CRN Rodent Behavior Assay Guidelines

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These guidelines offer general considerations for labs performing the most commonly used rodent behavioral assays of Parkinson's disease (PD), based on an April 2024 survey of the ASAP CRN. 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 use in the field.

As very few labs use species other than mice (i.e. rats and non-human primates), the assays below are focused on mice. General considerations and test protocols can be applied to rats if appropriate changes are made (e.g. the size of testing equipment).

For suggested protocols, please see the Behavioral Working Group collection on protocols.io.

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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 robust and interpretable.

Experimental Design

- **Take good notes**, including:
  - Date of each test
  - Order in which animals were tested
  - Arena in which each animal was tested
  - 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 as the test group. If possible, counterbalance and/or randomize the order of the groups.

- The experimenter should be **blinded** to groups (including sex, if possible) to avoid experimenter bias. This is easier with a second person to organize the testing cohorts.

- If using female mice, consider **monitoring the 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 when planning experiments.

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 outcomes.
  - 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 housing room and consider placing **"Do Not Disturb" cards** on your cages so you can control when the cage changes occur if possible.

Spontaneous Activity Assays

Spontaneous motor activity assays are behavioral tests designed to evaluate the natural, unprovoked movements and behaviors exhibited by an animal in the absence of specific external stimuli or tasks. This activity serves as a measure of the animal's general locomotion, exploratory tendencies, and overall level of activity in a given environment. It is often used to assess the effects of genetic modifications, environmental risk factors, or drug treatments on motor function in animal models of Parkinson's disease (PD).

Open Field

Description: The open field consists of an empty square arena with walls. A rodent is placed in the open field 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.

For an example open field protocol, see here.

Purpose: The open field is used to assess 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** – measurement of locomotor activity; indicates presence of bradykinesia/akinesia and rigidity
- **Velocity** – measurement of locomotor activity; indicates presence of bradykinesia/akinesia and rigidity; may also be an indication of anxiety
- **Time spent not in locomotion** – may indicate stereotyped behavior (i.e., time spent grooming) or presence of bradykinesia/akinesia
- **Rotation counts** – unilateral animal models of PD may display contraversive rotation which indicates unilateral loss of dopaminergic neurons
- **Time spent in center** (vs periphery of the arena) – increased thigmotaxis or reduction in exploratory behavior in the center of the arena indicates increased 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.

- 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. Additionally, precise quantification of stereotyped behaviors and fine movements may require a sophisticated software or machine learning toolbox like DeepLabCut.
- Interpretation of data should account for any potential confounds.
    - For example, low distance traveled may suggest motor impairments but taken with lower total time spent in the center may also be indicative of anxiety-like behavior.

Motor Function Assays

Motor function assays are behavioral tests designed to evaluate movement, coordination, balance, and strength in mice. Specifically, these tests are used to characterize the extent of motor deficits in animal models of Parkinson's disease (PD). These tests can be repeated over time to evaluate the progression of neurodegeneration and assess the efficacy of potential treatments.

Open Field

See above.

Rotarod

Description: The rotarod consists of a rod that rotates at a controlled speed. Rodents are placed on the rotating rod and must walk on the rod to prevent falling a short height. In some protocols the rod rotates at a consistent speed (RPM), while in others the rod accelerates over the course of the trial.

For an example rotarod protocol, see here.

Purpose: The rotarod is used to assess balance/coordination, motor learning, muscle strength, gait, fine motor skills, and akinesia (hypomimia/masked face) in PD models.

Primary outcome measures:

- **Average time to fall** – indicates motor coordination (average per day) or motor learning (individual trials over time)

Considerations

- Rotarod may be helpful for identifying general movement dysfunction but is likely more tied to cerebellar function (vs. loss of dopamine transmission).
- There is a learning component to the rotarod, which requires repeated testing/handling at multiple timepoints.

Challenging Beam

Description: The challenging beam consists of a beam with four sections that narrow as the beam progresses. The exact dimensions of the beams can vary. Example beam dimensions: 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 and placed 1 cm below the beam's upper surface.

For an example challenging beam protocol, see here.

Purpose: The challenging beam is used to assess bradykinesia (slowness), general locomotor activity, rigidity (stiffness, dystonia), balance/coordination, gait, catalepsy, fine motor skills, and anxiety.

Primary outcome measures:
- **Total time to traverse the beam** – indicates presence of bradykinesia/akinesia and rigidity
- **Latency to initiate movement** – longer latency to initiate movement may indicate anxiety-like behavior.
- **Number of steps on the beam** – measure of gait and balance/coordination; increased number of steps indicates impaired gait and coordination
- **Number of errors in each section of the beam** – increased errors indicates presence of rigidity, balance/coordination impairments, gait abnormalities, and/or poor fine motor skills; most errors are made in the last, most narrow section of the beam
- **Number of errors per step** – indicates balance/coordination impairments

Considerations
- The beam test requires a significant amount of training so that the mice willingly traverse across the beam. Mice that are highly anxious or skittish may dart off the beam altogether.
- 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.

