	Title: Acoustic startle and pre-pulse inhibition	
Standard Operating Procedure	Doc. Number: ESLIM_011_001 Rev No. 1	Date Issued: 22/02/2007

1.0 Purpose:

1.1 An acoustic startle model of sensorimotor gating, in which a weak acoustic stimulus (the pre-pulse) is used to decrease the reflex response (startle) produced by a second, more intense, stimulus (the pulse) in mice. Pre-pulse inhibition (PPI) provides an operational measure of sensorimotor gating which reflects the ability of an animal to inhibit sensory information properly. Several clinical studies have shown that schizophrenic patients have a reduced PPI. The lack of sufficient sensory gating mechanism is thought to lead to an overflow of the sensory stimulation and disintegration of the cognitive functions. The startle reflex paradigm is therefore largely used to assess the effects of putative anti-psychotics and to explore genetic and neurobiological mechanisms underlying behaviours of relevance to psychosis (Geyer, 1999; Ouagazzal et al., 2001).

2.0 Scope:

2.1 Individuals who have been trained, and are competent in performing the procedures described herein must follow this procedure.

2.2 Any queries, comments or suggestions, either relating to this SOP in general or to a specific problem encountered during a procedure, should be addressed to the Behavioural Neuroscience Project Leader

2.3 Any deviances from this protocol must be reported to Behavioural Neuroscience Project Leader.

3.0 Safety Requirements:

3.1 General laboratory procedures should be followed, which include: no eating, no chewing gum, no drinking, and no applying of cosmetics in the work area. Laboratory coats and gloves must be worn at all times in the work area, unless the protocol specifically describes the appropriate attire for the procedure.

4.0 Associated Documents:

5.0 Notes

5.1 The validity of results obtained from behavioural phenotyping is largely dependent on methods of animal husbandry. It is of vital importance that individuals following this procedure are experienced and aware of the animal's welfare.

5.2 The majority of mouse behavioural studies are age/sex/strain dependent. It is important to keep these parameters comparable throughout a single experiment.

5.3 Environmental factors may contribute to the levels of anxiety within the mouse. The temperature, humidity, ventilation, noise intensity and lighting intensity must be maintained at levels appropriate for mice. It is essential that the mice are kept in a uniform environment before and after testing to avoid anomalous results being obtained. In particular, the usual level of background noise can have an effect on results in this test.

5.4 It is recommended that all phenotyping experimentation is conducted at approximately the same time of day because physiological and biochemical parameters change throughout the day.

6.0 Quality Control:

6.1 The calibration of the sound and the movement sensors are fundamental in obtaining accurate test results and therefore must be frequently calibrated. Since both largely depend on the type of equipment used, the manufacturer's instructions for calibration must be followed rigorously.

6.2 **Sound calibration:**
Ideally, calibration of the sound is performed within the startle chamber in which the animal is tested. The applied sound pressure level should provide an accurate measure of the actual noise/tone intensity in dB. Furthermore, 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. Perform calibration of noise (white noise or pure tone signals) for Med Associate startle chambers by placing a microphone inside the Plexiglas cylinder

where the animal is confined during measurement. Startle chambers from San Diego Instruments require a sound level meter (Radio-Shack) to calibrate noise. A sound pressure level meter (Brüel & Kjaer) is used for GlaxoSmithKline apparatus.

6.3 **Movement sensor calibration:**
For Med Associate startle chambers the amplifiers of the single load cell platforms are calibrated in order to obtain a comparable startle magnitude across the devices used. Different weights (0, 40 and 200 grams) are positioned in the centre of the load cell platforms and their output measured with a voltage meter. The amplifier is balanced to 0V (for 0 gram weight) or adjusted to 2 and 10V (for 40 and 200 gram weights). For San Diego devices, the calibration of the startle enclosure is carried out with the standardisation unit and the unit force is set at 750 ± 10 for the mouse

7.0 Equipment:

7.1 The experimental apparatus consists of an outer chamber in which is a sound attenuated acoustic chamber with a load cell platform and an amplifier. A sound generator and the appropriate software regulate pulses from the amplifier. Various acoustic startle chambers are available (e.g. Med Associates Inc., San Diego Instruments, GlaxoSmithKline, etc.) that can be used.

7.2 Sound pressure level meter

7.3 Movement calibration unit

8.0 Supplies:

8.1 Tap water, 50% Ethanol

8.2 Tissue paper

9.0 Procedure:

9.1 General design

9.1.1 Acoustic startle reactivity and pre-pulse inhibition of startle are assessed in a single session. The PPI session is initiated with a 5 minute acclimation period followed by different trial types: acoustic startle pulse alone (white noise, 110dB/40msec); different pre-pulse trials in which either 10msec long P1, P2, P3, or P4 dB stimuli are presented alone or precede the pulse by 50msec, and finally one trial (NOSTIM) in which only the background noise is presented to measure the baseline movement in the Plexiglas cylinder.

