Video Article

Battery of Behavioral Tests Assessing General Locomotion, Muscular Strength, and Coordination in Mice

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Abstract

Behavioral testing is used in pre-clinical trials to assess the phenotypic effects and outcomes that a particular disease or treatment has on the animal's wellbeing and health. There are numerous behavioral tests that may be applied. We selected a test for general locomotion, the open field test (OFT); a test for muscular strength, the mesh test (MT); and a test for coordination, the rotarod test (RR). Testing can be accomplished on a weekly or monthly basis. As a test for general locomotion, the OFT works by objectively monitoring movement parameters while the mouse is in an open field apparatus. The field is generally a 2' x 2' box, and the movements are recorded through laser sensing or through video capture. The mouse is placed in the center of the open field and allowed to move freely for the test. The MT uses the latency for a mouse to fall off an inverted screen as a measure of muscular strength. A mouse is placed on a screen, which is inverted over a clear box, and is timed for their latency to fall. Three trials are performed, with the best of the three trials scored for that day. A score of 60 s is the maximum time a mouse is left inverted. Mice are given a 5-min rest period between mesh test trials. Lastly, an accelerated protocol on the RR assesses motor coordination and endurance. During a trial, a mouse walks on a rotating rod as it increases in speed from 4 rpm to 40 rpm over 5 min. The trial ends when the mouse touches the magnetized pressure sensor upon falling. Each mouse undergoes three trials, and the best trial is scored for that day. This combined behavioral data allows for the global assessment of mobility, coordination, strength, and movement of the test animals. At least two out of the three behavioral testing measures must show improvement for an animal to qualify as having overall improved motor function.

Video Link

The video component of this article can be found at https://www.jove.com/video/55491/

Introduction

The goal of behavioral testing is to measure phenotypic characterization, whether in studies for a new mouse model of diseases affecting the central nervous system or for a trial of a new treatment. There are a variety of behavioral tests to choose from. The rationale for using a combination of behavioral tests is often to fully assess the pre-clinical treatment outcomes. The battery of behavioral testing herein includes the open field test (OFT), the mesh test (MT), and the rotarod test (RR).

The OFT is used as a measure of general locomotor activity[1,2,3,4,5,6,7]. This assessment allows the mouse to move freely in an open field, and the measurements taken are the movements of the mouse over the course of the behavioral trial. The quantitative analyses made using an OFT include rearing, distance moved, time spent moving (*e.g.,* walking and running), and changes in activity over time (*e.g.,* jumping and slower/ hyperactive movements), which is why it is a useful and thorough analysis of rodent behavior.

The MT is used to assess the strength of the mice[1,2,8,9,10,11]. The use of all four limbs to hang off of a wire grid mesh allows for the non-invasive measurement of the ability of the mice to show sustained limb tension while opposing the effects of gravity. This assessment is done by placing a mouse on an inverted mesh and timing its latency to fall. This test assesses the muscular strength of the mouse by measuring how long it can hold itself onto the inverted screen before falling off.

The RR assesses the coordination of a mouse while using the accelerating protocol[1,2,7,8,9,12,13]. The rotarod apparatus consists of a cylindrical beam that rotates in the air starting at 4 rpm. The mouse can learn this motor coordination skill and is then challenged by slow, incremental increases up to 40 rpm. Classically, the constant speed protocol is a measure of muscular strength, whereas the accelerating protocol for the RR assesses coordination, endurance, and muscular strength[1,2,7,14].

The advantages of these three behavioral tests are that they are widely accepted throughout the rodent behavioral testing community[2,7,8,9], and when taken not just as the sum of the individual tests, but collectively, they can be used as a global assessment of phenotypic behavior

when investigating the different outcomes of experimental treatments. The OFT is one of the only tests for overall locomotor activity[2]. The RR is also one of the only ways to assess coordination[2], other than to use gait analysis software, which will not be discussed here. There are a few ways in which the muscular strength of a mouse can be assessed; however, using the MT, or a wire hang test, has been prevalently used in the literature and is accepted as a reliable method for testing muscular strength[2,8,9,10,11]. Many other techniques for measuring general locomotion and coordination are extremely time consuming and may not produce reliable data (*e.g.,* manually timing mouse movement or manually painting paws for gait analysis). The automated systems for the OFT and the RR increase the reliability and validity of the data produced and save time and effort.

