med associates inc

instrumentation and software for research

STARTLE REFLEX

SOF-825

USER'S MANUAL

DOC-040
Rev. 6.3

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notes

Table of Contents

CHAPTER 1 | INTRODUCTION

The startle reflex is a motor response to an intense and unexpected stimulus. The anatomy of the acoustic startle reflex is well-characterized and is often studied in humans as well as rodents to measure sensorimotor processing, emotional state, and/or attention. The amplitude of a study subject's response (usually a "jump") is quantified. This "jumping" reflex response can be altered in different strains of animals, by pre-pulse inhibition of startle (PPI), and in fear-potentiated startle (FPS). This Startle Reflex system allows users to obtain accurate measurement of the acoustic startle reflex in rodents.

The Startle Reflex software package is designed for use with Med Associates modules to produce stimuli, collect response data, plot data to the screen, and perform waveform analysis.

Please thoroughly read this manual prior to setup in order to gain an understanding of the Startle Reflex system.

CHAPTER 2 | HARDWARE AND CABLE OVERVIEW

Hardware Guide

Figure 2-1 - The Startle Reflex System

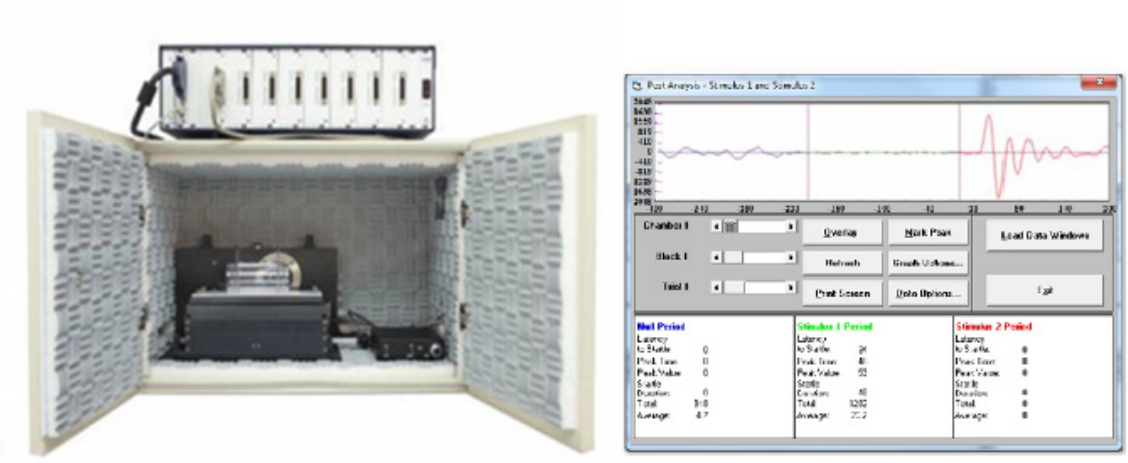


Figure 2-2 - Startle Reflex Cabinet with ANL-729, ANL-925E and ANL-925D

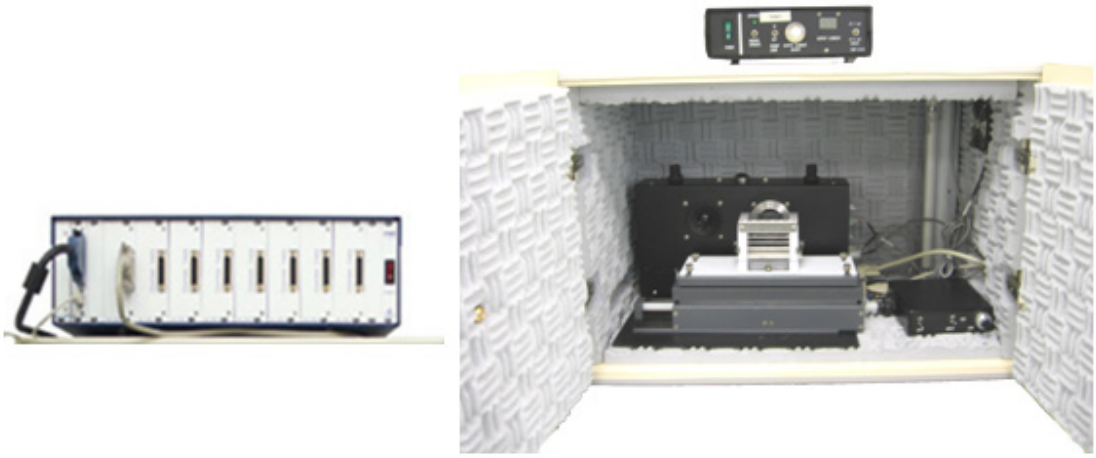
Figure 2-3 - Sound-Attenuating Cubicle (SAC)

Figure 2-4 - 255A Stimulus Connection Panel with Platform Table & Speakers

Figure 2-5 - PHM-250B Startle Load Cell Amplifier Front and Back Panel

Figure 2-6 - ENV-414S Constant Current Aversive Stimulator Front and Back Panel

Figure 2-7 - Startle Reflex System Assembled



Cable Guide

Table 2-1 - Startle Reflex Cable Guide

Part Number	Image
SG-244 68-pin NI PCI-6023E Interface Cable	
SG-USB-6 USB Cable	
SG-210CB-25 Serial Port Cable	

SG-216A-10 3-Pin Molex Cable	
SG-219G-10 DB-9 Shock Cable	
SG-250A PHM-250B to PHM-255A Cable	

CHAPTER 3 | SYSTEM SETUP

NOTE: Always turn off the power prior to performing work on the computer or interface cabinets. Serious damage or personal injury may occur if this precaution is not taken.

Software Installation

If the computer was purchased as part of Startle Reflex system from Med Associates, the driver and software installation and the DIG-744/DIG-744E PCI card installation was completed at the factory. If the computer was not purchased from Med Associates or was not a part of the system, follow the instructions below to install the drivers, software and DIG-744/DIG-744E PCI card.

Before beginning the software installation, phone, fax or e-mail Med Associates with the registration information in order to receive the software installation password. This password will be necessary during the software installation process.

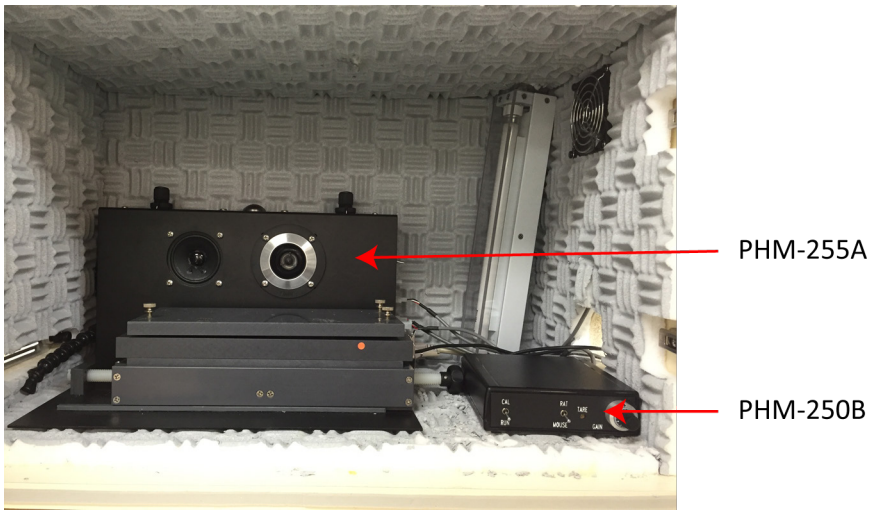
Insert the Startle Reflex CD into the CD drive and at the welcome screen click **Install**. If the CD does not auto-start, navigate to the CD drive in Windows Explorer and double click the "autorun.exe" file and click Install at the welcome screen. Successful installation of each component will be indicated by a green check mark, a red X will indicate an unsuccessful installation. When prompted enter the user name, company name and software installation password. When the installation completes, click **Finish**.

Once the software and driver installation is complete, turn off the computer and install the National Instruments PCI-6023E (DIG-744) or PCIe-6320 (DIG-744E) card, following the instructions included with the computer for installing PCI devices. After the card is installed, reboot the computer.

Hardware Installation

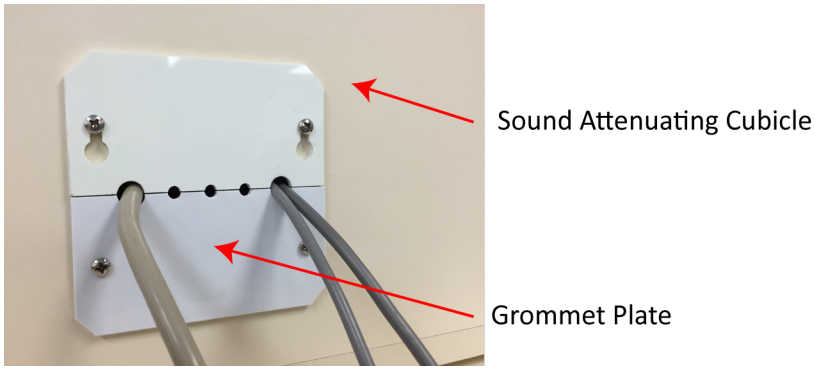
1. Place the PHM-255A Stimulus Connection Panel with Platform Table and Speakers, and the PHM-250B inside the Sound-Attenuating Cubicle (SAC) as shown in Figure 3-1.

Figure 3-1 - Startle Platform in SAC



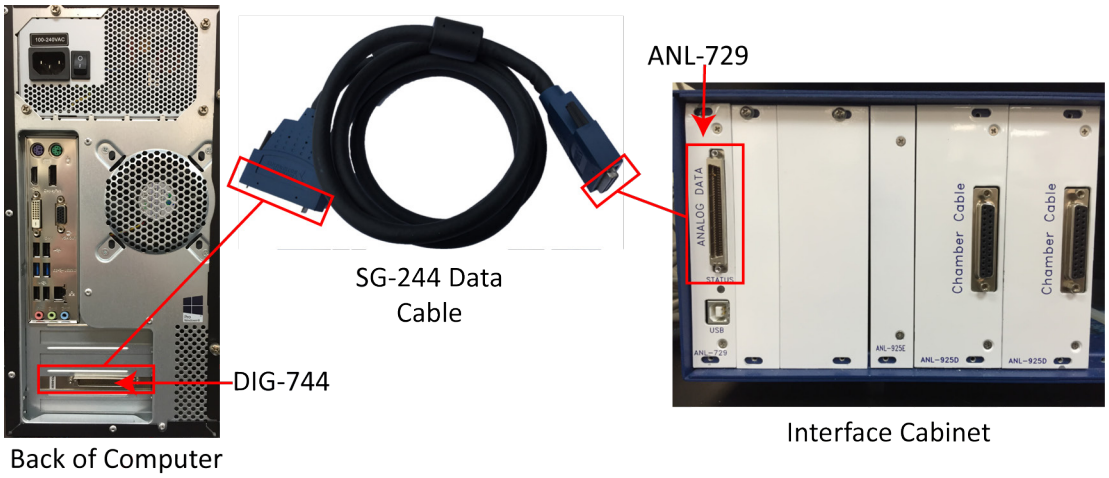
2. Loosen the two Philips screws on the grommet plate (right side of SAC) shown in Figure 3-2 and remove the top half. All cables should be routed into the SAC through this cable port.

Figure 3-2 - Cable Port



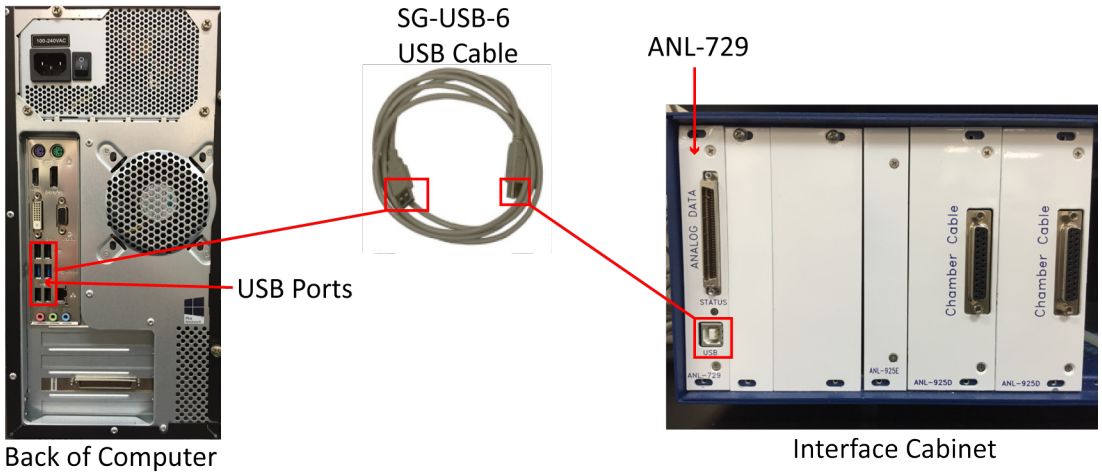
3. Using the SG-244 68-pin Interface Cable, connect the DIG-744 PCI card (back of computer) to the **ANALOG DATA** port on the ANL-729 card (Interface Cabinet). See Figure 3-3.

Figure 3-3 - Connect DIG-744 PCI Card to ANL-729 Card



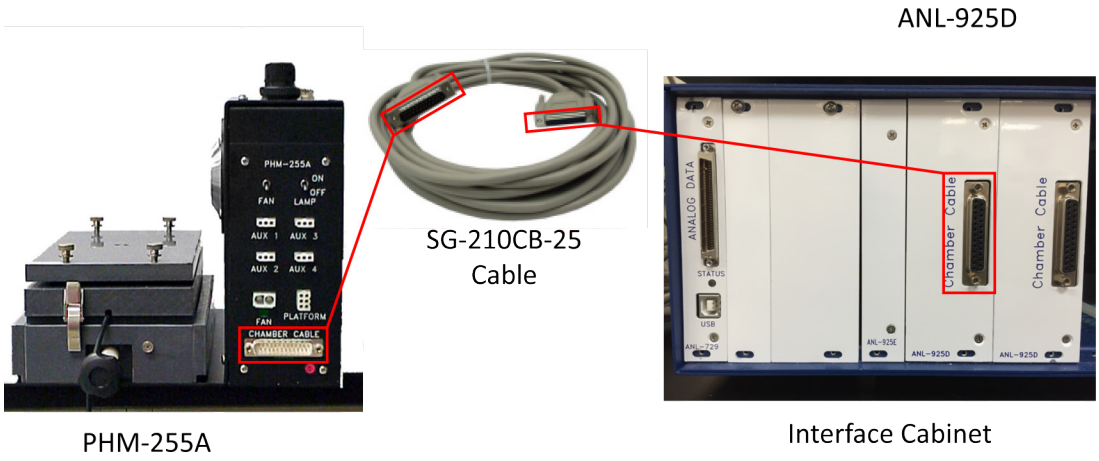
4. Using the SG-USB-6 Cable, connect any available USB port on the computer to the **USB** port on the ANL-729 card (Interface Cabinet) shown in Figure 3-4**.**

Figure 3-4 - Connect USB Port to ANL-729 Card



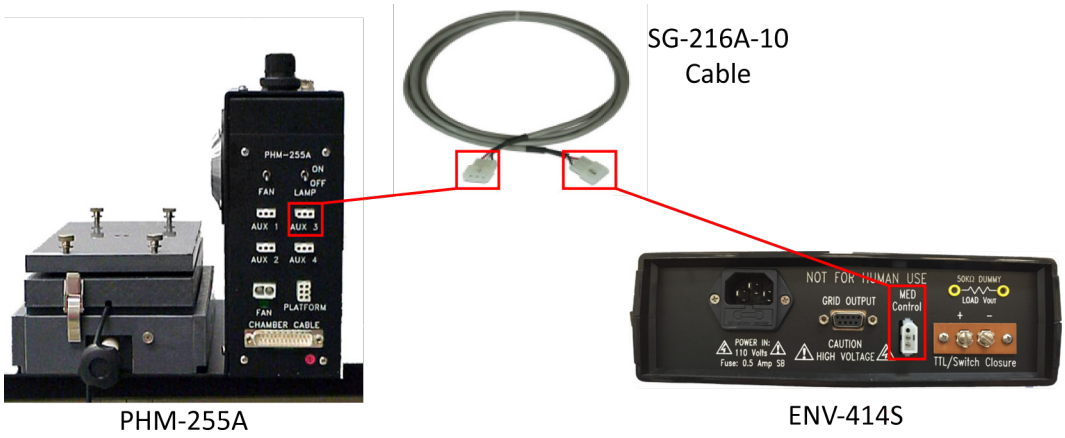
5. Using the SG-210CB-25 Cable, connect the **CHAMBER CABLE** port on the PHM-255A Stimulus Connection Panel to the **Chamber Cable** port on the ANL-925D card (Interface Cabinet). The leftmost ANL-925D card corresponds to Chamber 1; the second card corresponds to Chamber 2, etc (see Figure 3-5).

Figure 3-5 - PHM-255A Startle Platform to ANL-925D Card



6. (Optional) Using the SG-216A-10 Cable, connect the **AUX3** port on the PHM-255A Startle Connection Panel to the **MED CONTROL** port on the ENV-414S Aversive Stimulator shown in Figure 3-6.

Figure 3-6 - PHM-255A Startle Platform to ENV-414S Aversive Stimulator



7. Connect the Startle Platform Cable to the **LOAD CELL** port on the PHM-250B shown in Figure 3-7.

Figure 3-7 - Startle Platform to PHM-250B

PHM-255A PHM-250B

8. Using the SG-250A Cable, connect the **POWER/SIGNAL OUT** port on the PHM-250B to the **PLATFORM** port on the PHM-255A Stimulus Control Panel shown in Figure 3-8.

Figure 3-8 - PHM-255A Startle Platform to PHM-250B

PHM-255A PHM-250B

9. (Optional) Connect the Fluorescent Stimulus Light PHM-258L to the Fluorescent Lamp Controller PHM-258 shown in Figure 3-9.

   Connect Fluorescent Lamp Controller PHM-258 to an available **AUX** port on the PHM-255A Stimulus Control Panel shown in Figure 3-10.

Figure 3-9 - Connect PHM-258L Lamp to PHM-258 Lamp Controller

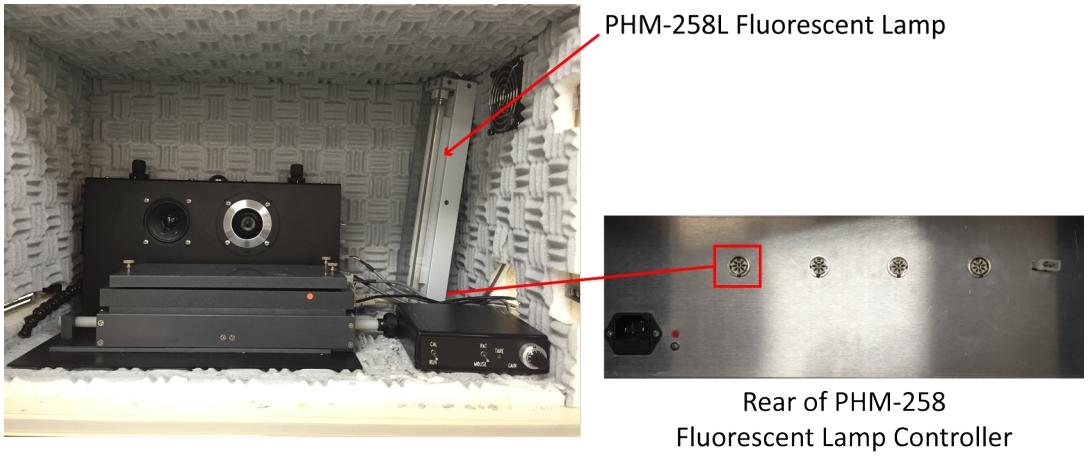
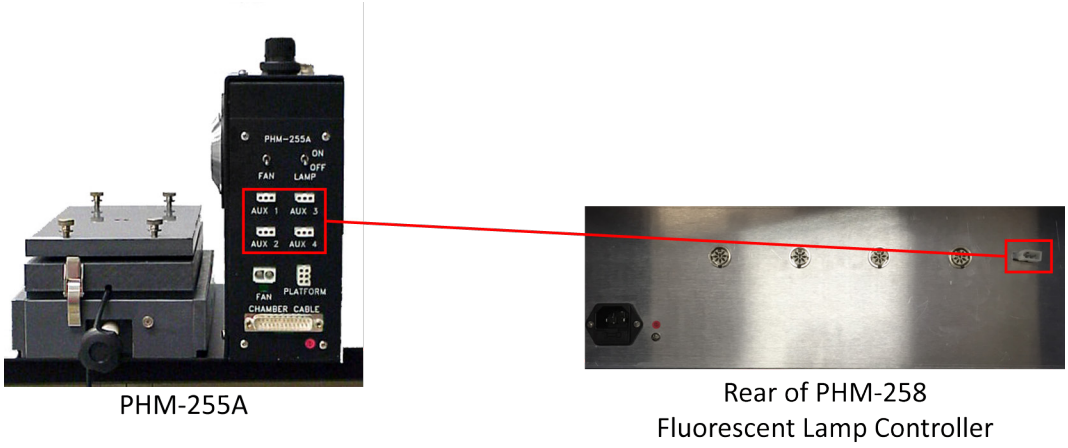
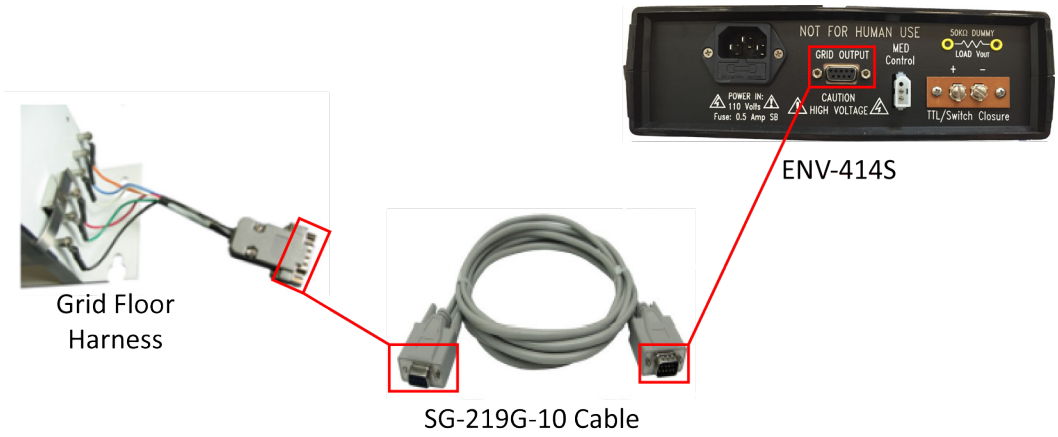


Figure 3-10 - Connect PHM-258 Lamp Controller to PHM-255A Stimulus Control Panel



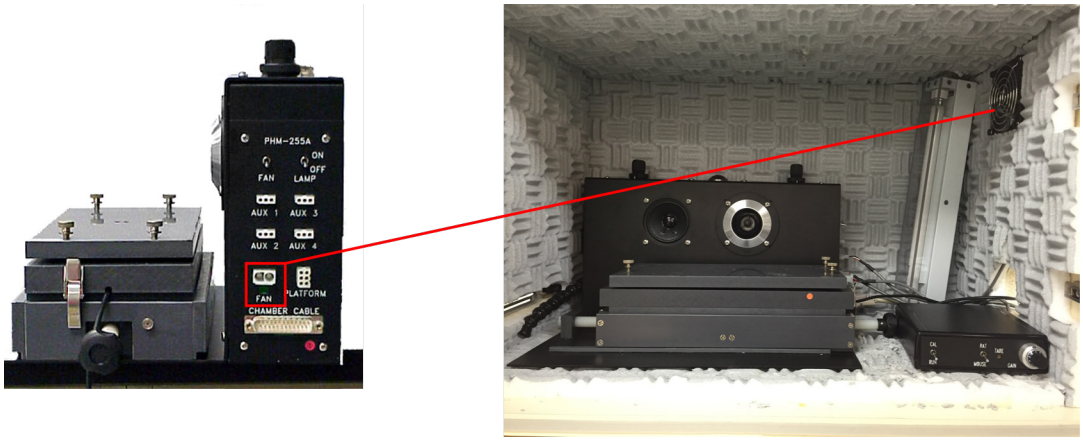
10. (Optional) Using the SG-219G-10 Cable, connect the **GRID OUTPUT** port on the ENV-414S Aversive Stimulator to the Grid Harness shown in Figure 3-11.

