LAB_068 Operant conditioning with positive reinforcement in rodents

I. OBJECTIVE

To describe the procedure for measuring reward related behaviours in mice and rats.

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.
- Rodents are usually on food restriction prior to and during testing (from 1 week to 2 months of training) and their body weight and condition must be monitored daily. Any animal losing condition or falling below 85% of their free feeding body weight should be returned to ab libitum food and monitored for full recovery.
- Variations to SOP. Operant testing can be incredibly variable with changes to stimulus, necessary responses, rewards given, and substances tested. Anything beyond what is detailed in this SOP must be indicated to the AEC for their approval.
- Turn on PC and perform a hardware check of operant chambers every day prior to bringing rodents into the behaviour room.
- It is important to perform operant testing at a similar time each day. This ensures that food availability is consistent each day for the rodents and reduces the effects of the circadian cycle on the behaviour.

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.
- Operant Chambers housed within sound-attenuated and ventilated cubicles with corresponding computers/software to run chambers. There are a variety of suppliers of testing equipment (Lafayette Instruments, Coulbourn Instruments, Med Associates etc.) which differ in design, but the key aspects of the test remain the same. The chamber typically consists of a wire rod floor, a collection tray for faeces and urine, a stimulus panel containing light/speakers, a manipulanda to record responses (nose-poke apertures, levers, touch screens) and a reinforcement delivery area (food magazine) for delivery of pellets or liquid rewards. Often most chambers also include an IP camera to allow the researcher to view the rodent's activity during sessions.
- Food pellets (20mg for mice, 45mg for rats) or liquid reward (water or milk)

IV. PREPARATION

1. Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work.
2. **Food restriction:** To motivate the rodents to perform in the operant chambers they will be food restricted to 85-95% of their free feeding body weight.
3. **Free feeding body weight** - The researcher will weigh the rodents daily at the same time (+/- 1 hour) for a minimum of 3 consecutive days while still on ad libitum food. The average of these 3 weights will be recorded as each rodent's "Free feeding" body weight and will be used to determine their percent of free feeding body weight (%FF) during food restrictions.
4. **Removal of food** – Once a free feeding body weight has been established, all food will be removed from the cages and a notification card attached which states "Do Not Feed" and includes contact information for the primary researcher as well as a backup researcher. UQBR staff are in daily communication and a feeding log is put in place to ensure complete transparency.
5. **1st week of food restrictions** – During the first week of food restrictions the rodents are weighed daily at the same time (+/- 1 hour) and their %FF calculated – they are not tested during this time. The rodents are fed enough standard rodent grain pellets in their home cage to ensure they reach the ideal weight (85-90%FF) by the end of the first week. Ideally the rodents should lose less than 5%FF on each day until they reach their ideal weight.
6. **Feeding window** – Daily food is supplied no later than 2 hours before light's out (or the rodent's awake cycle), and it is up to the rodent when to eat the food or over what length of time. **Please note:** the rodents also receive food daily during testing in the form of reward (pellets or milk).
7. **Reward habituation:** Rodents need to be habituated to the pellets or milk reward for at least 3 days prior to commencing testing to ensure they will take the rewards when earned.
8. Either dustless precision grain pellets which are used as rewards in the operant chambers are introduced with ~100 pellets per cage added to the daily feed, or milk reward is offered in small petri dish daily prior to daily feed, to habituate the rodents to the reward.

Please note – rodents will remain on food restrictions for the duration of operant testing. However, once they have acquired the task the %FF can be reduced if the behavioural responses in the operant chamber are maintained (90-100%FF).

9. Prepare operant chambers including disinfecting prior to first use.

10. Bring rodents into the room 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. Each animal is tested individually in its own operant chamber. However, there is often multiple chambers so that rodents can be tested simultaneously. Often multiple rodents are testing in the same chamber throughout the day, so it is important to clean the chamber thoroughly after each session.

It is preferable for each animal to be tested in the same chamber throughout the procedure.

2. Rodents are tested in daily sessions with either a finite number of trials (typically 100, but this can vary from 20 to unlimited) or a finite amount of time (typically 30min to 1 hour) in which the animal learns to respond to a stimulus with reinforcement.
    - The stimuli presented include light, sound, or olfactory cues.
    - The response can include nose poke into an aperture, level press, or touch on a screen.
    - Reinforcement is via food or liquid reward delivered to the food magazine for easy access.

Care must be taken to ensure that the delivery system is working correctly so that the rodent is rewarded with each successful trial.

3. While the trials are in progress, the researcher generally remains in the testing room and monitors the animal's performance from the screen. However, the equipment is fully automated so the researcher can leave for short periods of time (to take/remove other animals etc).

The rodents should not be left in the chamber for long periods beyond the end of their daily session. However, rodents should not be removed from the chamber until all other chambers have finished, unless this can be done without disturbing the other chambers.

4. At the end of the training session (maximum of 2 hours), the rodents are removed from the chamber and returned to their home cage.
5. Typically, rodents will go through several phases of training to acquire the operant task (which can take a few days or a few weeks).
6. Once animals have acquired the criteria for instrumental performance, contingencies may be altered, including manipulation of the stimulus (signal duration or intensity), use of distractors (lights or sounds), use of non-contingent trial (where no reward is given) to assess extinction.

Care must be taken to ensure that a reduction in rewards earned during a session will necessitate increased food given in the home cage.

7. Drugs/compounds can be given within appropriate times prior to introducing the rodents to the chamber 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 during training to improve/impair performance, or once criteria has been met, to assess the effects of the drug on other aspects.

VI. ANALYSIS

1. Software (and sometimes video recording equipment) is set up to record all responses within the chamber. There is a wide range of components that can be analysed, including response latencies, correct and incorrect responses.

Researchers should be trained on this software prior to using the equipment or testing rodents.

VII. REFERENCES

1. Toth LA, Gardiner TW. Food and water restriction protocols: physiological and behavioral considerations. Contemp Top Lab Anim Sci. 2000 Nov;39(6):9-17. https://www.ingentaconnect.com/content/aalas/jaalas/2000/00000039/00000006/art00003
2. Mitchell SJ, Bernier M, Mattison JA, Aon MA, Kaiser TA, Anson RM, Ikeno Y, Anderson RM, Ingram DK, de Cabo R. Daily fasting improves health and survival in male mice independent of diet composition and calories. Cell Metab. 2019 Jan 8;29(1):221-228.e3. https://www.sciencedirect.com/science/article/pii/S1550413118305126?via%3Dihub
3. Malkki HAI, Donga LAB, de Groot SE, Battaglia FP, NeuroBSIK Mouse Phenomics Consortium and Pennartz CMA (2010) Appetitive operant conditioning in mice: heritability and dissociability of training stages. Front. Behav. Neurosci. 4:171. https://www.frontiersin.org/articles/10.3389/fnbeh.2010.00171/full
4. Pritchett K, Mulder GB. Operant conditioning. Contemp Top Lab Anim Sci. 2004 Jul;43(4):35-6. https://www.ingentaconnect.com/content/aalas/jaalas/2004/00000043/00000004/art00008

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

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see "AEC Reviewed/Approved" date in this document's header).