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Forced Swim Test Protocol

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Protocol status: Working

We use this protocol and it's working

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Abstract

The following protocol is used to assess behavioral despair or learned helplessness using the University of Alabama at Birmingham's Neuroscience Behavioral Assessment Core.

Guidelines

The use of this protocol requires approval by the user's Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Materials

1. Chlorhexidine
2. Paper towels
3. Cylindrical tanks
4. Clean water
5. Ethovision version 17 software
6. Recovery cages
7. Heating pad
8. Fish net

Before start

You will need a test mouse whose behavior is not included in the experiment to calibrate detection settings.

Set Up

- 1 Bring animals into testing room for habituation (at least 30 minutes prior to start of experiment)
- 1 Set up test cylinders
 - Cylinder should be at least 50 cm tall and 20 cm wide for mice
- 1.1 Clean the cylinder (wipe out and spray with chlorohexidine)
- 1.2 Set up the number of cylinders you want to use (4 fit well under our camera setup)
- 1.3 Fill with clean water
 - Should be at 21° C
 - Fill water to level where subject cannot reach the bottom of the cylinder, but below level from which they could jump out
- 2 Make sure the EthoVision version 17 software dongle is in the computer
 - Shines a red light when working
- 3 Open EthoVision version 17 software
- 4 Start new experiment – New from Template
- 4.1 Name it
- 4.2 Tell it where to save
- 5 Experiment Settings
- 5.1 Select live tracking
 - Select the camera to be used
 - Center your arena under the camera



5.2 Select center point detection

5.3 Select the number of arenas that you will be using

5.4 Units should be cm, s, and deg

6 Arena settings

6.1 Grab a background image from the camera

- Ensure the cylinders are in their intended area, moving them after this step will affect analysis
- Mark placement with tape so the maze can be returned to the exact same location if accidentally between trials

6.2 Draw scale

- Measure the width of one cylinder
- Draw a scale bar over the area that you measured and indicate its length

6.3 Click first arena in the list on the right

- Select the ellipse drawing tool
- Trace one cylinder
- Drag arena label inside ellipse
- Repeat for every cylinder in the experiment

6.4 Validate setup

- If there are any overlapping zones, they will be considered invalid and must be redrawn without overlapping

7 Trial Control Settings

7.1 Begin condition should be on the start button with the condition of center-point being in the arena

7.2 Stop track with the condition being after 6 minutes

8 Detection Settings



- 8.1 Click advanced settings
- 8.2 Dynamic response
- 8.3 Select appropriate setting to indicate if subject is darker or lighter than the background
- 8.4 Place a test subject in the maze
- 8.5 Click and drag around the subject to create a box around the subject
- 8.6 The software will create an automatic threshold around the subject that moves with it
- 8.7 Fine tune it with the scrub bar to make it fit around the subject perfectly
- 9 Trial list
- 9.1 Add the desired number of trials

Running the Experiment

- 10 Acquisition
- 10.1 Software will load the first trial
- 10.2 Put subjects in center of cylinders
- 10.3 Press record



- The program will run for 6 minutes and automatically stop

10.4 You must watch subjects closely throughout the test. If the mouse dips below the water surface, remove this animal from the water, dry it, and place it in a recovery cage.



10.5 Remove subjects

10.6 Dry subject off with a clean towel and place in a clean cage over a heating pad set to high to avoid hypothermia

10.7 Remove all feces and bedding from water using a fish net

10.8 Repeat for all trials

Results and Analysis

11 Data Profile

11.1 Ensure that it is pulling data from all trials

12 Analysis Profile

12.1 Distance traveled

- Arena
- Statistics - total only

12.2 Velocity

- Arena
- Averaging interval - 1
- Statistics - mean

12.3 Movement

- Moving - cumulative duration
- Not moving - cumulative duration

12.4 Mobility State

- Highly mobile - cumulative duration



- Mobile - cumulative duration
- Immobile - cumulative duration

13 Results

13.1 Statistics and charts

13.2 Calculate to generate a file with the analysis parameters chosen

13.3 Export and save

Acknowledgements

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