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Assessment of mouse motor behaviour via open field test, narrowing beam assay, inverted wire hang, and gait analysis V.2

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We use this protocol and it's working

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Abstract

This protocol describes methods for assessment of mouse motor behaviour with 4 separate behavioural tasks: the open field test, the narrowing beam assay, the inverted wire hang, and gait analysis. The open field test measures an animals total locomotor behaviour, but can be confounded by stress and anxiety. The narrowing beam test provides a measure of skilled motor behaviour. The inverted wire hang measures limb strength and endurance. Gait analysis provides a multidimensional analysis of animal gait. Some or all of these behavioural assays may be used, depending on experimental question.

Before start

Unless necessary, keep animals on a reverse light cycle and perform behavioural tests during the mouse's dark phase. Ensure that animals have ample time to entrain to a new light cycle, if changing light cycle or transferring animals to new spaces.

Avoid conducting multiple behavioural assays in the same day and avoid conducting behavioural tests on cage change days.

Open field test

1 Set-up

The testing room should be lit by red light, and should be free of sources of non-red light (computer screens), sounds, and odours.

The open field consists 4 square arenas, each measuring 27 cm x 27 cm and arranged in a 2x2 grid, constructed out of white plastic. A camera mounted 1.83 m above the floor and centered over the middle of the open field can capture animal movement in the open field. Ensure that the floor of each arena is adequately illuminated by red lights; if necessary, place red light lamps around the arena to provide uniform illumination of the arena floors. EthoVision XT software can be used to capture and subsequently analyze the animal movement in the open fields.

2 Testing

On day of testing, transfer animals from holding room into the testing room, and allow them to acclimate to the space for at least 30 mins before beginning.

Use a peroxide-based cleaner to clean and deodourize open field. Transfer all animals from 1 cage into separate arenas of the open field. To mitigate confounds due to non-cagemate odours, we recommend only testing 1 cage of animals at a time (i.e. we don't recommend running animals from different cages at the same time).

Set up EthoVision software to collect data; each trial consists of a 2 minute habituation period, followed by a 10 min test period. Longer habituation or test times may be desirable in some cases.

Before starting test, ensure that the software is successfully detecting the animals' midpoint, nose and base of tail. If necessary, adjust contrast settings in EthoVision until this criterion is met. This is particularly important if using different strains of mice with differing coat colours.

Run open field test.

Once test is finished, transfer animals back to cage, then use a peroxide-based cleaner to clean and deodourize open field. You can now run the next cage of animals.

3 Data analysis

Use EthoVision XT software to extract relevant measures from animal traces, such as total distance moved during the trial period. Other measures, such as time spent in center vs. edges can also be useful to analyze.

Narrowing beam assay

4 Set-up

The narrowing beam consists of 4 segments, each 25 cm in length. The segments differ in width (3.5 cm, 2.5 cm, 1.5 cm, and 0.5 cm). Depending on the severity of the phenotype expected, it may be necessary to remove the 0.5 cm wide segment. A beam constructed out of a semi-transparent material (e.g. plexiglass) is best, as it can afford a view of both right and left limbs, even if recording the animal's side.

Assemble the narrowing beam and place over 4 empty cages on a table. Place the first 3 cages with the opening facing up underneath the widest segments. At the very end of the thinnest segment, position the last cage with opening facing down. Only ~1 cm of the thinnest beam should rest on the upside down cage. Ensure that the beam is level and stable, and does not sag due to light pressure. If it is not level or stable, adjust the positioning of the cages to make it level or stable.

On the last cage (the one with opening facing down), position another empty cage, with opening facing the beam. A mouse should be able to step off the beam into this cage.

Above the end of the thickest segment, position a lamp with a white light. This light will serve as an aversive stimulus to motivate animals to move down the beam.

Place a video camera on a tripod beside the table, with lens perpendicular to the beam and at the same height as the beam. Ensure that the entire beam is visible in the recording.

Note

To make the goal cage more enticing, create a dark space by wrapping the goal cage in aluminum foil.

