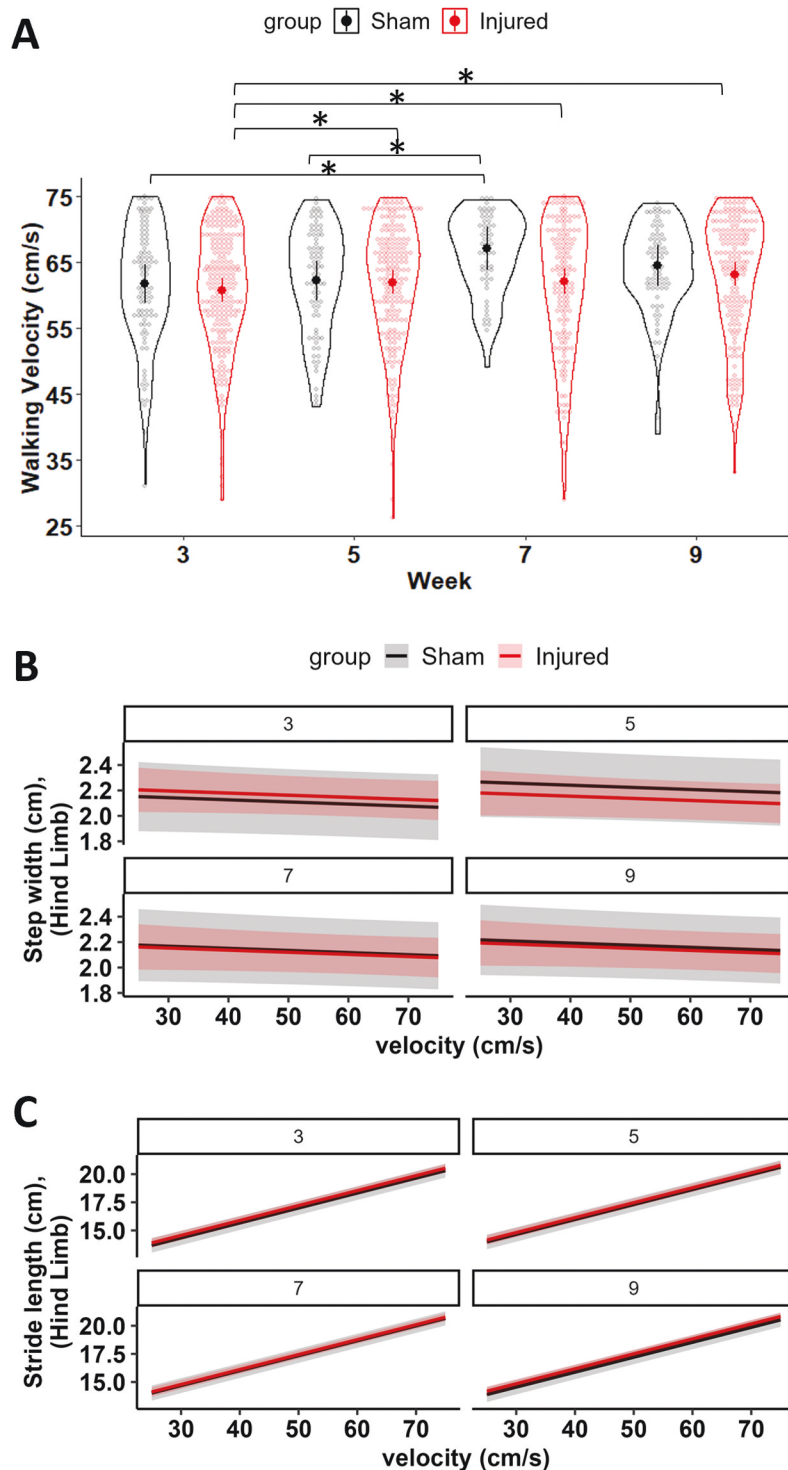
(a) Stance time imbalance of the hindlimb and (b) forelimb; (c) temporal symmetry of the hind and (d) forelimb; (e) spatial symmetry of the hindlimb and (f) forelimbs. Dashed line indicates no imbalance (0%) for stance time imbalance and no asymmetry (50%) for temporal and spatial symmetry, as defined by Table 1 and prior literature reporting symmetric and balanced, non-pathologic walking gait in rodents.<sup>28,42,43</sup> Violin plots show the spread of data for each trial with markers showing the mean and 95% CI. Asterisks (\*) symbolize significant deviations ( $p < 0.05$ ) from numerical definitions of (A, B) stance time imbalance at 0% and (C, D, E, F) symmetry at 50% (nonpaired least square means of the linear mixed-effects model).

injured animals. This is a common finding in rodent gait studies as rodent's growth plates do not fuse,<sup>47</sup> and they continue to grow over time. Thus, the changes in velocity, stride length, and duty factor are most likely associated with growth of the rodent's limb length.

