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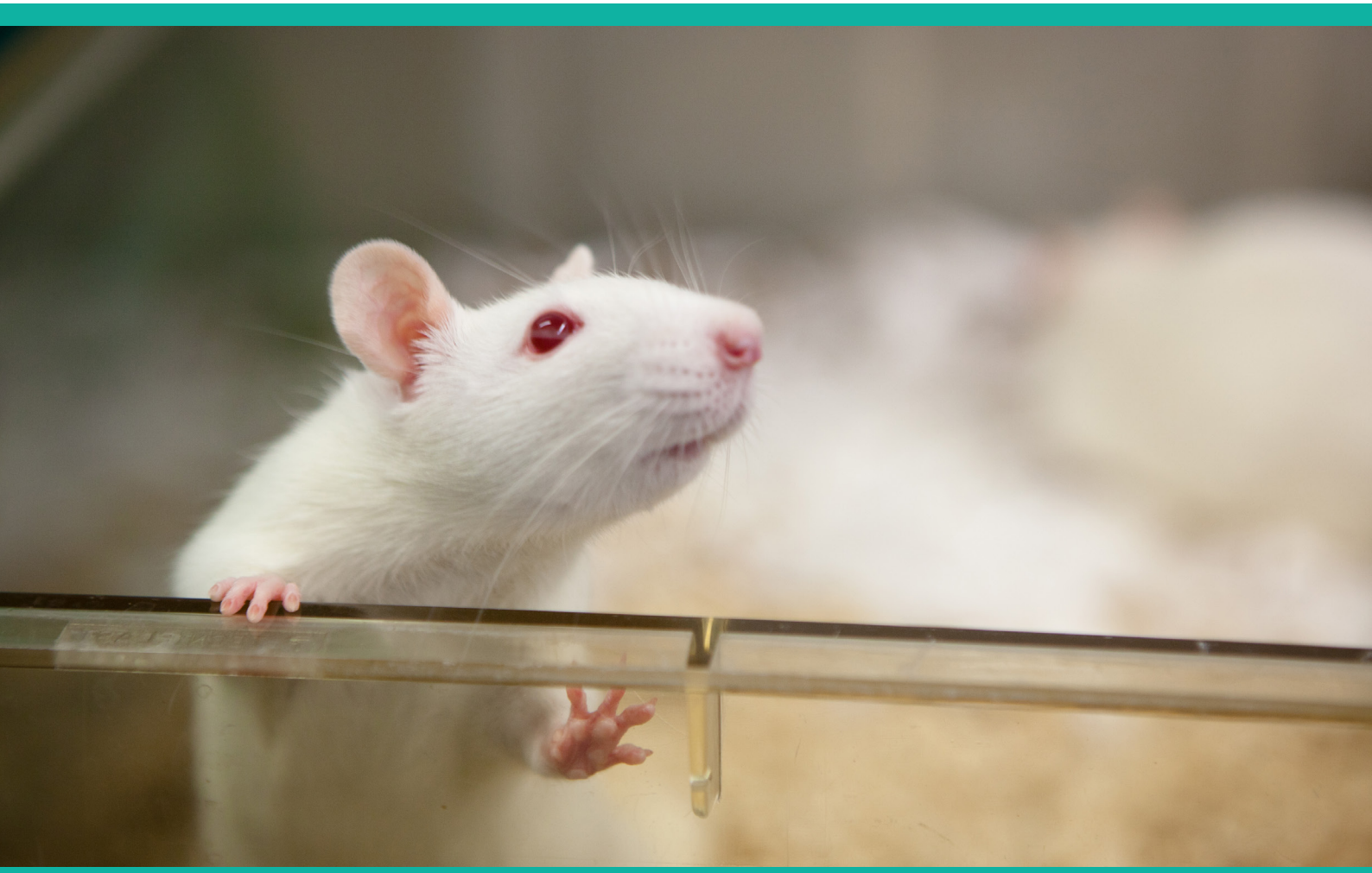
Measuring exploratory behavior with the open field test



This is a chapter from the e-book:

Basic behavioral neuroscience in rodents.

