In Vivo Murine Motor Function Assessments (righting reflex, mesh grip, balance beam, rota rod)

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The Author(s) of the SOP	Dr. Emma Sutton The Ottawa Hospital Research Institute / University of Ottawa
Working Group Members	Dr. Rashmi Kothary Rebecca Yaworski Ariane Beauvais
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Feedback	Please provide feedback on this SOP here.
Acknowledgement	When using any application of the methods in this SOP in research publications, reports, or presentations, please provide acknowledgment of the SOP as the source of the procedures. For example: "*This study was conducted in accordance with the NMD4C's BSTC Standard Operating Procedure (Version 1.0 NMD4C_SOP000X).*"

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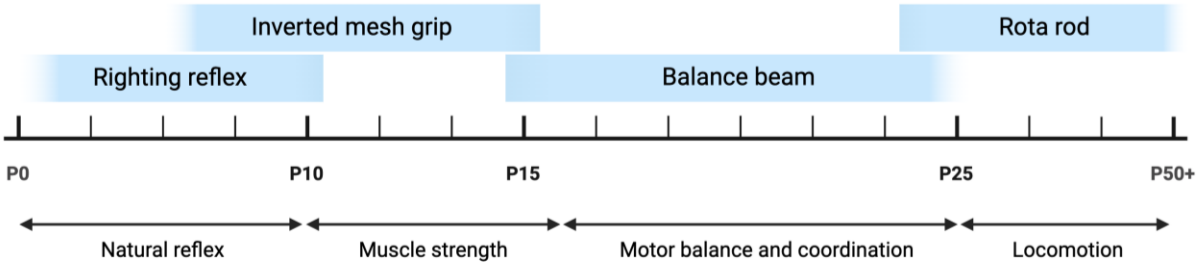
1 Objective

This SOP serves as a structured, standardised framework for evaluating motor function in mice using a variety of neuromuscular tests. This SOP details the most appropriate neuromuscular test to select dependent on postnatal age, how to perform each test maintaining quality monitoring methods and data analysis.

2 Applicability/Scope

This SOP applies to researchers working with murine models to identify any motor deficits that may or may not be present. This method is applicable to all NMD mouse models. It is designed to be used in studies involving assessment of neuromuscular deficit severity, or rescue thereof, following a therapeutic strategy. All motor function tests can be applied to monitor progression of motor problems.

3 Summary of Procedure



Assessments included in SOP:

3.1 Righting Reflex (PND 1-10)

Function: a natural reflex that helps an animal maintain balance and orientation.
Assessment: observe how long it takes a mouse pup to be able to flip onto its feet from a supine position.

3.2 Inverted Mesh Test (PND 5-15)

Function: forelimb and/or hindlimb muscle strength.
Assessment: observe how long a mouse can cling to an inverted or tilted surface.

3.3 Balance Beam (pen test) (PND 15-25)

Function: motor balance and coordination.
Assessment: observe how long the mouse can remain on the beam before falling.

3.4 Rota Rod (PND 25+)

Function: locomotion (neuromuscular coordination, balance and motor-planning).
Assessment: observe the ability of the mouse to stay on a horizontal rotating cylinder.

4 Definitions

Key terms or abbreviations used in the SOP:

- Latency: Cumulative time the mouse maintains its balance prior to falling
- RPM: revolutions per minute, speed of the rota rod defined by rotations per minute
- Trial: the event period during which the subject is assessed
- ITI: intertrial interval time, the period that elapses between consecutive trials
- Session: consists of three trials
- PND: postnatal day

5 Cautions

Environment: Environmental factors may contribute to the level of anxiety in test subjects. Confounding variables to consider include room temperature, humidity, lighting, noise, and time of day. It is vital these conditions remain constant for all sessions.

Test subjects: Each test is age dependant and should not be performed on mice younger than the specified age in the relevant section. The strain, sex and date of birth should be recorded and considered as confounding variables. Motor abilities are mostly age-dependent, only animals of the same age should be compared.

