Fear Conditioning

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Summary:

This procedure is used to evaluate aversive learning and memory. A neutral conditioned stimulus (CS) such as steady tone (85 dB, 2800 Hz, 30 sec), is paired with an aversive unconditioned stimulus (US) such as ae mild foot shock (0.75 mA, 2 sec). After conditioning, the spatial context or the CS (tone) elicits a central state of fear in the absence of the US (shock) that is expressed as reduced locomotor activity or total lack of movement (freezing). Immobility time is used as a measure of learning/memory performances.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Fear Conditioning Chamber	Med Associates	MED-FVC-SCT-M
Tracking Software	Med Associates	
A Frame insert	Med Associates	
NIR Video system	Med Associates	VFC
Lab coats/Gloves/PPE		
Acetic acid 5%	Fisher	Ricca Chemical 13016
Paper towels		
Disinfectant 10% Nolvasan		
Disinfectant Coverage Plus	Setris	

Protocol:

1. SET-UP (acclimation of mice, equipment preparation, software setup)

- **a.** Acclimate mice in an adjacent quite room for 30 min prior to testing.
- **b.** Wipe down testing arenas with 10% Nolvasan before each run.
- **c.** Open Ethovision software and the configuration file for the Y maze test and set the maze so that the arms correspond (1, 2, 3). The arm borders should not include the shadow of the vertical wall or the last few cm. at the end of the wall (mouse length) where there is shadow.
- **d.** Turn on the main blue power supply box first. This will automatically turn on four NIR light control boxes (NIR-100). White Light toggles should be switched to ON position for conditioning

and context trials and to Remote position before cue trails; NIR light toggles should be always set to ON.

e. Next, turn on all four A/S Aversive Stimulator boxes (ENV-414S). Ensure that DUMMY LOAD is set to OUT and that RANGE is set to 0-5 mA.

f. Turn on computer and monitor.

NOTES:

- Males are tested before females.
- Assign mice to a given chamber and keep the same chamber throughout testing.
- Group A mice should be obtained for their acclimation prior to calibrating cameras. Group B mice should be obtained for their acclimation prior to scenting pans for Group A.
- Group C (and additional groups) mice should be obtained for their acclimation once a previous group of mice have been removed from their chambers prior to cleaning the arenas.

2. PROCEDURE

DAY 1: Conditioning (5 min run): conditioning protocol consists of 120 seconds baseline period, 30 seconds of audible tone and 2 second foot shock that co-terminates with tone and 150 seconds with no stimuli at the end.

a. Open Video Freeze software, go to experiment set up window, enter experiment ID. Set trial to Conditioning. Open Conditioning protocol file. Select chambers. Set Default settings to: Motion Threshold (au): 18; Sample Rate (fps): 30 fps; Method: Linear; Min Freeze Duration (frames): 30. Click OK.

b. Cameras: switch the view to 4.

c. Ensure that all arenas are pushed all the way back inside their chambers and close door.

d. Click “Calibrate” and then click “Lock”.

e. Scent the drop pans with 70% alcohol and switch the White Light toggle to remote.

f. Weigh mice for their weekly body weight and place them in their assigned arenas. Switch the White Light toggle to ON and close doors. Click the “Record” button.

g. Once the 5 minute run is complete, open doors and slide the arenas forward slowly. Beware that animals will be timid, jumpy, and vocal. Cautiously, open the arena door and approach the animal slowly. Place all animals back in their home cages.

h. Thoroughly clean the arenas and drop pans with 10% Nolvasan before the next run. After the last run, clean arenas with Coverage Plus.

DAY 2: Context (5 min run): Context protocol is performed 24h following the conditioning protocol and it is similar to the Conditioning protocol except that the shock and the tone <u>are not used</u>.

a. Once the 5 minute run is complete, open doors and slide the arenas forward slowly. Beware that animals will be timid, jumpy, and vocal. Cautiously, open the arena door and approach the animal slowly. Place all animals back in their home cages.
b. Thoroughly clean the arenas and drop pans with 10% Nolvasan before the next run. After the last run, clean arenas with Coverage Plus.