For example, given the neurodegeneration of the Sandhoff disease mouse model, when performing this battery of tests, the OFT would be the first of these tests used to assess the motor abnormalities that are usually seen in this mouse model starting at 3 months of age. Deficits in motor coordination should also become apparent beginning at 12 weeks of age. Deficits in muscular strength should begin around 14 weeks of age, but they ultimately become much more significant by 16 weeks.

These tests are useful when studying diseases that cause significant motor impairments, including neurodegenerative diseases[9] such as Sandhoff disease[1,8,13], Parkinson's disease[7], Huntington's disease, multiple sclerosis. The tests can also be used to study aging and to make comparative geriatric assessments or measurements of health. Gait disturbances and changes in locomotion and balance are seen in many of these disease states. However, the usefulness of these tests is not limited to diseases with prevalent neuromuscular symptoms.

Please note that there have been no confounding variables noted with respect to motivation or the novelty of any of the described behavioral testing measures. It is imperative for certain disease states and pathologies that the researchers acquire preliminary behavioral data before the mouse shows any phenotypic symptoms; the researcher should then periodically (weekly) ensure that all motor impairments are accounted for. Many diseases have a quick onset of phenotypic symptoms, and without weekly testing, these symptoms would not be accounted for.

Protocol

All protocols herein described were performed in accordance with, and were approved by, the Queen's University Animal Care Committee. The frequency of behavioral testing can be weekly or monthly, or a combination of the two (*i.e.,* weekly when one expects to see the biggest difference, and monthly elsewhere).

NOTE: Murine behavioral studies are largely sex/age/strain- dependent, and therefore it is essential to collect the proper time points and to remain consistent across experimental groups. Environmental factors (*e.g.,* scents, noise, temperature, humidity lighting, *etc.*) greatly affect behavior and anxiety in mice. Try to reduce these environmental factors as much as possible.

1. Open Field Test

NOTE: Prior to behavioral testing, the animals should be habituated to the room where the testing is to take place for 10 - 15 min prior to the beginning of testing. Ensure that proper personal protective equipment and all local facility guidelines are followed for the behavioral testing protocols.

NOTE: No training prior to the test.

1. Ensure that the computer and OFT program are on, running, and properly connected to the camera or open field box. Click on the smartware program, the open field icon, and "OK." Chose the "digital analog converter" source from the camera options. Click on "detection" and make sure that the whole open field space is visualized and that the dimensions are 30 cm x 30 cm.
   NOTE: Equipment layout may vary depending on space constraints, but ensure that the computer and open field apparatus are near to each other in a well-lit room for proper video capture.
2. Click "calibration," "snapshot," and then "OK" to make sure that the mouse is being detected within the confines of the open field. Click "time" to pre-set an amount of time to test the mice; in this case, set 5 min.
3. Enter the subjects by clicking the "subjects" icon. Click "scheduler" and highlight all test subjects and then click the double green arrows and "phase one, session one;" this will bring in all of the subjects in order to begin collecting data.
4. Place a mouse in the center of the open field and click the "start" icon to prompt the program to begin recording. Test only one mouse at a time.
5. Once 5 min is complete, the program will automatically move to the next subject. In this case, remove the mouse that has completed the test and place the next subject into the open field. Click "start."
6. Wipe down the equipment. Repeat steps 1.3 - 1.4 for the remaining test subjects.
7. In order to analyze the data once all the subjects have gone through testing, click on the "analyze" icon at the top right of the screen. Click "analyze" again and then "summary report." Optionally, export the data onto a USB.

2. Mesh Test

NOTE: The surface area and size of the mesh on the MT may influence performance. On the MT, if a mouse falls off within the first 10 s during the first 2 collection time points, they will be put back on the mesh immediately for a second attempt. The mesh used herein was a wire mesh, with the mesh holes approximately 1.5 mm in diameter.

