med associates inc

instrumentation and software for research

ADVANCED STARTLE

SOF-828

USER'S MANUAL

DOC-005
Rev. 2.6

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notes

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CHAPTER 1 | INTRODUCTION

The Startle paradigm is used to study fear and anxiety in laboratory animals. Although the underlying circuits and systems controlling fear and anxiety are complex, the Startle paradigm itself is relatively simple: it is a form of Pavlovian conditioning where a central state of fear is inferred from an animal's behavioral response. This is done by measuring the amplitude of a simple brainstem reflex, the acoustic startle reflex, in the presence of a cue (the conditioned stimulus; CS) previously paired with a mild foot shock (the unconditioned stimulus; US). For example, when animals are repeatedly presented with a neutral stimulus (one that does not elicit any behavioral effect on its own), such as a light flash, a tone, or an odor, followed by a brief foot shock, the animal comes to associate the presentation of the cue with an impending aversive event (the shock). Eventually, presentation of the cue in the absence of the shock comes to elicit a constellation of behaviors that are typically used to define a state of fear in animals.

Using SOF-828 Advanced Startle to accomplish this, animals are placed in cages in an isolation chamber (the same chamber in which animals received the prior fear-conditioning) and presented with a number of startle-eliciting white-noise bursts. The cages are positioned on a sensitive load-cell device that transduces movement of the cage (produced by the animal's startle response) into an electrical signal that is amplified and quantified in arbitrary startle units. On some trials, the startle-eliciting stimulus is preceded by the cue previously paired with shock. In this case, the startle response is much larger (i.e., potentiated) than on trials without presentation of the cue and it is the difference (expressed either as an absolute value or a percent change in different cases) between the amplitude of startle on these two trial types that is our operational measure of fear.

CHAPTER 2 | DESCRIPTION OF ADVANCED STARTLE SOFTWARE

The Advanced Startle Software allows investigators to use both pre-programmed research experiments (for performing both **Prepulse Inhibition of Startle** (PPI) and **Fear-Potentiated Startle** (FPS) experiments), and user-defined research experiments based on the researcher's present needs. The experimental procedures are saved in text files (*.txt) that have been written in a tabular format. Because the files have been written using tab separated values they can be viewed and/or edited in Microsoft Excel or whatever spreadsheet software is most convenient. The purpose of the experimental procedure files is to initialize both **Control** and **Trial Variables** that the software uses to execute an experiment.

To understand what these variables represent, one should be familiar with the definition of an **Event Marker**. Understanding the Event Marker is critical to understanding what data will be recorded during a trial, and when they will be recorded. The Event Marker is the anchor for the timeline in a trial.

Variable Definitions

Trial Variables include either the suffix **CS** (Condition Stimulus) or **STL** (Startle), such as "AUX1 CS(sec)" or "AUX1 STL(sec)."

The **AUX** variables are used to control the four Auxillary Outputs. Any 28V active-low component can be connected to the Auxillary Outputs.

ACC/ITI is the Acclimation Period or Inter-Trial Interval. The ACC/ITI(sec) value determines when the Event Marker occurs; therefore, if ACC/ITI(sec) for a particular trial is 30 seconds, then the Event Marker will occur 30 seconds after the previous trial's Event Marker.

TONE and **NOISE** are the variables used for turning on Pure Tones and White Noise/Noise Bursts respectively.

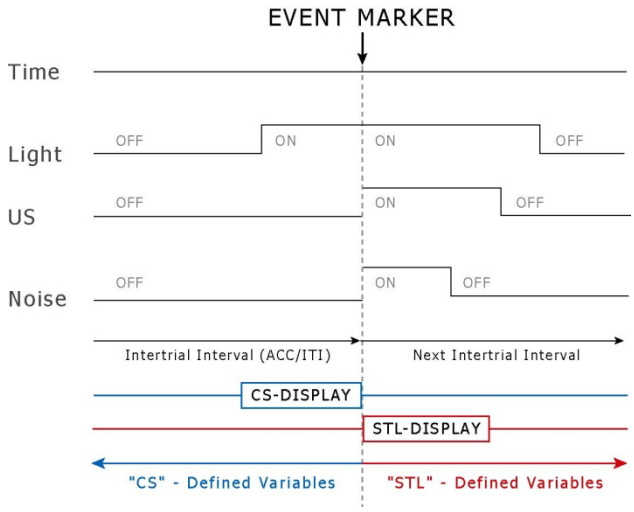
Timeline for Creating a Trial

Figure 2.1 on the next page illustrates the events occurring within any given trial. The **Event Marker** divides the trial into two parts and initiates STL-DISPLAY (one of two time periods during which the subject's activity will be captured).

All stimuli present PRIOR to the Event Marker include the suffix "**CS**." Therefore, if AUX1 CS(sec) is defined as 2.5, then this stimulus will turn on 2.5 seconds prior to the Event Marker and stay on for 2.5 seconds. All "CS" stimuli will terminate at the Event Marker (unless CS-STL DELAY(sec) is set to a value other than 0; see the upcoming discussion on CS-STL DELAY(sec)).

If a stimulus is to remain on AFTER the Event Marker, then include a value for that stimulus in the group of variables that have the suffix "**STL**." For instance, if the AUX1 stimulus is to remain on for 1.7 seconds past the Event Marker, define AUX1 STL(sec) as 1.7. In this example, the AUX1 stimulus would remain on for a total of 4.2 seconds (2.5 seconds before the Event Marker, and 1.7 seconds afterward).

Figure 2.1 - Event Marker and Trial Timeline



CS-DISPLAY(sec) & STL-DISPLAY(sec)

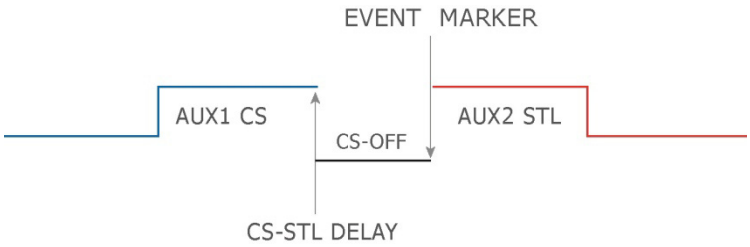
These two variables determine the time window during which the behavioral data will be recorded and displayed on the voltage graph in Advanced Startle or Startle Viewer. CS-DISPLAY(sec) records behavioral activity PRIOR to the Event Marker, and STL-DISPLAY(sec) records activity for a specified duration of time AFTER the Event Marker. STL-DISPLAY(sec) also includes the time data used to calculate peak-to-peak intervals. The total duration of behavioral recording (CS-DISPLAY(sec) + STL-DISPLAY(sec)) is a maximum of 32 seconds.

NOTE: While the Advanced Startle program will record and save all of the data to the .RAW file, it will not display more than 2 seconds of the data. To see all of the data at once the Startle Viewer needs to be used. **Chapter 7 | ADVANCED STARTLE DATA FILES** will have more information on how to read the data files.

CS-STL DELAY(sec)

The CS-STL DELAY(sec) sets the Controlled Stimulus minus Uncontrolled Stimulus (CS-US) trace interval, and overrides the values set for CS durations. If the CS-STL DELAY(sec) is set to 1.5 in the experimental procedure, for any given trial all CS-defined variables will terminate 1.5 seconds PRIOR to the Event Marker. If, for instance, the AUX1 CS(sec) is set to 2.5, and the CS-STL DELAY(sec) is set to 1.5, then the AUX1 CS(sec) stimulus will turn on for 1 second, and the turn off 1.5 seconds PRIOR to the Event Marker. In Figure 2.2, the AUX1 CS(sec) is turned off at the commencement of the CS-STL DELAY(sec), and no stimulus is present until the Event Marker, at which time the AUX2 STL(sec) stimulus turns on.

