Automated Rehabilitation Assessment System for Spinal Cord Injured Rats Using DeepLabCut-Based Ladder Test Analysis

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Abstract

Quantitative assessment of motor recovery after spinal cord injury (SCI) is essential for evaluating rehabilitation effects. Conventional behavioral tests, such as the BBB score and ladder rung walking test, are widely used in rodent SCI studies; however, they often rely on manual observation and subjective scoring. In this study, we propose an automated analysis framework for evaluating locomotor recovery in SCI rats using DeepLabCut-based markerless pose estimation. The system extracts anatomical keypoints from ladder walking videos and performs likelihood-based filtering, trajectory correction, ladder-coordinate transformation, and step/slip event detection. The DeepLabCut model used 14 anatomical keypoints, including facial landmarks, spinal points, tail-base points, and lower-limb joints, with a ResNet-50 backbone and a likelihood threshold of 0.6. A total of 200 behavioral videos were analyzed. The proposed framework provides quantitative metrics, including step count, slip count, slip ratio, hindpaw trajectory stability, and left-right hindlimb coordination, supporting objective and reproducible SCI rehabilitation assessment.

1. Introduction

Spinal cord injury (SCI) causes severe motor impairment and often leads to reduced hindlimb function and abnormal gait patterns. Electrical stimulation-based rehabilitation has been investigated as a promising strategy for promoting motor recovery after SCI. To evaluate its therapeutic effects, reliable and quantitative behavioral assessment is required in animal models.

In rodent SCI studies, the BBB locomotor rating scale and ladder rung walking test are commonly used to assess functional recovery. However, these methods often depend on visual observation and manual scoring, which may introduce inter-rater variability and time-consuming analysis. In particular, SCI rats frequently show hindlimb dragging, paw crossing, irregular stepping, and trunk imbalance, making consistent scoring difficult.

To address these limitations, this study proposes a DeepLabCut-based automated analysis framework for ladder rung walking test videos. The proposed method extracts keypoint trajectories from SCI rats, corrects unreliable detections, transforms trajectories into a ladder-centered coordinate system, and detects step and slip events. By converting behavioral videos into quantitative rehabilitation metrics, this framework aims to reduce subjectivity and improve reproducibility in SCI rehabilitation assessment.

2. Materials and Methods

2.1 Animals and Spinal Cord Injury Model

Twelve-week-old Sprague-Dawley rats were used in this study. The average body weight was 230–250 g, and each experimental group

consisted of five animals. The rats were introduced at 11 weeks of age and allowed to acclimate to the housing environment for one week. During this period, basic handling was performed to reduce stress during subsequent experiments. The animals were housed under controlled environmental conditions with a temperature of 22 ± 2℃, relative humidity of 50 ± 10%, and a 12−h light/dark cycle. Food and water were provided ad libitum.

The SCI model was established using an Infinite Horizon Impactor. The injury level was set at T9, and the impact force was 200 kDyn. Anesthesia was induced and maintained using isoflurane inhalation at approximately 2–2.5%. After surgery, body temperature was maintained using an infrared lamp. Antibiotic administration, analgesic treatment, and manual bladder expression were performed as postoperative care.

Item	Description
Animal	12-week-old SD rat
Body weight	230–250 g
Injury device	Infinite Horizon Impactor
Injury level	T9
Impact force	200 kDyn
Anesthesia	Isoflurane, 2−2.5%
Housing condition	22 ± 2℃, 50 ± 10% humidity, 12−h light/dark cycle

[Table 1. Experimental conditions]

2.2 Electrical Stimulation and Behavioral Assessment

Electrical stimulation was applied to the T9 region. The electrode was fabricated by depositing a 100−nm gold thin film on a 10−nm titanium thin film. Stimulation was initiated on the day of SCI surgery and applied for three consecutive days. Each stimulation session was performed once per day for 30 min. After electrode connection and stabilization, continuous electrical stimulation was delivered. Detailed stimulation parameters, including voltage, current, pulse width, and frequency, will be determined after optimization of the electrode and stimulation conditions.

