

# 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:**

mul(div(sumOfIncrements('HRWL\_FEA\_005\_001', 'HRWL\_FEA\_034\_001'), mul('HRWL\_FEA\_030\_001', '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:  
mul(div(incrementValue('HRWL\_FEA\_017\_001', 1), '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

[procedure](#)[Metadata](#)

**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

[procedure](#)[Metadata](#)

**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))

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# Experimenter ID Day 2 Cue

HRWL\_FEA\_076\_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: false

Is Annotated: false

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