Video Article

A Novel Single Animal Motor Function Tracking System Using Simple, Readily Available Software

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Abstract

We have recently demonstrated that implanting intracortical microelectrodes in the motor corteces of rats results in immediate and lasting motor deficits. Motor impairments were manually quantified through an open field grid test to measure the gross motor function and through a ladder test to measure the fine motor function. Here, we discuss a technique for the automated quantification of the video-recorded tests using our custom Capadona Behavioral Video Analysis System: Grid and Ladder Test, or BVAS. Leveraging simple and readily available coding software (see the **Table of Materials**), this program allows for the tracking of a single animal on both the open field grid and the ladder tests. In open field grid tracking, the code thresholds the video for intensity, tracks the position of the rat over the 3 min duration of the grid test, and analyzes the path. It then computes and returns measurements for the total distance traveled, the maximum velocity achieved, the number of left- and right-handed turns, and the total number of grid lines crossed by the rat. In ladder tracking, the code again thresholds the video for intensity, tracks the movement of the rat across the ladder, and returns calculated measurements including the time it took the rat to cross the ladder, the number of paw slips occurring below the plane of the ladder rungs, and the incidence of failures due to stagnation or reversals. We envision that the BVAS developed here can be employed for the analysis of motor function in a variety of applications, including many injury or disease models.

Video Link

The video component of this article can be found at https://www.jove.com/video/57917/

Introduction

There are many established methods to assess both functional and behavioral motor and cognitive impairments[1,2,3]. Some of the more commonly employed methods include testing fine motor function *via* paw placement, stepping, and limb coordination on a ladder test[4], testing gross motor function and stress behavior *via* the open field grid test[5,6], and testing for fear, depression, and despair *via* the forced swim test[7,8] or rotor rod[9]. However, many of these methods rely on human researchers to "score" the animal or to judge its performance subjectively. The need for a subjective human assessment can slow the generation and analysis of the data, as well as present the opportunity for an intentional or unintentional influence of research bias in the study[10]. Further, subjective assessment of the data also presents the risk of inaccurate data representation, be it through forgetfulness, poor motivation, improper training, or negligence[11].

We have recently reported the use of both an open field grid test and a ladder test in rats implanted with intracortical microelectrodes[12,13]. Due to the novelty of the findings in those studies, we immediately began employing those and additional functional testing in many ongoing studies in the laboratory. In anticipation of unintentional human-generated variability resulting from an increase in the number of subjective evaluators, and to improve the analysis throughput, we set out to create an automated, computer-assisted program to score behavioral testing, and greatly limit the potential for error.

Here, we report on the development of the BVAS. The BVAS uses computer analysis to score an open field grid test and a ladder test as metrics of gross and fine motor function, respectively. The results can be used to elucidate possible motor function deficits caused by injury or disease, regardless of the injury or disease model. The analysis codes can be adapted to account for changes in behavioral testing equipment or to score various metrics of motor function. Therefore, the BVAS can be implemented in many applications, beyond our intended use or the intended use of those currently employed by other laboratories.

Note that the open field grid and ladder tests require video recording. Therefore, each test will require a video camera [1080 p, minimum 15 frames per second (fps)], a laptop, and a room to store the video data. For both tests, place the camera in a centered position, allowing for the whole apparatus to be seen on the frame. Anchor the camera on a tripod or scaffolding so that it does not move during the testing. Keep the edges of the video frame as close to parallel with the edges of the testing apparatus as possible. Be sure the same personnel complete all testing and the room is well-lit with a temperature-controlled system. Use the same room for all animals throughout the course of the testing, with minimal changes to the room. Cereals or banana chips make good rewards to encourage the animals to complete the behavior tests.

Protocol

All procedures and animal care practices were approved by and performed in accordance with the Louis Stokes Cleveland Department of Veterans Affairs Medical Center Institutional Animal Care and Use Committee. The behavioral testing protocol closely follows previously published work[12,13].

1. Behavioral Testing: Filming the Tasks

NOTE: Here, the animals were tested for 8 weeks to detect any chronic behavioral changes. The study duration is dependent on the application/injury/disease model used for the study.

1. **Open field grid testing**
   NOTE: The grid test has a 1 m^2 area with 40 cm walls and was built in-house. The bottom is partitioned into nine squares *via* brightly colored tape (**Figure 1A**). For the use of the automated BVAS, it is important that the color of the line and the grid contrast with the rat. Here, white Sprague Dawley rats were used; thus, the grid background was painted black, and the lines were created with bright pink tape.
   1. Test the animals 2x per week for all tests including the week before beginning the study in order to create a baseline score for use in later calculations.
   2. Clean all the testing equipment with a chlorine dioxide-based sterilant at the beginning of each testing session and between each animal.
   3. Bring the animals to the room for testing and allow them to adjust to the room for 20 min prior to starting the trials.
   4. Begin filming and place the animal in the center of the grid, facing away from the researcher, to begin the grid test.
   5. Allow the animal to run freely for 3 min while being video recorded from above.
   6. Stop filming when the 3 min time period has ended and return the animal to its home cage. Clean the grid with a chlorine dioxide-based sterilant, taking care to fully dry the surfaces before testing. Test each animal 1x per session.

