Mouse Behavior Tracker: An economical method for tracking behavior in home cages

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Analysis of mouse behavior often requires expensive equipment and transfer of the mice to new test environments, which could trigger confounding behavior alterations. Here, we describe a system for tracking mouse behavior in home cages using a low-cost USB webcam and free software (Fiji and wrMTrck). We demonstrate the effectiveness of this method by tracking differences in distance traveled, speed, and movement tracks between wild-type mice and amyotrophic lateral sclerosis (ALS) model mice (SOD1^{G93A}).

Precise and accurate behavior analyses are essential for phenotyping disease-model mice and genetically modified mice (1). Although methods exist for measuring spontaneous behaviors of mice (actometers, running wheels, etc.), methods for quantifying distance traveled and tracking movement patterns in a home cage are limited. For example, running wheels allow measurement of the distance traveled in a home cage, but the results are complicated by the exercise effect of the activity in the wheel.

Commercial systems and custom systems can measure the distance traveled inside a cage and analyze mouse behaviors (Supplementary Table S1) (2–10); however, these systems use specialized cages, requiring animals to be transferred to a new environment for analysis. For some strains, altered behavior has been observed even after 3 weeks of habituation to a commercial metabolic cage system (11). Some commercial systems can analyze behavior in home cages, but one such system does not measure distance traveled (HomeCageScan; CleverSys Inc, Reston, VA), and another system monitors activity but does not accurately measure distance

traveled (Infrared Motion Detector; Starr Life Sciences Corp., Oakmont, PA).

Here, we address these limitations by developing a system to track mouse behavior using free public domain software for video tracking: wrMTrck and Fiji. wrMTrck was developed to track the behavior of *Caenorhabditis elegans* and has also been applied successfully to track melanoblasts, mitochondria, *Schistosoma mansoni*, *Xenopus tropicalis* tadpoles, and yeast (12–17). Our method, which we named Mouse Behavior Tracker, measures distance traveled, average speed, and movement patterns/tracks in home cages using economical USB-webcams, free software, and a standard desktop computer. When Mouse Behavior Tracker results were compared with a commercial system that uses a video tracking method, EthoVision XT (Noldus Information Technology Inc., Leesburg, VA), very similar results were obtained.

Materials and methods

Animal models

Animal experiments were performed in accordance with the animal care and

use protocol approved by the Institutional Animal Care and Use Committee of the University of Kansas Medical Center and its Guidelines for the Care and Use of Laboratory Animals. Three to six mice were analyzed for each genotype or coat color. Mice with black coat color—C57BL/6J wild-type male mice [JAX Stock No: 000664] and SOD1^{G93A} male mice [JAX Stock No: 004435; B6.Cg-Tg(SOD1*G93A)1Gur/J, high-copy number line on a pure B6 background]—were purchased from the Jackson Laboratory (JAX, Bar Harbor, ME) and analyzed in this study. Mice with brown coat color—Rosa tdTomato-EGFP, Wnt1-Cre male mice [Gt(ROSA)26Sor_tm4(ACTB-tdTomato,-EGFP)Luo_J (JAX Stock No: 007576) crossed with Tg(Wnt1-cre)11Rth Tg(Wnt1-GAL4)11Rth_J (JAX Stock No: 003829)]—and a littermate wild-type female mouse were analyzed in this study. White coat color mice—two female wild-type mice—were analyzed in this study. The mice used in this study had been bred in our institutional animal facility, kept on a 14 h light/10 h dark (6:00 am to 8:00 pm) cycle, and fed ad libitum with normal chow (53WU,

METHOD SUMMARY

Here, we present a new method for tracking mouse behavior in home cages. Our approach allows quantification of distance traveled, average speed, and movement tracks using a significantly lower-cost system compared with commercial alternatives.

PicoLab Rodent Diet 20; LabDiet, St. Louis, MO). No statistical methods were used to predetermine sample size. A method of randomization was not used for comparisons of disease-model to wild-type mice because the animals were separated by genotype.

Video recording

Mouse behavior was recorded using an economical webcam (Creative WebCam Instant Model VF 0040; Creative Technologies Ltd. Jurong East, Singapore) with the following settings: 320 × 240 pixels, 30 frames per second (fps), MJPEG compressor. Recordings were done between 12:00 PM and 4:00 PM under room ceiling fluorescent lighting as 20-min videos.