Differences in percentage stance time between left and right limb suggest an imbalance in the walking sequence. Symmetric and balanced walking patterns without gait imbalance should meet the numerical definition of a balanced gait sequence (stance time imbalance close to zero), as defined by Table 1 and prior

literature reporting symmetric and balanced, non-pathologic walking gait in rodents.<sup>28,42,43</sup> For our model, injured animals spent more time on their left hind limb than their right, resulting in imbalance at week 9 ( $1.11 \pm 0.85\%$ , 95% CI; Figure 3(a); Table 3). A similar gait change was seen at week 3, where injured animals spent more time on their left fore limb than their right ( $0.60 \pm 0.93\%$ , 95% CI; Figure 3(b); Table 3).

For spatial and temporal symmetries, a healthy walking gait should be symmetric and centered around 50%, as defined by Table 1 and prior literature reporting

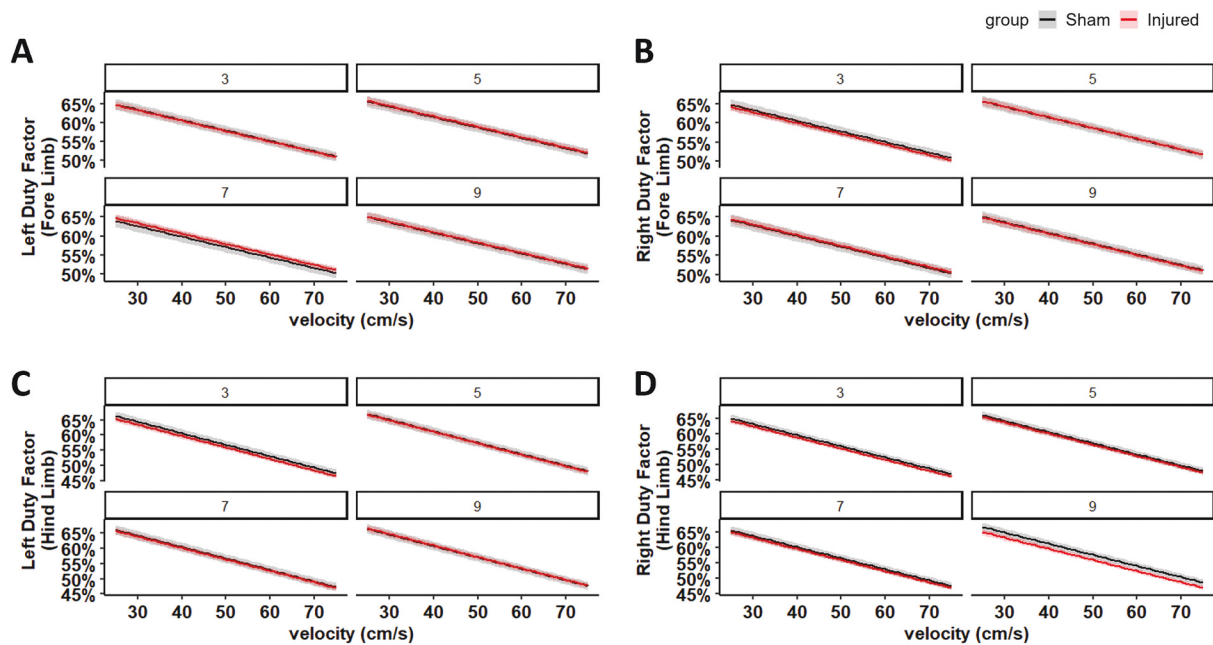


**Figure 4.** Velocity-dependent spatiotemporal gait patterns did not differ significantly between sham and injured.

(a) Violin plot of walking velocity (25–75 cm/s). Walking velocity differs significantly between weeks in sham and injured animals. Asterisks (\*) symbolize statistical significance ( $p < 0.05$ ) in walking velocity between weeks for each group (ANOVA with Tukey's test). (B) Hindlimb step width and (C) hindlimb stride length plotted over velocity. Lines represent the linear mixed-effects model with bars/bands representing the 95% confidence interval.

symmetric and balanced, non-pathologic walking gait in rodents.<sup>28,42,43</sup> In our model, the temporal symmetry of the hindlimbs stayed the same over time

(Figure 3(c); Table 3) except at week 9, where for the forelimb of injured animals was greater than 50%, indicating a potential right forelimb compensation



**Figure 5.** Duty factor across all hind and forelimbs did not differ significantly between sham and injured group. The duty factor of a) left forelimb, b) right forelimb, c) left hindlimb and d) right hindlimb of both sham and injured groups. Lines represent the linear mixed-effects model with bands representing the 95% confidence interval.