NOTE: Running these tests at the same time of day is recommended, since physiological and biochemical parameters fluctuate throughout the day. Bodyweight may influence performance in this test and therefore must be taken into consideration.

1. Place a mouse in the center of a mesh and invert the mesh at least 20 cm above a clear container. Once inverted, start the timer.

2. Time and record the time of latency to fall. If the mouse does not fall within 60 s, give it a score of 60 s and conduct no further trials. Allow the mouse to rest for at least 5 min. This is the end of trial 1.
3. Wipe down the equipment and during the 5-min inter-trial interval, test a second mouse, if required. Repeat steps 2.1 - 2.2 for trials 2 and 3, if necessary (as long as the mouse does not score 60 s, it should undergo three trials).

3. Rotarod Test

NOTE: The surface and diameter of the rod on the RT may influence performance. On the RT, if a mouse falls off within the first 10 s during the first 2 collection time points, put it back on the rod immediately for a second attempt.

NOTE: Running these tests at the same time of day is recommended, since physiological and biochemical parameters fluctuate throughout the day. Bodyweight may influence performance in this test and therefore must be taken into consideration.

1. Turn on the IITC rotarod and computer. Ensure that they are properly connected for data recording.
2. Make sure that the program is running the accelerated protocol. Set the desired length of the rotarod test to xxx (the valid range is 000 through 999); in this case, press 300*. Set the number of lanes to be used to xxx (the valid range is 001 through 005); in this case, press 005*. Set the starting speed to xxx rpms (enter 3005 for 5 rpms; the valid range is 001 through 045); in this case, press 004*.
3. Set the top speed to xxx rpms (enter 4,030 for 30 rpms; the valid range is 001 through 045); in this case, press 040*. Set the ramp speed to xxx in s (enter 5,030 for a 30-s ramp to top speed; the valid range is 000 through 999); in this case press 300*. Set the unit to mouse mode (enter 6001 for mouse forward and 6002 for mouse reverse); in this case, press 6001*.
4. Ensure that all magnetized lanes are balanced before beginning the trial. Place one mouse in each lane of the rotarod.
5. Press start to initiate the program. As the mice fall, remove them from their lanes promptly to ensure that they do not "trip" the other lanes.
6. Allow the mice to rest for a 10-min inter-trial interval. This is the end of trial 1. During the inter-trial interval, wipe down the equipment and begin the trial for the next set of mice.
7. Repeat steps 4 - 6 as necessary. If a mouse remains on the rotarod for the full 5-min trial, no further trials are required for that mouse.

Representative Results

The study animals may undergo the behavioral testing paradigm weekly or monthly to accommodate the necessary time points required for analysis. See **Figure 1** for examples on behavioral testing timing. In the representative data below, taken from Osmon *et al.* (2016), the mice underwent the battery of behavioral testing monthly, beginning at 12 weeks of age, until their humane endpoint around 40 weeks of age. The timing of behavioral testing is sometimes critical for an experiment, especially when the survival of untreated versus treated animals ranges drastically and comparisons are required.

Although these tests are not used solely for neurodegenerative disorders, we used Sandhoff mice (see **Figure 2**). This mouse model gets increasingly shaky and uncoordinated from 12 - 16 weeks of age if untreated and normally require euthanization at 16 weeks of age[1]. The data in **Figure 2D** demonstrates the use of the rotarod as a means to see how the treated mice are behaving compared to the normal heterozygous mice. The divergence of the two groups at 40 weeks denotes the beginning of the treatment group's decreased coordination in comparison to normal controls. In other mouse models where a mild phenotype is being examined, further expansion and specialization of this battery of tests is recommended.

When selecting the relevant results for publication, it is important to keep in mind two main factors. The first is that the metric used should be chosen to be specific to the animal model being used. In the OFT, the metric most commonly used is "time moving" (as seen in **Figure 2**); however, "distance travelled" is also a common metric for general locomotion. In the RR, "end rpm" and "distance travelled" are commonly used metrics. The second factor in selecting which metric should be chosen as a suitable outcome revolves around which metric reveals the most significant divergence or convergence in behavioral outcomes, depending on which outcomes are anticipated.

