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Elevated Zero Maze 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 anxiety-related behaviors using the Elevated Plus Maze at the University of Alabama at Birmingham's Neuroscience Behavioral Assessment Core.

Guidelines

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

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)

2 Set up maze under camera

2.1 Clean the maze with chlorohexidine

3 Make sure the EthoVision XT Version 17 software dongle is in the computer
- shines a red light when connected

4 Open EthoVision XT Version 17 software

5 Start new experiment

5.1 Name the file

5.2 Indicate where file will be saved

6 Experiment Settings

6.1 Select live tracking
- select the camera to be used
- center the arena under the camera

6.2 Select center point detection

6.3 Select the number of arenas that you will be using

6.4 Units should be cm, s, and deg

7 Arena settings

7.1 Grab a background image from the camera
- ensure the maze is in its intended area, moving it after this step will affect analysis
- mark placement with tape so the maze can be returned to the exact same lactaion if accidentally moved between trials

7.2 Draw scale
- measure diameter of the whole maze
- draw a scale bar over the area that you measured and indicate its length

7.3 Click first arena in the list on the right
- select the ellipse drawing tool
- trace the outer circle of the maze
- trace the inner circle of the maze
- drag the arena label inside the middle of the two circles

8 Draw zones

8.1 Select zone 1
- use closed polygon tool to trace one of the closed regions

8.2 Add a zone
- use closed polygon tool to trace the other closed region

8.3 Add a zone
- use closed polygon tool to trace one of the open regions

8.4 Add a zone
- use closed polygon tool to trace the other open region

8.5 Create 2 cumulative zones
- Closed - the two closed regions
- Open - the two open regions

8.6 Validate setup
- If you have any overlapping zones, they will be considered invalid and must be redrawn

9 Trial Control Settings

9.1 Begin condition should be on the start button with the condition of center-point being in the zone

9.2 Stop track with the condition being after 5 minutes

10 Detection Settings

10.1 Click advanced settings

10.2 Dynamic response

10.3 Select appropriate setting to indicate if subject is darker or lighter than the background

10.4 Place test subject in arena

10.5 Click and drag to create a box around the subject

10.6 The software will create an automatic threshold around the subject that moves with it

10.7 Fine-tune it with the scrub bar to make it fit around the subject perfectly

11 Trial list

11.1 Add the desired number of trials

Running the Experiment

12 Acquisition

12.1 Software will load the first trial

12.2 Put a subject in starting area
- use one of the enclosed arms
- keep starting area consistent across subjects

12.3 Press record
- the program will run for 5 minutes and automatically stop

12.4 Remove subject

12.5 Clean enclosure with chlorhexidine

12.6 Repeat for all trials

Results and Analysis

13 Data Profile

13.1 Ensure that it is pulling data from all trails

14 Analysis Profile

14.1 Parameters

14.2 Distance traveled
- arena (statistics - total only)

14.3 Velocity
- arena (avering interval - 1; statistics - mean)

15 In zone (open and closed cumulative zones)
- frequency
- latency to first
- cumulative duration

16 Results

16.1 Statistics and charts

16.2 Calculate to generate a file with the analysis parameters chosen

16.3 Export and save

17 Ensure the video files are exported and save.
- analysis can be repeated on video files if a problem occurs

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