**Table 3.** Deviations from symmetry and 95% confidence intervals of stance time imbalance, temporal symmetry, and spatial symmetry (%).

| Spatiotemporal gait measurements | Sham        |             |              |              | Injured             |                      |                      |                     |
|----------------------------------|-------------|-------------|--------------|--------------|---------------------|----------------------|----------------------|---------------------|
|                                  | Week 3      | Week 5      | Week 7       | Week 9       | Week 3              | Week 5               | Week 7               | Week 9              |
| <b>Hindlimb</b>                  |             |             |              |              |                     |                      |                      |                     |
| Stance time imbalance            | 0.65 ± 1.35 | 0.28 ± 0.28 | -0.09 ± 0.02 | -0.72 ± 1.39 | 0.51 ± 0.82         | 0.63 ± 0.84          | 0.36 ± 0.86          | <b>1.11 ± 0.85*</b> |
| Temporal symmetry                | 0.43 ± 2.83 | 0.86 ± 2.88 | -0.03 ± 3.00 | -1.32 ± 2.89 | -0.33 ± 1.72        | -1.08 ± 1.74         | -0.34 ± 1.80         | 0.83 ± 1.75         |
| Spatial symmetry                 | 0.15 ± 2.11 | 0.03 ± 2.15 | -0.15 ± 2.25 | -1.41 ± 2.17 | -1.13 ± 1.29        | <b>-1.51 ± 1.31*</b> | <b>-1.42 ± 1.35*</b> | -0.63 ± 1.32        |
| <b>Forelimb</b>                  |             |             |              |              |                     |                      |                      |                     |
| Stance time imbalance            | 0.16 ± 0.96 | 0.12 ± 1.00 | -0.15 ± 1.07 | 1.02 ± 0.51  | <b>0.60 ± 0.93*</b> | 0.23 ± 0.61          | 0.37 ± 0.23          | 0.37 ± 0.61         |
| Temporal symmetry                | 1.79 ± 1.81 | 1.17 ± 1.85 | 0.90 ± 1.93  | 0.76 ± 1.86  | 0.95 ± 0.95         | 0.57 ± 1.12          | 0.72 ± 1.16          | <b>1.73 ± 1.13*</b> |
| Spatial symmetry                 | 1.07 ± 1.67 | 0.72 ± 1.69 | 0.49 ± 1.75  | -0.52 ± 1.66 | 0.32 ± 0.32         | 0.11 ± 1.02          | 0.11 ± 1.05          | 0.80 ± 1.03         |

\*Asterisks symbolize significant deviations ( $p < 0.05$ ) from numerical definitions of normal stance time imbalance, temporal and spatial symmetry (nonpaired least square means of the linear mixed effects model).

(Figure 3(d); Table 3). On the other hand, for spatial symmetries, no significant shifts were detected in the forelimbs (Figure 3(f); Table 3), but the hindlimbs of the injured animals shifted significantly lower than 50% in weeks 5 and 7 indicating a potential right hindlimb compensation (Figure 3(e); Table 3).

## Discussion

This work demonstrates for the first time the GAITOR system being used in a rodent disc-associated LBP model. To date, no surgically induced animal models of disc-associated LBP have assessed changes in gait in female rats. In our female rat disc-associated LBP model, we did not see significant gait changes (Figures 3–5), despite observing disc degeneration,

evidenced by disc volume loss and pain-like behaviors in the form of axial hypersensitivity (Figure 2). Our results indicate that rats with induced lumbar disc degeneration do not have prominent spatiotemporal gait abnormalities up to 9 weeks post-injury.

Although the disc injury itself did not result in significant gait changes compared to sham, further analyses revealed mild deviations in forelimb and hindlimb stance time imbalance, forelimb temporal symmetry, and hindlimb spatial symmetry at some timepoints (Figure 3). These findings suggest that there could be subtle sidedness due to our disc injury, even though the comparisons between sham and injured groups did not yield significance. It is possible that a larger sample size is needed to detect these mild changes in gait between sham and

injured animals. In-depth gait analysis, including dynamic weight-bearing analysis, also have the potential to delineate and thoroughly characterize the subtle nuances inherent in asymmetric gait.

Other disc-associated LBP models that have analyzed gait used commercially available systems such as DigiGait<sup>13</sup> and CatWalk.<sup>14,15</sup> These two systems differ significantly and have their own pros and cons.<sup>27,32</sup> DigiGait employs a treadmill set for specific speeds for passive walk assessment, while CatWalk, like our GAITOR system, evaluates an animal's voluntary walk on a stationary floor within a fixed chamber.<sup>27</sup> Lai et al. used DigiGait and found that rats with injured discs have shorter swing duration and higher percentage of stride in stance.<sup>13</sup> Meanwhile, using the CatWalk system, Miyagi et al. observed longer stance time and duty cycle and shorter swing speeds and stride length in injured rats.<sup>14</sup> In contrast, Muralidharan et al. found no changes in the step width between the hind or fore paws, up to 7 weeks after disc injury using the CatWalk system which is most consistent with our results.<sup>15</sup> These mixed results could be attributed to the different modes of disc injury, study timespan, and age of animals. In addition, comparisons of our data with previous work are difficult because of the use of different gait analysis systems. The GAITOR system has robust analyses that account for velocity covariates, while previous studies mentioned above did not take into account velocity dependencies during analysis.<sup>13–15</sup> Spatiotemporal gait measurements such as step width, stride length, and duty factor vary significantly with velocity. The failure to account for the velocity covariate can greatly affect the statistical analysis and change the results of the study.<sup>28,48</sup> Moreover, changes in velocity will shift all of these measures, and thus, a shift in speed can be easily misinterpreted for a change in the gait pattern, when a velocity correction is not applied.<sup>49</sup> The recording speed or frame rate is another factor to consider with gait analysis.<sup>28</sup> The camera used with the GAITOR system has adjustable recording speeds and can be increased to enhance its sensitivity to subtle changes.<sup>28</sup> Our system and analysis outperform others due to our video recording speed set at 500 fps, which is significantly faster than the CatWalk XT system limited to 100 fps. This allows us to capture and detect subtle abnormalities in the temporal sequence, making our system potentially more reliable for precise assessments.

In humans, lumbar disc herniation, LBP, and lumbar spinal stenosis have unique gait alterations with more severe gait disturbances in herniation.<sup>50</sup> In rats, a lumbar disc herniation model can be simulated by applying an autologous nucleus pulposus derived from

rat caudal discs onto the left or right L5 dorsal root ganglion.<sup>26,51,52</sup> Using this disc herniation model, Allen et al. assessed gait in rats using the GAITOR system and observed significant differences in the symmetry and stance time imbalance in the experimental animals compared to sham.<sup>51</sup> These observations were expected as the herniation resulted in radiculopathy and mechanical hypersensitivity in the ipsilateral paw which may have caused gait imbalances and shift in symmetries. Since we did not observe significant changes in symmetry or stance time imbalance between sham and injured animals, our results confirm that our disc scrape injury method does not result in disc herniation or radiculopathy, supporting our previous work in which we did not observe any hind paw mechanical hypersensitivity after disc injury.<sup>16</sup>

In conclusion, our disc scrape injury does not significantly affect spatiotemporal gait patterns in female Sprague Dawley rats despite the presence of disc degeneration and axial hypersensitivity. In future investigations, it might be possible to add instrumental force panels underneath the arena floor to measure ground reaction forces<sup>53</sup> or to analyze gait kinematics<sup>54</sup> to fully ascertain whether disc injury results in gait alterations. Longer time-points beyond 9 weeks post-injury may also be needed to assess the longer-term impact of disc-injury on gait.

## Acknowledgments

We would like to acknowledge **Dr Heidi Kloefkorn** from Oregon State University for her advice and support with gait arena setup and AGATHA, **Evie Barnett** for collecting  $\mu$ CT data, **Anjeza Erickson** for  $\mu$ CT analysis, **Sydney Caparaso** for collecting grip strength data, and **Lydia Saltz** for performing open field test analysis.

## Funding

This work was supported by the National Science Foundation (NSF) under CAREER Grant [1846857].

## Conflict of interest

Dr. Kyle Allen is an editor for the Journal of Osteoarthritis and Cartilage

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Data availability statement

The data that support the findings of this study are available from the corresponding author, RAW, upon reasonable request.

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