Data collected could be analyzed using multiple statistical tests, but for consistent weekly or monthly behavioral tests using the same mice, a 2-way repeated-measures analysis of variance (2-way RM-ANOVA) is suggested. The repeated measures ANOVA enables the longitudinal assessment of results from the same animal, and allows for the comparison of changes in animal behavior over time, as well as between groups. The Bonferroni correction is also suggested. When comparing treatments within the same timepoint, a one-way ANOVA is suggested, as seen in **Figure 2**.

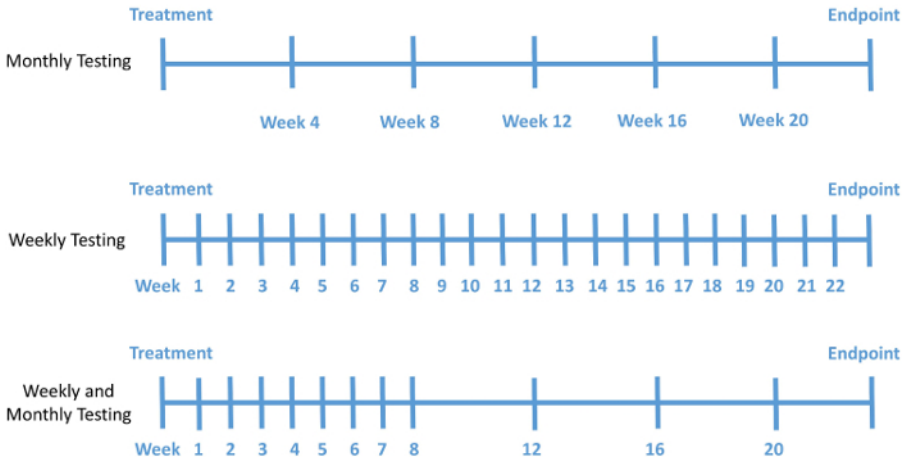

Figure 1: Examples of Possible Experimental Timeline for Behavioral Testing. Behavioral testing can be done with a variety of timelines, depending on the experimental need. Testing can be done on a monthly, weekly, or combinatorial basis. In the example provided, the study period lasts for 23 weeks, where week 0 is the treatment administration. When testing occurs on a monthly basis, the tests are performed on weeks 4, 8, 12, 16, and 20. When testing occurs on a weekly basis, the tests are conducted every week (weeks 1-22) before the 23-week endpoint. When testing occurs on a combinatorial basis, the tests are performed weekly on weeks 1 - 8, and then the tests are performed monthly on weeks 12, 16, and 20 before the 23-week endpoint. Please click here to view a larger version of this figure.