Experimenter: To reduce investigator variability the same individual should perform all sessions, on all mice, for a given motor function test. The experimenter should be blinded to treatment conditions where possible.

Equipment and Acclimation: Equipment must be standardised and universal for any group comparisons. Prior to transporting mice to the procedure room, equipment such as the rota rod, should be turned on such that test subjects can acclimate to the noise. Subjects should be left for a minimum of 10 minutes in the procedure room to acclimate with the surroundings.

6 Materials and Equipment Required

6.1 Instrumentation (Common)

- Weigh scale
- Stopwatch/ timer

6.2 Instrumentation (Test Dependant)

Righting reflex

- Suitable flat surface (figure 1)

Inverted mesh test

- Mesh wire screen consisting of 12 x 12 mm squares, using 1 mm diameter wire
- Protractor

Balance beam (pen test)

- Use of any pen or beam with a diameter of 1 cm (figure 2)

Rota rod

- A rotating rod with a diameter of 3 cm, with the rod elevated to a height of 16 cm from the base of the instrument.
- Model example: Ugo Basile, model 47600 (figure 3)

6.3 Consumables

- 70% ethanol for sanitising the equipment
- Paper towels

7 Step-by-Step Procedure

7.1 Righting reflex

a. Place pup on its back on top of a cotton sheet/paper towel and hold in position for 5 seconds[*tip1].
b. Release the pup and record the time it takes the pup to right/return to a supine position. Each trial is maxed at 1 minute, if the pup was unable to right itself, 60 seconds is recorded.
c. Repeat for a total of three trials per pup, take an average of the trials at the end.

7.2 Inverted mesh test

d. place the pup on the wire mesh, on a flat surface, allow pup to adjust to the environment for 5 seconds.
e. Using a protractor, begin to slowly lift the wire mesh noting the angle as you elevate. Invert to 180 degrees.
f. Record the length of time the mouse can maintain its grip while inverted
g. Alternatively, in weak mice with a neuromuscular phenotype, record the angle of the screen at which the pup falls off.
h. Repeat for a total of three trials, take an average of the trials at the end.

7.3 Balance beam (pen test)

i. Hold the pen horizontally, approximately 20 cm above a surface*[tip2].
j. Suspend the mouse by its tail close to the pen, enabling the mouse to grab the pen with its front paws.
k. Let go of the tail, the mouse should be balanced on the pen with all four limbs.
l. Record the latency to fall, time how long the mouse remains walking/balanced on the pen*[tip3].
m. If the mouse can remain on the pen for 1 minute, it is returned to the cage and latency to fall is recorded as 1 minute.
n. Repeat for a total of three trials, take an average of the trials at the end.

7.4 Rota rod

o. Sanitise the rod prior to starting and between test subjects with 70% ethanol.

7.4.1 Software setup

p. Create a new experiment, double click 'label 1' and name your experiment.
q. Select speed mode, click 'accelerated speed mode'.
r. Set ramp duration to 300 seconds, press ESC to enter, select no when prompted 'with reverse'.
s. Select speed settings, click set max value, set max speed to 40 RPM, press ESC to enter. Repeat to set min value at 4 RPM.
t. Create subject ID list, double click box in bottom right of main menu, and enter animal IDs with respect to lane number. Click ok to save. NOTE this must be entered for each set of test subjects after the 3 trials is completed for a set of mice.

u. Confirm configurations, select file, click configuration and ok. Select file again, click acquired data. Delete any previous data using the green bin bucket. Check the box that says 'refresh each measure'*tip4.