Figure 3-11 - ENV-414S Grid Output to Grid Harness



11. Connect the fan cable (inside the SAC) to the **FAN** port on the PHM-255A shown in Figure 3-12.

Figure 3-12 - Connect Fan to PHM-255A



12. It is now safe to apply power to the Startle Reflex Cabinet, ENV-414S (optional), PHM-258L (optional), and computer.

13. Replace the top half of the grommet plate and tighten the Philips screws.

CHAPTER 4 | CALIBRATION

After the Startle Reflex system has been properly installed (see Chapters 2 & 3), it is important to test the system to ensure that it is functioning properly. A complete calibration procedure is performed prior to shipping; however, it is necessary to perform the audio and load cell calibrations for each new protocol and/or set of animals.

Audio Calibration

Due to the fact that each speaker has a slightly different frequency response, and that different frequencies are produced at naturally different volumes, it is important to perform the audio calibration for each chamber. By using the Calibrate Audio utility and the ANL-929A-PC USB Microphone Package, each speaker's frequency response can be tested and the results can be used to correctly present the desired frequencies at the specified volume.

This system includes all components necessary for calibrating the speaker(s) to generate reproducible startle stimuli. For more detailed information regarding the installation and use of the ANL-929A-PC USB Microphone Package, refer to the **ANL-929A-PC User's Manual, DOC-089**.

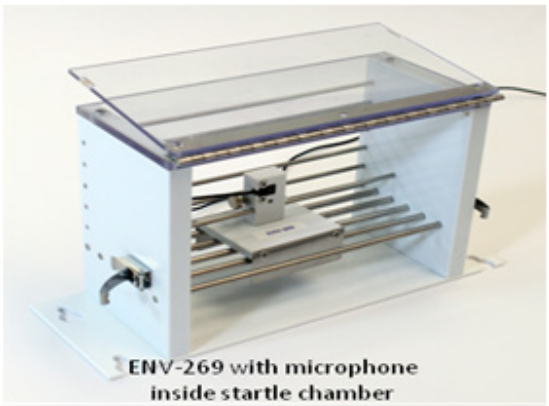
Calibrating Chamber 1

Before performing Audio Calibration, determine the sound parameters of the testing protocol. This includes the decibel (dB) levels for the white noise, background noise, and the dB level and frequency (Hz) for the pure tone(s).

Recommended audio calibration method:

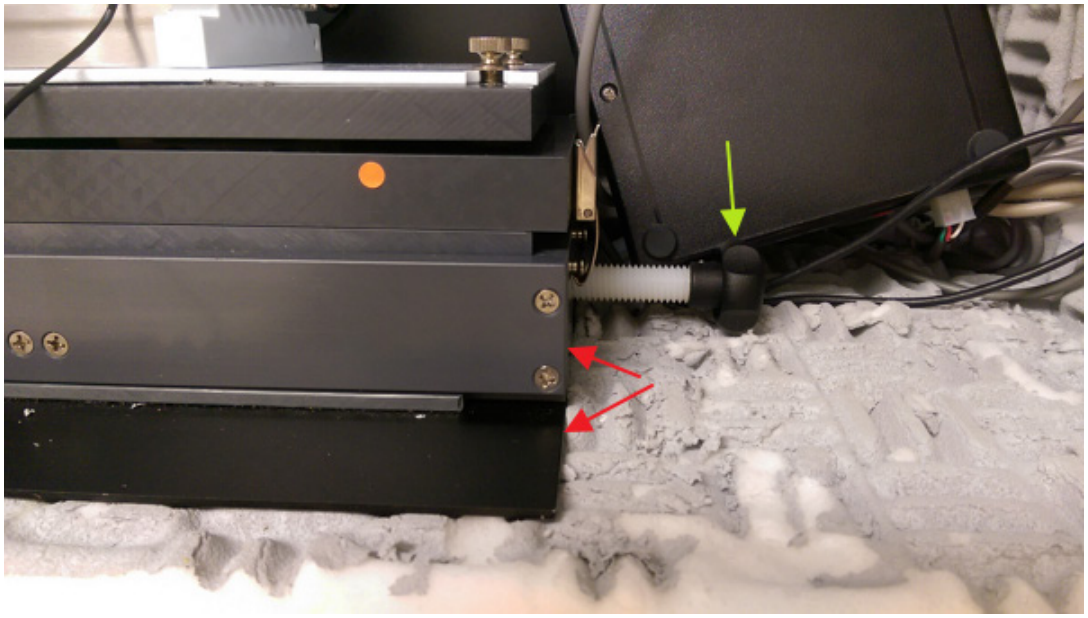
1. Install the ANL-929A-PC software and microphone according to the **ANL-929A-PC User's Manual, DOC-089.**
2. Place the ANL-929A-PC microphone inside the desired animal holder, using the microphone calibrator that came with your animal holder(s). Examples of microphone calibrator placement are shown inFigure 4-1.

Figure 4-1 - Microphone calibrators



3. Place the animal holder with the microphone calibrator on the startle platform inside Chamber 1, being sure to orient the holder so that the holes are facing towards the speaker at the back of the chamber. Align the right hand edge of the startle platform with the right hand edge of the base of the PHM-255A (red arrows, Figure 4-2). Use the platform adjustment knob (green arrow, Figure 4-2) to make this adjustment.

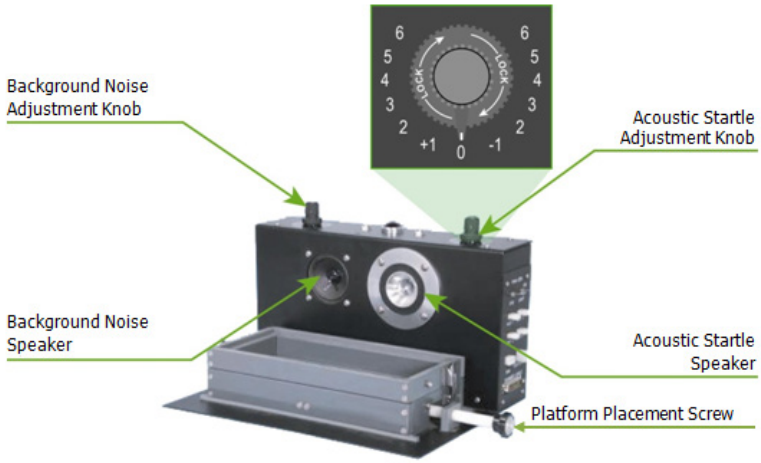
Figure 4-2 - Startle Platform Aligned With Base Plate



NOTE: Take careful note of the microphone and platform placement in Chamber 1. Be sure to orient the startle platform and microphone in **exactly the same position** in each chamber being calibrated.

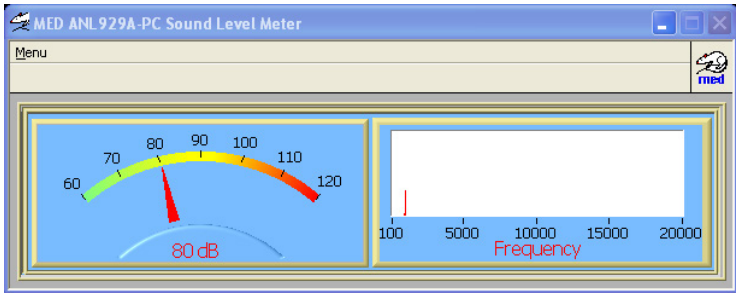
4. Set the background noise and acoustic startle dials on top of the PHM-255A to 0 and then close the doors on the startle chamber.

Figure 4-3 - PHM-255A Acoustic Startle Adjustment Knob



5. Launch the ANL-929A-PC Sound Level Meter software application (Figure 4-4) and drag it to a position near the bottom of the screen. Keep the application open and visible for the following steps.

Figure 4-4 - Sound Level Meter Software



6. Launch Startle Reflex and resize the application window so that the ANL-929A-PC Sound Level Meter software window is also visible. From the Startle Reflex main menu bar select **Hardware > Calibrate Audio**. The Calibrate Audio screen (Figure 4-5) will appear.

Figure 4-5 - Calibrate Audio Screen

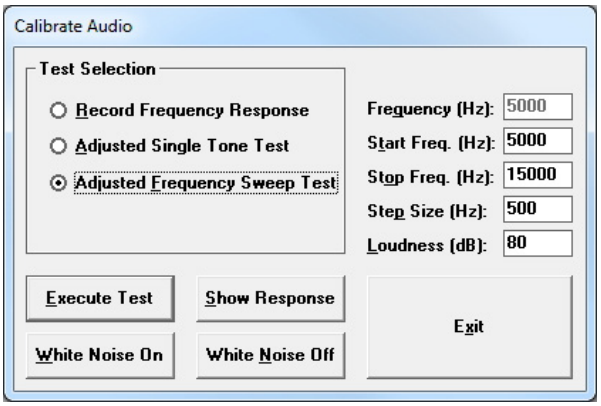


Table 4-1 - Calibrate Audio Screen Options

Item	Description
Record Frequency Response	Used to set the frequency offset to adjust for varying speaker frequency responses.
Adjusted Single Tone Test	Tests a single tone using the offset.
Adjusted Frequency Sweep Test	Used to confirm offsets applied during the Record Frequency Response test are correct. See **Pure Tone Calibration**.
Execute Test	Begins the selected test.
Show Response	Displays the offset table created from running the Record Frequency Response test.
White Noise On	Locks on the white noise.
White Noise Off	Turns white noise off.
Frequency (Hz)	Sets frequency used for Adjusted Single Tone Test.
Start Frequency (Hz)	Beginning (lowest) frequency being used in a protocol.
Stop Frequency (Hz)	Ending (highest) frequency being used in a protocol.
Step Size (Hz)	Size by which played frequency will be incremented. With a Step size of 500Hz there would be 20 steps. 15KHz – 5KHz = 10KHz / 500Hz = 20 steps. Played frequencies would be 5KHz, 5.5KHz, 6KHz, 6.5KHz…15KHz.
Loudness (dB)	Amplitude (volume) at which frequencies will be played.
Exit	Exits the Calibrate Audio Screen

White Noise Calibration

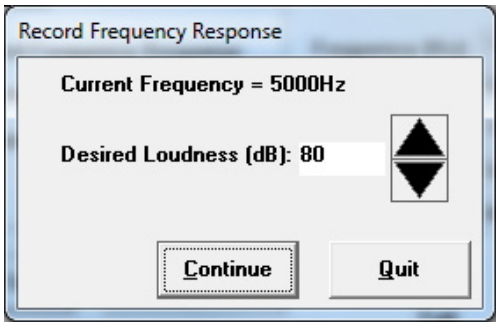
7. Set the **Loudness (dB)** to 80dB and click the **White Noise On** button. Verify the Sound Level Meter Software (Figure 4-4) displays approximately 80dB. Repeat these steps using 110dB. If the value displayed in the Sound Level Meter Software differs from the set value by a significant amount please contact technical support (see **Contact Information)** for detailed information on adjusting the internal trimming potentiometers.

NOTE: White Noise bursts and pure tones are both produced by the right-hand speaker on the PHM-255A.

Pure Tone Calibration

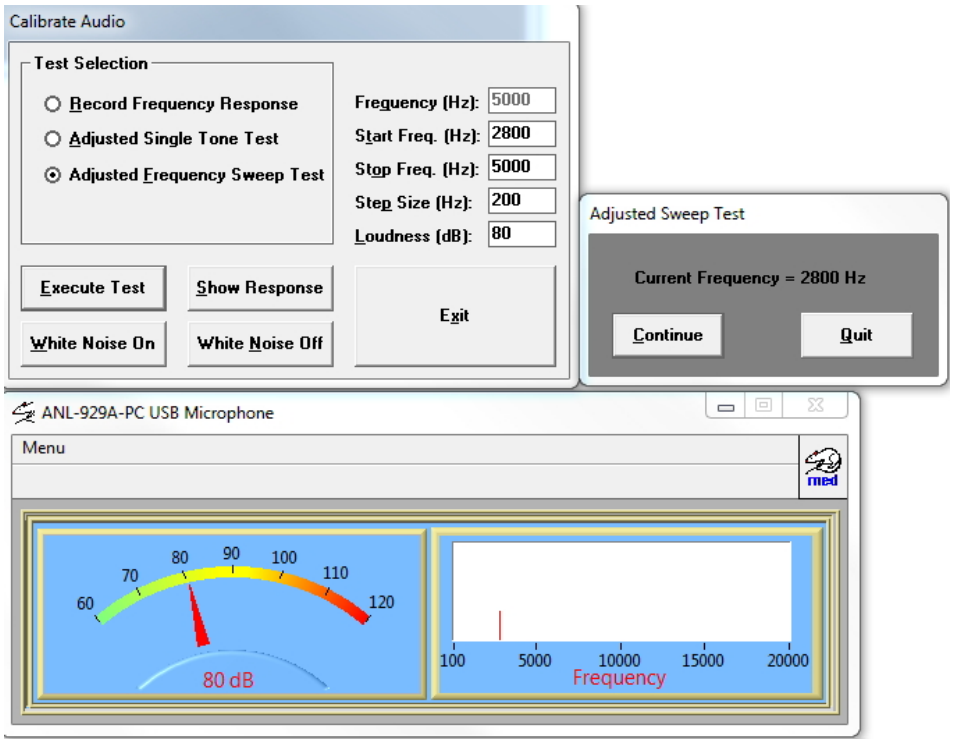
8. Enter the lowest frequency being used in the protocol in the **Start Freq (Hz)** box. Enter the highest frequency in the **Stop Freq (Hz)** box, the step size in the **Step Size (Hz)** box and the median loudness used in the protocol in the **Loudness (dB)** box. Refer to Figure 4-5.
9. Select the **Record Frequency Response** test and press **Execute Test**. The **Record Frequency Response** screen (Figure 4-6) will open.

Figure 4-6 - Record Frequency Response Screen



10. Use the Up/Down arrows to adjust the loudness displayed in the Sound Level Meter Software to match the **Desired Loudness (db)**. Press **Continue** when the two values are as close as possible. Pressing **Quit** will result in an incomplete calibration.
11. Select **Adjusted Frequency Sweep Test** and set the **Loudness (dB)** to the minimum pure tone dB level used in the protocol.
12. Press **Execute Test** and confirm the dB level shown in the Sound Level Meter Software approximates the desired pure tone dB level, see Figure 4-7. Press **Continue** to step through each frequency.

Figure 4-7 - Adjusted Frequency Sweep Test



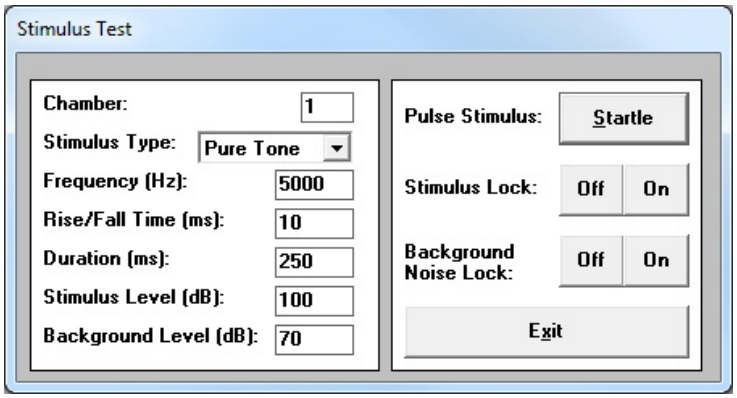
13. Enter the maximum pure tone dB level used in the protocol in the **Loudness (dB)** field. Repeat step 12. When complete click **Exit**.
14. If any of the values for the minimum or maximum dB levels significantly differ from the desired level, contact support (see **Contact Information**) for detailed instructions regarding adjusting the internal trimming potentiometers.

Background Noise Calibration

15. To calibrate Background Noise, click **Hardware > Stimulus** Test and set the dB level to 70dB in the **Background Level (dB)** field on the **Stimulus Test** screen (Figure 4-8).
16. Click **Background Noise Lock On.**
17. Verify the dB level using the Sound Level Meter software.
18. Repeat using a **Background Level (dB)** of 90dB. If the value displayed in the Sound Level Meter Software differs from the set value by a significant amount please contact technical support (see **Contact Information**) for detailed information on adjusting the internal trimming potentiometers.

NOTE: Background noise is produced by the left-hand speaker on the PHM-255A.

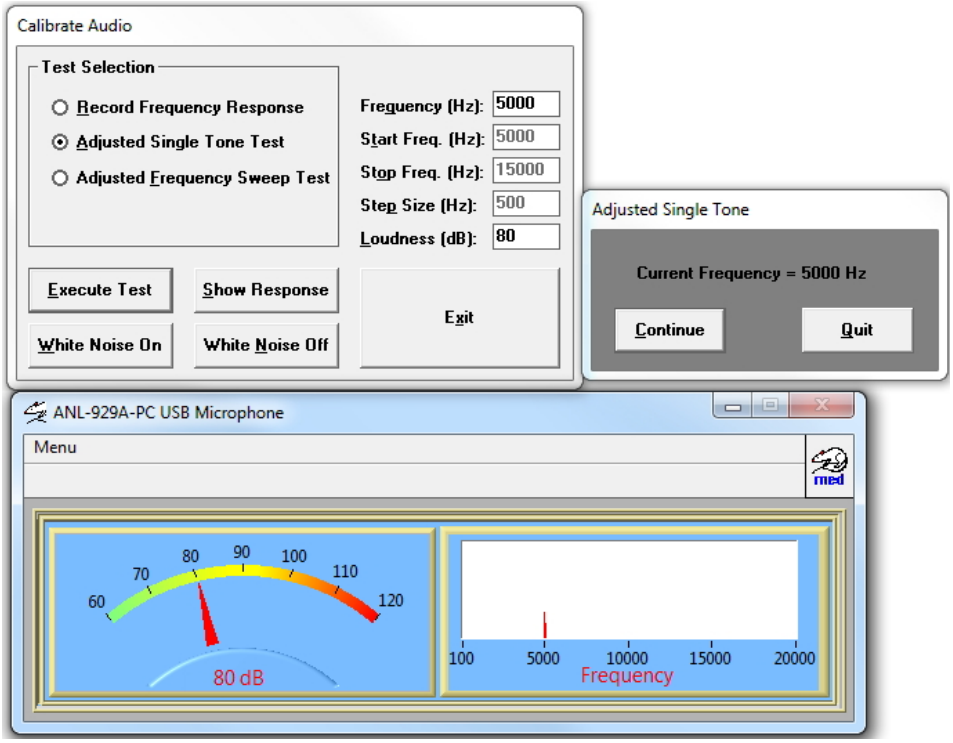
Figure 4-8 - Stimulus Test Screen



Calibrating Additional Chambers

1. Chamber 1 is used as a reference when calibrating additional chambers. For this reason it is important to position and orient the microphone in subsequent chambers precisely as it was in chamber 1.
2. To match additional chambers to Chamber 1, place the microphone and holder in the next chamber to be calibrated exactly as it was in Chamber 1 and select **Adjusted Single Tone Test** from the Calibrate Audio screen. Enter the median pure tone frequency that will be used in the **Frequency (Hz)** field and enter the desired volume in the **Loudness (dB)** field.
3. Select **Execute Test** and the screen shown in Figure 4-9 will appear.

Figure 4-9 - Adjusted Single Tone Test



4. A tone will now be generated at the desired Frequency (Hz). Using the Sound Level Meter software, compare the actual dB level being displayed to the set dB level.
5. Repeat steps 1 – 4 for each of the remaining chambers and use the acoustic startle adjustment knob (right hand knob, Figure 4-3) on the top of the PHM-255A to adjust the actual dB level until it is equal to the set dB level.

It is important to note that the human ear will incorrectly perceive certain frequencies to be louder than others, even though a dB meter will show the same loudness level for both.

Input Calibration

Proper calibration of the load cell ensures consistent measurements across multiple chambers. Startle Reflex software utilizes an analog-to-digital converter that converts the analog voltage signal from the startle sensor to a digital unit having a value between –2048 and +2048.