2. **Ladder testing**
   NOTE: The ladder test was built in-house and consists of two acrylic side walls, each 1 m in length, connected by 3 mm diameter rungs spaced at 2 cm intervals (**Figure 2**). The animals require a week of ladder training before beginning the official testing. There is no difference between the training and the testing protocol. Note that the training does not need to be filmed.
   1. For the use with the BVAS, cover the wall behind the ladder with a black poster board and delineate the level of the rungs, start, and finish lines with bright tape to create a contrast with the white rats.
   2. Test the animals 2x per week for all tests including the week before beginning the study to create a baseline score for use in later calculations.
   3. Clean all the testing equipment with a chlorine dioxide-based sterilant at the beginning of each testing session and between each animal.
   4. Bring the animals to the room for testing and allow them to adjust to the room for 20 min prior to starting the trials.
   5. Place the animal in a clean temporary cage before the ladder testing.
      NOTE: A temporary cage is a short-term cage to hold the animal while the animal's home cage is used at the end of the ladder. There is no age bias as all animals used in the study are the same age and complete testing over the same time period. The control used here is a naïve animal having never received surgery.
   6. Set the ladder apparatus up to span two cages; the start cage is a clean cage, and the end cage is the animal's living cage, an incentive to complete the run.
   7. Place the camera on a tripod, centered on the length of the ladder. Extend the tripod so the lens of the camera is at the height of the ladder rungs. Position the camera so that the rungs are exactly aligned with the lens as this is important for the slip detection algorithm in the BVAS code.
   8. Start the video recording and allow the rat to begin the run by holding their front paws over the first rung of the ladder. Allow the animal to move onto the ladder unassisted.
   9. Allow the animal to move from the start line to the finish line at their own pace while filming. End the video recording and remove the animal from the ladder once the animal has completed the run.
   10. Consider the run a failed run if the animal turns around or remains stagnant for 20 s. End the video recording and remove the animal from the ladder if the animal fails the run. Assign a penalty score for failed runs which is equivalent to the slowest time recorded during the pre-surgery baseline testing.
   11. Ensure that each animal completes 5 runs per testing day and give them a 1 min rest period between each trial.
       NOTE: In this study, the animals were tested 2x per week for 8 weeks. However, the timeframe is up to the discretion of the researcher.

2. File Storage and Naming

NOTE: The BVAS code uses specifically designated video file and folder naming conventions so that videos may be reliably parsed and analyzed properly. Different naming conventions are currently not supported. After the completion of a testing session, the video file is saved in the default location under a default name.

1. **Individual video naming**
   1. Name each video with the animal's designation for all grid testing videos.
   2. Collect 5 ladder videos per testing session for each animal. Select all five videos and rename the first video, using the animal's designation. For example, if the animal's name is "C1NS", the files are named "C1NS (1)", "C1NS (2)", "C1NS (3)", *etc.*

2. **Storage folder naming**
    1. Take the videos now named after each animal and place the files in their own storage folder. Name this folder using the following convention: "TestingWeek_TestMode_MM_DD_YY".
    NOTE: For example, if this folder holds the first week of grid videos and was filmed on January 1st, 2018, the folder holding these videos is named "Week1_GridTest_01_01_18".
    2. Place this specifically named folder in another folder that will denote the larger study that this set of experiments belongs to.
    NOTE: There are no naming requirements for the folder tree system to keep studies and testing modes organized. The only folder the system selects is the folder holding videos named in step 2.2.1.

3. **Spreadsheet file creation**
    1. Using an outside spreadsheet program (see the **Table of Materials**), create a new empty spreadsheet file to store the data for each study and testing mode.
    NOTE: There are no naming or storage location requirements for this file. Each study requires two spreadsheet files for use with the system, one for grid testing and one for ladder testing. Leave these files empty for now.
    2. Check to ensure these spreadsheet files are not open on any computers while the BVAS is running.
    NOTE: If the BVAS attempts to open a spreadsheet file that is already open, this will result in an error.

3. System Installation

NOTE: The BVAS was built and tested on PC operating systems and leverages Runtime components from simple and readily available coding software. Other system configurations are currently not supported.