Behavioral assessment was performed using the ladder rung walking test. The ladder apparatus was 100 cm in length and 8 cm in width, with side walls 85 cm in height. The apparatus contained 58 metal rungs with a diameter of 4 mm. One trial was defined as a round trip in which the rat moved from the starting point to the opposite end of the ladder and then returned to the starting point. Two trials were performed per session.

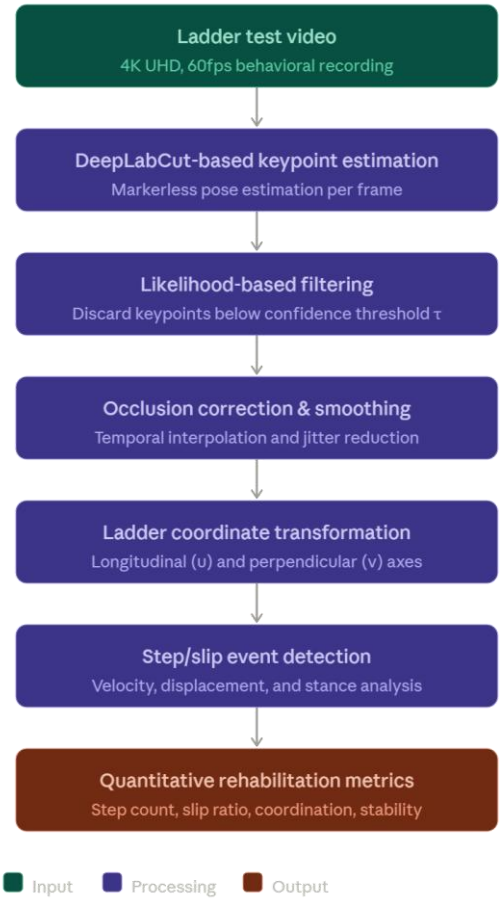
Behavioral videos were recorded using a National Geographic Hybrid 4K ActionCam mini. Videos were acquired at 4K UHD resolution and 60 frames per second and saved in mp4 format. The camera was placed 110 cm away from the apparatus and 85 cm above the floor. Fixed fluorescent lighting was used, and matte paint or felt materials were applied around the recording environment to reduce reflection and shadows.

Item	Description
Electrode structure	Titanium 10 nm + Gold 100 nm
Stimulation site	T9
Stimulation period	3 consecutive days from the day of surgery
Stimulation duration	Once daily, 30 min per session
Ladder size	100 cm × 8 cm
Side wall height	85 cm
Rungs	58 metal rungs, 4 mm diameter
Camera	National Geographic Hybrid 4K ActionCam mini
Video condition	4K UHD, 60fps, mp4

[Table 2. Rehabilitation and video acquisition conditions]

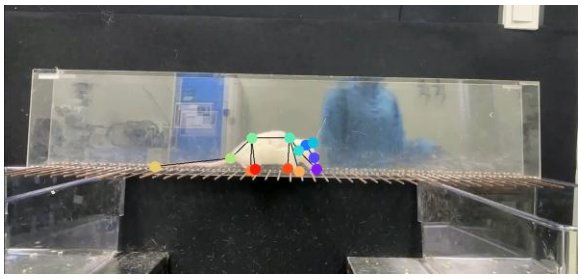
3. Proposed Automated Analysis Framework

The proposed system quantitatively analyzes locomotor recovery in SCI rats using ladder rung walking test videos. The overall workflow consists of video input, DeepLabCut−based keypoint estimation, likelihood−based filtering, occlusion correction, trajectory smoothing, ladder coordinate transformation, step/slip event detection, and rehabilitation metric computation.



[Fig 1. Proposed analysis pipeline]

DeepLabCut estimates the coordinates and likelihood values of predefined anatomical keypoints in each frame. In this study, the keypoint set includes not only the hindpaws but also trunk, forelimb, and tail–base points. This is important because SCI rats frequently exhibit hindlimb dragging, paw crossing, occlusion, and left–right limb ambiguity.



