LAB_074 Novelty Suppressed Feeding

I. OBJECTIVE

To describe the procedure for detecting anxiety-related behaviour in mice.

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, and intense lighting.
- 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, little brush, and paper towel for cleaning equipment.
- Arena – Square or rectangular opague boxes. Recommended dimentions are listed below.

	Dimentions
Mouse – Rectangular	~ 40 cm (Width) x ~ 60 cm (Length) x 20~50 cm (Height)
Mouse – Square	~ 50 cm (Length) x 20~50 cm (Height)

- Other items: Timers, petri dishes with two holes in the middle, white Whatman paper, rubber bands, standard bedding, balances.

IV. PREPARATION

- Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work.
- Food deprivation begins the evening before the testing day
- Prepare equipment items including disinfecting prior to first use.
- 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 around 1000 LUX and should remain the same for all rodents within an experiment.

2. Handling of rodents as per: LAB_006 Handling and Restraint in Mice and Neonates

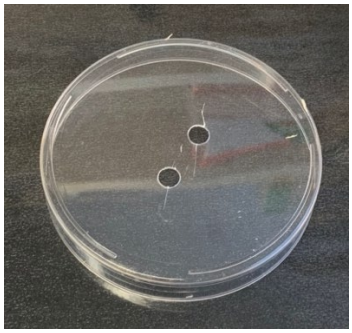
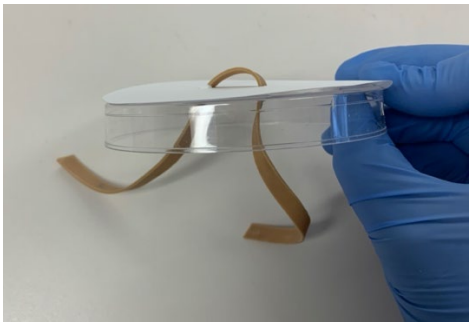
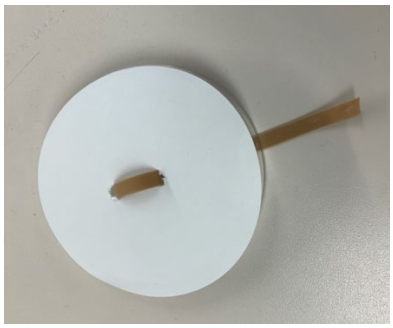
ONE DAY BEFORE THE TEST

Starting food deprivation 16 - 24 hours before the test

1. Using the sheet attached at the end to record the body weight of each mouse as "Pre-test body weight" right before food deprivation.
2. Transfer mice to a new cage with a new bottle of water and an empty food tray to start food deprivation. Write down "Food deprivation for 24h, NAME, DATE" on blank cards, and put the cards onto the front of each cage.

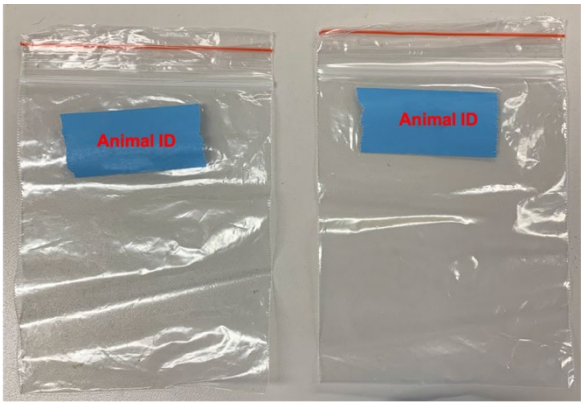
Things can be prepared before the testing day

1. Use rubber band to tie white filter paper to Petri Dishes (one Petri Dish for each cage).

2. Pick some large food pellets (one pellet for each cage) and put them together into one sachet. These pellets (A) will be tied to the petri dishes during the testing.

3. Weigh food pellets (Pre-test weight of food pellet) with similar size (one pellet for one mouse) using scales with two decimals. Put them into small sachets individually and write down the mouse ID on each sachet. These food pellets (B) will be used to measure home consumption right after the testing session to examine the change of appetite.



