Behavioral Testing - Open Field and Dyskinesia Scoring

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ABSTRACT

This protocol describes behavioral testing to assess motor deficits in mice. It includes open field locomotor testing and scoring of levodopa-induced dyskinesia.

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Open Field Locomotor Testing

1 CHAMBER SETUP

This chamber setup should be used for all steps below (Day1/2 Habituation and Day 3 Testing) and all steps are performed under the same general conditions (i.e. same room/area,

same time of day. same person running experiment)

To measure locomotor behavior, we obtain and analyze overhead video from sessions in which a mouse is placed in the open field (a clear, acrylic chamber, 25 cm in diameter). Video is acquired with overhead cameras that are triggered by software (Noldus Ethovision XT, Arduino, Raspberry Pi), and analyzed with behavioral tracking software (Noldus Ethovision) for several basic parameters.

1.1 Gather materials

- Open field chambers (can use up to 4) - clear, plastic cylinders (25 cm diameter)
- White surface (e.g. yoga mat) to place under the chambers - surface needs to contrast the fur color of the mice in order for the camera/software to effectively detect the mice
- 70% ethanol in spray bottle
- Camera, mounted above the chambers
- Data collection/analysis software (this protocol uses EthoVision: https://www.noldus.com/ethovision-xt)

1.2 Arrange cylinders in a 2x2 grid on your surface of choice under the camera

2 HABITUATION - Day 1 30m

Place mouse in the open field chamber for 30 minutes; clean chambers with ethanol between mice **00:30:00 per mouse (up to 4 mice at once)**

3 HABITUATION - Day 2 30m

Place mice in the open field chamber for 30 minutes; clean chambers with ethanol between mice **00:30:00 per mouse (up to 4 mice at once)**

4 TESTING SESSION - Day 3

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4.1 Recording setup - Using Noldus system (Ethovision XT)

- Choose a template file that contains the number of chamber that you wish to use (e.g. 1, 2, or 4 arenas)
- Arena Settings: Choose "Arena Settings" from the left sidebar, duplicate that last version of Arena Settings. Capture background video; this will open a live window with the overhead video. Adjust the chambers so they are captured by the camera in their entirety and are well-lit. Adjust the size, shape, and placement of the arenas, which are demarcated by oval shaded areas and a label, eg “Arena1” with an arrow pointing to the area of interest. Make sure no arena overlaps with any other, and that the arena for each chamber includes the entire bottom ring of the chamber, as well as some of the bottom side walls (for when an animal rears against the side wall).
- Detection Settings: Choose "Detection Settings" from the left sidebar, duplicate the last version of Detection settings. Click on grab background image. Now place your mouse or mice into the chambers. You will see a live behavioral detection signal on screen. Where a mouse is detected, it will appear yellow, and its center point will be marked with a red dot. In the Detection Settings dialog box, you can adjust the contrast and the size detection parameters to optimize detection of the mouse. Slide these around until you reliably detect each mouse, but do not pick up reflections on the side wall. Sometimes the detection will vary in different parts of the arena, due to shadows or lighting conditions, so make sure the mouse moves to different areas of the arena before accepting the settings.
- Trial Control Settings: Choose "Trial Control Settings" from the left sidebar. Choose the type of trial (eg “10 minute open field”) that you want. Testing sessions can be of variable length, but for basic characterization, 10-20 minutes is adequate.
- Trials List: Choose "Trials List" from the left sidebar. If new empty trials will be needed, push the button at the top to add trials. Otherwise, enter the information for each mouse into the Trials List spreadsheet, and into your lab notebook. At minimum note the name of the Noldus Template file, the Trial #, Arena #, and mouse name, as well as the manipulation that day in your lab notebook.

4.2 ⚠ 20m

Testing
A small dialog box at the right hand side of the screen will show a green button to “start trial”. When you are ready, push this button.

During the experiment, keep an eye on the detection of the mouse, and the panel at the bottom of the screen, which shows statistics regarding the detection of the mouse. **If an animal is detected <90% of the**

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time, the experiment will be unusable.

00:20:00 per mouse (up to 4 mice at once)

4.3 Cleanup

At the end of the experiment, replace each mouse in its respective home cage, and clean each chamber with 70% ethanol. Close the Noldus file.

5 ANALYSIS

Noldus experiments can be analyzed within Noldus or with other software after exporting the raw position (x,y) data.

5.1 Analysis using Noldus

- Use the Analysis tab on the left side bar to create and run simple analyses of your open field data.
- You can choose default metrics, such as rotational behavior, but set your own thresholds (ie count every full rotation, with a threshold of 180 degrees in one direction).
- You can export the data from these Analyses by choosing “export to Excel”. This will create an Excel file with your data in the Noldus folder on the computer.

5.2 Analysis with another progam

- Export the raw (x,y) data from Noldus
- Analyze in Excel, Matlab, etc.

This latter approach is most useful to detect specific user-defined events, such as movement starts (eg transitions from velocity <0.5 cm/sec to >2 cm/sec lasting >0.5 sec).

