Fear Conditioning IMPC_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.
   b. Set up conditioning arenas in the chambers ensuring that shocker, tone delivery and camera / beam break system are working correctly. If using video tracking equipment, ensure camera lenses are free of dust.
   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, audible tone, a single foot shock that co-terminates with the tone, and a period with no stimuli at the end.
   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.
   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. No CS or US stimuli are presented.
   e. Remove animals from arenas and place back in home cage. Clean arenas.
3. Day 2: Cue
   a. To be performed performed from 2 - 24 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 / beam break system are working correctly.

c. Remove animals from their cages and place in the arena. Animals are exposed to a baseline period without any stimulus followed by the presentation of the tone.

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** IMPC_FEA_001_001 | v1.0

<table>
<thead>
<tr>
<th>simpleParameter</th>
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<tbody>
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**Description:** conditioning_baseline_freeze_count

**Conditioning Baseline Freezing Time** IMPC_FEA_002_001 | v1.0

<table>
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**Unit Measured:** s

**Description:** conditioning_baseline_freezing_time

**Conditioning Baseline % Freezing Time** IMPC_FEA_003_001 | v1.1

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</table>
Unit Measured: %

Description: conditioning_baseline_freezing_time

Derivation: \( \text{mul}(\text{div}('IMPC\_FEA\_002\_001', 'IMPC\_FEA\_033\_001'), 100) \)

---

**Conditioning Baseline Average Motion Index**  IMPC\_FEA\_004\_001 | v1.0
simpleParameter


Description: conditioning_baseline_average_motion_index

---

**Conditioning Baseline Minimum Motion Index**  IMPC\_FEA\_005\_001 | v1.0
simpleParameter


Description: conditioning_baseline_minimum_motion_index

---

**Conditioning Baseline Maximum Motion Index**  IMPC\_FEA\_006\_001 | v1.0
simpleParameter


Description: conditioning_baseline_maximum_motion_index
Description: conditioning_baseline_maximum_motion_index

Context Freeze Count IMPC_FEA_007_001 | v1.0
simpleParameter


Description: context_freeze_count

Context Freezing Time IMPC_FEA_008_001 | v1.0
simpleParameter


Unit Measured: s

Description: context_freezing_time

Context % Freezing Time IMPC_FEA_009_001 | v1.1
simpleParameter


Unit Measured: %

Description: context_freezing_time

Derivation: \(\text{mul(div('IMPC_FEA_008_001', 'IMPC_FEA_049_001'), 100)}\)
Context Average Motion Index  IMPC_FEA_010_001 | v1.0

simpleParameter

Description: context_average_motion_index

Context Minimum Motion Index  IMPC_FEA_011_001 | v1.0

simpleParameter

Description: context_minimum_motion_index

Context Maximum Motion Index  IMPC_FEA_012_001 | v1.0

simpleParameter

Description: context_maximum_motion_index

Cue Baseline Freeze Count  IMPC_FEA_013_001 | v1.0

simpleParameter
**Cue Baseline Freezing Time**  
IMPC_FEA_014_001 | v1.0

*simpleParameter*

**Description:** cue_baseline_freeze_count

---

**Cue Baseline % Freezing Time**  
IMPC_FEA_015_001 | v1.1

*simpleParameter*

**Unit Measured:** %

**Description:** cue_baseline_freezing_time

**Derivation:** mul(div('IMPC_FEA_014_001', 'IMPC_FEA_057_001'), 100)

---

**Cue Baseline Average Motion Index**  
IMPC_FEA_016_001 | v1.0

*simpleParameter*

**Description:** cue_baseline_freezing_time
Description: cue_baseline_average_motion_index

Cue Baseline Minimum Motion Index  IMPC_FEA_017_001 | v1.0
simpleParameter


Description: cue_baseline_minimum_motion_index

Cue Baseline Maximum Motion Index  IMPC_FEA_018_001 | v1.0
simpleParameter


Description: cue_baseline_maximum_motion_index

Cue Tone Freeze Count  IMPC_FEA_019_001 | v1.0
seriesParameter


Description: cue_tone_freeze_count

Increments: Minimum 1
Cue Tone Freezing Time  IMPC_FEA_020_001  |  v1.0

seriesParameter


Unit Measured: s

Description: cue_tone_freezing_time

Increments: Minimum 1

Cue Tone % Freezing Time  IMPC_FEA_021_001  |  v1.1

simpleParameter


Unit Measured: %

Description: cue_tone_freezing_time

Derivation:
mul(div(sumOfIncrements('IMPC_FEA_020_001',1), 'IMPC_FEA_060_001'), 100)

