Motor behavioral assessment

In 1 collection

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ABSTRACT

This protocol describes motor behavioral assays used to assess motor function in a 6-OHDA mouse model of Parkinson's disease. These assays include open field, rotarod, cylinder, and balance beam tests.
Prior to each behavioral test, mice were habituated to the testing room for 30-40 min. All behavioral tests were performed 3-4 weeks following stereotaxic injections.

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Cylinder test: forelimb use asymmetry

1 Required materials:
- Clear plastic cylinder: 10.5 cm diameter, 14.5 cm height.
- Mirror.
- Camera.
- Video player software (e.g. VLC).
- 70% ethanol.

2 Set up behavior room:
- Clean cylinder with 70% ethanol.
- Place the camera in front of the cylinder with mirrors behind.
- Turn on camera; adjust zoom and focus and verifying an appropriate 360º vision of cylinder walls.

3 Habituation
- Bring mice to the behavior room in their home cages and leave undisturbed for at least 30-40 minutes.
- There must be **no prior habituation to the plastic cylinder**.

4 Testing:
- Place the mouse inside the cylinder and start video recording for 00:05:00

Expected result

Mice will explore the new space, rearing up and touching the cylinder walls with forelimb paws.

- After recording, return the mouse to home cage and clean the cylinder with 70% ethanol, before proceeding with another mouse.

5 Post-hoc scoring or analysis:
- Analyze the video frame by frame and count the number of fully extended digits wall touches executed with forelimb paws, discriminating between right and left touches.

Note

6-OHDA mouse model of Parkinson's disease or other unilateral manipulations/cranial injections: discriminate between ipsilateral and contralateral to the lesioned/injected site.

- Calculate the percentage of touches performed with the forelimb right vs left or contralateral vs ipsilateral to the injected brain side with respect to the total paw use.

Note

6-OHDA mouse model of Parkinson's disease: forelimb lateralization revealed by this test is conducted to verify unilateral DA-depletion. A contralesional paw use lower than 45% and 40% is expected for low dose 6-OHDA and high dose 6-OHDA-lesioned mice respectively.

Rotarod: balance and coordination

6 Required materials:

- Rotarod (Med Associates) connected to a PC with controlling 'RotaRod' software (mouse logo).
- Paper towels.
- 70% ethanol.

7 Set up behavior room:

- Clean each lane of the RotaRod with 70% ethanol.
- Cut paper towels to the size of lanes and place them there for ease of cleaning.
- Turn on RotaRod and corresponding 'RotaRod' software (mouse logo). Select the appropriate protocol. Hit 'Browse' option to navigate to your folder and create a file for the first group of mice to be tested (remember to create a new file for each group of mice).

Note

6-OHDA mouse model of Parkinson's disease: accelerated from 3-30 rpm in 00:05:00 (300s), 1 day test, three trials.

Note

This RotaRod model has 5 lanes. Test mice from same cage together.
If you have mice from more than one cage going at once, leave at least one empty lane between mice from different cages so you have some buffer lane if they wander into the next lane before you catch them.
Even if you are testing mice from the same cage, a buffer lane is always preferred when possible (<5 mice).

- Make sure nothing is blocking lane photobeams and push “Reset” buttons on rotarod, so each lane light is off (in the software, lanes will change from red to green. All must be green to start the test).

8 Habituation:

- Bring mice to the behavior room in their home cages and leave undisturbed for at least 30-40 minutes.

9 Testing: basic procedure for 1 day, 3 trials

9.1 Place mouse on the immobile rod, holding its tail for a few seconds to make sure it will not jump and to let it get its balance.

9.2 Wait 00:00:30 before starting rotation (first trial only). 30s

9.3 Hit 'Reset' button on software. Rod starts moving at 3 rpm (initial speed).

9.4 Then immediately press “On/Accel” button: acceleration program begins and automatically starts the time counting for each lane.

9.5 Remove the mouse if it falls (the photobem detection automatically stop the timer for that lane) or if continuous passive rotations occur (entire rotation of mouse body around the rod, with mouse holding on at one spot and riding around more than one rotation, without active intention to re-orient and continue walking forward). If passive rotations occurs, trip photobeam with your hand to stop the timer for that lane and then remove the mouse.

Although latency to fall (and the speed) will be automatically recorded and saved by the software, it is recommended to annotate it in real-time.

Note

Additional notes can be recorded regarding number of single rotations around the rod before re-engaging in walking or re-orienting.

9.6 Place removed mice into their home cages. Trial ends when all mice are collected. If any mice complete the 5 min (300 sec) program, stop the rotarod, remove the mice and give them the maximum score of 300 sec/30 rpm.

9.7 Clean rotarod (feces/urine) and replace paper towels if necessary (optional between trials for the same group of mice).

9.8 Let mice rest in their home cages for 00:10:00 between trials. 10m

9.9 Repeat steps from 9.1 to 9.8 for trials 2 and 3, except for step 9.2 (start the accelerated program immediately after placing mice on the rotarod, without 30 s of delay).

9.10 Once all three trials for a group of mice are finished, clean each lane of the rotaod with 70% ethanol, replace paper towels, and create a new file for the following group of mice.

9.11 Run again the previously described three trials per group of mice.

10 Analysis: latency to fall or time to reach maximum speed is recorded for the three trials and the average of the two best trials is reported.