[Fig. 2. Representative DeepLabCut keypoint estimation]

The DeepLabCut output for keypoint k at frame t can be represented as:

$$p_{t,k} = (x_{t,k}, y_{t,k}, l_{t,k})$$

where $x_{t,k}$ and $y_{t,k}$ denote the image coordinates, and $l_{t,k}$ denotes the likelihood value of the prediction. A keypoint with a likelihood lower than a predefined threshold is regarded as unreliable and treated as a missing value:

$$l_{\{t,k\}} < \tau$$

Short missing segments are corrected by temporal interpolation, and trajectory smoothing is applied to reduce frame–level jitter. The corrected keypoint trajectories are then transformed into a ladder–centered coordinate system. If the two endpoints of the ladder are denoted as L and R, the unit vector along the ladder direction is defined as:

$$u = \frac{R - L}{|R - L|}$$

Each keypoint position P_t is projected onto the longitudinal and perpendicular axes of the ladder as follows:

$$u_t = (P_t - L) \cdot u$$

$$v_t = (P_t - L) \cdot v$$

This transformation allows the analysis to separate forward progression along the ladder from vertical displacement relative to the ladder structure.

4. Step/Slip Detection and Rehabilitation Metrics

The primary target of the proposed analysis is the detection of step and slip events from hindpaw trajectories. A successful step was defined as an event in which the hindpaw maintained stable contact with a rung or support region and then progressed to the next position. A slip was defined as an event in which the hindpaw fell below the ladder, indicating loss of support during ladder walking.

Step detection was performed based on forward hindpaw movement, reduced velocity near the stance phase, and stable positioning near the

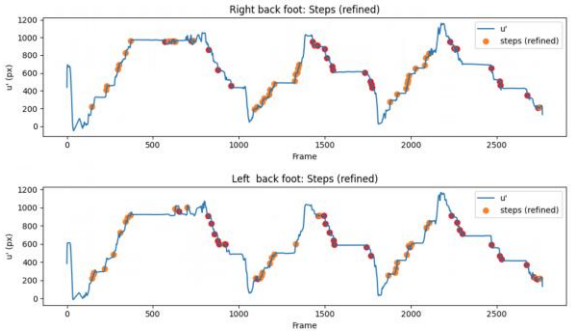
support region. Slip detection was performed based on downward displacement below the ladder structure and failure to maintain stable support.

Velocity was computed using the first difference of the smoothed trajectory:

$$\Delta u_t = u_t - u_{t-1}$$

$$\Delta v_t = v_t - v_{t-1}$$

The longitudinal coordinate u_t was mainly used to identify forward progression and repeated stepping patterns, whereas the perpendicular coordinate v_t was used to detect downward displacement related to slip events.



[Fig. 3. Automated step detection from refined hindpaw trajectories.]

The longitudinal hindpaw trajectories of the right and left hind paws were analyzed in the ladder-centered coordinate system. Orange markers indicate automatically detected step events after trajectory refinement.

The detected events were converted into quantitative rehabilitation metrics. The primary metrics included step count, slip count, slip ratio, hindpaw trajectory stability, left-right hindlimb coordination, and trunk stability.

Metric	Description
Step count	Number of successfully detected hindpaw steps
Slip count	Number of paw slips or support failures
Slip ratio	Proportion of slip events among total stepping attempts
Hindpaw trajectory stability	Stability of hindpaw movement trajectories
Left-right coordination	Timing and alternation pattern between left and right hindlimbs
Trunk stability	Stability of spine or tail-base movement

[Table 3. Quantitative rehabilitation metrics]

The slip ratio is defined as:

$$R_{slip} = \frac{N_{slip}}{N_{step} + N_{slip}}$$

This normalized metric allows more robust comparison than slip count alone, because animals with reduced movement may show fewer slips simply due to fewer stepping attempts.

