Auditory startle & Prepulse inhibition

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Summary:

The startle reflex measures the acoustic function of mice in response to an unexpected strong auditory stimulus. The prepulse inhibition (PPI) test measures the attenuation of the startle response by a less intense (weaker) auditory stimulus that precedes the startle. The response to the PPI test provides an operational measure of sensorimotor gating reflecting the ability of an animal to successfully integrate and inhibit sensory information.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
SR-LAB ABS Isolation Cabinet	San Diego Instruments	2325-4000
SR-LAB Control Unit	San Diego Instruments	
PC equipped w. SR Startle response system	San Diego Instruments	Model 6500-0091-R
Lab Coat/Gloves/PPE		
Paper towels		
Disinfectant -Coverage Plus	Steris	

Protocol:

1. SET-UP (acclimation of mice, software setup)

a. Acclimate mice in quite room for 30 min prior to testing. Mice are tested in the morning. Do not stimulate the mouse before starting the experiment. Do not change the cage on the day of the experiment.

b. Daily calibration of platform:

1. Turn on computer.

2. Turn on all Startle chambers by flipping the switch on the power strip under the table. The light inside each chamber should be on.

3. Screw vibration calibrator onto the platform in the first chamber.

4. Plug the long cord into the calibrator and the opposite end into the 12 Volt power input on the side of the chamber #2. The cable should be permanently kept in the input of the chamber. Warm up for 10-15 minutes.
5. Open Startle program using the desktop shortcut.
6. Click "RUN" option in the header row of the window.
7. Of the available options, select moto.SAS to create a calibration file.
8. Name output file name to include study name, date, and chamber number as follows: KOMPcal-9-25-13.

NOTE: the file name for each run of the calibration "experiment" must be different and may not include "." or "/".

9. Enter 1 as the selected chamber in the following window and a corresponding letter for each chamber if desired. Subject Identification click "Continue".
10. Before starting the data collection, be sure that the cord is not touching the platform (this specifically pertains to calibration of chambers 5 and 6).
11. Click on "Go" Is your "Subject ready" click "OK".
12. Press any key to bypass the five minute acclimation period.
13. Data collection will provide several columns of values, but the desired value is in the "Average" column. Write down average reading at trial 50.
14. The average value must be 800 ± 15 for 25 consecutive trials.
15. If the average is falling above or below the required range, turn the black dial on the right side of the chamber to adjust.
16. To turn the knob, first slide the unlock tab.
17. Clockwise adjustment will cause the average value to increase slightly.
18. Counterclockwise adjustment will cause the average value to decrease slightly.
19. Once the average falls within the range for 25 consecutive trials lock the adjustment knob into place. Record in Calibration record.
20. Click "Abort Session" to end data collection.
21. To begin calibration of the next chamber, click "Run", then "moto.SAS" in the program, name a new file following the same format and change the chamber number.
22. Once all chambers have been calibrated, close the program and unscrew the calibrator from the platform.

2. PROCEDURE

a. Set-up the experimental design to capture acoustic startle and acoustic pre-pulse inhibition in a single session (EXPERIMENTAL): (**1)** An initial 5 minute acclimation session (background noise (BN) at 65 dB. **(2)** A successive session in which trials of varying pulses are each presented 10 times in a pseudorandom order, with an intertribal (ITI) varying randomly between 10 and 20 seconds and includes: 20 msec duration, PP74, PP82, or PP90 dB of white noise stimuli, which are presented alone or precede the pulse by 100 msec (PP74 + pulse, PP82+ pulse, or PP90 + pulse) to derive the pre-pulse inhibition response. The intensities of the pre-pulse should be kept at levels above the background noise that do not elicit a significant startle response on their own, being approximately 2-20 dB above BN (65 dB). (74dB, 82 dB, 90 dB). **(3)** Startle pulse trials where 120 dB/40 msec of white noise is presented alone. **(4)** No stimulus (NOSTIM) trials in which a set background noise (BN) level is presented alone to measure baseline movement of

the animal in the chamber. **(5)** The response is recorded every millisecond for 65 ms after the onset of startle.

b. Turn ON computer and all startle chambers.

c. Click on STARTLE icon on desktop. Click "RUN" from menu on top of screen. CHOOSE SESSION screen will pop up. Click on EXPERIMENTAL session, click OK.

d. OUTPUT FILE screen will pop up. Enter output file name: (Can't use "." or"/"). Project-date-animal #'s , date then click "OK". This file name must be different for every run.

e. SUBJECT IDENTIFICATION window will pop up. Enter stock # -animal # of mouse ie BL-# for station 1 thru 6 accordingly in subject column then click "CONTINUE".

f. IS YOUR SUBJECT READY? Window will appear. Can load mice.

g. Load mice into appropriate testing station/chamber. Use the second slot for smaller animals and the third slot for larger. Animal must be able to turn around in restraint.

h. Go to computer, click "OK". Leave the room so that you do not create any noise while the mice are testing. Test duration is approximately 20-25 min.

i. At the end of test, remove each mouse at the end of the experimental session and record its weight before returning it to the relevant home cage.

j. Clean the animal holders inside and out with 10% Nolvasan. Change gloves for another group of mice.

k. At the end of the session, completely disinfect holders, chambers, and tabletop with Coverage Plus

3. MONTHLY CALIBRATION PROCEDURE

a. Turn ON computer and all startle chambers.

b. Remove the plug on the back of the first chamber and thread the sound meter wand and corresponding cable through the hole in the middle of the plug.

c. Replace plug onto back of chamber (sound meter wand should now be dangling inside the chamber).
NOTE: The meter display should always remain outside of the chamber.

d. Place one of the clear plastic barriers at the back of the subject tube) mode.

e. Place the sound meter wand inside the tube with the end of the wand facing the back of the chamber and the end of the wand connected to the cord facing the door of the chamber.
NOTE: Make sure that the tip of the wand is close to but not touching the plastic barrier and that the door does not push the wand against any other sides of the plastic tube or barrier.

f. Open the Startle program on the computer, select "Diagnostic", TEST: 65dB (background), 74dB, 82dB, 90dB and 120dB (startle).

g. Enter the corresponding number to the dB level specified by the experiment in the "Audio" field. If the measured value does not match the expected value corresponding to the particular dB level selected, adjust the small screw on the right-hand side of the chamber. Clockwise turning will cause the measured value to increase, while counterclockwise turning will cause it to decrease.
NOTE: Sound meter will pick up changes in noise in the room and surrounding areas, so be sure to remain quiet while testing.

h. To test another dB level for the same chamber, simply enter the corresponding number into the "Audio" field and press the number pad enter key again. Follow this procedure until all sound levels have been calibrated.
NOTE: Testing the 120 dB is very loud, do quickly. Get a reading and immediately turn off. If left on too long, this could blow the speakers.

i. To calibrate other chambers, simply remove the wand and sound meter from the subject tube in one chamber and place it in the same fashion into the next chamber.

j. After the sound and vibration have been calibrated for all chambers, proceed with experiment.