Figure 2.2 - Event Marker and AUX1 CS(sec)/AUX2 STL(sec) Timeline



Session Duration

Session Duration is the sum of ACC/ITI(sec) values for all the trials plus the STL-DISPLAY(sec) value. There is a minimum 2-second requirement between the offset of any post-Event Marker variable (e.g. STL-defined stimuli or STL-DISPLAY(sec)) and the onset of any pre-Event Marker variable (e.g. CS-defined stimuli or CS-DISPLAY(sec)).

CHAPTER 3 | HARDWARE CONFIGURATION AND CALIBRATION

Refer to the DOC-040 Startle Reflex User's Manual for hardware configuration instructions and for Audio Calibration and Input (load cell platform) Calibration procedures. The Hardware menu in the Startle Reflex software runs utilities for complete testing and calibration of all Startle hardware.

CHAPTER 4 | DEFINITIONS OF CONTROL AND TRIAL VARIABLES

Figure 4.1 contains definitions of the **Control Variables** that are used to initialize an experiment. Note that the Control Variables will remain constant throughout execution of the program.

Figure 4.1 - Advanced Startle Control Variable Definitions

		Control Variables Defined
1	STL-DISPLAY(sec)	The time during which activity (baseline or startle) is recorded. Enter the time value in seconds. This value must be greater than 0.
2	CS-DISPLAY(sec)	Records the subject's activity immediately prior to STL-DISPLAY(sec). Enter the time value in seconds. This value must be greater than 0. NOTE: The total duration of behavioral recording (CS-DISPLAY(sec) + STL-DISPLAY(sec)) is a maximum of 32 seconds.
3	CS-FREEZE(sec)	Records data but does not display it. This option is good for recording data to be stored in the RAW data file.
4	Rise-Time-Noise(ms)	This value sets the rise time of the white noise stimulus, in milliseconds. The recommended value is between 1 and 5ms, and not more than half the noise duration of each trial. When creating short audio events, keep in mind the rise time reduces the full volume duration of a noise burst. This value is used to help reduce pops from the speaker by increasing the amount of time it takes for the volume to go from off to full on. Example: If the NOISE-CS(sec) is set to 0.1s and the Rise-Time-Noise(ms) is set to 1ms, then the noise will actually only be on for 0.099s (0.1s - 0.001s = 0.099s).
5	Rise-Time-Tone(ms)	This value sets the rise time of the tone stimulus, in milliseconds. The recommended value is between 1 and 5ms, and not more than half the tone duration of each trial. When creating short audio events, keep in mind the rise time reduces the full volume duration of a tone burst. This value is used to help reduce pops from the speaker by increasing the amount of time it takes for the volume to go from off to full on.
6	LATENCY(ms)	Peak -to-peak values are recorded after specified value, in milliseconds. This value should remain at "0" to exclude this option.
7	BACKGROUND(dB)	Constant background noise. If a value is entered here, a noise will be present during the entire experimental session at the dB value entered here, and this variable should range from 70 to 115 db.
8	AUX1(1=Always on)	If "1" is entered here, then the stimulus corresponding to AUX1 on PHM-255C will be ON throughout the experiment.
9	AUX2(1=Always on)	If "1" is entered here, then the stimulus corresponding to AUX2 on PHM-255C will be ON throughout the experiment.
10	AUX3(1=Always on)	If "1" is entered here, then the stimulus corresponding to AUX3 on PHM-255C will be ON throughout the experiment.
11	AUX4(1=Always on)	If "1" is entered here, then the stimulus corresponding to AUX4 on PHM-255C will be ON throughout the experiment.
12	Peak Calc. Method	N/A, keep this value at 0!
13	Min Peak Value(Volts)	N/A, keep this value at 0!
14	Min Peak Time(ms)	N/A, keep this value at 0!
15	Reserved	N/A, keep this value at 0!
16	Reserved	N/A, keep this value at 0!
17	Reserved	N/A, keep this value at 0!
18	Reserved	N/A, keep this value at 0!
19	DAQ Gain(Volts)	This represents the maximum +/- voltage recorded from the load cell. Keep this at 10!
20	DAQ Frequency(Hz)	The number of samples taken per second; leave this at 1,000.
21	Reserved	N/A, keep this value at 0!
22	Reserved	N/A, keep this value at 0!

Figure 4.2 contains definition of the **Trial Variables** that are used to initialize an experiment. Note that the nature of these variables is to allow the control of the behavior of the program in any given trial, and due to this, at any given moment in the dynamic execution of the program the values may be different than they were at the beginning of the session.

Figure 4.2 - Advanced Startle Trial Variable Definitions

		Trial Variables Defined
1	ACC/ITI(sec)	Interval of time between the previous trial's Event Marker and this current trial's Event Marker. This variable value must be at least two seconds greater than the control variables, CS DISPLAY(sec) and STL DISPLAY(sec), for any trial.
2	AUX1 CS(sec)	The duration in seconds that the stimulus corresponding to AUX1 on the PHM-255C will be on prior to the Event Marker.
3	AUX2 CS(sec)	The duration in seconds that the stimulus corresponding to AUX2 on the PHM-255C will be on prior to the Event Marker.
4	AUX3 CS(sec)	The duration in seconds that the stimulus corresponding to AUX3 on the PHM-255C will be on prior to the Event Marker.
5	AUX4 CS(sec)	The duration in seconds that the stimulus corresponding to AUX4 on the PHM-255C will be on prior to the Event Marker.
6	TONE CS(sec)	The duration of the tone stimulus prior to the Event Marker. Tone and noise cannot both be active during the CS period of a trial. To use both audio events in a single trial, activate one as CS and the other as STL.
7	NOISE CS(sec)	The duration of the noise stimulus prior to the Event Marker. Tone and noise cannot both be active during the CS period of a trial. To use both audio events in a single trial, activate one as CS and the other as STL.
8	NOISE CS(dB)	The dB of the NOISE CS, and this variable should range from 70 to 115 db.
9	TONE CS(dB)	The dB of the TONE CS, and this variable should range from 70 to 115 db.
10	TONE CS(Hz)	The frequency of the TONE CS, this variable should range from 4,000 to 20,000.
11	TONE STL(Hz)	The frequency of the TONE STL (see below), this variable should range from 4,000 to 20,000.
12	TONE STL(dB)	The dB of the TONE STL, and this variable should range from 70 to 115 db.
13	AUX1 STL(sec)	The duration of the AUX1 stimulus (in seconds) that is turned on after the Event Marker.
14	AUX2 STL(sec)	The duration of the AUX2 stimulus (in seconds) that is turned on after the Event Marker.
15	AUX3 STL(sec)	The duration of the AUX3 stimulus (in seconds) that is turned on after the Event Marker.
16	AUX4 STL(sec)	The duration of the AUX4 stimulus (in seconds) that is turned on after the Event Marker.
17	TONE STL(sec)	The duration of the tone stimulus occurring after the Event Marker. Tone and noise cannot both be active during the STL period of a trial. To use both audio events in a single trial, activate one as CS and the other as STL.
18	NOISE STL(sec)	The duration of the noise stimulus occurring after the Event Marker. Tone and noise cannot both be active during the STL period of a trial. To use both audio events in a single trial, activate one as CS and the other as STL.
19	NOISE STL(dB)	The dB of the NOISE STL, this variable should range from 70 to 115 db.
20	Trial Comment 1	A description of the particular trial, use this to sort data for analysis. Enter any descriptor here because this cell is not read during execution.
21	Trial Comment 2	A secondary descriptor for sorting data by block or stimulus condition. Enter any descriptor here.
22	CS-STL DELAY(sec)	Turns off all CS durations prior to Startle Onset. Normally set to 0 for no delay. This value MUST be set to 0 if there are no Condition Stimulus events included in the trial.