Use the following calibration procedure to ensure that each Startle Reflex chamber produces similar data output.

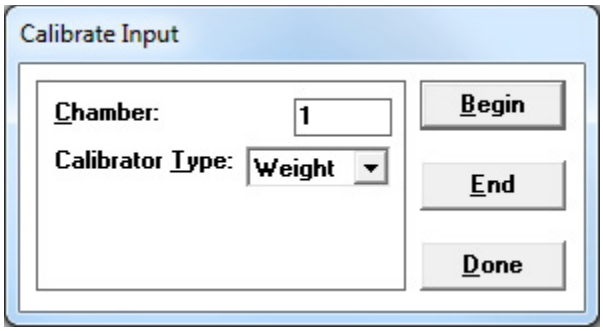
Note: Some older versions of the hardware require the use of a spinner calibrator. Please refer to **Chapter 9, Alternate Calibrate Input Utility** for input calibration using a spinner.

The input calibration procedure has two components:

- Adjust Tare (offset) so that the signal amplitude is zero over a range of gain settings. The Tare should be adjusted while only the animal holder is on the load cell platform. This ensures a consistent reading of zero is obtained with no applied force.
- Apply a known weight and adjust the gain so that the signal amplitude is at the same reference value in all chambers. This procedure ensures that a given amount of force will result in the same signal in all chambers.

1. From the **Hardware** Menu, select **Calibrate Input**. Enter the number of the chamber to calibrate in the **Chamber** field and select **Weight** from the **Calibrator Type** drop down menu (Figure 4-10).

Figure 4-10 - Calibrate Input

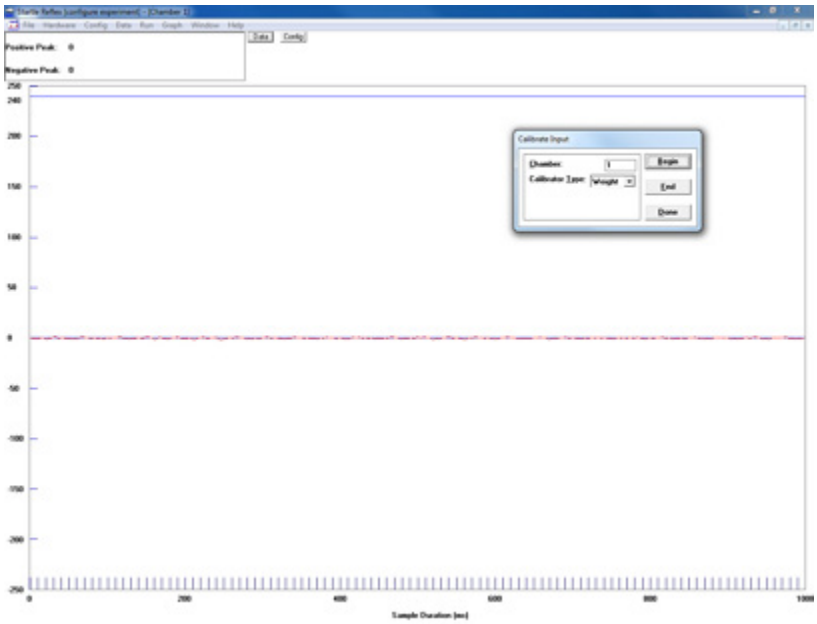


2. Set the **GAIN** knob on the front of the PHM-250B amplifier to **0.1** (Figure 4-11).

Figure 4-11 - PHM-250B amplifier

3. If working with mice, switch the **RAT/MOUSE** toggle switch to **MOUSE.** If working with rats, set the toggle switch to **RAT**.
4. Set the **CAL/RUN** switch to **CAL**.
5. Place the animal holder that will be used during the experiment on the load cell platform.
6. Click **Begin** (Figure 4-10). The screen shown in Figure 4-12will appear. The signal from the PHM-250B platform amplifier is displayed as a red line.

Figure 4-12 - Amplifier Signal at 0 Marker



7. Use a small screwdriver to adjust the **TARE** potentiometer located on the front of the PHM-250B amplifier until the signal is as close to zero as possible. Some slight fluctuations are normal, as the signal from the load cell is affected by environmental conditions such as air currents and vibrations.
8. Turn the **TARE** potentiometer slowly clockwise to increase the output amplitude and move the red line up. Turn the **TARE** potentiometer slowly counter-clockwise to decrease the output amplitude.

NOTES: Turn the **TARE** potentiometer gently and very slowly, as it is possible to turn it too far, and not be able to adjust the signal any further. If you have turned the potentiometer too far, you may hear a faint click upon each revolution. If this occurs, slowly turn in the opposite direction.

If the signal does not respond as the potentiometer is turned, ensure that the same chamber listed in the **Calibrate Input** dialog matches the chamber being tested. Trace the cable from the PHM-250B back to the interface cabinet to validate the chamber number. Reduce the gain, and turn the potentiometer more slowly.

9. Increase the **GAIN** knob on the front of the PHM-250B amplifier to 2.0.
10. Adjust the **TARE** potentiometer until the signal is as close to zero as possible.
11. Increase the **GAIN** knob on the front of the PHM-250B amplifier to 3.0.
12. Adjust the **TARE** potentiometer until the signal is as close to zero as possible.
13. Repeat steps 9-10, increasing gain in increments, until the amplifier is tared at the highest setting desired.

14. Decrease the **GAIN** to 1.0. The load cell signal should stay near zero, even while you are decreasing the gain.
15. To calibrate the platform for mice, place 40 grams of weight inside the animal holder (on the load cell platform). For rat calibration, use 300 grams.

NOTE: These are typical weights used, but any known weight can be used.

For a long-term experiment that begins with juvenile animals and ends with adult animals, be sure to calibrate using test weight that is appropriate for an adult animal.

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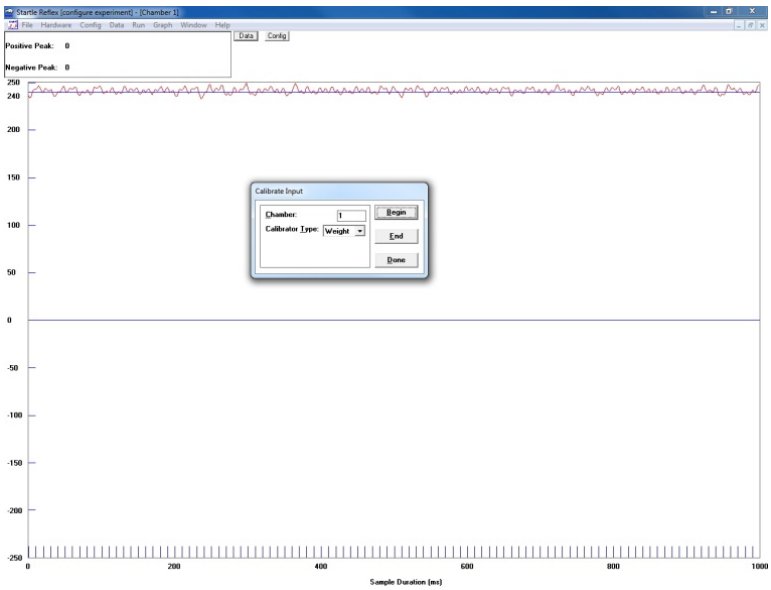
16. Adjust the gain knob on the front of the PHM-250B until the signal is in line with the 240 marker on the vertical scale on the left of the screen, as shown in Figure 4-13. (Vertical scale units are arbitrary.)
17. Remove calibration weight from the test platform and verify that the signal returns to zero.

Figure 4-13 - Amplifier Signal at 240 Marker



18. Set the **CAL/RUN** switch on the PHM-250B to **RUN** and click **DONE**.
19. Repeat this procedure for each Startle Platform.

NOTE: It is best to run a few test experiments with animals within an expected startle response range for your studies before acquiring data. This is to ensure your input calibration is within the ideal range for your particular experiments, as different individual animals or groups of animals may have different startle ranges or weights.

If gain is set too low:

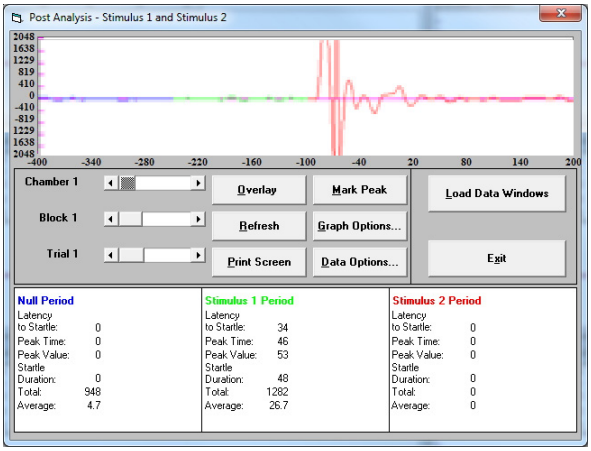
If gain is set too low, the system may report reduced or absent startle response. To fix this, re-calibrate using a decreased amount of test weight. Then, adjust gain knob on the PHM-250B until the signal is in line with the 240 marker on the vertical scale.

Figure 4-14 - Gain too low: Reduced or Absent Startle

If gain is set too high:

If gain is set too high, you may observe "clipping", which occurs when the amplifier is attempting to produce a response that exceeds its maximum voltage capability. To fix this, re-calibrate using increased test weight.

Figure 4-15 - Gain too high: Clipping



NOTE: If you have re-calibrated any one chamber with a different amount of test weight, you must re-calibrate all the other chambers with the same amount of test weight.

CHAPTER 5 | RUNNING EXPERIMENTS

The Startle Reflex system allows investigators to set up programs for performing basic startle reflex experiments as well as Prepulse Inhibition of Startle (PPI) and Fear-Potentiated Startle (FPS) experiments.

Sample Experiments

This chapter presents walkthrough examples of programming both PPI and FPS experiments in mice, and will serve to familiarize the user with the process of setting up and running such experiments. While the parameters used in these examples are based on guidelines devised by Dr. William A. Falls in the Psychology Department at the University of Vermont, Burlington, VT (see published references at the end of this chapter) they may not be suitable for the user's particular application. The user should conduct their own research to determine the appropriate parameters needed for their field of study.

Glossary of Important Terms

Acclimation Duration: The period (in minutes) during which the program will be running but no stimuli are presented and no data is collected.

Block: A phase of the experiment. An experiment contains up to 3 blocks. A block can contain up to 100 trials. An experiment that contains logical sequential components (eg. Training or Testing) can be separated into corresponding blocks.

Background Noise: A constant source of sound that is not the main stimulus. Background noise is optional during acclimation.

Experiment Table: Is used to configure the exact trial sequence and parameters for each trial. See Figure 5-5.

Inter-Trial Interval: The time (in seconds) from the end of the previous trial to the beginning of the next trial.

Null Period: Time period prior to any stimulus presentation during which data is collected. Data collected during the Null period determines whether the startle response is valid; in many trials, the subject moves prior to the startle stimulus presentation. Movement in the Null period will invalidate the response latency and amplitude in the ensuing Startle period.

Prepulse/Startle Delay: The time period (in milliseconds) between the onset of the prepulse and startle stimulus. There is a maximum of 1000 milliseconds (see Figure 5-4).

Prepulse Stimulus Duration: In prepulse inhibition of startle (PPI), the period (in milliseconds) during which a noise burst is presented prior to the startle stimulus.

Prepulse Stimulus Rise/Fall Time: Length of time (in milliseconds) for prepulse tone to rise to and fall from the maximum dB level; used to suppress pops in the speaker when there is a sudden change in signal.

Pre-Stimulus 2 Record: Duration of recording time prior to Stimulus 2. Similar to the Null Period, data collected during this period determines whether the startle response is valid.

Startle Period: The period (in milliseconds) during which the loud noise burst is presented.

Startle Stimulus Duration: The duration (in milliseconds) of the Startle Period.

Stimulus 1 Duration: In fear-potentiated startle (FPS), the duration of the Conditioned Stimulus tone.

Stimulus 2 Duration: In fear-potentiated startle (FPS), the duration of the aversive stimulus.

Data Acquisition Period: The amount of time that data will be recorded during each trial.

Tone: Sine wave that is produced at the specified frequency and volume.

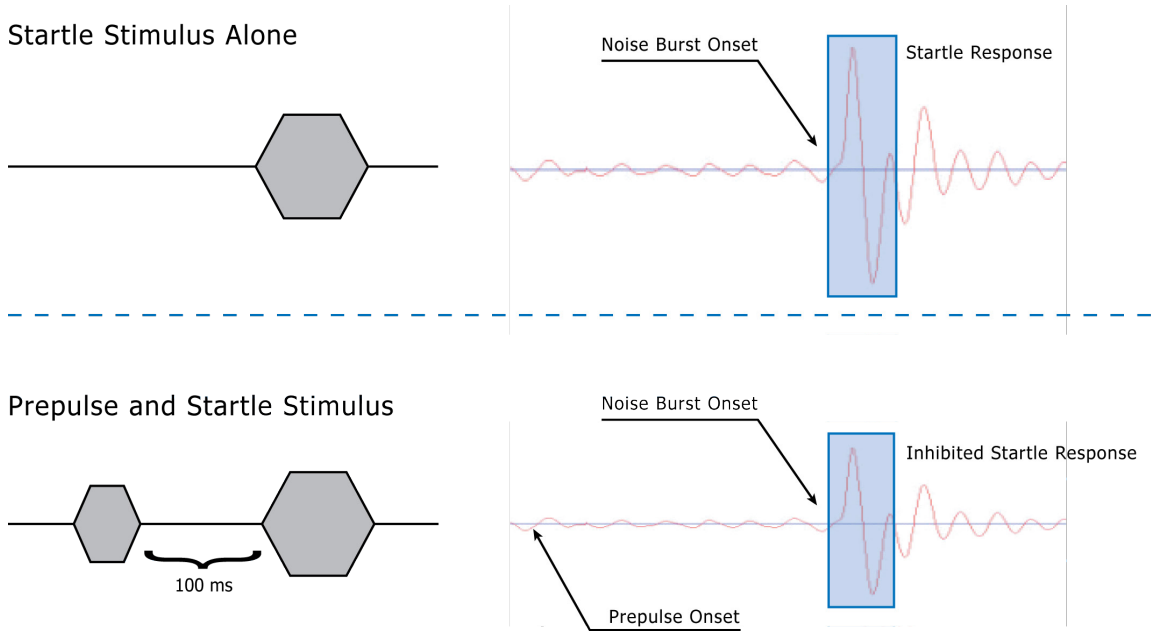
White Noise: Sound containing many frequencies at the same intensity.

Prepulse Inhibition (PPI)

Prepulse inhibition (PPI) of the startle response is a phenomenon in which a short pulse of sound presented just prior to a startle stimulus (such as a burst of white noise at a high decibel level) dampens the subsequent startle response.

This phenomenon has been observed in mice, rats and humans. When studied in rodents, PPI serves as a model for a number of disorders, including deficits in attention/sensory gating or sensorineural hearing loss.

Figure 5-1 - Prepulse Inhibition (PPI)

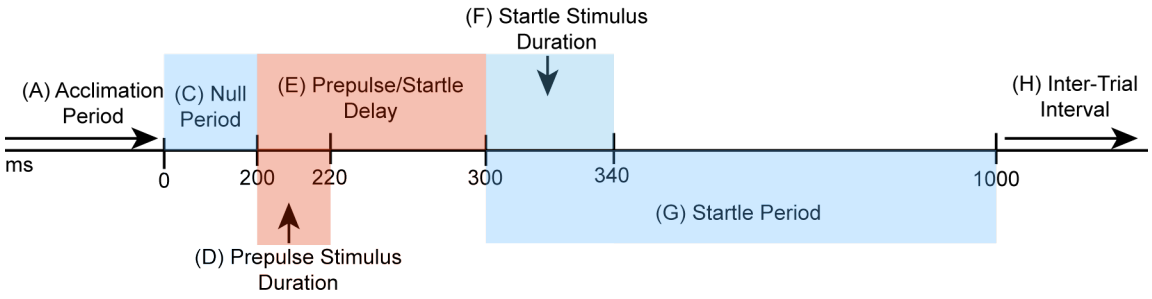


W.A. Falls 2003

PPI Timing

Figure 5-2 illustrates the framework for one trial of the sample PPI experiment that will be described in detail in the next section.

Figure 5-2 - Startle Reflex Timeline for PPI



Definitions of each time period depicted in Figure 5-2 are as follows:

(A) Acclimation = The period (in minutes) during which the program is running, but no stimuli are presented and no data are collected.

(B) Data Acquisition Period = User-set value that defines the time period in which data are recorded. It consists of the Null Period, the Prepulse/Startle Delay and the Startle Period.

Note: Start with Data Acquisition Period (Figure 5-3) at 1000 ms to ensure that data are collected after the startle stimulus is presented.

(C) Null Period = User-set value used to establish a baseline of the animal's current behavior. The Null Period is 200 ms in the example timeline above.

(D) Prepulse Stimulus Duration = User-set value that is the length of the prepulse tone. A 20 ms prepulse tone is used in the example above.

(E) Prepulse/Startle Delay = User-set value that is the time between the onset of the prepulse stimulus and the onset of the startle stimulus, see Figure 5-1. The Prepulse/Startle Delay must be greater than, or equal to the Prepulse Stimulus Duration. In the example above the Prepulse/Startle Delay is set to 100 ms.

(F) Startle Stimulus Duration = User-set value that determines the duration of the noise burst startle stimulus (Figure 5-5). It is set to 40 ms in the example above.

(G) Startle Period = The Startle Period begins at the start of the Startle Stimulus. The Startle Period duration is equal to the Data Acquisition Period minus the Null Period minus the Prepulse/Startle Delay. The Startle Period would be 700 ms in the example.

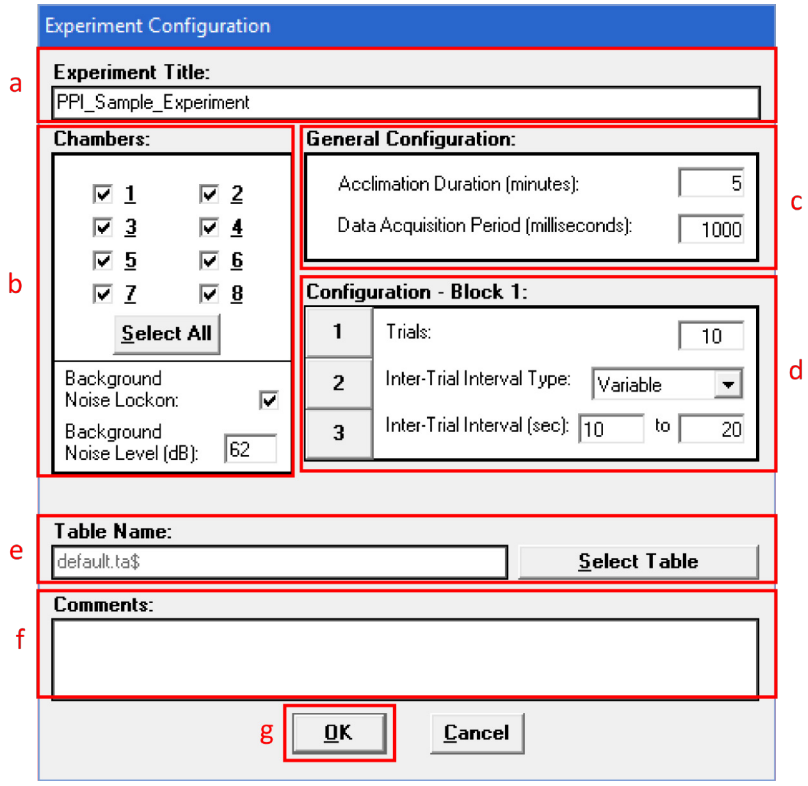
Prepulse Inhibition (PPI) Sample Experiment

1. Open the startle software and select **File** > **New** from the menu. This will open an **Experiment Configuration** screen (See Figure 5-3). This screen is used to establish the initial settings for the experiment, including Experiment Title, selection of Chamber(s) to be

used, Acclimation Duration and Data Acquisition Period, number of Trials per Block, length of Inter-Trial Interval (ITI), and a variable or fixed ITI.

Changes to this configuration can be made later by selecting **Config/Experiment Config** from the menu.

Figure 5-3 - Experiment Configuration (PPI)



a. Enter the name of the experiment in the **Experiment Title** field;

b. In the **Chambers** field:

   i. Select the chambers to be used in the experiment;

   ii. Select the **Background Noise Lockon** box;

   iii. Enter 62 in the **Background Noise Level (dB)** box;

c. In the **General Configuration** field:

   i. Enter 5 in the **Acclimation Duration (min)** box;

   ii. Enter 1000 in the **Data Acquisition Period (ms)** box;

d. In the **Configuration – Block (#)** field, the buttons marked 1, 2, and 3 correspond to different blocks, or phases, of the experiment. PPI has 3 phases and therefore information will be configured for Block 1, Block 2 and Block 3, with respect to number of trials per block, the ITI type and ITI Duration.

Block 1 represents an initial phase, consisting of 10 trials in which the startle-eliciting noise burst stimulus is presented alone (i.e. with NO prepulse), to determine a baseline response to a startle stimulus.

Block 2 is the actual PPI testing phase that has 28 trials. Four trials consist of the noise burst startle stimulus alone, 16 trials consist of a pre-pulse stimulus (at one of 4 different intensities) followed by the noise burst startle stimulus, four trials of prepulse alone (one at each intensity) and four null trials (no stimulus presented).

Block 3 is the same as Block 1 in order to ensure that no habituation has taken place.

i. To Configure Block 1: click on the **1** button, making sure the field title reads "Configuration – Block 1"; enter 10 in the **Trials** box, choose 'Variable' from the **Inter-Trial Interval Type** drop down menu and enter 10 and 20 in the **Inter-Trial Interval (sec)** boxes;

ii. To Configure Block 2: click on the **2** button, making sure the field title reads "Configuration – Block 2"; enter 28 in the **Trials** box, choose "Variable" from the **Inter-Trial Interval Type** drop down menu and enter 10 and 20 in the **Inter-Trial Interval (sec)** boxes;

iii. To Configure Block 3: click on the **3** button, making sure the field title reads "Configuration – Block 3"; enter 10 in the **Trials** box, choose 'Variable' from the **Inter-Trial Interval Type** drop down menu and enter 10 to 20 in the **Inter-Trial Interval (sec)** box.

