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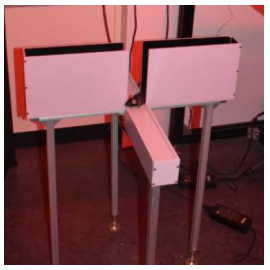
Elevated plus maze test for mice



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We use this protocol and it's working

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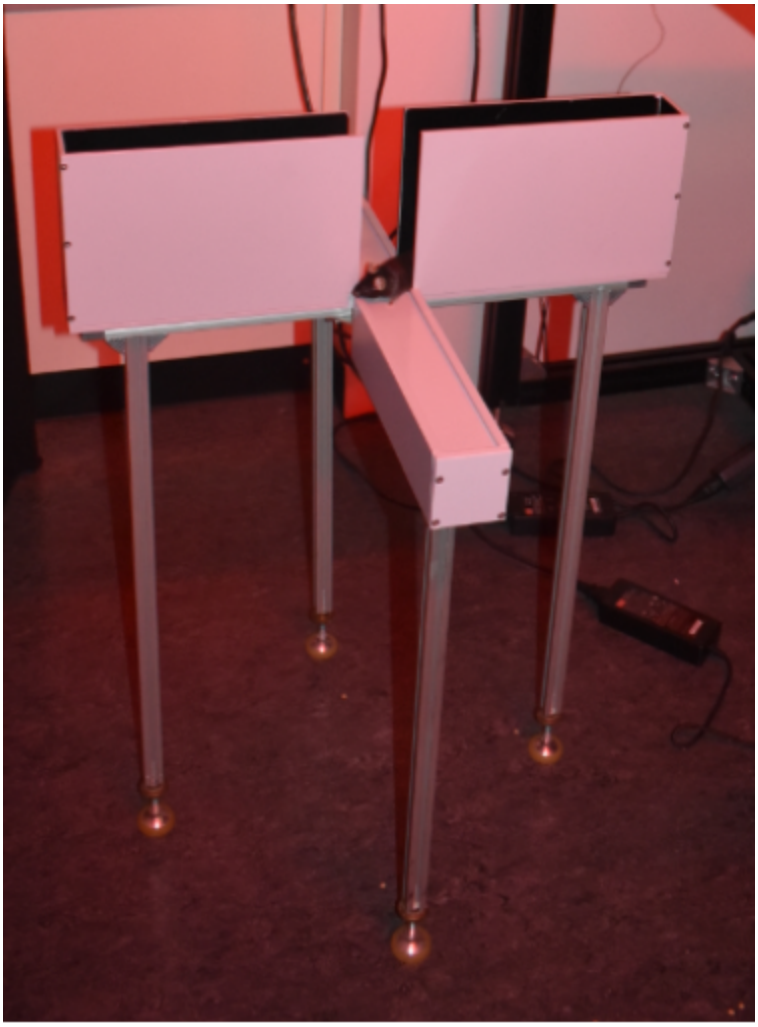
Abstract

The elevated plus maze is a classical test to screen anxiety-like behaviors in laboratory animals. This protocol is optimized to mice. The protocol also describes the use of Biobserve Viewer, which is is a software to track online the movement of animals.
Generally, less time spent in the open arms of the maze is associated with higher levels of anxiety.

Materials

- A 50-cm-high elevated plus maze setup. The arms of the maze are 25 cm long and 5 cm wide. The closed arms of the maze are covered by 16 cm high walls. The open arms have a 50mm high rim to avoid slipping.
- A webcam.
- White lights.



Biobserve Viewer

1 Open Biobserve Viewer. 30s

2 Make sure that the arena is well positioned in the middle of the view of the webcam. 1m

3 Go to Configuration and Experiment. Set “stop experiment after: “ to 10 minutes. If you are alone, it is best to tick “Delayed start”, so you can start the recording at the moment when the animal is put into the arena. 30s

4 At Camera, go to Capture settings and disable auto focus. 30s

5 At Filters and Objects, calibrate the camera. It is easiest if you highlight the center of the maze and tell the software its dimensions (5×5 cm). 2m

6 If you click on the Area configuration button, you can select the tracking area. Make sure that the whole arena is within the tracking area. 2m

7 You can make a background filter. With this, you can mask the walls and the background, so the tracking system will not confuse the mouse with dark areas. 5m

8 With a mouse which does not participate in your experiment, you can optimise the sensitivity of the background filter and the animal filter. Recommended values:
Background filter sensitivity: 20
Animal filter: 35
Min. animal size: 270 pixels 3m

9 Define the tracking zones in Zone Definition. I used the following zones:
center (5×5 cm)
closed arm north (5×25 cm)
closed arm south (5×25 cm)
open arm west (5×25 cm))
open arm east (5×15 cm) 10m

10 Reference point is the body. 1s

11 If you would like, you can define velocity classes. 3m

12 At Saving options, set the summary to 120 seconds (this will give you information about the mouse's behaviour in 2-min bins). 30s

13 Tick "Record movie" and set the frame rate to 25 fps. 30s

14 Define your output folders. The initial data path is to the folder where you want to save the videos and the .vrf files, and the initial export data path is where you will save the csv files. 2m

15 Save your settings. 1s

Behaviour 1h 4m 30s

16 Transport the mice to the behaviour room and put the mice in individual cages. 5m

17 Leave them in the lobby in darkness or with red light for at least 30 minutes. 30m

18 Turn on the red lights in the room.

19 Open Biobserve Viewer and position the maze.

20 Clean the maze with 70% ethanol. 1m

21 Turn on the white lights above the maze. Set the light intensity in the center of the maze to 50 lux with the luxmeter. 2m

22 Bring a mouse to the behaviour room. Open the cage and gently get the animal out. Do not chase it around in the cage. 2m

23 Place the animal in the center of the maze. Depending on your handling protocol, you can put it in by the tail or in the palm of your hand. 30s

24 Make sure to start the recording at the moment when you place the mouse on the maze. You can either do it by setting a delayed start in Biobserve, or with the help of another person.

25 Make a recording of 10 min. 10m

26 Make sure to save the video file.

27 After the video file is saved, go to Data analysis and click on the second icon on the left to save the results in .csv.

28 Pick the mouse out of the maze and put it back in the cage. 2m

29 Count poop pellets and take a note of them. 1m

30 Clean the maze with 70% ethanol. If you feel the necessity, you can rinse it with distilled water. 1m

Data analysis

31 We use DLC to track the position of the mice at any time point

We calculate time spend (percentage) in closed arm as the proxy for anxiety-related behavior