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Light-dark box test for mice

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We use this protocol and it's working

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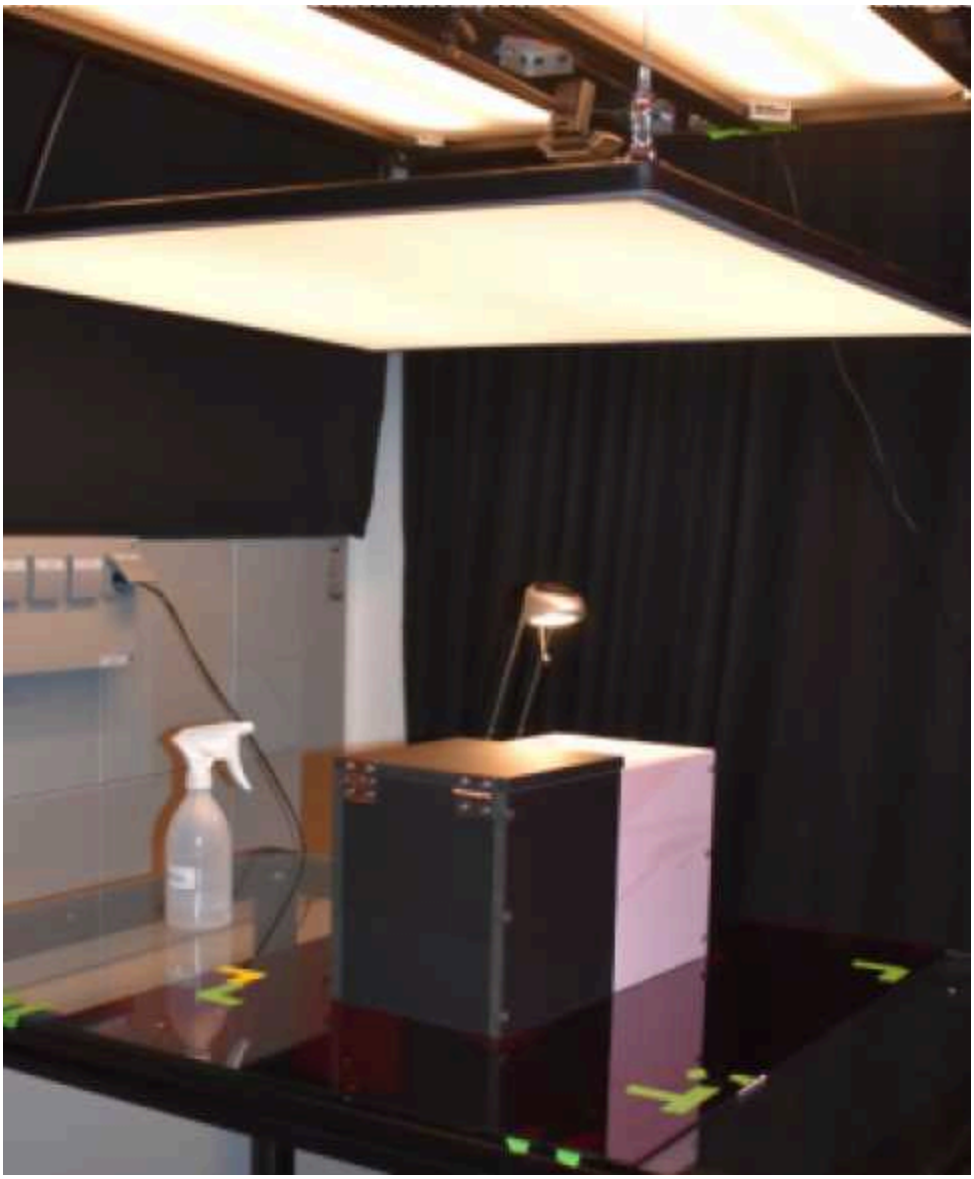
Abstract

This protocol describes the setup and execution of the light-dark box test for mice, a behavioral experiment used to assess anxiety-related responses. The protocol involves preparing a light-dark box in a controlled environment, utilizing specific lighting conditions and camera settings for optimal observation and recording.

Materials

- A 20×40×20 cm box with two equal sized compartments. One compartment is black and has a lid, the other one is white and open on the top. There is an 5×7 cm opening between the two compartments. The setup is open on the bottom.
- A flat small black tool which can cover the 5×7 cm opening.
- A transparent (glass) desk.
- An IR sensitive camera to capture the behavior from below the setup (a 1280×1024 pixels Basler camera and a Basler 2200000178 objective).
- Infrared (IR) lights.
- White lights.
- A large light filter that can homogenize the light.



Light/dark box test setup. The camera and the IR lights are below the table (not shown).

Troubleshooting

Pylon Viewer settings

1 Connect the camera to the computer and to a power source.

2 Open the Pylon Viewer and select the camera.

3 At AOI controls, crop the image, so that you only see the setup. You can only change the width and the height of the image when there is no continuous shot or recording.

4 With the help of a test mouse, you can set the image quality. On the camera, you can set the aperture and the focus. In Pylon Viewer, you can play around with Analog Controls > Gain (Raw), Black level (Raw), and Acquisition Controls > Exposure time (Raw) to achieve the best quality.

5 Go to Window and select Recording Settings. Set the Output format to .mp4, the fixed playback speed to 67 fps, and the Recording buffer size to 100 frames. Record a frame every 15 milliseconds. Stop the recording after 10 min. Set the output folder.

Behavior

6 Transport the mice to the behaviour room and put the mice in individual cages.

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

8 Turn on the red lights in the room. Turn on the IR lights below the table.

9 Place the light/dark box on the table.

10 Clean the box and the table with 70% ethanol.

11 Hang the big light filter above the arena. This is to have a homogenous background.

12 Turn on the white lights above the arena. Have 310-320 lux of light intensity in the light box. Probably you will need an additional lamp to achieve this intensity.

13 Open Pylon Viewer and check settings (see below).

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

15 Block the opening between the two compartments of the arena.

16 Place the animal in the dark compartment. Depending on your handling protocol, you can put it in by the tail or in the palm of your hand.

17 Close the dark compartment and wait 3 seconds before you unblock the opening.

18 Make sure to start the recording at the moment when the opening is free.

19 Make a recording of 10 min.

20 The video file is saved automatically. Rename it, so that you can easily identify your recording.

21 Pick the mouse out of the arena and put it back in the cage.

22 Count poop pellets and take a note of them.

23 Clean the arena and the table with 70% ethanol. If you feel the necessity, you can rinse the area with distilled water.

Data analysis

24 We use DeepLabCut to measure locomotion and the time spend in each compartment

Protocol references

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