Motion-capture Analysis of Mice Using a Video Recorded on an iPhone Camera

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Abstract

When focusing on quick movements in the analysis of animal behavior, a high-speed camera can be used as a powerful tool. There are many options for high-speed cameras to record movement. In recent years, the quality and sophistication of videos captured on cell phones have evolved so much that the iPhone's slow-motion video system can function as a tool for behavior analysis. Here, we describe a method to analyze the movement of the ankle joint and jump speed during the jumping action of mice, using an iPhone.

Keywords: Mouse, Jump, Speed, Ankle angle, Motor function, iPhone

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Background

Behavioral analysis is commonly performed in animals (Hart et al., 2009; Charles et al., 2016), and some studies have conducted gait analysis in mice, focusing on ankle motion for musculoskeletal research (Iwata et al., 2010; Suzuki et al., 2016; Suzuki et al., 2021). A camera is essential for this kind of analysis, and a high-speed camera is specifically useful for capturing quick movements (Iwata et al., 2010; Druelle et al., 2019; Pfeffer et al., 2019). Preparatory behavior just before a jump affects the jumping performance of animals, and analyzing the former is important for evaluating the latter (Bosco et al., 1982; Komi, 1984; Bosco et al., 1987; Bobbert and Casius, 2005). iPhone, a popular cell phone, has a high-speed camera mode (slow-motion video support). This mode can function as a tool for behavior analysis (Pittman and Ichikawa, 2013). In this protocol, we have introduced a method to evaluate the mouse jumping motion using an iPhone.

Materials and Reagents

1. Laboratory-bred mice
   Note: Both male and female mice were used for the test. The conditions described in this study (bar size and distance from the camera to bar) were suitable for over 12-week-old mice. Mice were housed in groups of 3–5 per cage and kept in a room with controlled temperature (~23 °C) and humidity (50–60%) under a 12-h light/dark cycle (lights on at 8:30 AM) with ad libitum *access to food and water.*

Equipment

1. iPhone 8 (MQ842J/A, Apple)
   Notes:
   a. *Slow-motion video support for 1,080 pixel at 120 or 240 frames per second (fps), video stabilization, and continuous autofocus video.*
   b. *The 240 fps slow-motion video support is available on almost all iPhone 6 and later models; therefore, it can be applied to models other than the iPhone 8.*
2. Tripod
3. Hair removal cream
4. Black marker pen
5. Steel jump bar with carbon plates
   Note: Dimensions: H: 0.8 cm (0.4 cm + 0.4 cm) × W: 4.5 cm × L: 100 cm.
6. Plastic ruler
7. Anesthesia machine with sevoflurane
   Note: This was used for a short time to mark the landmarks described below.

Software

1. Tracker (https://physlets.org/tracker/)
2. ImageJ software (https://imagej.nih.gov/ij/)
3. Microsoft Excel (Microsoft)

Procedure

*Note: This analysis was conducted to evaluate the jumping motion of mice. The mice were subjected to a training session. Five minutes before the beginning of the session, the mice were placed in the landing place (cage) for habituation. Then, at the beginning of the training session, the beam was placed in such a way that it touched the cage, and the mice were placed on the beam such that they could simply walk into the cage. After three successful entries at each distance, the beam was slowly moved further away from the cage in 5-cm increments. Through this process, mice could jump straight from the bar to the landing space (**Video 1**). The method for this long jump test has been described previously (Mittal et al., 2015).*

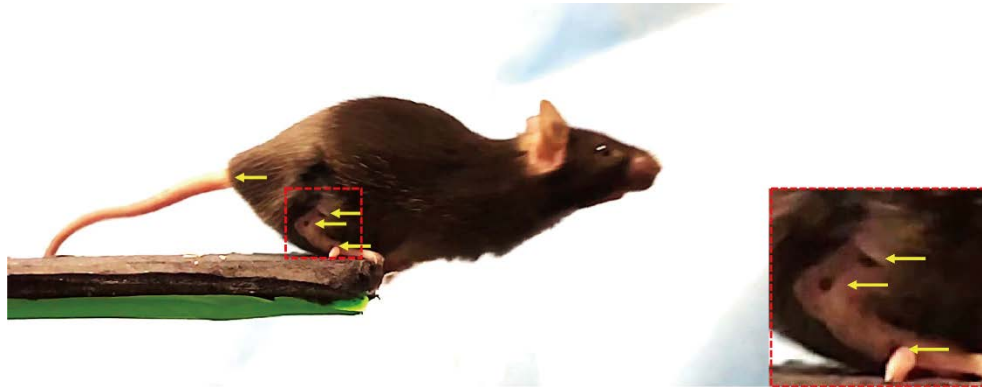**Video 1. Representative movie of a mouse jumping.**
This is an overview of the equipment setup. C57BL6J male mice jumped 35 cm from the bar into the landing space. For video camera analysis, the iPhone camera on the tripod was placed in front of the bar, as shown in **Figure 1.**
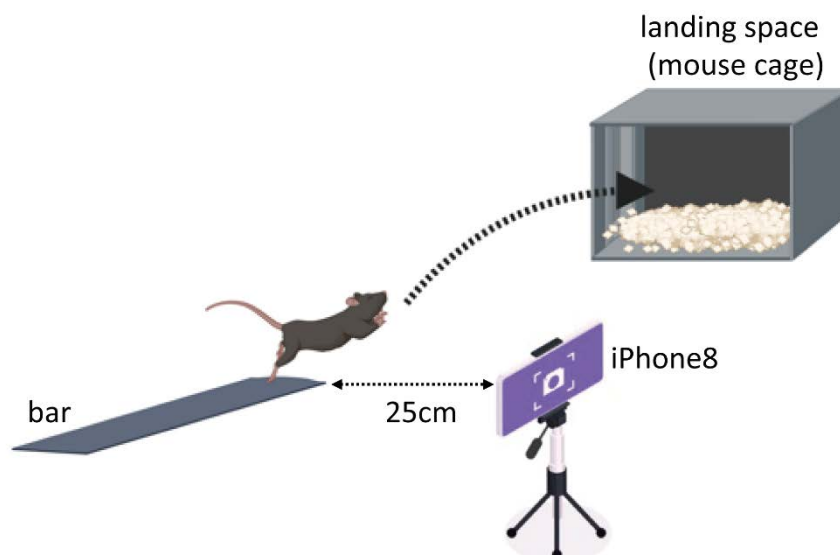
Figure 1. Schematic image of a video recorded using an iPhone camera (Created with BioRender.com)

1. Transport the mice from the breeding room to the laboratory in cages. Under anesthesia, shave the right hindlimbs of the mice using a hair removal cream, to analyze the motion of the ankle joint. Mark three

anatomical landmarks (the lower third of the tibia, fibula epiphysis, and fifth metatarsal head) with a black ink marker (Charles et al., 2016) (**Figure 2**). After this procedure, rest mice for 1 h to remove the effects of anesthesia.

Figure 2. Representative image of a mouse jumping.
Yellow arrows indicate the three ankle markers and tail ridge. The image on the right is an enlarged view of the hind limb (red dotted line).

2. Set up the jump bar 80 cm above the ground, and adjust the height of the camera vertically at the edge of the jump bar, such that the lens is at the same height as the jump bar. Adjust the distance between the jump bar and the camera, depending on the area to be photographed. For this analysis, we set the distance to 25 cm (**Figure 2**).

3. Set the iPhone to slow-motion video mode (Tap "setting" icon and select "General" → "Camera" → 1080p/240 fps). Let the mice jump from the jump bar to the landing space (**Figure 1**) (Mittal et al., 2015). Jumping is performed three times. Film all jumps, allowing a 3-min interval between jumps.
Note: The movie is excluded from evaluation if the mouse did not jump in a straight line from the jump bar to the cage.

Data analysis

1. Analyze the recorded videos using the Tracker software. Use a reference length in the movie as the calibration scale
Note: The thickness of the carbon plate was 4 mm, which was used as the calibration scale.

2. Manually define the coordinate positions of the markers for each image. The tracker displays the X- and Y-values of the marked coordinate positions. Reflect this information onto an Excel file, and analyze the data (**Figure 3**).

Figure 3. Representative image of marker identification.
The red, blue, and purple points indicate the ankle markers. The white points indicate the tail ridge.

3. Calculate the changes in the angle and angular velocity of the ankle joint, from the positional information of the three coordinates. Calculate the incident velocity and angle of incidence, based on the coordinates of the tail ridge and jump bar.
4. Analyze the centroid using ImageJ software by tracing the edge of the trunk and measuring the centroid to evaluate the change in potential energy (**Figure 4**). The tail is not included in the trace area, as the mice do not move their tails in the preparation phase.
   Note: How to use ImageJ: Briefly, open "Set measurement" and set "Centroid," then open "Analyze" and select "measure."
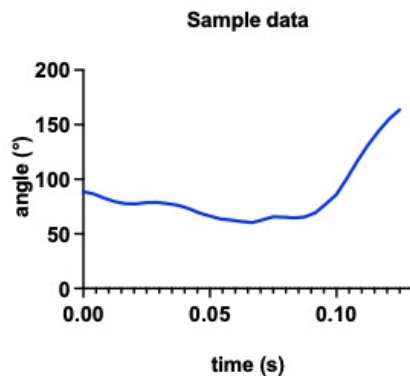

Figure 4. Representative image of centroid calculation.
The red line indicates the traced edge of the trunk in the preparation phase.

Representative data
Based on the video analysis data, graph the variation of ankle joint angle over time (**Figure 5**), and calculate the changes in angular velocity.

Figure 5. Represents the ankle angle of 12-week-old C57/BL6J mice.
It shows the change in ankle angle from the preparation phase of jumping to the take-off.

Acknowledgments

Competing interests

The authors declare that they have no competing interests.

Ethics

All animal experiments were performed with the approval of the Scripps Institutional Animal Care and Use Committee (protocol No. IACUC-09-0029).

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