

LAB_077 Contextual and Cued Fear Conditioning in Rodents (Expiry: Sept 2028)

I. OBJECTIVE

To describe the protocol for contextual and cued fear conditioning that assesses the ability of mice to learn and remember an association between environmental cues and aversive experiences. Using conditioning chambers, animals are given pairings of a conditioned stimulus (auditory or olfactory cue), and an aversive unconditioned stimulus (an electric foot shock). The animal can then associate the cue ('conditioned stimulus', CS) predicts an unpleasant event ('unconditioned stimulus', US) and learns to fear the CS alone. Animals will generally demonstrate a freezing response if it remembers and associated the cue with the aversive stimulus, which can be measured as an index of fear memory.

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, 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.
- Variations to SOP. Fear conditioning can be variable with changes to cue stimulus type, amount and intensity of shocks given, delay between trials, if you are performing extinction or recall testing. Anything beyond what is detailed in this SOP must be indicated to the AEC for their approval**.
- If wanting to use a novel scent, you MUST discuss this with the behaviour facility manager prior to starting your experiment.
- A 16000Hz tone has been tested and found effective; validated amplifier settings are available for this tone. If using a different sound cue, it is the user's responsibility to determine suitable amplifier settings using a decibel meter. The tone level should range between 75-80dB in each box (as too low may make it difficult for the animals to distinguish from background noise while too much loud may itself be aversive).
- For mice, the shock intensity commonly ranges between 0.3 to 0.7mA, depending on the paradigm being undertaken. Shock intensity must not exceed 1mA without permission from the AEC**.
- For rats, shock intensity must not exceed 1.5mA without permission from the AEC**.

III. SAFETY AND COMPLIANCE

- 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.

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).

IV. TRAINING CONSIDERATIONS

- All unsupervised animal procedures must be performed by appropriately trained personnel who have been deemed 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.
- Coulbourn Habitest Chambers housed within sound-attenuated cubicles or room with corresponding computers/software (FreezeFrame) to run chambers. The chamber consists of a removable grid floor, a collection tray for faeces and urine, cue LED light and/ or speakers, and a mounted video camera. There will be a Context A chamber and a Context B chamber, the exposure for the rodents in each will be specific for that context.
- If performing olfactory cue testing, you will also need various scents with common examples including lemon, almond and vinegar.

VI. PREPARATION

1. Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work**.
2. **Setting up chambers** - Open the cubicles. Wipe down the metal floor of the operant box with disinfectant. There should be no obvious odour of rodent or urine when you open the box. Remove any droppings.
 - A) **If using a sound cue** - ensure that the settings are producing the same volume, pitch and duration in each box. Operators must be trained in setting up amplifiers prior to starting the experiments.
 - B) **If using an olfactory cue** - Each set of boxes will have a different scent, which forms part of the contextual cue for the animal e.g. For Context A, the scent is lemon and for Context B the scent is vinegar. Please do not deviate from these scents, because lingering scents will affect other people's experiments. Open the cubicles. Wipe down the metal floor of the operant box with disinfectant. Wipe down the plastic surfaces inside the box with the scent cue.
3. **Software setup** – Ensure that the software has been setup correctly. Operators must be trained on this software prior to performing the experiment.
4. **Lighting** – If the animals are going into the room more than once (e.g. if you are doing fear conditioning and then extinction or recall testing), turn off the white lights in all rooms, leaving the red lights on. Also turn off computer monitors at the power button, leaving the computers on. This is so the animals can't see their surroundings as you take them into the room and will not form a memory about passing through this location.
5. **Configure the shockers** – Before every experiment, it is critical that you configure the shockers correctly. Use a digital multimeter to verify shock intensity before each use. Operators must be trained prior to performing the experiment.

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).

VII. PROCEDURE

- There are several variations on the fear conditioning protocol that affect its relevance to different brain regions/circuits, in relation to the intensity and longevity of the generated fear memory. Before you start, you need to decide the following parameters, with reference to the goals of your experiment and the part of the brain you are targeting:

- If you are using a sound cue: what volume, pitch, duration, scrambled or pure tone (standard is usually white noise at 70db)
- Number of shocks during experiment
- Intensity of the shocks (from 0.4mA to 1mA – standard is usually 0.7mA)
- Length of the pre-trial, inter-trial and post-trial intervals
- Which controls, if any, you need to use.

These details will need to be described in the animal ethics application.

Conditioning:

- Begin by loading the animals into their individual boxes - work quickly but be gentle. Ensure that the plastic gate is clipped closed, and that each cubicle is clipped closed.
- While the experiment is running, keep an eye on the screens – you should see all the animals visibly jump and panic when they are shocked.

If they appear not to respond, there is probably an equipment issue that has prevented them from being shocked. Immediately stop the experiment and check all systems. If no obvious issues can be found contact the behavioural facility staff.