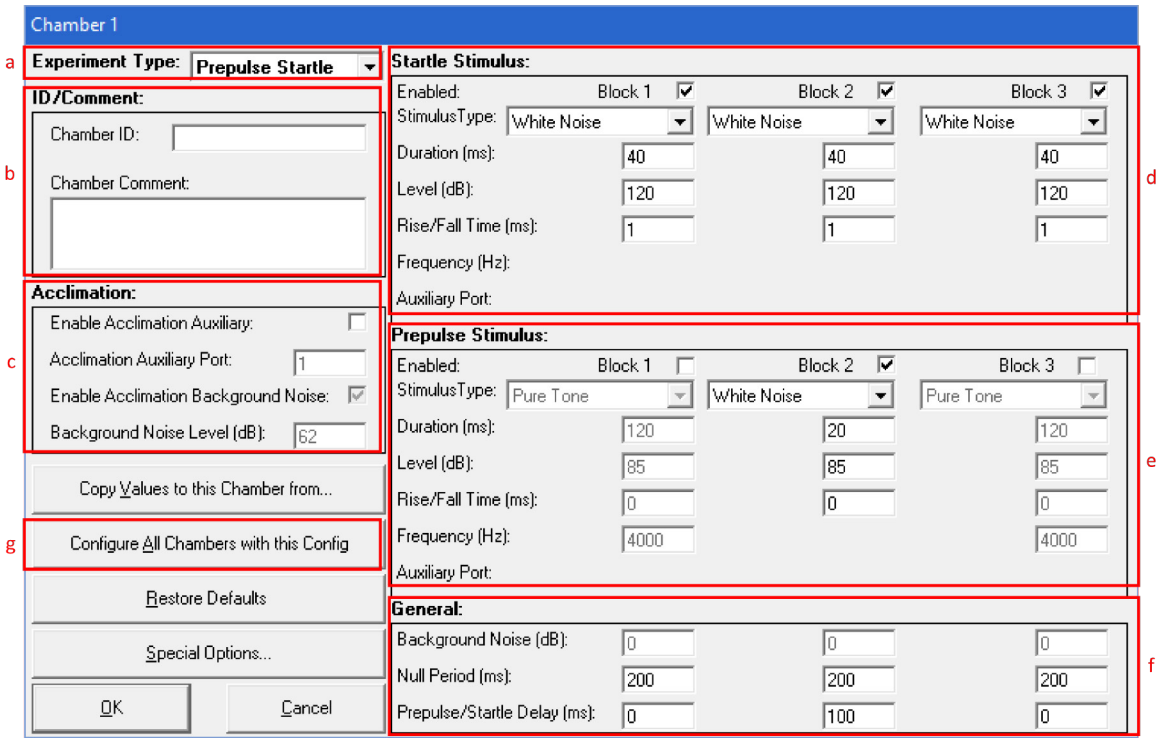
e. Entering a name in the **Table Name** field will not be done in this window, it will be done at a later step in which an Experiment Table is set up, and will be the default table for this example experiment.

f. In the **Comments** field, enter any information that will be helpful in identifying the parameters of this experiment.

g. Click **OK** and a "Save As" screen may appear. Click the **Cancel** button, as this information will be saved at a later step. The information will not be lost.

2. Select **Config** > **Chamber Config** from the menu. This will open a small "Chamber Config" window and "Chamber (#)" will be highlighted; click **OK**. This will open a **Chamber Configuration** screen, with "Chamber (#)" in the header, as shown in Figure 5-4. This screen is used to set up individual chamber parameters for the different blocks, or phases, of the PPI experiment.

Figure 5-4 - Chamber Configuration Screen for PPI



a. Choose “Prepulse Startle” from the **Experiment Type** drop-down menu;

b. In the **ID/Comment** field, enter any pertinent information, such as animal info, etc.

c. In the **Acclimation** field:

   i) Do not change the default settings, shown above.

d. In the **Startle Stimulus** field:

   i) Choose “White Noise” from the **Stimulus Type** drop down menus;

   ii) Enter 40 in the three **Duration (ms)** boxes;

   iii) Enter 120 in the three **Level (dB)** boxes;

   iv) Enter 1 in the three **Rise/Fall Time (ms)** boxes.

e. In the **Prepulse Stimulus** field:

   i) Enable only Block 2 by checking the **Block 2** box in the **Enabled** section, and unchecking Blocks 1 and 3;

   ii) Select “White Noise” from the **Stimulus Type** drop down menu;

   iii) Enter 20 in the **Duration (ms)** box;

   iv) Leave the default value in the **Level (dB)** box at this point; the order of the varying intensities will be set up later in these instructions;

   v) Enter 1 in the **Rise/Fall Time (ms)** box;

f. In the **General** field:

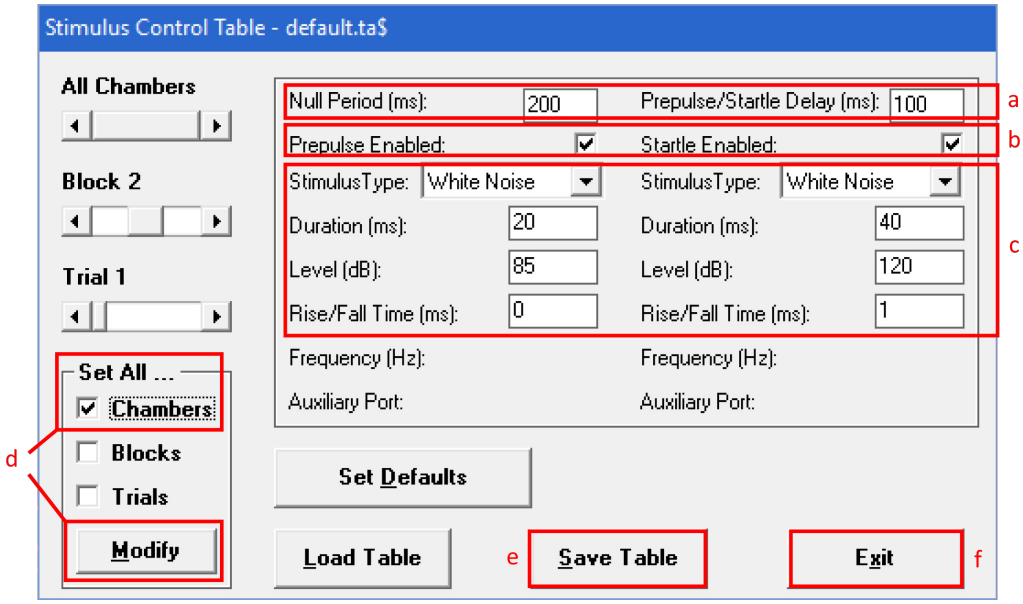
i) Enter 200 in the **Null Period (ms)** box for all three blocks;

ii) Enter 100 in the **Prepulse/Startle Delay (ms)** box for block 2.

g. Click the **Configure All Chambers with this Config** button and click **OK** in the confirmation window, then click **OK** in the lower left corner of the Chamber Configuration window.

3. Select **Config** > **Experiment Table** from the menu bar to open the **Experiment Table** screen shown in Figure 5-5. For this example, all values for Blocks 1 and 3 were set in the chamber configuration screen, only Block 2 needs to be configured. Use the selection bars on the left to select Block 2.

Figure 5-5 - Experiment Table Screen (PPI)



a. Ensure the **Null Period (ms)** is set to 200 and the **Prepulse/Startle Delay (ms)** is set to 100;

b. Ensure the **Prepulse Enabled** and **Startle Enabled** check boxes are checked;

c. Enter the individual trial parameters for Block 2 outlined in Table 6-1. The actual PPI experiment is set up in Block 2 and consists of 28 pseudo-randomized trials pairing different decibels of prepulse stimulus or no prepulse stimulus with the noise burst startle stimulus.

d. In the **Set All…** field, select **Chambers** and click the **Modify** button;

e. Click on the **Save Table** button, choose a file name and save the table;

f. Click the **Exit** button on the 'Stimulus Control Table' window.

4. Next select **Config > Experiment Config** from the menu. This will open the **Experiment Configuration** window (Figure 5-3).
   a. Click on the **Select Table** button to find and select the experiment table that was just set up, click **Open**, then **OK** on the Experiment Configuration window.
   b. Finally, select **File > Save Config** from the menu bar and choose a name for this program. It will be saved with an .exp extension.

Table 5-1 – Manually Entered Trial Parameters for PPI

	Trial	Pre-pulse Enabled	Startle Enabled	Stimulus Type		Duration (ms)		Level (dB)		Rise/Fall Time (ms)		Trial Type
				PP	S	PP	S	PP	S	PP	S	
Block 1	1	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	2	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	3	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	4	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	5	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	6	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	7	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	8	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	9	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	10	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
Block 2	1	√	√	WN	WN	20	40	67	120	1	1	Pre-pulse + Startle
	2	Un-√	Un-√	-	-	-	-	-	-	-	-	Null
	3	√	Un-√	WN	-	20	-	70	-	1	-	Pre-pulse Only
	4	√	√	WN	WN	20	40	73	120	1	1	Pre-pulse + Startle
	5	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	6	√	√	WN	WN	20	40	70	120	1	1	Pre-pulse + Startle
	7	√	√	WN	WN	20	40	76	120	1	1	Pre-pulse + Startle
	8	Un-√	Un-√	-	-	-	-	-	-	-	-	Null
	9	√	√	WN	WN	20	40	73	120	1	1	Pre-pulse + Startle
	10	√	√	WN	WN	20	40	67	120	1	1	Pre-pulse + Startle
	11	√	Un-√	WN	-	20	-	76	-	1	-	Pre-pulse Only
	12	√	√	WN	WN	20	40	70	120	1	1	Pre-pulse + Startle
	13	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	14	√	√	WN	WN	20	40	76	120	1	1	Pre-pulse + Startle
	15	√	√	WN	WN	20	40	67	120	1	1	Pre-pulse + Startle
	16	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	17	√	√	WN	WN	20	40	76	120	1	1	Pre-pulse + Startle
	18	Un-√	Un-√	-	-	-	-	-	-	-	-	Null
	19	√	√	WN	WN	20	40	73	120	1	1	Pre-pulse + Startle
	20	√	Un-√	WN	-	20	-	67	-	1	-	Pre-pulse Only
	21	√	√	WN	WN	20	40	70	120	1	1	Pre-pulse + Startle
	22	√	Un-√	WN	-	20	-	73	-	1	-	Pre-pulse Only
	23	√	√	WN	WN	20	40	70	120	1	1	Pre-pulse + Startle
	24	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	25	√	√	WN	WN	20	40	76	120	1	1	Pre-pulse + Startle
	26	Un-√	Un-√	-	-	-	-	-	-	-	-	Null
	27	√	√	WN	WN	20	40	67	120	1	1	Pre-pulse + Startle
	28	√	√	WN	WN	20	40	73	120	1	1	Pre-pulse + Startle

WN = White Noise; PP = Pre-pulse; S = Startle

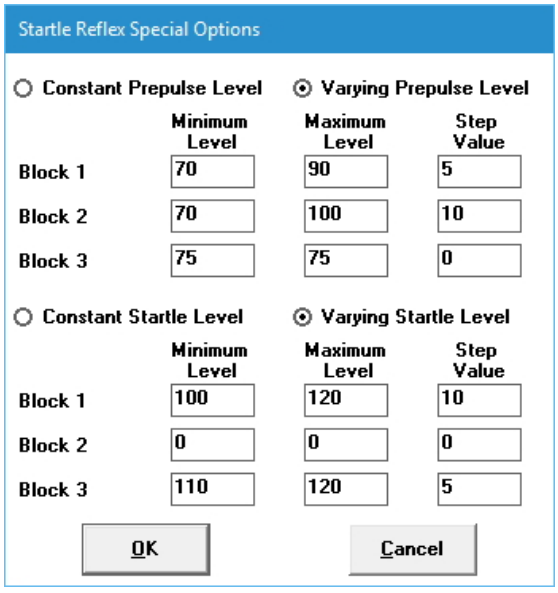
	Trial	Pre-pulse Enabled	Startle Enabled	Stimulus Type PP	Stimulus Type S	Duration (ms) PP	Duration (ms) S	Level (dB) PP	Level (dB) S	Rise/Fall Time (ms) PP	Rise/Fall Time (ms) S	Trial Type
Block 3	1	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	2	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	3	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	4	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	5	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	6	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	7	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	8	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	9	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only
	10	Un-√	√	-	WN	-	40	-	120	-	1	Startle Stimulus Only

WN = White Noise; PP = Pre-pulse; S = Startle

Special Options Utility

In the lower left corner of the Chamber Configuration window (**Config > Chamber Config**) is a "Special Options" button. When this button is clicked, it brings up a **Special Options** screen (Figure 5-6) with "Startle Reflex Special Options" in the header. This option allows the user to vary levels for the Prepulse and Startle stimuli in Prepulse Inhibition experiments.

Figure 5-6 - Startle Reflex Special Options



If only the **Varying Prepulse Level** radio button is selected, the program will increment the Prepulse Level on each trial as set in the **Minimum Level**, **Maximum Level**, and **Step Value** boxes for the relevant Blocks. For example, using the values in Figure 5-6, for a Prepulse Inhibition program, for Block 1, the first trial would have a Prepulse Level of 70 dB, the next trial would have a Prepulse with a value 5 dB higher (75 dB) and so on, until trial 5, which would

reach the maximum value set, 90 dB. If there are more than 5 trials in Block 1, the Prepulse Level will return to 70 dB and continue to cycle through the settings as just described.

If only the **Varying Startle Level** radio button is selected, the Startle Level will vary according to the settings depicted in Figure 5-6, in the same fashion as described for "Varying Prepulse Level".

If both the **Varying Prepulse Level** and **Varying Startle Level** are selected for the settings depicted in Figure 5-6, the trials begin with the minimum Level Prepulse and Startle Levels and increment through all the Prepulse Levels as set, then it will increment to the next Startle Level and again, increment through all the Prepulse Levels as set. For example: the first trial in Block 1 will have a Prepulse Level of 70 dB and a Startle Level of 100 dB, the second trial will have a Prepulse Level of 75 dB and a Startle Level of 100 dB…the fifth trial with have a Prepulse Level of 90 dB (Maximum Level set) and the startle Level will still be 100 dB. The sixth trial the Prepulse Level will be at the Minimum Level setting, 70 dB while the Startle Level will be at the next increment level of 110 dB. Trials 7 – 10 will have the Prepulse Level increment as just described, with the Startle Level remaining at 110 dB. This pattern will continue until trial fifteen, when the Prepulse Level and the Startle Levels will reach the Maximum Levels set: 90 dB and 120 dB, respectively. If there are more than 15 trials, the pattern will repeat, starting with the Minimum Level values set.

If a combination of varying and constant blocks are desired, set the varying blocks as described above and set constant blocks by entering "0" in the **Step Value** boxes for the blocks that should be kept constant. If a Stimulus in a block should be disabled, enter "0" in the **Maximum Level** and **Minimum Level** boxes for the appropriate block.

When finished setting values in the Special Options window, click the **OK** button. This saves the settings. If "Varying Prepulse Level" has been selected, the **Level (dB)** box in the "Prepulse Startle" field of the associated **Chamber Configuration Window** will be "grayed-out". If "Varying Startle Level" was selected, then the same will be true for the **Level (dB)** boxes in the "Startle Stimulus" field of the associated **Chamber Configuration Window**.

Fear-Potentiated Startle (FPS)

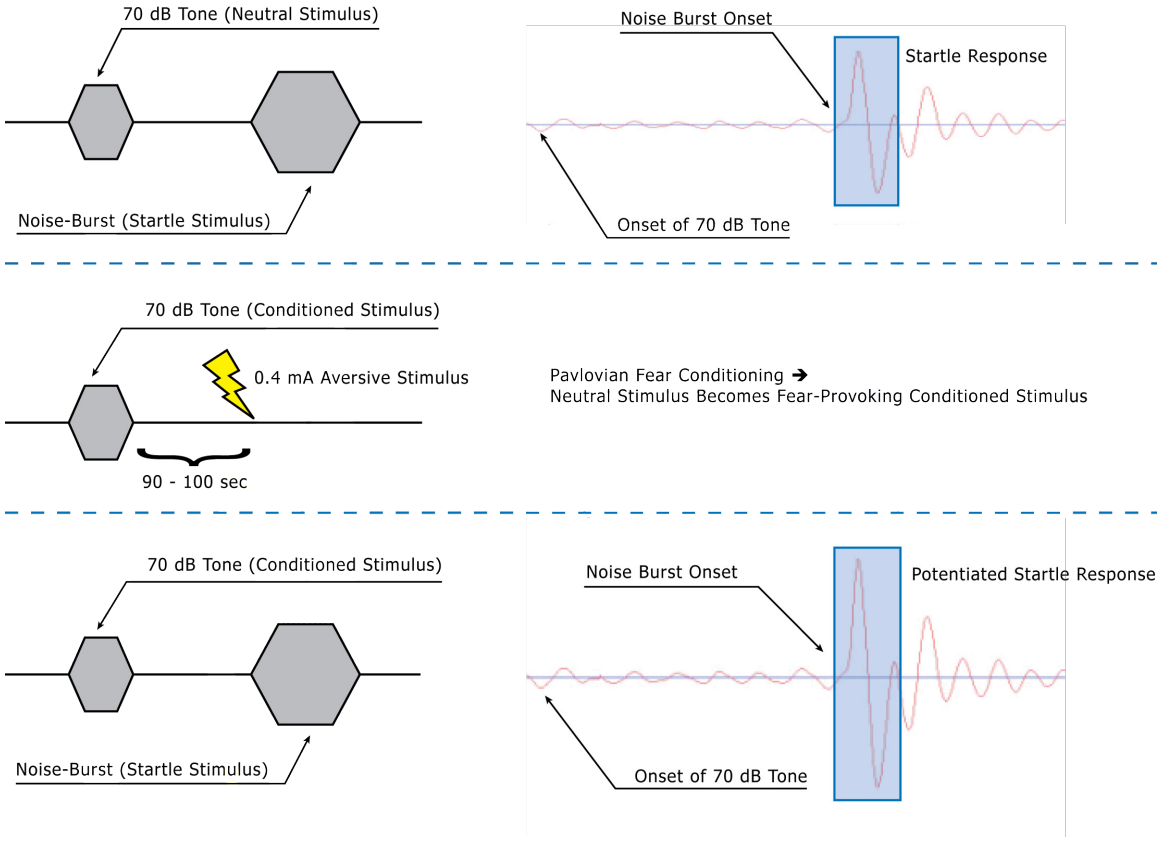
Fear-Potentiated startle (FPS) is a phenomenon in which the startle response to an acoustic stimulus is increased (potentiated) in the presence of a Conditioned Stimulus (CS). The CS is a normally neutral stimulus (usually a tone) that has been introduced together with an aversive stimulus (a shock) during training. This Pavlovian conditioning is a learned response.

FPS studies begin with a training protocol, consisting of a simple pairing of a tone with an aversive stimulus. Once training is complete, testing is performed. Testing consists of the presentation of the CS tone with the white noise burst startle stimulus at pseudo-random intervals.

The fear-potentiated acoustic startle reflex is observed in both rodents and humans, thus making it a useful tool for investigating learning and memory, including long-term memory.

Both FPS training and FPS testing sample experiments are described in detail below (from W.A. Falls 2002).

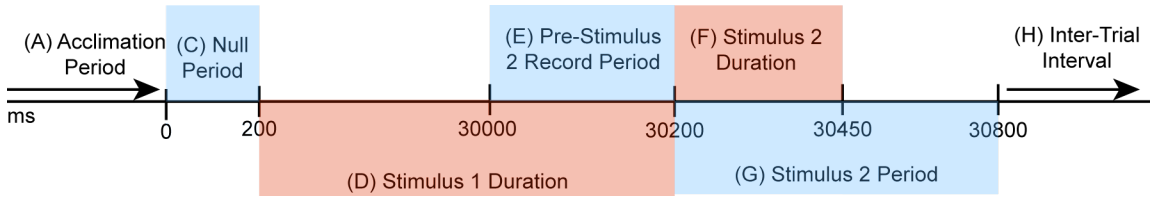
Figure 5-7 - Fear-Potentiated Startle (FPS)



W.A. Falls 2002

FPS Timing

Figure 5-8 - Startle Reflex Timeline for Potentiated Experiments



Definitions of the time periods in Figure 5-8:

(A) Acclimation = The period (in minutes) during which the program is running, but no stimuli are presented and no data are collected.

(B) Data Acquisition Period = A user-set value that defines the time period in which data is recorded. It consists of the Null Period, the Pre-Stimulus 2 Record Period, and the Stimulus 2 Period.

Note: Start with a Data Acquisition Period (Figure 5-9) of 1000 ms to ensure that data is collected after the startle stimulus is presented.

(C) Null Period = A user-set value used to establish a baseline of the animal's current behavior. The Null Period is 200 ms in the example timeline above.

(D) Stimulus 1 Duration = A user-set value that defines the duration of the Conditioned Stimulus tone, set to 30000 ms in the sample timeline.

(E) Pre-Stimulus 2 Record = A user-set value that defines the duration of the Duration of recording time prior to Stimulus 2, set to 200 ms in the sample timeline.

Note: Data recorded during Pre-stimulus 2 Record will be defined as "Stimulus 1 Time Period" when saving data later.

(F) Stimulus 2 Duration = A user-set value that defines the duration of the Aversive Stimulus, set to 250 ms in the sample timeline.

(G) Stimulus 2 Period = A system-calculated value that is equal to the Data Acquisition Period (1000 ms) minus the Null Period (200 ms) minus the Pre-Stimulus 2 Record (200 ms) = 600 ms in the sample timeline above.