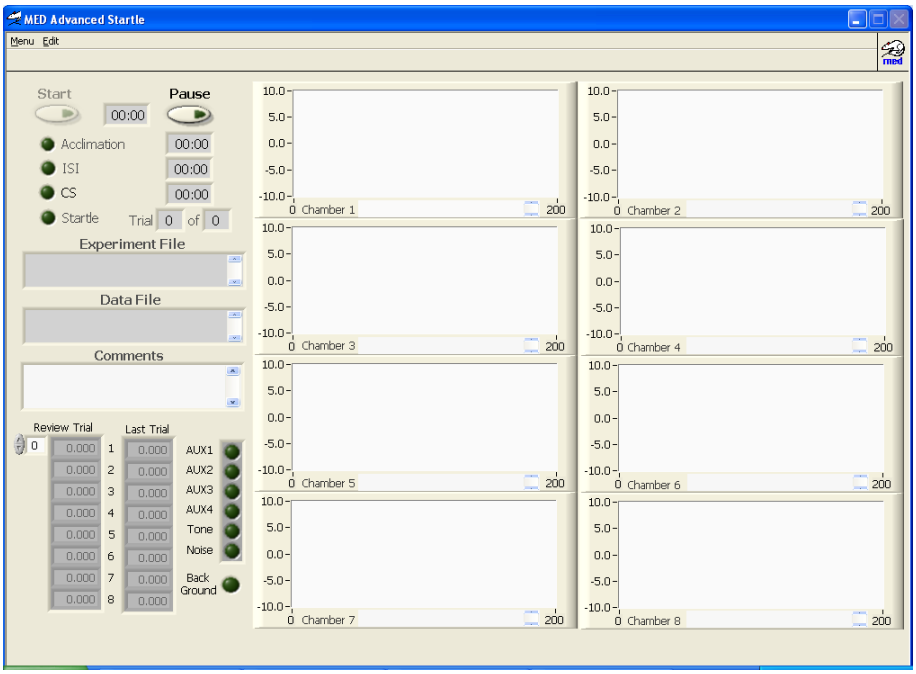
CHAPTER 5 | PRE-PROGRAMMED EXPERIMENTAL PROCEDURES

Loading and Executing a Pre-Programmed Experiment File

The installation of the Advance Startle program included a folder called "C:\Program Files\MED Associates\Advanced Startle\Tables". The following experimental programs/tables are listed in the folder. These are the files used to execute the following pre-programmed experiments.

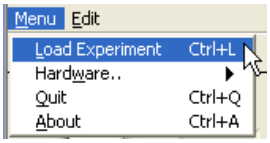
Open the Advanced Startle software and the main screen shown in Figure 5.1 will appear.

Figure 5.1 - Advanced Startle Main Screen



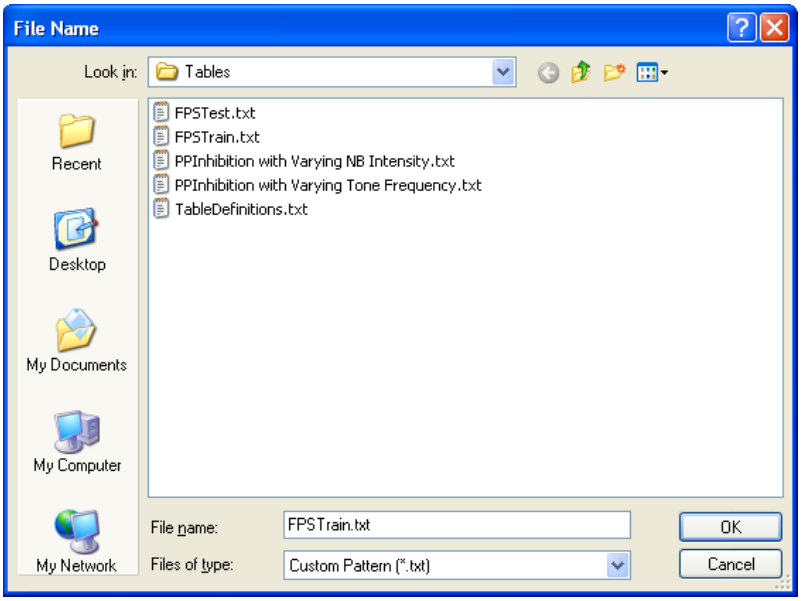
Select **Menu**| **Load Experiment** as shown below, and a "File Name" dialog box, as shown in Figure 5.3 will open.

Figure 5.2 - Menu | Load Experiment



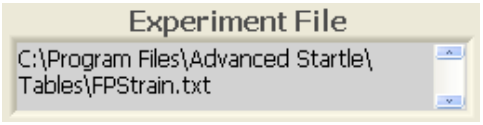
Choose the directory and experiment file to execute and click **OK** (all experiment files will have the file extension ".txt").

Figure 5.3 - Select the File to Execute



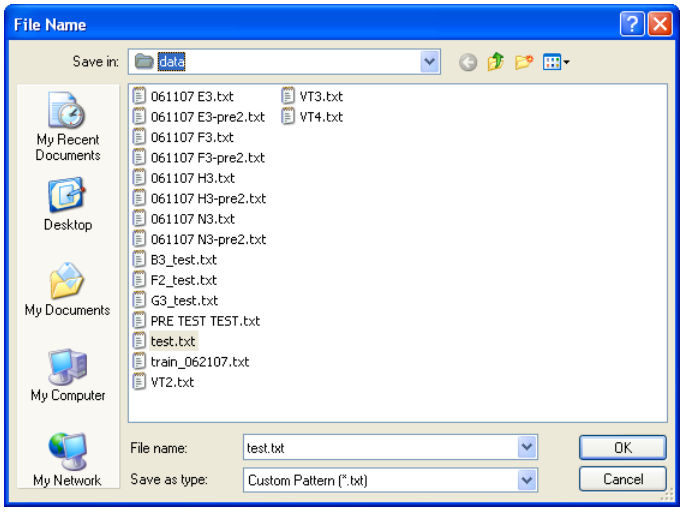
The name of the file selected (shown with its full path) will appear in the "Experiment File" box just under the "Trial X of X" boxes in the left part of the display.

Figure 5.4 – Experiment File Box



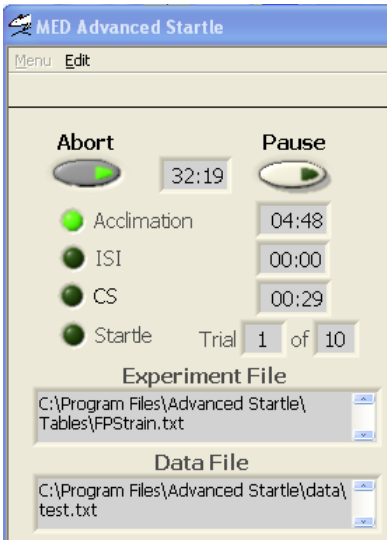
Click the **Start** button, and another "File Name" dialog box will appear, as shown in Figure 5.5. Enter a name for the saved data file (a folder in the C:\Program Files\MED Associates\Advanced Startle directory called "data" has been provided to store data files). The file will get a .txt extension by default. Clicking the **OK** button will automatically begin the experiment.