Dyskinesia Scoring

6 We use a single dyskinesia scoring system (Cenci and Lundblad, 2007) to quantify levodopa-induced dyskinesia (LID).

For scoring large cohorts of animals in which you wish to monitor LID over time or in response to manipulations use "High Throughput Dyskinesia Scoring" **go to step #7**

For scoring 1-2 mice while synchronously collecting other data (e.g. in vivo physiology) use "Low Throughput Dyskinesia Scoring" **go to step #8**

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Cenci MA, Lundblad M (2007). Ratings of L-DOPA-induced dyskinesia in the unilateral 6-OHDA lesion model of Parkinson's disease in rats and mice.. Current protocols in neuroscience.
https://doi.org/10.1002/0471142301.ns0925s41

7 High Throughput Dyskinesia Scoring

High throughput (1) is most appropriate for large cohorts of animals in which you wish to monitor LID over time or in response to manipulations. You can run up to 20 animals/session with one rater.

7.1 Prior to the day of scoring, weigh each of the mice and create a table with the mouse name/code, weight, and volume of levodopa to be injected. Our default dosing is:

- 5 mg/kg of levodopa
- 2.5 mg/kg of benserazide

Give as a small volume of 1 mg/mL solution of levodopa (+ 0.5 mg/mL of benserazide) in normal saline.

7.2 On testing day, assemble empty cages (top and bottom, but no food hopper), which you will need for scoring (1 per mouse) and obtain a timer

7.3 Use tape and a Sharpie pen to label each cage with the code of the mouse to be placed in that cage. [For blinding, have a colleague flip the cage cards and write codes on the back of the cards, eg “A2” for the mouse with the #2 toe tattoo in that cage, and “A4” for the mouse with the #4 tattoo in that cage.]

7.4 Make up your levodopa, as per your calculations above (**go to step #7.1**)

7.5 Set up a table in your lab notebook with time across the top, and mouse codes along the left.

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7.6 Remove first mouse from its home cage, inject with appropriate dose of levodopa intraperitoneally, and place in clean testing cage

7.7 Start timer.

7.8 Now repeat the injection process **every minute until you have injected all of your mice** in order of the testing cages (maximum of 20 mice in 20 minutes).

7.9 When the timer hits 20 minutes, you will commence testing. Each cage will be scored for 1 minute every 20 minutes (at 20, 40, 60, 80, 100, and 120 minutes).

7.10 Get close to the cage, and observe the mouse.

7.11 ⚠

Following the guidelines described in Cenci & Lundblad, 2007 #8, score each of three body segments: axial, limb, and orofacial (ALO)
For each segment:

- 0 = no abnormal movements (no dyskinesia), then the score is 0.
- 1 = some abnormal movements, present <50% of the time
- 2 = abnormal movements between 50-99% of the time
- 3 = continuous abnormal movements
- 4 = continuous abnormal movements that additionally cannot be interrupted by tapping on the cage (uninterruptible)

Note the "ALO" scores in your notebook.
Beyond the Cenci criteria:

- If a mouse is rotating but all 4 limbs are on the floor, that is a rotation, not axial dyskinesia.
- If the mouse is on 2 feet and its trunk is twisted, it's axial dyskinesia.
- Brief chewing of the floor material does not count as orofacial dyskinesia.

Keep an eye on the timer so you move to the next mouse each 1 minute.

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7.12 After 120 minutes have passed and you have scored all your cohort, then return each mouse to its home cage.

8 Low Throughput Dyskinesia Scoring

Low throughput is appropriate for 1-2 mice that are typically being run on a timed optical manipulation or in which you are acquiring synchronous in vivo physiological data.

8.1 Weigh mouse

8.2 Prepare levodopa:
- 5 mg/kg of levodopa
- 2.5 mg/kg of benserazide

Give as a small volume of 1 mg/mL solution of levodopa (+ 0.5 mg/mL of benserazide) in normal saline.

8.3 Start associated experiment (e.g. physiology) as necessary

8.4 Inject mouse with appropriate dose of levodopa intraperitoneally; mark your file and note the time in your notebook.

8.5 Sit adjacent to the mouse and score the mouse live.

You may like to set up a side camera so you can also score video posthoc, though this has a lower sensitivity as it is challenging to score the mouse when it turns away from the camera.

Use the ALO system to score every minute until dyskinesia appears
Note the exact time that rotations begin, and the exact time that dyskinesia appears.

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ALO Scoring (based on Cenci and Lundblad 2007):
Score each of three body segments: axial, limb, and orofacial (ALO). For each segment:

- 0 = no abnormal movements (no dyskinesia), then the score is 0.
- 1 = some abnormal movements, present <50% of the time
- 2 = abnormal movements between 50-99% of the time
- 3 = continuous abnormal movements
- 4 = continuous abnormal movements that additionally cannot be interrupted by tapping on the cage (uninterruptible)

Beyond the Cenci criteria:

- If a mouse is rotating but all 4 limbs are on the floor, that is a rotation, not axial dyskinesia.
- If the mouse is on 2 feet and its trunk is twisted, it's axial dyskinesia.
- Brief chewing of the floor material does not count as orofacial dyskinesia.

8.6 Once dyskinesia appears, score every 1, 2, or 5 minutes until dyskinesia is absent for 5 minutes,

8.7 Once dyskinesia is absent for 5 minutes, score for 1 minute every 5 minutes.

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