

Submission ID #: 69798

Scriptwriter Name: Sulakshana Karkala

Project Page Link: <https://review.jove.com/account/file-uploader?src=21288438>

Title: Deep-Learning Based Multi-Joint Synchronous Tracking for Objective Quantification of Hindlimb Locomotor Kinematics in Rats

Authors and Affiliations:

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **YES, all done**
- 3. Filming location:** Will the filming need to take place in multiple locations? **NO**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **YES**

Current Protocol Length

Number of Steps: 17

Number of Shots: 35

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Penghao Liu:** We developed an AI-driven treadmill to objectively track rat movements, helping us precisely evaluate spinal cord injury recovery.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Penghao Liu:** Unlike traditional footprint methods that miss complex joint kinematics, our markerless AI system directly tracks multi-joint movements.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.4. **Penghao Liu:** Our system allows researchers to precisely measure continuous multi-joint trajectories, force distribution, and movement smoothness in real time.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.5. **Penghao Liu:** An important consideration is thoroughly acclimating the rat to the treadmill to ensure stable, analyzable gait patterns.
 - 1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

1.6. **Penghao Liu:** We can synchronize this system with EEG or EMG to decode the neural mechanisms driving these precise movements.

1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your article address?

- 1.3. **Penghao Liu, Neurosurgeon & Physician scientist of Xuanwu Hospital, Capital Medical University**: (authors will present their testimonial statements live)

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What value do you expect this video article to have for other researchers working in your field?

- 1.4. **Penghao Liu, Neurosurgeon & Physician scientist of Xuanwu Hospital, Capital Medical University**: (authors will present their testimonial statements live)

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Beijing Institute for Brain Research

Protocol

2. Synchronized Treadmill Gait Acquisition in Rats and Video Recording Setup

Demonstrator: Penghao Liu

2.1. To begin, turn on the Industrial Personal Computer and launch the video acquisition and analysis software [1]. ~~Create a directory for the day's experiment that includes the experiment name, animal identification, experimental group, speed, incline, and weight reduction percentage [2].~~

2.1.1. WIDE: Talent switching on the IPC and launching the software.

AUTHORS: Please note that JoVE requires the opening shot to be a wide, non-screen capture. This shot was added specifically to meet that requirement

2.1.2. ~~SCREEN: Talent creates a new folder with the experiment name, animal identification, group, speed, incline, and weight reduction percentage.~~

~~This was included in the 3.2.2 section~~

2.2. ~~Use an aluminum profile bracket to fix a high-speed industrial camera equipped with a manual focus lens [1].~~ Position the camera lens perpendicular to the treadmill longitudinal axis to obtain a strictly lateral view [2-TXT].

2.2.1. ~~Talent mounting the high-speed industrial camera onto the aluminum profile bracket.~~ Author's NOTE: This was not essential in our steps and was not filmed.

2.2.2. Talent adjusting the camera so that it is perpendicular to the treadmill. **TXT: Maintain horizontal working distance of 15 cm**

2.3. Next, record the body weight of each rat with an electronic scale [1]. Include only rats with matched body weights to minimize body size effects [2].

2.3.1. Talent placing a rat on the electronic scale and recording the weight.

2.3.2. Shot of similarly sized rats.

2.4. Put on gloves and gently grasp the rat [1]. Loop the elastic chest strap around the front armpit and attach it to the adjustable slide rail [2]. Then adjust the strap so that one finger can be inserted without restricting breathing [3].

2.4.1. Talent wearing gloves and holding the rat securely.

2.4.2. Talent looping the elastic chest strap around the rat and securing it to the slide rail.

- 2.4.3. Talent inserting one finger between the strap and the rat to check looseness.
- 2.5. On the touch control screen, set the **speed** to 150 millimeters per second and the **incline** to 0 degrees [1]. Conduct a 10-minute acclimation session to adapt the rat to the body weight support level [2-TXT].
 - 2.5.1. Shot of the touchscreen interface as the speed and incline are being set.
 - 2.5.2. Shot of the rat walking on the treadmill during acclimation. **TXT: Monitor rats for signs of stress**
- 2.6. Confirm successful acclimation when the rat maintains a continuous and uniform stride for at least 60 seconds without paw dragging and with a naturally hanging tail [1]. Exclude animals that fail to meet these criteria after the maximum acclimation period [2].
 - 2.6.1. Shot of the rat demonstrating continuous uniform strides with no paw dragging and a relaxed tail.
 - 2.6.2. Shot of a rat being removed from the setup.
- 2.7. Now, enter the parameters for the formal experiment on the touchscreen [1]. Sequentially input the desired speed and incline then select the treadmill belt direction [2].
 - 2.7.1. Talent accessing the parameter settings menu on the touchscreen.
Videographer: Please capture the screen of the instrument for this shot
 - 2.7.2. Shot of the speed, incline, and direction fields being entered and confirmed.
Videographer: Please capture the screen of the instrument for this shot
- 2.8. After stabilization, select **Start Recording** to begin data acquisition [1]. Continuously capture at least 5 complete gait cycles [2].
 - 2.8.1. Talent clicking **Start Recording**.
 - 2.8.2. Shot of the rat walking while the recording indicator is active.
- 2.9. At the end of each trial, reduce the speed to 0 millimeters per second [1]. Unclip the chest strap and return the rat to its corresponding cage [2].
 - 2.9.1. Talent reducing the speed to 0 millimeters per second.
Videographer: Please capture the screen of the instrument for this shot
 - 2.9.2. Talent unclipping the strap and placing the rat back into its cage.
- 3. Gait Video Processing and Integration with Electrophysiological Recording in Rats**
 - 3.1. Extract video segments of approximately 10 seconds that contain the target gait with at least 10 stable gait cycles [1].
 - 3.1.1. SCREEN: JOVE_SCREEN_69798_STEP3.1.1.Video_Editing 00:20-00:40.

- 3.2. Register the rat information including identification number, group, and experimental conditions [1]. Import the .mov (*dot-M-O-V*) file into the analysis software [2].
 - 3.2.1. SCREEN: JOVE_SCREEN_69798_STEP3.2.1-3.2.2.IMPORT_FILE 00:10-00:30.
 - 3.2.2. SCREEN: JOVE_SCREEN_69798_STEP3.2.1-3.2.2.IMPORT_FILE 00:50-01:00.

NOTE: Show 3.4 here
- 3.3. Export the spatial position coordinates of each joint over time as a .csv(*dot-C-S-V*) file for further analysis [1].
 - 3.3.1. SCREEN: JOVE_SCREEN_69798_STEP3.4.1-3.4.2_Gait_Dynamics 01:50-02:05.
Author's NOTE: Move 3.3 after 3.4
- 3.4. Normalize each gait cycle from 0 to 100 percent to standardize cycle length [1]. Generate representative images to illustrate gait dynamics [2].
 - 3.4.1. SCREEN: JOVE_SCREEN_69798_STEP3.4.1-3.4.2_Gait_Dynamics 00:20-00:35.
 - 3.4.2. SCREEN: JOVE_SCREEN_69798_STEP3.4.1-3.4.2_Gait_Dynamics 01:30-01:40.
- 3.5. After completing animal modeling, implant the electrophysiological recording device [1].
 - 3.5.1. Shot of the implanted electrophysiological recording device secured on the animal.
- 3.6. For brain signal recording, place electrodes on the skull surface, epidural space, or cerebral cortex to record brain signals [1]. For spinal cord recording, insert recording electrodes into the epidural space of the intervertebral foramen [2]. For electromyography recording, bury bipolar silver wires into the target muscles to record muscle electrical activity [3].
 - 3.6.1. Shot of electrodes positioned on the skull surface for electroencephalography recording. **Author's NOTE: Spinal cord electrodes, muscle electrodes for 3.6.2 and 3.6.3 were both recorded in 3.7.1, together with brain electrodes. Video editor: The authors has written 3.7.1, but I think they meant 3.6.1, please check the footage and determine**
 - 3.6.2. Shot of electrode placement at the intervertebral foramen for spinal recording.
 - 3.6.3. Shot of bipolar silver wires implanted into muscle tissue.
- 3.7. Allow the animal to recover for 5 to 7 days after implantation [1]. Check the wound and gait daily to ensure no signs of infection or pain before treadmill testing [2].
 - 3.7.1. Shot of the recovered animal in its cage during the recovery period.
 - 3.7.2. Talent inspecting the surgical site and observing gait.
- 3.8. Prepare the equipment and animal before the synchronized experiment [1]. Then synchronize the electrophysiological data acquisition with the movement video to

ensure both share the same timestamp [2]. Align the neural signals with corresponding video frames during analysis to visualize electrophysiological patterns at different gait phases [3].

- 3.8.1. Shot of the fully prepared treadmill, camera, and implanted animal ready for recording.
- 3.8.2. SCREEN: JOVE_SCREEN_69798_STEP3.8.2-3.8.3_SyncroElectro_Signal 00:05-00:20.
- 3.8.3. SCREEN: JOVE_SCREEN_69798_STEP3.8.2-3.8.3_SyncroElectro_Signal 00:45-00:55.

Results

4. Results

AUTHORS: Please note that the results section is limited to 200 words. If you would like to emphasize any additional information, please replace the existing statements to meet the word limit

4.1. The rats with spinal cord injury exhibited significant loss of swing and irregular iliac displacement curves [1].

4.1.1. LAB MEDIA: Figure 2A,B. *Video editor: show the rat images (Top most)*

4.2. Spinal cord injury rats showed increased joint angle fluctuations compared with healthy rats [1].

4.2.1. LAB MEDIA: Figure 2A,B. *Video editor: Show the middle panel graphs for both A and B.*

4.3. The joint range of motion heatmap showed a widespread decrease in color scale in spinal cord injury rats compared with healthy rats [1]. The trajectory activity map showed a significant reduction in range of motion of each joint and impaired continuity in spinal cord injury rats [2].

4.3.1. LAB MEDIA: Figure 3A. *Video editor: Emphasize the heatmap for the SCI group*

4.3.2. LAB MEDIA: Figure 3B *Video editor: Highlight the panel for SCI group*

4.4. The point cloud map showed increased dispersion and a leftward shift of the center of mass in spinal cord injury rats [1]. The height waterfall chart showed a bimodal distribution along the X-axis between groups and a chaotic overall difference distribution in spinal cord injury rats [2].

4.4.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the panel for SCI group*

4.4.2. LAB MEDIA: Figure 3D. *Video editor: Show the top graph and then the bottom graph*

4.5. The vertical height peak distribution of the toes was shifted to the right in spinal cord injury rats [1]. The velocity range chart showed that the combined velocity range in each frame was narrowed in the injury group [2].

4.5.1. LAB MEDIA: Figure 3E. *Video editor: Highlight the blue columns for SCI rat*

4.5.2. LAB MEDIA: Figure 3F. *Video editor: Highlight the blue columns for SCI rat*

4.6. The phase plane area [1] and the estimated peak propulsion force index was reduced in spinal cord injury rats [2]. Movement smoothness indicators were also decreased in spinal cord injury rats [3].

- 4.6.1. LAB MEDIA: Figure 3G. *Video editor: Highlight the blue columns for SCI rat*
- 4.6.2. LAB MEDIA: Figure 3H. *Video editor: Highlight the blue columns for SCI rat*
- 4.6.3. LAB MEDIA: Figure 3I. *Video editor: Highlight the blue columns for SCI rat*