1. **Installing latest BVAS version**
    1. Run the installer by double-clicking on the installer icon labeled '*BVASX.XXinstaller*'. "X.XX" is the current version number, currently '*BVAS3.30installer*'.
    NOTE: This is open source software. Contact the corresponding author for the most recent version.
    2. Follow the on-screen instructions to install the BVAS.
    NOTE: The installer will check the system for the correct version of the software (*e.g.*, MATLAB Runtime) and if it is not found, it will install the software from the internet.
    3. Once installed, launch the program by clicking the '*BVAS.exe*' file.

4. System Use

1. **Opening the program**
    1. Double-click on the BVAS program icon to bring up the BVAS main menu which allows the following options: '*Grid Analysis*', '*Ladder Analysis*', '*Ladder Review*', or '*Quit*'.
    NOTE: There is also a drop-down menu located in the top left corner labeled '*Email Settings*' (**Figure 3A**).

2. **Email settings**
    1. Click on the '*Email Settings*' drop-down menu and first select '*Change email destination*'.
    2. Enter a preferred email address to receive updates on the analysis and select '*OK*'. Select 'Cancel' to not change the email currently saved.
    3. Click the '*Send Test Email*' button to send a test email to the email currently saved in the system. Close the '*Email Sent!*' window pop-up signifying the test's completion.

3. **Open Field Grid Test Analysis**
    1. Select '*Grid Analysis*' from the top menu screen.
    2. Ensure the toggle button labeled '*Single Video/Whole Session/Plot Data*' is displaying Whole Session to complete the analysis on a whole set of testing session videos.
    3. Utilize the '*…*' button next to the upper input box to browse the file system to find the file of videos to analyze. Select the folder labeled in the style discussed in step 2.2.1.
    4. Utilize the … button next to the lower input box to browse the file system to select the spreadsheet file in which to store the data.
    NOTE: Note that, as well as outputting the data to a spreadsheet file, the complete path data for each video is stored in a data file (.mat) in the same directory that holds the videos chosen in step 4.3.3.
    5. Ensure the toggle button mentioned in step 4.3.2 is set to '*Single Video*' to complete the analysis on a single video.
    6. Utilize the … button next to the upper input box to browse the file system to find the file of the video to analyze.
    7. Utilize the … button next to the lower input box to browse the file system to select a directory to save the data file (.mat) that results from the single video analysis.
    NOTE: The data file will be named after the video, which is named after the animal.
    8. Select '*Output Data*' from the toggle mentioned in step 4.3.2. to output the data from a previously analyzed grid video. Examples of this plotted data are shown in **Figures 1B** and **1C**.
    9. Utilize the … button next to the upper input box to browse the file system to select a previously created grid video data file. Select '*Go!*' to create the plot and data figure.
    NOTE: This function also creates a text file, named the same and located in the same directory as the data file, which has the animal's run data in it.

10. Once both necessary files have been located, select '*Go!*' to begin the video analysis. Note that the '*Return*' button will reopen the top menu and the '*Quit*' button will close the program completely. Choose the '*Debug Display*' toggle to show a visual representation of the tracking algorithm (**Figures 3C** and **3D**).
11. Select '*Go!'* to start the analysis process. Examine the images of each video on the reviewer screen. Use the '*Next*' and '*Previous*' page buttons if there are more than five videos.
12. To bring up a manual selection menu, select the '*Edit*' button if there are no green lines or the green line does not appear to be in the correct square around the testing area. To edit the corners, select the 'Edit Corners' button.
NOTE: The '*Next Frame*' button changes the representative image to the next frame of the video in question. Select the '*Next Frame*' button if the frame given automatically is obstructed or out of focus.
13. Follow the instructions to the right of the representative image to select the testing area and hit '*Enter*' to complete the selection process.
NOTE: The selected green lines will now be shown on the representative image.
14. Select '*Done*' to save the selection and return to the reviewer screen.
15. Select the center '*Done*' button to start the complete analysis process once the grid area for each video is selected correctly. Allow the program to run and, upon completion, a success prompt will appear.
NOTE: The full analysis of a session of videos can take some time, approximately 10 min per animal.

4. **Ladder test analysis**
NOTE: The protocol for the initial ladder test analysis is very similar to the protocol outlined in steps 4.3.10–4.3.15. The notable differences prior to those steps are as follows:
    1. Select '*Ladder Analysis*' from the top menu to open the ladder analysis menu.
    2. View the two options in the toggle, '*Single Video*' and '*Whole Session*' (**Figure 3B**).
    3. With '*Whole Session*', select the upper input … button to select the folder with ladder videos named in step 2.2.1.Select the lower … button to select a directory to save a folder with data from the ladder videos to review later.
    4. If '*Single Video*' is selected, select a single video file with the upper … button and a location the same as in step 4.4.3.
    5. Select '*Go!*' to start the analysis process and bring up the ladder test area reviewer screen.
    6. Follow steps 4.3.10–4.3.15 the same way for the ladder as for the grid. Allow the program to run and, upon completion, a success prompt will appear.
    NOTE: If something goes wrong during the analysis, an email is sent to the email address entered in step 4.2.2. Upon completion of an analysis of any kind, a completion email is sent to the same email address.

