# Novel Object Recognition test

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Ref: Denninger JK, JOVE 2018.

## Summary:

The novel object recognition test is a commonly used behavioral assay for the investigation of various aspects of learning and memory (recognition) in mice. This test is completed over 2 days. The first day, mice are acclimated to the arena for 10 minutes and placed back in their home cage. The second day, mice are placed in the arena and presented with 2 identical objects for 10 minutes and returned to their home cage. After 2 hours, the mice are placed again in the same arena for 10 minutes and one of the 2 objects is replaced by a novel object. The camera and software record the position, area entries, distance, area latency, and object time investigation. The amount of time taken to explore the new object provides an index of recognition memory.

## Reagents and Materials:

| Reagent/Material | Vendor | Stock Number |
|---|---|---|
| Plexiglas test arena (40 x 60 x 19 cm) | | |
| Plexiglas Divider | | |
| Sample objects | See Supplementary Notes | |
| Holding cage | | |
| Camera (GigE or WebCams) | Bassler or Microsoft, etc | |
| Tracking Software (EthoVision XT) | Noldus | |
| Double stick square adhesive pads | | |
| Animal marker- black | Stoelting | 50451-1 |
| Lab Coat/Gloves/PPE | | |
| Paper towels | | |
| Disinfectant | Rescue-Veterinary use | |
| Dinsinfectant | Nolvasan 10% | |

## Protocol:

**1. SET-UP** (acclimation of mice, software setup)

**a.** Acclimate mice in the testing room for 30 min but not more than one hour prior to testing; record time of transfer.

**b.** Turn the side overhead white lights off; center overheard lights should be off. Turn on/connect the camera to the ethernet port of computer.

**c.** Open Ethovision software and the configuration file for the Novel Object test and align the arena with the zone map of software (boarders should match). Use this method to position the yellow toys and Lego block in prevision to running the identical and novel trials.

**d.** The arena should be 44 inches above ground. The arena size (60 x 40) is reduced to 40 x 40 cm by inserting a Plexiglas dividing wall.

**SUPPLEMENTARY NOTES:**

- The camera is mounted at an angle of approximately 90 degrees and centered to facilitate the best viewing conditions.
- The novelty objects should be simple, slightly larger than a normal mouse, can be climbed on, and should be 2 sets of objects with distinct features from one another (color, texture, size). Our test objects are towers made of plastic blogs (Lego) and small rubber toys.
- If white mice are being run, they need to have a black stripe painted from head to rump so that the tracking will work. Use the animal marker paint. If the experiment includes mice of various colors, all mice should go through the marking process even if they don't need the mark for tracking.

## 2. PROCEDURE

### *Day 1. Acclimation (10 min)*

**a.** Make sure the arena and divider are clean and aligned according to the zone map of software. **NOTE: there are no objects in the arena during acclimation.**

**b.** Place the subject mouse in the center arena (top) and allow acclimation for 10 minutes.

**c.** To start the run, click on the red record button in the "Playback Control" menu (found beneath the live camera image). Timing is not critical because the tracking will not begin until the mouse has been detected in the test arena. The program will stop collecting data after 10 min.

**d.** Return mouse to its home cage.

**e.** Clean the arena with disinfectant, allow complete drying prior to testing additional mice.

### *Day 2. Trial 1: Identical Objects (10 min)*

**a.** Mice are to be tested ~24 hours following the acclimation trial.

**b.** Make sure the test arena is clean and aligned according to the zone map of software.

**c.** Place the 2 identical toys (e.g., rubber yellow toys) in the left and right zones according to the zone map. Affix the toys to the arena floor with double stick square pads.

**d.** Prior to starting the run, ensure the correct mouse ID is listed under "Trial Status" tab (found under the "Analysis Results and Scoring" header, beneath the live camera image).

**e.** To start the run, click on the red record button in the "Playback Control" menu (found beneath the live camera image). Timing is not critical because the tracking will not begin until the mouse has been detected in the test arena.

**f.** Quickly retrieve the subject mouse and place in the middle of the top of the center zone.
**g.** After 10 minutes, the program will automatically stop the data collection (tracking).
**h.** Manually stop the video recording by hitting the same record button.
**i.** Return the mouse to its home cage.
**j.** Clean the arena with Rescue disinfectant and allow complete drying prior to running additional mice.

***Day 2 Trial 2: Novel Objects (10 min)***

**a.** Mice are tested *2 hours* after Trial 1 or Identical Object run.
**b.** Make sure the test arena is clean and aligned according to the zone map.
**c.** Place 1 yellow rubber toy in the **left zone** according to the zone map. Affix the toy to the arena floor with a double stick pad.
**d.** Place the green/purple Lego block in the **right zone** according to the zone map. Affix the Lego block to the arena floor with a couple of double stick tape.
**e.** Prior to starting the run, ensure the correct mouse ID is listed under "Trial Status" tab (found under the "Analysis Results and Scoring" header, beneath the live camera image).
**f.** To start the run, click on the red record button in the "Playback Control" menu (found beneath the live camera image). Timing is not critical because the tracking will not begin until the mouse has been detected in the test arena.
**g.** Click the red record button to start recording the video.
**h.** Quickly retrieve the subject mouse from its cage and place in the middle of the top of the center zone.
**i.** After 10 minutes, the program will automatically stop the data collection (tracking).
**j.** Manually stop the video recording by hitting the same record button.
**k.** Return the mouse to its home cage.
**l.** Clean the arena with Rescue disinfectant and allow complete drying prior to running additional mice.

***Clean-up***

**a.** Return all mice to their respective home cage.
**b.** Clean the test arena and objects with 70% ethanol then with wipe down with Nolvasan 10%. Repeat the process for subsequent mice.