# Acoustic Startle and Pre-pulse Inhibition (PPI) IMPC_ACS_002

## Purpose

The acoustic startle response is characterized by an exaggerated flinching response to an unexpected strong auditory stimulus (pulse). This response can be attenuated when it is preceded by a weaker stimulus (pre-pulse) and is the principle underlying pre-pulse inhibition (PPI). PPI has been described in numerous species, including mice and humans and provides an operational measure of sensorimotor gating reflecting the ability of an animal to successfully integrate and inhibit sensory information. Several clinical studies have shown that a number of human disorders have impaired PPI including: schizophrenia, Huntington's disease, fragile X syndrome, and autism. The acoustic startle and PPI paradigm is therefore largely used to assess sensorimotor gating and the effects of a number of treatment modalities such as putative anti-psychotics, and to explore genetic and neurobiological mechanisms underlying behaviors of relevance to psychosis (Geyer, 1999; Ouagazzal et al., 2001).

Ontological description: MP:0002067 - abnormal sensory capabilities/reflexes/nociception.

## 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

## Equipment

The experimental apparatus consists of an outer attenuated chamber that serves to prevent external noise or vibrations interfering with experiment. Within this chamber a load cell platform that records the startle response is linked to the transducer and amplifier, which calibrates the load cell platform. An animal holder rests upon the load cell platform. A sound generator and the appropriate software regulate pulses from the amplifier.

## Procedure

1. Transport mice (in their rack if possible) to the testing suite and leave undisturbed for a minimum of 30 minutes in the antechamber. Take care not to stimulate the mouse before starting the experiment. Do not change the cage on the day of the experiment.

2. The session is initiated with a 5 minute acclimation period (only background noise is on). In addition, it is an option to acclimate to the startle pulse in which 110-120 dB/40-60ms of white noise is presented alone, 5 times. These will be excluded from the statistical analysis.
3. The session is then continued by presentations of different trial types, each of which should be presented 6-10 times in pseudorandom order, with an inter-trial interval (ITI) varying randomly between 20 and 30 seconds (or 10 and 20 if preferred). The trials are:

a. Different pre-pulse trials of 20 ms duration of white noise stimuli which are presented alone (PP1, PP2, PP3, or PP4 dB; a minimum of 3 different pre-pulses should be utilized) or precede the pulse by 50-120 ms (PP1 + pulse, PP2 + pulse, PP3 + pulse, or PP4 + pulse) to derive the pre-pulse inhibition response. The intensities of the pre-pulse should be kept at levels above the background noise (BN) that do not elicit a significant startle response on their own, being approximately 2-20dB above BN (e.g. PP1=BN+5dB, PP2=BN+10, PP3=BN+15 and PP4=BN+20).
b. Startle pulse trials where 110-120 dB/40-60 ms of white noise is presented alone.
c. No stimulus (NOSTIM) trials in which only background noise is presented to measure baseline movement of the animal in the chamber.
d. The BN set will vary according to the apparatus used (generally around 65-70dB depending on the noise of the environment).

4. Startle response is recorded every millisecond for 65-100 ms after the onset of startle, i. e. 40-60 ms during the startle plus 25-40 ms after the startle ended; 65-100 ms from the end of the previous ITI for NOSTIM (see Fig. 1).

