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|-----------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| <br><small>CREATE CHANGE</small> | UQ Animal Ethics Committee - Standard Operating Procedure<br><b>LAB_062 Novel Object/Location Recognition for Rodents</b><br>Institutional author: <b>Queensland Brain Institute</b><br>AEC Reviewed and Approved: July 2026<br>SOP Expiry: July 2029 | Version #2.0 |
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## LAB\_062 Novel Object/Location Recognition for Rodents (Expiry: July 2029)

### I. OBJECTIVE

To describe the procedure for measuring recognition memory in 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 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 from females, 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.
- Any changes to the process detailed in this SOP must be fully described in project applications and receive AEC approval \*\*.
- All substances administered to rodents as part of this procedure must be clearly detailed as a 'substance' in approved projects \*\*.
- Any supporting SOPs referenced in this procedure (e.g. handling or substance administration SOPs) must also be described in the approved project ethics \*\*.

### III. SAFETY AND COMPLIANCE

- Correct PPE use is essential when handling laboratory rodents
- The person undertaking this task must ensure all relevant approvals are in place, training has been undertaken, and risk assessments have been performed. If unsure, consult your supervisor.
- Facility protocols should be followed.
- Possible risks include mouse/rat bite injury, repetitive task musculoskeletal injury and psychosocial harm.
- All accidents, injury or near misses are to be reported immediately to the Facility Manager and recorded on a UQ OHS Incident Report Form.

### IV. TRAINING CONSIDERATIONS

- All unsupervised procedures must be performed by appropriately trained personnel who have been deemed to be competent in the procedure.

### V. EQUIPMENT

- PPE\*

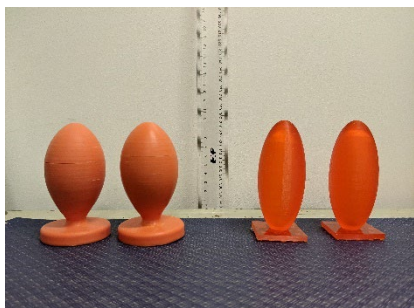
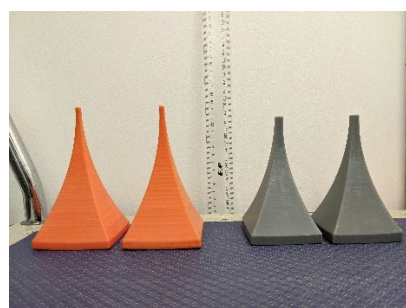
*Minimum PPE is gloves and gown, additional PPE may be required based on facility or additional risk e.g. working with infectious animals.*

- Appropriate trolley for transporting cages.
- Disinfectant\* and paper towel for cleaning equipment.

#### Conditions:

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- 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.

## VI. PREPARATION

1. Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work\*\*.
2. Prepare and disinfect equipment items 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.*

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## VII. 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. This is particularly important for albino mice where light intensity above 400Lux may induce retinal damage.*
2. Rodents will undergo separate Habituation, Training and Testing sessions. Training sessions can be repeated daily for up to 6 days to ensure that recognition of original objects and/or their location is obtained.
3. Drugs/compounds\*\* can be given at appropriate times depending on absorption prior to introducing the rodents to the arena. Drug administrations should be performed as per the relevant substance administration SOP.  
*This can be done for either or both training and testing days depending on the 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. Place rodent in an empty arena, on the outer edge facing in towards the centre.
6. Trials last for 10 mins to allow time for the rodent to explore the arena.
7. At the end of the trial, remove the rodent and return them to their home cage.
8. Stop recording and make sure to save the video file or activity monitor output.
9. Remove scat and thoroughly disinfect the arena and allow to dry completely.

### Training

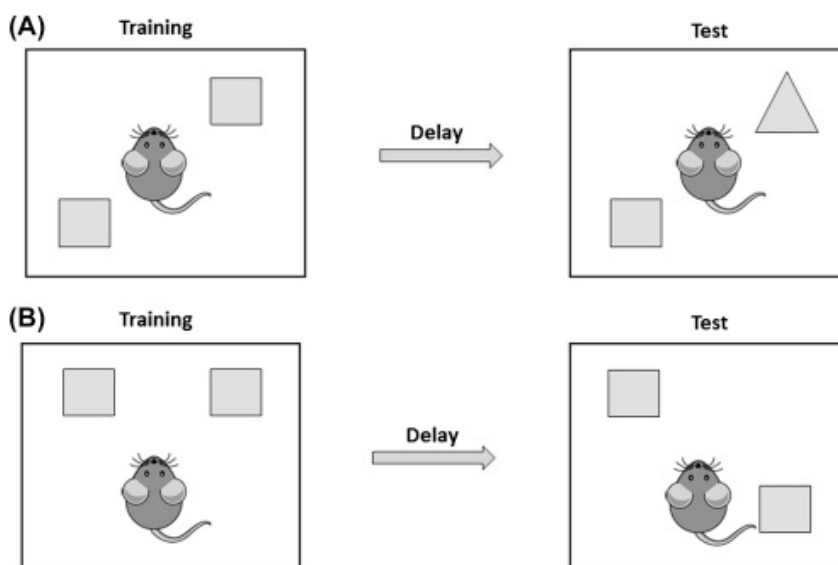
10. Either 90 mins (short-term memory) or twenty-four hours (long-term memory) after habituation, training the rodents to the presence of the objects takes place.
11. Two identical objects are placed into the arena, separated from the edges of the arena and each other (see images below).
12. Follow steps 4-6 above, 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 long term memory testing to ensure the rodents to retain the information sufficiently.*
13. At the end of the trial, remove the rodent and return them to the home cage.
14. Stop recording and make sure to save the video file or activity monitor output.
15. Remove scat and thoroughly disinfect the arena and allow to dry completely.

### Testing

16. 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.
17. 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).

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18. Follow steps 4-6 above, then place the rodent in the arena and allow them to explore for a 10 min trial.
19. At the end of the trial, remove the rodent and return them to the home cage.
20. Stop recording and make sure to save the video file or activity monitor output.
21. Remove scat and thoroughly disinfect the arena and allow to dry completely.

## VIII. 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  $\leq 2\text{cm}$ .

### 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 ( $\geq 50\%$  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.

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## IX. REFERENCES

1. Leger, M. et al (2013). Object recognition test in mice. *Nat Protoc* (8), 2531–2537.  
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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>
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*The development of this Standard Operating Procedure is duly attributed to Dr. Daniel Blackmore, whose contribution warrants formal acknowledgment. Dr Blackmore is a Research Fellow, an Affiliate of Clem Jones Centre for Ageing and Dementia Research, and the Animal Behaviour Facility Manager at the Queensland Brain Institute, 2026.*

| Version # | Reviewing AEC                                                                                             | AEC Review Date                 | Outcome                |
|-----------|-----------------------------------------------------------------------------------------------------------|---------------------------------|------------------------|
| 1         | Anatomical Biosciences AEC, Health Sciences AEC, Molecular Biosciences AEC and Laboratory Biomedicine AEC | March 2025 (one year extension) | Approved to March 2026 |
| 2         | Anatomical Biosciences AEC, Health Sciences AEC, Molecular Biosciences AEC and Laboratory Biomedicine AEC | July 2026                       | Approved to July 2029  |

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