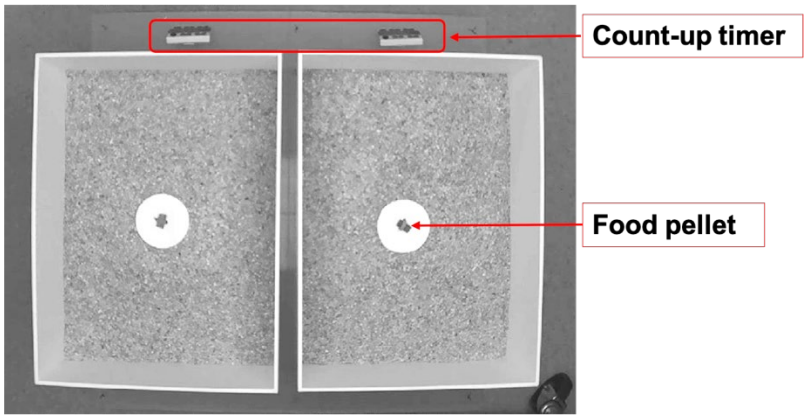
ON THE TESTING DAY

Preparation before the start

1. Adjust the light to approximately 1000 lux.
2. Transfer the mice to the behavioural room at least half an hour before the test to habituate animals to the new environment.
3. Prepare a holding cage and a new home cage for each cage of mice.

   Holding cage – No water, food or white tissue.

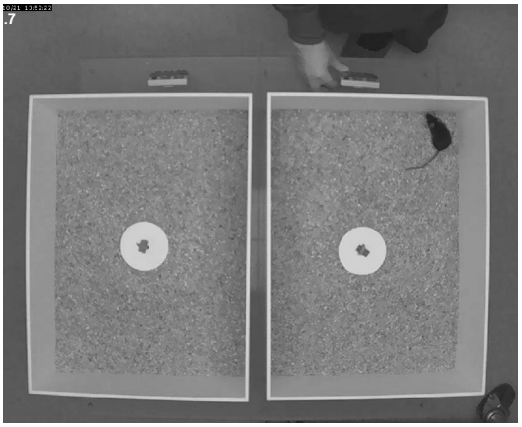
   New home cage – With food and water.
4. Make sure you have enough bedding for the test.
5. Prepare the testing boxes (two animals can be tested at the same time, one animal per home cage is run at a time)
   - Put testing boxes under the camera.
   - Cover the bottom of each boxes with 2cm of wooden bedding.
   - Tie down a single food pellet (A) onto the white filter paper on the Petri Dish. And put it in the center of the box. Make sure the Petri Dish is hidden by the wooden bedding, only white filter paper and the food pellet is visible.
   - Put one count-up timer in front of each box to record the latency to eat during the test.



   - One additional count-down timer for measuring food consumption in the old home cage within 5 min.

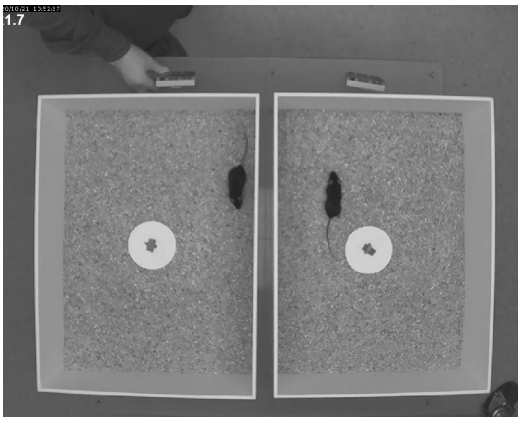
6. Transfer two cages of mice from their old home cage to their holding cages. Leave the holding cages under the light.
7. Put the old home cages to a relatively dimly lighted place.

Starting the test -Testing two mice at the same time



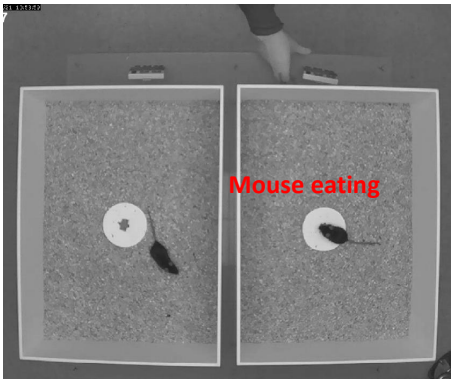
1. Start recording.