Figure 5.5 – Enter a Data File Name



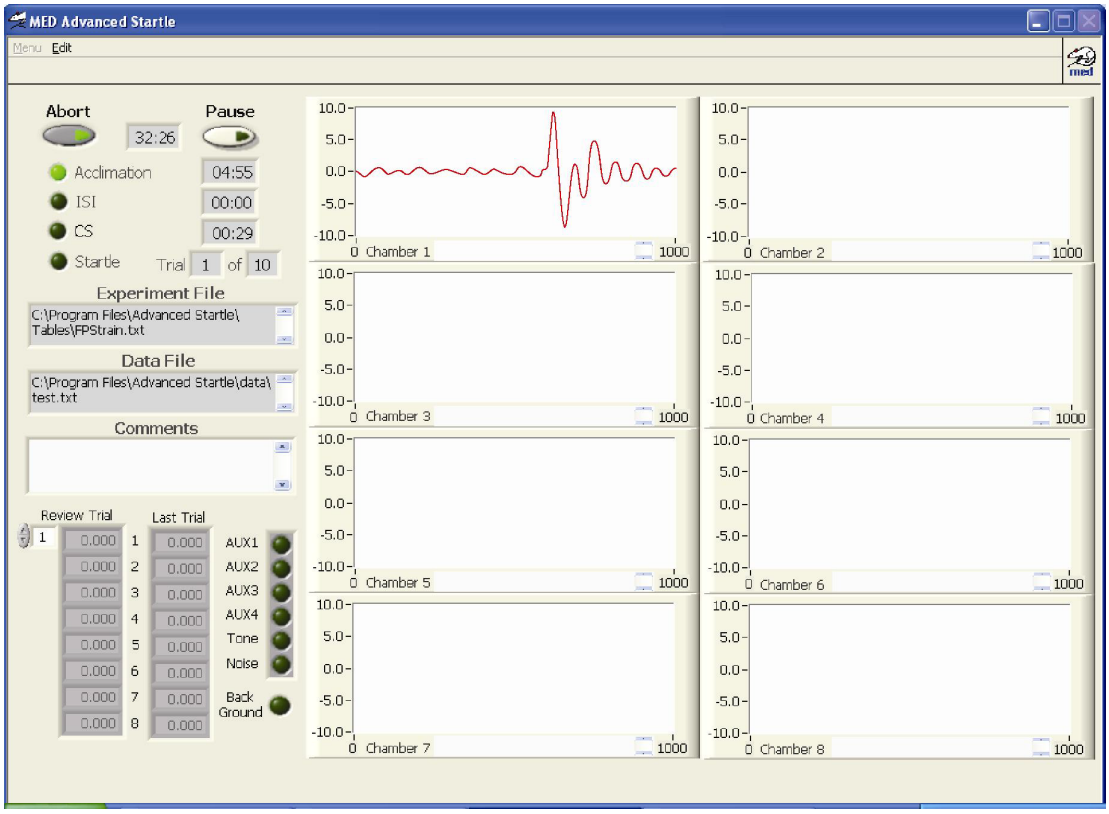
During the experiment, the program file selected is listed in the "Experiment File" box, and the data file to save data in is listed in the "Data File" box, as shown in Figure 5.6. Data will be saved to the data file specified, even if the experiment is aborted before the session has expired.

Figure 5.6 - Experiment and Data File Names



Advanced Startle includes a total of eight startle-response display windows; one window corresponds to each of eight chambers. The startle response (in volts) for each trial is displayed in the corresponding chamber window.

Figure 5.7 - Advanced Startle Screen with Response in Display Window

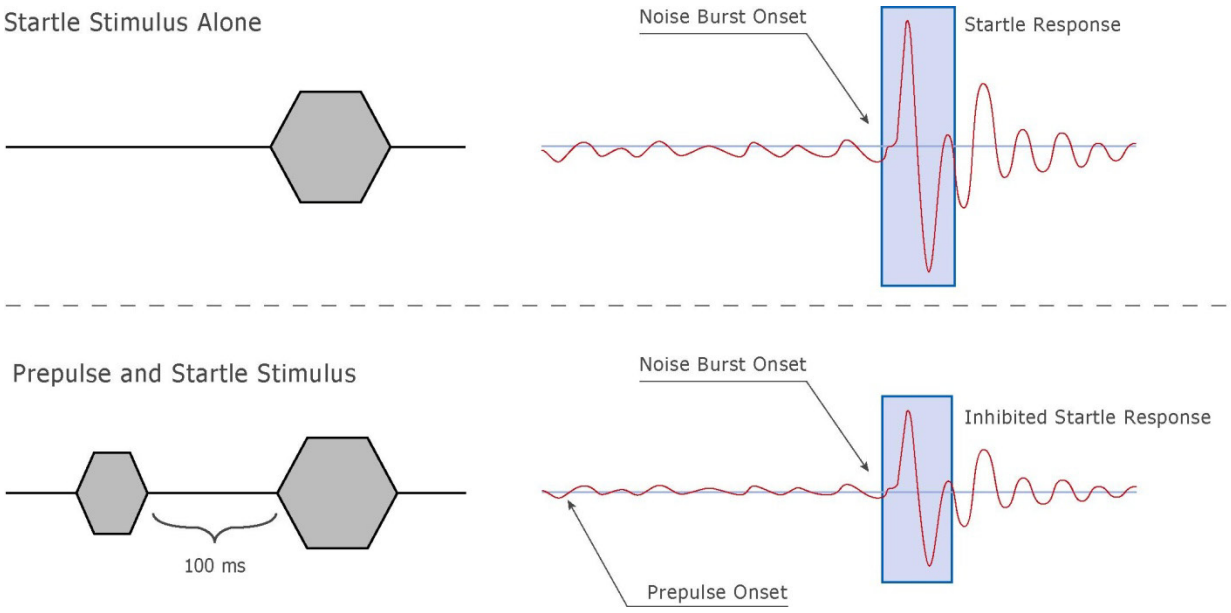


Please note that only the first trial will be displayed initially. To see the results of the current trial (its number is shown in the Trial XX of XX boxes in the top left part of the display), toggle the "Review Trial" control in the lower left corner to find the trial of interest. Alternatively, the trial number can be entered into the text box provided, then hit "Enter" to bring up the data recorded for that particular trial. The Review Trial feature shows the maximum startle output for a selected trial per chamber. The data column to the right displays the data for the most recent trial.

Prepulse Inhibition of Startle (PPI)

PPI of the startle response (modeled in Figure 5.8) is a behavioral phenomenon in which a short tone presented just prior to an acoustic startle stimulus (a sound presented at a fairly high amplitude) will serve to dampen the subsequent startle response. PPI has been observed in mice[1], rats[2], Rhesus Macaques[3-4], and humans[5-7]. Rodent testing of PPI is used in the testing of therapeutic compounds[8-9,] and has been used as a screening technique for transgenic/knock-out mice[10].

Figure 5.8 - Prepulse Inhibition of Startle (PPI)



(Figure courtesy of William A. Falls, PhD, University of Vermont)

Experiment Initiated by the "PPInhibition with Varying Tone Frequency.txt" Procedure

Earlier, it was stated that all experiment files contain data to set the values for both Control and Trial Variables while the program is in execution. As noted earlier, these files are in a text file with the experimental parameters separated by tabs, so they can be viewed in a software spreadsheet program like Microsoft Excel. Shown in Figure 5.9 is the "PPInhibition with Varying Tone Frequency.txt" experiment file as it appears when it is opened in Microsoft Excel. The experiment is controlled by the values entered in the "PPInhibition with Varying Tone Frequency.txt" file. A description of the variables in the experiment follows:

Figure 5.9 - PPInhibition with Varying Tone Frequency.txt Experiment Table

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	STL-DISPLAY(sec)	0.7	ACC/ITI(sec)	60	4	6	5	7	8	4	4	5	8
2	CS-DISPLAY(sec)	0.3	AUX1 CS(sec)	0	0	0	0	0	0	0	0	0	0
3	CS-FREEZE(sec)	0	AUX2 CS(sec)	0	0	0	0	0	0	0	0	0	0
4	RISE-TIME-NOISE(ms)	1	AUX3 CS(sec)	0	0	0	0	0	0	0	0	0	0
5	RISE-TIME-TONE(ms)	1	AUX4 CS(sec)	0	0	0	0	0	0	0	0	0	0
6	LATENCY(ms)	0	TONE CS(sec)	0	0.1	0	0.1	0.1	0	0	0.1	0.1	0
7	BACKGROUND(dB)	0	NOISE CS(sec)	0	0	0	0	0	0	0	0	0	0
8	AUX1(1=Always on)	0	NOISE CS(dB)	0	0	0	0	0	0	0	0	0	0
9	AUX2(1=Always on)	0	TONE CS(dB)	0	70	0	70	70	0	0	70	70	0
10	AUX3(1=Always on)	0	TONE CS(Hz)	0	20000	0	12000	4000	0	0	20000	4000	0
11	AUX4(1=Always on)	0	TONE STL(Hz)	0	0	0	0	0	0	0	0	0	0
12	Peak Calc. Method	0	TONE STL(dB)	0	0	0	0	0	0	0	0	0	0
13	Min Peak Value(Volts)	0	AUX1 STL(sec)	0	0	0	0	0	0	0	0	0	0
14	Min Peak Time(ms)	0	AUX2 STL(sec)	0	0	0	0	0	0	0	0	0	0
15	Reserved	0	AUX3 STL(sec)	0	0	0	0	0	0	0	0	0	0
16	Reserved	0	AUX4 STL(sec)	0	0	0	0	0	0	0	0	0	0
17	Reserved	0	TONE STL(sec)	0	0	0	0	0	0	0	0	0	0
18	Reserved	0	NOISE STL(sec)	0.01	0	0	0.01	0.01	0	0.01	0.01	0	0.01
19	DAQ Gain(Volts)	10	NOISE STL(dB)	100	0	0	100	100	0	100	100	0	100
20	DAQ Frequency(Hz)	1000	Trial Comment 1	NULL	20k Tone	NULL	12k Tone	4k Tone	NULL	NULL	20k Tone	4k Tone	NULL
21	Reserved	0	Trial Comment 2	100dB NB	NULL	NULL	100dB NB	100dB NB	NULL	100dB NB	100dB NB	NULL	100dB NB
22	Reserved	0	CS-STL DELAY(sec)	0	0.09	0	0.09	0.09	0	0	0.09	0.09	0

Column **A** lists the titles of **Control Variables** that stay constant throughout the experiment. The values of these variables are found in the corresponding row in column **B** (for instance, STL-DISPLAY(sec) is set to 0.7 (in seconds), and this means that the startle response will be recorded for 700 milliseconds for every trial). Column **C** lists the **Trial Variables**, and these are the parameters that define the stimulus presentations and Inter-Trial Interval (ITI) durations; their values are found in the corresponding row in Columns **D** and greater (**E**, **F**, etc.), which describe individual trials for the experiment.

The other Control Variables for this experiment are as follows:

RISE-TIME-NOISE(ms)/RISE-TIME-TONE(ms) is set at 1 millisecond, which is the rise time of the noise/tone stimulus.

LATENCY(ms) is set to 0 milliseconds. Essentially, Latency is the amount of time set between the onset of a "response" and when a "peak" load value will be considered as the result of the startle stimulus. If the amount of time between the onset of a response and a peak is less than the Latency value, the peak is not counted as a response.

DAQ Gain(Volts) is the maximum +/- voltage recorded from the load cell, and typically should be left at 10 volts.

The **DAQ Frequency(Hz)** is set to 1000; this is the sample frequency for the program, and is generally left at 1000 samples/second.

A trial is an event during which a response is recorded, whether it is a baseline measurement of activity or a startle response. Columns **D** (and **E**, **F**, etc.) in the above list are the corresponding parameter values for every trial. In other words, these columns are user-defined, and each column identifies the stimulus properties used during a single trial. Therefore, the number of columns that contain values (from columns **D**, **E**, etc.) determines the total number of trials in a particular experiment.

The protocol for this experiment has randomly intermixed trials that receive a noise burst only with those that receive a prepulse tone before the delivery of the noise burst; the experiment shows 60 trials (columns **D** through **BK**). The first trial, shown in column **D**, is a trial that receives a noise burst only. This is true because only the NOISE STL(sec) and NOISE STL(dB) Trial Variables have been given values.

The **Trial Comment** cells allow the user to define a particular trial using an identifier. The identifier is a sequence of alphanumeric characters (no more than 8 digits and/or letters), and is intended to define a unique tag to help sort the data once it has been imported into a spreadsheet program like Microsoft Excel. The Trial Comment cells will be present in the table of the Startle Viewer and in the .txt data files that can be used for data analysis. Therefore, use identifiers that will allow the data to be sorted easily for analysis. The computer program does not read the Trial Comment cells during the execution of the experiment, so values entered here will not impact the procedure. Note that the Trial Comment 1 entry for the first trial (column **D**) is NULL, and the Trial Comment 2 entry is 100dB NB. These values can be used to sort the trials in the experiment that receive only a noise burst at 100dB.

The first trial (column **D**) occurs 60 seconds (1 minute) after the experiment is initiated, the next trial (column **E**) occurs 4 seconds later, the third trial (column **F**) occurs 6 seconds after that, etc. These values are defining the **ACC/ITI(sec)** Trial Variable, which sets the Inter-Trial Interval; i.e., the amount of time between event markers. The ACC/ITI(sec) value set in the first trial is used to define the length of the Acclimation Period for the test subject.

The second trial (column **E**), is one in which there will be a prepulse tone with no noise burst, since the **TONE CS(sec)**, **TONE CS(dB)** and the **TONE CS(Hz)** Trial Variables have non-zero values. In the case of the second trial, a 70 dB tone (set in TONE CS(dB)) will be emitted from the speaker at 20000 Hz (set in TONE CS(Hz)) for duration of 0.01 seconds (set value of TONE CS(sec) = 0.1 seconds – set value in CS-STL DELAY(sec)= 0.09 seconds = 0.01 seconds).

This is a functional description of how the software will reference an experimental (.txt) file during the execution of the experiment. Let us now consider the Fear-Potentiated Startle experiment, and its experiment tables for training and testing of a subject.

Experiment Initiated by the "PPInhibition with Varying NB Intensity.txt" Procedure

The "PPInhibition with Varying NB Intensity.txt" has 48 trials total. The experiment starts off with 10 noise burst only (i.e. with No Prepulse) trials (columns **D** through **M**), to determine a baseline response to a startle stimulus.

The experiment then has 28 trials (columns **N** through **AO**) of which 4 of the trials (columns **R**, **Z**, **AC**, **AK**) consist of the noise burst startle stimulus alone, 16 of the trials (columns **N**, **Q**, **S**, **T**, **V**, **W**, **Y**, **AA**, **AB**, **AD**, **AF**, **AH**, **AJ**, **AL**, **AN**, **AO**) consist of a pre-pulse stimulus (at one of 4 different intensities 67dB, 70dB, 73dB, and 76dB) followed by the noise burst startle stimulus, 4 of the trials (columns **P**, **X**, **AG**, **AI**) are prepulse only (one at each intensity), and 4 null (no stimulus presented) trials (columns **O**, **U**, **AE**, **AM**).

The experiment then ends with 10 noise burst only trials (columns **AP** through **AY**) in order to ensure that no habituation has taken place.

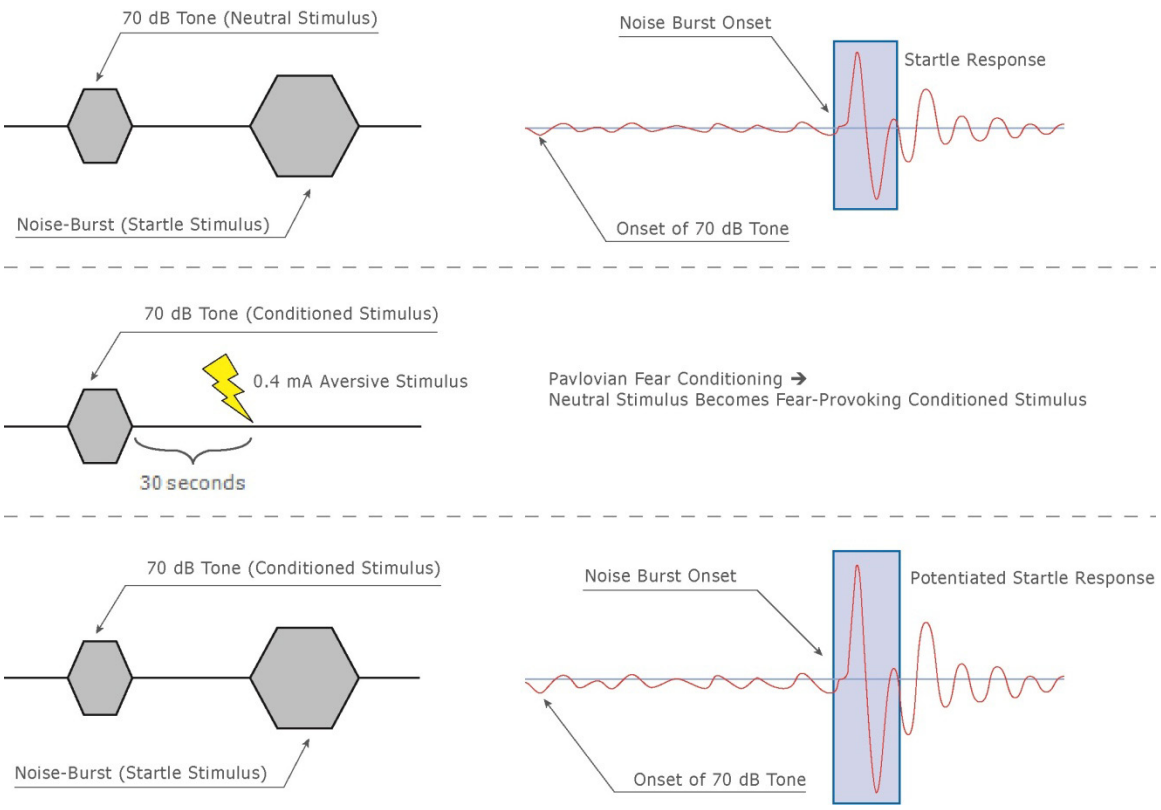
Figure 5.10 - PPInhibition with Varying NB Intensity.txt Experiment Table

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	STL-DISPLAY(sec)	0.7	ACC/ITI(sec)	300	15	12	18							15	12	19	17	14	11
2	CS-DISPLAY(sec)	0.3	AUX1 CS(sec)	0	0	0	0							0	0	0	0	0	0
3	CS-FREEZE(sec)	0	AUX2 CS(sec)	0	0	0	0							0	0	0	0	0	0
4	RISE-TIME-NOISE(ms)	1	AUX3 CS(sec)	0	0	0	0							0	0	0	0	0	0
5	RISE-TIME-TONE(ms)	1	AUX4 CS(sec)	0	0	0	0							0	0	0	0	0	0
6	LATENCY(ms)	0	TONE CS(sec)	0	0	0	0							0	0	0	0	0	0
7	BACKGROUND(dB)	62	NOISE CS(sec)	0	0	0	0							0.1	0	0.1	0.1	0	0.1
8	AUX1(1=Always on)	0	NOISE CS(dB)	0	0	0	0							67	0	70	73	0	70
9	AUX2(1=Always on)	0	TONE CS(dB)	0	0	0	0							0	0	0	0	0	0
10	AUX3(1=Always on)	0	TONE CS(Hz)	0	0	0	0							0	0	0	0	0	0
11	AUX4(1=Always on)	0	TONE STL(Hz)	0	0	0	0							0	0	0	0	0	0
12	Peak Calc. Method	0	TONE STL(dB)	0	0	0	0							0	0	0	0	0	0
13	Min Peak Value(Volts)	0	AUX1 STL(sec)	0	0	0	0							0	0	0	0	0	0
14	Min Peak Time(ms)	0	AUX2 STL(sec)	0	0	0	0							0	0	0	0	0	0
15	Reserved	0	AUX3 STL(sec)	0	0	0	0							0	0	0	0	0	0
16	Reserved	0	AUX4 STL(sec)	0	0	0	0							0	0	0	0	0	0
17	Reserved	0	TONE STL(sec)	0	0	0	0							0	0	0	0	0	0
18	Reserved	0	NOISE STL(sec)	0.04	0.04	0.04	0.04							0.04	0	0	0.04	0.04	0.04
19	DAQ Gain(Volts)	10	NOISE STL(dB)	120	120	120	120							120	0	0	120	120	120
20	DAQ Frequency(Hz)	1000	Trial Comment 1	NULL	NULL	NULL	NULL	U	U	U	U	U	U	67dB NB	NULL	70dB NB	73dB NB	NULL	70dB NB
21	Reserved	0	Trial Comment 2	120dB NB	120dB NB	120dB NB	120dB NB							120dB NB	NULL	NULL	120dB NB	120dB NB	120dB NB
22	Reserved	0	CS-STL DELAY(sec)	0	0	0	0							0.08	0	0.08	0.08	0	0.08

Fear-Potentiated Startle (FPS)

Fear-potentiated startle (FPS)[3-4] occurs when the startle response to an acoustic stimulus is increased relative to baseline startle responding via Pavlovian conditioning. The general procedure for producing FPS involves preceding an aversive stimulus (such as a shock or air puff) with a tone (or some other neutral stimulus). After several presentations of this stimulus pairing, the presentation of the tone elicits a fear response. Once conditioning has occurred, the tone is then presented before an acoustic startle stimulus, increasing the intensity of the startle response. FPS is a typical reflex that has been observed in a variety of species, thus making it a useful tool for investigating many learning and memory processes.

Figure 5.11 - Fear-Potentiated Startle (FPS)



(Figure created by modifying previous figure from William A. Falls, PhD, University of Vermont)

Experiment Initiated by the "FPSTrain.txt" Procedure

The Fear-Potentiated Experiment protocol describes two separate procedures. The first procedure is used to create Pavlovian conditioning in a test subject. This experiment is described in the "FPSTrain.txt" Experiment Table (shown in Figure 5.12).

This experiment consists of 10 trials (columns **D** through **M**). Inspection of the table reveals the fact that the only Trial Variable that changes from trial to trial is the ACC/ITI(sec) variable; it ranges from 90 to 300 seconds.

Each trial consists of a 30s Prepulse (70dB at 12KHz) followed by a 0.25s foot shock. The foot shock is controlled by an ENV-414S connected to the AUX 1 output on the PHM-255A. The amplitude of the foot shock is controlled by the ENV-414S.

One special thing to note about this experiment is that the CS-DISPLAY(sec) is set to 30.2 seconds. This is so the program can record a 200ms NULL Period before the 30s Prepulse or CS Tone.

NOTE: While the Advanced Startle program will record and save all of the data to the .RAW file, it will not display more than 2 seconds of the data. To see all of the data at once the Startle Viewer needs to be used. **Chapter 7 | ADVANCED STARTLE DATA FILES** will have more information on how to read the data files.