Balance beam: balance and coordination

11 Required materials:

- Balance beam stands.
- Mirrors.
- Goal cage.
- Camera and tripod.
- Lamp.
- Beams (square): 19 mm, 12 mm and 6 mm cross section. Each beam has two lines marked in black separated by 80 cm lenght.
- Video player software (e.g. VLC).
- 70% ethanol.

12 Set up behavior room:

- Wipe down beams and goal box with 70% ethanol.
- Assemble balance beam stand with goal box, inside a closed space (e.g. water tank).
- Place some bedding (preferentially from the home cage) in the goal box to make it more enticing for the mouse.
- Attach a camera/tripod to the the tank wall at the side opposite the goal box. Connect the camera to a PC.
- Turn on the lamp and shine the bright light onto the initial segment of the beam, the end opposite to the goal box (this encourages the mouse to cross the beam to reach a darker goal box).
- Place mirrors on each side of the apparatus and angle them so that the camera can capture the middle 80cm (comprised between the two black markers) of the different sized balance beams at both sides.

13 Habituation:

- Bring mice to the behavior room in their home cages and leave undisturbed for at least 30-40 minutes.

14 **Training: Day 1**

Video recording of training session is not required.

Only medium size beam (12 mm) is used for training.

14.1 Place mouse in goal box for 00:03:00

14.2 Pick the mouse from goal box and place it on the medium size beam (12 mm) at 6 inches from the goal box. Make sure the mouse is facing the goal box and is well-balanced before you release its tail. Let the mouse walk along the beam and enter the goal box, then leave it inside for 00:00:30 .

> **Note**
>
> During training, if the mouse turns around or does not move, use your hand to prod it along the beam in the right direction.

14.3 Remove feces/urine on the beam, if any, while the mouse is in the goal box.

14.4 Repeat 14.2 step three more times by taking the mouse out of the goal box and then placing it at:

- 12 inches from the goal box
- In the middle of the beam
- At the far end of the beam

Allow the mouse to stay inside the goal box for 00:00:30 each time.

14.5 After a mouse completes the training, if another mouse from the same home cage is going to be trained, keep that same home cage bedding in goal box and just remove feces/urine on the beam.

When all mice from a given home cage were trained, clean the beam/goal box with 70% ethanol, and remove the bedding. Replace with bedding from the home cage of the following mice to train.

14.6 After all mice complete the training, clean the beam/goal box with 70% ethanol, and remove the bedding.

15 Testing: Day 2

Setup is the same as day 1, but **all different sized beams are used (19 mm, 12 mm and 6 mm).** Wipe down beams and goal box with 70% ethanol prior to start.

15.1 Start recording the video with the camera.

15.2 Place a mouse on the 19 mm beam (large sized beam) at the farthest position from the goal box. Make sure the mouse is facing the goal box and is well-balanced before you release its tail. Encourage the mouse to move along the beam if it does not do so on its own after a few seconds, but just until it reaches the first marker.
The mouse must cross the 80 cm distance between the two markers on the beam in less than 00:01:00 . The maximal score for latency to cross the beam is 60s and it is considered an unsuccessful trial.

15.3 Once crossed the beam and reached the goal box, leave the mouse inside for 00:00:30
If it the maximal score 60s was reached, gently grab the mouse from the beam and place it on the goal box also for 00:00:30

15.4 Repeat the trial with same beam.

15.5 Repeat steps 11.2 to 11.4 with the 12mm (medium) and 6mm (small) beams, going from largest to smallest, for a total of 6 trials per mouse (2 trials per beam size).

15.6 Once finished testing one mouse, clean the beam/goal box with 70% ethanol, and replace the bedding (for mice from the same home cage it is not necessary to clean and change the bedding, just clean excess of urine or feces that can be on the beam).

15.7 After all mice complete the test, stop video recording, clean the beam/goal box with 70% ethanol, and discard the bedding.

16 Post-hoc analysis:
The following data is expressed as the average of the two trials per beam size
- Latency to cross the beam: corresponds to the latencty to walk the 80cm between the two markers in each beam, with a maximum score of 60 seconds (unsuccessful trial).
- Number of hind foot slips made along the way (If the trial is unsuccessful because the mouse remains immobile for most of the time and not engaged in the walking task, do not include the number of slips for the average).

Open field: locomotion

10m

17 Required materials:
- Open field chamber: 50 cm x 50 cm square box.
- Overhead mounted camera.
- Video analysis software.
- 70% ethanol.

18 Set up behavior room:
- Set up lighting minimizing total room light, reducing shadows within the chamber, and ensuring camera is able to detect the mice.
- Clean chamber with 70% ethanol.
- Place chamber under camera.
- Turn on camera, adjust zoom and focus.

19 Habituation:
- Bring mice to the behavior room in their home cages and leave undisturbed for at least 30-40 minutes.
- No previous habituation to the open field arena is required.

20 Testing:

20.1 Start recording video before the mouse is in the chamber to collect the background images or just save a background image before starting the video.

20.2 Place mouse in chamber.

20.3 Record video for the desired length of time (e.g. 00:10:00). 10m

20.4 Remove mouse from chamber and return it to home cage.

20.5 Clean the chamber between mice with 70% ethanol and allow to fully dry before starting another trial.

21 Post-hoc analysis:

- Run tracking EthoVision software to calculate the desired measures (e.g. total distance traveled, velocity, etc.).

Equipment	
EthoVision	NAME
Software	TYPE
Noldus	BRAND
RRID:SCR_000441	SKU
https://www.noldus.com/ethovision-xt	LINK
RRID:SCR_000441	SPECIFICATIONS