**CRN Behavior Good Practices**

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**Purpose**

These guidelines offer general considerations for labs performing behavioral assays in rodent models of Parkinson’s disease.

Note that this guide discusses important considerations for certain assays in general behavior, motor, and cognitive domains. While there are many symptoms important to the diagnosis and progression of Parkinson’s disease, we highlight these domains and subsequent assays because of their widespread focus and use in the field. For similar reasons, we highlight assays used for rodents. We plan to expand this guide to non-human primates.

**README:** Note that this file uses the navigation pane. Click View🡪 Navigation Pane to navigate to the section of interest.

**Important General Considerations**

It is important to recognize that each institution has different requirements and systems in place for animal husbandry. These must be adhered to throughout all behavioral assays and new protocols should receive institutional approval before being performed.

Below are considerations to aid in producing behavioral data that is relevant as well as interpretable.

Experimental design

* As with other scientific methods, consistency is key for reproducible data. Keep the following in mind and be sure to take notes:
  + Date of each test and order of mice tested in which arena
  + Time each trial began
  + Light intensity (i.e. lux) in each arena should be equal
  + Temperature, especially in water maze
  + Unusual behavior- excessive grooming, jumping, tail shaking, etc.
  + Unusual environmental changes (e.g. extra noisy in the corridor, etc.)
* Strain differences may influence the baseline behavior. Due to this, it is advisable to perform small pilot studies to confirm that the “normal” protocol will still work for the wildtype/control groups.
* Appropriate control groups should be included and tested at the same time the test group is. If possible, counterbalance the order of the groups and randomize the order.
* Stay blind to groups (including sex if possible) to avoid experimenter bias.
  + This is easier done with a second person to organize the testing cohorts.
* If using female mice, consider monitoring estrus cycle.
* Circadian rhythms can influence behavior.
  + Behavioral tests, especially those relating to anxiety-like behavior, should be done in the first half of the light cycle or during the dark cycle.
  + Testing that occurs over multiple days should be done at the same time of day.

**\*\*Note what time the housing room lights turn on/off.**

Animal handling and husbandry

* **Stress affects behavior!** It is important to minimize the stress of the mice while you perform these behavioral tests for ethical concerns but also because it can drastically alter data.
  + Mice should be brought from the housing room to the behavior room 1 hour before the start of testing to allow habituation to the room.
  + Handling animals should always be done with care.
* Mice have very sensitive olfactory systems.
  + It is important to clean any traces of other mice in between testing. Cleaning solution may be determined based on the policies of the animal housing facility.
  + Avoid wearing fragrances while testing.
* Consider when cage changes occur in the holding room and consider placing “Do Not Disturb” cards on your cages so you can control when the cage changes occur if possible.

**Below are important considerations for the most commonly used behavioral assays within the CRN based on the** [**April 2024 survey**](https://docs.google.com/spreadsheets/d/1kKg3J9eEuz0B72rVo5gH6ufh8Fxnati_/edit?usp=drive_link&ouid=112457132543319954566&rtpof=true&sd=true)**. For suggested protocols, please see the** [**Behavioral Working Group collection**](https://www.protocols.io/view/behavior-working-group-suggested-protocols-dhvx367n) **on protocols.io.**

**Tests for General Activity**

### **Open Field**

*Description*: The open field consists of a empty square arena with walls in which a rodent is placed in and allowed to explore freely for a set period of time. While the size, color, and length of test vary widely, the open field is one of the most common assays used to assess general locomotor activity.

*Purpose:* Within the CRN, the open field is used to model the following symptoms associated with PD: general locomotor activity, anxiety, bradykinesia (slowness), dyskinesia, gait, balance/coordination, rigidity (stiffness, dystonia), motor asymmetry, stereotyped behavior, and akinesia (hypomimia/masked face). The open field is also sometimes used to habituate animals to the arena used for novel object recognition (see below).

*Primary outcome measures:*

* Total distance moved – indicate levels of locomotion
* Mean/maximum velocity (cm/s) – indicate levels of locomotion, anxiety
* Time spent in center – typically used to indicate levels of anxiety

*Considerations*

* Behavior within the open field is spontaneous and thus can be subject to many variables, planned and unplanned. Consistency is critical to compare results from one day to another.
* Interpreting data should be done while accounting for any potential confounds.
  + E.g. Lower amounts of total distance travel may suggest motor impairments but taken with lower total time spent in the center may demonstrate anxiety-like behavior.

**Tests for Motor Function**

### Open Field- See General Activity

### **Rotarod**

*Description*: The rotarod consists of a rotating rod that rodents are placed on and must learn to walk on or otherwise fall a short height. Some protocols may call for a consistent speed of rotation (RPM) while others make the rotarod accelerate over time.