Figure 5.12 - FPSTrain.txt Experiment Table

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	STL-DISPLAY(sec)	0.6	ACC/ITI(sec)	300	157.5	157.5	180	90	157.5	135	112.5	135	112.5
2	CS-DISPLAY(sec)	30.2	AUX1 CS(sec)	0	0	0	0	0	0	0	0	0	0
3	CS-FREEZE(sec)	0	AUX2 CS(sec)	0	0	0	0	0	0	0	0	0	0
4	RISE-TIME-NOISE(ms)	3	AUX3 CS(sec)	0	0	0	0	0	0	0	0	0	0
5	RISE-TIME-TONE(ms)	3	AUX4 CS(sec)	0	0	0	0	0	0	0	0	0	0
6	LATENCY(ms)	0	TONE CS(sec)	30	30	30	30	30	30	30	30	30	30
7	BACKGROUND(dB)	0	NOISE CS(sec)	0	0	0	0	0	0	0	0	0	0
8	AUX1(1=Always on)	0	NOISE CS(dB)	0	0	0	0	0	0	0	0	0	0
9	AUX2(1=Always on)	0	TONE CS(dB)	70	70	70	70	70	70	70	70	70	70
10	AUX3(1=Always on)	0	TONE CS(Hz)	12000	12000	12000	12000	12000	12000	12000	12000	12000	12000
11	AUX4(1=Always on)	0	TONE STL(Hz)	0	0	0	0	0	0	0	0	0	0
12	Peak Calc. Method	0	TONE STL(dB)	0	0	0	0	0	0	0	0	0	0
13	Min Peak Value(Volts)	0	AUX1 STL(sec)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
14	Min Peak Time(ms)	0	AUX2 STL(sec)	0	0	0	0	0	0	0	0	0	0
15	Reserved	0	AUX3 STL(sec)	0	0	0	0	0	0	0	0	0	0
16	Reserved	0	AUX4 STL(sec)	0	0	0	0	0	0	0	0	0	0
17	Reserved	0	TONE STL(sec)	0	0	0	0	0	0	0	0	0	0
18	Reserved	0	NOISE STL(sec)	0	0	0	0	0	0	0	0	0	0
19	DAQ Gain(Volts)	10	NOISE STL(dB)	0	0	0	0	0	0	0	0	0	0
20	DAQ Frequency(Hz)	1000	Trial Comment 1										
21	Reserved	0	Trial Comment 2										
22	Reserved	0	CS-STL DELAY(sec)	0	0	0	0	0	0	0	0	0	0

Experiment Initiated by the "FPSTest.txt" Procedure

The second procedure contains 27 Trials and is used to test a subject that has been trained using the "FPSTrain.txt" procedure.

This experiment is described in the "FPSTest.txt" Experiment Table (shown in Figure 5.13). The procedure begins with 9 noise-burst only trials (columns **D** through **L**) that are followed by 18 trials (columns **M** through **AD**) of randomly selected noise-bursts and pre-pulses for the remainder of the experiment.

One special thing to note about this experiment is that the CS-DISPLAY(sec) is set to 30.2 seconds. This is so the program can record a 200ms NULL Period before the 30s Prepulse or CS Tone.

NOTE: While the Advanced Startle program will record and save all of the data to the .RAW file, it will not display more than 2 seconds of the data. To see all of the data at once the Startle Viewer needs to be used. **Chapter 7 | ADVANCED STARTLE DATA FILES** will have more information on how to read the data files.

Figure 5.13 - FPSTest.txt Experiment Table

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	STL-DISPLAY(sec)	0.6	ACC/ITI(sec)	300	60	60	60	60	60	60	60	60	60
2	CS-DISPLAY(sec)	30.2	AUX1 CS(sec)	0	0	0	0	0	0	0	0	0	0
3	CS-FREEZE(sec)	0	AUX2 CS(sec)	0	0	0	0	0	0	0	0	0	0
4	RISE-TIME-NOISE(ms)	3	AUX3 CS(sec)	0	0	0	0	0	0	0	0	0	0
5	RISE-TIME-TONE(ms)	3	AUX4 CS(sec)	0	0	0	0	0	0	0	0	0	0
6	LATENCY(ms)	0	TONE CS(sec)	0	0	0	0	0	0	0	0	0	30
7	BACKGROUND(dB)	0	NOISE CS(sec)	0	0	0	0	0	0	0	0	0	0
8	AUX1(1=Always on)	0	NOISE CS(dB)	0	0	0	0	0	0	0	0	0	0
9	AUX2(1=Always on)	0	TONE CS(dB)	0	0	0	0	0	0	0	0	0	70
10	AUX3(1=Always on)	0	TONE CS(Hz)	0	0	0	0	0	0	0	0	0	12000
11	AUX4(1=Always on)	0	TONE STL(Hz)	0	0	0	0	0	0	0	0	0	0
12	Peak Calc. Method	0	TONE STL(dB)	0	0	0	0	0	0	0	0	0	0
13	Min Peak Value(Volts)	0	AUX1 STL(sec)	0	0	0	0	0	0	0	0	0	0
14	Min Peak Time(ms)	0	AUX2 STL(sec)	0	0	0	0	0	0	0	0	0	0
15	Reserved	0	AUX3 STL(sec)	0	0	0	0	0	0	0	0	0	0
16	Reserved	0	AUX4 STL(sec)	0	0	0	0	0	0	0	0	0	0
17	Reserved	0	TONE STL(sec)	0	0	0	0	0	0	0	0	0	0
18	Reserved	0	NOISE STL(sec)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
19	DAQ Gain(Volts)	10	NOISE STL(dB)	105	110	100	110	100	105	100	105	110	105
20	DAQ Frequency(Hz)	1000	Trial Comment 1	NULL	NULL	NULL	NULL	NULL	NULL	NULL	NULL	NULL	12k Tone
21	Reserved	0	Trial Comment 2	105dB NB	110dB NB	100dB NB	110dB NB	100dB NB	105dB NB	100dB NB	105dB NB	110dB NB	105dB NB
22	Reserved	0	CS-STL DELAY(sec)	0	0	0	0	0	0	0	0	0	0

CHAPTER 6 | CUSTOMIZING TABLES IN MICROSOFT EXCEL

Modifying experimental parameters to create experiments is easy with Advanced Startle. All experimental tables conclude with ".txt," but it is recommended that tables be created using a spreadsheet program such as Microsoft Excel and then saved as a "tab-delimited, text format (.txt)" file. To modify the value for any parameter in the table, enter the desired value in the correct row/column location, and save the file as described. Then open Advanced Startle, and select the new experiment file using the **Main Menu | Load Experiment** menu choice.

Extracting Data from a text file into Microsoft Excel

A spreadsheet program can be used to open data saved as an ASCII text file (.txt). This guideline illustrates an example of how to open ASCII data using Microsoft Excel 2007.

1. In Excel click on the Microsoft logo in the upper left hand corner and select **Open**. Locate where the file has been saved. Select **Text Files (*.prn; *.txt; *.csv)** in the file-type drop down menu.
2. Choose the desired data file table to edit and click **Open**. This will automatically open the first of three windows for the Text Import Wizard.
3. The data file is tab-delimited, so make sure to select "**Delimited**" in the Original data type box.
4. Make sure that **Start the import at row:** is set to "1," and the **File origin:** is set to "437 : OEM United States." Click **Next** to bring up the second of the three-step process.
5. Make sure that the **Tab** Delimiters check box is checked, and then click **Next**.
6. The final stage of the import wizard should now be open. Make sure that "**General**" is selected in the Column data format box and click **Finish**.

The desired data file table should appear in an Excel spreadsheet upon completing the Text Import Wizard.

CHAPTER 7 | ADVANCED STARTLE DATA FILES

Advanced Startle creates two data files when recording data from an experiment.

The first file type has a .RAW extension. It is a binary data file that contains all of the data from all of the trials that were run during the experiment. The data from all eight chambers is saved in this data file even if you don't have eight chambers or are not running animals in all eight chambers. The data in this file can only be read by the Startle Viewer program (see Chapter 8| Using Startle Viewer).