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INTRODUCTION

The behavior of mice and rats is often highly incentivized by their natural curiosity. Curiosity is part of an essential survival mechanism, which is driven by novelty-seeking and investigative behavior of the surrounding environments. This so-called *motivational state* initiates behavior and helps achieve desired goals. Hunger is another example of a motivational state that initiates (exploratory) behavior, as food seeking and eating behavior are essential for survival.

WHY DO WE MEASURE EXPLORATORY AND LOCOMOTOR BEHAVIOR?

In order understand the (behavioral) phenotype of a rodent model, exploratory and locomotor behavior are commonly studied in behavioral batteries. For example, when comparing different strains of mice or different effects of drug treatments. If locomotor activity or ability is affected due to a treatment, then measuring behavior that relies on the ability of the animals to move is automatically confounded.

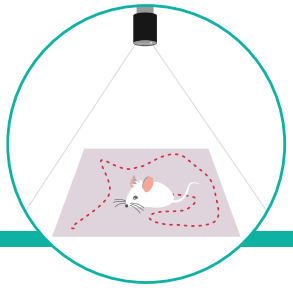
HOW DO WE MEASURE EXPLORATORY BEHAVIOR?

The open field test is one of the most commonly used platforms to measure exploratory and locomotor behavior in animals and was introduced by Hall and Ballachey in 1932. As a fast and relatively easy test, it provides a variety of behavioral information ranging from general ambulatory ability to information about the emotional state of the animal.

An open field is, simply put, a box with an open area, which makes exploratory behavior easy to analyze in other spaces as well. Home cage monitoring is a great example of this. This, however, does differ in interpretation of the behavior, as the home cage is a familiar and safe area, whereas a standard open field is a novel arena.

In other tests (such as the elevated plus maze, three chamber test and Barnes maze) exploratory behavior can be measured as well. However, interference in the behavioral readouts may occur due to the enriched environment of these arenas, whereas a standard open field is as it was stated: an un-enriched open area solely meant for the exploration by the animal.

OPEN FIELD



A common first question that comes up when planning an open field is if the arena should be round or square.

Intuitively a round arena seems more appropriate, since the lack of corners creates an *equal* and *unenriched* space to explore. Corners can be perceived as safe and sheltered, thus might be more attractive to visit for the animals, which could skew results. In practice this is hardly the case as showed by Grabovskaya and Salyha, showing virtually no differences in many of the main readout parameters of the open field, which commonly are:

- Total distance moved
- Time in zone (outer versus inner)
- Zone crossings
- Defecations and urinations
- Stretch attend postures
- Rearing

MOUSE OPEN FIELD

Ranges from 25 x 25 cm to 80 x 80 cm

30 - 40 cm raised walls

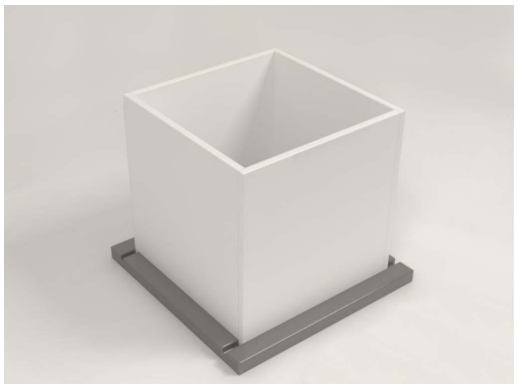
RAT OPEN FIELD

Ranges from 60 x 60 cm to 120 x 120 cm

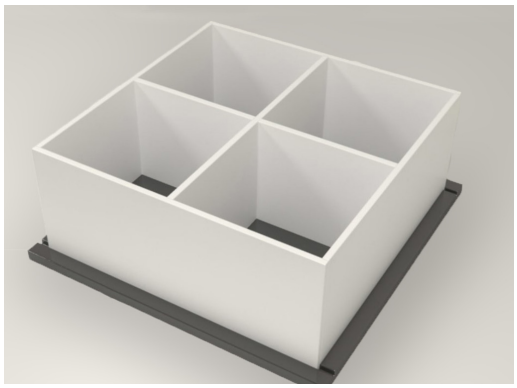
40 - 50 cm raised walls

Generally the dimensions of the open field (smaller or larger) do not affect locomotor behavior, however rats should be tested in a larger arena than mice.

