FEAR & ANXIETY

Anxiety & fear

INTRODUCTION

Anxiety is a behavioral component that plays a role in any behavioral test, since animals constantly take decisions while interacting with their environment. Without a clear motivational state, animals resort to approaching a pleasant situation, and explicitly avoiding unpleasant situations. In some situations, opposing motivations conflict with each other and have to be resolved by the animal: approaching a mating partner in a potentially unsecure environment, or staying in a known safe and sheltered environment. Resolving this conflict between unpleasant versus pleasant is a key component of anxiety tests. And although some form of anxiety-like behavior can be measured in almost any behavioral paradigm, it is important to take validated and robust tests as your main readout parameter.

Fear is defined as a negative emotional state associated with the perception of imminent or present threat to wellbeing or survival. It is a defensive reaction, in order to escape and avoidance impending identifiable danger. An important difference between fear and anxiety is that anxiety is associated with the perception of potential or ambiguous threat. Like fear, it is a defensive reaction, but characterized by a feeling of apprehension, uncertainty, worry, uneasiness, or tension

stemming
from the anticipation of potential threat or negative outcomes. When animals (and humans) face an unambiguous situation; they can avoid the threatening stimulus or escape to safety.

WHY DO WE TEST ANXIETY AND FEAR

Anxiety serves as an important characteristic for the general phenotype of the animal. Highly anxious animals perform any behavioral test much differently compared to animals that display less anxiety-like behavior. Anxiety tests also often have a distinct learning curve, while fear is highly preserved in all species of animals (since it plays an essential role in survival). Anxiety is thus an important characteristic to phenotype in experimental animals, however is does require proper justification since anxiety (and fear) tests are generally not very pleasant for the experimental animals. Pharmacological interventions are often aimed at anxiolytic or anxiogenic effects, for example with a novel drug that is aimed at decreasing anxiety, which has to be validated in a robust anxiety test.

Like anxiety, fear has a primal role in ensuring survival. However, as these behavioral subsets turn into a chronic condition, this can interfere with daily functioning and manifest into a disorder. This poses a large threat to mental health. Treatments for these conditions are primarily tested in animals (rodent) models. Due to a large degree of cross-species preservation in the neural circuitry underlying these behaviors, rodent models of fear (and anxiety) provide great translational value for the human condition.

WHAT TESTS CAN WE USE TO MEASURE ANXIETY AND FEAR?

There are several well documented, robust, behavioral tests for anxiety-like behavior which make use of the choice between an unprotected versus a protected area.

- **Center versus border exploration** of the open field test, as described in the previous section, can also be used to measure anxiety-like behavior apart from locomotor and/or exploratory behavior.

- **Aversion towards open spaces and thigmotaxis** (wall hugging) serve as the contrasts in unpleasant versus pleasant.

- **Freezing behavior** in the open field is a good indication of fearful behavior in the open field.

What is Thigmotaxis?

Rodents tend to move in contact with vertical surfaces, and prefer to eat in a corner rather than in an open space. The motivational state for these types of behavior is presumably an aspect of taking cover from predators. The tendency to remain to vertical surfaces, or "Wall hugging", is referred to as 'Thigmotaxis'. This type of behavior is commonly associated with anxiety and serves as a very important motivator and interpretation of the results in an open field. This thigmotactic response makes it so that the animals spend more time in the corners and/or at the border of the open field, making this readout an important factor in the analysis of the open field test.

Please visit the previous chapter for the testing protocol of the open field test.

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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 x 25 cm to
50 x 50 cm
15 - 30 cm raised walls
Raised ±50 cm from the floor

RAT ELEVATED PLUS MAZE

Ranges from 25 x 25 cm to
50 x 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 XT 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.

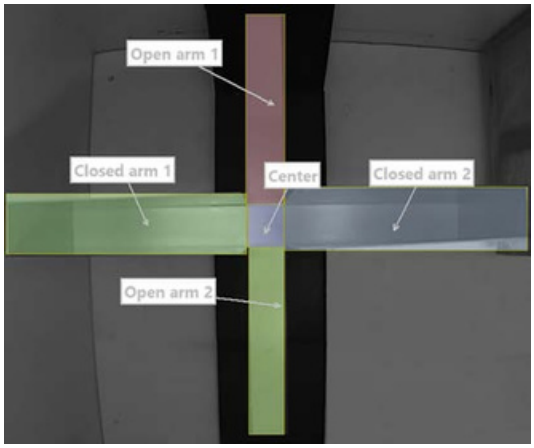
A standard elevated plus maze.
Credits: He, S. and Corscadden, L. (2022). Maze Engineers.

- 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 faecal pellets present and manually record the numbers for further analysis.
- Remove all faecal 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 XT

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 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:

$$\frac{\text{Time spent in open arms}}{\text{Total time in all arms}} (\text{x } 100)$$

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 XT. These behaviors give additional information on the anxiety-like and fearful phenotype.

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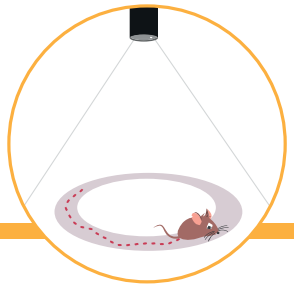
ELEVATED ZERO MAZE



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), while also finding consistent outcomes over multiple sessions, while the elevated plus maze is advised to be only performed once per mouse to avoid learning and habituation to the maze, potentially masking 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

- 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 XT 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 zero maze.
Credits: He, S. and Corscadden, L. (2022). Maze Engineers.

- 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 faecal pellets present and manually record the numbers for further analysis.
- Remove all faecal 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 XT

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.

INTERPREATION OF 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 XT. These behaviors give additional information on the anxiety-like and fearful phenotype.

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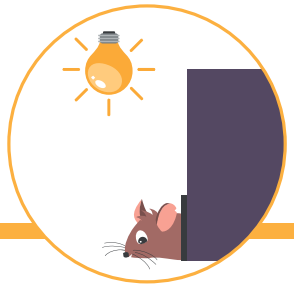
LIGHT-DARK BOX



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 XT 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 faecal pellets present and manually record the numbers for further analysis.
- Remove all faecal 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 XT

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

INTERPRETATION OF 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.
Credits: He, S. and Corscadden, L. (2022). Maze Engineers.