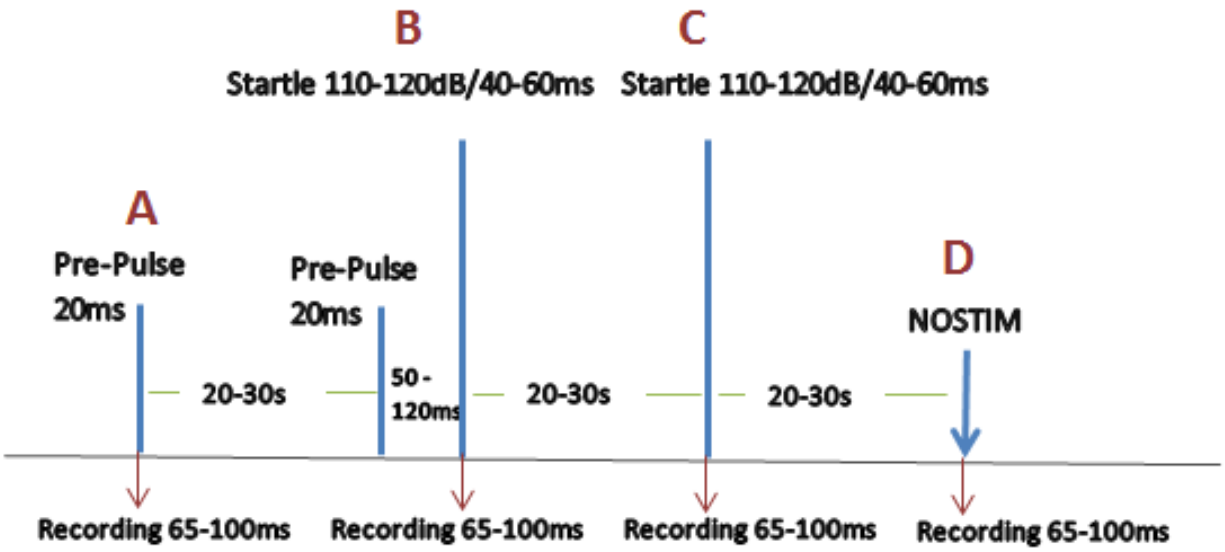


Fig.1. The different type of trials of the acoustic startle & pre-pulse inhibition test. A: pre-pulse alone (PP1, PP2, PP3 and PP4), B: startle preceded by pre-pulse (PP1_S, PP2_S, PP3_S and PP4_S), C: startle alone and D: NOSTIM.

5. Ensure that all apparatus are functioning correctly
6. Place each mouse onto the load cell platform inside the sound attenuated acoustic chamber and secure the door close.
7. Run the experimental session according to the experimental design described above.
8. Remove each mouse at the end of the experimental session and record its weight before returning it to the relevant home cage.
9. Wipe clean the animal holders and allow time to dry before loading another test cohort.
10. At the end of the experimentation, save the data for detailed analysis of acoustic startle and acoustic pre-pulse inhibition responses.

**Data collection.** The maximal peak amplitude is used to determine the acoustic startle response. Basal startle responses S and PP-S, are calculated respectively as the average responses to the pulses presented alone and the average responses to the combined pre-pulse-pulses. The amount of pre-pulse inhibition (PPI) is calculated as a percentage score for each acoustic pre-pulse trial type:  % PPI= 100 x (S – PPi_S)/S.  The global level of PPI is also calculated as the mean %PPI for the different prepulse responses: 100 x [S?(PP1_S + PP2_S + PP3_S + PP4_S)/4]/S.

# Notes

Illumination and noise levels in the holding room should be comparable to the housing suite during acclimation and testing to minimize their effects on behavioral outcome.

The maximal voltage change is to be used as the startle response over the recording interval. The background noise is on throughout the experiment and therefore between the prepulse and the startle.

The information about the date of the experiment, that is the date when the measurement is performed, is an important parameter which is to be submitted in the Experiment xml file (dateOfExperiment="2013-02-28").

## Data QC

The calibration of the sound and the movement sensors are important for obtaining valid test results and therefore must be routinely calibrated, e.g. at least monthly. Each depends on the type of equipment used therefore follow manufacturer guidelines for effective calibration and provide that the sensitivity of the instrument be high enough to avoid inaccuracy on measures of prepulse inhibition.  It is recommended that the maximum signal peak of the startle response with a standard unit (e.g. male mouse C57) be in a range at about 800-1200 (higher is not a problem).