Cue Tone Average Motion Index  IMPC_FEA_022_001  |  v1.0

seriesParameter


Description: cue_tone_average_motion_index

Increments: Minimum 1
Cue Tone Minimum Motion Index IMPC_FEA_023_001 | v1.0

**seriesParameter**


Description: cue_tone_minimum_motion_index

Increments: Minimum 1

Cue Tone Maximum Motion Index IMPC_FEA_024_001 | v1.0

**seriesParameter**


Description: cue_tone_maximum_motion_index

Increments: Minimum 1

Conditioning: Date and time IMPC_FEA_025_001 | v1.0

**procedureMetadata**


Description: conditioning_date_and_time

Conditioning: Room acclimation time IMPC_FEA_026_001 | v1.0
**Conditioning: White house light level in arena**  
IMPC_FEA_02

**Conditioning: Olfactory cue**  
IMPC_FEA_028_001  
v1.0
Conditioning: Mouse arena shape

Description: conditioning_mouse_arena_shape
Options: Rectangle, Square,

Conditioning: Mouse arena dimension

Unit Measured: cm
Description: conditioning_mouse_arena_dimension
Options: 30.5 L X 24.1 W X 24.5 H cm, 16.6 L X 17 W X 25 H cm, 25 L x 21 W x 19 H cm, 25 x 25 x 19 cm, 17 x 17 x 25 cm, 30.5 cm L X 24.1 cm W X 21 cm H,

Conditioning: Mouse arena colour

Description: conditioning_mouse_arena_colour
Options: Metallic, White and Clear, Clear, White, Metallic, clear,
**Conditioning: Mouse arena floor** IMPC_FEA_032_001 | v1.0

*procedureMetadata*

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

**Description:** conditioning_mouse_arena_floor

**Options:** Metal grill, Clean saw dust, Steel rods,

**Conditioning: Length of time baseline** IMPC_FEA_033_001 | v1.0

*procedureMetadata*

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

**Unit Measured:** s

**Description:** conditioning_length_of_time_baseline

**Options:** 240, 150, 120,

**Conditioning: Tone stimulus intensity (dB; conditioned stimulus)** IMPC_FEA_034_001 | v1.0

*procedureMetadata*

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

**Unit Measured:** dB
**Conditioning: Tone stimulus frequency (Hz; conditioned stimulus)** IMPC_FEA_035_001 | v1.0

- **Description**: conditioning_tone_stimulus_frequency_hz_conditioned_stimulus
- **Options**: 10, 2.8, 4, 2

---

**Conditioning: Length of time tone stimulus** IMPC_FEA_036_001 | v1.0

- **Description**: conditioning_length_of_time_tone_stimulus
- **Options**: 20, 30

---
Conditioning: Shock stimulus intensity (unconditioned stimulus) IMPC_FEA_037_001 | v1.1

procedureMetadata


Unit Measured: mA

Description: conditioning_shock_stimulus_intensity_unconditioned_stimulus

Options: 0.4, 0.5, 0.75,

Conditioning: Length of time shock stimulus IMPC_FEA_038_001 | v1.0

procedureMetadata


Unit Measured: s

Description: conditioning_length_of_time_shock_stimulus

Options: 0.5, 1, 2,

Conditioning: Length of time no stimulus IMPC_FEA_039_001 | v1.0

procedureMetadata


Unit Measured: s
**Description:** conditioning_length_of_time_no_stimulus

**Options:** 120, 150,

---

**Conditioning: Total time**  IMPC_FEA_040_001 | v1.0

**procedureMetadata**

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

**Unit Measured:** s

**Description:** conditioning_total_time

**Options:** 380, 300,

---

**Context: Date and time**  IMPC_FEA_041_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**  IMPC_FEA_042_001 | v1.0

**procedureMetadata**

**Req. Analysis:** false  **Req. Upload:** true  **Is Annotated:** false
Unit Measured: min
Description: context_room_acclimation_time
Options: 30, 60,

Context: White house light level in arena  IMPC_FEA_043_001  |  v1.2
procedureMetadata


Unit Measured: Lux
Description: context_white_house_light_level_in_arena
Options: 26, 100, 200, 40,