1. acquisition
Pronunciation link: <https://www.merriam-webster.com/dictionary/acquisition>
IPA: /ˌækwəˈzɪʃən/
Phonetic Spelling: ak·wuh·zish·uhn
2. aluminum
Pronunciation link: <https://www.merriam-webster.com/dictionary/aluminum>
IPA: /əˈluːmənəm/
Phonetic Spelling: uh·loo·muh·nuhm
3. perpendicular
Pronunciation link: <https://www.merriam-webster.com/dictionary/perpendicular>
IPA: /ˌpɜːpənˈdɪkjələr/
Phonetic Spelling: per·puhn·dik·yuh·ler
4. longitudinal
Pronunciation link: <https://www.merriam-webster.com/dictionary/longitudinal>
IPA: /ˌlɑːndʒəˈtuːdənəl/
Phonetic Spelling: lon·juh·too·duh·nuhl
5. acclimation
Pronunciation link: <https://www.merriam-webster.com/dictionary/acclimation>
IPA: /ˌækləˈmeɪʃən/
Phonetic Spelling: ak·luh·may·shuhn
6. acclimation
Pronunciation link: <https://www.merriam-webster.com/dictionary/acclimation>
IPA: /ˌækləˈmeɪʃən/
Phonetic Spelling: ak·luh·may·shuhn
7. electrophysiological
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophysiology>
IPA: /ɪˌlektroʊˌfɪziəˈləːdʒɪkəl/
Phonetic Spelling: ih·lek·troh·fiz·ee·uh·loj·ih·kuhl
8. electrophysiological
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophysiology>
IPA: /ɪˌlektroʊˌfɪziəˈləːdʒɪkəl/
Phonetic Spelling: ih·lek·troh·fiz·ee·uh·loj·ih·kuhl
9. epidural
Pronunciation link: <https://www.merriam-webster.com/dictionary/epidural>
IPA: /ˌɛprɪˈdʊrəl/
Phonetic Spelling: ep·ih·door·uhl

10. intervertebral
Pronunciation link: <https://www.merriam-webster.com/dictionary/intervertebral>
IPA: /ˌɪntərˈvɜːtəbrəl/
Phonetic Spelling: in·ter·ver·tuh·bruhl
11. electromyography
Pronunciation link: <https://www.merriam-webster.com/dictionary/electromyography>
IPA: /ɪˌlektroʊmaɪˈɑːɡrəfi/
Phonetic Spelling: ih·lek·troh·my·og·ruh·fee
12. electroencephalography
Pronunciation link: <https://www.merriam-webster.com/dictionary/electroencephalography>
IPA: /ɪˌlektroʊənˌsefəˈlɑːɡrəfi/
Phonetic Spelling: ih·lek·troh·en·sef·uh·log·ruh·fee
13. synchronize
Pronunciation link: <https://www.merriam-webster.com/dictionary/synchronize>
IPA: /ˈsɪŋkrəˌnaɪz/
Phonetic Spelling: sing·kruh·nyze
14. electrophysiological
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophysiology>
IPA: /ɪˌlektroʊˌfɪziəˈlɑːdʒɪkəl/
Phonetic Spelling: ih·lek·troh·fiz·ee·uh·loj·ih·kuhl
15. displacement
Pronunciation link: <https://www.merriam-webster.com/dictionary/displacement>
IPA: /dɪsˈpleɪsmənt/
Phonetic Spelling: dis·plays·muhnt
16. fluctuation
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluctuation>
IPA: /ˌflʌktʃuˈeɪʃən/
Phonetic Spelling: fluk·choo·ay·shuhn
17. trajectory
Pronunciation link: <https://www.merriam-webster.com/dictionary/trajectory>
IPA: /trəˈdʒektəri/
Phonetic Spelling: truh·jek·tuh·ree
18. dispersion
Pronunciation link: <https://www.merriam-webster.com/dictionary/dispersion>
IPA: /dɪˈspɜːʒən/
Phonetic Spelling: dih·spur·zhuhn
19. bimodal
Pronunciation link: <https://www.merriam-webster.com/dictionary/bimodal>
IPA: /ˌbaɪˈmoʊdəl/
Phonetic Spelling: bye·moh·duhl
20. propulsion
Pronunciation link: <https://www.merriam-webster.com/dictionary/propulsion>
IPA: /prəˈpʌlʃən/
Phonetic Spelling: pruh·pul·shuhn

21. velocity

Pronunciation link: <https://www.merriam-webster.com/dictionary/velocity>

IPA: /vəˈlɑːsəti/

Phonetic Spelling: vuh·loss·uh·tee

22. electrophysiology

Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophysiology>

IPA: /ɪˌlektroʊˌfɪziˈɑːlədʒi/

Phonetic Spelling: ih·lek·troh·fiz·ee·ol·uh·jee