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3D Kinematic Analysis for the Functional Evaluation in Rat Model of Sciatic Nerve Crush Injury --Manuscript Draft--

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May 15, 2019

Editors,
JoVE
Dear Editor:

We have enclosed our manuscript titled, “**A three-dimensional kinematic analysis method for functional evaluation in the rat sciatic nerve crush injury model.**” for your consideration to be published in *Journal of Visualized Experiments*.

The Sciatic Functional Index (SFI) has always been considered the gold standard of functional evaluation; however, its intrinsic shortcomings may impede further application in relevant studies. Kinematic analysis is becoming accepted as a reliable tool for functional evaluation in recent research.

In this manuscript, we introduced a brand-new kinematic analysis method that uses a three-dimensional motion capture apparatus containing cameras and data processing software. We described this method in detail and took the rat with sciatic nerve crush injury model for example to present several representative results.

We believe that our manuscript is within the scope of *JoVE* and would be of interest to its readership. We look forward to hearing from you.

Sincerely,

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TITLE:**3D Kinematic Analysis for the Functional Evaluation in the Rat Model of Sciatic Nerve Crush Injury****AUTHORS AND AFFILIATIONS:**

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KEYWORDS:

rat, sciatic nerve injury, three-dimensional, kinematic analysis, functional evaluation, center of gravity

SUMMARY:

We introduce a kinematic analysis method that uses a three-dimensional motion capture apparatus containing four cameras and data processing software for performing functional evaluations during fundamental research involving rodent models.

ABSTRACT:

Compared to the Sciatic Functional Index (SFI), kinematic analysis is a more reliable and sensitive method for performing functional evaluations of sciatic nerve injury rodent models. In this protocol, we describe a novel kinematic analysis method that uses a three-dimensional (3D) motion capture apparatus for functional evaluations using a rat sciatic nerve crush injury model. First, the rat is familiarized with treadmill walking. Markers are then attached to the designated bone landmarks and the rat is made to walk on the treadmill at the desired speed. Meanwhile, the posterior limb movements of the rat are recorded using four cameras. Depending on the software used, marker tracings are created using both automatic and manual modes and the desired data are produced after subtle adjustments. This method of kinematic analysis, which uses a 3D motion capture apparatus, offers numerous advantages, including superior precision and accuracy. Many more parameters can be investigated during the comprehensive functional

evaluations. This method has several shortcomings that require consideration: The system is expensive, can be complicated to operate, and may produce data deviations due to skin shifting. Nevertheless, kinematic analysis using a 3D motion capture apparatus is useful for performing functional anterior and posterior limb evaluations. In the future, this method may become increasingly useful for generating accurate assessments of various traumas and diseases.

INTRODUCTION:

The Sciatic Functional Index (SFI) is the benchmark method for carrying out functional sciatic nerve evaluations¹. The SFI has been widely adopted and is frequently used within various functional evaluation studies on rat sciatic nerve injuries²⁻⁶. In spite of its popularity, there are several problems with SFI, including automutilation⁷, joint contracture risk, and smearing of the footprints⁸. These problems seriously affect its prognostic value⁹. Therefore, an alternative, less error-prone method is required as a substitute for the SFI.

One such alternative method is kinematic analysis. This includes comprehensive gait analysis using tracking markers attached to bony landmarks or joints. Kinematic analysis is increasingly used for functional evaluations⁹. This method is progressively being recognized as a reliable and sensitive tool for functional evaluation¹⁰ without the shortcomings attributed to the SFI^{11,12}.

In this protocol, we describe a series of kinematic analyses that use a 3D motion capture apparatus consisting of a treadmill, four 120 Hz charged coupled device (CCD) cameras, and data processing software (see **Table of Materials**). This kinematic analysis method differs from general video walking or gait analysis^{13,14}. Two cameras are positioned in different directions to record posterior limb movements from a single side. Subsequently, a 3D digital model of the posterior limb is constructed using computer graphics⁹. We can calculate designated joint angles, such as hip, knee, ankle, and toe joint, by closely recapitulating the actual limb dimensions. Additionally, we can determine various parameters such as stride/step length and the ratio of the stance phase to the swing phase. These reconstructions are based on a completely reconstructed 3D digital model of the posterior limbs, generated from data transmitted by two sets of cameras. Even the imaginary center of gravity (CoG) trajectory can be calculated automatically.

We used this 3D motion capture apparatus to introduce and assess multiple kinematic parameters that reveal functional changes over time within the context of the rat sciatic nerve crush injury model.

PROTOCOL:

The protocol was approved by the animal experimentation committee of Kyoto University, and all protocol steps were performed in accordance with the Guidelines of the Animal Experimentation Committee, Kyoto University (approval number: MedKyo17029).

1. Familiarizing rats with treadmill walking

1.1. Set up two transparent plastic sheets on both sides of the treadmill to let a 12-week-old male Lewis rat walk in a straight, frontward direction, then turn on the electric shock grid.

1.2. Have each rat walk on the treadmill. Gradually accelerate the treadmill to the desired speed (20 cm/s or 12 m/min) and let the rat walk normally at this speed for 5 min. After each walking session, provide a 1–2 min rest break. Repeat this process 3x per day, 5 days per week, for 1 week.

NOTE: Start the treadmill walking 1 week before step 2.

1.3. House rats in groups of three per cage with a 12 h light-dark cycle and feed them commercial rat food and tap water ad libitum.

