3 Tests to measure anxiety in rodents



This is a chapter from the e-book:

Basic behavioral neuroscience in rodents



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3.2

Elevated plus maze

The elevated plus maze arena is entirely raised from the floor (hence the *elevated* component) and shaped as a *plus-sign*. This creates four arms, of which two opposing arms are enclosed with raised walls, and the other two arms are completely open. The elevated plus maze is thought of as one of the most robust and reliable anxiety tests. Although it makes use of the same behavioral contrasts as the open field (aversion of open spaces/ thigmotaxis), the spaces are much more defined.



Mouse elevated plus maze
Ranges from 25×25 cm to 30×50 cm
15-30 cm raised walls
Raised ±50 cm from the floor

Rat elevated plus maze
Ranges from 25×25 cm to 50×50 cm
30-40 cm raised walls
Raised ±65 cm from the floor

Protocol suggestion

- Transport the animals, preferably in their home cages, into the testing room and allow the animals to acclimate to this room for a minimum of 30 minutes prior to starting the test.
- Remove a single animal from the home cage with your preferred handling technique: tail handling, full hand handling, tube handling. Place the animal in the center square of the elevated plus maze. Recording/tracking automatically starts in EthoVision if this option has been selected. Otherwise, do not forget to concurrently activate your video recording. It is normal for the animals to immediately move into an enclosed arm of the maze.



A standard elevated plus maze.

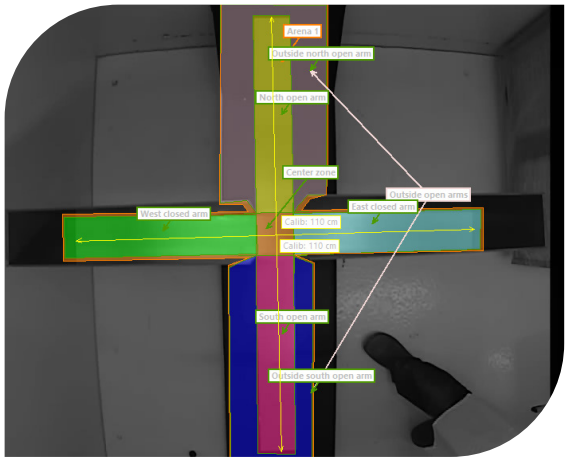
- Preferably leave the testing room to allow free and uninterrupted movement of the subject animal. Record/track the animals for 5 minutes.
- After the testing time is finished, gently pick up the animal, again using your preferred handling technique, and return it to its home cage.
- Before cleaning the arena, visually count the fecal pellets present and manually record the numbers for further analysis.

- Remove all fecal pellets and wipe up all spots of urination. Spray the entire maze with 30-70% ethanol and wipe down with a clean paper towel. Allow the ethanol solution to completely dry prior to testing other animals.

Setup in EthoVision

In arena settings, 5 zones are required: central square, open arm 1, open arm 2, closed arm 1 and closed arm 2.

Group the open and closed arms together with a zone label to automatically extract a total time on open versus closed arms.



A simple EthoVision arena setup in an elevated plus maze with zones for the open arms, closed arms and a central square.

Interpretation of the results

Time spent on the open arms of the maze is related to lower anxiety-like behavior. **Time spent in the closed arms** is related to an increase in anxiety-like behavior, since this part of the maze is enclosed and sheltered with raised walls.

However, this test should preferably only be performed once, since rats and mice show less exploration of the open arms upon repeated testing. This avoidance can also not be rescued by anxiolytics compounds and thus reflects a lack of motivation as opposed to increased anxiety. Visualizing time spent in open versus closed arms is generally done with an open arm ratio:

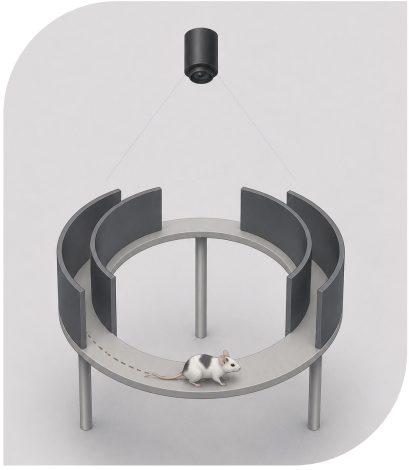
$$\frac{\text{Time spent in open arms}}{\text{Total time in all arms}} \text{ (x100)}$$

Total arm transitions says something about general activity on the maze, while additional behaviors such as **rearing** and **freezing** can also be automatically detected and scored in EthoVision. These behaviors give additional information on the anxiety-like and fearful phenotype.

3.3

Elevated zero maze

The elevated zero maze is a variation on the elevated plus maze, and makes use of the same behavioral contrast. This shape was created to eliminate the center region of the plus maze. It is an elevated ring-shaped arena with two open and two closed quadrants (with elevated walls).



Mouse elevated zero maze
50 cm diameter
15 cm raised walls
Raised 60 cm from the floor

Rat elevated zero maze
100 cm diameter
30 cm raised walls
Raised 60 cm from the floor

Tucker and McGabe describe an experiment in which they compare the elevated plus maze and elevated zero maze in male and female C57BL/6J mice [14]. They found that the elevated zero maze encourages greater exploration of the anxiogenic regions (open quadrants). It also produced consistent outcomes across multiple sessions. The elevated plus maze, by contrast, is advised to be performed only once per mouse, to avoid learning and habituation to the maze, which could otherwise mask anxiety-like behavior.

