LAB_077 Contextual and Cued fear conditioning in rodents

I. OBJECTIVE and SUMMARY

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 parings of a conditioned stimulus (auditory or olfactory cue), and an aversive unconditioned stimulus (an electric foot shock). The animal can then associate that 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 associates the cue with the aversive stimulus, which can be measured as an index of fear memory.

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.
- Experimenters need to be devoid of strong-smelling deodorants or perfumes and must be experienced at handling rodents and trained on the behavioural paradigm and equipment used.
- 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 new 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 sound should not be much quieter than about 75-80dB in each box (as it may be difficult for the animals to distinguish from background noise) or much louder (as a deafening sound may itself be aversive).

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.
- 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/speakers, and a mounted 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 lemon and vinegar.

IV. 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. Remove all pans and floors and wash thoroughly between animals and wipe down the metal grid thoroughly. There should be no obvious odour of rodent or urine when you open the box.
   - **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 place.
5. **Configure the shockers** – Before every experiment, it is very important that you configure the shockers correctly. Use a voltmeter to verify shock intensity before each use. Operators must be trained prior to performing the experiment.

V. PROCEDURE

1. 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:

2. 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.
3. 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.

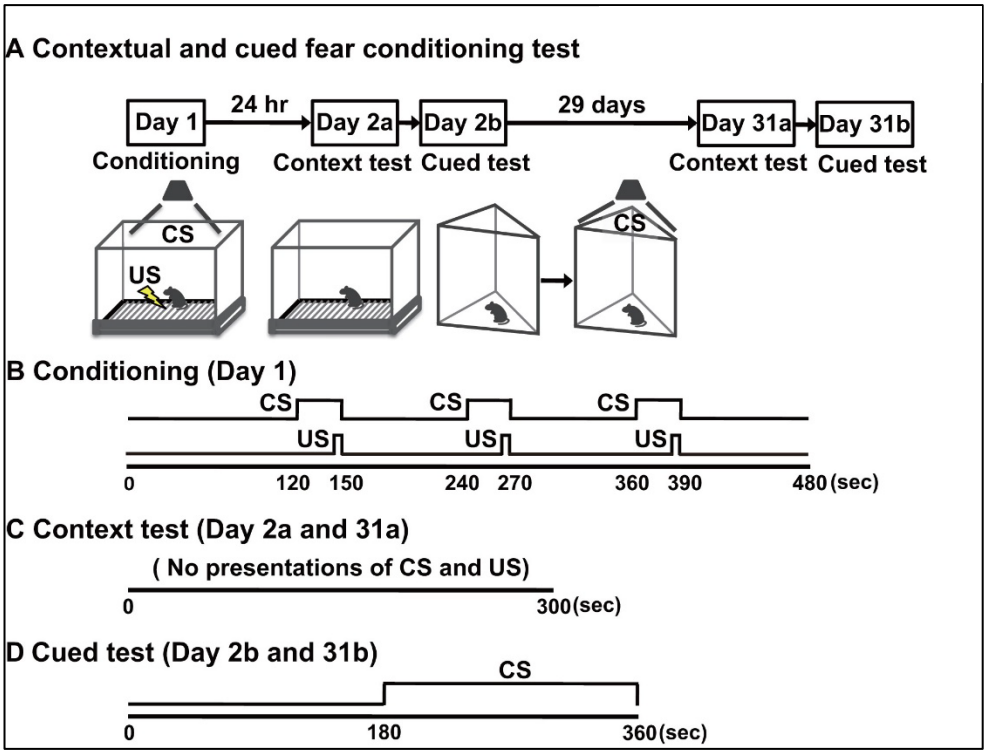

Figure 1. Example of a contextual and cued fear conditioning protocol. CS is conditioned stimulus; US is unconditioned stimulus.

4. 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.
5. Turn off computer monitors again before taking the animals back through ante rooms. Return the animals to the colony room.
6. Completely remove all urine and droppings and wipe down the operant boxes, grid floors and trays with disinfectant. If the next batch of animals smell urine from the previous batch, they will immediately fear the context.

Extinction:

7. 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.
8. To test retention of the fear memory, all rodents are returned to Context B for final assessment of freezing following the CS.

Modifications:

9. 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 to attenuate fear response (or forget) or enhance fear response (improve fear memory)

VI. ANALYSIS

1. FreezeFrame 4 Software (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.

VII. 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

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