**Metadata With Example Values**

| Metadata | Example |
| --- | --- |
| Light level in chamber | The amount of light in the sound attenuated chamber. E.g. 70 (lx) |
| Date and time | Day and time the mice were tested. |
| Startle stimulus | The intensity of the startle pulse. E.g. 110 (dB). |
| Background noise | The intensity of the background noise. E.g. 65 (dB). |
| Pre-pulse stimulus 1 (db) | The intensity of the PP1 pulse. E.g. 70 (dB). |
| Pre-pulse stimulus 2 (db) | The intensity of the PP2 pulse. E.g. 75 (dB). |
| Pre-pulse stimulus 3 (db) | The intensity of the PP3 pulse. E.g. 80 (dB). |
| Pre-pulse stimulus 4 (db) | The intensity of the PP4 pulse. E.g. 85 (dB). |
| Inter PP-S stimulus interval | Time between the pre-pulse and the startle stimulus. E.g. 50 ms. |
| Inter-trial interval | Time between each trial. Can be a fixed value or a random value in a fixed range. E.g. Random 10-20 sec (if stimulus order is random), or 20 sec. |
| Number of trials | Number of repetition for each different type of trial. E. g. 6. |
| In-chamber adapt time | Duration of the in-chamber acclimation period with only background noise on. E.g. 300 sec. |
| Stimulus order | The order in which the trials are presented. E.g. Pseudo random. |

| | |
|---|---|
| Mouse chamber ID | ID of the chamber used when more than 1 is used. E. g. chamber 1. |
| Equipment ID | ID of the machine used when more than 1 is used  having same model and manufacturer. E.g. machine 1, machine 2, machine Minnie, machine Mickey Mouse, etc. |
| Equipment Manufacturer | Manufacturer of the equipment. E.g. San Diego Instruments. |
| Equipment Model | Model of the equipment. E.g. SR-LAB Startle Response System. |
| Software version | Version of the software used to generate the pulses and collect the responses. Animal Startle SR-9020 |
| Mouse chamber dimension | The internal diameter of the attenuated chamber.<br><br>E.g. 3 (W, cm) x 3.5 (ID, cm). |
| Sound generator manufacturer | Manufacturer of the sound generator. E.g. San Diego Instruments. |
| Sound generator model | Model of the sound generator. E.g. SR-LAB Startle Response System. |
| Sound-proof box dimension | Sound-proof box dimension.<br><br>E.g. 33 (L, cm) x 43 (W, cm) x 33 (H, cm) |
| Experimenter ID | An ID of any format to be used coherently both inside the same procedure and for all procedures. E.g. Harw_001, or 1/2/3. |
| Date equipment last calibrated | Most recent date in which the equipment (or any part of) used in the procedure was subject to a calibration event. |

## Parameters and Metadata

### Response amplitude - BN IMPC_ACS_001_001 | v1.5

simpleParameter

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

**Description:** response_amplitude_bn

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### Response amplitude - PP1 IMPC_ACS_002_001 | v1.4

simpleParameter

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

**Description:** response_amplitude_pp1

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### Response amplitude - PP2 IMPC_ACS_003_001 | v1.5

simpleParameter

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

**Description:** response_amplitude_pp2

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## Response amplitude - PP3 IMPC_ACS_004_001 | v1.4

simpleParameter

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

**Description:** response_amplitude_pp3

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## Response amplitude - PP4 IMPC_ACS_005_001 | v1.5

simpleParameter

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

**Description:** response_amplitude_pp4

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## Response amplitude - S IMPC_ACS_006_001 | v1.4

simpleParameter

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

**Description:** response_amplitude_s

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## Response amplitude - PP1_S IMPC_ACS_007_001 | v1.5

simpleParameter

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

**Description:** response_amplitude_pp1_s

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## Response amplitude - PP2_S IMPC_ACS_008_001 | v1.5

simpleParameter

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

**Description:** response_amplitude_pp2_s

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## Response amplitude - PP3_S IMPC_ACS_009_001 | v1.5

