\*Title: **Acoustic startle and pre-pulse inhibition**

\*Centre: IMPC

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{Sections:}

## \*1. Purpose:

The acoustic startle response is characterized by an exaggerated flinching response to an unexpected strong auditory stimulus (pre-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.

## \*2. Experimental Design

Minimum number of mutant animals: 7 mice for each sex.

Age of animal at test: over 8 weeks 8 weeks, but not greater than 12 weeks, ideal age: 10 weeks.

Sexual dimorphism: yes, the results of this test tend to be sexually dimorphic.

## \*3. 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.

## \*4. 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. Switch on the computer and set-up the experimental design to capture acoustic startle and acoustic pre-pulse inhibition in a single session, as described below.
3. 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.
4. 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 intertrial interval (ITI) varying randomly between 20 and 30 seconds (or 10 and 20 if preferred). The trials are:
5. 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 80-120 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 2-20 dB above BN (e.g. PP1=BN+5dB, PP2=BN+10, PP3=BN+15 and PP4=BN+20). At least three of these are needed for the test.
6. Startle pulse trials where 110-120 dB/40-60 ms of white noise is presented alone.
7. No stimulus (NOSTIM) trials in which only background noise is presented to measure baseline movement of the animal in the chamber.
8. The BN set will vary according to the apparatus used (generally around 65-70dB depending on the noise of the environment).
9. 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).

**B**

**C**

Startle 110-120dB/40-60ms

Startle 110-120dB/40-60ms

**A**

**D**

Pre-Pulse 20ms

NOSTIM

Pre-Pulse 20ms

20-30sec

80 -120ms

20-30sec

20-30sec

Recording 65-100ms

Recording 65-100ms

Recording 65-100ms

Recording 65-100ms

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.

1. Ensure that all apparatus are functioning correctly.
2. Place each mouse onto the load cell platform inside the sound attenuated acoustic chamber and secure the door close.
3. Load additional mice for the experimental session in the same way ensuring that the identification number of each mouse and the chamber number in which it is placed are noted.
4. Run the experimental session according to the experimental design described above.
5. Remove each mouse at the end of the experimental session and record its weight before returning it to the relevant home cage.
6. Wipe clean the animal holders and allow time to dry before loading another test cohort.
7. 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-to-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 – PP-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.

## \*5. 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.

### Data QC

The calibration of the sound and the movement sensors are important for obtaining valid test results and therefore must be routinely calibrated. Each depends on the type of equipment used therefore follow manufacturer guidelines for effective calibration.

1. **Sound calibration:** This can be completed within each of the startle chambers by applying varying sound intensities to ensure that the noise/tone intensity in decibels (dB) is accurate. The measurement should display the actual frequency of tone/frequency spectrum of white noise in order to obtain exact information about the precision of the tone/composition of the noise. Calibration of noise (white noise or pure tone signals) for Med Associate startle chambers is performed by placing a microphone within the Plexiglas cylinder in which the mouse is placed for acoustic startle and PPI assessment. Startle chambers from San Diego Instruments use a sound level meter to calibrate noise intensities.
2. **Movement sensor calibration:** The startle chambers amplifiers of the single load cell platforms are calibrated to attain a comparable startle magnitude across the device (Med Associates Inc.), by positioning different weights (0, 40 and 200 grams) in the center of the load cell platform and measuring their output with a voltage meter. The amplifier is balanced to 0V (0 gram weight) or adjusted to 2V and 10V (for 40 and 200 gram weights). Calibration of the startle enclosure for San Diego Instruments is carried out using a standardization unit with the unit force set at 750 ± 10 for the mouse.

### Metadata With Example Values

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| --- | --- | --- | --- |
| **Metadata** | **Example** | **Required for data upload** | **Required for data analysis** |
| Light level in chamber (lx) | 60-70 | NO | NO |
| Date and time |  | YES | NO |
| Startle stimulus (db) | 110 | YES | YES |
| Background noise (db) | 65 | YES | YES |
| Pre-pulse stimulus 1 (db) | 70 | YES | YES |
| Pre-pulse stimulus 2 (db) | 75 | YES | YES |
| Pre-pulse stimulus 3 (db) | 80 | YES | YES |
| Pre-pulse stimulus 4 (db) | 85 | YES | YES |
| Intertrial interval | 10-20 sec., (or 20-30 sec.) | YES | YES |
| Number of trials | 6 | YES | YES |
| In-chamber adapt time | 300 sec | YES | YES |
| Stimulus order | Pseudo random | YES | YES |
| Mouse chamber ID |  | NO | NO |
| Equipment ID |  | YES | NO |
| Equipment Manufacturer | O’hara Co. Ltd. | YES | YES |
| Equipment Model | SR-4020 | YES | YES |
| Software version | Animal Startle SR-9020 | YES | YES |
| Mouse chamber dimension | internal diameter  S: 3 cm, M: 3.5 cm | YES | NO |
| Sound generator |  | YES | NO |
| Sound-proof box dimension | 330(L)x430(W)x330(H) | YES | NO |
| Experimenter ID |  | YES | NO |
| Date equipment last calibrated |  | NO | NO |

## \*6 . Measured Parameters - list

{Placed in Parameters spreadsheet}

## \*7. MetaData Parameters - list