LAB_062 Novel Object/Location Recognition for Rodents (Expiry: March 2026)

I. OBJECTIVE

To describe the procedure for measuring recognition memory of rodents using novel objects or novel locations in an open field arena.

NB: The use of (*) indicates this statement is dependent on the facility procedures
NB: The use of (**) indicates this statement is dependent on AEC Approvals

II. COMMENTS / RECOMMENDATIONS

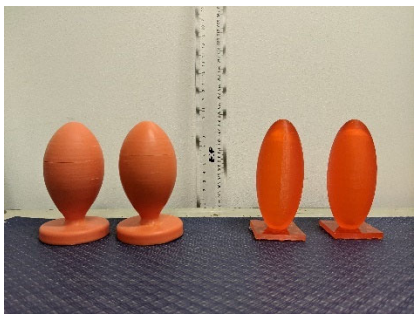
- Behavioural assessments are ideally performed in a dedicated behavioural suite.
- The environment should be free from uncontrolled external stimuli that may influence the animal's behaviour such as human traffic, unnecessary noise, intense lighting. Similarly, it is important that assessments are controlled for those stimuli which cannot be removed, such as such as time of day and light or dark phase.
- Male and female rodents should be tested separately, with one sex in the room at a time. Where possible males should be tested first, preferably on separate days but with at least thorough cleaning between the sexes. This is unless rodents are already housed within wire top cages or equivalent and both sexes are present in the home room.

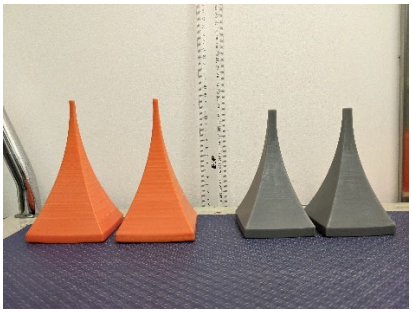
III. EQUIPMENT

- PPE*

Minimum PPE is gloves and gown, additional PPE may be required based on facility or additional risk e.g. working with infectious animals.

- Appropriate trolley for transporting cages.
- Disinfectant* and paper towel for cleaning equipment.
- Suitable objects – need to be easily cleaned to remove rodent scent, chew resistant, non-absorbent, adhere to PC2 restrictions (no drink cans), and novel and interesting to the rodents. Need 2 identical objects for both tests, as well as 1 novel object for object recognition. Examples of suitable objects are shown in the images below.





- Home cage bedding can be used in the base of the arena to reduce cleaning requirements between rodents/trials. Shake bedding to equally distribute any odour cues.
- Arena – the open field can consist of any square or circle box, ideally within the ranges specified in the table below.

  To aid in spatial orientation within the arena, one of the walls could be a different colour or pattern.

Arena dimensions	Mouse	Rats
Height (cm)	30	50
Minimum width/diameter (cm)	30	60
Maximum width/diameter (cm)	50	120

- Video recording equipment connected to a computer for video capturing and use of tracking software should be used to measure animal movement.

To facilitate automatic tracking with video recording equipment, use diffuse lighting to minimise reflections.

- A curtain or screened off area for experimenter to be hidden from the rodents during testing, if available.

IV. PREPARATION

1. Check AEC approvals** to ensure that the correct procedure and personnel are approved for the planned work.
2. Prepare equipment items including disinfecting prior to first use.
3. Bring rodents into the room (with lighting levels pre-set at the level required for the experiment) for at least 30 mins prior to start of experiment.

Length of habituation time in the testing room should be consistent for all rodents within an experiment.

V. PROCEDURE

1. Record light levels in the middle of the arena, for reproducibility and consistency.

Lux range should be between 30-100 LUX and should remain the same for all rodents within an experiment.

2. Rodents will undergo Habituation, Training and Testing on 3 consecutive days. Training can be repeated daily up to 6 days to ensure recognition of original objects and/or their location is obtained.
3. Drugs/compounds** can be given within appropriate times prior to introducing the rodents to the arena depending on absorption. Drug administrations as per the relevant SOP for injection type (examples below):
   LAB_028 Injections - Intra-peritoneal (IP) in Mice, Rats and Neonates
   LAB_029 Injections - Intramuscular (IM) in Mice and Rats
   LAB_030 Injections - Intravenous (IV) tail vein, in Mice and Rats

This can be done for either or both training and testing days depending on aspect of memory you wish to manipulate.

Habituation

4. Start recording and identify subject/s within the camera view or set up activity monitor to start recording once presence of rodent is detected.
5. Handling of rodents as per: LAB_006 Handling and Restraint in Mice and Neonates
   LAB_039 Handling and Restraint in Rats and Neonates
6. Place rodent in an empty arena, on the outer edge facing in towards the centre.
7. Trials last for 10 mins to allow time for the rodent to explore the arena.
8. At the end of the trial, remove the rodent and return them to the home cage.
9. Stop recording and make sure to save the video file or activity monitor output.
10. Remove scat and thoroughly disinfect the arena and allow to dry completely.