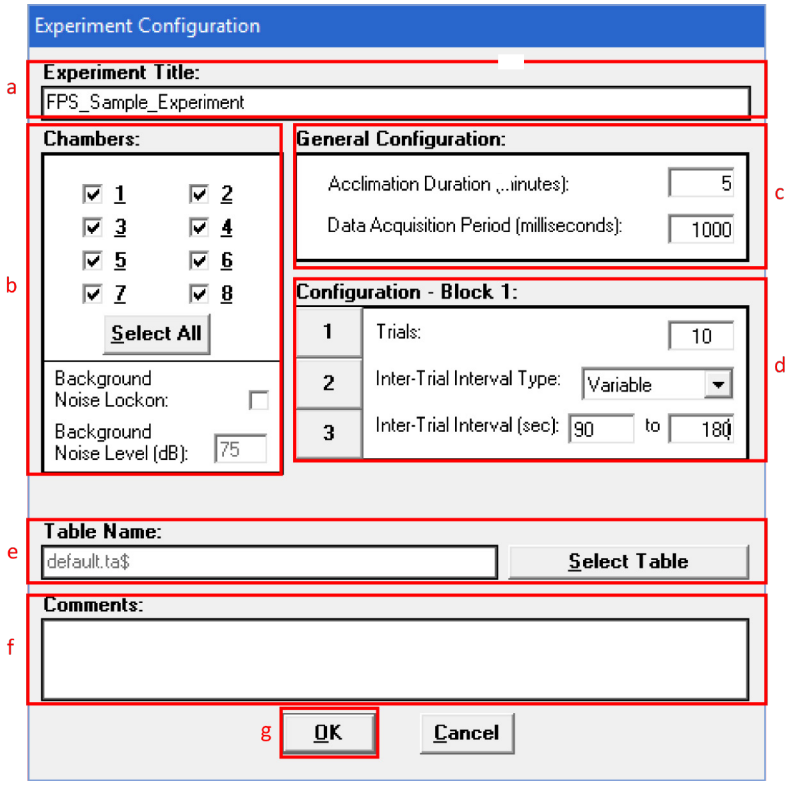
(H) Inter-Trial Interval = Latency between successive trials. May be variable or fixed.

Fear-Potentiated Startle (FPS) Training Sample Experiment

1. Open the Startle Reflex software and select **File > New**. This will open the **Experiment Configuration** window, shown in Figure 5-9. This window is used to define the initial settings for the experiment, such as experiment title, chamber(s) to be used, length of acclimation, Data Acquisition Period, number of trials per block and length of inter-trial interval (ITI), as well as choosing a variable or fixed ITI. Changes to this configuration may be made later by selecting **Config > Experiment Config**.

NOTE: Do not use the **Hardware > Training** option to set up this training program, see Chapter 4 for use of this utility.

Figure 5-9 - Experiment Configuration Screen for FPS



a. Enter a name for the experiment in the **Experiment Title** field;

b. In the **Chambers** field:

   i) Select the chambers to be used in the experiment;

   ii) De-select (uncheck) the **Background Noise Lockon** box;

c. In the **General Configuration** field:

   i) Enter 5 in the **Acclimation Duration (min)** box (this is the period during which the program will be running but no stimuli are presented);

ii) Enter 1000 in the **Data Acquisition Period (ms)** box;

d. In the **Configuration – Block (#)** field,

i) The buttons marked 1, 2, and 3 are to select different blocks, or phases, of the experiment. FPS training has only 1 phase and therefore information will be configured for Block 1 with respect to number of trials per block, the ITI type and duration. Training will consist of a 5 min acclimation period (set up in previous step) followed by 10 trials in which the Conditioned Stimulus (CS), a tone (70 dB; 12000 Hz) lasting 30 seconds, is paired with the unconditioned stimulus, a 0.4 mA shock lasting 0.25 second. Pairings will be presented at a variable interval.

ii) To Configure Block 1:

(1) Click on the **1** button, make sure the field title reads "Configuration – Block 1";

(2) Enter 10 in the **Trials** box;

(3) Select 'Variable' from the **Inter-Trial Interval Type** drop down menu;

(4) Enter 90 and 180 in the **Inter-Trial Interval (sec)** boxes.

e. If the **Table Name** field is not set to "default.ta$", select the "default.ta$" table.

f. Enter any information that will be helpful to identify the parameters of this experiment in the **Comments** field;

g. Click **OK** and a 'Save As' box will pop-up, provide a meaningful experiment name and click **Save**.

2. Next, Select **Config > Chamber Config** to open the "Chamber Config" screen, "Chamber (#)" will be highlighted; click **OK**. The **Chamber Configuration** screen, Figure 5-10, is used to set up individual chamber parameters for the different blocks, or phases, of the FPS experiment (FPS training has only 1 phase).

a. Select 'Potentiated Startle' from the **Experiment Type** drop down menu;

b. Enter any pertinent information, such as animal info, etc. in the **ID/Comment** field;

c. No steps are required in the **Acclimation** field.

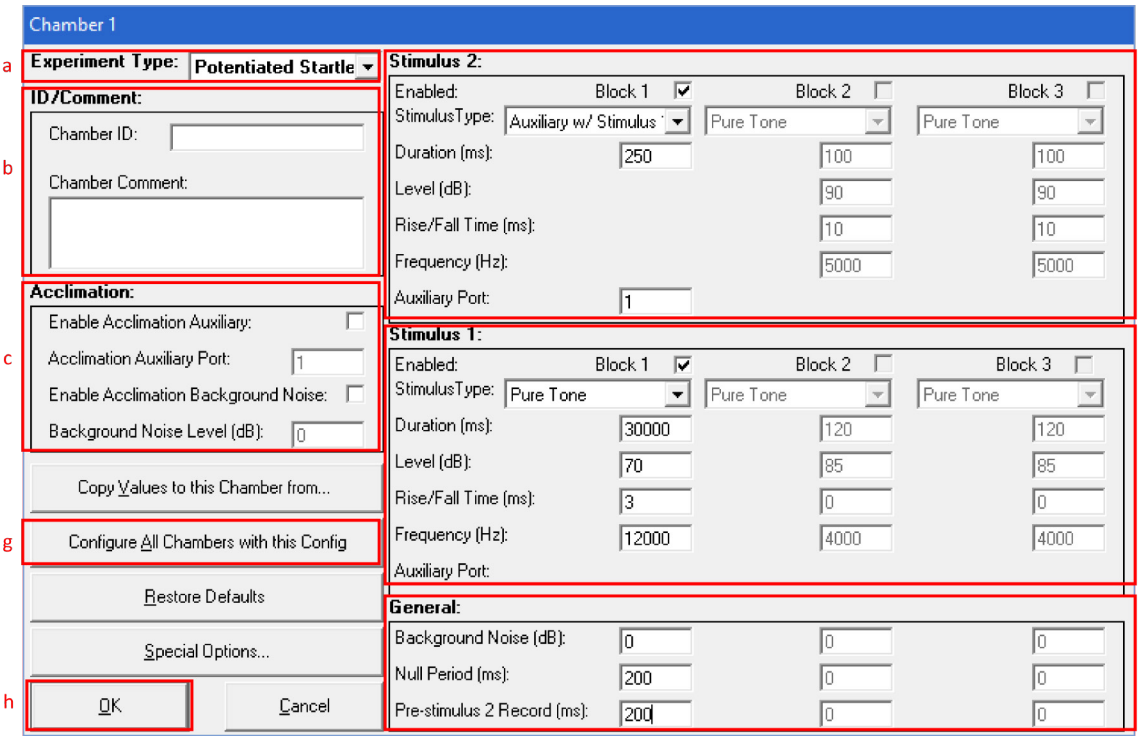
d. In the **Stimulus 2** field:

i) Verify that 'Auxiliary w/ Stimulus 1' is selected from the **Stimulus Type** drop down menu;

ii) Enter 250 in the **Duration (ms)** box;

iii) Enter the auxiliary port number on the PHM-255A that the ENV-414S unit is connected to.

Figure 5-10 - Chamber Configuration (FPS)



e. In the **Stimulus 1** field:

   i) Enter 30000 in the **Duration (ms)** box;

   ii) Enter 70 in the **Level (dB)** box;

   iii) Enter 3 in the **Rise/Fall Time (ms)** box;

   iv) Enter 12000 in the **Frequency (Hz)** box.

f. In the **General** field:

   i) Enter 0 in the **Background Noise (dB)** box;

   ii) Enter 200 in the **Null Period (ms)** box;

   iii) Enter 200 in the **Pre-stimulus 2 Record (ms)** box.

g. Click **Configure All Chambers with this Config**, click on **OK** in pop-up window, and then click **OK** button in lower left corner of the Chamber Configuration window.

h. Click **OK** to exit the Chamber Configuration screen.

Fear-Potentiated Startle (FPS) Testing Sample Experiment

The following is a sample FPS testing experiment. Such a testing experiment would normally follow the preceding training component.

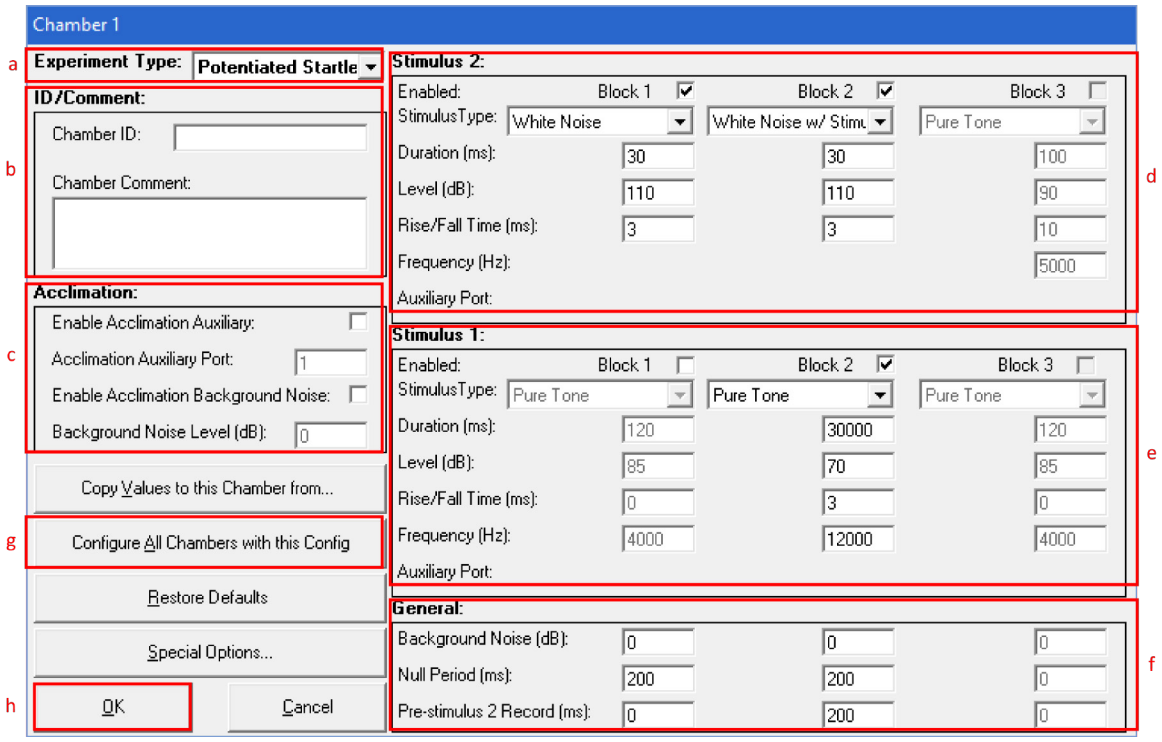
1. Open the startle software and select **File > New**. This will open an **Experiment Configuration** window (see Figure 5-9).
   a. Enter a name for the experiment in the **Experiment Title** field;
   b. In the **Chambers** field:
      i) Select the chambers to be used in the experiment;
      ii) De-select (uncheck) the **Background Noise Lockon** box;
   c. In the **General Configuration** field:
      i) Enter 5 in the **Acclimation Duration (min)** box (this is the period during which the program will be running but no stimuli are presented);
      ii) Enter 1000 in the **Data Acquisition Period (ms)** box;
      iii) This FPS testing experiment example will have two phases and therefore information will need to be configured for Block 1 and Block 2. Block 1 will be a 9 trial leader sequence phase to establish a stable startle baseline. The trials will consist of 3 presentations of stimuli at 3 different volumes in a pseudo-random order, without presentation of the conditioned stimulus (CS) tone. Block 2 has 3 different noise burst startle stimuli, each presented 6 times. Half of the block 2 trials will present a CS tone.
      iv) To Configure Block 1:
         (1) In the "Configuration Block (#)" field, click on the **1** button, be sure the field title reads "Configuration – Block 1";
         (2) Enter 9 in the **Trials** box;
         (3) Select 'Fixed' from the **Inter-Trial Interval Type** drop-down menu;
         (4) Enter 60 in the **Inter-Trial Interval (sec)** box.
      v) To Configure Block 2:
         (1) Click on the **2** button, be sure the field title reads "Configuration – Block 2";
         (2) Enter 18 in the **Trials** box;
         (3) Select 'Fixed' from the **Inter-Trial Interval Type** drop-down menu;
         (4) Enter 60 in the **Inter-Trial Interval (sec)** box.
   d. Again, selecting a name in the "Table Name" field will not be done in the **Experiment Configuration** window, it will be done at a later step in which an Experiment Table is set up, and will be the default table for this example experiment.

e. In the **Comments** field, enter any information that will be helpful to identify the parameters of this experiment.

f. Click **OK** and a "Save As" box will pop-up, provide a meaningful experiment title and click **Save**.

2. Next, Select **Config > Chamber Config**. This will open a small "Chamber Config" window and "Chamber (#)" will be highlighted; click **OK**. This will open a **Chamber Configuration** window, with "Chamber (#)" in the header (see Figure 5-11).

Figure 5-11 - FPS Testing Chamber Configuration Window



a. Verify that 'Potentiated Startle' from the **Experiment Type** drop down menu;

b. Enter any pertinent information, such as animal info, etc. in the **ID/Comment** field

c. No steps are required in the **Acclimation** field.

d. In the **Stimulus 2** field:

i) Enable Block 1 and Block 2 by selecting both **Block 1** and **Block 2** boxes in the **Enabled** section;

ii) For Block 1, choose 'White Noise' from the **Stimulus Type** drop down menu and for Block 2, choose 'White Noise w/ Stimulus 1' from the drop down menu;

iii) Enter 30 in the **Duration (ms)** box for both Blocks 1 and 2;

iv) Enter 110 in the **Level (dB)** box for both Blocks 1 and 2 and 3 in the **Rise/Fall Time (ms)** box for both Blocks 1 and 2.

e. In the **Stimulus 1** field:

i) Enable only Block 2 by checking the **Block 2** box in the **Enabled** section, and un-check **Block 1**;

ii) Select 'Pure Tone' from the **Stimulus Type** pull down menu;

iii) Enter 30000 in the **Duration (ms)** box;

iv) Enter 70 in the **Level (dB)** box;

v) Enter 3 in the **Rise/Fall Time (ms)** box;

vi) Enter 12000 in the **Frequency (Hz)** box.

f. In the **General** field:

i) Enter 200 in the **Null Period (ms)** box for both blocks;

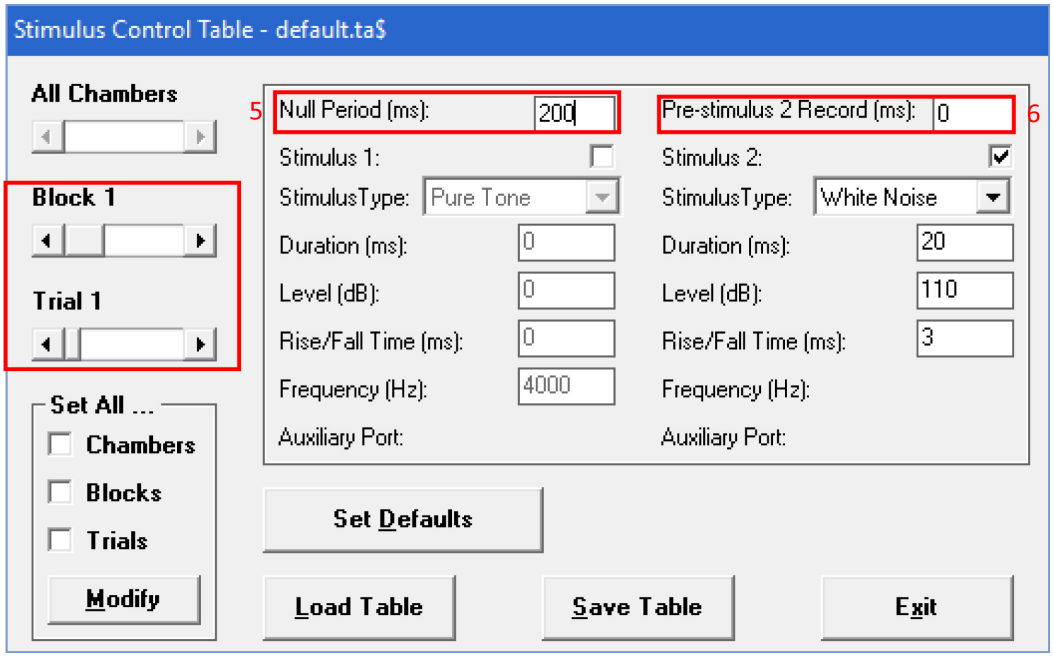
ii) For Block 1 enter 0 in the **Pre-stimulus 2 Record (ms)** box and enter 200 for Block 2.

g. Click the **Configure All Chambers with this Config** button, then click on **OK** in the confirmation window;

h. Click **OK** in lower left corner of the Chamber Configuration window.

3. Next, select **Config > Experiment Table**. This will open an **Experiment Table** screen (Figure 5-12), with "Stimulus Control Table – default.ta$." in the header. The **Experiment Table** screen is where the exact trial sequence and parameters for each trial will be set up. In the upper left corner of the window are selection bars to be used to advance through blocks and trials.

Figure 5-12 - Experiment Table Window



4. Ensure that the appropriate Block and Trial # appear above the selection bars for the Block and trial being set up;
5. Enter 200 in the **Null Period (ms)** box;
6. Verify that the **Pre-stimulus 2 Record (ms**) box has 0 for both Block 1 and Block 2;
7. Block 1, the Leader Sequence phase, is used to establish a stable startle baseline. In this block the 3 sets of the 3 intensities of noise burst startle stimuli ('Stimulus 2') are presented. Check only the **Stimulus 2** checkbox and enter the values from Table 5-2.

Table 5-2 - Trial Parameters for Block 1 (Leader Sequence Phase)

Trial #	Stimulus 1					Stimulus 2			
	Type	Duration (ms)	Level (dB)	Rise/Fall Time (ms)	Frequency (Hz)	Type	Duration (ms)	Level (dB)	Rise/Fall Time (ms)
1	NA	NA	NA	NA	NA	WN	30	105	3
2	NA	NA	NA	NA	NA	WN	30	110	3
3	NA	NA	NA	NA	NA	WN	30	100	3
4	NA	NA	NA	NA	NA	WN	30	110	3
5	NA	NA	NA	NA	NA	WN	30	100	3
6	NA	NA	NA	NA	NA	WN	30	105	3
7	NA	NA	NA	NA	NA	WN	30	100	3
8	NA	NA	NA	NA	NA	WN	30	105	3
9	NA	NA	NA	NA	NA	WN	30	110	3

NA = Not Applicable WN = White Noise

8. For entering individual Trial Parameters for Block 2, enter the values from Table 5-3. Trials that have a stimulus 1, highlighted rows in Table 5-3, need to have a Pre-stimulus 2 Record of 200 ms. See Figure 5-13.

Figure 5-13 - FPS Testing Block 2 Experiment Table Window

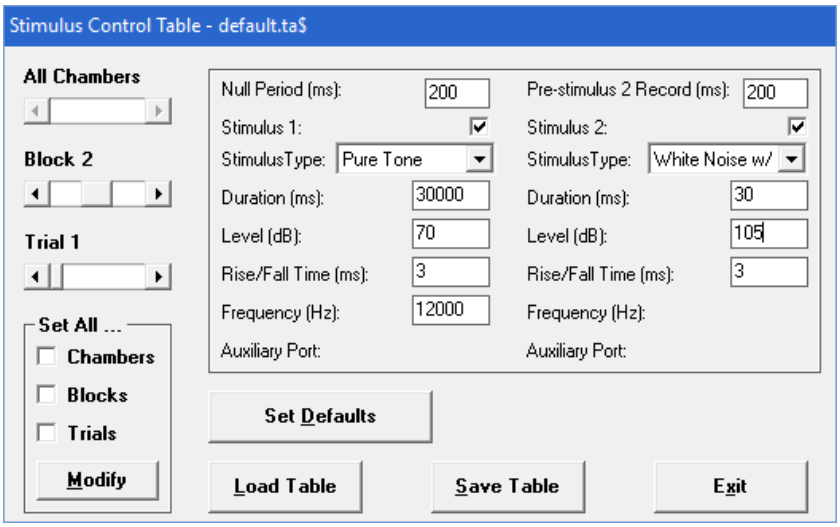


Table 5-3 - Trial Parameters for Block 2 (Testing Phase)

Trial #	Stimulus 1					Stimulus 2			
	Type	Duration (ms)	Level (dB)	Rise/Fall Time (ms)	Frequency (Hz)	Type	Duration (ms)	Level (dB)	Rise/Fall Time (ms)
1	PT	30000	70	3	12000	WN	30	105	3
2	NA	NA	NA	NA	NA	WN	30	110	3
3	PT	30000	70	3	12000	WN	30	100	3
4	NA	NA	NA	NA	NA	WN	30	100	3
5	PT	30000	70	3	12000	WN	30	110	3
6	NA	NA	NA	NA	NA	WN	30	105	3
7	NA	NA	NA	NA	NA	WN	30	110	3
8	PT	30000	70	3	12000	WN	30	110	3
9	NA	NA	NA	NA	NA	WN	30	100	3
10	PT	30000	70	3	12000	WN	30	105	3
11	PT	30000	70	3	12000	WN	30	100	3
12	NA	NA	NA	NA	NA	WN	30	105	3
13	PT	30000	70	3	12000	WN	30	100	3
14	NA	NA	NA	NA	NA	WN	30	105	3
15	PT	30000	70	3	12000	WN	30	110	3
16	NA	NA	NA	NA	NA	WN	30	100	3
17	NA	NA	NA	NA	NA	WN	30	110	3
18	PT	30000	70	3	12000	WN	30	105	3

NA = Not Applicable PT = Pure Tone WN = White Noise

9. In the "Set All…" field, select **Chambers** and click the **Modify** button.
10. Click on the **Save Table** button and choose a file name and save the table; click the **Exit** button on the 'Stimulus Control Table' window.
11. Next select **Config > Experiment Config.** This will open the **Experiment Configuration** window.
12. Click on **Select Table,** find and select the experiment table that was just set-up, click **Open**; click the **OK** button of the Experiment Configuration window.
13. Finally, select **File > Save Config** from the menu bar and choose a name for this program (it will be saved with an .exp extension).