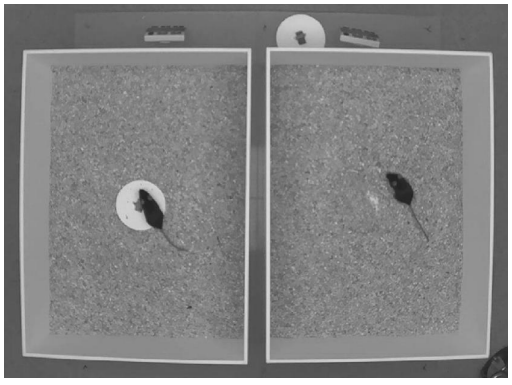
2. Put one mouse to the corner of one testing box. Start the count-up timer in front of the box immediately after that.



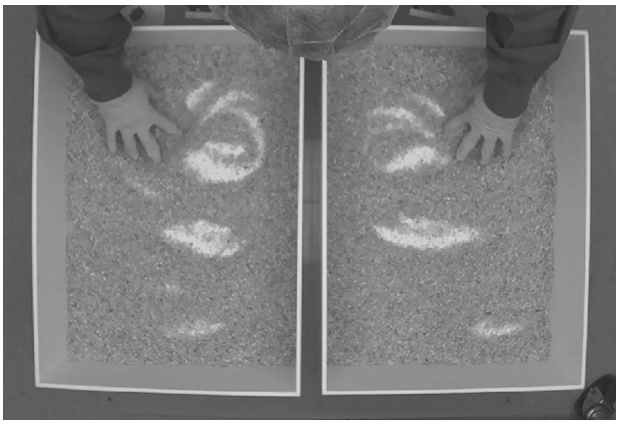
3. Quickly wipe your hands with 80%v/v ethanol and then put the second mouse to the corner of another testing box. Start the other count-up timer immediately after that.

4. Observe the mice carefully and stop the timer once they start to eat the food from its forepaws while standing on or near the petri-dish. This is very hard to distinguish from grooming or sniffing from videos, so you have to observe carefully during the test and record the time manually. Once the animal starts to eat, take out the Petri-dish from the testing box carefully and queitly. The animal should be left in the testing box until the other mouse starts to eat to avoid interrupting the approaching behaviour of the other mouse (right image).





5. Record the time on the timer (Latency to eat) on the sheet. **The maximum time of the test is 10 min.** If the animal doesn't eat by 10 min. Stop experiment and record 10 min as latency to eat.
6. Put food pellets with the matching animal ID to the old home cages. Put the two mice into their old home cage and start a 5 min count-down timer. Record the weights of the food pellets as "Post-test weight of food pellet" after 5 min. Then dispose of the food pellets. Record the weights of the two mice as "Post-test body weight".



During home cage consumption test, mix the bedding to avoid accumulation of urine at one place. Flatten bedding, attach a new piece of food to the petri-dish and place back into the appropriate box ready for next animal.

NOTE: Same bedding can be used for mice from one home cage. After finishing mice from one home cage, dispose of the test bedding, wipe the box with 70% ethanol, wipe the box with dry paper towel to remove ethanol residue and put new bedding to the box.

7. Put the two tested mice to their new home cages respectively and start testing next animals.

VI. ANALYSIS

1. Latency to eat

   The Kaplan–Meier estimator is particularly useful, because it allows for censoring animals that do not eat during the test. When dealing with multiple variables, the logrank test (often called Mantel–Cox) is used to compare two Kaplan–Meier curves.

2. Home cage feeding

   Subtract pellet weight at the end of home cage feeding from pellet weight at the start of home cage feeding. Divide the amount eaten (in mg) by the animal's weight. Plot the results and perform analysis of variance (ANOVA) to statistically compare different genotypes and/or treatments.

3. Animal weight lost

   Subtract weight prior to deprivation from weight following the novelty-suppressed feeding test. Calculate the percentage of weight lost from the weight prior to deprivation. Most mouse strains lose about 10% of their body weight with a 24-h deprivation.

4. Videos can be used to analyse the total distance travelled during testing and the number of entries to the white filter paper area.

VII. REFERENCES

1. Samuels BA, Hen R. Novelty-suppressed feeding in the mouse. In Mood and anxiety related phenotypes in mice 2011 (pp. 107-121). Humana Press. https://doi.org/10.1007/978-1-61779-313-4_7

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
1	LBM	06/10/2022	06/10/2025

Attachment: Recording sheet

Project : **Date:**

Animal ID	Pre-test body weight / g	Post-test body weight / g	Pre-test weight of food pellet / g	Post-test weight of food pellet / g	Latency to eat (min:sec)	Note