5. **Ladder image review**
NOTE: The following protocol is to review previously analyzed ladder videos to confirm any failures and paw slips. Note, spreadsheet files for a ladder crossing are not generated to view until '*Ladder Image Review*' (steps 4.5.1–4.5.5) is completed.
    1. From the top menu, select '*Ladder Image Review*'. This brings up the Ladder Review file select screen.
    2. Select the top … button to select the folder of the data files.
    NOTE: The files are saved in the location chosen in step 4.4.2 and are named in the following format: "M1_D1_Y1 Ladder Video Session HH_MM M2_D2_YYYY". M1, D1, and Y1 are the date of the session being analyzed. "Ladder Video Session" will read "Single Video Analysis" if only a single video was analyzed. HH_MM and M2_D2_YYYY are the time and date that the review session was started.
    3. Select the bottom … to select the spreadsheet file created for the study.
    4. Select '*Go!'* to start the manual review process.
    5. View the automatically opened ladder data reviewer menu. If a slip is detected, use the '*Next Frame*' and '*Last Frame*' buttons to check any consecutively recorded frames if available.
    6. Utilize the slip toggle to denote what foot of the rat has slipped. Leave the toggle on '*No Slip*' if the detection is a false positive. Click '*Save/Continue*' to move on to the next detection (**Figure 2A**).
    7. In the case of failure, use the toggle to denote whether the failure is due to reversal (where the rat turned around during the run) or stagnation (where the rat failed to complete the run in a timely manner).
    8. Click '*Save/Continue*' to move on to the next detection. On the last detection, click '*Save/Continue*' to complete the analysis and save the data to the spreadsheet file chosen earlier. Dismiss the completion notice.
    NOTE: After the review is completed, the folder that was reviewed is renamed "REVIEWED M1_D1_Y1 Ladder Video Session HH_MM M2_D2_YYYY".

5. Output Data Analysis

1. **Grid spreadsheet file**
    1. Locate the created data in the spreadsheet file from each grid video.
    NOTE: The columns are left to right as follows: animal name, date of test, week of study number, total distance traveled, total time stopped, maximum velocity achieved, right turns, left turns, and grid line crosses (**Figure 1D**).
    2. Use these metrics to quantify the gross motor performance and create comparison plots between animals and time points.

2. **Ladder spreadsheet file**
    1. Locate the created data in the spreadsheet file from each ladder video following the failure and paw slip review.
    NOTE: The columns are left to right as follows: animal name, run number 1–5, week of study number, date of test, run type (either success or failure), time of run for successes, percent completion, paw slips on each paw, and failure mode. The time column is blank for a failure and the failure mode column will read either reversal or stagnation (**Figure 2B**).
    2. Use these metrics to quantify the fine motor performance and create comparison plots between animals and time points.

Representative Results

Following the methods presented here, rats completed the open field grid and ladder tests 2x per week. The data were analyzed both by using BVAS and manually with a stop-watch by trained and novice reviewers. The results presented are an average of the raw weekly scores from a single non-implanted control animal over an 8 week study, where week "0" corresponds to the baseline testing. Note there was no testing during week 1 as this was a rest week for the surgery animals. Because the open field grid is tested 1x per day, the ladder is tested 5x per day, and there are two testing days per week, there is a sample of 16 trials for open field grid whereas there are 80 trials for the ladder test in the same 8 week time period.

BVAS *vs.* manual analysis:
To confirm the consistency of the BVAS system and validate it against manual analysis, the results for grid lines crossed and ladder crossing time were compared to the manual results from three expert reviewers (*n* = 3) as a "gold standard." Novice users (*n* = 7) also reviewed the data both manually and using the BVAS. For the validation, each reviewer examined the same one day of experiments for one animal (grid testing = one video/trial; ladder testing = five independent trials). The results for the open field grid test showed that expert reviewers were more consistent than novice reviewers were (a variance of 0 *versus* a variance of 17.1, respectively), but when using the BVAS, there was zero variance for both expert and novice users (**Figure 4A**). Similarly, the results for the ladder test showed that expert reviewers were more consistent than novice reviewers were (~3.5x larger: a variance of 0.120 *versus* a variance of 0.414, respectively), but when using the BVAS, there was again zero variance for both expert and novice users (**Figure 4B**).