Tracking mouse behavior using Fiji

The analysis protocol described here was developed for macOS 10.12 using Fiji (version 1.51h, <https://fiji.sc/>) running on a MacBook Pro (Apple Inc., Cupertino, CA) (CPU: Intel Core i7 2.8 GHz; flash storage: 751 GB; RAM: 16GB 1.6 GHz DDR3; graphics: NVIDIA GeForce GT 650M 1024 MB; operating system: macOS 10.12.6). The RAM size of 16 GB was sufficient to process 30-fps videos up to 30-min long. The combination of Fiji, wrMTrck plugin, and the Mouse Behavior Tracker macro was confirmed to function properly on Mac OS X 10.9, 10.11, and macOS 10.12. Mouse behavior was tracked using the publicly available free Fiji software, the wrMTrck plugin for ImageJ (wrMTrck.zip developed by Jesper Søndergaard Pedersen; www.phage.dk/plugins/wrmtrck.html), and our Mouse Behavior Tracker macro. The Mouse Behavior Tracker source codes for the AVI (MJPEG) file format and MP4 file format are included with the protocol in the Supplementary Material. The same video data files were analyzed using EthoVision XT (Noldus

Figure 1. The Mouse Behavior Tracker method. Schematic diagram of the video recording setup showing the side view (A) and the top-down view (B), with dimensions. (C) A mouse being recorded by a Creative WebCam Instant Model VF 0040. (D) A screenshot of the Creative camera software showing a recording of a mouse. Representative images are shown from a raw video of a C57BL/6J wild-type mouse recorded at 30 frames per second (fps) (E), an average intensity-projected image showing the cage without the mouse (F), the differential binary-image video showing the mouse after subtracting the projected empty cage image from the raw video (G), the tracking video showing the mouse with a tracking number assigned by Mouse Behavior Tracker (H), and movement tracks of the mouse for a 1-min period (I). Scale bar in (E): 10 cm.

Information Technology Inc., Leesburg, VA) for validation.

Statistics

All statistics were performed using GraphPad Prism software version 6 (GraphPad Software, Inc., La Jolla, CA). Significance was assessed by an unpaired *t*-test with a two-tailed *P* value. The *P* value and the *n* value are described in the text, and the data are shown as mean ± SEM.

A detailed protocol is available in the online Supplementary Material.

Results and discussion

Mouse behavior was recorded in a home cage with an inner lid, without the filter tops, using a webcam positioned above the cage on a chromatography column stand with adjustable clamps. An economical USB webcam provided sufficient sensitivity, resolution, and frame rate (Creative

live webcam instant VF 0040, 320 × 240 pixels, 30 fps) to record mouse behavior in the home cage (Figure 1, Supplementary Video S1). File sizes for 20-min videos averaged 123 MB. Therefore, a desktop computer (Dell Precision PWS390; Dell, Inc., Round Rock, TX) with standard hard disk storage space was sufficient for the recording (CPU: Intel Core2 Duo 6600, 2.4 GHz; RAM: 2 GB 2.3 GHz; internal hard disk: 148 GB; operating system: Windows XP Professional Version 2002 Service Pack 3).

The 20-min videos were analyzed using Fiji (ImageJ package software) running on an iMac 14.2 (Apple Inc.) with RAM size increased to 24 GB (CPU: Intel Core i5, 3.2 GHz; hard disk size: 1 TB; RAM: 24GB, 1.6 GHz DDR3; operating system: Mac OS X 10.9.5). Videos were Z-projected in Fiji using the “Average Intensity Type” to generate an image of the cage without a mouse (Figure 1F). This cage image was subtracted from each frame of the 20-min video to generate a video of a differential binary image of mouse behavior without the cage (Figure 1G). The differential binary image video was processed using the “Gaussian Blur” filter to remove shadows resulting from the wire lid of the cage above the animal. Resulting time-lapse video was thresholded and tracked using Fiji and the wrMTrck plugin for ImageJ. The wrMTrck plugin is based on the Object Tracker

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plugin developed by Wayne Rasband, who developed ImageJ at the NIH. The two plugins use the Pythagorean theorem to calculate the distance traveled by a tracked object. The 20-min video was trimmed down to 1 min to show representative results of a movement track and a tracking video (Figure 1H, Supplementary Video S2). A representative result of a movement track generated from a 20-min video is shown in Figure 3C. These results suggest that Mouse Behavior Tracker is capable of tracking mouse behavior in videos ranging from 1 min to 20 min. A Fiji macro was created for these processes (described in the Supplementary Material). Mouse Behavior Tracker generates the following data: (i) tracking data (distance traveled and average speed) as raw and summarized data in Microsoft Excel files, (ii) video of mouse tracking with the tracking object number on the tracked mouse, and (iii) movement tracks (Figure 1I). Mouse Behavior Tracker gave identical results when the same video of a mouse with a black coat color was analyzed three times. Thus, Mouse Behavior Tracker allowed us to quantify mouse activity in a home cage.

One commercial system, EthoVision XT, uses video tracking methods and quantifies distance traveled in home cages. Therefore, we compared Mouse Behavior Tracker and EthoVision XT by analyzing distance traveled in the same video using the two programs. The distance traveled results were very similar, with the ratio of the results obtained from the 2 programs (EthoVision XT result divided by Mouse Behavior Tracker result) being 0.98 ± 0.03 (mean $\pm$ SEM, $n = 6$ wild-type C57BL/6J mice) (Figure 2); this demonstrates the accuracy of Mouse Behavior Tracker for distance traveled measurements.

