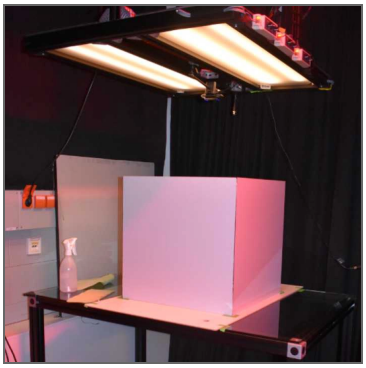

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Protocol status: Working
We use this protocol and it's working

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Open field test for mice

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ABSTRACT

The open field test is a classical test for the quantification of locomotor activity and anxiety-like behaviors in laboratory animals. This protocol is optimized for mice, and it includes the automatic analysis of their movement by Biobserve Viewer. Higher amount of time spent next to the walls of the arena is associated with higher levels of anxiety.

MATERIALS

- A 50x50(x50) cm opaque arena.
- A webcam.
- White lights.

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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 20 minutes. If you are alone, it is best 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 whole arena and tell the software its dimensions (50x50 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. If you would like to measure the mouse's average distance from the walls, then the tracking area should be equivalent to the floor of the arena. 2m

7 You can make a background filter. With this, you can mask the walls, so the tracking system will not confus the mouse with dark areas. 3m

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:
50x50 cm is the whole arena
25x25 cm in the middle is the center
12.5x12.5 cm in the middle is the minimal center 5m

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 300 seconds (this will give you information about the mouse's behaviour in 5-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 Place a 50x50 cm arena on the table.

20 Clean the arena and the table with 70% ethanol. 1m

21 Turn on the white lights above the arena. Set the light intensity in the bottom of the arena to 300 lux with th 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 arena. Depending on your handling protocol, you can put it in by the ta or in the palm of your hand. 30s

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

25 Make a recording of 20 min. 20m

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 arena and put it back in the cage. 2m

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

30 Clean the arena and the table with 70% ethanol. If you feel the necessity, you can rinse the area with distille 1m water.

Data analysis

31 Deep lab cut