One obvious difference between the elevated zero maze and elevated plus maze is the starting point. The central square is removed in the elevated zero maze taking away the availability of a logical starting point while also removing any ambiguity in the interpretation of the time spent in the maze.

Protocol suggestion



A standard elevated zero maze.

- Transport the animals, preferably in their home cages, into the testing room and allow the animals to acclimate to this room for a minimum of 30 minutes prior to starting the test.
- Remove a single animal from the home cage with your preferred handling technique: tail handling, full hand handling, tube handling. Place the animal in the center square of the elevated zero maze. Recording/tracking automatically starts in EthoVision if this option has been selected. Otherwise, do not forget to concurrently activate your video recording. It is normal for the animals to immediately move into an enclosed arm of the maze.
- Preferably leave the testing room to allow free and uninterrupted movement of the subject animal. Record/track the animals for 5 minutes.
- After the testing time is finished, gently pick up the animal, again using your preferred handling technique, and return it to its home cage.
- Before cleaning the arena, visually count the fecal pellets present and manually record the numbers for further analysis.

- Remove all fecal pellets and wipe up all spots of urination. Spray the entire maze with 30-70% ethanol and wipe down with a clean paper towel. Allow the ethanol solution to completely dry prior to testing other animals.

Setup in EthoVision

In arena settings, 4 zones are required: open quadrant 1, open quadrant 2, closed quadrant 1 and closed quadrant 2. Group the open and closed quadrants together with a zone label to automatically extract a total time on open versus closed quadrants.

Interpretation of the results

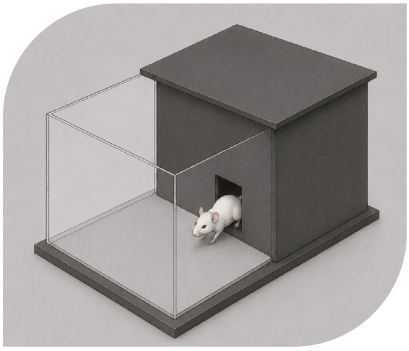
Like the elevated plus maze, the **total time spent on the open quadrants** of the maze is related to a lower anxiety-like behavior. Additionally, **total distance moved** is also a common parameter measured in the zero maze, especially since this test can be performed multiple times within the same mouse.

Total quadrant transitions says something about general activity on the maze, while additional behaviors such as **rearing** and **freezing** can also be automatically detected and scored in EthoVision. These behaviors give additional information on the anxiety-like and fearful phenotype.

3.4

Light-dark box

The light dark box uses the aversion of rodents towards brightly illuminated areas as a behavioral contrast to test unconditioned anxiety. Originally described in 1980 by Crawley and Goodwin [4], and since then adopted as one of the most used tests to measure anxiety-like behavior. The box consists of a small (one third of the box) dark safe compartment and a larger (two thirds of the box) illuminated compartment, a sliding door separates the two compartments.



Mouse light-dark box
60 cm long x 20 cm wide
20-26 cm high

Rat light-dark box
105 cm long x 35 cm wide
35-46 cm high

Removable roof & sliding door
Black IR material

Protocol suggestion

- Transport the animals, preferably in their home cages, into the testing room and allow the animals to acclimate to this room for a minimum of 30 minutes prior to starting the test.
- Remove a single animal from the home cage with your preferred handling technique: tail handling, full hand handling, tube handling.
- Animals can be placed either in the middle of the lit chamber [5,6], or of the dark chamber [7,8]. However, since latency to enter the light chamber can be used as an anxiety-index, the door between the chambers can be used to prevent the animal immediately moving to the other chamber. In this case, place the animal in the dark chamber with the door closed.
- Recording/tracking automatically starts in EthoVision if this option has been selected. Otherwise, do not forget to concurrently activate your video recording.
- After placement in the box, or after the door opens, allow the animal to move freely between the two chambers for 10 minutes. Preferably leave the testing room during this time.
- After the testing time is finished, gently pick up the animal, again using your preferred handling technique, and return it to its home cage.
- Before cleaning the arena, visually count the fecal pellets present and manually record the numbers for further analysis.
- Remove all fecal pellets and wipe up all spots of urination. Spray the entire maze with 30-70% ethanol and wipe down with a clean paper towel. Allow the ethanol solution to completely dry prior to testing other animals.

Setup in EthoVision

In EthoVision, in the arena settings tab, draw two zones: light compartment and dark compartment.

Interpretation of the results

- **Latency to enter** the dark or the light compartment for the first time and the **total time spent** in the lit versus dark compartment are used as readouts for bright-space anxiety.
- **Total transitions** (from light to dark compartment and vice versa) gives an indication of activity\exploration because of habituation over time.

In general, control mice spend more time in the bright chamber compared to anxious mice. Similarly control mice generally show a higher number of transitions between the chambers, while the latency to enter the bright chamber is lowest in this group.



An example of a light-dark box for mice.

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