LAB_068 Operant Conditioning with Positive Reinforcement in Rodents (Expiry: Sept 2029)

I. OBJECTIVE

To describe the procedure for measuring reward related learning 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 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.
- Rodents are usually on mild food restriction prior to and during testing (from 1 week to 2 months) 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 daily until full recovery.
- While food restriction is the most common approach, this is not mandatory with alternate approaches available that include palatable rewards or short-cycle intermittent food access. In all instances physiological impacts must be monitored.
- 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.
- All substances (not including routine diet or rewards) administered to rodents as part of this procedure must be clearly detailed as a 'substance' in approved projects **.
- Supporting SOPs referenced or used in this procedure (e.g. handling or substance administration SOPs) must also be identified 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.
- 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 lights/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. Most chambers also include an infrared camera to allow the researcher to view the rodent's activity during sessions.
- Food pellets (20mg for mice, 45 mg for rats) or liquid reward (flavoured milk) as defined in approved project application**

VI. 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 - 90% of their free feeding body weight.
    - **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.
    - **Removal of ad libitum food access**– Once a free feeding body weight has been established, all ad libitum 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. The 'do not feed restriction' only pertains to the ad libitum food supply; animals will receive a restricted food ration supplied daily. UQBR staff are in daily communication, and a feeding log is put in place to ensure complete transparency.
    - **1st week of food restrictions** – During the first week of food restrictions the rodents receive a restricted, pre-weighed ration of standard rodent grain pellets in their home cage daily. Ration size should be set to ensure that mice lose ~3-5% FF mass each day and they reach the ideal weight (85-90% FF) by the end of the first week. Rodents are weighed daily at the same time (+/- 1 hour) and their %FF mass monitored – they are not tested during this time.
3. **Feeding windows** – Daily food rations during the weight loss, training and testing periods are supplied no later than 2 hours before light's out (or the start of 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 will also receive additional calories daily during training/testing periods in the form of rewards (food pellets or flavoured milk).

4. **Reward habituation:** Rodents need to be habituated to the reward pellets or flavoured milk, which are used positively reinforce behaviours in the operant chambers, for at least 3 days prior to commencing testing. Either ~100 dustless precision grain reward pellets per cage are added to the daily feed ration, or the flavoured milk reward is offered in small petri dish daily prior to the 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).
5. Prepare and disinfect the operant chambers prior to first use.
6. 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.

VII. PROCEDURE

1. Each animal is tested individually in its own operant chamber. However, there are often multiple chambers in the behavioural suite so that several rodents can be tested simultaneously. Often different rodents are tested 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 trained or tested in daily sessions with either a finite number of trials (typically 100, but this can vary from 20 to 200) or a finite amount of time (typically 30 min to 1 hour; maximum 2 h per daily session) in which the animal learns to respond to a stimulus with positive reinforcement.
   - The stimuli presented include light, sound, or olfactory cues.
   - The response can include nose poke into an aperture, lever press, or touch on a screen.
   - Reinforcement is via a 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 the room for short periods of time (to take out /bring in 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 daily 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 prior to introducing the rodents to the chamber . Drug administrations should be delivered as per the relevant SOP for administration type**.

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.

VIII. ANALYSIS

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.

IX. REFERENCES

1. Toth LA, Gardiner TW. Food and water restriction protocols: physiological and behavioural considerations. Contemp Top Lab Anim Sci. 2000 Nov;39(6):9-17. https://www.ingentaconnect.com/content/aalas/jaalas/2000/00000039/00000006/art00003
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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

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Version #	Reviewing AEC	AEC Review Date	Outcome
1.0	Laboratory Biomedicine AEC	06/10/2022	Approved to 06/10/2025
2.0	Health Sciences AEC, Laboratory Biomedicine AEC, Molecular Biosciences AEC, Anatomical Biosciences AEC	September 2026	Approved (with administrative updates) to September 2029.