Context: Olfactory cue  IMPC_FEA_044_001  |  v1.0
procedureMetadata


Description: context_olfactory_cue
Options: Alcohol, None,

Context: Mouse arena shape  IMPC_FEA_045_001  |  v1.0
procedureMetadata
### Context: Mouse arena shape

**Options:** Rectangle, Square,

---

### Context: Mouse arena dimension

**IMPC_FEA_046_001 | v1.0**

**Unit Measured:** cm

**Description:** context_mouse_arena_dimension

**Options:**
- 30.5 cm L X 24.1 cm W X 24.5 cm H,
- 16.6 cm L X 17 cm W X 25 cm H,
- 25 L x 21 W x 19 H cm,
- 25 x 25 x 19 cm ,
- 17 x 17 x 25 cm,
- 30.5 cm L X 24.1 cm W X 21 cm H,

---

### Context: Mouse arena colour

**IMPC_FEA_047_001 | v1.0**

**Description:** context_mouse_arena_colour

**Options:** Metallic, White and Clear, Clear, White, Metallic, clear,

---

### Context: Mouse arena floor

**IMPC_FEA_048_001 | v1.0**
procedureMetadata

**Context: Total time** IMPC_FEA_049_001 | v1.0

*Description:* context_total_time

*Options:* 360, 300,

---

**Cue: Date and time** IMPC_FEA_050_001 | v1.0

*Description:* cue_date_and_time

*Options:* Context + 2-4h, Context + 4h, Context + 24h,

---

**Cue: White house light level in arena** IMPC_FEA_051_001 | v1.1
**Unit Measured:** Lux

**Description:** cue_white_house_light_level_in_arena

**Options:** 0, 16, 14-18,

---

### Cue: Olfactory cue IMPC_FEA_052_001 | v1.0

**Description:** cue_olfactory_cue

**Options:** 5% acetic acid, Vanillin, Cinnamon,

---

### Cue: Mouse arena shape IMPC_FEA_053_001 | v1.0

**Description:** cue_mouse_arena_shape

**Options:** A frame ceiling, Cylinder, Rectangle,

---

### Cue: Mouse arena dimension IMPC_FEA_054_001 | v1.0
**procedureMetadata**

**Unit Measured:** cm

**Description:** cue_mouse_arena_dimension

**Options:**
- 30.5 cm L x 24.1 cm W x 24.5 cm H,
- 20 cm dia x 30 cm H,
- 25 L x 21 W x 19 H cm,
- 25 x 25 x 19 cm,
- 17 x 17 x 25 cm,
- 30.5 cm L x 24.1 cm W x 21 cm H,

---

**Cue: Mouse arena colour** IMPC_FEA_055_001 | v1.0

**procedureMetadata**

**Description:** cue_mouse_arena_colour

**Options:** Black and white, Red, Clear, Beige, black and white,

---

**Cue: Mouse arena floor** IMPC_FEA_056_001 | v1.0

**procedureMetadata**

**Description:** cue_mouse_arena_floor

**Options:** PVC, Plexi glass, Polyethylene, Perspex,
**Cue: Length of time baseline**  IMPC_FEA_057_001 | v1.0

*procedureMetadata*

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Unit Measured: s

Description: cue_length_of_time_baseline

Options: 120,

**Cue: Tone stimulus intensity (conditioned stimulus)**  IMPC_FEA_058_001 | v1.1

*procedureMetadata*

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Unit Measured: dB

Description: cue_tone_stimulus_intensity_conditioned_stimulus

Options: 85, 80, 75, 77, 70,

**Cue: Tone stimulus frequency (Hz; conditioned stimulus)**  IMPC_FEA_059_001 | v1.0

*procedureMetadata*

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Unit Measured: kHz

Description: cue_tone_stimulus_frequency_hz_conditioned_stimulus
**Cue: tone response measurement time** IMPC_FEA_060_001 | v1.0

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

**Unit Measured:** s  
**Options:** 120, 180,

**Cue: Length of time no stimulus** IMPC_FEA_061_001 | v1.0

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

**Unit Measured:** s  
**Description:** cue_length_of_time_no_stimulus  
**Options:** 120, 0, 220,

**Cue: Total time** IMPC_FEA_062_001 | v1.0

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

**Unit Measured:** s
Description: cue_total_time
Options: 480, 300, 420, 240,

Mouse arenaID IMPC_FEA_063_001 | v1.0
procedureMetadata


Description: mouse_arenaid
Options: Arena 1, Arena 2, Arena 3, Arena 4, Arena 5, Arena 6, Arena 7, Arena 8,