5. Preliminary Results

To evaluate the feasibility of the proposed framework, preliminary validation was performed using 50 ladder rung walking test videos. The automated detection results were compared with manual counting performed by a human evaluator. Manual counting was used as the reference standard for both step and slip events.

DeepLabCut successfully tracked the predefined anatomical keypoints from ladder walking videos, including hindpaw, trunk, and tail-base landmarks. The keypoint overlay results confirmed that markerless pose estimation could provide usable trajectory information from behavioral videos. After likelihood-based filtering, interpolation, and smoothing, the refined hindpaw trajectories were used for event-level analysis.

The proposed algorithm achieved approximately 81% agreement for step counting and 85% agreement for slip counting compared with manual human counting. Slip events were defined as cases in which the hindpaw fell below the ladder during walking. These preliminary results indicate that the proposed method can detect major gait events with reasonable consistency compared with manual evaluation.

Detection target	Manual reference	Number of videos	Agreement
Step events	Human manual counting	50	81%
Slip events	Human manual counting	50	85%

[Table 4. Preliminary comparison between manual counting and automated detection]

The step detection result shown in Fig. 3 demonstrates that the proposed algorithm can identify repeated stepping patterns from refined hindpaw trajectories. The 85% agreement in slip detection suggests that the proposed framework can capture support failure events associated with ladder walking impairment. These results support the feasibility of using DeepLabCut-based trajectory analysis for objective rehabilitation assessment in spinal cord injured rats.

6. Conclusion

In this study, we proposed a DeepLabCut-based automated analysis framework for evaluating locomotor recovery in spinal cord injured rats using ladder rung walking test videos. The proposed system estimates 14 anatomical keypoints using a ResNet-50-based DeepLabCut model and processes the extracted trajectories through likelihood filtering, interpolation, smoothing, ladder coordinate transformation, and step/slip event detection.

Preliminary validation using 50 videos showed that the automated detection results achieved 81% agreement for step counting and 85% agreement for slip counting compared with manual human counting. These results indicate that the proposed framework can reduce the subjectivity and labor intensity of conventional behavioral evaluation while providing quantitative metrics for rehabilitation assessment.

Future work will focus on improving event detection accuracy, validating the system across larger experimental groups, and comparing the proposed automated metrics with conventional behavioral scores such as the BBB score.

7. Reference

[1] D. M. Basso, M. S. Beattie, and J. C. Bresnahan, "A sensitive and reliable locomotor rating scale for open field testing in rats," Journal of Neurotrauma, vol. 12, no. 1, pp. 1–21, 1995.

[2] G. A. Metz and I. Q. Whishaw, "The ladder rung walking task: a scoring system and its practical application," Journal of Visualized Experiments, no. 28, e1204, 2009.

[3] A. Mathis, P. Mamidanna, K. M. Cury, T. Abe, V. N. Murthy, M. W. Mathis, and M. Bethge, "DeepLabCut: markerless pose estimation of user-defined body parts with deep learning," Nature Neuroscience, vol. 21, pp. 1281–1289, 2018.

[4] Y. Sato, T. Kondo, M. Shinozaki, R. Shibata, N. Nagoshi, J. Ushiba, M. Nakamura, and H. Okano, "Markerless analysis of hindlimb kinematics in spinal cord-injured mice through deep learning," Neuroscience Research, 2022.

[5] A. Aljovic, S. Zhao, M. Chahin, C. de la Rosa, V. Van Steenbergen, M. Kerschensteiner, and F. M. Bareyre, "A deep learning-based toolbox for Automated Limb Motion Analysis (ALMA) in murine models of neurological disorders," Communications Biology, vol. 5, article no. 131, 2022.

[6] S. Wang et al., "Epidural electrical stimulation effectively restores locomotion function in rats with complete spinal cord injury," Neural Regeneration Research, vol. 16, no. 3, pp. 573–579, 2021.