STANDARD OPERATING PROCEDURE

TITLE: Pre-Pulse Inhibition and Startle Response
CATEGORY: Behavioral Assay

Introduction

Goal: Prepulse inhibition (PPI), also termed startle reduction or reflex modification, is a phenomenon in which a weak stimulus (prepulse) suppresses the startle response of a subsequent, stronger startling stimulus (pulse). Impairment in PPI is believed to be linked to a dysfunction in the sensorimotor gating mechanism; clinical studies in humans have shown that patients with schizophrenia, schizotypal personality disorder, obsessive-compulsive disorder and Huntington's disease have impaired PPI. The parts of the brain that believed to be involved in PPI include the nucleus accumbens, hippocampus, amygdala, medial prefrontal cortex, pedunculopontine tegmental nucleus, and ventral and caudodorsal striatum. Several neurotransmitters such as dopamine, acetylcholine, serotonin, glutamate, GABA and norepinephrine also have an effect on PPI.

Materials

- Subjects – any species of rat or mouse may be used. Subjects will require acclimation to the apparatus before conducting the experiment
- Apparatus – Acoustic Startle Reflex Starter Package for Rat or Mouse (Product Number: MED-ASR-PRO1) and Standard Acoustic Startle Sound Attenuation Cubicle (Product Number: ENV-022S) from Med Associates Inc.
- Software- Startle Reflex Software (Product Number: SOF-825)
- Ethanol – 20% or greater ethanol is used between trials to eliminate residue in the holding chambers

Test Settings

Room should be dark and quiet. The apparatus should be placed on a steady bench top and calibrated according to guideline provided by manufacture.

Detailed Standard Operation Procedure

The following are intended for training purposes and should be used as a guideline to help run the Pre-Pulse Inhibition and Startle Response experiment.

The subjects were acclimated to the animal holder in the startle box over 3 days for 15min each day prior to the experiment. In the beginning of the experiment, each animal was introduced into the animal holder for 5 minutes without any stimulus. After this initial acclimation period, each animal was given three blocks of different startle sounds. Block 1 was for habituation of the startle pulse, Block 2 was for testing Pre-Pulse Inhibition, and Block 3 was for testing the Acoustic Startle Response.
In the first block, each animal was given five 120 dB Startle Pulses lasting 40ms (no prepulses) with randomly variable inter-trial intervals of 10-20s. In the second block, the animals encountered the same Startle Pulses with the addition of prepulse tones prior to them. Each subject was given 5 exposures to each of 5 different prepulses (0, 69, 73, 77, and 81dB), presented in random order for a total of 25 trials. Duration of the prepulse tone was 20ms for all pre-pulses followed by a fixed interval of 100ms (startle delay). After each prepulse tone, the animals received 40ms of 120 dB Startle Pulse alone and the startle response of the animals was recorded. Inter-trial intervals for all 25 trials of Block 2 were randomly variable from 10-20secs. In the third block, each animal received 25 different trials with 10-20secs randomly variable inter-trial intervals. Five different intensities of startle pulses (0, 90, 100, 110, 120 dB) lasting 40ms were used for this block. Each animal receive five times of different startle pulses in random order.
Background noise was set at 65 dB for the entire experiment and the peak to peak amplitude of startle response in each trial was recorded for analysis. The animal holder of the apparatus was cleaned with 20% alcohol between each animal. All the experiments were run during the animals' dark cycle.

Data Extraction and Analysis

If the data has been saved as a Microsoft Access Database File, it can be imported into Microsoft Excel by using a Query.

1) Start Microsoft Excel. Go to “Data”. Click on “From Access” button on the top left of the screen. Select the data source (place you have saved your data on your computer). Click on “Open” button. Then select the “Trial Data” and Click “OK”.An Import

Data window will be appear. Select "Table", "Existing worksheet", and cell "A1". Then Click on OK.

2) All the information will be on the Excel file including Chamber No, Block No, Trial No, Trial Type, Null Period, S1 Stim Type, S1 Stim Duration, S1 Level, S1 Rise/Fall, S1 Frequency, S1 Aux Port, S1-S2 Delay, S2 Stim Type, S2 Stim Duration, S2 Level, S2 Rise/Fall, S2 Frequency, S2 Aux Port, Background Level, Null Latency to Startle, Null Peak Time, Null Peak Value, Null Peak to Peak Value, Null Duration, Null Total, Null Average, S1 Latency to Startle, S1 Peak Time, S1 Peak Value, S1 Peak to Peak Value, S1 Duration, S1 Total, S1 Average, S2 Latency to Startle, S2 Peak Time, S2 Peak Value, S2 Peak to Peak Value, S2 Duration, S2 Total, S2 Average.
3) Average all the peak to peak values in each level of Prepulse (S1 Level) and report as a result of PPI. Percentage of PPI was calculated by:
   [(Startle response with Startle pulse alone – Startle response with Prepulse plus Startle pulse)/ Startle response with Startle pulse alone] X 100
4) For Startle Response just report the startle amplitude for the sessions with no S1 stimulus (S1 level=0). Graph the results.