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Assessment of hindlimb motor recovery after severe thoracic spinal cord injury in rats: classification of CatWalk XT® gait analysis parameters

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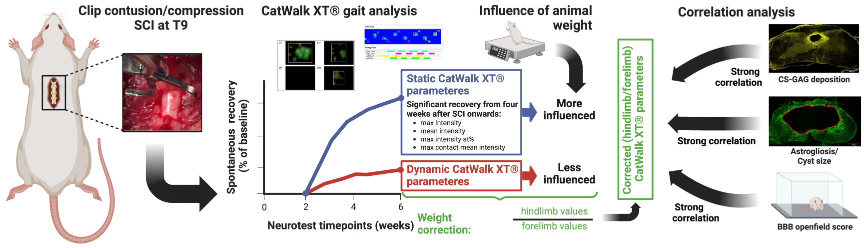
From the Contents

Graphical Abstract

Static CatWalk XT gait analysis parameters with weight correction are useful for assessing functional outcome after severe thoracic spinal cord injury in rats



Abstract

Assessment of locomotion recovery in preclinical studies of experimental spinal cord injury remains challenging. We studied the CatWalk XT® gait analysis for evaluating hindlimb functional recovery in a widely used and clinically relevant thoracic contusion/compression spinal cord injury model in rats. Rats were randomly assigned to either a T9 spinal cord injury or sham laminectomy. Locomotion recovery was assessed using the Basso, Beattie, and Bresnahan open field rating scale and the CatWalk XT® gait analysis. To determine the potential bias from weight changes, corrected hindlimb (H) values (divided by the unaffected forelimb (F) values) were calculated. Six weeks after injury, cyst formation, astrogliosis, and the deposition of chondroitin sulfate glycosaminoglycans were assessed by immunohistochemistry staining. Compared with the baseline, a significant spontaneous recovery could be observed in the CatWalk XT® parameters max intensity, mean intensity, max intensity at%, and max contact mean intensity from 4 weeks after injury onwards. Of note, corrected values (H/F) of CatWalk XT® parameters showed a significantly less vulnerability to the weight changes than absolute values, specifically in static parameters. The corrected CatWalk XT® parameters were positively correlated with the Basso, Beattie, and Bresnahan rating scale scores, cyst formation, the immunointensity of astrogliosis and chondroitin sulfate glycosaminoglycan deposition. The CatWalk XT® gait analysis and especially its static parameters, therefore, seem to be highly useful in assessing spontaneous recovery of hindlimb function after severe thoracic spinal cord injury. Because many CatWalk XT® parameters of the hindlimbs seem to be affected by body weight changes, using their corrected values might be a valuable option to improve this dependency.

Key Words: Basso, Beattie, and Bresnahan rating scale; behavioral assessment; CatWalk XT® gait analysis; contusive and compressive injury; hindlimb motor function; histological changes; spinal cord injury; spontaneous recovery; thoracic; weight

Introduction

According to a report from the global burden of diseases, injuries, and risk factors study in 2016, spinal cord injuries (SCI) were the fourth leading contributor to global neurological disability-adjusted life-years (DALYs) in all five high-income regions, including the United States and Western Europe (GBD 2016 Neurology Collaborators, 2019). It is well understood that these injuries and their complications lead to a series of devastating physical and psychological health problems for the patients and a significant burden to society (Lee et al., 2014; Hunt et al., 2021). Unfortunately, while advances have been made in the fields of prevention, surgery, and intensive care after SCI, no well-established neuroprotective or neuroregenerative treatment currently exists to mitigate secondary injury processes such as demyelination, inflammation, and scar formation or to replenish lost neural tissue and connections (Gill et al., 2016). This is particularly disenchanting because various experimental treatments have been assessed in the last decades, with mostly promising preclinical results in animal models but failure in clinical trials (Reinhardt et al., 2020).

Such failures of clinical translation have been attributed to e.g., the trauma models used in animal experiments to mimic SCI: Despite a contusion and compression injury being the most common trauma to the spinal cord in the clinical setting, insufficiently realistic hemi- or transection injuries are often applied in preclinical studies (Shende and Subedi, 2017; Timotius et al., 2021). In addition to the injury model, behavioral assessments play a significant role in the design of preclinical studies: Only with such assessments, functional recovery and thus the overall effect of any experimental treatment can be observed and validated (Bhimani et al., 2017). Because rats are commonly used for preclinical SCI studies, the Basso, Beattie, and Bresnahan (BBB) open field rating scale has been the primary behavioral assessment in many cases: It comprehensively covers several aspects of locomotor recovery such as joint movements, weight-bearing, coordination, and trunk stability (Basso et al., 1995). Although the BBB score is typically rated by trained, blinded observers, it still requires a learning curve and remains a rather subjective system. Thus, relying on the BBB open field rating scale in preclinical experiments might have complicated clinical translation of promising treatments for SCI.

The CatWalk XT® gait analysis system is a more recent behavioral assessment platform for rodents, providing more than 100 specific parameters of motor function, coordination, and gait (Hamers et al., 2001). It is thought to allow a more objective analysis of functional recovery. Although it has been used in several experimental SCI studies, the possibility of subjectively selecting

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its most favorable outcomes and the heterogeneity of presented parameters in the context of different SCI models hinder its comprehensibility and generalizability (Datto et al., 2015; Imani et al., 2016; Jalan et al., 2017).

Thus, our current study aims to describe the locomotion changes within commonly used parameters of the CatWalk XT® system after a clinically relevant clip compression/contusion injury on the thoracic spinal cord in rats. Moreover, we examine the correlation of those CatWalk XT® parameters with the BBB open field score but also with cyst formation, astrogliosis, and the deposition of chondroitin sulfate glycosaminoglycans (CS-GAG deposition) as typical histological changes in the injured spinal cord, providing more evidence for their use in the context of preclinical SCI studies.

Methods

Study design and experimental groups

A total of 12 female Wistar rats (specific-pathogen-free grade, ~250 g, 12 weeks old; Charles River Laboratories, Sulzfeld, Germany) were housed in cages (2–3 rats/cage) with a 12/12-hour light/dark cycle, at 26°C and with food and water *ad libitum* were used for this study. Animals were randomly assigned to either the SCI group (n = 7) or the sham group (n = 5) by the random number table method. After surgery, animals were housed alone for 3 days (during wound healing), before shared living conditions (2–3 animals per cage) were restored to avoid social anxiety. Behavioral assessment was performed at baseline and then weekly until the 6th week after surgery (neurotest (N) time points 1–6 (N1–N6)). Animals were weighed at baseline, daily for the first week after surgery, every other day for the second week, and twice a week thereafter until the end of the experiment and the respective change in weight compared to the initial weight was calculated and given as a percentage:

$$\text{Weight change (\%)} = \frac{\text{Present weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

Six weeks after SCI, all animals were sacrificed and perfused and their spinal cords were extracted for histological analyses (**Figure 1**). All experiments met the requirements of "the animal protection law and animal research regulation of the Federal Republic of Germany" and were approved by the Animal Care Committee of the federal government (file reference G-68/18; approval date: May 8, 2018).

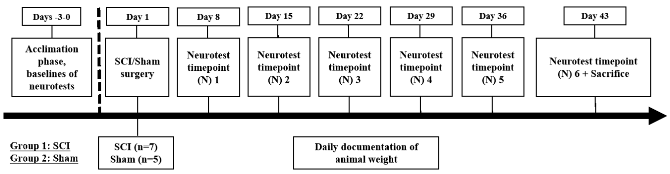

Figure 1 | A time-line diagram illustrating the study design and workflow.

Severe thoracic spinal cord injury models

Animals were anesthetized with isoflurane (3.5% induction concentration, 1.5% maintenance concentration; Baxter International, Frankfurt, Germany) on a heating pad at 36°C. After skin disinfection, the T9 level was located by palpating the T2 spinous process and the 12th rib. A T9 laminectomy was performed with a small rongeur until the T9 and T10 nerve roots were visualized exiting the spinal cord. For animals of the SCI group, a 28-g modified aneurysm clip (Fehlings Laboratory, Toronto, Canada) was slid around the spinal cord, at the level between the T9 and T10 nerve roots, snap-shot, and left on compressing the T9 spinal cord for 60 seconds as previously described (Zhang et al., 2021). After removal of the clip, the contusion/compression SCI was directly visible. For animals of the sham group, only a T9 laminectomy and exposure of the T9 and T10 nerve roots were performed. Muscle and skin layer were sutured separately, and the incision was disinfected afterwards. After surgery, all animals were subjected to an intensive postoperative care regime for 5 days which included the subcutaneous injection of buprenorphine (0.05 mg/kg; Bayer AG, Leverkusen, Germany) and meloxicam (2 mg/kg; CP-Pharma, Burgdorf, Germany) as analgesic medication on the back twice a day. An antibiotic prophylaxis (moxifloxacin, 4 mg/kg p.o., Bayer AG) was given for 7 days, and bladders were manually voided twice a day until bladder function was restored.

Behavioral assessments

All animals were adapted to the BBB open field and the CatWalk XT® automated gait analysis system (Noldus Information Technology, Wageningen, The Netherlands) before their baseline tests which then were performed 3 days before the SCI or sham operation (baseline (BL)). Both neurotests were repeated weekly at the same time of day (time points N1–N6).

For the CatWalk XT® automated gait analysis, animals were positioned at one of the ends of the horizontal glass walkway and ran spontaneously towards the other end. Both forelimbs (FL) and hindlimbs (HL) parameters of the runs were recorded. Data were integrated and analyzed with the CatWalk XT® software (version 10.5; Noldus Information Technology). To improve the quality of the analysis, animals had to perform at least three uninterrupted runs for each trial at the respective time points. A maximum run variation of 60%, a camera gain of 16.99 decibels (dB), and a detection threshold of 0.1 arbitrary units (a.u.) were set as default detection conditions for all runs. Every run was first automatically classified by the CatWalk XT® software and then manually reviewed to exclude irrelevant data such as contacts of the nose, the abdomen, or the tail with the walkway. With the CatWalk XT® software, the major parameters (the definition of these parameters is given in **Additional Table 1**) related to, e.g., gait, kinetic or coordination, were calculated for every run, and the results were averaged for every time point per animal (**Additional Tables 2–3**). In addition, to minimize the influence of body weight changes during the experiment, we calculated corrected values (H/F) of the CatWalk XT® parameters by dividing the respective absolute values of the hindlimbs (H), which are typically impaired after thoracic SCI, by the values of the unaffected forelimbs (F) (**Additional Tables 4–5**).

The BBB open field rating scale score (BBB score) was performed in all animals at the same time points (BL, N1–N6) to assess the general recovery of hind limb locomotion. The animals were placed into an open field and recorded with a camera for 4 minutes. The videos were then reviewed by two blinded observers, and hindlimb joint movement, coordination, and weight support were evaluated using a rating scale from 0 points (no movement of any kind) to 21 points (normal locomotion) (Basso et al., 1995). The results of both observers were averaged for every animal.

Tissue processing and histological assessment

All animals were euthanized with isoflurane (5%) and transcardially perfused with 50 mL 0.1M cold phosphate-buffered saline followed by 150 mL 4% paraformaldehyde. The spinal cords were extracted, cut into 2 cm pieces centered around the lesion, fixed in 4% paraformaldehyde for 24 hours and cryoprotected with 30% sucrose for 48 hours. The pieces were then embedded in tissue embedding medium (Sakura Finetek Europa B.V., Staufen, Germany) on dry ice and cut into a thickness of 30 µm with a cryostat (Leica CM3050S, Leica Biosystems, Nussloch, Germany). The obtaining spinal cord cross-sections were then stored at –80°C until further processing.

For histological assessment, immunofluorescence staining was performed. The spinal cord cross-sections were stained with the primary antibodies anti-glial fibrillary acidic protein (GFAP; MAB360, 1:1000; Millipore, Burlington, MA, USA) for astrogliosis assessment, and anti-chondroitin sulfate proteoglycan (CSPG; MAB1581, 1:400; Millipore) for CS-GAG deposition evaluation, diluted in blocking solution (5% milk powder, 1% bovine serum albumin, 0.3% Triton-X) at 4°C overnight. Alexa Fluor 568 goat anti-mouse (1:500; Invitrogen, Waltham, MA, USA) was used as the secondary antibody and applied at room temperature for 1 hour. After removal of the secondary antibody and washing with PBS, the spinal cord cross-sections were covered with mounting medium (10981, Merck KGaA, Darmstadt, Germany) and directly subjected to imaging analysis.

All images were taken with a confocal laser scanning microscope (LSM 700; Carl Zeiss Microscopy GmbH, Esslingen, Germany) and analyzed with the image processing software ImageJ (version 1.52s; National Institute of Health, Bethesda, MD, USA). For the assessment of histological changes, images of 11 spinal cord cross-sections with the following distances to the lesion epicenter were used: 0 µm (epicenter), ±240 µm, ±480 µm, ±720 µm, ±960 µm, and ±1200 µm. Regions of interest were drawn around the entire/remaining spinal cord, and the immunointensity of the staining was output and averaged per animal and treatment group to represent the extent of astrogliosis and CS-GAG deposition. In addition, the total volume (mm^3) of the post-traumatic intramedullary cyst, visible on GFAP-stained cross-sections, was calculated by multiplying the cyst area with the distance between two analyzed sections (240 µm) and averaged per animal and treatment group.

Data processing and statistical analysis

The CatWalk XT® parameters, the body weight, the immunointensity of the GFAP-staining (astrogliosis), the CSPG-staining (CS-GAG deposition), and the cyst volume are presented as the mean with 95% confidence interval (CI). The BBB scores are shown as the median with interquartile range. A two-way repeated measure analysis of variance with post hoc Tukey's HSD (honestly significant difference) test was used for the statistical comparison of neurotest results between groups and time points. Means between the sham and SCI groups in the histological assessments were compared using unpaired t-test.

To better understand the influence of weight changes on the CatWalk XT® parameters in SCI animals, they were first grouped into "parameters more related to animal weight" (including max intensity, mean intensity, max contact max intensity, max contact mean intensity, print area, and max contact print area), and "parameters less related to animal weight" (including kinematic measurements, e.g., regularity index, stride length, base of support, and swing speed). Then, their absolute and corrected (H/F) hindlimb values were correlated with the weight of the respective animals at the corresponding time points using Pearson's correlation. For statistical comparison, the resulting r values were transformed into z values using Fisher's z transformation (Fisher, 1915), the z values where then averaged for the "parameters more related to animal weight" and "parameters less related to animal weight" groups and analyzed using paired two-sample t-test. Finally, the mean z values and their 95% CI were transformed back to r values.

To assess the correlation of the different CatWalk XT® parameters with the averaged BBB scores of the time points N2–N6, and the post-traumatic histological changes (cyst formation, astrogliosis, and CS-GAG deposition) of the last time point (N6), the respective Pearson's correlation coefficients were calculated (presented as r values with 95% CI). For statistical analysis, Fisher's z transformation was performed, the resulting z values were averaged for all four correlation analyses (BBB scores, cyst formation, astrogliosis, and CS-GAG deposition) and compared using one-way analysis of variance with *post hoc* Tukey's HSD (honestly significant difference) test. Then, the mean *z* values and their 95% CI were transformed back to r values.

Normality assumption was confirmed prior to all parametric analyses using Shapiro-Wilk normality tests and a *P*-value of $P < 0.05$ was considered significant. Depending on the respective absolute values of the *r* value, the correlation between the variables was classified as negligible (0.00–0.30), low (0.30–0.50), moderate (0.50–0.70), strong (0.70–0.90) or very strong (0.90–1.00) (Hinkle et al., 2003). All statistical analyses were performed using the software GraphPad Prism (version 7.0; GraphPad, San Diego, CA, USA).

Results

BBB open field rating scale scores

The BBB score was used to evaluate the general locomotor recovery of the hindlimbs. For all baseline tests and all neurotest time points of the sham group, the maximum of 21 points was achieved (**Figure 2**). However, in the SCI group, the BBB score significantly dropped from 21 points at baseline to 0 (0, 0) points ($P < 0.001$) 1 week after injury. During the natural course of recovery, the BBB score significantly increased 4 weeks after injury (3.5 [2.0, 3.5] points; N1 *vs.* N4; $P < 0.001$), further increased to 5.5 (4.0, 5.5) points (N1 *vs.* N5; $P < 0.001$) after 5 weeks and reached a peak of 6.5 (6.0, 7.0) points at the end of the experiment (N1 *vs.* N6; $P < 0.001$). These results indicated a gradually spontaneous recovery of the general motor function of the hindlimbs after severe thoracic contusion/compression SCI. Nevertheless, no SCI animal was able to bear its own weight, corresponding to a BBB score of 9 points, during 6 weeks of the experiment.

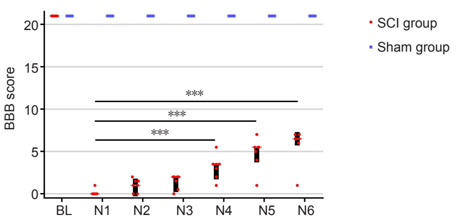

Figure 2 | Weekly BBB open field rating scale scores of the SCI and sham animals from baseline (BL) until the end of the experiment (N6).
A steep decrease of the BBB score from baseline to N1 could be observed. During the following weeks, the BBB scores gradually increased and reached a significant improvement compared to the first post-injury value after 4 weeks and thereafter (N1 *vs.* N4; N1 *vs.* N5; N1 *vs.* N6; $***P < 0.001$ each; two-way repeated measure analysis of variance followed by post hoc Tukey's HSD (honestly significant difference) test). The data are shown as the median with interquartile range (IQR). *n* = 7 in SCI and *n* = 5 in sham, respectively. BBB: Basso, Beattie, and Bresnahan; SCI: spinal cord injury. N1–N6: 1, 8, 15, 22, 29, 36, and 43 days after injury.

CatWalk XT® automated gait analysis results

During the first behavioral assessment 7 days after injury (N1), none of the animals in the SCI group were able to finish a compliant CatWalk XT® run due to their inability to fully support their weight. Therefore, this time point was not included in the statistical analysis.

In contrast to previous reports in a cervical clip compression/contusion SCI model, dynamic CatWalk XT® parameters, such as the regularity index, stride length (H/F) and swing speed (H/F), indicated insignificant spontaneous recovery (**Figure 3A–D**; **Additional Tables 2** and **3** for absolute and **Additional Tables 4** and **5** for corrected values) after the thoracic compression/contusion injury. Only the dynamic parameter base of support (H/F) showed significant spontaneous improvement compared with the first available post-injury value (N2) at the end of the experiment ($P < 0.001$; **Figure 3B**; **Additional Tables 2** and **3** for absolute and **Additional Tables 4** and **5** for corrected values). However, the paw intensity parameters, which directly represent the pressure of the hindlimbs on the runway, displayed an ability to elaborate differences in spontaneous recovery already in earlier post-injury stages from 4 weeks after SCI onwards (**Figure 3E–H**): In particular, a significant increase in stepping pressure compared with the first available post-injury value (N2) and thus measurable spontaneous recovery could be observed for the parameters max intensity (H/F) ($P < 0.001$), mean intensity (H/F) ($P < 0.001$), max intensity at % (H/F) ($P < 0.001$), and max contact mean intensity (H/F) ($P < 0.001$), starting from timepoint N4 and lasting until the end of the experiment (**Additional Table 2** for absolute and **Additional Table 4** for corrected values). CatWalk XT® automated gait analysis results of animals in the sham group are shown in **Additional Tables 3** and **5**.

Post-traumatic cyst-formation, astrogliosis and CS-GAG deposition

The volume of a post-traumatic intramedullary cyst and the extent of post-traumatic astrogliosis were assessed on GFAP-stained spinal cord cross sections 6 weeks after injury (**Figure 4A**), while CSPG-stained sections were used to quantify the extent of post-traumatic CS-GAG deposition (**Figure 4B**). Intramedullary cysts could only be observed in SCI animals with a mean volume of 12.5 (7.61, 17.3) mm^3, and no cyst-formation was present in the sham group.

The average immunointensity of CSPG was higher in the SCI group than in the sham group (38.3 [28.3, 48.4] *vs.* 27.9 [26.4, 29.4]; $P = 0.061$), without reaching statistical significance. However, the average immunointensity of GFAP and thus the astrogliosis was significantly higher in the SCI group compared with the sham group (23.5 [19.4, 27.6] *vs.* 16 [10.5, 21.5]; $P = 0.016$; **Figure 4C**).

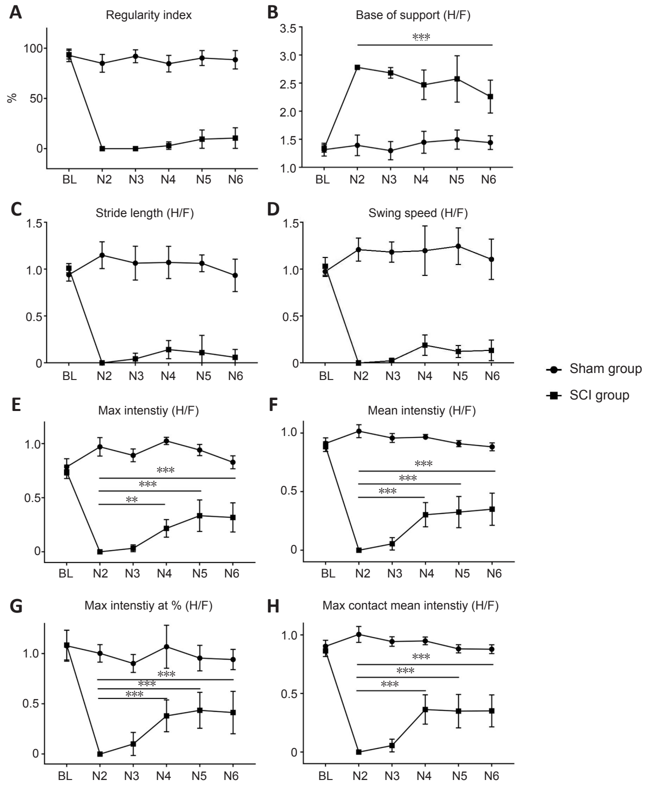

Figure 3 | The CatWalk XT® automated gait analysis parameters regularity index (A), base of support (H/F) (B), stride length (H/F) (C), swing speed (H/F) (D), max intensity (H/F) (E), mean intensity (H/F) (F), max intensity at % (H/F) (G), and max contact mean intensity (H/F) (H) are displayed for animals in the SCI and sham groups from baseline (BL) until the end of the experiment (N6).
In the SCI group, a similar pattern of severe deterioration from the baseline values after the injury could be observed in all presented CatWalk XT® parameters. Spontaneous recovery, however, was only apparent in the dynamic parameter base of support (N2 *vs.* N6; $***P < 0.001$) whereas all print parameters indicated significant improvements from week 4 after the injury (N4) onwards (N2 *vs.* N4; $**P < 0.01$ and N2 *vs.* N5 and N2 *vs.* N6; $***P < 0.001$ each; two-way repeated measure analysis of variance followed by post hoc Tukey's HSD (honestly significant difference) test). Data are presented as the mean with 95% confidence interval (CI). *n* = 7 in SCI and *n* = 5 in sham, respectively. H/F: corrected hindlimb (H) values (divided by the unaffected forelimb (F) values); SCI: spinal cord injury. N1–N6: 1, 8, 15, 22, 29, 36, and 43 days after injury.

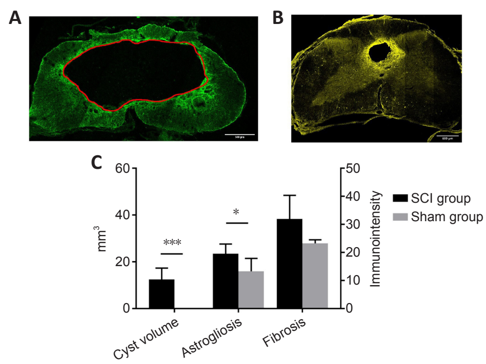

Figure 4 | Histological changes after SCI.
(A) Glial fibrillary acidic protein-stained (green) spinal cord cross-section of an SCI animal six weeks after the injury. The intramedullary cyst is framed with a red line. Scale bar: 500 µm. (B) Chondroitin sulfate proteoglycan-stained (yellow) spinal cord cross-section of an animal in the sham group six weeks after the injury. Scale bar: 500 µm. (C) The estimated cyst volume, immunointensity of astrogliosis and chondroitin sulfate glycosaminoglycans are depicted for the SCI (*n* = 7) and sham (*n* = 5) group 6 weeks after injury. A significantly increased cyst volume ($***P < 0.01$) and astrogliosis ($*P < 0.05$) can be observed in SCI animals (unpaired *t*-test). Data are presented as the mean with 95% confidence interval (CI). SCI: spinal cord injury.

Effects of weight changes on the CatWalk XT® parameters

Because the CatWalk XT® paw intensity parameters directly measure the pressure of each paw on the runway, we determined whether the weight change of the animals would affect the efficacy of the CatWalk XT® to describe spontaneous recovery after thoracic contusion/compression SCI.

The rate of weight change compared to baseline showed a sharp decrease right after the surgery in both SCI and sham animals, with the lowest values being –15.4% (–19.4%, –11.3%) after 5 days and –12.7% (–17.7%, –7.74%) after 2 days, respectively (**Figure 5A**). However, the weight of the sham animals gradually recovered to the baseline level approximately 2 weeks after laminectomy, whereas it took the SCI animal almost 4 weeks to reach baseline levels and thus recover from injury. After restoring the baseline value, the animal weights further increased and were highest 42 days after injury (23.53% [21.5%, 25.6%] in the sham group and 10.1% [3.68%, 16.5%] in the SCI group). In the SCI group, the largest change of weight in one specific animal was 40.08% (from –18.76% to 21.32%) of the baseline weight and the smallest change of weight was 24.87% (from –13.76% to 11.11%).

Because these differences in weight change could potentially bias the different CatWalk XT® parameters after SCI, the rat parameters were grouped into "parameters more related to animal weight" and "parameters less related to animal weight". We then performed a correlation analysis with the animals' weight, using both, their corrected (H/F) but also absolute values and, after z transformation, compared the mean results of both groups, respectively. For parameters "more related to animal weight", the correlation of the animal weight with the absolute hindlimb values was significantly stronger than with the corrected (H/F) hindlimb values (r = 0.50 [0.39, 0.59] *vs*. r = 0.36 [0.29, 0.43]; P = 0.0012; **Figure 5B**). For parameters "less related to animal weight", the correlation of the animal weight with the absolute hindlimb values was also stronger than with the corrected (H/F) hindlimb values (r = 0.29 [0.20, 0.37] *vs*. r = 0.21 [0.15, 0.27]), without reaching a statistical significance (P = 0.073; **Figure 5C**). These results indicated that CatWalk XT® parameters more related to animal weight, specifically the print intensity parameters, might be prone to additional bias due to the pronounced weight loss after the clip contusion/compression SCI. Their absolute values should thus be interpreted with caution.

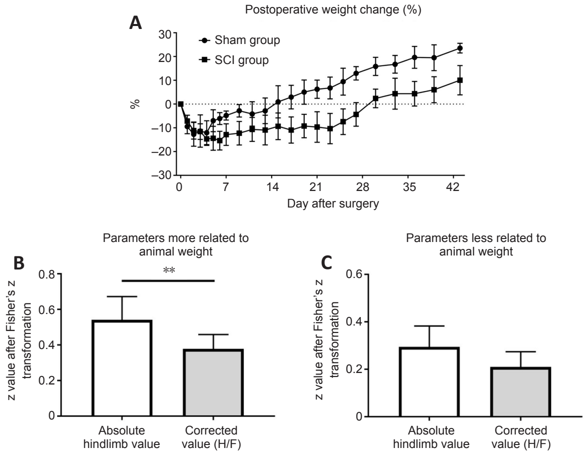

Figure 5 | Postoperative weight change and its effect on the CatWalk XT® parameters.

Percentage of weight change from the baseline (dotted line; day 0 after surgery) until the end of the experiment (day 42 after surgery) for animals in the SCI (n = 7) and sham (n = 5) groups (A). The mean correlation between the animals' weights and the absolute hindlimb values is significantly higher than between the animals' weights and the corrected values (H/F) for CatWalk XT® "parameters more related to animal weight" (B), but not for CatWalk XT® "parameters less related to animal weight" (C). (**P < 0.01; unpaired t-test). Data are presented as the mean with 95% confidence interval (CI). H/F: Corrected hindlimb (H) values (divided by the unaffected forelimb (F) values); SCI: spinal cord injury.

Correlation of the CatWalk XT® parameters with the BBB scores and histological post-injury changes

To verify the consistency of the corrected CatWalk XT® parameters (**Additional Tables 4** and **5**) in evaluating spontaneous recovery after a severe thoracic contusion/compression SCI, the correlation of those CatWalk XT® parameters with the BBB scores and the post-traumatic histological changes was examined.

Pearson correlation coefficients for the BBB scores and the corrected values (H/F) of the CatWalk XT® parameters for time points N2 to N6 in all SCI animals were calculated (**Figure 6**). The CatWalk XT® parameter max intensity showed the highest correlation with the BBB scores over the duration of the experiment (r = 0.869 [0.732, 0.938]), followed by print length (r = 0.843 [0.684, 0.926]), print width (r = 0.827 [0.655, 0.918]), and mean intensity (r = 0.775 [0.562, 0.891]). On the other hand, the kinematic parameters regularity index (r= 0.35 [–0.03, 0.642]), swing speed r = 0.482 [0.129, 0.726]), and stride length (r = 0.586 [0.268, 0.788]) were less correlated with the BBB scores after the thoracic compression/contusion SCI. These findings suggested at least a comparable ability of the CatWalk XT® gait analysis to the BBB rating scale score in describing the spontaneous recovery of the hindlimbs after a severe thoracic contusion/compression SCI, with a better performance of paw intensity parameters.

Pearson correlation coefficients were calculated for the correlation between corrected CatWalk XT® parameters and the post-traumatic histological changes cyst volume, astrogliosis and CS-GAG deposition as well (**Figure 6**). However, because the histological analyses were performed after the end of the experiment, this was only possible for the last neurotest timepoint (N6). The mean correlation between all CatWalk XT® parameters and the cyst volume was r = 0.799 (0.753, 0.837). The highest r value was found for the parameter mean intensity (0.872 [0.434, 0.977]), while the lowest r value belonged to the parameter max contact at % (0.578 [–0.214, 0.911]). As for the correlation of the CatWalk XT® parameters and the astrogliosis, the mean r value was 0.841 [0.783, 0.883], ranging from 0.663 [–0.08, 0.932] (max contact at %) to 0.951 [0.567, 0.984] (regularity index). The pattern of the correlation between the CatWalk XT® parameters and the CS-GAG deposition was similar to the one of the cyst volume, with an overall correlation coefficient of 0.78 [0.724, 0.826], ranging from 0.59 [–0.196, 0.915] (swing speed) to 0.908 [0.563, 0.983] (max intensity). When compared to the average correlation (r value) between the CatWalk XT® parameters and the BBB scores at the time point N6 (0.703 [0.613, 0.776]), the correlation between the CatWalk XT® gait analysis and the post-traumatic histological changes cyst formation (P = 0.024), astrogliosis (P = 0.037) and CS-GAG deposition (P = 0.013) was found to be significantly stronger. These findings suggested a close relationship between the CatWalk XT® gait analysis and histological post-injury changes, highlighting the relevance of this behavioral assessment to evaluate the spontaneous recovery of the hindlimbs after severe thoracic SCI.

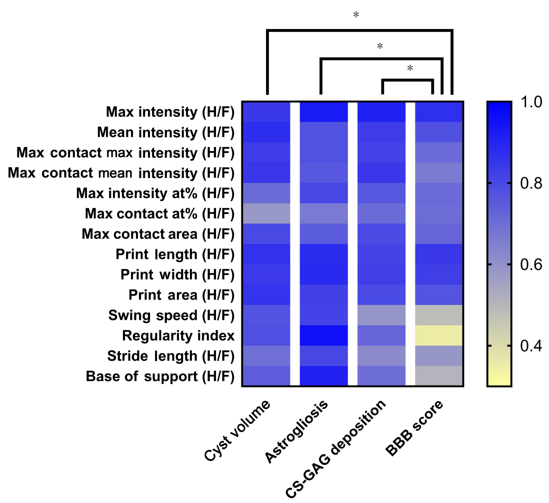

Figure 6 | Heat map of the correlation between the CatWalk XT® parameters and the Basso, Beattie, and Bresnahan (BBB) open field rating scale scores or post-injury histological changes such as cyst volume, astrogliosis and chondroitin sulfate glycosaminoglycans.

The value of the correlation coefficient r is represented by the color scale on the right (between 0–1.0). Data are presented as the mean with 95% confidence interval (CI). H/F: Corrected hindlimb (H) values (divided by the unaffected forelimb (F) values).

Discussion

In our current study, we aimed to assess the consistency of the CatWalk XT® gait analysis system in a clinically relevant SCI model. Such an injury model should include a sudden contusion after the trauma and a lasting compression, like what is observed after spinal trauma in human patients due to, e.g., a burst fracture or a dislocation of the spine (Badhiwala et al., 2021). Furthermore, the preservation of reticulospinal, vestibulospinal (lateral to ventrolateral white matter) and the rubrospinal tracts (dorsolateral white matter) is highly relevant for the functional recovery in human patients, and injury models involving sharp cuts such as the dorsal or unilateral hemisection models should thus be avoided (Asboth et al., 2018; Younsi et al., 2020). Therefore, a contusion/compression injury model such as the one used in our experiment is desirable and better reflects the clinical reality of human SCI patients.

When compared to previous study on a severe cervical contusion/compression SCI with the same clip snap contusion force (28 g) and duration of compression (1 minute) (Zheng et al., 2021), the thoracic injury used in our current experiment led to a more pronounced disability of the hindlimbs throughout the process of spontaneous recovery as measured by the BBB score and CatWalk XT® gait analysis: Hardly any animal could regain the ability to bear their own weight 6 weeks after injury. It was reported that after a complete spinal transection, rodents can still regain part of the hindlimb locomotion because the spinal cord below the lesion level can circuit through the central pattern generator, which located in the lower thoracic and lumbar region (Rossignol et al., 2009; Guertin, 2013). Considering the activity of the central pattern generator in the spontaneous recovery after SCI, Li et al. (2017) suggested that a lesion closer to the T12 vertebra could have more impact on the neuronal regeneration. Moreover, when exposed to a dorsal hemisection SCI at the C4 or the T7 level, the sensitivity of different CatWalk XT® parameters, along with their time of onset and duration varied between cervical and thoracic SCI models. For instance, print-width was significantly impaired by a cervical injury only, while base of support was sensitive towards both injury levels but remained affected for a longer time after a T7 SCI (Fagoe et al., 2016).

Previous studies have also raised such concern that the weight of used experimental animals could bias the measurement of different CatWalk XT® parameters (Koopamns et al., 2007; Gabriel et al., 2009). Parkkinen et al. (2013) found in their cerebral ischemia rat model that several parameters, such as intensity and maximum contact area, were more independent on the change of the body weight during the process of observation, while dynamic parameters were less influenced. To address this issue in a model of severe thoracic SCI, we investigated the corresponding changes of the CatWalk XT® parameters in dependency of the weight changes of the animals during a 6-week-long observation. As an example, the correlation between "hindlimb max contact max intensity" and the absolute weight was strong, similar to several other print parameters, such as "max intensity" and "mean intensity", which showed at least a medium correlation (r values between 0.4–0.6). Correlation between the kinematic CatWalk XT® parameters and the weight, however, mostly was poor (*r* values below 0.4).

To further assess the dependency of the CatWalk XT® parameters on the body weight, we categorized them into two groups ("parameters more related to animal weight" and "parameters less related to animal weight"). As a peculiarity of the thoracic SCI which, in contrast to experimental models of cervical SCI or traumatic brain injury, only affects the hindlimbs, we were able to calculate "corrected" values for the CatWalk XT® parameters in our present study: We divided the affected hindlimb CatWalk XT® parameters by the ones of the unaffected forelimbs, thus minimizing the impact of the weight change on the CatWalk XT® assessment. As a result, the group of "parameters more related to animal weight" showed a significantly lower correlation with the body weight, suggesting that the potential bias caused by the weight change throughout the duration of the experiment could be improved by using the forelimb measurement as an internal control. However, this method is not applicable for kinetic and coordination CatWalk XT® parameters (e.g., "regularity index" or "stride length").

In our previous study on the efficacy of various CatWalk XT® parameters in describing spontaneous recovery after the clip compression/contusion SCI on the C6 level, we introduced a correlation analysis with histological post-injury changes (Zheng et al., 2021). For the cervical SCI, we found that the CatWalk XT® parameters had a generally high correlation with the BBB score, and especially with the percentage of preserved tissue. In our current study, we further extended the correlation analysis by two more histological post-injury changes: astrogliosis, characterized by GFAP-immunointensity, and CS-GAG deposition, characterized by CSPG-immunointensity. Furthermore, for the statistical analysis, we chose Spearman and Pearson correlation instead of a linear regression which was used to evaluate the correlation among variables in previous studies (Crowley et al., 2018; Aceves et al., 2020). For the thoracic SCI, we found a strong correlation ($r > 0.6$) between various major CatWalk XT® parameters and the BBB scores as well as the histological assessments. More importantly, the CatWalk XT® parameters tested in our study seemed to be more strongly correlated with astrogliosis and CS-GAG than with the BBB scores. Specifically, the CatWalk XT® parameters regularity index, stride length, base of support, and swing speed showed a medium or even poor correlation with the BBB scores, supporting the notion that the BBB open field rating scale is a rather subjective assessment. Our hypothesis, therefore, is that due to the severe thoracic SCI (represented by maximum BBB scores < 8 throughout the experiment), the BBB open field rating scale was not able to differentiate slight changes in the recovery of coordination or even more general locomotion, which on the other hand was doable with CatWalk XT® gait analysis. Furthermore, our results suggest that static print parameters (intensity or size measurement of the prints) could be a more stable and safe option to assess the functional recovery in animals that undergo a severe thoracic compression/contusion SCI.

Unlike human patients, animals show an alarming variation in behavioral assessment results after experimental SCI, which also affects the CatWalk XT® gait analysis and could strongly affect the outcome evaluation of preclinical studies. Aceves et al. (2020) reported a reference change value of up to 136.6% for the stance duration in a cervical SCI model, while other parameters, such as base of support, stride length, and swing duration/speed had relatively lower reference change values (about 30%). Hence, they proposed that more "incompliant" CatWalk XT® runs with high reference change values should be taken into consideration, especially during the early phase after the injury. Timotius et al. (2021) published their findings on the best combination of CatWalk XT® parameters to predict locomotion recovery in 2021. In their study, a linear discriminant analysis of nine ranking gait parameters between uninjured and SCI rats was conducted, in which the parameter-weights of the linear combination were given based on an unbiased machine learning method. Although the power of linear discriminant analysis was weakened in a cervical SCI model and in the early stage after injury, it still showed a significant sensitivity while assessing the recovery process of thoracic SCI models, regardless of multiple variables, including sex, strain, weight, or different injury types (Timotius et al., 2021).

Our current study has several limitations that must be noted: First, since the introduction of the BBB open field score system (Basso et al., 1995), most researchers including Basso et al. themselves, have been regarding the BBB open field scale scoring system as parametric. However, the meaning of the individual, non-integer BBB scores is undeniably hard to interpret by this definition. Hence, whether the BBB open field scale is parametric or non-parametric remains debatable. Because of the limited number of animals in our experiment, it was not possible to analyze the BBB scores with chi-square tests which would have been required for a non-parametric assumption. As a compromise, we decided to regard the BBB open field scale as parametric system but to present its results with median and interquartile range values. Second, the SCI had deprived all animals to bear their own weight until the end of the experiment and thus resulted in difficulties classifying and distinguishing the hindlimb CatWalk XT® parameters during spontaneous recovery. Nevertheless, we observed that although totally paralyzed in the hindlimbs, starting from the second week after the injury, the SCI animals were able to finish three CatWalk XT® runs, dragging their lower body part around and leaving big contact signals in the acquisition videos. These were labeled as "abdomen" and later discarded from the analysis so that the system was still capable of detecting the exerted pressure of the hindlimbs.

In conclusion, our results indicate that the CatWalk XT® gait analysis could be regarded as an objective and consistent tool to assess the impairment and recovery of locomotor function after thoracic contusion/compression SCI in rats. The weight gain of the animals has an impact on the evaluation of the CatWalk XT® parameters, however, it can be attenuated by using the corrected values (H/F). The CatWalk XT® parameters we used, had a good correlation with the BBB scores, and an even better correlation with the histological assessment of astrogliosis, cyst formation, and CS-GAG deposition. Among all the CatWalk XT® parameters, static parameters, such as paw intensity and paw size measurement, seemed more sensitive to our severe thoracic SCI model in rats than dynamic parameters, such as swing speed or stride length.

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Additional files:
Additional Table 1: *Description of the used CatWalk XT® parameters.*
Additional Table 2: *CatWalk XT® automated gait analysis parameters (absolute values from hindlimbs) of animals in the SCI group assessing motor function for all available time points.*
Additional Table 3: *CatWalk XT® automated gait analysis parameters (absolute values from hindlimbs) of animals in the sham group assessing motor function for all available time points.*
Additional Table 4: *CatWalk XT® automated gait analysis parameters (corrected values from hindlimbs) of animals in the SCI group assessing motor function for all available time points.*
Additional Table 5: *CatWalk XT® automated gait analysis parameters (corrected values from hindlimbs) of animals in the sham group assessing motor function for all available time points.*

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Additional Table 1 Description of the used CatWalk XT® parameters

Parameter	Description
Regularity index (%)	Describes the exclusive use of normal step sequence patterns during uninterrupted locomotion.
Average speed (cm/s)	Measures the average speed of the recorded run.
Base of support (cm)	Describes the average width between the hind paws.
Stride length (cm)	Measures the distance between successive placements of the same paw.
Swing speed (cm/s)	Denotes the speed of the paw during a swing.
Max intensity	Describes is the maximum intensity of a complete paw.
Mean intensity	Measures the mean intensity of a complete paw.
Mean intensity of most 15 prints	Is defined as the mean intensity of the 15 pixels with the highest intensity of a paw.
Max contact max intensity	Measures the maximum intensity of a paw at maximum contact.
Max contact mean intensity	Describes the mean intensity of a paw at maximum contact.
Max intensity at % (%)	Max intensity at s is the time in seconds since the start of a run that the maximum intensity is measured. Max intensity at % is max intensity at s relative to the stand of a paw.
Print length (cm)	Describes the length (horizontal direction) of the complete paw print.
Print width (cm)	Denotes the width (vertical direction) of the complete paw print.
Print area (cm^2)	Measures the surface area of the complete paw print.
Max contact area (cm^2)	Is defined as the maximum area of a paw that comes into contact with the glass plate.
Max contact at %	Max contact at s is the time in seconds since the start of a that a paw makes maximum contact with the glass plate. Max contact at % is max contact at s relative to the stand of a paw.
Body speed (cm/s)	Is calculated by dividing the distance that the animal's body traveled from one initial contact of a paw to the next by the time to travel that distance.
Step cycle (s)	Describes the time in seconds between two consecutive initial contacts of the same paw.
Duty cycle (%)	Expresses the stand as a percentage of the step cycle.

Additional Table 2 CatWalk XT® automated gait analysis parameters (absolute values from hindlimbs) of animals in the SCI group assessing motor function for all available time points

CatWalk XT® parameters	BL	N2	N3	N4	N5	N6
Regularity index (%)	93.61 (89.32, 97.90)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	3.07 (-0.62, 6.76)	9.50 (0.39, 18.62)	10.60 (0.32, 20.87)
Average speed (cm/s)	27.31 (23.80, 30.81)	0.00 (0.00, 0.00)	8.36 (2.33, 14.39)	14.18 (9.85, 18.52)	16.88 (12.12, 21.64)	11.81 (8.12, 15.50)
Base of support (cm)	2.62 (2.44, 2.79)	7.00 (7.00, 7.00)	7.00 (6.99, 7.00)	5.90 (4.94, 6.85)	5.58 (4.67, 6.49)	5.88 (5.11, 6.65)
Stride length (cm)	11.06 (10.35, 11.77)	0.00 (0.00, 0.00)	0.94 (-0.22, 2.10)	2.32 (1.15, 3.50)	3.42 (1.44, 5.40)	3.28 (0.78, 5.77)
Swing speed (cm/s)	77.70 (72.33, 83.07)	0.00 (0.00, 0.00)	3.30 (-0.48, 7.08)	10.66 (4.63, 16.69)	10.22 (4.80, 15.64)	12.99 (4.48, 21.51)
Max intensity	107.91 (97.30, 118.52)	0.00 (0.00, 0.00)	7.18 (-0.04, 14.39)	36.78 (23.70, 49.86)	38.20 (17.43, 58.97)	51.34 (27.11, 75.58)
Mean intensity	58.84 (56.64, 61.04)	0.00 (0.00, 0.00)	5.18 (0.13, 10.23)	19.59 (12.52, 26.67)	16.65 (7.37, 25.94)	20.02 (9.09, 30.94)
Mean intensity of most 15 prints	90.20 (81.50, 98.91)	0.00 (0.00, 0.00)	5.64 (0.10, 11.17)	29.42 (19.11, 39.73)	34.62 (16.30, 52.94)	50.51 (28.76, 72.27)
Max contact max intensity	100.02 (90.80, 109.24)	0.00 (0.00, 0.00)	6.63 (-0.01, 13.26)	32.15 (15.02, 49.27)	40.35 (18.99, 61.71)	40.80 (21.06, 60.53)
Max contact mean intensity	56.79 (54.45, 59.13)	0.00 (0.00, 0.00)	4.98 (0.12, 9.84)	14.62 (6.34, 22.91)	17.65 (10.91, 24.39)	17.84 (8.93, 26.74)
Max intensity at % (%)	65.54 (60.58, 70.50)	0.00 (0.00, 0.00)	3.64 (-0.52, 7.80)	15.47 (6.50, 24.44)	16.15 (9.09, 23.22)	16.30 (8.54, 24.05)
Print length (cm)	1.48 (1.26, 1.70)	0.00 (0.00, 0.00)	0.05 (0.00, 0.10)	0.48 (0.23, 0.74)	0.51 (0.19, 0.84)	0.64 (0.36, 0.91)
Print width (cm)	1.46 (1.31, 1.62)	0.00 (0.00, 0.00)	0.05 (0.00, 0.11)	0.41 (0.20, 0.61)	0.51 (0.23, 0.79)	0.52 (0.30, 0.73)
Print area (cm^2)	1.11 (0.96, 1.26)	0.00 (0.00, 0.00)	0.01 (0.00, 0.03)	0.28 (0.09, 0.47)	0.42 (0.15, 0.70)	0.41 (0.21, 0.62)
Max contact area (cm^2)	0.59 (0.48, 0.70)	0.00 (0.00, 0.00)	0.01 (0.00, 0.03)	0.30 (0.14, 0.45)	0.35 (0.14, 0.56)	0.35 (0.14, 0.56)
Max contact at % (%)	33.63 (29.28, 37.98)	0.00 (0.00, 0.00)	1.64 (-0.35, 3.63)	8.98 (4.48, 13.49)	10.75 (5.48, 16.02)	11.57 (5.54, 17.60)
Body speed (cm/s)	27.19 (23.77, 30.60)	0.00 (0.00, 0.00)	2.90 (0.07, 5.74)	5.25 (2.78, 7.72)	5.05 (2.40, 7.70)	4.83 (2.45, 7.20)
Step cycle (s)	0.41 (0.37, 0.46)	0.00 (0.00, 0.00)	0.02 (0.00, 0.05)	0.40 (-0.04, 0.84)	0.55 (0.21, 0.88)	0.29 (0.01, 0.57)
Duty cycle (%)	61.47 (58.30, 64.64)	0.00 (0.00, 0.00)	1.91 (-0.37, 4.19)	12.86 (6.26, 19.46)	11.38 (5.35, 17.41)	13.46 (4.07, 22.85)

Values are shown as mean with 95% confidence interval. BL = baseline test; Nx = neurotest timepoint x (in weeks) after spinal cord injury (SCI).

Additional Table 3 **CatWalk XT® automated gait analysis parameters (absolute values from hindlimbs) of animals in the sham group assessing motor function for all available time points**

CatWalk XT® parameters	BL	N2	N3	N4	N5	N6
Regularity index (%)	92.98 (86.72, 99.23)	85.02 (76.18, 93.87)	92.05 (85.67, 98.43)	84.73 (76.47, 92.98)	90.31 (82.83, 97.80)	88.61 (79.44, 97.79)
Average speed (cm/s)	28.13 (24.20, 32.07)	20.03 (13.12, 26.93)	24.61 (19.21, 30.00)	11.94 (7.55, 16.33)	22.16 (13.77, 30.54)	21.63 (15.22, 28.04)
Base of support (cm)	2.66 (2.48, 2.83)	2.58 (2.31, 2.85)	2.46 (2.25, 2.66)	2.80 (2.47, 3.14)	2.69 (2.47, 2.91)	2.69 (2.47, 2.91)
Stride length (cm)	11.14 (10.15, 12.14)	11.27 (9.34, 13.20)	11.95 (10.88, 13.01)	12.27 (10.59, 13.96)	12.02 (10.05, 14.00)	11.68 (9.97, 13.40)
Swing speed (cm/s)	79.32 (70.22, 88.42)	80.67 (69.60, 91.74)	81.39 (76.07, 86.71)	88.73 (77.65, 99.81)	86.33 (71.69, 100.98)	83.75 (75.69, 91.82)
Max intensity	97.14 (80.79, 113.50)	142.54 (126.34, 158.75)	152.69 (140.13, 165.24)	148.40 (135.23, 161.57)	178.23 (167.89, 188.57)	201.59 (195.00, 208.17)
Mean intensity	54.73 (50.63, 58.82)	65.95 (62.11, 69.79)	68.42 (66.09, 70.75)	67.62 (64.64, 70.60)	73.40 (71.25, 75.54)	86.28 (82.66, 89.91)
Mean intensity of most 15 prints	95.54 (74.79, 116.30)	110.65 (95.85, 125.46)	129.76 (119.32, 140.21)	128.24 (116.83, 139.65)	156.47 (146.72, 166.21)	181.69 (173.19, 190.19)
Max contact max intensity	101.96 (83.63, 120.30)	136.80 (126.10, 147.50)	130.44 (113.74, 147.14)	155.16 (149.51, 160.81)	160.13 (150.26, 170.01)	167.59 (156.01, 179.17)
Max contact mean intensity	59.06 (54.14, 63.98)	64.75 (62.35, 67.16)	62.43 (58.49, 66.36)	64.27 (60.78, 67.76)	68.56 (66.49, 70.62)	76.33 (73.41, 79.25)
Max intensity at % (%)	65.82 (59.72, 68.13)	63.93 (59.72, 68.13)	58.20 (51.99, 64.42)	62.19 (56.06, 68.32)	59.59 (51.67, 67.50)	59.80 (53.45, 66.16)
Print length (cm)	1.54 (1.32, 1.75)	1.86 (1.72, 2.00)	1.93 (1.74, 2.11)	2.41 (2.28, 2.54)	2.20 (2.05, 2.35)	1.96 (1.81, 2.11)
Print width (cm)	1.48 (1.27, 1.69)	1.89 (1.75, 2.02)	1.74 (1.58, 1.90)	2.16 (2.08, 2.25)	2.01 (1.90, 2.21)	1.77 (1.65, 1.89)
Print area (cm^2)	1.07 (0.83, 1.31)	1.42 (1.16, 1.68)	1.43 (1.11, 1.76)	1.76 (1.63, 1.89)	1.55 (1.35, 1.75)	1.38 (1.20, 1.57)
Max contact area (cm^2)	0.63 (0.46, 0.80)	0.99 (0.80, 1.18)	0.99 (0.75, 1.22)	1.39 (1.08, 1.70)	1.43 (1.24, 1.62)	1.03 (0.92, 1.13)
Max contact at % (%)	36.65 (31.36, 41.94)	37.84 (31.31, 44.37)	38.16 (33.41, 42.90)	33.69 (28.79, 38.59)	37.05 (32.38, 41.72)	33.19 (25.90, 40.49)
Body speed (cm/s)	28.23 (24.26, 32.20)	25.18 (19.39, 30.96)	25.66 (20.86, 30.46)	19.66 (16.20, 23.12)	22.38 (14.95, 29.82)	22.60 (16.50, 28.71)
Step cycle (s)	0.47 (0.43, 0.51)	0.66 (0.29, 1.04)	0.59 (0.47, 0.70)	0.60 (0.40, 0.80)	0.68 (0.49, 0.87)	0.63 (0.57, 0.69)
Duty cycle (%)	60.97 (58.25, 63.70)	71.95 (66.39, 77.52)	68.54 (64.02, 73.07)	73.11 (67.38, 78.85)	70.58 (64.57, 76.59)	65.34 (58.72, 71.97)

Values are shown as mean with 95% confidence interval. BL = baseline test; Nx = neurotest time point x (in weeks) after spinal cord injury (SCI).

Additional Table 4 CatWalk XT® automated gait analysis parameters (corrected values from hindlimbs) of animals in the SCI group assessing motor function for all available time points

CatWalk XT® parameters	BL	N2	N3	N4	N5	N6
Regularity index (%)	93.61 (89.32, 97.90)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	3.07 (-0.62, 6.76)	9.50 (0.39, 18.62)	10.60 (0.32, 20.87)
Average speed (cm/s)	27.31 (23.80, 30.81)	0.00 (0.00, 0.00)	8.36 (2.33, 14.39)	14.18 (9.85, 18.52)	16.88 (12.12, 21.64)	11.81 (8.12, 15.50)
Base of support (H/F)	1.34 (1.27, 1.40)	2.78 (2.78, 2.79)	2.68 (2.59, 2.78)	2.47 (2.21, 2.73)	2.57 (2.16, 2.99)	2.26 (1.97, 2.55)
Stride length (H/F)	1.01 (0.96, 1.06)	0.00 (0.00, 0.00)	0.04 (-0.02, 0.10)	0.14 (0.04, 0.24)	0.11 (-0.08, 0.29)	0.06 (-0.03, 0.14)
Swing speed (H/F)	1.03 (0.94, 1.13)	0.00 (0.00, 0.00)	0.02 (0.00, 0.05)	0.19 (0.08, 0.30)	0.12 (0.06, 0.19)	0.14 (0.03, 0.25)
Max intensity (H/F)	0.74 (0.68, 0.80)	0.00 (0.00, 0.00)	0.03 (0.00, 0.06)	0.22 (0.14, 0.30)	0.33 (0.19, 0.48)	0.32 (0.18, 0.45)
Mean intensity (H/F)	0.88 (0.84, 0.93)	0.00 (0.00, 0.00)	0.05 (0.00, 0.11)	0.30 (0.20, 0.41)	0.33 (0.19, 0.41)	0.35 (0.21, 0.49)
Mean intensity of most 15 prints (H/F)	0.79 (0.73, 0.84)	0.00 (0.00, 0.00)	0.03 (0.00, 0.06)	0.21 (0.13, 0.29)	0.30 (0.16, 0.43)	0.30 (0.18, 0.43)
Max contact max intensity (H/F)	0.73 (0.66, 0.80)	0.00 (0.00, 0.00)	0.03 (0.00, 0.06)	0.27 (0.15, 0.39)	0.33 (0.19, 0.48)	0.31 (0.18, 0.43)
Max contact mean intensity (H/F)	0.86 (0.82, 0.91)	0.00 (0.00, 0.00)	0.06 (0.00, 0.11)	0.36 (0.24, 0.49)	0.35 (0.21, 0.49)	0.35 (0.22, 0.49)
Max intensity at % (H/F)	1.09 (0.94, 1.23)	0.00 (0.00, 0.00)	0.10 (-0.01, 0.22)	0.38 (0.22, 0.54)	0.44 (0.26, 0.61)	0.41 (0.20, 0.62)
Print length (H/F)	0.83 (0.73, 0.93)	0.00 (0.00, 0.00)	0.02 (0.00, 0.04)	0.26 (0.13, 0.39)	0.25 (0.11, 0.39)	0.29 (0.16, 0.41)
Print width (H/F)	0.93 (0.84, 1.01)	0.00 (0.00, 0.00)	0.02 (0.00, 0.05)	0.27 (0.14, 0.39)	0.30 (0.15, 0.45)	0.27 (0.16, 0.39)
Print area (H/F)	0.81 (0.69, 0.93)	0.00 (0.00, 0.00)	0.01 (0.00, 0.01)	0.10 (0.04, 0.17)	0.11 (0.05, 0.17)	0.21 (0.11, 0.31)
Max contact area (H/F)	0.63 (0.54, 0.72)	0.00 (0.00, 0.00)	0.01 (0.00, 0.01)	0.16 (0.05, 0.28)	0.17 (0.06, 0.29)	0.16 (0.09, 0.24)
Max contact at % (H/F)	0.81 (0.70, 0.92)	0.00 (0.00, 0.00)	0.03 (-0.01, 0.06)	0.34 (0.19, 0.50)	0.30 (0.16, 0.45)	0.24 (0.12, 0.35)
Body speed (H/F)	0.98 (0.96, 1.00)	0.00 (0.00, 0.00)	0.09 (0.00, 0.17)	0.31 (0.19, 0.44)	0.35 (0.21, 0.50)	0.36 (0.20, 0.53)
Step cycle (H/F)	1.02 (0.98, 1.07)	0.00 (0.00, 0.00)	0.07 (-0.02, 0.15)	0.39 (0.09, 0.68)	0.58 (0.23, 0.93)	0.43 (0.04, 0.82)
Duty cycle (H/F)	1.01 (0.95, 1.08)	0.00 (0.00, 0.00)	0.02 (0.00, 0.05)	0.26 (0.13, 0.39)	0.26 (0.13, 0.38)	0.17 (0.05, 0.28)

Values are shown as mean with 95% confidence interval. BL = baseline test; Nx = neurotest timepoint x (in weeks) after spinal cord injury (SCI); H/F = hindlimbs/forelimbs.

Additional Table 5 CatWalk XT® automated gait analysis parameters (corrected values from hindlimbs) of animals in the sham group assessing motor function for all available time points

CatWalk XT® parameters	BL	N2	N3	N4	N5	N6
Regularity index (%)	92.98 (86.72, 99.23)	85.02 (76.18, 93.87)	92.05 (85.67, 98.43)	84.73 (76.47, 92.98)	90.31 (82.83, 97.80)	88.61 (79.44, 97.79)
Average speed (cm/s)	28.13 (24.20, 32.07)	20.03 (13.12, 26.93)	24.61 (19.21, 30.00)	11.94 (7.55, 16.33)	22.16 (13.77, 30.54)	21.63 (15.22, 28.04)
Base of support (H/F)	1.32 (1.20, 1.43)	1.39 (1.21, 1.58)	1.30 (1.14, 1.46)	1.45 (1.25, 1.64)	1.49 (1.32, 1.67)	1.44 (1.32, 1.56)
Stride length (H/F)	0.94 (0.87, 1.01)	1.15 (1.01, 1.29)	1.07 (0.88, 1.25)	1.07 (0.90, 1.24)	1.06 (0.97, 1.15)	0.93 (0.76,1.11)
Swing speed (H/F)	0.98 (0.92, 1.03)	1.21 (1.09, 1.33)	1.18 (1.08, 1.29)	1.12 (0.93, 1.46)	1.25 (1.05, 1.44)	1.11 (0.89, 1.32)
Max intensity (H/F)	0.79 (0.71, 0.86)	0.97 (0.89, 1.06)	0.89 (0.83, 0.95)	1.03 (0.99, 1.06)	0.94 (0.89, 0.99)	0.83 (0.77, 0.89)
Mean intensity (H/F)	0.91 (0.87, 0.96)	1.02 (0.96, 1.07)	0.96 (0.92, 1.00)	0.97 (0.94, 0.99)	0.91 (0.88, 0.94)	0.88 (0.85, 0.92)
Mean intensity of most 15 prints (H/F)	0.82 (0.74, 0.90)	1.00 (0.95, 1.06)	0.94 (0.89, 1.00)	1.08 (1.02, 1.13)	0.97 (0.92, 1.03)	0.88 (0.82, 0.95)
Max contact max intensity (H/F)	0.78 (0.69, 0.86)	0.96 (0.86, 1.05)	0.89 (0.83, 0.96)	1.01 (0.97, 1.04)	0.90 (0.84, 0.95)	0.84 (0.79, 0.89)
Max contact mean intensity (H/F)	0.90 (0.85, 0.95)	1.00 (0.94, 1.07)	0.94 (0.90, 0.98)	0.95 (0.92, 0.98)	0.88 (0.85, 0.92)	0.88 (0.84, 0.92)
Max intensity at % (H/F)	1.08 (0.93, 1.23)	1.00 (0.92, 1.09)	0.90 (0.81, 0.99)	1.07 (0.86, 1.28)	0.96 (0.83, 1.08)	0.94 (0.84, 1.04)
Print length (H/F)	0.83 (0.73, 0.93)	1.03 (0.95, 1.12)	0.99 (0.88, 1.09)	1.07 (0.90, 1.24)	1.04 (0.96, 1.12)	0.96 (0.87, 1.05)
Print width (H/F)	0.90 (0.81, 0.99)	1.08 (0.94, 1.21)	0.98 (0.89, 1.07)	1.09 (0.96, 1.21)	1.04 (0.96, 1.11)	0.93 (0.86, 1.00)
Print area (H/F)	0.78 (0.66, 0.89)	0.94 (0.94, 1.03)	0.90 (0.79, 1.02)	1.11 (1.00, 1.23)	1.04 (0.92, 1.16)	0.85 (0.71, 0.98)
Max contact area (H/F)	0.61 (0.48, 0.74)	0.79 (0.70, 0.88)	0.76 (0.62, 0.90)	0.98 (0.88, 1.07)	0.86 (0.75, 0.97)	0.68 (0.55, 0.80)
Max contact at % (H/F)	0.88 (0.73, 1.03)	0.82 (0.67, 0.97)	0.84 (0.71, 0.97)	0.71 (0.48, 0.94)	0.78 (0.59, 0.97)	0.74 (0.59, 0.89)
Body speed (H/F)	0.97 (0.93, 1.01)	1.03 (0.96, 1.10)	1.01 (0.98, 1.05)	1.01 (0.86, 1.17)	1.02 (0.93, 1.12)	0.93 (0.83, 1.03)
Step cycle (H/F)	0.97 (0.90, 1.03)	1.15 (1.00, 1.30)	1.03 (0.94, 1.13)	1.06 (0.80, 1.32)	1.08 (0.90, 1.27)	1.02 (0.91, 1.13)
Duty cycle (H/F)	1.02 (0.95, 1.10)	1.14 (1.02, 1.26)	1.08 (1.02, 1.14)	1.21 (0.97, 1.45)	1.09 (0.98, 1.19)	1.16 (1.01, 1.32)

Values are shown as mean with 95% confidence interval. BL = baseline test; Nx = neurotest time point x (in weeks) after laminectomy; H/F = hindlimbs/forelimbs.