9.1.2 Ideally for San Diego Instrument chambers, the pre-pulse intensities (P1 to P4) are set at 70, 80, 85 and 90dB, and the background noise at 65dB. However, different background levels and only 3 pre-pulse intensities (instead of 4) can be used. Nevertheless, the intensities of the pre-pulse should be kept at 5, 15, 20 and for the 4^{th} pre-pulse if used, 25dB above the background.

9.1.3 The test session begins with five presentations of the startle pulse alone trial that are excluded from statistical analysis. Then, each acoustic or NOSTIM trial is presented 10 times in random order. The inter-trial intervals (ITIs) vary between 20 and 30seconds. The test is conducted with the house light on. The startle response is recorded every millisecond (ms) for 65ms after the onset of startle. The maximal peak-to-peak amplitude is used to determine the acoustic startle response.

9.2 Experimental setting and testing

9.2.1 Transport animals for testing to the antechamber (pre-test room) and leave undisturbed for 30 minutes before the test.

9.2.2 Switch on the computer and set up the experimental design to observe the trials as described in 9.2.4 – 9.2.5.

Note: Acoustic startle reactivity and pre-pulse inhibition of startle are assessed in a single session.

9.2.3 The session is initiated with a 5 minute acclimation period without a pre-pulse stimulus, followed by 5 presentations of the startling pulse alone (white noise 110dB/40msec). These are excluded from the statistical analysis.

9.2.4 The session is then continued by presentations of different trial types. Pulses should be presented 10 times in pseudorandom order with an inter-trial delay varying between 20 and 30 seconds. The trials are:

9.2.4.1 Trials in which only the startling pulse is presented as white noise 110dB/40msec.

9.2.4.2 Six or eight different pre-pulse trials, of 10msec duration, of P1, P2, P3, or P4 dB white noise stimuli presented alone or preceding the pulse by 50msec.

9.2.4.3 A trial in which only background noise (BN) is presented to measure baseline movement of the animal in the chamber

Note: Different background levels can be used, however, the intensities of the pre-pulse must be kept at levels above the background which do not elicit a significant startle response on their own (e.g. 5, 15, 20 and 25dB above background; the maximal meaningful pre-pulse intensity should be determined experimentally for each startle chamber setup).

9.2.5 Startle response is recorded every millisecond for 65ms after the onset of startle.

9.3 Ensure that all apparatus are functioning correctly (see section 6.0).

9.4 Place each mouse onto the load cell platform inside the sound attenuated acoustic chamber and secure the door close.

9.5 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.

9.6 Run the experimental session according to the experimental design described in 9.2.4 – 9.2.5.

9.7 At the end of each run, animals are labelled (if necessary) and put back into their home cages.

9.8 Clean the load cell platform thoroughly with water, 50% ethanol and again water after each run.

9.9 Once all animals are tested, save data from the experimental sessions onto a disc and analyse.

9.10 Turn off the chambers and clean the equipment.

10.0 Data Records and Reports:

10.1 The maximal peak-to-peak amplitude is used to determine the acoustic startle response.

10.2 Basal startle responses are calculated as the average responses to the pulses presented alone or combined pre-pulse-pulses: BN, P1, P2, P3, (P4 if used), ST110, PP1, PP2, PP3, (PP4 if used).

10.3 The amount of PPI is calculated as a percentage score for each acoustic pre-pulse trial type:
% PPI= 100 x [(mean amplitude startle response for the PULSE alone - mean amplitude startle response for PRE-PULSE/PULSE)] / mean amplitude startle response for PULSE alone.
The global level of PPI is also calculated as the mean %PPI for the different pre-pulses.

10.4 **Parameters recorded:**

Startle amplitude - BN
Startle amplitude - P1
Startle amplitude - P2
Startle amplitude - P3

Startle amplitude - P4
Startle amplitude - ST110
Startle amplitude - PP1
Startle amplitude - PP2
Startle amplitude - PP3
Startle amplitude - PP4
%Pre-pulse inhibition - PP1
%Pre-pulse inhibition - PP2
%Pre-pulse inhibition - PP3
%Pre-pulse inhibition - PP4
%Pre-pulse inhibition - Global

11.0 Supporting information

11.1 Geyer MA (1999) Assessing pre-pulse inhibition of startle in wild type and knockout mice. Psychopharmacology 147:11-13

11.2 Ouagazzal AM, Jenck F, Moreau JL (2001) Drug-induced potentiation of pre-pulse inhibition of acoustic startle reflex in mice: a model for detecting anti-psychotic activity? Psychopharmacology 156:273-283.

11.0History Review:
12.0
In the present version it is described that 3 or 4 pre-pulses can be used and the pre-pulse intensities are noted P1 to P4.

In the previous version " EMPReSS SOP:10_005, Revision 0", it was defined that 4 pre-pulse intensities are used.

12.0 Emergency Procedures: