Fear Conditioning ICS_FEA_001

Purpose

The fear conditioning is an associative learning paradigm for measuring aversive learning and memory.

In the fear conditioning procedure, a neutral conditioned stimulus (CS) such as light or tone is paired with an aversive unconditioned stimulus (US) such as mild footshock. Concomitantly, animals associate the background context cues with the CS. After conditioning, the CS or the spatial context elicits a central state of fear in the absence of the US, 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 10
- **Sex:** We would expect the results of this test to show sexual dimorphism

Procedure

3.1 Conditioning

Turn on the equipment and check that all components are working correctly

a. Ensure that the CS (light/auditory cue) are connected correctly and deliver the appropriate stimulus (for the tone, 10 kHz, 80dB for 20 s for the ICS).
b. Ensure the shocker is working correctly and set the shock level at 0.4 mA for 1s (for ICS). *The CS co-terminate with the US*.
c. Ensure that activity/freezing detection module is working correctly.

- If using a beam break system, ensure that beams are not interrupted before putting the mouse.

d. Put new sawdust in each cage
e. Animals are transported to the testing room and tested immediately according to the design described bellow. *(only animals to be tested are transported depending on the number of stations available). If more than 1 station is available, test mice from different experimental groups simultaneously, and counterbalance the position between groups.*

f. The mouse is allowed to acclimate for 4 min, then a light/tone (~10 kHz, 80-dB) CS is presented for 20 s and co-terminated by a mild (1 s, 0.4 mA) footshock (US). Animals are left in the cage another 2 minutes, before replacing them in their home cages.
g. At the end of the run take the animals to the housing facility
h. Wipe the apparatus clean and allow time for it to dry
i. Repeat the same procedure for the next runs as described above
j. Once the experiment is finished, clean the equipment.
k. Testing is conducted during the light phase of the cycle with 1 hour gap from the light /dark change.

a. The activity/immobility is recorded as index of baseline and conditioning. *The mouse is considered freezing/immobile when there is a complete lack of movement for at least 2 consecutive seconds.*

> *Males and females must be run in separate tests; ideally males are tested first, then followed by females.*

3.2 Contextual testing

b. Context testing is performed ~24 hours following the conditioning session.
c. Check that all modules are connected properly. Only the hose light is connected, the cues (light/auditory) and shocker are disconnected.
d. Put new sawdust in each cage
e. Animals are placed back into the same chamber that was used for the conditioning and allowed to explore for 6 minutes without presentation of the light/auditory CS.
f. The movement/immobility of the animal is monitored to detect freezing behavior consequent to recognition of the chamber as the spatial context (contextual learning). *T he mouse is considered freezing when there is a complete lack of movement for at least 2 consecutive seconds.*
g. At the end of the run take the animals to the housing facility
h. Wipe the apparatus clean and allow time for it to dry
i. Repeat the same procedure for the next runs as described above
j. Once the experiment is finished, clean the equipment.

1. Cued testing

a. Cue testing is performed ~5 hours after the context testing
b. Animals are tested in a novel test chamber of distinct appearance (wall color, odor and floor texture).
c. Change the contextual environment of the chambers using the cue testing accessories (floor and black walls). The sawdust should be removed and replaced by a tissue paper soaked in lemon juice.

d. Check if the house light, auditory/visual cues and activity detector are working properly *(the shocker is disconnected)*.
e. The mouse is allowed to habituate for 2 minutes then presented with light/auditory cues for 2 minutes. This sequence is repeated once again.

k. The movement of the animal is monitored to detect freezing behavior consequent to cue presentations. *The mouse is considered freezing when there is a complete lack of movement for at least 2 consecutive seconds.*

f. At the end of the run take the animals to the housing facility
g. Wipe the apparatus clean and allow time for it to dry
h. Repeat the same procedure for the next runs as described above
i. Once the experiment is finished, clean the equipment.

Notes

Abbreviation

US = Unconditioned stimulus

CS = Conditioned stimulus

Data Collection

- Duration of immobility/activity is recorded during each testing session.

- Percentage of inactivity (freezing) during re-exposition to the context or to the auditory /visual cues is calculated in blocks of 2 min and used as an index of learning/memory performance.

Data are expressed as mean ± s.e.m. Between groups comparisons are made using repeated measures Anova, unpaired Student's t-test or one factor ANOVA followed by Student-Newmann-Keuls test.

IMPC Parameters (+ontology annotations)

1. Duration of immobility during the first 4 min (presented per 2-min blocks) before presentation of the CS-US, as index of baseline activity before conditioning.

Annotations: hyperactivity (MP:0001399); hypoactivity (MP:0001402)

2. Duration of freezing and percentage of freezing during the context testing session (presented per 2-min blocks) as index of contextual freezing performance.

Annotations: abnormal contextual conditioning (MP: 0001469)

3. Duration of freezing and percentage of freezing during the cue testing session (presented per 2-min blocks) as index of contextual freezing performance.

Annotations: abnormal cued conditioning behaviour (MP:0001454)

Data Analysis, annotation and display (+statistics)

Presentation of data as mean±SEM

Data for evolution over time (to be presented as time bins per 2-min periods) as shown in the graphs bellow.

Statistical analysis of the data using repeated measures ANOVA for parameters which evolve with time. One factor or t-tests for analyzing global parameters or at each time point.

Parameters and Metadata

Freezing duration habituation ICS_FEA_002_001 | v1.6

seriesParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: s

Increments: 1, 2,

Freezing duration post-us ICS_FEA_003_001 | v1.4

seriesParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: s

Increments: 1, 2,

Freezing duration context ICS_FEA_004_001 | v1.2

seriesParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: s

Increments: 1, 2, 3,

Context freezing total ICS_FEA_005_001 | v1.1

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: s

Derivation: meanOfIncrements('ICS_FEA_004_001',1)

% Freezing duration context-1 ICS_FEA_006_001 | v1.3

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: %

Derivation: mul(div(incrementValue('ICS_FEA_004_001',1),120),100)

% Freezing duration context-2 ICS_FEA_007_001 | v1.3

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: %

Derivation: mul(div(incrementValue('ICS_FEA_004_001',2),120),100)

% Freezing duration context-3 ICS_FEA_008_001 | v1.4

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: %

Derivation: mul(div(incrementValue('ICS_FEA_004_001',3),120),100)

% Context freezing total ICS_FEA_009_001 | v1.4

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: %

Derivation:
div(sum(mul(100, div(incrementValue('ICS_FEA_004_001', 1), 120)), mul(100, div(incrementValue('ICS_FEA_004_001', 2), 120)), mul(100, div(incrementValue('ICS_FEA_004_001', 3), 120))), 3)

Freezing duration pre-cue ICS_FEA_010_001 | v1.2

seriesParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: s

Increments: 1, 2,

Freezing duration cue ICS_FEA_011_001 | v1.2

seriesParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: s

Increments: 1, 2,

% Freezing duration cue-1 ICS_FEA_012_001 | v1.2

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: %

Derivation: mul(100, div(incrementValue('ICS_FEA_011_001', 1), 120))

% Freezing duration cue-2 ICS_FEA_013_001 | v1.2

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: %

Derivation: mul(100, div(incrementValue('ICS_FEA_011_001', 2), 120))

Equipment manufacturer ICS_FEA_014_001 | v1.0

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Options: Coulbourn,

Equipment model ICS_FEA_015_001 | v1.0

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Options: Coulbourn polymodal cages,

Experimenter ID ICS_FEA_001_001 | v1.0

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

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% Freezing duration habituation-1 ICS_FEA_016_001 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: %

Derivation: mul(100, div(incrementValue('ICS_FEA_002_001', 1), 120))

% Freezing duration habituation-2 ICS_FEA_017_001 | v1.1

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: %

Derivation: mul(100, div(incrementValue('ICS_FEA_002_001', 2), 120))

% Freezing duration pre-cue-1 ICS_FEA_018_001 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: %

Derivation: mul(100, div(incrementValue('ICS_FEA_010_001', 1), 120))

% Freezing duration pre-cue-2 ICS_FEA_019_001 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: %

Derivation: mul(100, div(incrementValue('ICS_FEA_010_001', 2), 120))

% Cue freezing total ICS_FEA_020_001 | v1.1

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: %

Derivation:
mul(div(add(incrementValue('ICS_FEA_011_001', 1), incrementValue('ICS_FEA_011_001', 2)), 240), 100)

% Pre-cue freezing total ICS_FEA_021_001 | v1.2

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: %

Derivation:
mul(div(add(incrementValue('ICS_FEA_010_001', 1), incrementValue('ICS_FEA_010_001', 2)), 240), 100)
