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Protocol status: Working
We use this protocol and it's working

Created: May 15, 2023

Motor Behavior Assays (Mouse)

Kelsey Barcomb¹, Beatriz E Nielsen¹, Christopher Ford¹

¹University of Colorado Anschutz Medical Campus

Team Edwards



kelsey.barcomb

ABSTRACT

This protocol describes motor behavioral assays used to assess motor function in different models of Parkinson's disease.

These assays include open field, pole, rotarod, cylinder, balance beam tests. It includes an example experimental time course used to perform identical behavioral assays across two institutions in a progressive DA loss model; and the assays (and its variants) conducted to examine motor function in a partial and more complete DA depletion induced by 6-OHDA (acute model).

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PROTOCOL integer ID: 81907

Keywords: ASAPCRN, Mouse, Behavior, Motor

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Open Field Test

1 Required materials

- Open field chambers - either clear 25 cm diameter cylinders or gray 50 cm x 50 cm square boxes (if chambers are open ended on both sides, use gray silicone mats underneath)
- Overhead mounted camera
- Video analysis software

2 Basic procedure

2.1 Set up behavior room

- Set up lighting with the goals of (a) minimizing total room light, (b) reducing shadows within the chambers, and (c) ensuring camera is able to detect the mice; bright overhead lights should remain off; adding a red filter to the light source is a good option for maintaining low light
- Clean chambers and silicone mats with 70% ethanol
- Place chambers under camera; a 2 x 2 configuration is recommended when multiple mice are being tested at once
- Turn on camera; adjust zoom and focus
- If using simultaneously tracking with EthoVision, set up the software

2.2 Habituation

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

2.3 Testing

- Start recording video *before* mice are in the chamber to collect the background images
- If performing a multi-day testing paradigm, it is recommended to use a Sharpie to mark the tails with the corresponding chamber number
- Place mice in chambers
- Collect video for desired length of time (usually 10-30 minutes, see use cases below)
- Remove mice from chambers and return them to their home cage
- Save video file and (if desired) count the number of fecal deposits in the chamber

2.4 Cleanup

- Between groups of mice, clean chambers and silicone mats with 70% ethanol; allow to fully dry before starting another trial
- At the end of the day, wipe everything down with a sterilant such as Clidox

Note

Avoid using Clidox before placing mice in the chambers - it can irritate their skin

2.5 Analysis

- Run tracking software to calculate the desired measures, usually total distance traveled and velocity. We typically use EthoVision or ezTrack.

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

Note

ezTrack (RRID:SCR_021496) available for free from
<https://github.com/deniseceilab/ezTrack>

- Depending on main research question, you may want to gather additional parameters such as total time in center, rearing/stereotyped behaviors, total fecal deposits, or binned distance over time.

3 Common use cases for open field test

3.1 Open field test for stress

- One week before OFT, move mice to a clean cage and limit interaction with other animal staff (e.g. place on "Do Not Disturb" status)
- For each of the three days leading up to OFT, the researcher who will perform OCT should handle mice for 30-60 seconds
- On the day of testing, set up OFT chambers as normal, using the 50 x 50 cm square chambers; if using real-time tracking, set up to measure time in center vs. borders
- Record locomotion in the chamber for 30 minutes
- Compare percentage of time in center vs. border zones

3.2 Open field test for parkinsonian motor deficits

- Plan for a 3-day paradigm
- Set up the chambers as normal each day and label the tails of the mice 1-4 with a Sharpie; each mouse should stay in its same chamber location each day

Note

Either the cylinder or square chambers can be used

- Days 1 and 2, habituate animals to the chamber by running a 30 minute OFT; these trials do not need to be recorded or analyzed
- Day 3, run the OFT for 20 minutes; measure total distance and velocity

Pole Test

4 Required materials

- 50-60 cm pole with attachable base
- Gauze
- Clear chamber (optional)
- Silicone mat
- Camera (the camera on a laptop is sufficient)