7.4.2 Placement on rods

v. Prior to placing the mice on the rods, confirm the trip plates are in the active position.
w. Place each mouse on its own rod, be sure all subjects are facing the same way then start the rod spinning, ensure it starts at 4 RPM.
x. As the mice fall off the rod, each lane's trip plate with stop the timer and display the latency in seconds. Do not reset the trip plate until all mice have completed the trial.
y. Return mice to their cage once the trial is complete.
    i. Mice that fall from the rod before the experimenter can press go are automatically given a zero (0 second latency to fall) and placed back in their cage until the next trial.
    ii. Mice that maintain their balance for the maximum 300 seconds are placed back in their cage upon completion during the ITI until the next trial. Each ITI is 15 minutes, in this time you can test another cage of subjects.
    iii. Ensure each subject is placed back in the same lane, on the same rod in the same sequential order or placement for all three trials.
z. At the end of the experiment the animals are returned to their housing room and the instrument is thoroughly sanitised with 70% ethanol.

7.4.3 Data analysis

aa. Confirm all lanes recorded data as expected.
bb. Data should be analysed by someone blinded to the treatment group. Data is analysed as latency to fall (sec) over each trial and the average of latency to fall over the three trials.

8 Quality Assurance and Quality Control

- Repeat for a total of three trials, take an average of the trials at the end.
- A single mouse should only undergo one motor function test a day, comprising of three trials.
- Three biological replicates recommended, include untreated control groups in addition to the experimental group.
- The rota rod software can be used to minimise human error if applicable.

9 Safety Precautions

- All personnel who perform activities with this SOP must have the basic training in laboratory safety procedure.
- Remain up to date in training with this SOP.
- Animal training must be up to date and signed off by your facility administrator.

10 References

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11 Attachments/Checklists

Figure 1 righting reflex

Figure 2 balance beam

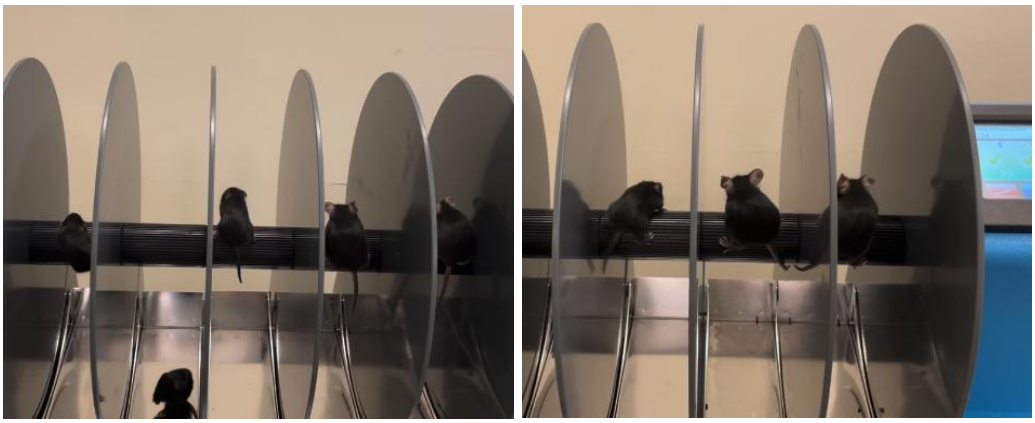

Figure 3 rota rod

12 Examples & Tips

The righting reflex has no learning component therefore it can easily be performed on younger mice and repeated throughout the experimental period.

Tip 1: Holding the mouse in position for 5 seconds ensures it is fully on its back and that you have not influenced its motion when the pup is trying to right itself, a lack of care here (motion of your hand) may encourage/provide advantage for the pup.

The mesh wire

Tip 2: Ensure the mouse is safe and secured when falling off the pen. Hold the pen over an empty cage, containing bedding or padding to reduce fall impact. The cage will also contain the mouse, preventing escape.

The balance beam is best performed with two people. One to start/stop the timer and the other to conduct the test.

Tip 3: If the mouse is healthy or undergoing therapeutic intervention it can be tricky to keep the mouse on the pen i.e. the mouse immediately runs to the end of the pen and onto your hand. Learning to quickly alternate the hand holding the pen is needed. If you have exhausted the trial attempt, use your expertise and record the mouse as 1 minute.

Tip 4: You can record data in the machine and transfer it onto a USB at the end of your experiment. Alternatively, you can record the data manually.