The second file type has a .TXT extension. Below is a sample data file:

C:\Program Files\MED Associates\Advanced Startle\Tables\TableDefinitions.txt
C:\Users\Sample.txt

2/5/2003
11:34:57 AM

				Chamber Number							
Trial Number	Startle Start	Comments		1	2	3	4	5	6	7	8
1	0:10	CS1	NB	0.005	0.688	1.025	0.01	0.005	1.108	0.815	0.742
2	0:20	CS2	NB	0.005	0.229	0.347	0.005	0.005	0.322	0.259	0.254
3	0:30	CS3	AUX1	0.005	0.151	0.225	0.005	0.005	0.215	0.166	0.146
4	0:40	CS4	Text	0.005	0.146	0.229	0.005	0.005	0.205	0.181	0.22
5	0:51	CS5	Trial 5	0.005	0.127	0.195	0.01	0.005	0.171	0.137	0.137
6	1:01	CS6	NB	0.005	0.083	0.386	16.196	0.005	0.127	0.117	0.166
7	1:12	CS7	NB	0.005	0.107	0.093	0.005	0.005	0.151	0.112	0.098
8	1:23	CS8	NB	0.005	0.093	0.083	0.005	0.005	0.122	0.073	0.146
9	1:33	CS9	NB	0.005	0.112	0.151	0.005	0.005	0.156	0.127	0.132
10	1:44	CS10	Last Trial	0.005	0.103	0.142	0.005	0.005	0.146	0.112	0.103

This data file contains the number of trials that were run, the time of the beginning of each trial (Startle Start), and any comments (Trial Comment 1 and Trial Comment 2) that were saved along with the experiment. The data file also contains the max Peak-to-Peak voltage that was recorded during the trials for each chamber.

The data in this file is read by the Startle Viewer and displayed in the table at the top of the program.

CHAPTER 8 | USING STARTLE VIEWER

The Advanced Startle Software also comes with the Startle Viewer program. This software allows the researcher to examine the details of the startle reflex and manually adjust the scale of the primary dependent measure. Use Startle Viewer to quantify the amplitudes and frequencies of the startle response.

Menu Selections

After opening Startle Viewer, three options appear in the upper left corner of the screen. These options (and corresponding keystroke commands) are:

File (Alt+F):

- Open (Ctrl+O):
  - New File (Ctrl+F): This menu option allows the User to open a new or different data file and import the data. Readable data files have a ".RAW" extension.
  - Chamber (Ctrl+H): This menu option allows the User to import the data from a different chamber. The selected data file remains the same.
- Save (Ctrl+S): Saves the Startle Viewer file to a specified directory.
- Quit (Ctrl+Q): Exits Startle Viewer.
- About (Ctrl+A): Provides information regarding the Startle Viewer program.

Edit (Alt+E):

- Cut (Ctrl+X)
- Copy (Ctrl+C)
- Paste (Ctrl+V)

Export (Alt+P): Saves the ".RAW" file in ASCII tabbed-file format

- Compression (Ctrl+M): Select how the file is compressed. Compressing using 2X, 5X, or 10X saves every second, fifth, or tenth data point. None means the file will not compress.
- Range (Ctrl+R)
  - Chamber (Ctrl+R): Saves all the trial information for that chamber.
  - Trial (Ctrl+T): Saves only the data for that trial.

Opening a Data File

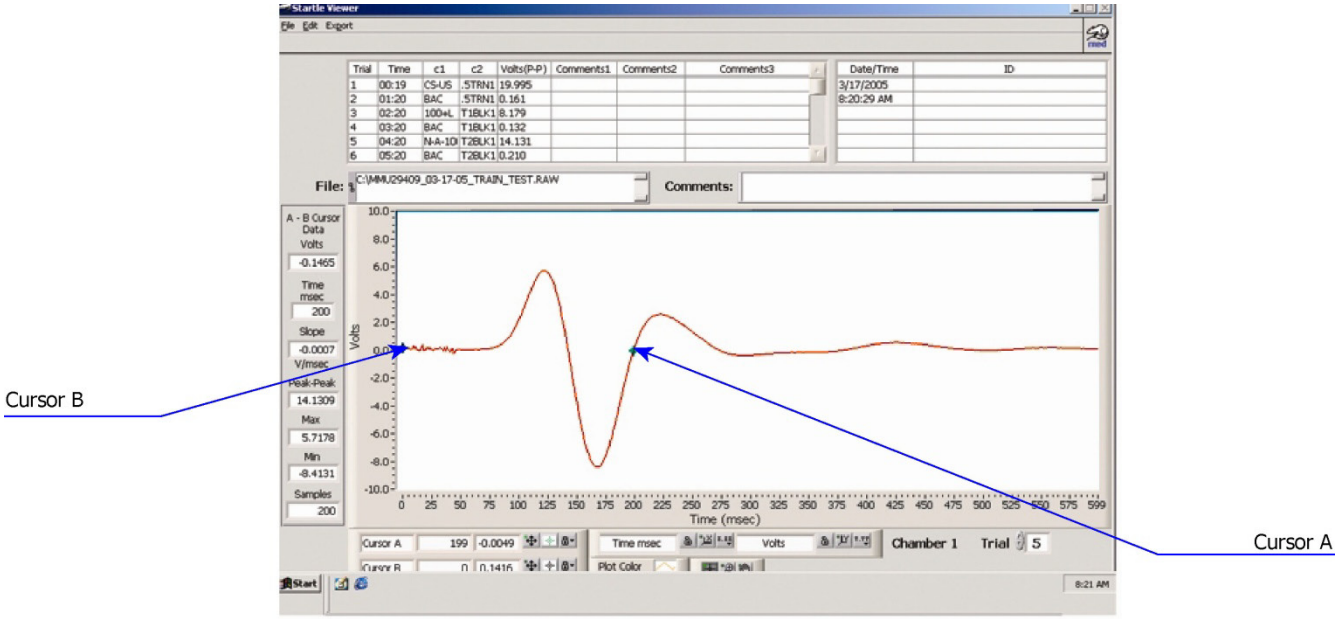
A window automatically appears upon opening Startle Viewer that prompts the user to select a data file. Find the appropriate directory, and select the data file to view. Readable data files have a ".RAW" extension. Another window will appear after opening the desired data file, and select the appropriate chamber to import the data from that chamber.

The Table Display

Startle Viewer allows the user to view the startle load cell output, in volts, for any particular trial. The Startle Viewer table window lists the maximum startle output by trial number and any comments (Trial Comment 1 & Trial Comment 2) that were provided for that particular trial. Note that .RAW data files are matched to .TXT files with the same name, and the .TXT file contains the data that is displayed in the table at the top of the program.

Viewing a Particular Startle Response

Figure 8.1 - Startle Viewer Window



The primary feature of Startle Viewer is the voltage graph (Figure 8.1). Select the particular trial to view on the graph by either double-clicking on the trial number in the table window, or by using the up/down switch in the lower right corner of the screen labeled "Trial."

Use Cursors "A" and "B" to pinpoint and quantify certain locations on the graph. For instance, to quantify the peak-to-peak frequency (in milliseconds), place cursor A on one peak and cursor B on the subsequent peak. Doing this also allows the user to quantify maximum peak amplitude within a specified period of time. Another benefit of Startle Viewer is that it determines the slope of a line (in volts per millisecond) for any selected peak amplitude.

The X-axis and Y-axis can be modified by clicking on the icons in the lower portion of the screen. The precision and notation of each dimension can be altered, and the overall scale of the graph can be modified using the "magnifying-glass" icon and the zoom function.

Startle Viewer can be used to generate data regarding startle latency (a dependent measurement taken in milliseconds), which is defined as the time period in between the white-noise (startle stimulus) presentation and the maximal startle response. Select the specific trial to examine and the startle response will appear in the main display. Fix cursor "A" on time point zero and move cursor "B" to the peak-positive amplitude for that trial. Note the difference between cursor positions along the X-axis.

APPENDIX A| REFERENCES

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APPENDIX B| CONTACT INFORMATION

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

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