2. Performing the sciatic nerve crush injury

2.1. Place the rat in an anesthesia induction chamber and introduce 5% isoflurane inhalation solution.

2.2. Provide an intraperitoneal injection of a combination anesthetic prepared with 0.15 mg/kg medetomidine hydrochloride, 2 mg/kg midazolam, and 2.5 mg/kg butorphanol tartrate to the rat. Check for the lack of pedal reflexes. Then shave an area from the left greater trochanter to the mid-thigh with an electric shaver.

2.3. Spread out a piece of aseptic cloth, place the rat on it, and have it lie in the left lateral position. Place sterile surgical instruments on the cloth as well.

2.4. Create a straight incision from the greater trochanter to the mid-thigh with a surgical no. 10 blade. Then perform a blunt dissection between quadriceps femoris and biceps femoris using a surgical hemostat to expose the sciatic nerve.

2.5. Detach the sciatic nerve from the surrounding tissue with two pairs of microforceps and crush the sciatic nerve for 10 s, using a standard surgical hemostat, to create a 2 mm long crush injury at the site directly below the gluteal tuberosity.

2.6. Perform a 9-0 nylon epineural stitch at the proximal end of the injury using a pair of microforceps and then close the muscle and skin with 4-0 nylon sutures.

2.7. Provide an intraperitoneal injection of an anesthetic antagonist prepared with 0.3 mg/kg atipamezole hydrochloride to the rat, to wake it up within 10 min. After the rat recovers from anesthesia, observe the left toe movements while the rat is suspended by the base of its tail. If the toe does not spread at all, the surgery was successful.

2.8. House the rats individually after the surgery with a 12 h light-dark cycle and feed them commercial rat food and tap water ad libitum.

3. Attaching the markers

3.1. Place the trained rat in an anesthesia induction chamber and introduce a 5% isoflurane inhalation solution. Check for the lack of the pedal reflex by pinching the toe.

3.2. Allow the rat to be continuously anesthetized using an anesthetic mask (2% isoflurane inhalation solution). While the rat receives stable anesthesia, shave an area from the lower back to the bilateral malleoli using an electric shaver.

CAUTION: To prevent exposing researchers to the leaking isoflurane, make sure that the mask tightly covers the head and face of the rat.

NOTE: To prevent injury to the rat, shave the hair off as gently as possible.

3.3. Place the rat in the prone position. Use a black marker pen to mark the following bone landmarks on the shaved skin: A line through the spinous processes from the lumbar to sacral vertebrae, the anterior superior iliac spines, the greater trochanters, the knee joints, the lateral malleoli, the fifth metatarsophalangeal joints, and the tip of the fourth toe.

NOTE: The line through the spinous processes is used for determining whether the bilateral markers are axially symmetric.

3.4. Use a liquid adhesive to attach hemispheric markers to these bone landmarks, except for the line through the spinous processes from the lumbar to the sacral vertebrae, and the tip of the fourth toe. Use distinct colors for every other landmark to avoid confusion. The tip of the fourth toe is marked with pink ink.

CAUTION: Take care not to drip adhesive onto the exposed skin of the operator.

3.5. After placing all the markers, put the rat back into the cage. Do not put the rat on the treadmill until it fully recovers from anesthesia.

NOTE: Reduced consciousness may seriously influence normal walking if the rat does not fully recover from anesthesia.

4. Calibration and software setup

4.1. Set up two transparent plastic sheets on both sides of the treadmill and place the calibration box in the middle of the treadmill. Open the recording software and then click on the **Calibration Image** icon on the display (Supplementary File 1).

4.2. Click the **Recording** icon to record 1–2 s of video from four directions using 120 Hz CCD cameras. Click on the **Recording** icon again to stop the recording.

NOTE: The video will be saved automatically once the recording stops.

4.3. Open the video file in the calculation software. Click and drag the characteristic points of the calibration box 3D models on the bottom right corner of the screen to the corresponding markers on the four pictures, which are automatically transformed from the video in the calibration pattern (Supplementary File 2). Then click on the **Save** icon.

NOTE: Do not change the positions of the cameras after the calibration is completed.

5. Recording the walking

5.1 Take the calibration box out of the treadmill, turn on the electric shock grid, and place the fully awake rat on the treadmill. Open the recording software and input the basic information about the rat, including its serial number, walking speed, and the name of the principal operator.

5.2 Turn on the treadmill and set the speed to 20 cm/s. After the rat adapts to the speed and is able to walk normally, click the **Recording** icon on the display to record the walking rat with the four cameras. Once enough steps are recorded (>10), click on the icon again to stop recording, and turn off the treadmill.

NOTE: The video will be saved automatically once the recording stops.

5.3 Put the rat back into the anesthesia induction chamber for anesthetization. While the rat is under continuous anesthesia (administered via the anesthetic mask), remove the hemispheric markers.

NOTE: Remove the markers as gently as possible to avoid causing pain to the rat.

5.4 At the designated time (e.g., 1 week, 3 weeks, or 6 weeks after surgery), perform the kinematic measurement on the rat by repeating steps 3.1–5.3. Make the kinematic measurement only once, at the beginning of the experiment, for the rats that did not receive surgery (i.e., the control group).

6. Marker tracing

6.1 Open the calculation software and open the video file on the interface.

6.2 Click and drag the **bilateral control bar** on the progress bar of the video to ensure that only a 10-step treadmill walking record is displayed (Supplementary File 3). Click and drag each **characteristic point** from the 3D model on the bottom right corner of the screen to the corresponding marker on each of the four initial pictures of the videos that were taken by the cameras (Supplementary File 4).