Running PPI or FPS Experiments

To run experimental sessions with the PPI program or either the training or testing FPS programs outlined in this chapter, select **File > Load Config** and select the appropriate .EXP file. Once the experiment file is open, click **Run > Begin Experiment**.

Saving Data from PPI or FPS Experiments

At the conclusion of each experiment it is important to click **File > Save Data** to open the "Save As" window and provide a unique data file name for each experiment's data. **If a unique data file name is not supplied, the previous experiment's data will be overwritten**.

After saving the data file see Chapter 7, **Data Management**, for other data saving and exporting options.

References

For PPI:

Jaworski DM, Boone J, Caterina J, Soloway P, Falls WA. (2005). Prepulse inhibition and fear-potentiated startle are altered in tissue inhibitor of metalloproteinase-2 (TIMP-2) knockout mice. Brain Research. Volume 1051, Issues 1-2, 27 July; 81-89.

For FPS:

Falls, W.A. (2002). Fear-Potentiated Startle in Mice, Current Protocols in Neuroscience Supplement 19, Unit 8.11B; J. Crawley, Editor, John Wiley & Sons.

CHAPTER 6 | MENU SELECTIONS

File Menu Options

New: Closes the current experiment and brings up the Experiment Configuration screen.

Load Config: Allows the user to load pre-determined Experiment Configuration parameters so that new parameters do not have to be entered every time an experiment is run.

Save Config: Saves the current configuration to an .EXP Experiment Configuration file. It is recommended that the Experiment Configuration files be named to reflect the protocols (configuration) that they apply to. This will allow the user to recognize and use these files at a later time.

Save As Default Config: Makes the current Experiment Configuration file the default configuration to be loaded when the Startle Reflex software begins.

Print Config: Prints experiment and chamber configurations either to a text file or to the default Windows printer.

Save Data: Stores data collected from the current experiment for future analysis with the Startle Reflex software. When saving data, Startle Reflex generates a .DAT file and a .EXP file with the same name as the .DAT file. This .EXP file is used by Startle Reflex when opening .DAT files during analysis and must not be confused with the .EXP Experiment Configuration file generated when **Save Config** is selected. The .EXP file generated when saving session data cannot be used as an Experiment Configuration file for running an experiment. It is recommend that .DAT and .EXP data files be stored in a separate folder from the Experiment Configuration .EXP files to avoid confusion between file types.

Load Data: Retrieves and displays data files saved from a previous experiment. Specify the experiment filename either with its .DAT extension or with no extension.

Save Data as…: Allows the user to save raw or analyzed data in a standard ASCII format for use in third party spreadsheet or database software. For detailed information on exporting data files see **Chapter 7, Data Management.** For information on displaying and adjusting data display see the **Data Menu Options** section of this chapter.

Print Data: Displays the Print Data dialog box. Specify sets and ranges of data to print to the default Windows printer. The Print Data dialog functions identically to the Save as ASCII dialog described in the previous section.

Exit: Exits the program. If an experiment has been run, but not saved, the user is prompted to save the current experiment and data file.

Hardware Menu Options

Configure Hardware: Allows the user to select which amplifier is to be used. Click the **Configure ADC Device** button to check the status of the DIG-744 data acquisition card, see Figure 6-2. Please note, Startle Reflex will use the first device listed on the **ADC Device Properties** screen.

The ADC Device Properties screen enables the user to name, test, detect and remove the DIG-744 data acquisition card(s) as desired.

Figure 6-1 - Hardware Configuration Screen

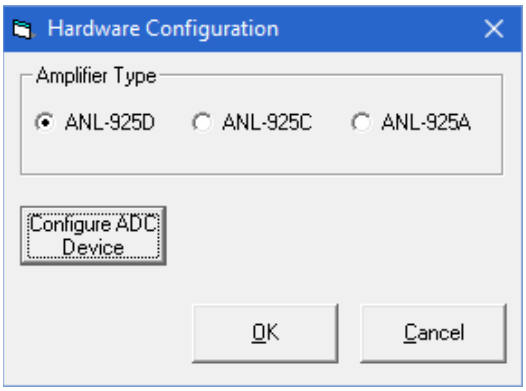
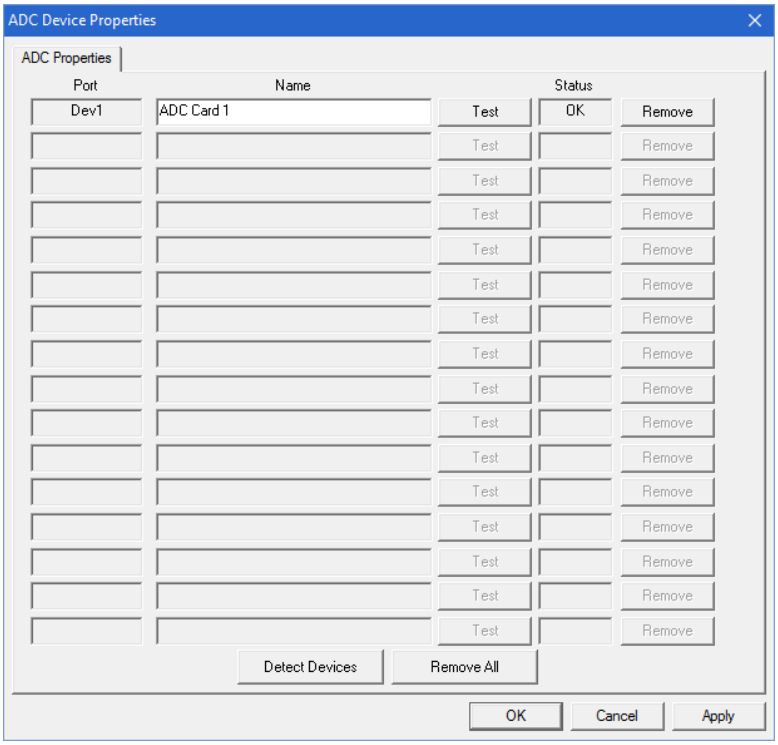


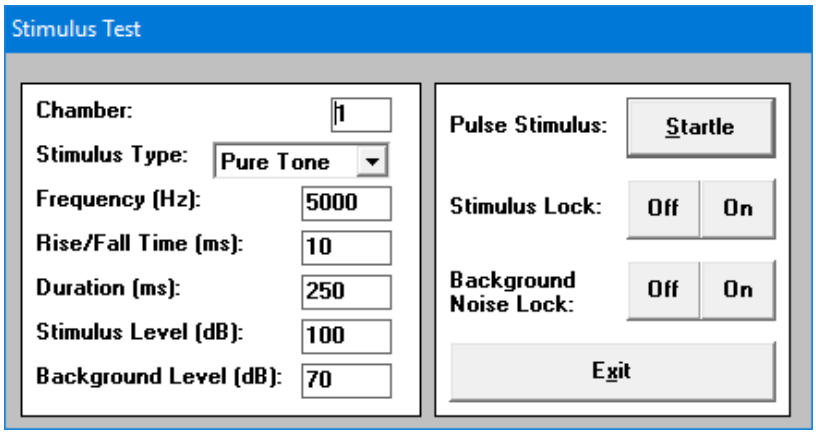
Figure 6-2 - ADC Device Properties Screen



Calibrate Audio: For the speakers to generate reproducible audio startle stimuli, audio calibration must be performed. The audio calibration should be performed each time the audio values used in protocols are changed. This requires the measurement of output volume over a range of frequencies at specific volume levels. This information is then saved to a data file that will ensure accurate audio output levels during subsequent experiments. See **Chapter 5, Calibration** for audio and input calibration procedures.

Stimulus Test: The Stimulus Test utility is used to test attachment of all audio and auxiliary stimuli (Figure 6-3).

Figure 6-3 - Stimulus Test Screen



Menu options for testing stimulus outputs:

Table 6-1 - Stimulus Test Options

Option	Description
Chamber	Enter the number of the chamber to test. If using the ANL-925D, this number should remain "1".
Stimulus Type	Select a Stimulus Type (Pure Tone, White Noise, or any of the four Auxiliary outputs).
Frequency (Hz)	Enter a test frequency of 100 to 32,767 Hz in 1 Hz increments.
Rise/Fall Time (ms)	Enter a Rise/Fall Time of 0 to 250 ms in 1 ms increments.
Duration (ms)	Enter a stimulus Duration of 1 to 250 ms in 1 ms increments.
Stimulus Level (dB)	Enter a Startle pulse level of 40 to 130 dB in 1 dB increments.
Background Level (dB)	Enter a Background noise level of 30 to 100 dB in 1 dB increments.
Pulse Stimulus	Create the specified Startle pulse.
Stimulus Lock	Click these buttons to lock on and turn off the Startle stimulus output in the chamber.
Background Noise Lock	Click these buttons to lock on and turn off the background noise speaker in the chamber.
Exit	Returns to the main program.

Calibrate Input: Use this menu option to test or fine-tune the sensitivity of the Startle Reflex platform hardware. See **Chapter 5, Calibration** for audio and input calibration procedures.

Training: Use this menu option to condition an animal to expect an aversive stimulus after a Pure Tone stimulus. The duration for the Auxiliary stimulus (shock) can be longer than 250 ms during training. See Figure 6-4.

Figure 6-4 – Training Screen

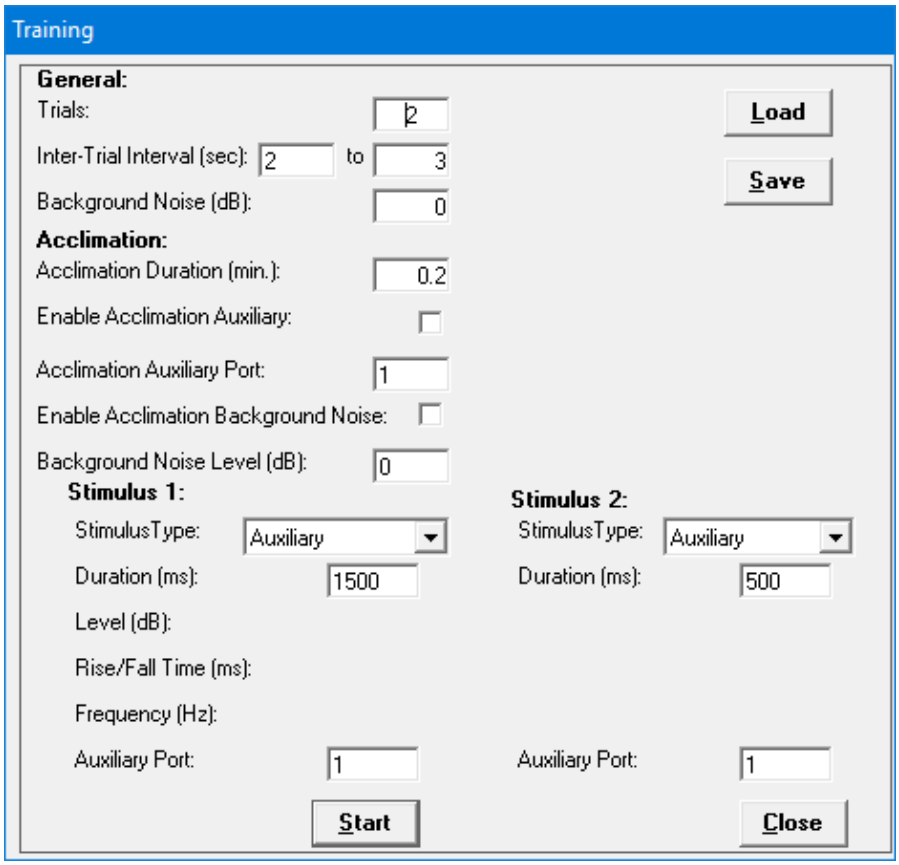


Table 6-2 - Training Options

Option	Description
Trials	The number of trials for this training session. The valid range is 1 to 100 trials.
Inter-Trial Interval (sec)	Enter an Inter-Trial Interval in seconds. The Inter-Trial Interval is the time from the end of the previous trial to the beginning of the next trial. The valid range is 1 to 999 seconds in 1-second intervals.
Background Noise (dB)	Enter the Background Noise level to be used during the trial period. The valid range is from 30 to 100 dB.
Acclimation Duration (min)	Enter the duration of the acclimation period in minutes. The valid range is from 0 to 99.0 in 0.1 min increments.
Enable Acclimation Auxiliary	Check this box to have Auxiliary stimulus during the acclimation period.
Acclimation Auxiliary Port	If the acclimation auxiliary is selected, enter the auxiliary port number. Valid ports are 1 to 4.
Enable Acclimation Background Noise	Check this box to have background noise during the acclimation period.
Background Noise Level (dB)	If Background Noise is selected, enter the dB level of the noise. The valid range is from 30 to 100 dB.

Option	Description
Stimulus Type	Select Pure Tone, White Noise or Auxiliary. The Stimulus 2 may also include the Stimulus 1 signal, if they are not conflicting signals. **Note**: For Stimulus 2 the Auxiliary or Auxiliary w/Stimulus 1 option should be selected for aversive stimulus presentation.
Duration (ms)	Enter a stimulus duration from 1 to 32767 ms in 1 ms increments.
Level (dB)	Enter an audio stimulus level from 40 to 130 dB in 1 dB increments.
Rise/Fall Time (ms)	Rise/Fall Time is used to suppress pops in the speaker when there is a sudden change in the signal. Enter a Rise/Fall Time of 0 to 50ms in 1ms increments.
Frequency	Enter a Pure Tone Frequency value from 100 to 32,767 Hz in 1 Hz increments.
Auxiliary Port	Enter the port number to be turned on during an auxiliary stimulus. Valid ports are 1 to 4.
Load	The Load button is used to retrieve a saved training setup from a file. Specify the training filename with its .TRN extension or with no extension.
Save	The Save button is used to save any training setup to a binary file. Specify the file with its .TRN extension or with no extension. Filenames with no extension receive the .TRN extension by default.
OK	Start the training session.
Cancel	Closes the Training windows or if a training session has already been started, it cancels the training session and closes the training window.

Config Menu Options

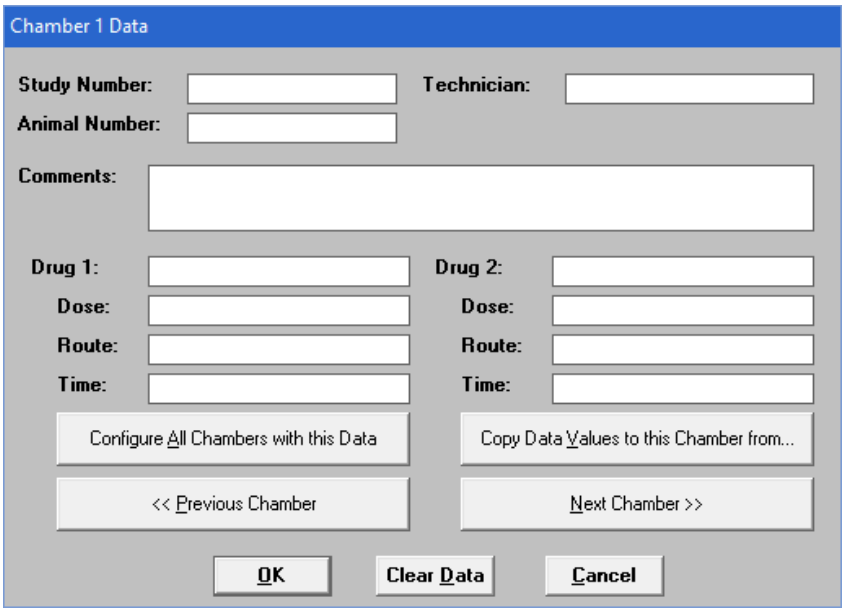
The Config Menu selections determine experiment and data configurations. These are described in greater detail in the **Sample Experiments** section of Chapter 5. The Config and Experiment Table menu items are active once an experiment is loaded.

Experiment Config: Use this option to display and edit the global experiment configuration options. These settings can be modified at any time prior to running an experiment.

Chamber Config: After a chamber is selected, the Chamber Configuration screen for that chamber is displayed. The Chamber Config screen may also be accessed by clicking the **Config** button located in the Chamber 1 display of the open experiment. This screen is used to set up individual chamber parameters for the different blocks, or phases, of an experiment.

Chamber Data: Select the chamber to edit and then click **OK,** or click the **Data** button in the Chamber 1 display of the open experiment configuration to open the **Chamber Data** screen, see Figure 6-5. Use the **Chamber Data** screen to add additional experiment information.

Figure 6-5 - Chamber Data Screen



Experiment Table: This screen is where the exact trial sequence and parameters for each trial can be set up. In the upper left corner of the screen are selection bars to be used to advance through blocks and trials. Any changes made to this table may affect the Experiment Configuration and Chamber Configuration data.

Data Menu Options

Options: The Data Options screen (Figure 6-6) is used to adjust how data will be saved to your text file under the Computed Values option (see File Menu Selections earlier in this chapter). See Table 6-3 for a list of parameters that can be adjusted in the data file.

Figure 6-6 - Data Options

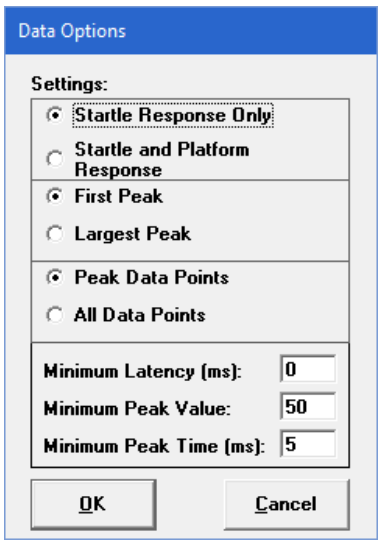


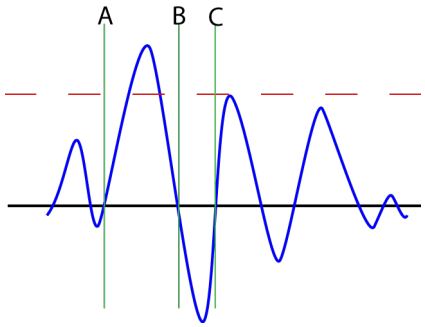
Table 6-3 - Data Options

Option	Description
Startle Response Only	The Total and Average values computed are based on the first positive wave that meets the minimum wave criteria. The area between lines A and B in Figure 6-7.
Startle and Platform Response	The Total and Average values computed are based on the first positive wave that meets the minimum wave criteria and the following negative wave ("peak-to-peak" or "peak-to-trough" detection).The area between lines A and C in Figure 6-7.
First Peak Largest Peak	Reports the Peak Time and Peak Values for either the first data peak or the largest data peak.
Peak Data Points All Data Points	Gives the Duration, Total and Average for either all of the data points or only the peak data points.
Minimum Latency (ms)	This value is used when searching for the startle response. If the start of a wave (ie. the first positive value after a negative value) occurs before the minimum latency time, it is not counted as the startle response.
Minimum Peak Value	This value is used when searching for the startle response. If a peak occurs below the minimum peak value, it is not counted as the startle response.
Minimum Peak Time (ms)	This value is used when searching for the startle response. If a peak occurs before the minimum peak time, it is not counted as the startle response.
OK	Save all changes and return to the main screen.
Cancel	Ignore all changes and return to the main screen.

Load Data Options: Load Data Options is used to retrieve previously saved data options from a file. Specify the data options filename with its .DA$ extension or with no extension.

Save Data Options: Save Data Options is used to save the data options setup to a binary file. Once you have made changes to Data Options screen, you must then Save Data Options to make the changes effective. Specify the file with its .DA$ extension or with no extension. Filenames with no extension receive the .DA$ extension by default.

Figure 6-7 - Startle and Platform Response Example



Run Menu Options

Begin Experiment: When the experiment and chamber configurations have been completed, select this option to begin the experiment.

Abort Experiment: Choose this menu option if a running experiment must be aborted prior to completion. Data collected from completed trials will not be lost and can be saved.

Post Analysis: Select this option to view and analyze data (Figure 6-8).

Figure 6-8 - Post Analysis

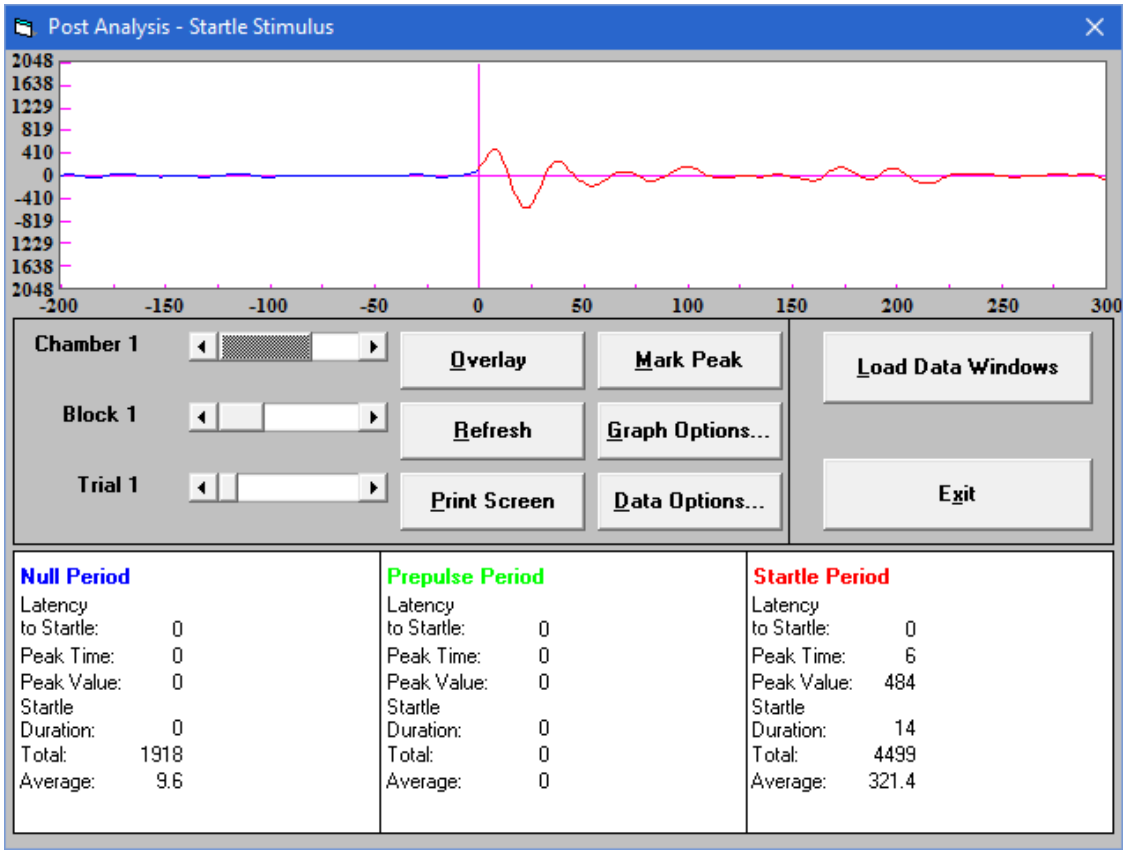


Table 6-4 - Post Analysis Options

Option	Description
Chamber Block Trial	Use these scroll bars to specify which chamber, block and trial to view. The data and graphs will be displayed accordingly.
Overlay	Click this button to superimpose the current graph over the graph display. This feature allows the user to directly compare two or more graphs. To clear the graph, click Refresh.
Mark Peak	Click this button to display the peak time and value on the graph. Click this button again or click Refresh to clear the display.