Therefore, where possible, behavior metrics for both the open field grid and ladder test were quantified manually by the same expert user and compared to the results generated using the BVAS. Here, the results from all included animals throughout the duration of the experiments were evaluated. All error is reported as a standard error of the mean (SEM) unless reported otherwise. The difference between the two methods ranged from an average of 0.64 ± 0.06 s for the ladder test and 3.56 ± 0.53 lines for the grid test. The average difference for the open field grid test over the course of 8 weeks between the two methods was 11.13 ± 3.03%. The average difference for the ladder test over the course of 8 weeks between the two methods was 9.05 ± 1.07%. The percent difference between the two methods was calculated following **Equation 1**.

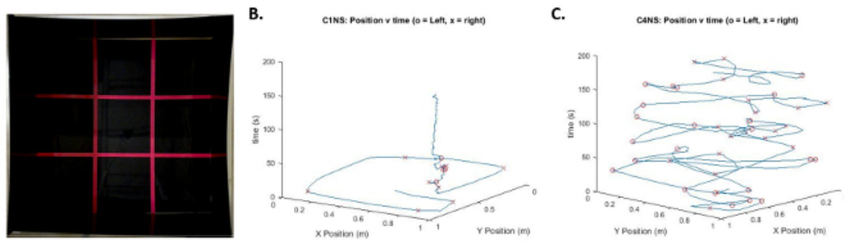
Equation 1: $$\%\ difference\ between\ methods = \frac{(manual\ score - BVAS\ score)}{manual\ score} * (100)$$

Note also for the ladder test timing, the manual data collection is only precise to 1 s (which can change based on the device used to keep time), while the BVAS is precise to the inverse of the frame rate of the video being reviewed. For example, if the video is filmed at 15 frames per second, the BVAS data is precise to 1/15th of a second.

Open field grid test:
Following previously published protocols[12,13], animals were allowed to run freely in an open field grid test for 3 min to measure their gross motor function and stress behavior. The video recorded during the testing was analyzed using the BVAS to quantify the raw scores for the maximum velocity, the total distance, the number of gridlines crossed, and the percentage of right turns achieved by the animal (**Figure 5**). While this data is only representative of one animal, trends were seen over the course of the study of increased maximum velocity achieved, increased total distance traveled, and an increased number of gridlines crossed. Unsurprisingly, when compared to each other, the total distance traveled, and the total gridlines crossed had a strong positive correlation. Comparing the manual quantification to the BVAS gridline quantification revealed comparable results (**Figure 5C**). For this particular animal, the percentage of right turns largely hovered between 40% and 50% (**Figure 5D**). Of note, the metrics for the total distance traveled, maximum velocity, and turn direction were unable to be quantified manually, and represent another added feature of the automated BVAS program.

Ladder test:
As previously reported[4,14], the ladder testing was completed to measure the coordinated grasp and fine motor function. The video recorded during the testing was analyzed using the BVAS to quantify the raw scores for the time to cross and the number of paw slips (**Figure 6**).In this animal, there was a decreasing trend in the time to cross the ladder, followed by an increase in time starting around week 4. Comparing the manual quantification to the BVAS crossing time quantification revealed comparable results (**Figure 6A**). As the animal presented was a non-surgical control, paw slips were infrequent, and comparable in number between the left and right paw (**Figure 6B**).

A. B. C.

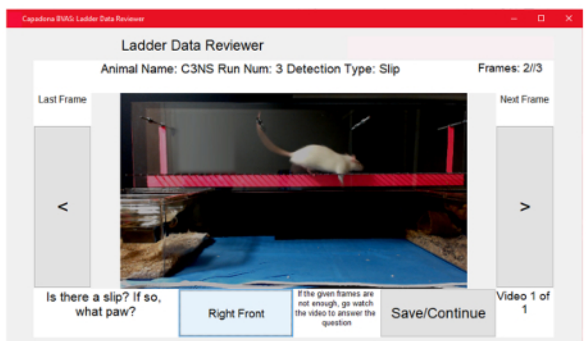


D.

Animal	Date	Week	Total_Distance	Time_Stopped	Max_Vel	Rights	Lefts	Line_Crosses
C1NS	07/24/17	Week6	4.449130018	24.1259968	0.329844714	10	3	10
C4NS	07/31/17	Week7	21.82123653	7.1303837	0.477236583	19	22	54

Figure 1: Example of open field grid test results. (**A**) This panel shows the behavioral testing 1 m^2 open testing area with a black background and pink grid lines. (**B**) This is an example of a plot of a single animal's 3-min grid run. This animal made one circuit of the grid and then remained mostly stationary for the remainder of the time. The red O's and X's denote where the turn detection algorithm detected a left (O) or a right (X) turn. (**C**) This is a second example of a plot of a single animal's 3-min grid run. This animal was extremely active for the entire 3-min testing period. The red O's and X's denote where the turn detection algorithm detected a left (O) or a right (X) turn. (**D**) This is an example of the output in the data file for the two runs seen in panels **B** and **C**. Please click here to view a larger version of this figure.

A.



B.

Animal	Run_Number	Date	Week	Run_Type	Time_for_Successes	Percent_Completion	Right_Front_Slips	Left_Front_Slips	Right_Rear_Slips	Left_Rear_Slips	Failure_Mode
C3NS	3	07_26_17	Test	Success	2.9795704	100	1	0	0	0	

Figure 2: Example of a ladder data reviewer screen. (**A**) This panel shows the ladder data reviewer screen, complete with a screenshot demonstrating the ladder test set-up. This screen is where the user will confirm detected slips and failures. This example shows a positively detected right front paw slip. (**B**) This is an example of the output in the data file for the ladder run that was examined in panel **A**. Please click here to view a larger version of this figure.

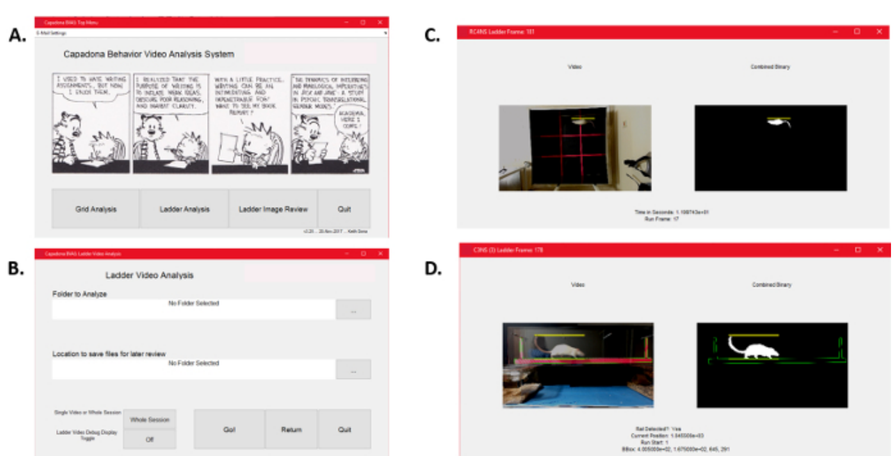


Figure 3: Example of BVAS analysis screens. (**A**) This panel shows the top menu of the BVAS. Note the four buttons along the bottom and the **Email Settings** drop-down menu in the top left corner. (**B**) This is an example of a file selection screen, in this case for the ladder video analysis. (**C**) This is an example of a debug analysis viewer for a grid test video. This demonstrates the detection algorithm during the analysis of a grid test. (**D**) This panel shows an example of a debug analysis viewer for a ladder test video. This demonstrates the detection algorithm during the analysis of a ladder test. Please click here to view a larger version of this figure.

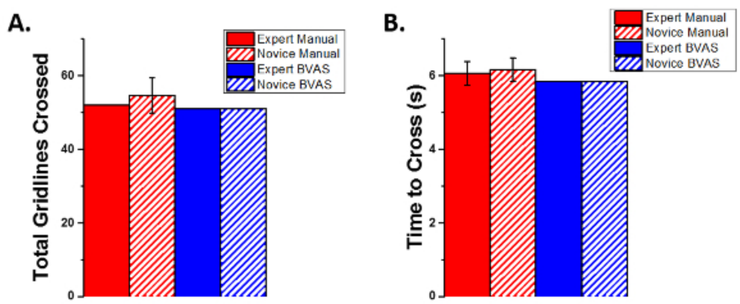


Figure 4: Example of expert and novice testing results. These panels show the results of a manual and a BVAS expert and novice testing for (**A**) the number of gridlines crossed on the open field grid test and (**B**) the time to cross on the ladder test. For both tests, the results showed that expert users had a lower variance than the novice users for the manual analysis, while using the BVAS resulted in zero variance, regardless of the user expertise. The standard deviation is reported. Please click here to view a larger version of this figure.

