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Y Maze - Novel Arm

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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 spatial memory function using the Y Maze (novel arm variant) 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.

Materials

1. Chlorhexidine
2. Paper towels
3. Y maze
4. Ethovision version 17 software

Before start

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

This test requires each mouse to go through the maze twice. During the first trial, the novel arm should be blocked so the mouse cannot access it. After running through the maze once (learning run), there should be a 10-minute to 1-hour interval before the mouse is put back in the maze. Prior to their second run, the novel arm block should be removed.

The optimal time between trials varies based on mouse genetic background.

Set Up

- 1 Bring animals into testing room for habituation (at least 30 minutes prior to start of experiment)
- 2 Set up maze
 - 2.1 Clean the maze (wipe out and spray with chlorohexidine)
- 3 Plug the EthoVision XT Version 17 software dongle 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
 - Indicate 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 location if accidentally moved between trials

7.2 Draw scale

- Measure the bottom arm of the Y
- Draw a scale bar over the area that you measured and indicate its length (in cm)

7.3 Click first arena in the list on the right

- Select the closed polygon tool
- Trace the outside barrier of the entire maze

8 Draw zones

8.1 Select zone 1

- Rename "baseline 1"
- Used closed polygon tool to trace one arm of the maze
- This will be one of two arms open in the learning run

8.2 Add a zone

- Rename "baseline 2"
- Used closed polygon tool to trace another arm
- This will be one of two arms open in the learning run

8.3 Add a zone

- Rename "novel"
- Used closed polygon tool to trace the last arm
- This arm will be closed during the learning run and open during the probe run

8.4 Create cumulative zone

- Baseline – include baseline 1 and baseline 2

8.5 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 arena

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 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 enough trials for each subject to go twice

Running the Experiment

12 Acquisition



- 12.1 Software will load the first trial
- 12.2 Put a subject in starting area
 - At the outside edge of one of the baseline 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 Distance traveled
 - Arena
 - Statistics - total only
- 14.2 Velocity
 - Arena
 - Averaging interval – 1
 - Statistics – mean
- 14.3 In zone
 - Select the baseline cumulative zones and the novel zone



- Frequency
- Latency to first
- Cumulative duration

15 Results

15.1 Statistics and charts

15.2 Calculate to generate a file with the analysis parameters chosen

16 Export and save

- ## 17 Ensure the video files are exported and saved
- analysis can be repeated from video files if a problem occurs

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