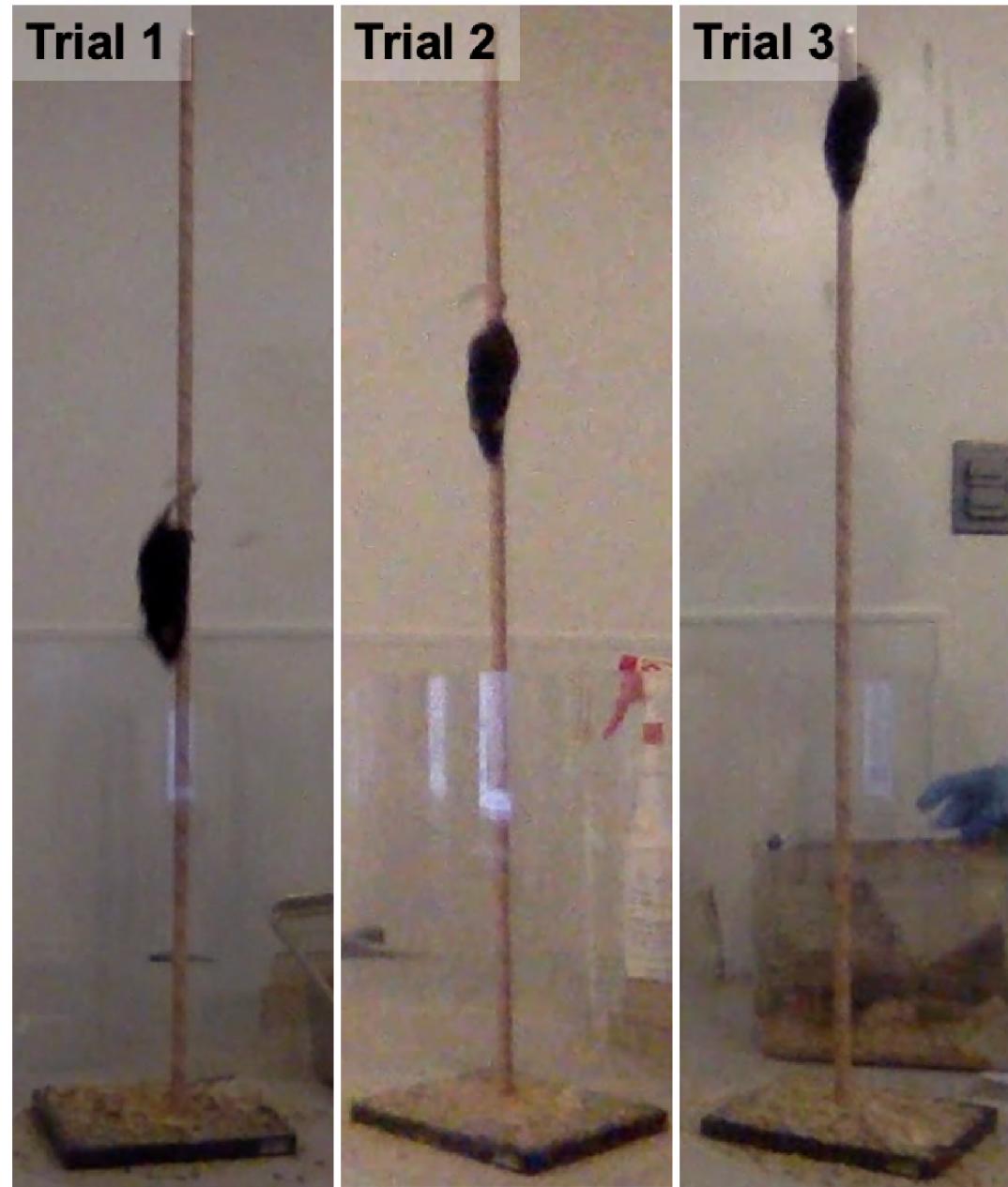
5 Basic procedure (3 day paradigm).

For each of the following days of training/testing, the room setup is as follows:

- Turn on dim lighting (bright overhead lights should remain off)
- Wrap pole with gauze; stretching taut while wrapping will reduce the tendency of the nails of the mice getting caught in the fibers of the gauze
- Screw pole into base
- Wipe down pole, base, clear chamber, and silicone mat with 70% ethanol
- Place clear chamber on silicone mat and place pole inside the chamber
- Position camera in front of apparatus

5.1 Training Day 1 - perform three trials of training as described below; recording video and analyzing trials are **not** required

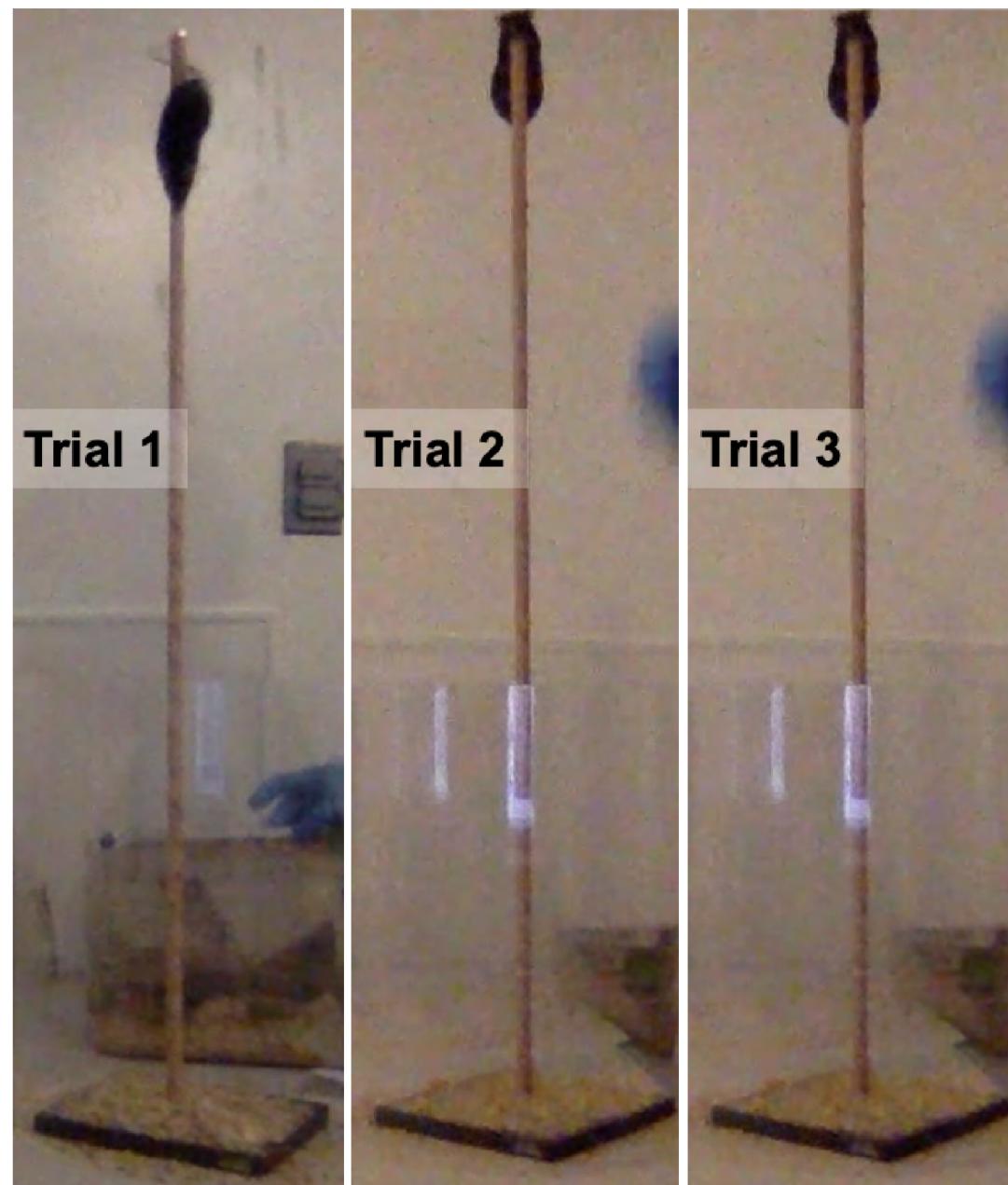
- Habituate mice to the room in their home cage for at least 30 minutes
- Start training on the first cage and prepare the apparatus by covering the base of the pole with home cage bedding; if desired, use a Sharpie to label the tails of the mice to keep track of their order.
- Perform trial one: Grab the first mouse from the first cage and place approximately halfway up the pole with their nose facing down towards the base. Allow the mouse to travel down the pole and dismount onto the base; if they are struggling or not completing the task correctly, you may need to tap their backside to nudge them in the correct direction and/or help them maintain a downward orientation. Return mouse to its home cage and repeat for each subsequent mouse in that cage; start a timer for 5 minutes.
- Perform trial two: Repeat the same procedure as for trial one, but starting the mice at 3/4 of the way up the pole rather than halfway (still facing downward).
- Perform trial three: Repeat the same procedure as for trial one, but starting the mice at the top of the pole (still facing downward).
- If there are additional cages to be tested, clean apparatus by discarding the bedding and wiping down all components thoroughly with 70% ethanol.
- Repeat procedure on each subsequent cage.
- After the last cage, clean everything with Clidox (or similar disinfectant).



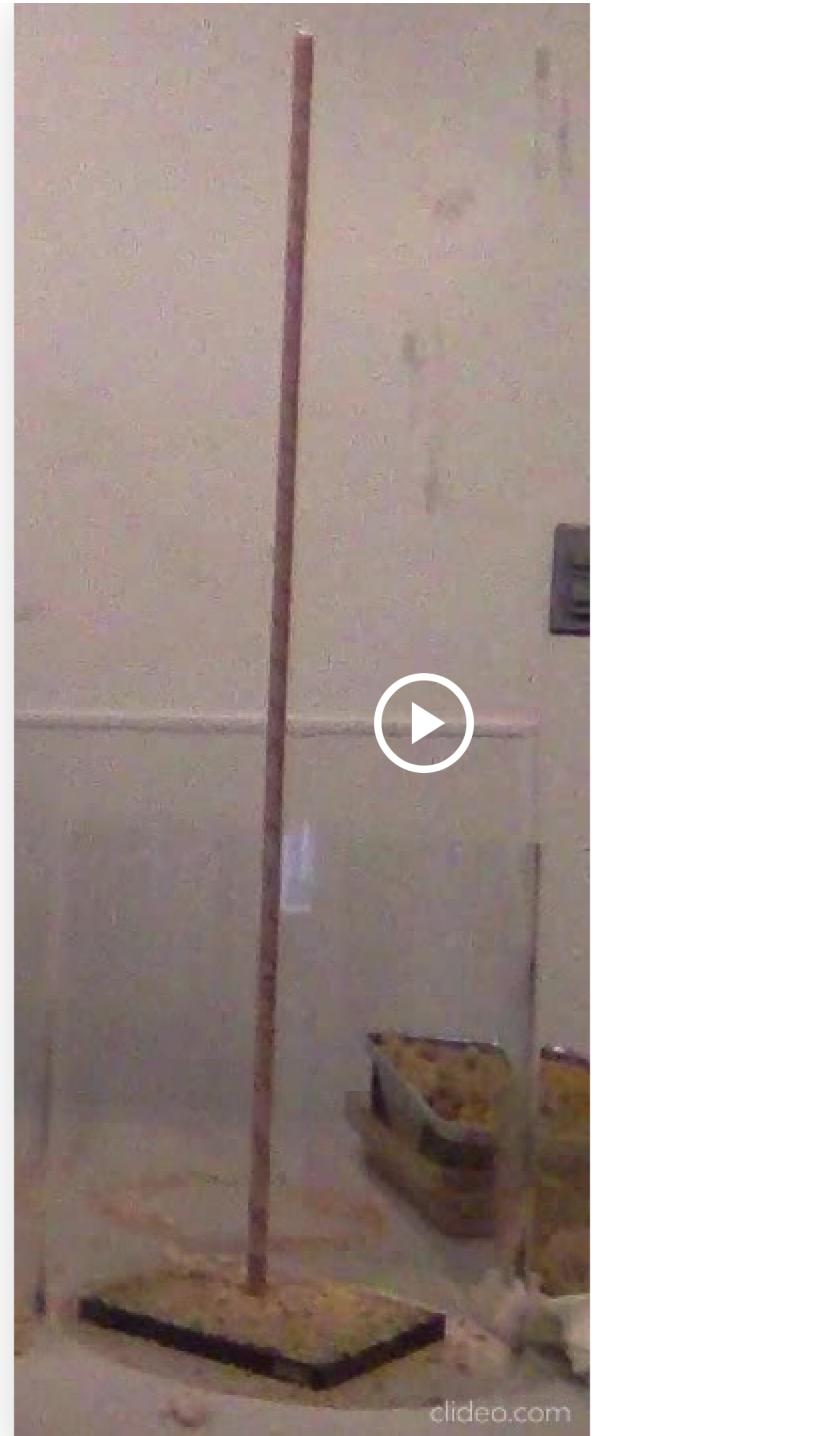
- 5.2** Training Day 2 - perform three trials of training as described below; recording video and analyzing trials are not required, but are suggested for trials 2 and 3
- Habituate mice to the room for at least 30 minutes
 - Start training on the first cage and prepare the apparatus by covering the base of the pole with home cage bedding; if desired, use a Sharpie to label the tails of the mice to keep track of their order.
 - Perform trial one: Grab the first mouse from the first cage and place at the top of the pole, facing down. Allow the mouse to travel down the pole and dismount onto the base; if they are struggling or not completing the task correctly, you may need to tap their backside to nudge

them in the correct direction and/or help them maintain a downward orientation. Return mouse to its home cage and repeat for each subsequent mouse in that cage; start a timer for 3-5 minutes.

- Perform trial two: Repeat the same procedure as for trial one, but starting the mice at the top of the pole and facing upwards. Recording video is recommended.
- Perform trial three: Repeat the same procedure as for trial one, but starting the mice at the top of the pole and facing upwards (identical to trial two). Recording video is recommended.
- If there are additional cages to be tested, clean apparatus by discarding the bedding and wiping down all components thoroughly with 70% ethanol.
- Repeat procedure on each subsequent cage.
- After the last cage, clean everything with Clidox (or similar disinfectant).



- 5.3** Test Day - perform three trials of training as described below; recording video and analyzing trials is **required** for all 3 trials
- Habituate mice to the room for at least 30 minutes
 - Start testing on the first cage and prepare the apparatus by covering the base of the pole with home cage bedding; if desired, use a Sharpie to label the tails of the mice to keep track of their order.
 - Perform test trial one: Start recording video. Grab the first mouse from the first cage and place at the top of the pole, facing UP. Allow the mouse to travel down the pole and dismount onto the base; do NOT assist during the test phase. Return mouse to its home cage and repeat for each subsequent mouse in that cage; stop video and start a timer for 5 minutes.
 - Perform test trial two: Repeat the same procedure as for trial one.
 - Perform test trial three: Repeat the same procedure as for trial one.
 - If there are additional cages to be tested, clean apparatus by discarding the bedding and wiping down all components thoroughly with 70% ethanol.
 - Repeat procedure on each subsequent cage.
 - After the last cage, clean everything with Clidox (or similar disinfectant).



5.4 Analysis - For each full trial (i.e. trial in which mouse was placed on the top of the pole facing upward and not assisted in completing the trial), determine the following measures by analyzing the video recordings:

1. Total time on pole: number of seconds from when the mouse was placed on the pole until the mouse touched its two front paws to the base

2. Time to orient: number of seconds from when the mouse was placed on the pole until the mouse oriented fully downward, as defined by having all four paws rotated down
3. Descent time: number of seconds from when the mouse is oriented downward until the mouse touched its two front paws to the base

Depending on the desired analysis, any of these three measures can be used as the average of all three trials, the best trial (i.e. fastest run), or the sum of the two best (i.e. drop the worst).

Note

For mice that have successfully completed the trial with no errors, Total Time = Orient Time + Descent Time.

If a mouse re-orient upward during the course of a trial then the total time and descent time are not accurate measures to use and that trial should be discarded. If such errors are common, it is recommended to use the best trial or the sum of the two best.

Rotarod Test

6 Required materials

- Rotarod
- Camera (laptop camera is sufficient)

Equipment

RotaRod for Mouse

NAME

Behavioral Apparatus

TYPE

Ugo Basile

BRAND

47650

SKU

<https://ugobasile.com/products/categories/motory-coordination/rotarod-for-mice>

LINK

RotaRod has slots for simultaneously testing up to 5 mice; options for constant speed or ramp up to 80 RPM.

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7 Basic procedure for a 1 day test

7.1 Set up behavior room

- Turn on dim lighting (bright overhead lights should remain off)
- Clean each slot and tray of the RotaRod with 70% ethanol, allow to fully dry before beginning test
- Turn on RotaRod, select "Ramp" setting and set to 5-80 RPM over 300 seconds
- Place paper towel in each tray for the slots that will be used
- Position camera in front of apparatus (a laptop camera is sufficient)

7.2 Habituate mice to behavior room in their home cage for at least 30 minutes

7.3 Hit "Run" on the RotaRod to begin the rotation; load mice onto apparatus and begin the video recording

Note

The mice may try to grab the sides of the RotaRod slots as you load them onto the rod. Orienting them down to get into the slot and then lifting them to the rod will reduce this tendency.

7.4 Once all mice have been loaded, wait 30 seconds to make sure all of the mice continue to walk as the rod rotates, then hit "Start" to initiate the ramp process.

7.5 Observe the mice as they perform the RotaRod task and notate the following (either in real-time or by watching the video recording later):

- End point - time in seconds at which the mice stop successfully completing the task, either due to a fall or no longer continuing to keep up with the rotation (e.g. holding on at one spot and riding around the rod for one or more rotations)

- Fall point - time in seconds at which the mice fall off the rod (note, this may be the same as the end point)
- Depending on the analysis, you may want to make additional notations regarding number of times that the mouse rotates with the rod before falling or changes in orientation.

Note

During the start of the task, the mice may reorient on the wheel such that they are walking backwards. This behavior is not considered as the "end point", even if the mouse rotates with the rod in order to re-orient and continue walking forward.

- 7.6** Use an intertrial interval of 5 minutes and perform the task 2 more times for a total of 3 trials. Mice should remain in the same slot for each trial and cleaning the apparatus between trials is not required.
- 7.7** After completing the three trials for a group, if another group will be run then wipe down the apparatus with 70% ethanol and replace the paper towels in the trays. At the end of the day, clean the apparatus using Clidox (or similar disinfectant).
- 7.8** Analysis - determine Latency to End (in seconds) for each mouse in each trial.
For each mouse, the following measures of performance may be used depending on the goals of the analysis:
 - average of 3 trials
 - sum of two best trials (drop lowest)
 - maximum trial time
 - time course across all 3 trials (recommended if assessing improvement over time)

Example Behavior Timeline - Motor Function Battery

20m

- 8** The following timeline was used for behavioral testing for a Parkinson's Disease model. In this study, 25 cm clear cylinders on top of silicone mats were used for the open field testing and the pole test.

- 8.1** Day 1 - Training (OFT/Pole)
 - Behavior room setup
 - 30 minute room habituation

- Open Field Test - 30 minute open field habituation (not analyzed)
- Pole Test - Training Day 1 (see above; not analyzed)

8.2 Day 2 - Training (OFT/Pole)

- Behavior room setup
- 30 minute room habituation
- Open Field Test - 30 minute open field habituation (not analyzed)
- Pole Test - Training Day 2 (see above; recorded but not analyzed)

8.3 Day 3 - Testing (OFT/Pole)

- Behavior room setup
- 30 minute room habituation
- Open Field Test - **20 minute** open field test (measure total distance and velocity)
- Pole Test - Test Day using 3 trials, 5 minutes intertrial interval (measure Total Time, Orient Time, and Descent Time)

8.4 Day 4 - Testing (RotaRod)

- Behavior room setup
- 30 minute room habituation
- RotaRod Test - 3 trials, 5 minutes intertrial interval (measure Latency to End)

8.5 Day 5 (optional) - Testing (RotaRod)

- Behavior room setup
- 30 minute room habituation
- RotaRod Test - 3 trials, 5 minutes intertrial interval (measure Latency to End)

8.6 Day 6 (optional) - Testing (RotaRod)

- Behavior room setup
- 30 minute room habituation
- RotaRod Test - 3 trials, 5 minutes intertrial interval (measure Latency to End)

Cylinder test: forelimb use asymmetry

5m

9 Required materials:

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

- 10 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 360° vision of cylinder walls.
- 11 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**.
- 12 Testing: 5m
- 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.
- 13 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.

Balance beam: balance and coordination

4m

- 14** 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 length.
 - Video player software (e.g. VLC).
 - 70% ethanol.

- 15** 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 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.

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

- 17** **Training: Day 1**
- Video recording of training session is not required.
Only medium size beam (12 mm) is used for training.

- 17.1** Place mouse in goal box for  00:03:00

3m

- 17.2 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 30s.

 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.

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

- 17.4 Repeat 17.2 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.

- 17.5 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.

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

18 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.

- 18.1** Start recording the video with the camera.

- 18.2** Place a mouse on the 19 mm beam (large sized beam) at the farthest position from the goal box 1m
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.

- 18.3** Once crossed the beam and reached the goal box, leave the mouse inside for  00:00:30 1m
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

- 18.4** Repeat the trial with same beam.

- 18.5** Repeat steps 18.2 to 18.4 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).

- 18.6** 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).

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

19**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 latency 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).