6.3 Click on the **Automatically Tracing** icon to start the automatic marker tracing process (Supplementary File 5, Supplementary File 6). If the system does not accurately trace a marker, click on the **Manually Digitize** icon to switch to the manual tracing mode (Supplementary File 7).

click on the **tracing characteristic point** in the 3D model, and then on the **responding marker** in the picture.

6.4. Once the marker is clicked, ensure that the picture switches to the next frame of the video. Now continuously click on the marker until the marker tracing process is completed. Once finished, click on the **Save** icon.

7. 7. Kinematic analysis

7.1. Open the analysis software and then open the processed video file on the interface.

7.2. Click on the **Setting** icon and select and add designated parameters such as the ankle angle, toe angle, and pelvic shift (X and Z axes) to the **Display List** in the pop-up window on the right (**Supplementary File 8**). Click on **OK**, so that curves representing the value changes in the parameters appear on the interface.

7.3. Click on the **Measurement** icon and select **Smooth processing** in its pull-down menu. Enter **20 Hz** in the pop-up window to remove frequencies greater than 20 Hz within the curves (**Supplementary File 9**).

7.4. Ensure that there are five panels on the interface: the walking video of the rat, the dynamic 3D model, curves that represent value changes in the parameters from the 10-step cycle, curves that represent mean value changes in parameters, and histograms and schematic diagrams that represent the ratio of the stance and swing phase (**Supplementary File 10**).

7.5. Right click the panel for curves representing mean value changes in parameters and select **Data Output** in the pull-down menu (**Supplementary File 11**). This will produce the average values of the posterior limb joint angles, including the ankle and toe angles, pelvic shift, and any other desired parameters in 10-step cycle periods.

REPRESENTATIVE RESULTS:

We selected four parameters to investigate functional changes over time in a rat sciatic nerve crush injury model. These were the ratio of the stance-to-swing phase, center of gravity (CoG) trajectory, ankle angles, and toe angles in the 'toe off' phase⁹. Twenty-four rats were randomly assigned to one of four groups: the control group (C), rats at the first (1w), third (3w), and sixth (6w) week following left sciatic nerve crush injury.

By means of 3D kinematic analysis, the mean ratio of the stance or swing phase in the 10-step cycle was automatically calculated and represented on the interface (**Figure 1A–D**). We found that the ratio of the stance-to-swing phase was recovered following surgery.

The CoG is a virtual point that can be traced with a virtual marker by the 3D motion capture apparatus. It is located at the cross point of two lines connecting either of the two anterior superior iliac spines to their contralateral greater trochanters. Thus, the real time pelvic shift in

the coronal plane (X and Z axes) results in a simultaneous shift of the CoG as a 3D constructed pelvic model is used. This shift can also be automatically measured. The CoG trajectory is described as the changing curve of the pelvic shift mean value in the X and Z axes of the 10-step cycle. The normal CoG trajectory shape resembles the infinity sign (∞). We found that the CoG trajectory shape did not return to an approximately normal shape until 6 weeks after surgery (Figure 2A–D).

The normal ankle and toe angles in the “toe off” phase reach maximum value during the terminal stance of the step cycle¹⁵, but these parameters might be falsely reported if the rat has received surgery. Nevertheless, the 3D kinematic analysis allowed us to determine the angles in the “toe off” phase by referring to the video. The mean value of the ankle or toe angle in the “toe off” phase was calculated from the 10-step cycle. The results suggested that the ankle and toe angles, in the “toe off” phase improved in an upward direction after surgery. (Figure 3A–B).

FIGURE AND TABLE LEGENDS:

Figure 1: Bilateral stance and swing phase. The right swing (magenta), right stance (red), left swing (azure), and left stance (blue) phases are represented by their respectively colored bars. Yellow bars symbolize double support phases. Panels A–D show each bilateral stance and swing phase in 10-step cycle periods for the control group (A), 1w (B), 3w (C), and 6w (D) groups. C = control; 1w = 1 week post-surgery; 3w = 3 weeks post-surgery; 6w = 6 weeks post-surgery.

Figure 2: CoG trajectories. Panels A–D show representative average CoG trajectories during 10-step cycle periods for the control group (A), 1w (B), 3w (C), and 6w (D) groups. C = control; 1w = 1 week post-surgery; 3w = 3 weeks post-surgery; 6w = 6 weeks post-surgery.

Figure 3: Ankle and toe angles. Panels A and B show changes over time in the ankle and toe angles in the 10 “toe off” phases for the control group, 1w, 3w, and 6w groups (**p < 0.01, compared to the control group, ** p<0.01, compared to adjacent group. Error bars = standard error of the mean (SEM); C = control; 1w = 1 week post-surgery; 3w = 3 weeks post-surgery; 6w = 6 weeks post-surgery.

DISCUSSION:

In this protocol, a stable and continuously walking rat is the most vital component of kinematic analysis. The treadmill speed was set to 20 cm/s. This walking speed is by no means considered “high” if rats move without space constraints¹⁶. Nevertheless, this speed is too fast for untrained rats to stably walk on the treadmill and would likely result in an abnormal gait and nonuniform movements. These events may seriously affect data reliability and authenticity. However, treadmill speeds lower than 20 cm/s can cause the rats to intermittently stop walking, potentially producing large deviations and reduced data reliability. Therefore, training the rats to be able to steadily walk in a straight, frontward direction on the treadmill is extremely important if one is to achieve precise kinematic analysis.