Equipment ID IMPC_FEA_064_001 | v1.0
procedureMetadata


Description: equipment_id
Options: Machine A, Machine B, Machine 1, Machine 2,

Equipment Manufacturer IMPC_FEA_065_001 | v1.0
procedureMetadata


Description: equipment_manufacturer
Options: Med Assoicates, Ugo Basile, San Diego Instruments,

Equipment Model | equipment_model | IMPC_FEA_066_001 | v1.0

Description: equipment_model
Options: MED-FVC-SCT-M, Freeze monitor, FearCS Mouse-46152, MED-VFC2-SCT-M, 46000, MED-VFC-NIR-M SOP #1, MED-VFC-NIR-M SOP #2, MED-VFC-NIR-M SOP #3,

Tracking Method | tracking_method | IMPC_FEA_067_001 | v1.0

Description: tracking_method
Options: Video tracking, Beam break,

Software | software | IMPC_FEA_068_001 | v1.0

Description: software
**Sound generator manufacturer**  IMPC_FEA_069_001 | v1.0

**Description:** sound_generator_manufacturer

**Options:** Med Associates, Ugo Basile, San Diego Instruments,

---

**Sound generator model**  IMPC_FEA_070_001 | v1.0

**Description:** sound_generator_model

**Options:** VFC-100, other, 46000-165,

---

**Sound-proof box dimension**  IMPC_FEA_071_001 | v1.0

**Description:** sound_proof_box_dimension

**Options:** 71 cm L X 63.5 cm W X 35.5 cm H, 71 cm L X 59.7 cm W X 31.7 cm H, other, 47 cm L X 35 cm W X 49 cm H,
Experiment ID | IMPC_FEA_072_001 | v1.0
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procedureMetadata


Description: experimenter_id

Experiment ID day 2 | IMPC_FEA_073_001 | v1.0
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procedureMetadata


Description: experimenter_id_day_2

Experiment ID day 2 cue | IMPC_FEA_074_001 | v1.0
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procedureMetadata


Description: experimenter_id_day_2_cue

Date equipment last calibrated (shock) | IMPC_FEA_075_001 | v1.0
**Date equipment last calibrated (tone)**  
IMPC_FEA_076_001 | v1.0

**Freeze definition**  
IMPC_FEA_077_001 | v1.1

**Conditioning: Near infra red light arena**  
IMPC_FEA_078_001 | v1.0
**Context: Near infra red light arena**  
IMPC_FEA_079_001 | v1.0

**Cue: Near infra red light arena**  
IMPC_FEA_080_001 | v1.0

**Conditioning: Start time of baseline**  
IMPC_FEA_081_001 | v1.0
Conditioning: Start time of each tone stimulus  IMPC_FEA_0

Unit Measured: s
Description: conditioning_start_time_of_baseline

Conditioning: Start time of each shock stimulus  IMPC_FEA_083_001 | v1.0

Unit Measured: s
Description: conditioning_start_time_of_each_shock_stimulus

Conditioning: Start time of no stimulus  IMPC_FEA_084_001 | v1.0

Unit Measured: s
Description: conditioning_start_time_of_each_shock_stimulus
Cue: Start time of baseline  IMPC_FEA_085_001 | v1.0

**Description:** conditioning_start_time_of_no_stimulus

---

Cue: Start time of each tone stimulus  IMPC_FEA_086_001 | v1.0

**Description:** cue_start_time_of_each_tone_stimulus

---

Cue: Start time of no stimulus  IMPC_FEA_087_001 | v1.0

**Description:** cue_start_time_of_no_stimulus

---
Unit Measured: s
Description: cue_start_time_of_no_stimulus

Cue: arena rotation  IMPC_FEA_088_001 | v1.0

procedureMetadata


Description: cue_arena_rotation

Options: 90 degrees,

Conditioning Tone Freeze Count  IMPC_FEA_089_001 | v1.0

simpleParameter


Description: conditioning_tone_freeze_count

Conditioning Tone Freezing Time  IMPC_FEA_090_001 | v1.0

simpleParameter


Unit Measured: s
Description: conditioning_tone_freezing_time
### Conditioning Tone % Freezing Time
**IMPC_FEA_091_001** | v1.1