simpleParameter

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

**Description:** response_amplitude_pp3_s

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## Response amplitude - PP4_S IMPC_ACS_010_001 | v1.6

simpleParameter

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

**Description:** response_amplitude_pp4_s

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## Light level in chamber IMPC_ACS_011_001 | v1.0

procedureMetadata

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

**Unit Measured:** Lux

**Description:** light_level_in_chamber

**Options:** 70, 60, 360, 400, 2, 95, 0, 150,

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## Experimenter ID IMPC_ACS_014_001 | v1.0

procedureMetadata

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

**Description:** experimenter

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## Startle stimulus IMPC_ACS_015_001 | v1.2

procedureMetadata

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

**Unit Measured:** dB

**Description:** startle_pulse

**Options:** 110, 120,

## Background noise IMPC_ACS_016_001 | v1.1

procedureMetadata

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

**Unit Measured:** dB

**Description:** background_noise

**Options:** 70, 65, 50, 53,

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## Pre-pulse stimulus 1 IMPC_ACS_017_001 | v1.1

procedureMetadata

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

**Unit Measured:** dB

**Description:** pre_pulse_stimulus_1

**Options:** 74, 67, 70, 55, 56,

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## Pre-pulse stimulus 2 IMPC_ACS_018_001 | v1.1

procedureMetadata

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

**Unit Measured:** dB

**Description:** pre_pulse_stimulus_2

**Options:** 69, 75, 78, 80, 65, 82, 58, 77,

---

## Pre-pulse stimulus 3 IMPC_ACS_019_001 | v1.1

procedureMetadata

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

**Unit Measured:** dB

**Description:** pre_pulse_stimulus_3

**Options:** 82, 73, 80, 85, 70, 90, 65,

---

## Pre-pulse stimulus 4 IMPC_ACS_020_001 | v1.2

procedureMetadata

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

**Unit Measured:** dB

**Description:** pre_pulse_stimulus_4

**Options:** 90, 85, 81, 75,

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## Inter-trial interval IMPC_ACS_021_001 | v1.3

procedureMetadata

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

**Unit Measured:** s

**Description:** inter_trial_interval

**Options:** random 20-30, random 10-20, 50, 25,

---

## Number of trials IMPC_ACS_022_001 | v1.0

procedureMetadata

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

**Description:** number_of_trials

**Options:** 48, 105, 6, 10, 90, 80, 60, 8,

---

## In-chamber adapt time IMPC_ACS_023_001 | v1.1

procedureMetadata

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

**Unit Measured:** s

**Description:** in_chamber_adapt_time

**Options:** 5, 300,

## Stimulus order IMPC_ACS_024_001 | v1.0

procedureMetadata

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

**Description:** stimulus_order

**Options:** Pseudo-random, Random,

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## Mouse chamber ID IMPC_ACS_025_001 | v1.1

procedureMetadata

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

**Description:** mouse_chamber_id

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## Equipment ID IMPC_ACS_026_001 | v1.0

procedureMetadata

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

**Description:** equipment_name

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## Equipment manufacturer IMPC_ACS_027_001 | v1.0

procedureMetadata

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

**Description:** equipment_manufacturer

**Options:** Med Associates Inc., VT, USA, San Diego Instruments, O'hara Co. Ltd.,

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## Equipment model IMPC_ACS_028_001 | v1.0

procedureMetadata

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

**Description:** equipment_model

**Options:** Acoustic startle chambers MED-ASR-PRO1, SR-LAB Startle Response System, SR-4020, Med Associates Acoustic Startle Model - Startle Cubicle (ENV 022S), Med Associates Acoustic Startle Model - Startle Cubicle (ENV 022S) (reconfigured),

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## Software version IMPC_ACS_029_001 | v1.1

procedureMetadata

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

**Description:** software_version

**Options:** Med Associates Startle Reflex Vers. 6.00, SR LAB software, Animal Startle SR-9020, Med Associates Startle Reflex Vers. 5.94, Med Associates Startle Reflex Vers. 6.01,