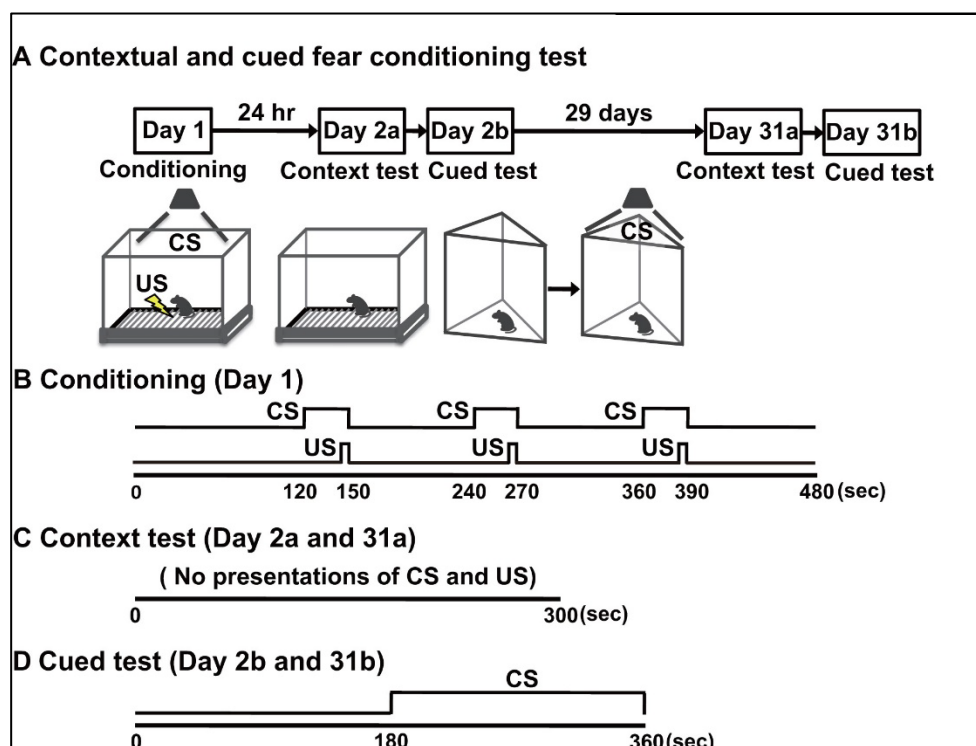


Figure 1. Example of a contextual and cued fear conditioning protocol. CS is conditioned stimulus, US is unconditioned stimulus.

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).

3. When testing is complete, caution should be taken when removing the animals from their boxes to their home cages. Animals will be very fearful and will not want to be picked up. Do not fully open the plastic gate – hold it just open far enough to admit your hand as some animals will jump. Alternatively, you can use a beaker or a container to remove them from the boxes. Animals that are already aggressive may bite.
4. Turn off computer monitors again before taking the animals back through ante rooms. Return the animals to the colony room.
5. Completely remove all urine and droppings and wipe down the operant boxes, grid floors and trays with disinfectant. If the next group of animals smell urine from the previous batch, they will immediately fear the context.

Extinction:

6. To test extinction/retention of fear, first perform fear conditioning in Context A, then perform extinction training in Context B with only CS (no unconditioned stimulus). A retention control group can be included by placing them in Context B without CS and US.
7. To test retention of the fear memory, all rodents are returned to Context B for final assessment of freezing following the CS.

Modifications:

8. 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 and AEC approval**.

This can be done to attenuate fear response (or forget) or enhance fear response (improve fear memory)

VIII. ANALYSIS

1. FreezeFrame 4 or later (and sometimes video recording equipment) is set up to record freezing behaviour. *Researchers should be trained on this software prior to using the equipment or testing rodents.*

IX. REFERENCES

1. Shoji, H., Takao, K., Hattori, S., & Miyakawa, T. (2014). Contextual and cued fear conditioning test using a video analyzing system in mice. Journal of visualized experiments : JoVE, (85), 50871.
<https://www.jove.com/t/50871/contextual-cued-fear-conditioning-test-using-video-analyzing-system>
2. Chang, C. H., Knapska, E., Orsini, C. A., Rabinak, C. A., Zimmerman, J. M., & Maren, S. (2009). Fear extinction in rodents. *Current protocols in neuroscience, Chapter 8*, Unit 8.23.
<https://doi.org/10.1002/0471142301.ns0823s47>
3. Pietersen, C.Y., Bosker, F.J., et al., (2006). Fear conditioning and shock intensity: the choice between minimizing the stress induced and reducing the number of animals used. *Laboratory Animals* (40) 2, 180-5.
<https://www.ncbi.nlm.nih.gov/pubmed/16600077>

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 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).

Version #	Reviewing AEC	AEC Review Date	Outcome
1.0	Laboratory Biomedicine AEC (ratified by MBS, ABS and HS AECs)	October 2022	Approved
2.1	Molecular Biosciences AEC, Laboratory Biomedicine AEC, Anatomical Biosciences AEC, Health Sciences AEC	September 2025	Approved

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).