- **simpleParameter**

- **Unit Measured:** %
- **Description:** conditioning_tone_freezing_time
- **Derivation:** `mul(div('IMPC_FEA_090_001', 'IMPC_FEA_036_001'), 100)`

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### Conditioning Tone Average Motion Index
**IMPC_FEA_092_001** | v1.0

- **simpleParameter**

- **Description:** conditioning_tone_average_motion_index

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### Conditioning Tone Minimum Motion Index
**IMPC_FEA_093_001** | v1.0

- **simpleParameter**

- **Description:** conditioning_tone_minimum_motion_index
### Conditioning Tone Maximum Motion Index

**IMPC_FEA_094_001**

- **v1.0**
- **simpleParameter**

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

**Description:** conditioning_tone_maximum_motion_index

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### Conditioning Shock Average Motion Index

**IMPC_FEA_095_001**

- **v1.0**
- **simpleParameter**

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

**Description:** conditioning_shock_average_motion_index

---

### Conditioning Shock Minimum Motion Index

**IMPC_FEA_096_001**

- **v1.0**
- **simpleParameter**

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

**Description:** conditioning_shock_minimum_motion_index
**Conditioning Shock Maximum Motion Index** IMPC_FEA_097_001 | v1.0

simpleParameter

**Description:** conditioning_shock_maximum_motion_index

---

**Conditioning Post-shock Freeze Count** IMPC_FEA_098_001 | v1.0

simpleParameter

**Description:** conditioning_post_shock_freeze_count

---

**Conditioning Post-shock Freezing Time** IMPC_FEA_099_001 | v1.0

simpleParameter

**Unit Measured:** s

**Description:** conditioning_post_shock_freezing_time
Conditioning Post-shock % Freezing Time  IMPC_FEA_100_001
| v1.1

simpleParameter


Unit Measured: %

Description: conditioning_post_shock_freezing_time

Derivation: \( \text{mul}(\text{div}('IMPC_FEA_099_001', 'IMPC_FEA_039_001'), 100) \)

Conditioning Post-shock Average Motion Index  IMPC_FEA_101_001 | v1.0

simpleParameter


Description: conditioning_post_shock_average_motion_index

Conditioning Post-shock Minimum Motion Index  IMPC_FEA_102_001 | v1.0

simpleParameter


Description: conditioning_post_shock_minimum_motion_index
**Conditioning Post-shock Maximum Motion Index**  IMPC_FE
A_103_001 | v1.0

simpleParameter


Description: conditioning_post_shock_maximum_motion_index

---

**Freeze definition (minimum motion index)**  IMPC_FEA_104_001
| v1.0

procedureMetadata


Description: freeze_definition_minimum_motion_index

Options: 18, 30,

---

**Difference in Context and Conditioning Baseline % Freezing**  IMPC_FEA_105_001 | v1.0

simpleParameter


Derivation:
sub(mul(div('IMPC_FEA_008_001', 'IMPC_FEA_049_001'), 100), mul(div('IMPC_FEA_002_001', 'IMPC_FEA_033_001'), 100))
Difference in Cue Tone and Cue Baseline % Freezing

\[
\text{ImpC_FEA_106_001} | \text{v1.0}
\]

**simpleParameter**

**Req. Analysis:** false  \hspace{1cm} **Req. Upload:** false  \hspace{1cm} **Is Annotated:** true

**Derivation:**
\[
\text{sub(mul(div('IMPC_FEA_020_001', 'IMPC_FEA_060_001'), 100), mul(div('
\text{IMPC_FEA_014_001}', 'IMPC_FEA_057_001'), 100))}
\]

---

Difference in Conditioning Post-shock and Conditioning Baseline % Freezing

\[
\text{IMPC_FEA_107_001} | \text{v1.0}
\]

**simpleParameter**

**Req. Analysis:** false  \hspace{1cm} **Req. Upload:** false  \hspace{1cm} **Is Annotated:** true

**Derivation:**
\[
\text{sub(mul(div('IMPC_FEA_099_001', 'IMPC_FEA_039_001'), 100), mul(div('
\text{IMPC_FEA_002_001}', 'IMPC_FEA_033_001'), 100))}
\]

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Cue: Length of time tone stimulus

\[
\text{IMPC_FEA_108_001} | \text{v1.0}
\]

**procedureMetadata**

**Req. Analysis:** true  \hspace{1cm} **Req. Upload:** false  \hspace{1cm} **Is Annotated:** false

**Unit Measured:** s

**Options:** 20, 120,