Training

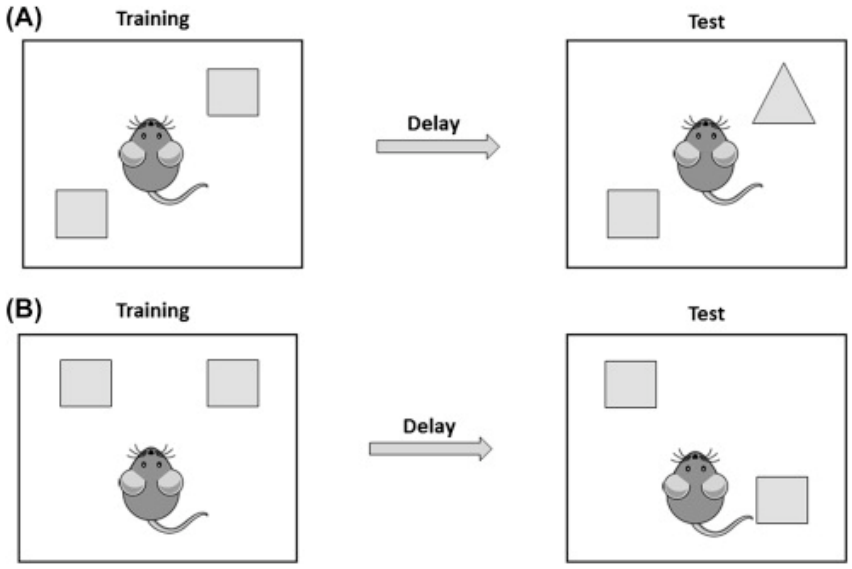
11. Either 90 mins (short-term memory) or Twenty-four hours (long-term memory) after habituation, training the rodents to the presence of the objects takes place.
12. Two identical objects are placed into the arena, separated from the edges of the arena and each other (see images below).
13. Follow steps 3-5 above, then place the rodent in the arena with the objects to allow them to explore for a 10 min trial.

Depending on the strain, training over consecutive days may be required for the rodents to retain the information sufficiently.

14. At the end of the trial, remove the rodent and return them to the home cage.
15. Stop recording and make sure to save the video file or activity monitor output.
16. Remove scat and thoroughly disinfect the arena and allow to dry completely.

Testing

17. Either 90 mins (short-term memory) or Twenty-four hours (long-term memory) after the last training session measuring of the rodent's recognition memory takes place.
18. For object recognition memory (A), one of the identical objects is removed and replaced with a novel object in the same location as the previous object. For location recognition memory (B), one of the objects is moved to a new location in the arena (see images).



19. Follow steps 3-5 above, then place the rodent in the arena and allow them to explore for a 10 min trial.
20. At the end of the trial, remove the rodent and return them to the home cage.
21. Stop recording and make sure to save the video file or activity monitor output.
22. Remove scat and thoroughly disinfect the arena and allow to dry completely.

VI. ANALYSIS

Several different parameters can be analysed during the novel object/location recognition. Please note, 'inspection' of the object is defined as approaching, sniffing, and looking at an object from ≤ 2cm.

Habituation

- Latency to enter the centre of the arena

- Time spent in the centre of the arena

Training

- Latency to inspect either object
- Time spent inspecting each object
- Time spent in the centre of the arena

Testing

- Latency to inspect either object
- Latency to inspect the novel object/location
- Time spent inspecting each object (≥ 50% of time inspecting the familiar object suggests memory deficit)
- Distance travelled
- Number of entries into centre
- Number of inspections of each object

A key parameter is the percent of time inspecting: $T_F/(T_N+T_F) \times 100$, where T_F = time spent inspecting familiar object and T_N = time spent inspecting novel object or object in novel location. Please note: Controls must have greater than 50% of time spent inspecting the novel object or location to be a valid result.

VII. REFERENCES

1. Leger, M. et al (2013). Object recognition test in mice. *Nat Protoc* (8), 2531–2537. https://doi.org/10.1038/nprot.2013.155
2. Lueptow L. M. (2017). Novel Object Recognition Test for the Investigation of Learning and Memory in Mice. *J Visual exp: JoVE*, (126), 55718. https://doi.org/10.3791/55718
3. Takahashi, Y. et al (2013). The diurnal variation of performance of the novel location recognition task in male rats. *Behav Brain Res* (256), 488-493 https://doi.org/10.1016/j.bbr.2013.08.040
4. Vogel-Ciernia, A. and Wood, M.A. (2014) Examining Object Location and Object Recognition Memory in Mice. *Curr Protoc Neurosci 69:8.31.1-8.31.17* https://currentprotocols.onlinelibrary.wiley.com/doi/10.1002/0471142301.ns0831s69