

Fear Conditioning HRWL_FEA_001

Purpose

This procedure is used for measuring aversive learning and memory. A neutral conditioned stimulus (CS) such as steady tone is paired with an aversive unconditioned stimulus (US) such as one or more mild foot shocks. 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.

Experimental Design

- **Minimum number of animals** : 7M + 7F
- **Age at test**: Week 11
- **Sex**: We would expect the results of this test to show sexual dimorphism

Equipment

- Fear conditioning chambers
- Arenas and components for cue and conditioning phases
- Computer with recording software
- Olfactory substance

Procedure

1. Day 1: Conditioning
 - a. Transport animals to the testing area and allow to acclimatise for 30 minutes.
 - b. Set up conditioning arenas in the chambers ensuring that shocker, tone delivery and camera are working correctly.
 - c. Remove the animals from their cages and place in the arenas. Animals are conditioned using the training protocol which consists of a baseline period and a repetition of an audible tone, a single foot shock, and a stimulus-free period three times.
 - d. Remove animals from arenas and place back in home cage. Clean arenas.
2. Day 2: Context
 - a. To be performed 24 hours after conditioning.
 - b. Transport animals to the testing area and allow to acclimatise for 30 minutes.
 - c. Set up conditioning arenas in the chamber as on day 1 except for shocker and tone delivery system which are not used.
 - d. Remove animals from their cages and place in the arena. Freezing is monitored for 5 minutes with no CS or US stimuli.
 - e. Remove animals from arenas and place back in home cage. Clean arenas.

3. Day 2: Cue
 - a. To be performed 4 hours after the context protocol.
 - b. Set up cue arenas using different floors, walls, olfactory cues and / or lighting from the conditioning context. Ensure that tone delivery and camera are working correctly.
 - c. Remove animals from their cages and place in the arena. Animals are exposed to a baseline period without stimuli followed by a repeat of the tone and stimulus-free period three times.
 - d. Remove animals from arenas and place back in home cage. Clean arenas.
4. At the end of the cue protocol animals are placed back into their holding room and data is analyzed by collecting the number of freezing episodes and the total freezing /immobility time.

Parameters and Metadata

Conditioning Baseline Freeze Count HRWL_FEA_001_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: conditioning_baseline_freeze_count

Conditioning Baseline Freezing Time HRWL_FEA_002_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: conditioning_baseline_freezing_time

Conditioning Baseline % Freezing Time HRWL_FEA_003_001 | v1.

1

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: %

Description: conditioning_baseline_freezing_time

Derivation: $\text{mul}(\text{div}(\text{'HRWL_FEA_002_001'}, \text{'HRWL_FEA_027_001'}), 100)$

Conditioning Tone Freeze Count HRWL_FEA_004_001 | v1.0

seriesParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: false

Description: conditioning_tone_freeze_count

Increments: Minimum 1

Conditioning Tone Freezing Time HRWL_FEA_005_001 | v1.0

seriesParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: false

Unit Measured: s

Description: conditioning_tone_freezing_time

Increments: Minimum 1

Conditioning Tone % Freezing Time HRWL_FEA_006_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: false

Unit Measured: %

Description: conditioning_tone_freezing_time

Derivation:

$$\text{mul}(\text{div}(\text{sumOfIncrements}(\text{'HRWL_FEA_005_001'}, \text{'HRWL_FEA_034_001'}), \text{mul}(\text{'HRWL_FEA_030_001'}, \text{'HRWL_FEA_034_001'})), 100)$$

Conditioning Post-shock Freeze Count HRWL_FEA_007_001 | v1.0

seriesParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: conditioning_post_shock_freeze_count

Increments: Minimum 1

Conditioning Post-shock Freezing Time HRWL_FEA_008_001 | v1.

0

seriesParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: conditioning_post_shock_freezing_time

Increments: Minimum 1

Conditioning Post-shock % Freezing Time HRWL_FEA_009_001

| v1.1

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: false

Unit Measured: %

Description: conditioning_post_shock_freezing_time

Derivation:

$$\text{mul}(\text{div}(\text{sumOfIncrements}(\text{'HRWL_FEA_008_001'}, \text{'HRWL_FEA_034_001'}), \text{mul}(\text{'HRWL_FEA_033_001'}, \text{'HRWL_FEA_034_001'})), 100)$$

Context Freeze Count HRWL_FEA_010_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: context_freeze_count

Context Freezing Time HRWL_FEA_011_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: context_freezing_time

Context % Freezing Time HRWL_FEA_012_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: %

Description: context_freezing_time

Derivation: $\text{mul}(\text{div}(\text{'HRWL_FEA_011_001'}, \text{'HRWL_FEA_044_001'}), 100)$

Cue Baseline Freeze Count HRWL_FEA_013_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: cue_baseline_freeze_count

