Novel object recognition: a simple but powerful test



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Basic behavioral neuroscience in rodents



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Novel object recognition

The novel object recognition test is designed to study recognition memory in rodents using one or more familiar objects and one novel object. The test is typically performed in an open field arena. Animals tend to spend more time investigating the novel object as a result of the natural curiosity of rodents. This must be compared to a *baseline* or *training* environment, where animals are habituated to the testing arena and a set of familiar objects. If during the novel object recognition test, exploration of the novel and familiar objects is the same, this can be interpreted as a memory or cognitive deficit.



Mouse open field
Ranges from 25×25 cm to 80×80 cm
30-40 cm raised walls

Rat open field
Ranges from 60x60 cm to 120x120 cm
40-50 cm raised walls

In addition to the novel object recognition test, the novel location recognition test can also be performed, which measures spatial memory. Since in the latter test the object is moved (not replaced by another object), studies suggest that while object recognition is hippocampus-independent, location recognition is, in fact, dependent on hippocampal functioning.

This has long been the general consensus, however, as we gain a larger understanding in the neurobehavioral field due to technological advances, recently studies have shown, like one study by Cinalli Jr *et al.*, that the mouse hippocampal CA1 region and Perirhinal cortex play complementary roles in spontaneous object recognition [32].

> ***Important!***
> *This test requires that the animals are already habituated to the arena they are tested in. This is usually done in an open field arena, thus this protocol follows the protocol of the open field test in chapter 1.*

Protocol suggestion - square arena

Phase 1 - Learning objects and locations, same day as the open field test

- 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.
- Place two similar objects in opposing corners of the arena, randomize these corners for all animals tested. Objects used can be anything from Lego Duplo blocks and small toys that are easy to clean, to simple glass or (3D printed) plastic objects.
- 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 if this option has been selected. Otherwise, do not forget to concurrently activate your video recording.

- 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 how long they were tested in the open field test.
- 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 floor, walls and objects 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.

Phase 2, option 1 - Novel object recognition test, approximately 24 hours after phase 1

- 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.
- Again place two objects in opposing corners of the arena (these must be the same corners as phase 1!), one object is the same as in phase 1, but the other object must be of a different shape (similar in size). Randomize which object is replaced for all animals tested.
- 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 if this option has been selected Otherwise, do not forget to concurrently activate your video recording.
- 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 how long they were tested in the open field test.
- 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 floor, walls and objects 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.

Phase 2, option 2 - Novel object recognition test, approximately 24 hours after phase 1

- 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.
- Again place the same objects in the arena (these must be the same objects as phase 1). However, one object must remain in a similar corner/position of the arena, while the other object must be moved to a different location. Randomize which object is moved for all animals tested.
- 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 if this option has been selected. Otherwise, do not forget to concurrently activate your video recording.

- 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 how long they were tested in the open field test.
- 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 floor, walls and objects 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 subsequent animal.

> ***Important!***
>
> *If you want to test novel object recognition and novel location within the same group of animals: phase 1 must be repeated before going on to either of the options. Thus if you have done novel object recognition, you have to re-train the animals as was done in phase 1 before performing a novel location task (and vice versa).*

Setup in EthoVision

General arena zones are similar to the open field. Exploration of the object is usually defined as the animal's nose being in the zone of 2-3 cm around the object. Some researchers exclude data points in which both the nose and the center point were detected in this zone, effectively excluding climbing behavior and exploration around the object instead of the object itself. Other researchers use a different approach, describing donut shaped object zone between 0.75 and 1.5 times the radius of the object defined as the zone of exploration.

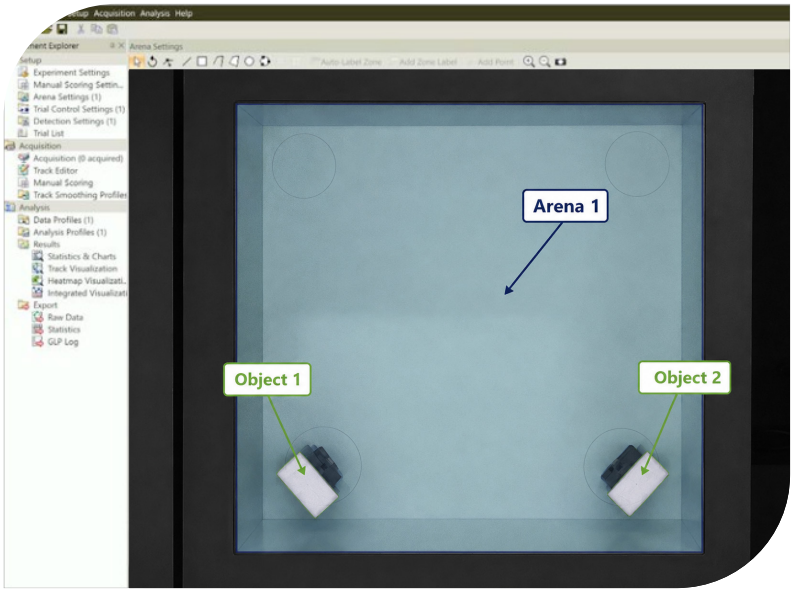


Image credits: Alicia Brantley, PhD. Mouse Behavior Core, Scripps Research.

EthoVision allows you to define areas and objects within your video image by simply drawing zones around them. If you want object exploration to be defined as being within 2 cm of the object, for example, you can draw a zone that is the same shape as your object, plus a 2 cm circumference. Donut shapes are also possible by excluding the center of the circle.

When zones are defined, EthoVision automatically registers when the animal enters the zone, and which body points are in that zone. You can also filter out data points in which the animal has both its nose and body center in the zone (e.g. indicating the animal is climbing on the object).

Interpretation of the results

The most important results from the novel object or novel location test include **the exploration times of the objects** and **the frequency of each object exploration**. These are generally expressed as the percentage of exploration time spent on novel object:

$$\frac{\text{Time spent exploring novel objects}}{\text{Time spent exploring any object}} \text{ (x100)}$$

The **preference for novelty** is a positive value if there is preference for novelty, and is zero if there is no preference:

$$\frac{\text{Time novel - Time familiar}}{\text{Time novel + Time familiar}} \text{ (x100)}$$

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