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# OPEN ACCESS

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

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## Open field locomotion test

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**ABSTRACT** 

This protocol describes open field locomotion test.

**ATTACHMENTS** 

730-1841 docx

**MATERIALS** 

### **Apparatus**

- 1. ANY-maze software (Stoelting, Wood Dale, IL)
- 2. Open field boxes
- 3. Camera (either attached to tripod or mounted to the ceiling).

Set up

Place the open field boxes right below the camera attached to the ceiling. 2 Connect the camera to the computer via USB. 3 Clean the boxes with 70% ethanol. 4 Open the ANY-maze software on computer. 5 To create a new protocol, select "New Experiment". 5.1 Change the name of the protocol under the "APPARATUS" section. 6 Add a new video source by selecting the green "+ (add item)" button in the header section. 6.1 Add item > New video source > Select the camera from the drop-down menu. 7 Adjust the camera/open field box so that the whole boxes can be seen on the screen.

#### Note

It may be beneficial to use small blocks (roughly the size of the animal) when adjusting the box/camera to ensure the animals will be detectable in all locations within the box.

- 8 Add a new apparatus by selecting the green "+ (add item)" button in the header section.
- Add item > New apparatus > Name the apparatus based on which box it represents (e.g., "Top Left") > Set the length of the ruler line based on the dimensions of the box (e.g., 500 mm) > Use the rectangle tool to draw the outline of the perimeter of the box.
- Add a grid over the box by selecting the "Grid settings" option in the header section. Select "Include a square grid spaved at (cm)" and set desired spacing (e.g., 5 cm).
- **8.3** Repeat the above steps in "8.1" for each box.
- 9 To create zones within each box, select the green "+ (add item)" button in the header section.

#### Note

**Important**: a grid must be placed over each apparatus to create designated zones.

- 9.1 Add item > New zone > Name the zone (e.g., center) > select the squares within each apparatus that represent the zone. Optional: select "Highlight this zone during tracking when animal is in it" to be able to visualize when the animals are within each zone during testing.
- **9.2** Go to 'Set zone entry settings' then check 'use the centre of the animal'.

9.3	Repeat for each desired zone.
10	Set the animal colour under the "Animal colour" tab in "TRACKING AND BEHAVIOR".
11	If desired, select "detect peiods when the animal is immobile" and "detect period when the animal is freezing" under the "Immobility detections" and "Freezing detection" tabs, respectively.
12	Set the test duration by selecting "Stages" > "First Stage" under the "TESTING" tab.
12.1	Set the test duration to specified time (e.g., 10 min).
13	Go to "Treatment groups" to set how ANY-maze should assign animals to treatment groups, or if treatment groups are not desired.
14	If using the animals ID is needed, go to "Animal ID" below "Treatment groups" and select "Use my IDs to refer to animals".
15	Go to "EXPERIMENT" at the top and select "Add Animals" and the number of animals to add.  Note  If "Use my IDs to refer to animals" was selected, enter the animals ID.

# **Testing**

**16** Go to "TESTS" tab at the top.

17 Untested animals should populate immediately and the apparatuses should be "Ready..."

#### Note

If the animals do not auto-populate, add an animal by pressing the green '+' button. Do this for all boxes.

To begin testing, place an animal in its designated box and then press the green play button.



#### Note

- 1. **Do not** cross over the box being recorded as the tracking system can pick up the movements.
- 2. Ensure the software is tracking the animal on the screen after starting the test.
- **19** Repeat step 18 for each animal.
- Either hide behind a curtain and remain silent or step out of the room so as not to distract/disturb the animals being tested.
- Once the session has ended, remove each animal from the box and return to its home cage.

Clean each box with 70% ethanol before testing the next back of animals.

### **Data retrieval**

- Go to the "PROTOCOL" at the top and scroll down to "Results, reports, and data" at the bottom under "ANALYSIS AND RESULTS".
- 24 Select "Data page" and under "Apparatus measures" choose the desired parameters to be displayed.
- Go to the "DATA" tab and the top and the chosen parameters will be displayed.
- 26 Either press "Save" to save results to the computer or select "Send as e-mail" to email a .CSV file with the results for analysis offline.