Additionally, operators should not ignore the needs for reconfirmation and fine adjustments during the kinematic analysis process. We found that the swing phase accounted for 25% of the

step cycle in normal rats. This means that posterior limb movements during the swing phase accelerated to the point where the camera system was not able to accurately capture the movements continually and over time. Also, excessively bright or dim ambient light, stains on the transparent treadmill sheets, and abnormal movement patterns that incidentally occur while walking could result in an exaggerated deviation of the tracing labels from the markers attached to the rats. These factors could reduce the accuracy of the motion capture process. Manual adjustments were introduced into the marker tracing system to address this issue. Using manual adjustment, evident deviations or subtle motion capture losses can be immediately rectified during the marker tracing process. Moreover, reconfirmation of changes in the curves of multiple parameters processed using the kinematic analysis software aided in searching for and correcting flaws in the marker tracing process. Reconfirmation also allowed us to generate the most reliable and authentic data.

Compared to the kinematic analysis, the shortcomings of the SFI are mainly derived from its low accuracy and reliability, rather than from interferences produced by the abovementioned factors. A previous study also noted that the SFI method is neither reliable nor reproducible when applied during the early post-injury period¹⁷. On the other hand, the high accuracy and reliability of kinematic analysis has been widely recognized. However, many previous applications were only capable of observing and measuring designated angles, especially ankle angles^{10,15,18–20}. The limitations of two-dimensional (2D) video analysis prevents investigation of additional parameters during functional evaluations.

Three-dimensional kinematic analysis overcomes all the shortcomings of SFI and enables investigation of many additional parameters. The 3D digital model is constructed from images captured by four cameras. Consequently, this apparatus can measure or calculate parameters more accurately than conventional 2D kinematic methods. Therefore, kinematic analysis that uses the 3D motion capture apparatus holds enormous promise as a potential substitute for other functional evaluation methods.

However, the 3D kinematic analysis method has several limitations. Training rodents, attaching markers, and tracing examination processes are complicated and time-consuming. In order to obtain reproducible and reliable data, the operator should be well-acquainted with the critical steps required. The skin shifting that occurs during rodent walking is particularly likely to produce data deviations²¹. Furthermore, the high cost of 3D kinematic analysis equipment may impede its popularization and limit usage in relevant studies.

Previous studies have found that the 3D kinematic analysis achieved accurate and valid results in the context of the rat sciatic nerve injury model^{9,22}. Consequently, we have reasons to believe that this method may be a useful tool for functional evaluations of various trauma or disease states that involve the posterior limbs, including disorders of the central and peripheral nervous system and musculoskeletal diseases. Moreover, by altering the marker positioning, this method may be used to functionally evaluate anterior limb movements. Although these hypotheses require further verification through future experiments, we believe that kinematic analysis using a 3D motion capture apparatus may inspire more promising functional evaluation methods and

play an important role in research and clinical applications.

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DISCLOSURES:

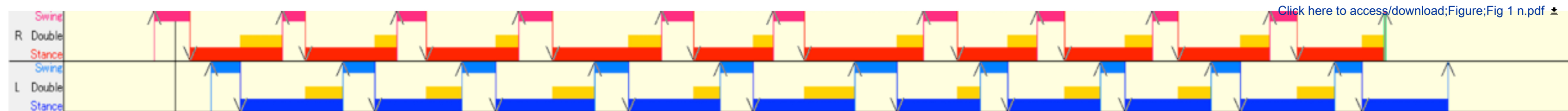
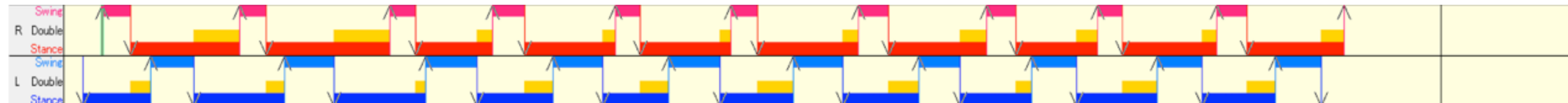
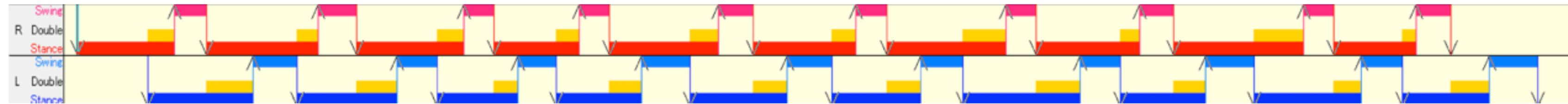
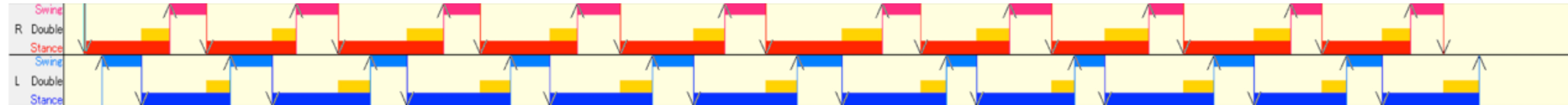
The authors have nothing to disclose.