Cue Baseline Freezing Time HRWL_FEA_014_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: cue_baseline_freezing_time

Cue Baseline % Freezing Time HRWL_FEA_015_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: %

Description: cue_baseline_freezing_time

Derivation: $\text{mul}(\text{div}(\text{'HRWL_FEA_014_001'}, \text{'HRWL_FEA_052_001'}), 100)$

Cue Tone Freeze Count HRWL_FEA_016_001 | v1.0

seriesParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: cue_tone_freeze_count

Increments: Minimum 1

Cue Tone Freezing Time HRWL_FEA_017_001 | v1.0

seriesParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: cue_tone_freezing_time

Increments: Minimum 1

Cue Tone % Freezing Time HRWL_FEA_018_001 | v1.3

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: false

Unit Measured: %

Description: cue_tone_freezing_time

Derivation:

$\text{mul}(\text{div}(\text{incrementValue}(\text{'HRWL_FEA_017_001'}, 1), \text{'HRWL_FEA_056_001'}), 100)$

Conditioning: Date and time HRWL_FEA_019_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: conditioning_date_and_time

Conditioning: Room acclimation time HRWL_FEA_020_001 | v1.0

[procedureMetadata](#)

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: min

Description: conditioning_room_acclimation_time

Options: 30,

Conditioning: White house light level in arena HRWL_FEA_021_001 | v1.0

[procedureMetadata](#)

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: Lux

Description: conditioning_white_house_light_level_in_arena

Options: 26,

Conditioning: Olfactory cue HRWL_FEA_022_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: conditioning_olfactory_cue

Options: None,

Conditioning: Mouse arena shape HRWL_FEA_023_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: conditioning_mouse_arena_shape

Options: Square,

Conditioning: Mouse arena dimension HRWL_FEA_024_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: cm

Description: conditioning_mouse_arena_dimension

Options: 16.6 L X 17 W X 25 H cm,

Conditioning: Mouse arena colour HRWL_FEA_025_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: conditioning_mouse_arena_colour

Options: Clear,

Conditioning: Mouse arena floor HRWL_FEA_026_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: conditioning_mouse_arena_floor

Options: Metal grill,

Conditioning: Length of time baseline HRWL_FEA_027_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: conditioning_length_of_time_baseline

Options: 150,

Conditioning: Tone stimulus intensity (dB; conditioned stimulus) HRWL_FEA_028_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Unit Measured: dB

Description: conditioning_tone_stimulus_intensity_db_conditioned_stimulus

Options: 70,

Conditioning: Tone stimulus frequency (Hz; conditioned stimulus) HRWL_FEA_029_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: false

Is Annotated: false

Unit Measured: kHz

Description: conditioning_tone_stimulus_frequency_hz_conditioned_stimulus

Conditioning: Length of time tone stimulus HRWL_FEA_030_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: conditioning_length_of_time_tone_stimulus

Options: 5,

Conditioning: Shock Stimulus intensity (unconditioned stimulus) HRWL_FEA_031_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Unit Measured: mA

Description: conditioning_shock_stimulus_intensity_unconditioned_stimulus

Options: 0.5,

Conditioning: Length of time shock stimulus HRWL_FEA_032_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: conditioning_length_of_time_shock_stimulus

Options: 0.5,

Conditioning: Length of time no stimulus HRWL_FEA_033_001 |

v1.0

[procedureMetadata](#)

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: conditioning_length_of_time_no_stimulus

Options: 150,

Conditioning: No. of stimulus presentations HRWL_FEA_034_001

| v1.0

[procedureMetadata](#)

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Description: conditioning_no_of_stimulus_presentations

Options: 3,

Conditioning: Total time HRWL_FEA_035_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: conditioning_total_time

Options: 616.5,

Context: Date and time HRWL_FEA_036_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: context_date_and_time

Options: Conditioning +24h,

Context: Room acclimation time HRWL_FEA_037_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: min

Description: context_room_acclimation_time

Options: 30,

Context: White house light level in arena HRWL_FEA_038_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: Lux

Description: context_white_house_light_level_in_arena

Options: 26,

Context: Olfactory cue HRWL_FEA_039_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: context_olfactory_cue

Options: None,

Context: Mouse arena shape HRWL_FEA_040_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: context_mouse_arena_shape

Options: Square,

Context: Mouse arena dimension HRWL_FEA_041_001 | v1.0

[procedureMetadata](#)

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: cm

Description: context_mouse_arena_dimension

Options: 16.6 cm L X 17 cm W X 25 cm H,

Context: Mouse arena colour HRWL_FEA_042_001 | v1.0

[procedureMetadata](#)

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: context_mouse_arena_colour

Options: Clear,

Context: Mouse arena floor HRWL_FEA_043_001 | v1.0

[procedureMetadata](#)