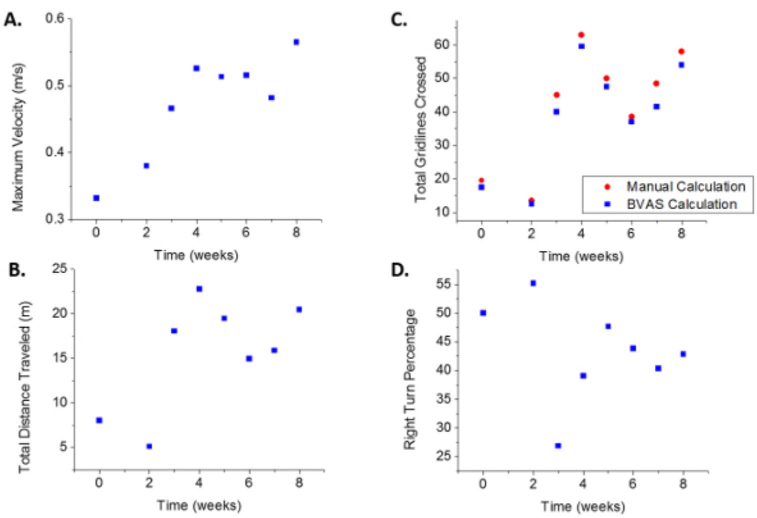


Figure 5: Representative open field grid test results. These panels show representative open field grid test results for a non-implanted control animal for (**A**) the maximum velocity achieved, (**B**) the total distance traveled, (**C**) the total number of gridlines crossed, and (**D**) the percentage of right turns made by the animal. Please click here to view a larger version of this figure.

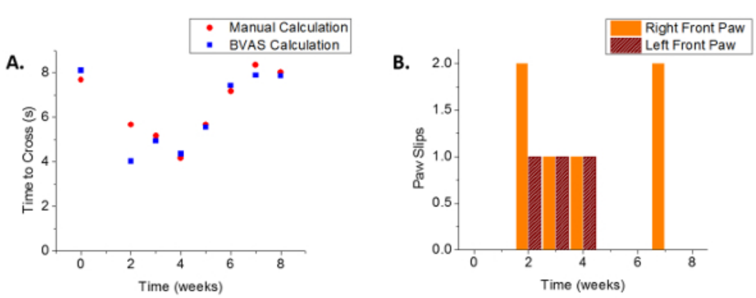


Figure 6: Representative ladder test results. These panels show representative ladder test results for a non-implanted control animal for (**A**) the time to cross the ladder, and (**B**) the number of right- and left-front-paw slips. Please click here to view a larger version of this figure.

Discussion

The most critical portion of the protocol to ensure a strong analysis is the consistent filming. If the videos are well lit and filmed at the correct position as discussed in the first section of the protocol, the system will be able to do a precise analysis. As with any image-processing problem, the work done in preprocessing will make the post-processing more accurate and simple. As such, making sure that the apparatus and animals are well-lit during the testing and any shadows or other motion in the frame is kept to a minimum will mean the BVAS can function at a higher level of accuracy.

The protocol presented here can be used to efficiently and reproducibly analyze results from both gross and fine motor function testing in rodents *via* an open field grid and a ladder test. Additionally, it reduces the possibility of human error or bias in the analysis process as the data analysis is largely completed by the self-functioning computer without any user input. Because of this feature, the BVAS system can be used with the same level of accuracy by both expert and novice users. The BVAS program is self-checking, easy to implement, and inexpensive to use.

Further, the code can be adapted to fit a researcher's individual needs. For example, the open field grid code can be used to analyze a variety of metrics including the total distance traveled, the maximum speed achieved, and the number of left and right turns, and could likely be easily modified for the tracking in a water maze or forced swim task. Additional modifications can be made to the code to account for varying room lighting, differences in grid and ladder line color, and differences in animal color. Although the testing apparatus and the BVAS code presented here were designed for their use in rat studies, we expect that either could be scaled up or down to be used with various sized animals, although this has not been verified to date.

As with all behavior testing and data analysis, it is important to maintain as much consistency as possible throughout the study and subsequent analyses. Although the presented BVAS greatly reduces any reliance on human input for the data analysis, human variance can come into play with the researcher working with the animals and handling the procedures[15]. Furthermore, changes in the testing location[16] or housing and husbandry conditions[17] can also influence the results. While the BVAS can be updated to account for lighting and camera angle, factors such as smells, personnel, or diet can only be accounted for at the time of the study. Therefore, researchers should take caution to remain as consistent as possible in the animal testing and housing conditions, testing personnel, and analytical methods, among others.

The BVAS is novel because of its compound detection system. Each frame of the video is passed through multiple image filters to create binary masks all calibrated to look for the rat in the frame. Each possible rat shape is then rated by the system on its likelihood of being the rat. This rating factors in size, the number of filters the shape is detected on, and the last known location and the predicted location of the animal based on the previous trajectory. This makes for a strong animal detection that can overcome most issues that arise based on shadows and changes in the lighting. Unlike current commercially available animal tracking systems, this method does not require any modifications to the animal to make it more visible and uses a single standard-speed webcam for the video recording. The BVAS system is also an improvement over conventional manually quantified behavior testing because all the metrics that are outputted are objectively measured from the videos. A human quantified behavioral study will have some subjectivity in nature as the researcher decides what constitutes a paw slip or grid line cross.