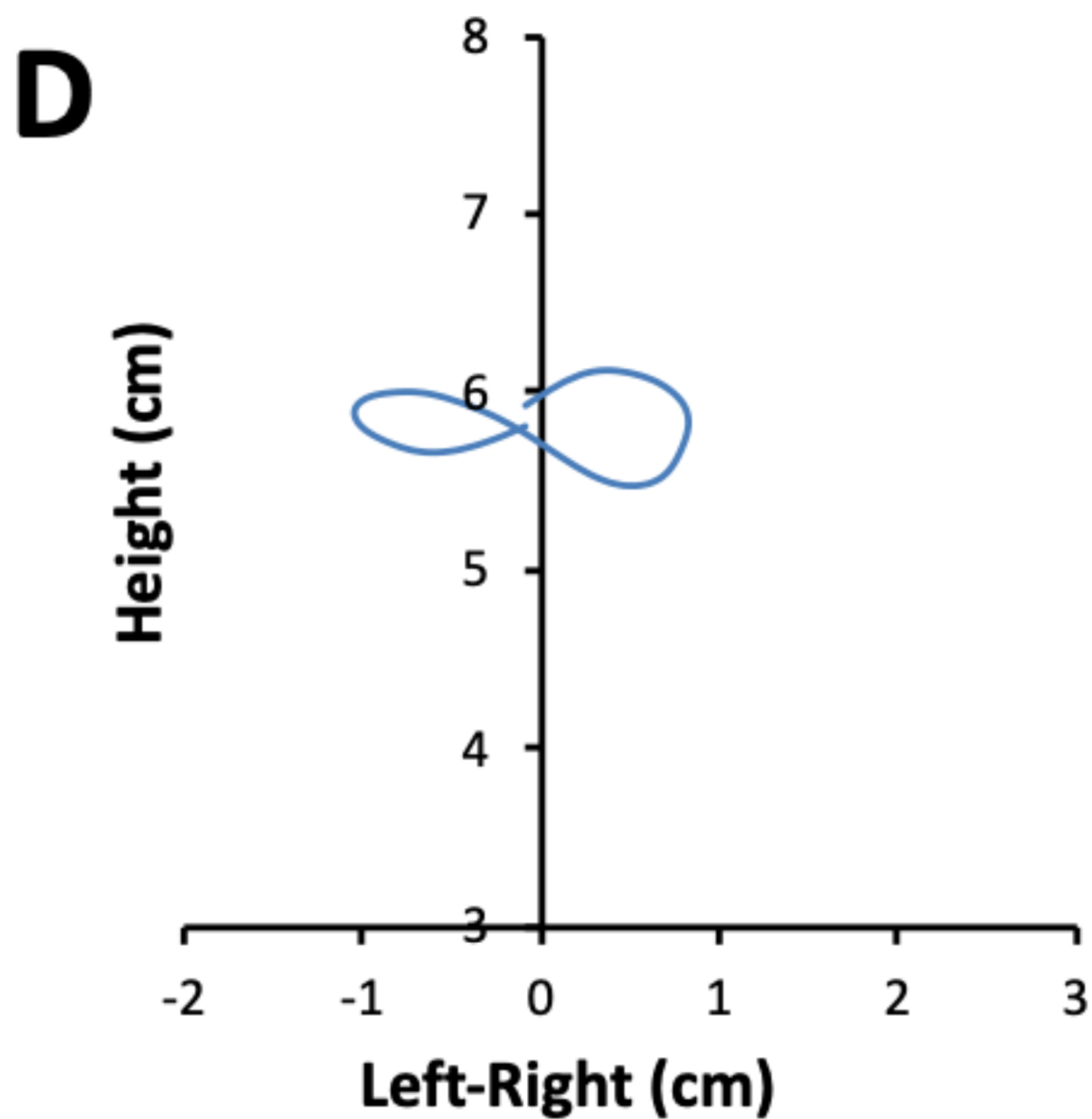
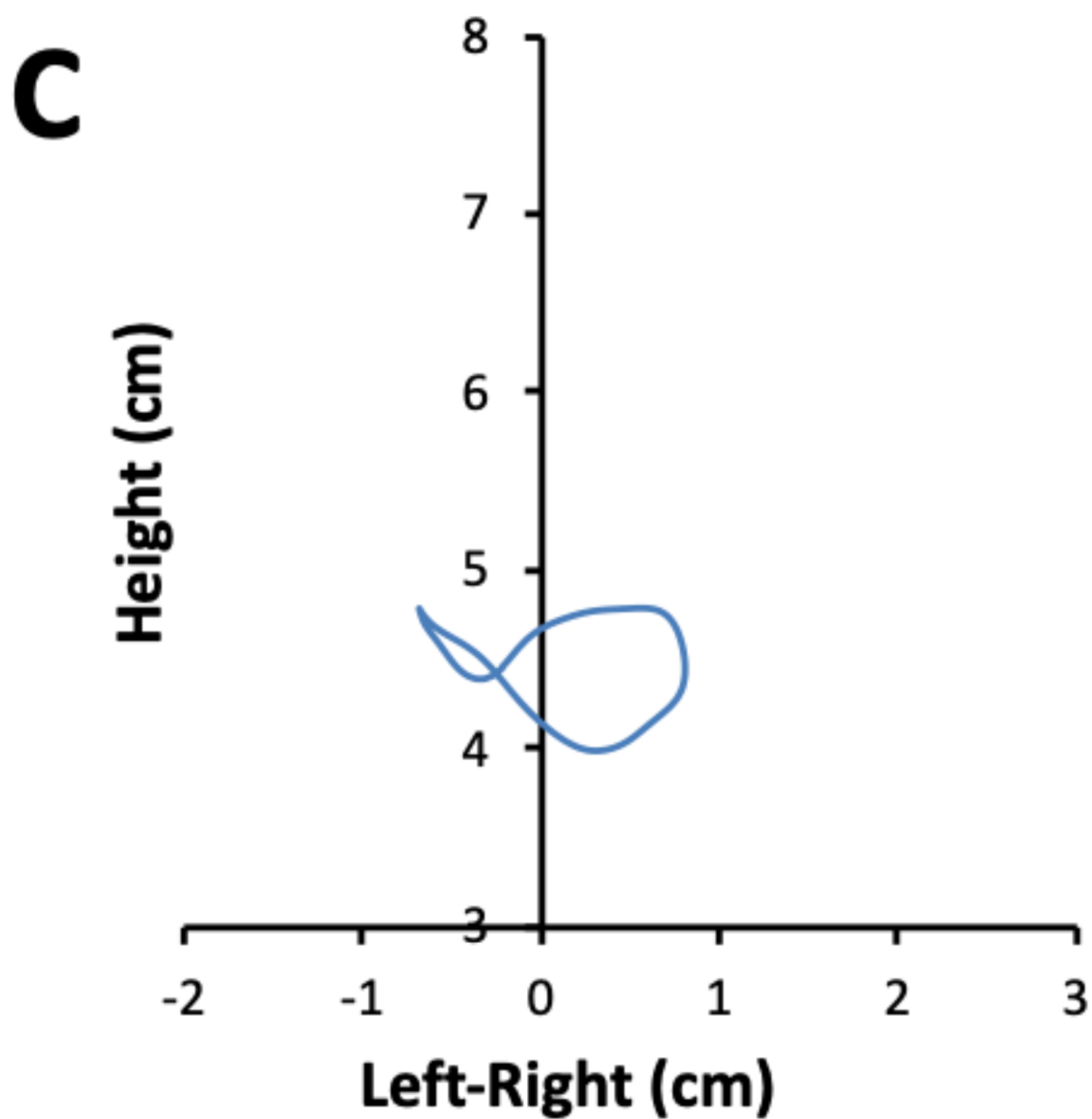
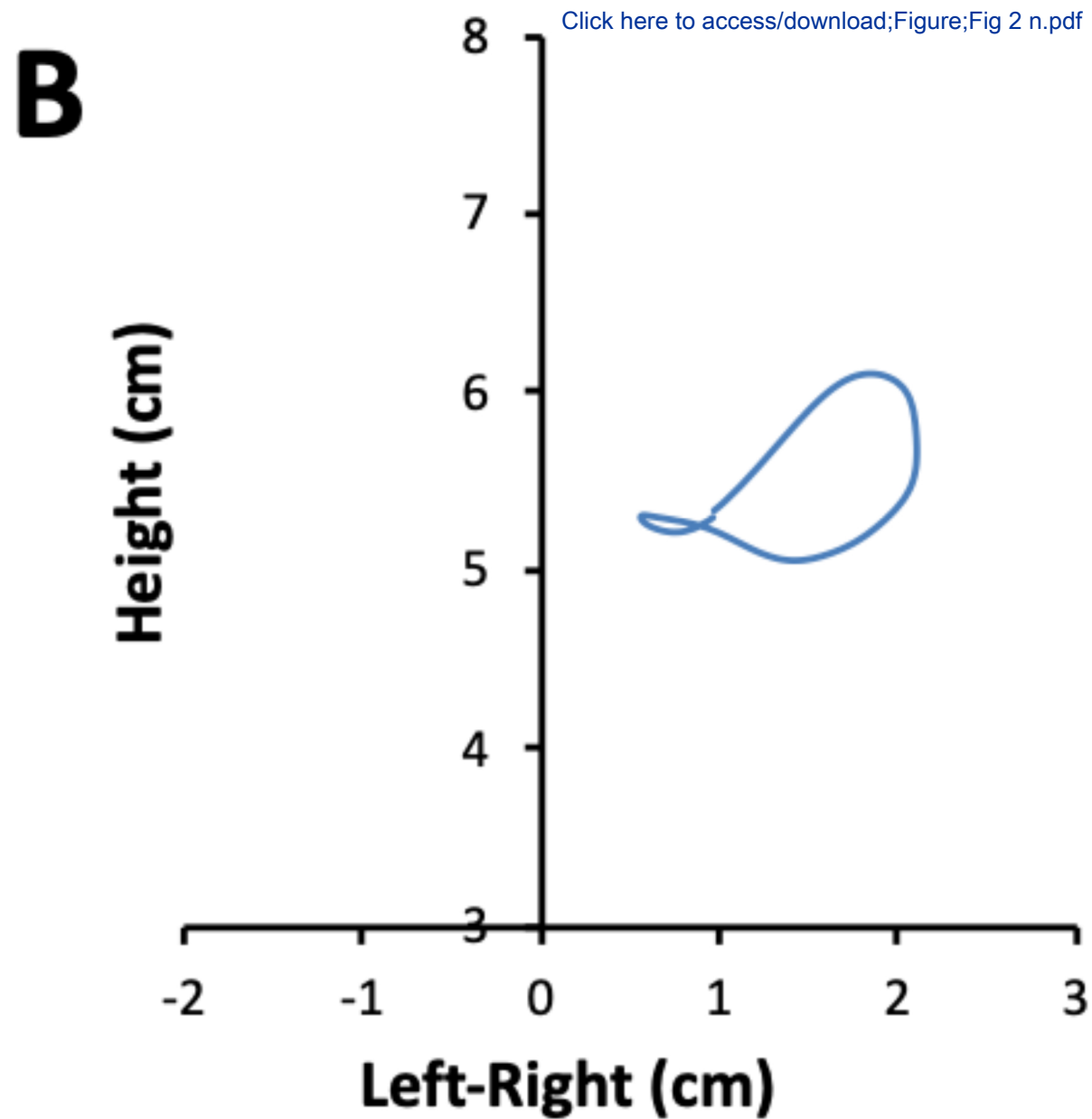