Pole Test

Description: The vertical pole test assesses ability to turn and descend. The pole is typically 1 cm in diameter; the height can vary, but is typically 30cm to 50cm. 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.

For an example pole test protocol, see here.

Purpose: The pole test is used to assess sensorimotor coordination, balance/coordination, muscle strength, rigidity (stiffness, dystonia), bradykinesia (slowness), and anxiety.

Primary outcome measures:
- **Average time to turn** – indicates impairment of balance/coordination and/or fine motor skills.
- **Average time to reach the bottom** – indicates the presence of bradykinesia/akinesia and rigidity.

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.
- The material of the pole should have enough friction for the animal to hold onto. Although wood has enough friction, it 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, which can be replaced between animals.

Cognitive Function Assays

Cognitive function assays are behavioral tests used to evaluate learning, memory, attention, and decision-making in laboratory animals. These assays are particularly important in studies of Parkinson's disease, as cognitive deficits such as impaired executive function and attention are common non-motor symptoms of the disease. These assays help identify the progression of cognitive decline and evaluate potential therapeutic strategies, including pharmacological treatments and neuroprotective agents. Standardized testing conditions and careful control of confounding factors are critical for obtaining reliable data in cognitive studies.

Novel Object Recognition

Description: The novel object recognition assay uses an open field arena to examine an animal's cognitive ability to detect change in their environment. The test can be performed by placing a new object in the arena or by moving the location of an object. Rodents are habituated to two identical objects. After varying amounts of time (usually 1 hr – 24 hrs), rodents are returned to the altered arena. Video tracking is often used to determine how long a mouse spends exploring each object.

For an example novel object recognition protocol, see here.

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

Primary outcome measures:

- **Total distance moved** – measurement of locomotor activity; indicates presence of bradykinesia/akinesia and rigidity
- **Velocity** – measurement of locomotor activity; indicates presence of bradykinesia/akinesia and rigidity; may also be an indication of anxiety
- **Time spent exploring familiar and novel objects** – indicates reference/recognition memory and working memory
- **Time spent exploring objects in familiar and novel locations** – indicates reference/recognition memory, working memory, and spatial memory

Considerations

- Pilot trials are required to ensure an animal does not have an inherent bias towards one object vs another.
- Some strains of mice have greater difficulty discerning objects from each other and may need simpler object options.
- This test requires video recording in order to be able to use nose-point tracking and interaction with the objects in the arena.
- Mice must be well habituated to the arenas. Typically, a 5–10-minute habituation session is included prior to the testing. Most labs prefer performing the habituation 24 hours prior to the test. Otherwise, they may not spend significant time exploring the objects.

Operant Conditioning

Description: Rodents can be trained to perform tasks using an operant chamber. Operant chamber conditioning consists of training sessions that orient animals to the components of the chamber, the availability of rewards, and teach rodents to respond (via nose pokes or lever pressing) to a stimulus to receive a food reward. Ultimately, there is a testing phase that can assay different aspects of behavior. There are many possible variations. For example, the experimenter can choose specific cues (such as auditory, visual, or olfactory cues), specific rewards (such as water, food), response types (such as nose pokes, lever presses, or movement to a specific location in the operant box), and the reward schedule (such as fixed ratio “FR” or random ratio “RR” schedules of reward for a certain # of responses, delays between response and reward, or the probability of reward for each response). Operant conditioning training may be followed by specific operant learning tasks, such as probabilistic reversal learning or delay discounting.

Labs can choose to purchase modular systems that include the operant box and user-friendly tools to design and implement tasks or can build their own systems and program them using relatively simple electronics and code.

For an example operant conditioning training protocol, see here.
For an example probabilistic reversal learning protocol, see here.

Purpose: Operant conditioning can be used to assess cognitive impairment (executive function, attention/working memory) and apathy/anhedonia. In modeling Parkinson’s Disease, operant tasks can be useful in assessing multiple cognitive-behavioral features, such as changes in learning, motivation, decision-making, behavioral flexibility and impulsivity.

Primary outcome measures:

- **Latency to respond to cue** – indicates bradykinesia and motivation (apathy)
- **Percentage of correct responses** – indicates cognitive impairments/learning; a “correct” trial may be one in which the action is rewarded, or in probabilistic tasks, a “correct” trial is one associated with a higher probability of reward
- **Rate of correct responses across trials** – indicates changes to learning, decision-making, and behavioral flexibility (reversal learning)
- **Number of errors** – indicates difficulty in decision-making or learning; may also indicate difficulty perceiving the cue or processing the outcome
- **Number of days to reach criterion** – indicates cognitive impairments/learning

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 and at separate times.
- This assay requires food restriction and may not be suitable for all animal models.
- This is a time-intensive assay.