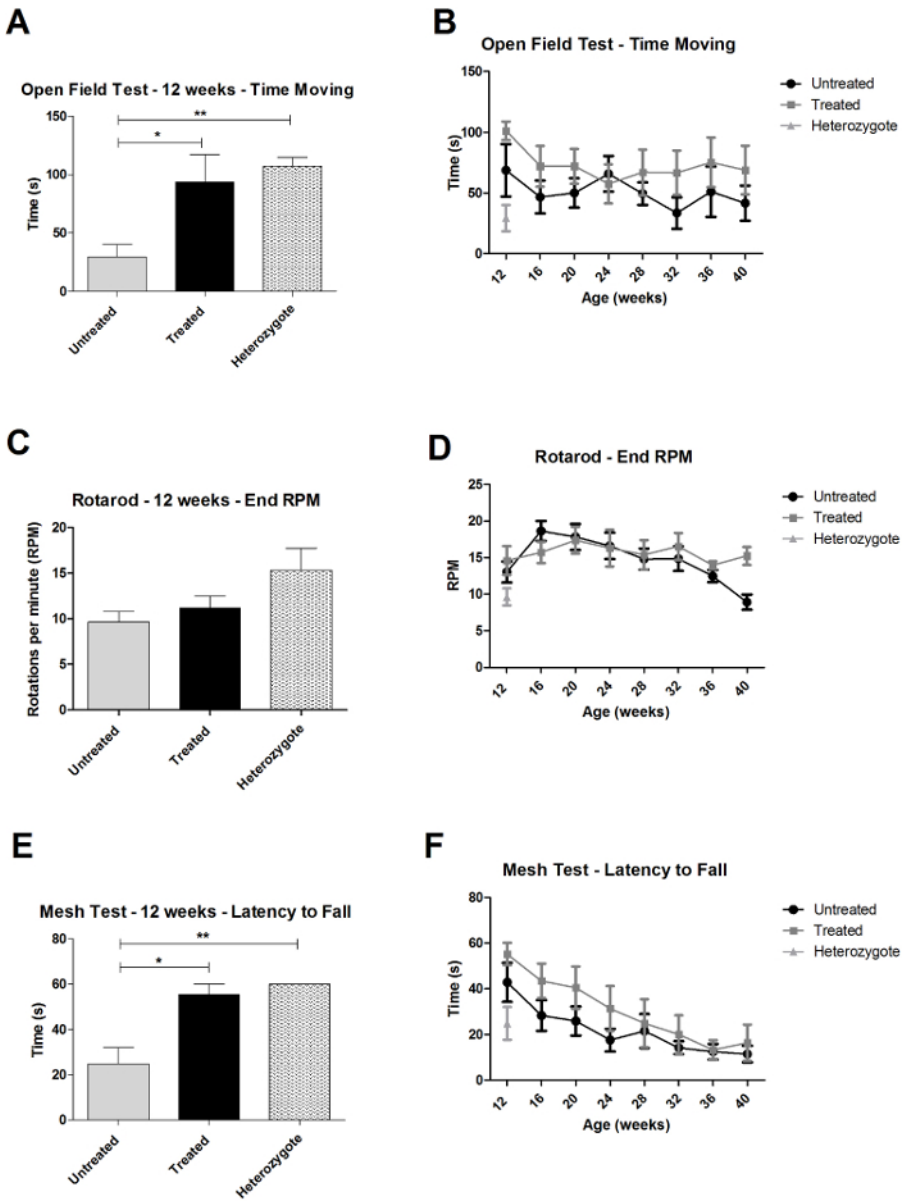

Figure 2: Monthly Behavioral Testing Results Where Treated Animals Outperform Untreated Animals. At 12 weeks, treated animals outperform untreated animals on "time moving" in the OFT **(A)** and on the "latency to fall" in the MT **(E)**. There are no statistical differences between the treated and untreated animals in the "rotarod end rpm" at 12 weeks **(C)**. Over monthly behavioral testing, the treated mice perform similarly to the normal heterozygous controls in the OFT **(B)**, the RR **(D)**, and the MT **(F)**. All statistical analyses were performed using statistical software (*e.g.* GraphPad), one-way ANOVA tests were performed on the week 12 analysis (**2A, C**, and **E**) and 2-way RM-ANOVA tests were performed on the monthly analysis (**2B, D,** and **F**). All error bars are the SEM. This figure modified from Osmon *et al.*[1] and reprinted with permission from Mary Ann Liebert Publishers, Inc. Please click here to view a larger version of this figure.

Discussion

For all tests, Clean and disinfect the surface to minimize contagions and distractors to the mice. Also, make certain that the mice are given sufficient rest periods between trials and between behavioral testing when using the OFT, RR, and MT.

In the OFT, it is critical that each mouse is placed in the middle of the open field and that the program is started upon the release of the mouse into the center. Be sure that there are no hand movements the sensors may register upon starting the program.

For the MT, it is important to have a barrier between the mesh area where the mouse will hang and the sides of the container they are falling into. It has been noted that when the mice can reach the side of the container, they slowly release their front paws and then their back paws to jump down. Taping a barrier between the walls and the center of the mesh greatly reduces this behavior and allows for a more accurate measure of muscular strength.