An important consideration to make when choosing for a round or square arena, is any further testing within the same arena. Particularly involving the exploration of objects, such as in the novel object or novel place recognition test. Specific corner placements of these objects in a square arena makes its location distinctly recognizable for the animal. A round arena can be used in this context, but can become tricky when objects have to be placed in a specific place in the arena relating to a



A standard square open field. Credits: He, S. and Corscadden, L. (2022). Maze Engineers.



A partitioned open field can also be used, which is used to conduct multiple tests simultaneously, increasing the throughput of your testing. Credits: He, S. and Corscadden, L. (2022). Maze Engineers.

specific position in the surrounding area (testing room), since the animals need a point of reference in order to successfully recognize an object. A square arena could thus be better suited for this purpose.

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 middle of the open field arena . 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 to the periphery walls of the maze.
- Preferably leave the testing room to allow free and uninterrupted movement of the subject animal. Record/track the animals for 5 or 10 minutes, depending on your preference, previous experiments, or examples from literature that you wish to replicate.
- 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 fecal pellets and wipe up all spots of urination. Spray the floor and walls of the 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

EthoVision XT makes it possible to automatically track all movements and behaviors in the open field. Drawing zones, and subsequently dividing them accordingly: center, border, etc. This makes extracting the data from the open field test as simple as a few clicks. In EthoVision XT, two main zones are of importance: the center of the arena and the borders. With a square shaped arena is can be advised to also include the corners as zones.

With multiple body point detection in EthoVision XT, additional behavior such as rearing and grooming can be detected. This gives additional information on the behavioral phenotype in the open field, and adds resolution to the exploratory and/or locomotor behavioral profile.

INTERPRETATION OF THE RESULTS

- **Total distance moved** - In the open field Total distance moved gives an indication on locomotor behavior. This is usually presented as an absolute value (in cm). Another indicator that can be used for this type of behavior is time moving versus time not moving, which can be presented as a percentage or ratio. For example:

$$\frac{\text{Time moving}}{\text{Total time}} \text{ (x 100)}$$

- **Zone crossings** - These crossings also give an indication on locomotor behavior, however this adds information based on if the animals spends a lot of time exploring one particular zone, or that this is more distributed over the entire arena. The latter can be visualized in EthoVision XT with either a heat map or with track visualization.
- **Time in zone (outer vs inner)** - This is a parameter that gives an indication about the willingness of the animal to spend time in an open area versus an area closer to the walls (giving an indication on thigmotaxis). Thus depending on your preferred readout, you can present this data either as time in inner (or center) zone divided by the total time, or the time in the outer zone (close to the walls) divided by the total time. For example:

$$\frac{\text{Time in center}}{\text{Total time}} \text{ (x 100)} \quad \text{or} \quad \frac{\text{Time in outer zone}}{\text{Total time}} \text{ (x 100)}$$

- **Defecations** - Defications are related to emotionality. In general, an increased amount of defecations can be used to indicate increased levels of anxiety in the subject, and are thus presented as a cumulative value. This can be manually scored within EthoVision XT.
- **Rearing behavior** - Rearing can automatically be detected and scored by EthoVision XT and is displayed as a cumulative value. This type of behavior consists of subject animals standing on both hind paws in a vertical upright position. It is considered an exploratory behavior, but has also been used as a measure of anxiety. Depending on the test situation, rearing can be considered to be anxiolytic or anxiogenic. It is often used to discriminate anxiety-linked behavior from simple ambulatory behavior.