DAY 2: Cue (5 min run): Cue protocol is used 2 hours following the context protocol and it consists of 120 seconds without any stimulus followed by 180 second presentation of the tone. This trail will be performed in the dark with only the NIR Light ON. Set up cue arenas using different floors, walls (insert A frame) and olfactory cues (acetic acid) from the conditioning context. Ensure that tone delivery and camera system are working correctly.

a. Open Video Freeze software, go to experiment set up window, enter experiment ID. Set trial to Cue. Open Cue protocol file. Select chambers. Make sure default settings are entered as follows: Motion Threshold (au): 18; Sample Rate (fps): 30 fps; Method: Linear; Min Freeze Duration (frames): 30. Click OK. Cameras: switch the view to 4.
b. Ensure that all arenas are pushed all the way back inside their chambers and close the doors.
c. Click "Calibrate" and then click "Lock" .
d. Scent the drop pans with 5% acetic acid. Spray the acetic acid solution through the whole pan then with a cotton ball, spread the solution so that it covers the whole pan. Leave the White Light toggle on the REMOTE position.
e. Load mice in their assigned arenas, closing the door immediately. Click the "Record" button.
f. After the 5 minute run is complete, open doors and pull the arenas forward slowly. Beware that animals will be timid, jumpy, and vocal. Cautiously, open the arena door and approach the animal slowly. Place all animals back in their home cages.
g. Thoroughly clean the arenas and drop pans with 10% Nolvasan before the next run. After the last run, remove A-Frame and plastic floors from all arenas and wipe down all items (including arenas and drop pans) with Coverage Plus.
NOTE: because of the lingering smell of acetic acid, there should be a 2day period between sets of testing.

3. CALIBRATIONS

1.0 Camera Calibration for Conditioning and Context Trails

1. Go to File > Chamber Configuration and then double click on specific Camera Id/Box configuration.
2. In Configuration Setup window, click on "Calibrate Camera" button.
3. Ensure that all chambers are pushed all the way back inside their chambers and close doors. Make sure the camera view shows the entire arena.
4. Slide Brightness, Gain and Shutter controls back to 0.
5. Next increase Brightness until a single narrow peak appears in the top left Grayscale Histogram Display.
6. Move the Shutter slider to the right until the right-most edge of the histogram curve is at the right-most edge of the Grayscale Histogram Display.

7. Now, decrease Brightness until the left-most edge of the histogram curve is at the left-most edge of the Grayscale Histogram Display. This will again create a gap at the right edge.
8. Increase the Gain slider until the gap disappears and ensure that Grayscale Histogram doesn't contain "jagged bumps". This is achieved by either decreasing Gain or increasing Shutter the average light intensity should be set for 130 (130.0-130.9).
9. Click "OK" then a Calibration parameters changed box will appear, select "Yes" to save the new calibration settings.
10. Repeat the procedure with each camera.

2.0 Camera Calibration for Cue Trials (performed before each trials)

1. Prior to beginning camera calibration, place A-Frames and plastic floors in all arenas. Switch the White Light toggle to REMOTE. Only the NIR lights are required for "A-Frame" configuration camera calibration.
2. Go to File > Chamber Configuration and then double click on specific Camera Id/Box configuration.
3. In Configuration Setup window, click on "Calibrate Camera" button.
4. Ensure that all chambers are pushed all the way back inside their chambers and close doors. Make sure the camera view shows the entire Fear Conditioning chamber.
5. Increase the brightness all the way up and decrease the shutter until the average light intensity is 130 (130.0-130.9).
6. Click "OK" then a Calibration parameters changed box will appear, select "Yes" to save the new calibration settings.
7. Repeat the procedure with each camera.

3.0 Shock Calibration (performed monthly)

1. Connect ENV-420 box to computer using USB cable and plug the adaptor into wall outlet.
2. Open ENV-420 Amp Meter software. Click on Run switch.
3. Connect black and red crocodile clips to two adjacent wires of the grid floor.
4. On the ENV-414S push down MANUAL OPERATE switch and hold it. ENV420 Amp Meter window should read out the output current in milliamps. It should be set at 0.75 mA.
5. To adjust the output, unlock the Output Current Adjust knob first and turn it around until desired current is reached. Lock it again.
6. Repeat the procedure with each arena.

4.0 Shock Calibration (performed monthly):

1. Connect ANL-929A box to both computer using USB cable and plug the adaptor into wall outlet.
2. Plug the microphone with its holder ENV-269 into the box and position it into the center on the arena.
3. Open ANL-929A-PC USB Microphone software and ensure that it works fine (detects microphone).

4. Open Video Freeze and go to Experiment > Start > … > Sound Calibration. Input Test as Experiment Id and select all 4 boxes under configuration. Click OK.
5. Lock > Record. The sound will come on and the sound intensity will be shown on ANL-929A-PC window. It should read 85 dB at ~ 2800 Hz.
6. If needed, intensity is adjusted by turning the little screw found on the front of a corresponding VFC-100 card.

5.0 Light Calibration (performed monthly):

1. Power on the TR-74Ui Illuminance UV Recorder.
2. Position the two sensors into the center on the arena.
3. Close area door and sound acoustic chamber. The light should read ~200lux.
4. If needed, intensity level is adjusted by turning the little screw found on the front of a corresponding NIR-100V above the #3 dial.