Alone, each of these tests is a good measure for general locomotion (open field test), muscular strength (mesh test) and coordination (rotarod)[2,7,8]. However, taken together, the complete collection of these three behavioral tests allows for the broad assessment of locomotion in mice. This group of tests independently confirms the lack of confounding effects in motor impairment on complex motor functioning. The main limitation of these methods is their general assessment of locomotion, which is problematic for extremely specific deficits in behavioral functioning. In addition, these tests, like all behavioral tests, are subject to changes in behavior due to external stressors, such as sound, temperature changes, and light cycles. It is prudent to maintain a consistent external environment for all test subjects. This battery of behavioral testing can be expanded to include tests for ataxia and memory. Other methods for studying muscular strength[2,8,9] include conducting constant-speed RR to correlate muscular strength results with the MT. For more specific assessments on muscular strength, coordination, or ataxia, there are grip strength[2] and weights tests[15] and balance beam or gait analysis assays[2], respectively.

Disclosures

The authors have nothing to disclose.

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References

1. Osmon, K.J., *et al.*, Systemic Gene Transfer of a Hexosaminidase Variant Using an scAAV9.47 Vector Corrects G_{M2} Gangliosidosis in Sandhoff Mice. *Human Gene Therapy.* **27** (7), 497-508 (2016).
2. Brooks, S.P., & Dunnett, S.B. Tests to assess motor phenotype in mice: a user's guide. *Nature Reviews, Neuroscience.* **10**, 519-529 (2009).
3. Ma, M., Basso, D.M., Walters, P., Stokes, B.T., & Moser, P. Behavioural and histological outcomes following graded spinal cord contusion injury in the C57Bl/6 mouse. *Exp. Neurol.* **169**, 239-254 (2001).
4. Menalled, L.B. *et al.* Early motor dysfunction and striosomal distribution of huntingtin microaggregates in Huntington's disease knock-in mice. *J.Neurosci.* **22**, 8266-8276, (2002).
5. Grusser, C., & Grusser-Cornehls, U. Improvement in motor performance of Weaver mutant mice following lesion of the cerebellum. *Behav. Brain Res.* **97**, 189-194 (1998).
6. De Leonibus, E. *et al.* Spatial deficits in a mouse model of Parkinson's disease. *Psychopharmacology (Berl.).* **194**, 517-525 (2007).
7. Morris, M., Koyama, A., Masliah, E., Mucke, L. Tau Reduction Does Not Prevent Motor Deficits in Two Mouse Models of Parkinson's Disease. *PLoS ONE.* **6** (12): e29257, 1-7 (2011).
8. Abo-ouf, A. *et al.* Deletion of tumour necrosis factor-α ameliorates neurodegeneration in Sandhoff disease mice. *Human Molecular Genetics.* 1-16 (2013).
9. Dumont, M. Behavioral Phenotyping of Mouse Models of Neurodegeneration. *Neurodegeneration : Methods and Protocols, Methods in Molecular Biology.* **793**, 229-237 (2011).
10. Cabe, P.A., Tilson, H.A., Mitchell, C.L., & Dennis, R. A simple recording grip strength device. *Pharmacol. Biochem.* **8**, 101-102 (1978).
11. Barnēoud, P., Loliver, J., Sanger, D,J,., & Moser, P. Quantitative motor assessment in FALS mice: a longitudinal study. *Neuroreport.* **8**, 2861-2865 (1997).
12. Lui, Y. *et al.* Mouse model of G_{M2} activator deficiency manifests cerebellar pathology and motor impairment. *Proc. Natl. Acad. Sci.* **94**, 8133-8143, (1997).
13. Sango, K., *et al.* Mouse models of Tay-Sachs and Sandhoff disease differ in neurologic phenotype and ganglioside metabolism. *Nature Genetics.* **11**, 170-176 (1995).
14. Jones, B. J., & Roberts, D.J. A rotarod suitable for quantitative measurements of motor inco-ordination in naïve mice. *Nauyn-Schmiedebergs Arch. Pharmacol.* **259**, 211, (1968).
15. Deacon, R.M.J. Measuring the Strength of Mice. *Journal of Visualized Experiments : JoVE.* **76** (2013).