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: context_mouse_arena_floor

Options: Metal grill,

Context: Total time HRWL_FEA_044_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: context_total_time

Options: 300,

Cue: Date and time HRWL_FEA_045_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: cue_date_and_time

Options: Context + 4h,

Cue: White house light level in arena HRWL_FEA_046_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: Lux

Description: cue_white_house_light_level_in_arena

Options: 16,

Cue: Olfactory cue HRWL_FEA_047_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: cue_olfactory_cue

Options: Vanillin,

Cue: Mouse arena shape HRWL_FEA_048_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: cue_mouse_arena_shape

Options: Cylinder,

Cue: Mouse arena dimension HRWL_FEA_049_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: cm

Description: cue_mouse_arena_dimension

Options: 20 cm dia X 30 cm H,

Cue: Mouse arena colour HRWL_FEA_050_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: cue_mouse_arena_colour

Options: Black and white,

Cue: Mouse arena floor HRWL_FEA_051_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: false

Is Annotated: false

Description: cue_mouse_arena_floor

Cue: Length of time baseline HRWL_FEA_052_001 | v1.0

[procedureMetadata](#)

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: cue_length_of_time_baseline

Options: 180,

Cue: Tone Stimulus intensity (conditioned stimulus) HRWL_

FEA_053_001 | v1.0

[procedureMetadata](#)

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Unit Measured: dB

Description: cue_tone_stimulus_intensity_conditioned_stimulus

Options: 70,

Cue: Tone stimulus frequency (conditioned stimulus) HRW

L_FEA_054_001 | v1.0

[procedureMetadata](#)

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Unit Measured: kHz

Description: cue_tone_stimulus_frequency_conditioned_stimulus

Cue: Length of time tone stimulus HRWL_FEA_055_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: cue_length_of_time_tone_stimulus

Options: 5,

Cue: Length of time no stimulus HRWL_FEA_056_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: cue_length_of_time_no_stimulus

Options: 55,

Cue: No. of stimulus presentations HRWL_FEA_057_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Description: cue_no_of_stimulus_presentations

Options: 3,

Cue: Total time HRWL_FEA_058_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: cue_total_time

Options: 360,

Mouse arenaID HRWL_FEA_059_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: mouse_arenaid

Options: Arena 1, Arena 2, Arena 3, Arena 4,

Equipment ID HRWL_FEA_060_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: equipment_id

Options: Machine A,

Equipment Manufacturer HRWL_FEA_061_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: equipment_manufacturer

Options: Ugo Basile,

Equipment Model HRWL_FEA_062_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: false

Is Annotated: false

Description: equipment_model

Tracking Method HRWL_FEA_063_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Description: tracking_method

Options: Video tracking,

Software HRWL_FEA_064_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Description: software

Options: Anymaze,

Sound generator manufacturer HRWL_FEA_065_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: sound_generator_manufacturer

Options: Ugo Basile,

Sound generator model HRWL_FEA_066_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: sound_generator_model

Sound-proof box dimension HRWL_FEA_067_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: false

Is Annotated: false

Description: sound_proof_box_dimension

Experimenter ID Day 1 HRWL_FEA_068_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: experimenter_id_day_1

Experimenter ID Day 2 Context HRWL_FEA_069_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: false

Is Annotated: false

Description: experimenter_id_day_2_context

Date equipment last calibrated (shock) HRWL_FEA_070_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: date_equipment_last_calibrated_shock

Date equipment last calibrated (tone) HRWL_FEA_071_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Description: date_equipment_last_calibrated_tone

Freeze definition HRWL_FEA_072_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Unit Measured: s

Description: freeze_definition

Options: 1,

Difference in Context and Conditioning Baseline % Freezing HRWL_FEA_073_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: %

Derivation:

sub(mul(div('HRWL_FEA_011_001', 'HRWL_FEA_044_001'), 100), mul(div('HRWL_FEA_002_001', 'HRWL_FEA_027_001'), 100))

Difference in Cue Tone and Cue Baseline % Freezing HRWL_FEA_074_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: %

Derivation:

sub(mul(div(incrementValue('HRWL_FEA_017_001', 1), 'HRWL_FEA_056_001'), 100), mul(div('HRWL_FEA_014_001', 'HRWL_FEA_052_001'), 100))

Difference in Conditioning Post-shock and Conditioning Baseline % Freezing

HRWL_FEA_075_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: %

Derivation:

```
sub(mul(div(sumOfIncrements('HRWL_FEA_008_001', 'HRWL_FEA_034_001'), mul('HRWL_FEA_033_001', 'HRWL_FEA_034_001')), 100), mul(div('HRWL_FEA_002_001', 'HRWL_FEA_027_001'), 100))
```

Experimenter ID Day 2 Cue

HRWL_FEA_076_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: false

Is Annotated: false