Option	Description
Refresh	Click this button to clear and redraw the current graph. Selecting this button also has the effect of clearing any superimposed graphs displayed using the Overlay button.
Graph Options	Click this button to display the graph options dialog box. Refer to Graph Options in the following section.
Print Screen	Click this button to print the screen to your default Windows printer.
Data Options	Click this button to display the Data Options dialog box. Refer to Data Options in the previous section.
Load Data Windows	Click this button to load the raw Null, Prepulse, and Startle time period data of a particular trial into display windows.
Exit	Select this button to return to the main program.

Graph Menu Options

Print Active: Prints the active window to the default Windows printer.

Refresh All: Refreshes all the chamber display windows. Refer to Refresh Active described below.

Refresh Active: Use this option to redraw the graph and calculated data for the active window. This is useful if chamber graph windows become cluttered or erased when another window passes over a graph.

Options: Displays the Graph Options dialog (Figure 6-9).

Figure 6-9 - Graph Options

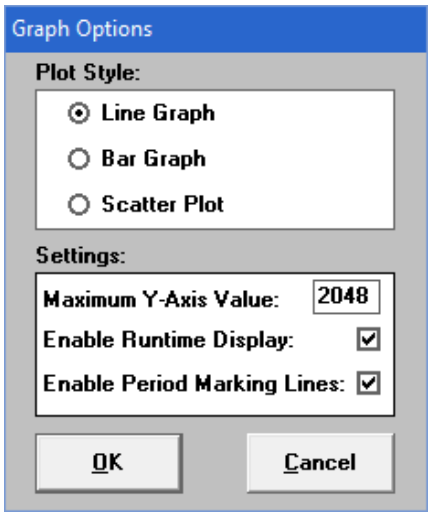


Table 6-5 - Graph Options

Option	Description
Plot Style	Select either line graph, bar graph, or scatter plot.
Maximum Y Axis Value	Specify the largest Y value desired on the graphs. Graphs will be scaled accordingly.
Enable Runtime Display	Only disable Runtime Display when running experiments with exceptionally short Trial Intervals. Disabling the runtime display ensures the most accurate interval timing possible. If the user specifies a Trial Interval that may be too short to enable runtime graphing, the program will automatically prompt the user and disable runtime graphing.
Enable Period Marking Lines	Turn on or off the vertical marking lines that clearly mark the starting and stopping points of the Null Period, Stimulus 1 Period and the Stimulus 2 Period.
OK	Save all changes and return to the main screen.
Cancel	Ignore all changes and return to the main screen.

Window Menu Options

Cascade: Arranges the windows in a cascade fashion.

Tile: Arranges the windows in a tile fashion.

Arrange Icons: Orders the windows that have been reduced to icons in the bottom of the screen, from left to right.

About Menu Selection

This displays the Startle Reflex copyright and version screen.

CHAPTER 7 | SAVING AND EXPORTING DATA

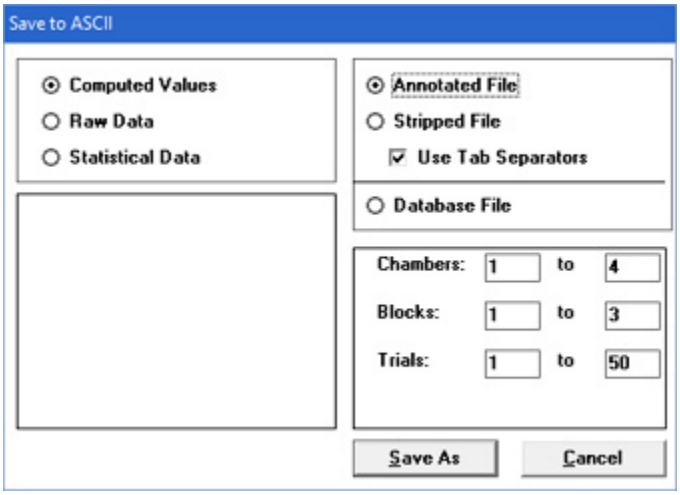
At the conclusion of each experiment it is important to click **File > Save Data** to open the "Save As" window and provide a unique data file name for each experiment's data. **If a unique data file name is not supplied, the previous experiment's data will be overwritten**.

After the data file has been saved, Startle Reflex offers different options for exporting the data for use in spreadsheet and database applications. With the experiment's data open in Startle Reflex click **File > Save Data as …** to access the following options.

Computed Values

The Computed Values option, Figure 7-1, saves the data as an annotated or stripped text file. The annotated format is recommend as it includes the experiment information while the stripped format does not. The Computed Values option also includes the option to save the data into a Microsoft Access® database file (*.mdb file) by selecting the **Database File** radio button. See the **Opening A Startle Database With Excel** section for information on importing the *.mdb file into Microsoft Excel™. The user may also select to only export a portion of the data file by exporting the data for only selected chambers, blocks or trials. See Table 7-1 for more information.

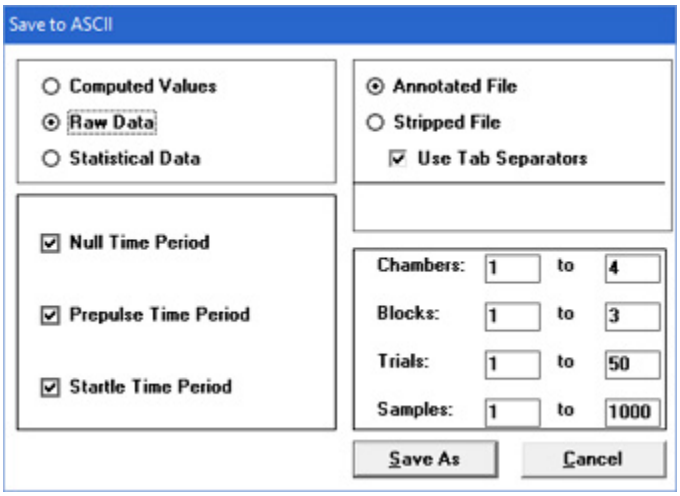
Figure 7-1 - Computed Values Export Option



Raw Data

The **Raw Data** export option, Figure 7-2, exports the actual raw, unprocessed data to a text file. The Raw Data option allows the user to export in the annotated (recommended) or stripped format, select which data sampling (time periods) period(s) to export, and select which chambers, blocks and trials to export. See Table 7-1 and **Importing a Text Data File Into Excel** for more information.

Figure 7-2 – Raw Data Export Option



Statistical Data

Figure 7-3 illustrates the **Statistical Data** export option. Similar to the other export options, the user can select an annotated or stripped data file format, select which chambers, blocks and trials to export, and it provides the option to calculated statistical values such as averages, standard deviation and standard error of the mean. See also Table 7-1 and **Importing a Text Data File Into Excel**.

Figure 7-3 - Save Data As Option With Statistical Values

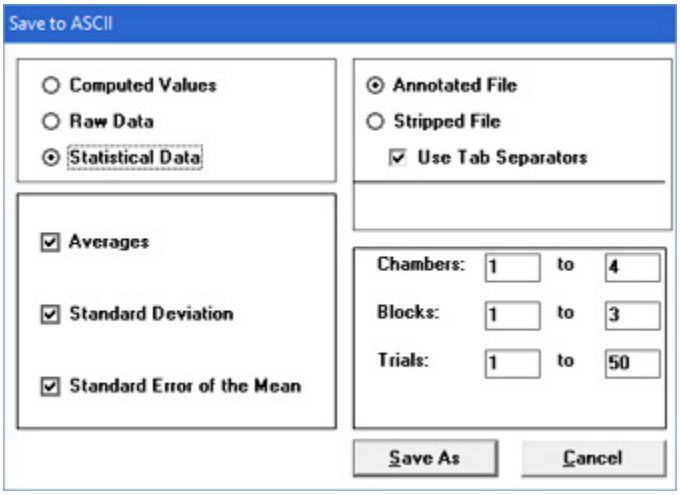


Table 7-1 - Save Data As Export Options

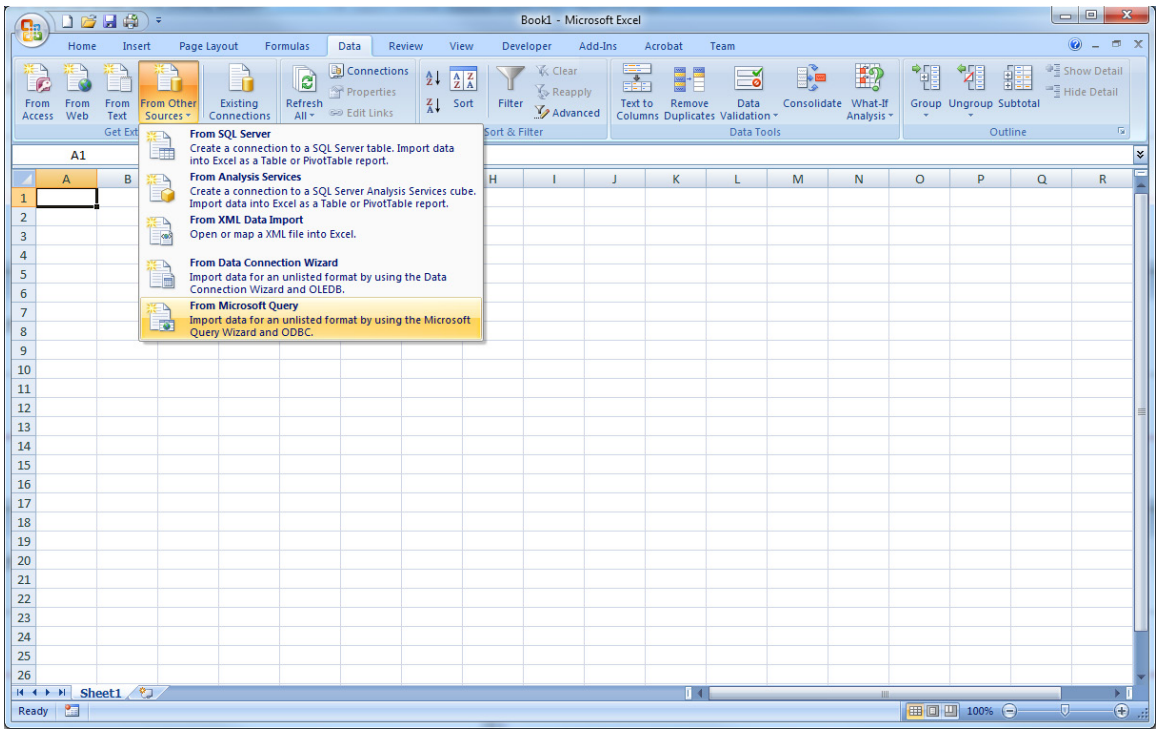
Option	Description
Computed Values	Choose Computed Values to save a text file containing latency to startle, peak time, peak value, duration, total and average for each sample period.
Raw Data	Select Raw Data to save actual unprocessed data values. See **Chapter 9, Startle Units Explained**.
Statistical Data	Select Statistical Data to save averages, standard deviation, and standard error of the mean for each sample period.
Null Period Prepulse Time Period Startle Time Period	If Raw Data has been selected, check the desired sample period(s) to save to disk.
Averages Standard Deviation Standard Error of the Mean	If Statistical Data has been selected, check the desired statistic(s) to save to disk.
Annotated File	Annotated files include file information and headings.
Stripped File	Select **Use Tab Separators** to insert a tab between each field instead of a space. In order to open a Tab Separated file, right click on the file name and select Open with Excel.
Database File	The Database File format saves data in a Microsoft Access® Database (*.mdb) file, and is only available if the Computed Values option was selected.
Chambers	Specify the chamber or range of chambers to save as ASCII. Specify the bottom of the range first, followed by the top of the range. For example: Chambers 3 to 6.
Blocks	Specify the block or range of blocks to save as ASCII. If the user has selected to run only 1 block of trials then block 1 should be selected, otherwise, specify the bottom of the range first, followed by the top of the range. For example: Blocks 1 to 2.
Trials	Specify the trial or range of trials to save as ASCII. Specify the bottom of the range first, followed by the top of the range. For example: Trials 45 to 73.
Samples	If Raw Data has been selected, specify the sample or range of samples to save as ASCII. Specify the bottom of the range first, followed by the top of the range. For example: Samples 100-250. **Note**: The maximum range value must be equal to or less than the number of samples.
Save As	Brings up the Save As file dialog. Enter the desired filename.
Cancel	Returns to the main menu without saving the data to disk.

Opening A Startle Database With Excel

If the **Computed Values** export option was used to save the data as a Microsoft Access® database file (*.mdb), it can be opened in Microsoft Excel™ by using the instructions below to create a query to import the data.

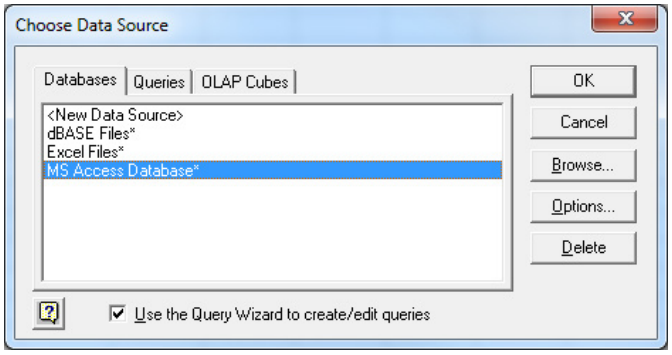
Start Microsoft Excel™. Click the **Data** tab and in the **Get External Data** section click the drop down arrow for **From Other** Sources and select **From Microsoft Query** as shown in Figure 7-4.

Figure 7-4 - New Database Query



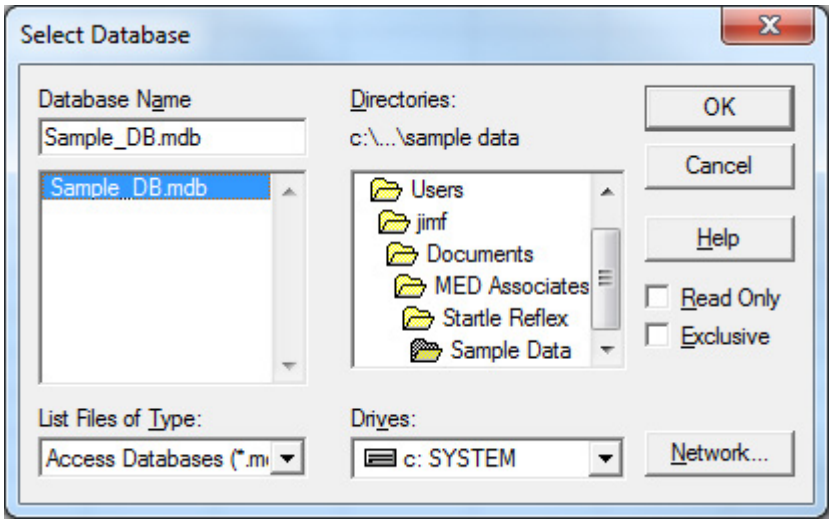
Select **MS Access Database** from the list of databases (Figure 7-5), and click the **OK** button.

Figure 7-5 - Choose Data Source



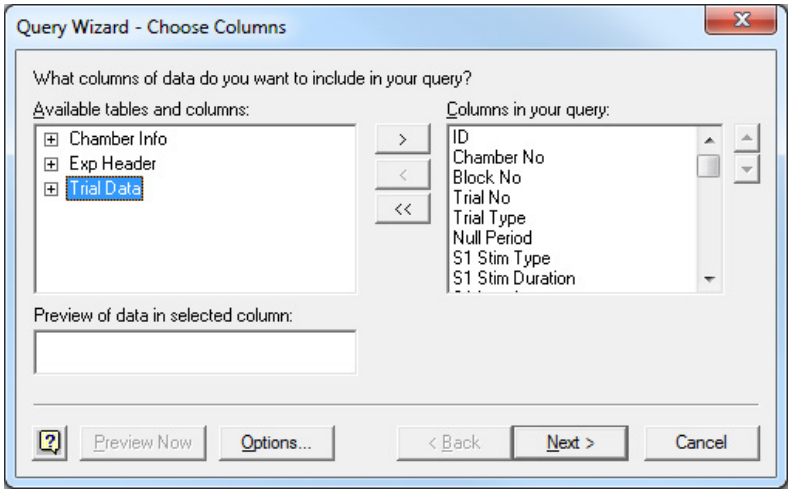
Navigate to the Startle directory and select the desired database as shown in Figure 7-6 and click **OK**.

Figure 7-6 - Select Database



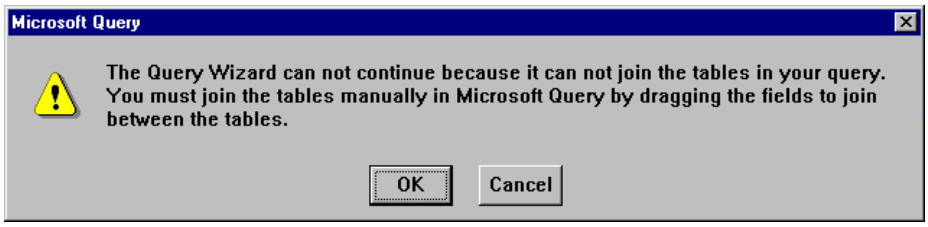
Click **Trial Data** and then the **>** button to add all of the database columns to the **Columns in your query:** list (Figure 7-7). Click on an item in the **Columns in your query:** list and click **<** to remove it from the query. If unsure, include them all, as fields may be deleted later. Select an item and use the up and down arrows on the right to change the order in which the items appear. Click **Next**.

Figure 7-7 - Query Wizard



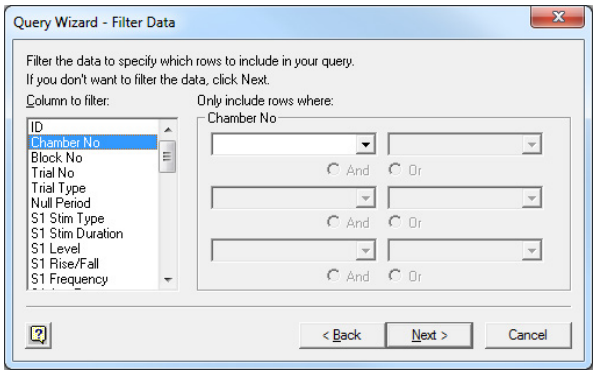
If the message shown in Figure 7-8 appears, click the **OK** button.

Figure 7-8 - Microsoft Query Message



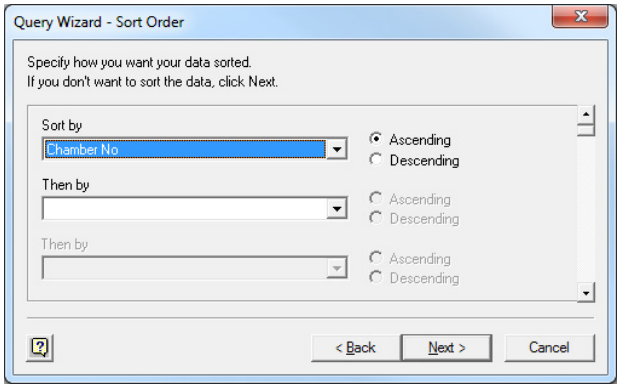
The next display, as shown in Figure 7-9, allows the data to be filtered to only present certain data. Leave blank to display all data. Click **Next**.

Figure 7-9 - Filter Data Query Wizard



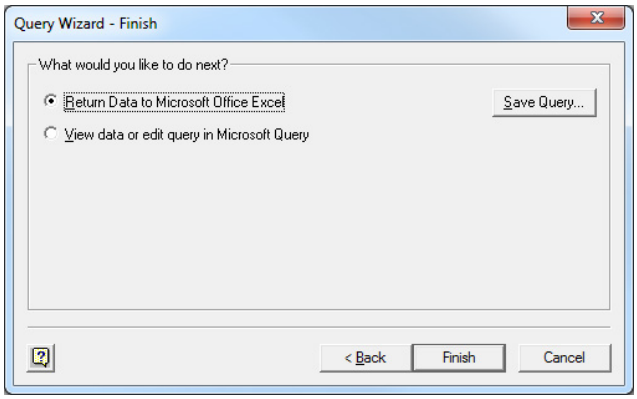
The data may then be sorted based on multiple criteria, see Figure 7-10. Click **Next**.

Figure 7-10 - Sort Order



On the Finish screen select **Return Data to Microsoft Office Excel** and click **Finish** or **Save Query** to save the query for reuse and then **Finish**. Refer to Figure 7-11.

Figure 7-11 - Finish Screen



By saving the query, it will be able to be rerun in Excel at a later date, with a new database. The only time these steps will need to be gone through again is if the data fields returned to Excel need to be modified.