*Purpose:*  Within the CRN, the rotarod is used to assess balance/coordination, general locomotor activity, motor learning, muscle strength, gait, fine motor skills, and akinesia (hypomimia/masked face).

*Primary outcome measures:*

* Average time to fall- analyze this as an average per day or can be shown as individual trials to demonstrate motor learning

*Considerations*

* Rotarod is helpful for general movement dysfunction but is likely more tied to cerebellar function).
* There is a learning component and does require careful, repeated handling. This would be especially important if the mice are to be tested at multiple timepoints/

### **Challenging Beam**

*Description:* Like the open field, the challenging beam test can take many forms and differ in size and shape. Here we focus on the one that is flat and consists of 4 sections that narrow as it progresses.

Example beam dimensions: Beam consists of four sections and is constructed of Plexiglas. Each section is 25 cm and the total length is 1 m. The beam starts with a 3.5 cm width and narrows down to .5 cm, in 1 cm increments. The underhanging ledges are 1 cm wide placed 1 cm below the beam’s upper surface.

*Purpose*: Within the network, the challenging beam is used to assess bradykinesia (slowness),

general locomotor activity, rigidity (stiffness, dystonia), balance/coordination, gait, catalepsy, cognitive impairment (Attention/working memory), fine motor skills, and anxiety.

*Primary outcome measures:*

* Total time to traverse the beam
* Number of steps are the beam
* Number of errors in each section of the beam- typically most errors are made in the last, most narrow section
* Number of errors/step

*Considerations*

* The beam test requires significant amount of training so that the mice traverse willingly across the beam.
* This test requires video recording in order to be able to manually collect all the primary outcome measures. Significant time scoring the videos is then needed. Machine learning may eventually help reduce this testing burden.
* Mice that are highly anxious or skittish may dart off the beam altogether.

### **Pole Test**

*Description*: The vertical pole test assesses a mouses ability to turn and descend. The height of the pole can vary- from 30cm to 50cm but is typically 1cm in diameter. To perform this test, mice must be handled carefully and trained to hold onto the pole with all 4 paws with their head facing up towards the ceiling. The experimenter then trains the mouse over 1-3 days so that the mouse turns and climbs down the entire length of the pole.

*Purpose*: Within the CRN, the pole test is used to assess sensorimotor coordination, balance/coordination, muscle strength, rigidity (stiffness, dystonia), bradykinesia (slowness), general locomotor activity, and anxiety.

*Primary outcome measures:*

* Average time to turn
* Average time to reach the bottom

*Considerations*

* While anxiety-like behavior may be demonstrated on the pole test, we recommend using another assay to verify this phenotype.
* The pole test can generate highly variable outputs and requires fine motor ability to perform well.
* Heavier mice may require more strength to hold onto the pole and thus may perform poorly.
* Some poles are wooden and have enough friction for mice to hold onto. Wood is difficult to sterilize so many facilities do not allow it. Medical gauze can be used to create a surface for the mice to hold onto more securely.

**Tests for Cognitive Function**

## **Novel Object Recognition**

*Description:* The novel object recognition assay uses the open field arenas to examine a mouse’s cognitive ability to detect change in their environment, either in a new object or in a new location of an object. Mice are habituated to the two identical objects. After varying amount of time (usually 1hr – 24 hrs), they are then placed back in the arena with the new objection or new location. Video tracking is often used to determine how long a mouse spends exploring each object.

*Purpose:* The novel object is used within the CRN to assess cognitive impairment reference/recognition memory, attention/working memory, and spatial memory.

*Primary outcome measures:*

* Total distance moved
* Mean/maximum velocity (cm/s)
* Time spent in Object 1 and Object 2 (nose-point tracking)

*Considerations*

* Pilot trials are required to ensure there is no inherent bias towards one object vs another. Similarly, some strains of mice have greater difficulty discerning objects from each other and may need “easier” options.
* Mice must be well habituated to the arenas. Otherwise, they may not spend significant time exploring the objects.

## **Operant Chamber Conditioning**

*Description*: Operant chamber conditioning consists of significant amounts of training to teach rodents to respond (via nosepokes or lever pressing) to a stimulus in order to receive a food reward. There are many variations of testing.

*Purpose:* Operant chamber conditioning is used within the CRN to assess cognitive impairment (executive function, attention/working memory) and apathy/anhedonia.

*Primary outcome measures:*

* Latency to respond
* Percentage of correct responses
* Number of errors
* Number of days to reach criterion

*Considerations*

* To promote consistent results, each mouse should be trained in the same operant chamber at the same time of day, throughout the study.
* Male and female mice should be trained in separate operant chambers.
* This assay requires food restriction and may not be suitable for all animal models.
* This is a time-intensive assay.