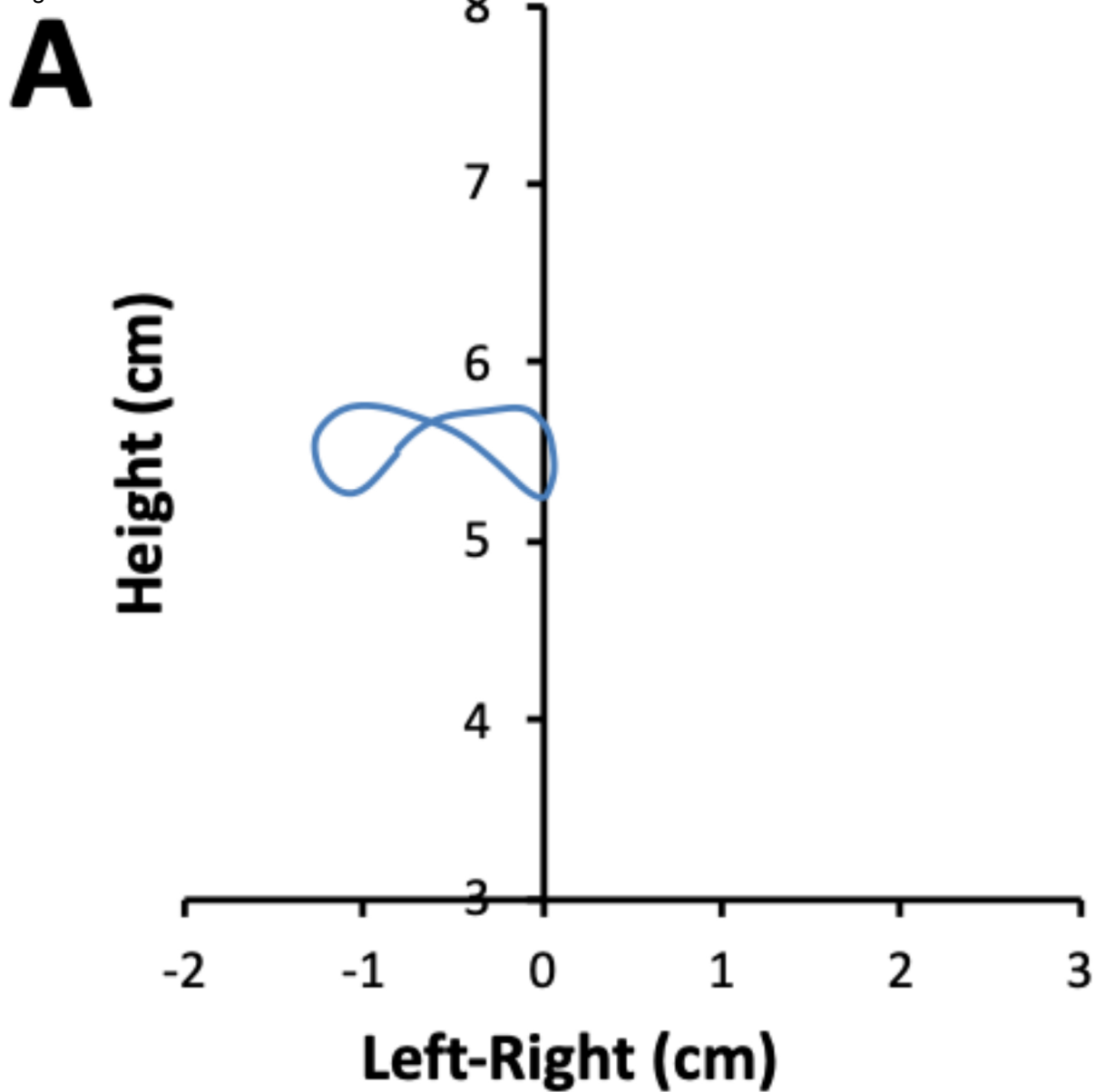
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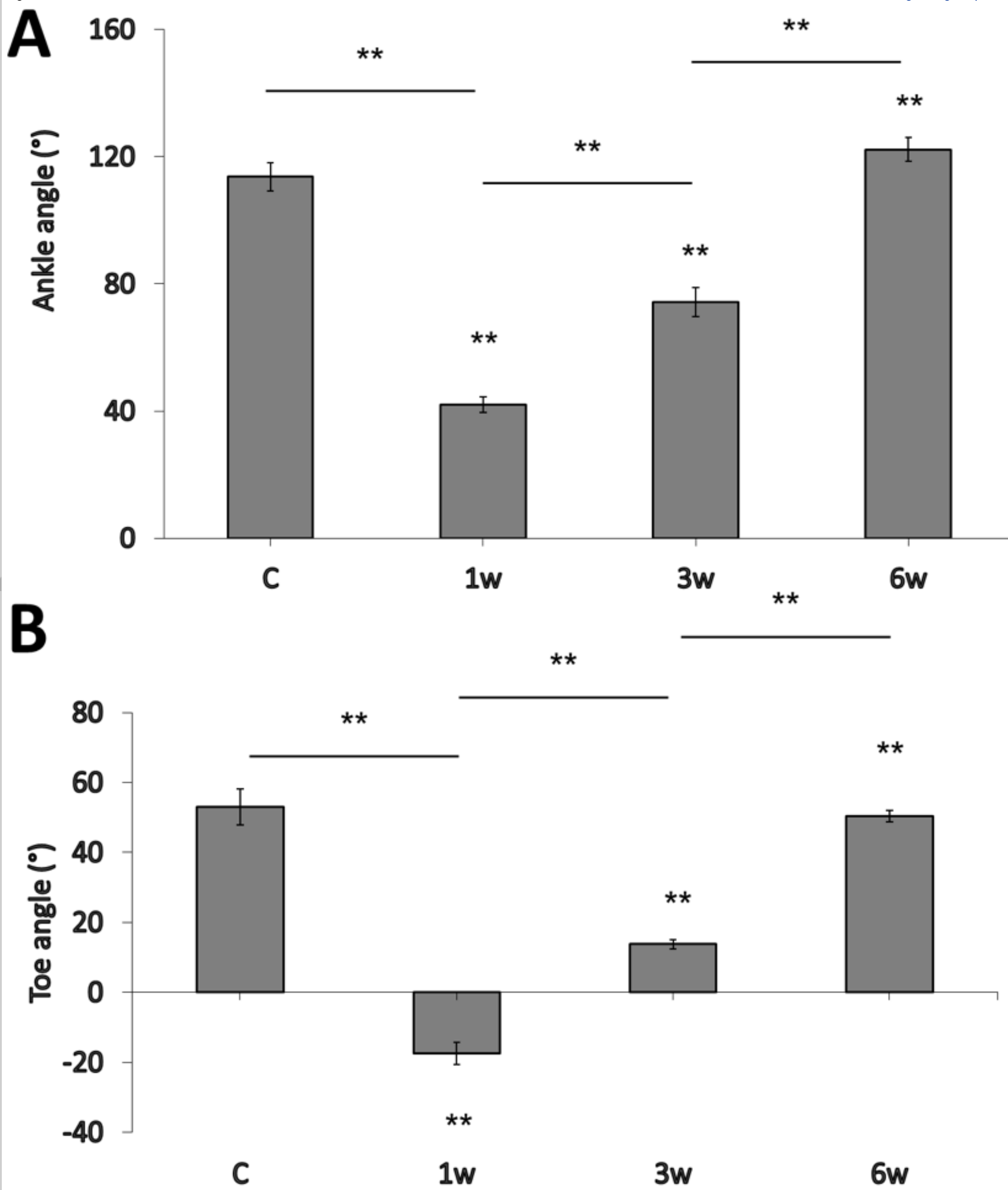
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Figure 1

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Name of Material/Equipment	Company	Catalog Number
9-0 nylon suture	Bear Medic Corporation.	T06A09N20-25
Anesthetic Apparatus for Small Animals	SHINANO MFG CO.,LTD.	SN-487-0T
ISOFLURANE Inhalation Solution	Pfizer Japan Inc.	(01)14987114133400
Kine Analyzer	KISSEI COMTEC CO.,LTD.	N.A.
Liquid adhesive	KANBO PRAS CORPORATION	PT-B180
Micro forceps	BRC CO.	16171080
Motion Recorder	KISSEI COMTEC CO.,LTD.	N.A.
Standard surgical hemostat	Fine Science Tools, Inc.	12501-13
Surgical blade No.10	FEATHER Safety Razor CO., LTD	100D
Surgical hemostat	World Precision Instruments	503740
Three-dimensional motion capture apparatus (KinemaTracer for Animal)	KISSEI COMTEC CO.,LTD.	N.A.
Three-dimensional(3D) Calculator	KISSEI COMTEC CO.,LTD.	N.A.
Treadmill	MUROMACHI KIKAI CO.,LTD	MK-685

Comments/Description

A analysis software

A recording software

A 3D motion analysis system that consists of cameras

A marker tracing software

a treadmill with affialiated the electrical schocker, transparent sheats and a speed control apparatus

ARTICLE AND VIDEO LICENSE AGREEMENT

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Author(s):	Tianshu Wang, Akira Ito, Junichi Tajino, Hiroshi Kuroki, Tomoki Aoyama

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Detailed Response to Reviewers

We sincerely thank the editor and reviewers for their thoughtful comments, which helped us improve our manuscript. The manuscript has been revised to address the concerns. The specific responses to the editor and reviewers can be found below. "Page X, Line XXX" mentioned below are from the revised manuscript with tracked changes.

Editorial Comments

1.As your article contains detailed, step-by-step, descriptions of software usage, the inclusion of supplemental screen capture or screenshots for the software usage would greatly expedite the scripting and production. You can either take screenshots of the software GUI or use screen capture software (<https://www.jove.com/video/5848/screen-capture-instructions-for-authors?status=a7854k>). Please include the manuscript number in these supplemental files and number the files in order of appearance: JoVE60267R1_screenfile1, etc..

[Response]

As suggested by editor, we added screenshots into supplemental files and included the manuscript number. (Page 4, Line 176; Page 5, Line187, 220; Page 6, Line 223, 226, 228,241, 246, 251, 254)

2. The editor has formatted the manuscript to match the journal's style. Please retain.

[Response]

Yes, we have done.

3. Please address minor specific comments marked in the manuscript.

[Response]

As suggested by editor, we revised them or added supplementary text into the manuscript. (Page 1, Line 2; Page 2, Line 70; Page 3-4, Line 97, 99, 118, 128-134; Page 5, Line 186; Page 6, Line 261)

4. Please include all the surgery materials as well in the materials table.

[Response]

We added all the surgery materials in the materials table.

5. Once done, please ensure that the protocol highlight is no more than 2.75 pages including headings and spacings.

[Response]

The aim of this protocol is to introduce a 3D kinematic analysis method, and it might not be much worth adding such general surgery into the protocol highlight. Moreover, if we included the section of surgery in the highlight, it would be more than 2.75 pages. Thus, we felt sorry that we have to delete the text on surgery from the highlight.