Excel will now prompt the user (Figure 7-12) for a location on the spreadsheet to place the returned data. Click on the **OK** button to open the data in Excel.

Figure 7-12 - Returning External Data to Microsoft Excel

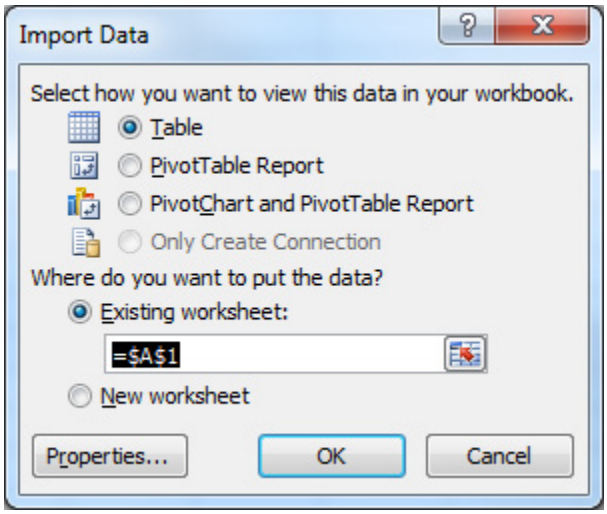
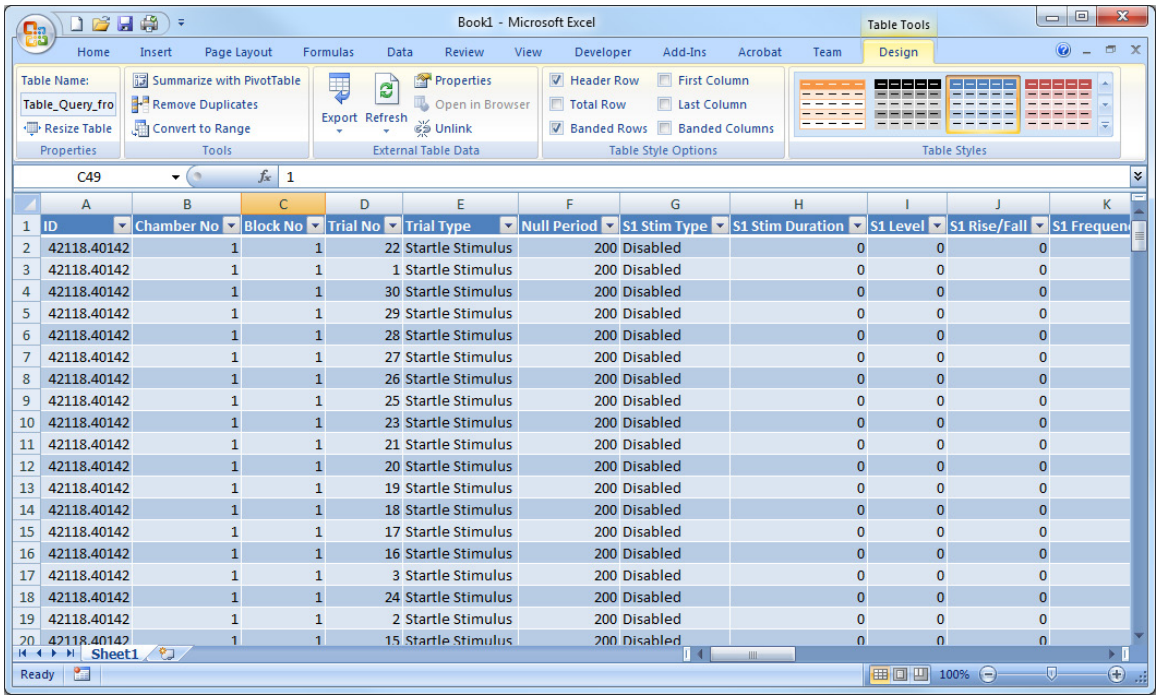


Figure 7-13 shows what the returned data looks like in Excel.

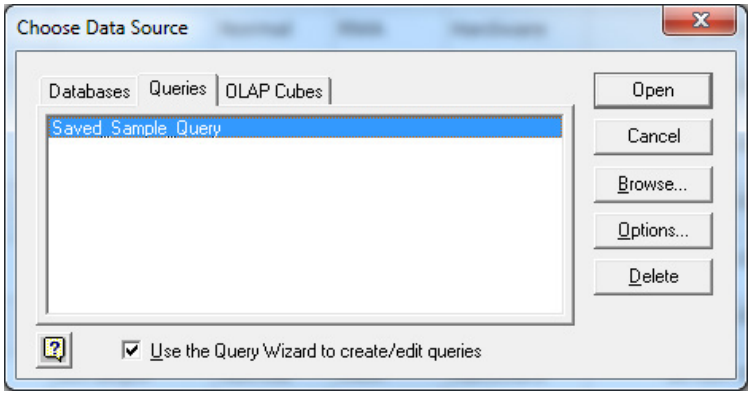
Figure 7-13 - Queried Data in Excel from MS Access Database



To run a saved query, go to **Data > Get External Data > From Microsoft Query** as shown in Figure 7-4.

Select the saved query from the Queries tab as shown in Figure 7-14 and click the **Open** button.

Figure 7-14 - Selecting Saved Query to Run



Select the data table(s) to work with, the filter(s) to apply and the sort order to use as shown in Figure 7-7.

Importing a Text Data File Into Excel

A spreadsheet program may be used to open data saved as an ASCII text file. This section illustrates how to open ASCII data using Microsoft Excel™. The **Use Tab Separators** option must be used when saving the data for these steps to work.

1. In Excel, select **File** > **Open**. Locate where the file has been saved. Select **All Files (*.*)** in the file type drop down menu.
2. Select the desired data file to open and click **Open**.
3. This will open step 1 of the **Text Import Wizard**, Figure 7-15. Be sure **Delimited** is selected and click **Next**. In step 2 of the import wizard, Figure 7-16, check the box for Tab Delimiters and click **Finish**.

Figure 7-15 - Text Import Wizard Step 1

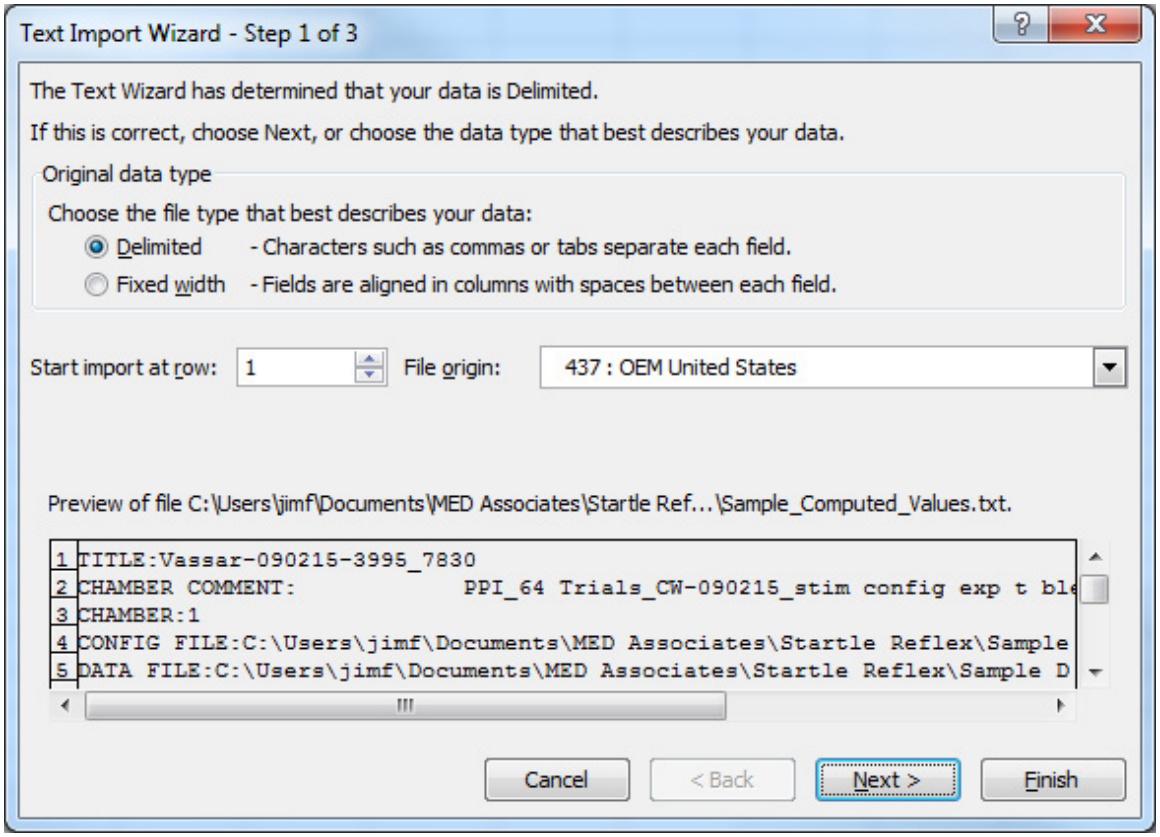
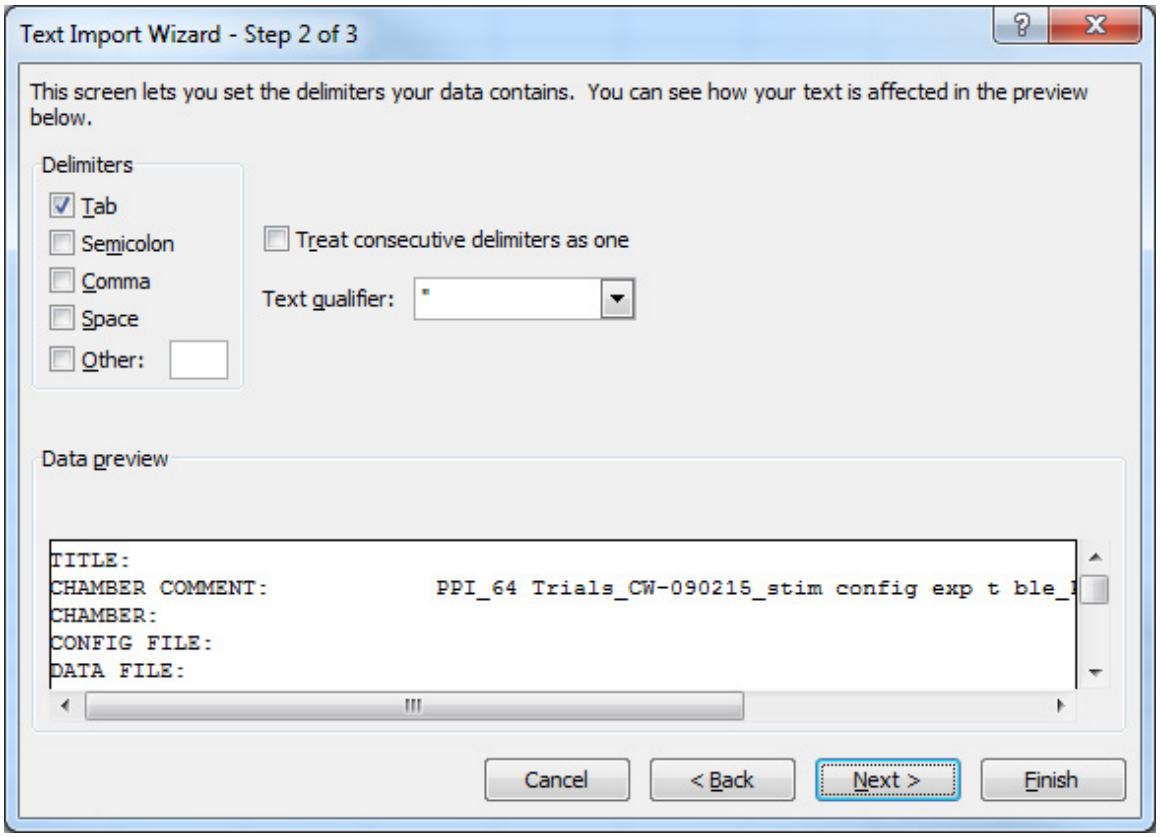


Figure 7-16 - Text Import Wizard Step 2



4. The data will appear in Excel in different formats depending on how the data was exported. The format will vary depending on whether a stripped or annotated file was saved and whether computed values, raw data or statistical data was exported. See the **File Formats** section for examples.

File Formats

Sample PPI experiment raw data saved in the stripped format:

```
1      (chamber)
1      (block)
1      (trial)
[NEWLINE]
Condition: Startle Stimulus
[NEWLINE]
23     (Null Time Period - First value)
345    (Null Time Period - Second value)
234    (Null Time Period - Third value)
[NEWLINE]
23     (Prepulse Time Period - First value)
345    (Prepulse Time Period - Second value)
234    (Prepulse Time Period - Third value)
[NEWLINE]
23     (Startle Time Period - First value)
345    (Startle Time Period - Second value)
234    (Startle Time Period - Third value)
```

Sample computed values saved in the stripped format:

```
1            (chamber)
1            (block)
[NEWLINE]
95           (Latency to Startle  - Trial 1 - Null Time Period)
122          (Peak Time - Trial 1 - Null Time Period)
120          (Peak Value - Trial 1 - Null Time Period)
52           (Startle Duration  - Trial 1 - Null Time Period)
3816         (Total - Trial 1 - Null Time Period)
73.4         (Average - Trial 1 - Null Time Period)
55           (Latency to Startle  - Trial 1 - Prepulse Time Period)
78           (Peak Time - Trial 1 - Prepulse Time Period)
88           (Peak Value - Trial 1 - Prepulse Time Period)
43           (Startle Duration  - Trial 1 - Prepulse Time Period)
2327         (Total - Trial 1 - Prepulse Time Period)
54.1         (Average - Trial 1 - Prepulse Time Period)
37           (Latency to Startle  - Trial 1 - Startle Time Period)
55           (Peak Time - Trial 1 - Startle Time Period)
457          (Peak Value - Trial 1 - Startle Time Period)
32           (Startle Duration  - Trial 1 - Startle Time Period)
9479         (Total - Trial 1 - Startle Time Period)
296.2        (Average - Trial 1 - Startle Time Period)
21           (Latency to Startle  - Trial 1 - Null Time Period)
22           (Peak Time - Trial 1 - Null Time Period)
[NEWLINE]
[NEWLINE]
2            (chamber)
1            (block)
[NEWLINE]
0            (Latency to Startle  - Trial 2 - Null Time Period)
0            (Peak Time - Trial 2 - Null Time Period)
```

CHAPTER 8 | ALTERNATE CALIBRATE INPUT UTILITY

The Calibrate Input utility is used to adjust the sensitivity of the response platform using the spinner and solenoid accessories offered for older systems.

Figure 8-1 - Calibrate Input Screen for Spinner Calibrators

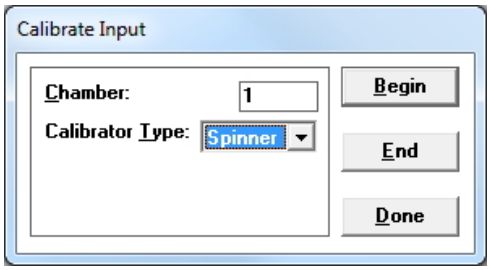
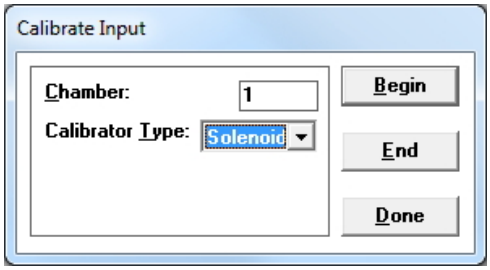


Figure 8-2 - Calibrate Input Screen for Solenoid Calibrators



Menu options for input calibration:

Table 8-1 - Calibrate Input Options

Option	Description
Chamber	Enter the chamber number of the platform to be calibrated.
Calibrator Type	Select the type of calibrator being used.
Output Level (dB)	If speaker calibrator is selected, enter the output level for the calibration woofer. The default value is 75 dB, but a smaller value may be used if calibrating the system for mice.
Frequency (Hz)	If the speaker calibrator is selected, enter the pulse frequency for the calibration woofer. This value must fall within the response range of both the speaker and platform. 10 Hz works well.
Begin	This button turns on the calibrator while sampling and graphing data. Observe and modify the Startle Platform Amplifier gain until it is at the appropriate level for the experiment.
End	Stop the calibrator and data collection graphing.
Done	Stops data collection and graphing. Returns the user to the main program.

The response platform generates an analog signal that is digitized and brought into the software. A preamplifier boosts the signal before it reaches the analog to digital converter in the computer. The gain of the preamplifier must be set to produce a signal in the desired amplitude range.

The Calibrate Input Utility is used to simulate the pressure generated on the platform by an animal's response by placing the calibrator on the response platform, securing it firmly to the platform with the thumbscrews, and driving it with the appropriate signal. The resulting signal is displayed on the screen. Adjusting the gain of the amplifier affects the signal's amplitude. It is important to have the platform responses calibrated as closely as possible in order to have consistent amplitude measures between subjects. The trick is to not only have equal amplitudes between platforms for a given force, but to maximize the response, within the dynamic range of the system, without clipping (off the scale, so to speak) the signal. This provides accuracy and resolution.

To run the calibration procedure,open Startle Reflex and click **File > New** from the main menu. Specify the chambers to be calibrated by selecting their check boxes in the upper left hand corner of the Experiment Configuration window, see Figure 5-3, and click the **OK** button. An experiment that is already set up with the desired number of chambers may also be loaded. When the chamber display windows have been loaded, select **Hardware > Calibrate Input** from the main menu.

Begin by entering the chamber number to calibrate in the **Chamber** box. Next, select the type of calibrator that is going to be used. Place the calibrator on the response platform. Connect the calibrator's cable to the **AUX 1** port on the PHM-255A in the selected Chamber.

Click the **Begin** button. The calibrator applies a 10 Hz modulated force to the platform and the software plots the response data. Observe the data. The response data window displays the average Positive Peak value and the average Negative Peak value of ten responses. Adjust the amplifier gain so that the average amplitude is at the desired level. When satisfied with the platform’s gain adjustment, use the positive peak value as a standard for calibrating all the other platforms. The gain settings between amplifiers should be close. If the amplifier settings vary considerably, please contact Med Associates, Inc.

NOTE: The value that is chosen to calibrate to is dependent upon the subject types and the resolving power that should be obtained. We suggest that some pre-study information be gathered, such as the average amplitude of a CD - 1 male mouse startle with the platform gain set at 5, or the average amplitude of a Sprague-Dawley rat with the platform gain set to 0.1. The important thing is to set all platforms to the same value and maintain this value throughout the duration of the protocol.

Repeat this procedure for each additional chamber. When all chambers have been calibrated, click the **Done** button on the **Calibrate Input** dialog box.

CHAPTER 9 | STARTLE UNITS EXPLAINED

Researchers studying the acoustic startle reflex often wonder what units their data should be expressed in. Most commercial systems designed to measure the acoustic startle reflex use a sensor to convert the animal's movement into a digital signal that is analyzed by the computer. Various methods exist for calibrating such sensors, and various types of sensors can be used, but usually startle responses are quantified in terms of "Arbitrary Units," "Startle Units," "Startle Response", "A/D Units," or some other similar designation (e.g. Bortolato *et al*, 2007; Jaworski *et al*, 2005; Meloni *et al*, 2006; Risbrough & Geyer, 2005; Vinkers *et al*, 2007; Winslow, Noble, & Davis, 2007). The within-subjects design of startle experiments indicates that such modes of quantitation are usually acceptable, independent of the specific vendor of the startle apparatus. The most important consideration for the researcher is that a standard calibration procedure be adopted that ensures similar sensitivities among all the startle sensors in his/her system.

Q: What are the units for data gathered using SOF-825 Startle Reflex software?

We are often asked what units the actual data from the startle sensor are expressed in. Startle Reflex software utilizes a highly sensitive analog-to-digital converter that converts the analog voltage signal from the startle sensor to a digital unit having a value between –2048 and +2048. The converter can handle voltages between –10 Volts and +10 Volts. In most cases, people simply leave their startle responses expressed on a scale of 0 to 2048 "Arbitrary Units" or "Startle Response Units" (e.g. Bortolato *et al*, 2007; Jaworski *et al*, 2005; Meloni *et al*, 2006). However, these values can be easily converted to a dimensioned quantity, such as Voltage.

Recalling the principles of operation described above, you can simply divide your startle response by 2048, and multiply this number by 10.

$$\frac{\text{Startle Response } (\textit{in arbitrary Startle Units})}{2048} \times 10 \text{ Volts} = \text{Startle Response } (\textit{in Volts})$$

This process would yield the actual voltage measured by the analog-to-digital converter. This value can serve to instill confidence that similar sensitivities are achieved across all of your startle chambers. However, it is still difficult to compare actual data from different publications, unless you know specific calibration procedures that were used to generate the data of interest. We advocate publishing as much detail as possible regarding calibration methods. This allows researchers in different labs to make the most informative conclusions about the reported data, regardless of the units that the data are expressed in.

References:

Bortolato M, Frau R, Orru M, Piras AP, Fa M, Tuveri A, Puligheddu M, Gessa GL, Castelli MP, Mereu G, Marrosu F. Activation of GABA(B) receptors reverses spontaneous gating deficits in juvenile DBA/2J mice. *Psychopharmacology (Berl)*. 2007 Jun 29; DOI 10.1007/s00213-007-0845-5.

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Meloni EG, Jackson A, Gerety LP, Cohen BM, Carlezon WA. Role of the bed nucleus of the stria terminalis (BST) in the expression of conditioned fear. *Ann. N. Y. Acad. Sci*. 1071:538-541, 2006.

Risbrough VB, Geyer MA. Anxiogenic treatments do not increase fear-potentiated startle in mice. *Biol. Psychiatry*. 57(1):33-43, 2005.

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Winslow JT, Noble PL, Davis M. Modulation of fear-potentiated startle and vocalizations in juvenile rhesus monkeys by morphine, diazepam, and buspirone. *Biol. Psychiatry*. 61(3):389-395, 2007.

CHAPTER 10 | CONTACT INFORMATION

Please contact Med Associates, Inc. for information regarding any of our products.

Visit our website at www.med-associates.com for contact information.

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