5 Habituation and training

We recommend at least 2 sessions of habituation and training, conducted on separate days. The goal of training session is to ensure that animals are performing the task at the

following criterion: the animal successfully moves across the beam from the starting position and into the goal cage, without stopping or falling off the beam.

Transfer animals from holding room into the testing room, and allow them to acclimate to the space for at least 30 mins.

Turn on the lamp. It is not necessary to record these sessions.

For each cage of animals to test, remove some soiled bedding from the home cage and place inside the goal cage.

For each animal in the cage:

1. Remove first animal, gripping by the base of the tail. Place onto the end of the thickest segment, with all 4 limbs on the beam and the tail hanging off the beam.
2. Allow animals to explore the beam. If animals turn around on the beam, climb off the beam, or fall off the beam, reposition them at the starting position.
3. Once the animal has successfully moved across the beam into the goal cage, allow the animal to rest in the goal cage for 30s, then place at the starting position again.
4. At this point, you may start encouraging the animal to move along the beam by gently pushing on their rear when the animal pauses for more than 3s.
5. Repeat steps 3-4 until the animal has completed 3 successful beam traversals with minimal encouragement.

Use a peroxide-based cleaner to sterilize and deodorize. Clean beam between each animal, or between each trial if animal has defecated or urinated on the beam. Between each cage, change bedding in the goal cage and clean the entire set-up (beam, goal cage, and table).

By the end of the training sessions, full-functioning animals should rapidly move across the beam each trial without difficulty or pausing.

6 Test day

Test sessions can start at least 1 day after training day, though it may be necessary to collect at least 2 sessions worth of data before any experimental manipulation(s), if possible.

Transfer animals from holding room into the testing room, and allow them to acclimate to the space for at least 30 mins.

Turn on the lamp and set up video camera. Ensure that the camera can record the entire length of the beam and that the beam is in focus. For each animal, we recommend

recording the entire session, then cropping the video into individual trials.

Test sessions can take place in same manner as habituation and training sessions. For each cage, remove some soiled bedding from the home cage and place inside the goal cage.

For each animal:

1. Gripping by the base of the tail, place the mouse onto the end of the thickest segment, with all 4 limbs on the beam and the tail hanging off the beam.
2. Allow animal to move across the beam. If the animal pauses for more than 3s, encourage the animal to move across the beam by gently pushing on their rear. If the animal turns around on the beam or climbs off the beam, return the animal to the starting position. If the animal falls off the beam, the trial is finished; place the animal into the goal cage (failed trial). If the animal fails to traverse the beam in 120s, but did not turn around, climb off, or fall off, place the animal into the goal cage (failed trial)
3. Once the animal has successfully moved across the beam into the goal cage from starting position, or has fallen off the beam, allow the animal to rest in the goal cage for 30s, then place at the starting position again.
4. Repeat steps 1-3 until the animal has completed 3 trials with either a successful traversal or a failed traversal (fall off or timed out). This may take more than 3 attempts to achieve.

Use a peroxide-based cleaner to sterilize and deodorize. Clean beam between each animal, or between each trial if animal has defecated or urinated on the beam. Between each cage, change bedding in the goal cage and clean the entire set-up (beam, goal cage, and table).

7 Data analysis

Videos of the entire session can be cropped into individual successful and/or failed trials for scoring. Ideally, an experimenter blinded to the experimental conditions should score the videos.

Score animals on time to cross the beam, discounting time spent stationary on the beam (i.e. only count time spent moving). Play video at 1x speed for this.

Score animals on performance in task. The specific nature of the scoring system may depend on the model being used. The following scoring system may be appropriate for models of cerebellar ataxia.

- (7) traverses the beam successfully, with no more than 4 foot slips and does not grip the side of the beam

- (6) traverses the beam successfully, using hindlimbs to aid in more than 50% of strides
- (5) traverses the beam successfully, using hindlimbs to aid in less than 50% of strides
- (4) traverses the beam successfully, using a hindlimb at least once to push forward, but without bearing load on limb
- (3) traverses beam successfully, by dragging hindlimbs without using them to push forward
- (2) moves at least 1 body length, but fails to traverse beam in 120 s trial period or falls off
- (1) fails to traverse beam or falls off, and does not move more than 1 body length.

For scoring animal performance, play video at 0.25x to 0.5x speed. For both measures (traversal time and score), take average of 3 trials.

Inverted wire hang

8 Set-up

For this assay, you will need a piece of wire mesh (about 20 cm x 20 cm) with 6 mm mesh, and a 0.5 m tall cylinder. The diameter of the cylinder should be small enough that the wire mesh can be placed on the top. You will also need a source of clean bedding and a stopwatch.

As animals may climb under the wire mesh even when inverted, create a 2.5 cm border of tape around the edge of the wire mesh to prevent animals from climbing onto the top of the mesh.

9 Testing

On day of testing, transfer animals from holding room into the testing room, and allow them to acclimate to the space for at least 30 mins.

Add clean bedding to the bottom of the cylinder.

For each mouse:

1. Remove mouse from its cage, and place into the center of the mesh square
2. Quickly invert the mesh and place on the top of the cylinder. Start the stopwatch.
3. Stop the stopwatch when the animal falls to the bottom of the cage or the if the trial lasts for longer than 120s.
4. Allow the animal to rest for 120s between trials
5. Repeat steps 2-4, until a total of 3 trials have been conducted.

After each cage of animals, discard bedding in cylinder and clean the cylinder with a peroxide-based cleaner. After cleaning, add more clean bedding before starting on a new cage of animals.

10 Data analysis

To quantify animal performance in inverted wire hang, take average of the latency to fall for the 3 trials. If animals manage to hang on for >120s, assign a score of 120s.

Gait analysis

11 Set-up

For gait analysis, we have used the MouseWalker hardware and analysis pipeline (Mendes et al., *BMC Biol* 13: 50).

The hardware consists of a clear acrylic platform, 80 cm long, with a 5.3 cm wide corridor flanked by 12.5 cm high walls. LED lights positioned around the platform enable tracking of animal contact (e.g. footfalls) with the surface, through frustrated total internal reflection (fTIR). A simple video camera (e.g. a smartphone camera) placed underneath the platform captures the fTIR signal, tracking contact with the platform as the mouse moves over the platform. MouseWalker software can then be used to analyze the data, providing multiple parameters of animal gait.

12 Habituation and training

We recommend at least 2 sessions of habituation and training, conducted on separate days. The goal of training session is to ensure that animals readily move through the corridor with minimal pausing.

Transfer animals from holding room into the testing room, and allow them to acclimate to the space for at least 30 mins.

Use a peroxide-based cleaner to clean and deodourize MouseWalker apparatus. Ensure that lights on the platform are on. There is no need to record the session, but habituation to the LED lights is important.

For each animal,

1. Place the animal on one side of the corridor, in a region not captured by the camera.
2. Allow the animal to explore the space for at least 3 min.
3. Once the animal has moved across the corridor, over the camera's field of view, gently lift the mouse up and turn the mouse around.
4. Continue this process until the mouse has completed 3 traversals of the camera's field of view.

Between each animal, clean and deodourize MouseWalker apparatus using a peroxide-based cleaner.

13 Testing

Transfer animals from holding room into the testing room, and allow them to acclimate to the space for at least 30 mins.

Use a peroxide-based cleaner to clean and deodourize MouseWalker apparatus. Ensure that lights on the platform are on and that the camera is recording.

For each animal,

1. Place the animal on one side of the corridor, in a region not captured by the camera.
2. Once the animal has moved across the corridor, over the camera's field of view, gently lift the mouse up and turn the mouse around.
3. Continue this process until the mouse has completed 3 continuous traversals of the camera's field of view.

Between each animal, clean and deodourize MouseWalker apparatus using a peroxide-based cleaner.

14 Use MouseWalker software (Mendes et al., *BMC Biol* 13: 50) to analyze the data.

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