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## Mouse chamber dimension IMPC_ACS_030_001 | v1.2

procedureMetadata

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

**Unit Measured:** cm

**Description:** mouse_chamber_dimension

**Options:** 3 x 3.5, 4 x 9, 12 x 3.8, 7.5 x 3.0, 3.8 x 7, 6 x 6 x 4.8,

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## Sound generator manufacturer IMPC_ACS_031_001 | v1.1

procedureMetadata

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

**Description:** sound_generator

**Options:** Med Associates Inc., VT, USA, San Diego Instruments, O'hara Co. Ltd.,

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## Sound-proof box dimension IMPC_ACS_032_001 | v1.2

procedureMetadata

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

**Unit Measured:** cm

**Description:** sound_proof_box_dimension

**Options:** 38.1 x 35.6 x 45.7, 33 x 43 x 33, 28.5 x 28.5 x 30, 50.8 x 33 x 30.5cm, 32.2 x 34.5 x 46.3, 63.5 x 40 x 42,

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## % Pre-pulse inhibition - PPI1 IMPC_ACS_033_001 | v1.7

simpleParameter

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

**Unit Measured:** %

**Description:** _pre_pulse_inhibition_pp1

**Derivation:**
mul(div(sub('IMPC_ACS_006_001', 'IMPC_ACS_007_001'), 'IMPC_ACS_006_001'), 100)

---

## % Pre-pulse inhibition - PPI2 IMPC_ACS_034_001 | v1.7

simpleParameter

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

**Unit Measured:** %

**Description:** _pre_pulse_inhibition_pp2

**Derivation:**
mul(div(sub('IMPC_ACS_006_001', 'IMPC_ACS_008_001'), 'IMPC_ACS_006_001'), 100)

---

## % Pre-pulse inhibition - PPI3 IMPC_ACS_035_001 | v1.7

simpleParameter

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

**Unit Measured:** %

**Description:** _pre_pulse_inhibition_pp3

**Derivation:**
mul(div(sub('IMPC_ACS_006_001', 'IMPC_ACS_009_001'), 'IMPC_ACS_006_001'), 100)

---

## % Pre-pulse inhibition - PPI4 IMPC_ACS_036_001 | v1.8

simpleParameter

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

**Unit Measured:** %

**Description:** _pre_pulse_inhibition_pp4

**Derivation:**
mul(div(sub('IMPC_ACS_006_001', 'IMPC_ACS_010_001'), 'IMPC_ACS_006_001'), 100)

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## % Pre-pulse inhibition - Global IMPC_ACS_037_001 | v1.6

simpleParameter

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

**Unit Measured:** %

**Description:** _pre_pulse_inhibition_global

**Derivation:**
ifElse(equals('IMPC_ACS_020_001', null),mul(div(sub('IMPC_ACS_006_001', div(sum ('IMPC_ACS_007_001', 'IMPC_ACS_008_001', 'IMPC_ACS_009_001'), 3)), 'IMPC_ACS_006_001'), 100),mul(div(sub('IMPC_ACS_006_001', div(sum ('IMPC_ACS_007_001', 'IMPC_ACS_008_001', 'IMPC_ACS_009_001', 'IMPC_ACS_010_001'), 4)), 'IMPC_ACS_006_001'), 100))

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## Date equipment last calibrated IMPC_ACS_038_001 | v1.2

procedureMetadata

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

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## Inter PP-S stimulus interval IMPC_ACS_013_001 | v1.0

procedureMetadata

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

**Unit Measured:** ms

**Options:** 50, 120, 100, 80,

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# Sound generator model IMPC_ACS_039_001 | v1.0

procedureMetadata

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

**Options:** PHM 255A, SR-LAB Startle Response System, SR1040, SR-LAB CONTROL SRC000444, SR-LAB CONTROL SRC000495,