The system requires a somewhat specific setup to work to its fullest capabilities. While it is possible to adapt the system to changes in the apparatus, it would currently require some fluency in computer coding to edit the code. With some coding fluency, troubleshooting is made easy because the error notice emails include a full error information readout. If the videos fed into the system are not at the ideal resolution or frame rate (1080 p, 15 fps), the user is alerted *via* a warning message. This change will affect the accuracy of the system, but it still can run on lower resolution videos. Any lighting or shadow problems that cause an error during the analysis will lead to a dynamic error message sent to the user-entered email. The user can then use the debug viewer to watch the frame by frame analysis to understand what issue arose. The error message also includes an error report, so if it is a coding issue, it can be addressed simply.

In the future, the BVAS system may be able to be further adapted without any knowledge of the syntax from coding software. The addition of a tracking options menu could allow the user to select the color of the animal and grid lines simply and conveniently. The ladder test tracking will also increase in accuracy with the possible addition of a cascade object detector in the code and a better ladder lighting rig to accompany the ladder test and filming apparatus. Therefore, we expect that the BVAS system described here can be readily implemented into an array of behavioral and motor function tasks spanning a wide variety of disease and injury models.

Disclosures

The authors have nothing to disclose.

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References

1. Beery, A. K., Kaufer, D. Stress, social behavior, and resilience: insights from rodents. *Neurobiology of Stress.* **1**, 116-127 (2015).
2. Crawley, J. N. Behavioral phenotyping of rodents. *Comparative Medicine.* **53**, 140-146 (2003).
3. Wolf, A., Bauer, B., Abner, E. L., Ashkenazy-Frolinger, T., Hartz, A. M. A Comprehensive Behavioral Test Battery to Assess Learning and Memory in 129S6/Tg2576 Mice. *PLoS One.* **11**, e0147733 (2016).
4. Metz, G. A., Whishaw, I. Q. Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. *Journal of Neuroscience Methods.* **115**, 169-179 (2002).
5. Bailey, K. R., Crawley, J. N. Anxiety-Related Behaviors in Mice. in *Methods of Behavior Analysis in Neuroscience.* edited by Buccafusco, J. J., chapter 5, CRC Press/Taylor & Francis. Boca Raton, FL (2009).
6. Prut, L., Belzung, C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *European Journal of Pharmacology.* **463**, 3-33 (2003).
7. Porsolt, R. D., Bertin, A., Jalfre, M. Behavioral despair in mice: a primary screening test for antidepressants. *Archives Internationales de Pharmacodynamie et de Thérapie.* **229**, 327-336 (1977).
8. Porsolt, R. D., Brossard, G., Hautbois, C., Roux, S. Rodent models of depression: forced swimming and tail suspension behavioral despair tests in rats and mice. *Current Protocols in Neuroscience.* **Chapter 8**, Unit 8 10A (2001).

9. Dunham, N. W., Miya, T. S. A note on a simple apparatus for detecting neurological deficit in rats and mice. *Journal of the American Pharmaceutical Association.* **46**, 208-209 (1957).
10. Forstmeier, W., Wagenmakers, E. J., Parker, T. H. Detecting and avoiding likely false-positive findings - a practical guide. *Biological Reviews of the Cambridge Philosophical Society.* **92**, 1941-1968 (2017).
11. Reason, J. Human error: models and management. *The Western Journal of Medicine.* **172**, 393-396 (2000).
12. Goss-Varley, M. Rodent Behavioral Testing to Assess Functional Deficits Caused by Microelectrode Implantation in the Rat Motor Cortex. *Journal of Visualized Experiments.* **In Press** (2018).
13. Goss-Varley, M. *et al.* Microelectrode implantation in motor cortex causes fine motor deficit: Implications on potential considerations to Brain Computer Interfacing and Human Augmentation. *Scientific Reports.* **7**, 15254 (2017).
14. Metz, G. A., Whishaw, I. Q. The ladder rung walking task: a scoring system and its practical application. *Journal of Visual Experiments.* (28), e1204 (2009).
15. Chesler, E. J., Wilson, S. G., Lariviere, W. R., Rodriguez-Zas, S. L., Mogil, J. S. Influences of laboratory environment on behavior. *Nature Neuroscience.* **5**, 1101-1102 (2002).
16. Crabbe, J. C., Wahlsten, D., Dudek, B. C. Genetics of mouse behavior: interactions with laboratory environment. *Science.* **284**, 1670-1672 (1999).
17. Richter, S. H., Garner, J. P., Auer, C., Kunert, J., Wurbel, H. Systematic variation improves reproducibility of animal experiments. *Nature Methods.* **7**, 167-168 (2010).