We also analyzed mice with brown or white color coats using these two software programs. Both programs could track mice with brown coats. Mouse Behavior Tracker gave identical results when the same video of a mouse with brown coat color was analyzed three times. However, the EthoVision XT quantified 20% less distance traveled compared with the Mouse Behavior Tracker results using the settings for tracking mice with black coat color. The ratio of the results obtained from EthoVision XT compared

Figure 2. Evaluation of Mouse Behavior Tracker method. The distance traveled by mice in 20-min-long videos recorded at 30 frames-per-second (fps) was analyzed using the Mouse Behavior Tracker macro or the EthoVision XT software. (A) The graph shows the distance traveled by C57BL/6J wild-type mice, and each line represents one video analyzed using the two programs. (B) The distance-traveled ratio was obtained by dividing EthoVision XT results by Mouse Behavior Tracker results. The graph shows mean $\pm$ SEM (red whiskers). Very similar results were obtained using the two programs.

to Mouse Behavior Tracker was 0.71 ± 0.1 (mean $\pm$ SEM, $n = 3$ mice). The difference in distance traveled quantified by the two software programs requires further investigation. Mice with white coats could not be tracked by either Mouse Behavior Tracker or EthoVision XT using the settings that track mice with black coats. The inability to track white mice is likely due to the reduced contrast between the white coat color and the corn cob bedding in the cages.

Finally, Mouse Behavior Tracker was used to analyze the behavior of amyotrophic lateral sclerosis (ALS) model mice (SOD1^{G93A}; 139–155 days old) and age- and sex-matched wild-type controls (C57BL/6J; 141–168 days old) in their home cages. The mean distance traveled during a 20-min recording was 13.79 ± 2.03 m (mean $\pm$ SEM, $n = 5$ mice) for symptomatic-stage SOD1^{G93A} mice. However, wild-type mice traveled a significantly longer distance of 37.24 ± 4.68 m ($n = 6$ mice; $P = 0.0021$, unpaired t -test) (Figure 3A). In addition, the average speed of SOD1^{G93A} mice was also significantly slower than that of wild-type mice (SOD1^{G93A}: 1.86 ± 0.07 cm/s, $n = 5$ mice; wild-type: 3.49 ± 0.43 cm/s, $n = 6$ mice; $P = 0.0076$, unpaired t -test) (Figure 3B). Furthermore, the movement pattern reflected behavior differences between the two mouse strains. SOD1^{G93A} mice moved slowly with alternating lateral displacements of the body, which were represented by characteristic zigzag tracks at the 30-fps video sampling rate

Figure 3. Amyotrophic lateral sclerosis (ALS) model SOD1^{G93A} mice are less active than wild-type mice and show tottering behavior.

Graphs show distance traveled (A) and average speed (B) during the 20 min of recording time. Symptomatic-stage SOD1^{G93A} male mice (SOD1 139–155 days old) traveled a shorter distance at a slower speed than age- and sex-matched wild-type mice (WT, 141–168 days old). A total of $n = 5$ –6 mice were analyzed. The graph shows mean $\pm$ SEM (red whiskers), and each dot represents one mouse. Asterisks indicate significance by an unpaired t -test ($P < 0.01$). (C) Representative movement tracks indicate a higher activity level for wild-type mice than SOD1^{G93A} mice during the 20-min recordings at 30 frames per second (fps). (D) The tracks of a wild-type mouse show a smooth and balanced movement pattern during a 1-min period; however, the tracks of a SOD1^{G93A} mouse indicated tottering behavior with a zigzag pattern during the same amount of time. These tracks are portions of the tracks shown in (C).

(Figure 3D, Supplementary Videos S3 and S4). This tottering movement of SOD1^{G93A} mice may be related to gait differences reported previously for this ALS animal model (18–21). Meanwhile, wild-type mice moved quickly and in a balanced manner, yielding smooth tracks without zigzag tracks. These results demonstrated that there are differences in the activity levels (distance traveled and average speed) and movement patterns between SOD1^{G93A} mice and wild-type mice in their home cages.

Measurement of spontaneous activity in non-exercised control animals is seldom undertaken; however, it is essential to compare activity of sedentary animals in home cages with exercised animals with running wheels or forced exercise activities. This is partly due to a lack of simple and economical methods for measuring

the distance traveled of sedentary animals in their home cages. Systems to analyze home-cage mouse behavior have been developed to quantify behavioral phenotypes (4) and social interactions (7), and mouse behavior has been analyzed using time-lapse VCR systems (22,23); however, these systems do not measure the distance traveled in home cages. Mouse Behavior Tracker is an efficient and low-cost method for measuring distance traveled and movement speed, as well as displaying movement tracks in home cages. Furthermore, this system generates tracks for detecting abnormal behavior, including tottering. Finally, home cage-based analysis is relevant for studies of aging, disease models, genetic manipulation, and pharmacological treatments. It should be noted that for long-term observation over many days or weeks, a commercially available system would be more suitable.

Author contributions

H.N. designed the experiment. S.T., N.N., and H.N. performed the experiments and analyzed the data. J.R. contributed essential technical

expertise on video recording methods. S.T. and H.N. wrote the manuscript, and all authors reviewed the manuscript.

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Competing interests

The authors declare no competing interests.

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